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Investigation into the fate of ^{14}C-labelled xenobiotics (naphthalene, phenanthrene, 2,4,5,2′,4′,5′-hexachlorobiphenyl, octachlorostyrene) in Bermudian corals

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ABSTRACT: Uptake and elimination of 4 labelled lipid-soluble xenobiotics (naphthalene, phenanthrene, 2,4,5,2′,4′,5′-hexachlorobiphenyl (PCB), octachlorostyrene) were studied in 19 anthozoans and 1 hydrozoan common to Bermudian waters. The concentration of radioactivity in the tissues was determined using liquid scintillation counting. All organisms tested took up radioactivity from the water. However, elimination rates were very slow compared to those of other marine organisms. Naphthalene was the most rapidly eliminated compound of the 4 tested. There was a much higher concentration of phenanthrene in the tissues than octachlorostyrene throughout the entire experiment. Tissue samples from *Diploria strigosa* taken 9 mo after exposure to PCB contained 84 % of the original radioactivity. No general trend or correlation between species and amount of radioactivity taken up was observed.

INTRODUCTION

Coral reefs are unusually diverse and complex. They often play a vital role as a fishery resource, as barriers against coastal erosion, and as asset to tourist industries. Primary components of the coral reef ecosystem are hermatypic (reefbuilding) corals. In addition to their structural role, they provide shelter, substrate, and nutrients for the reef community (Odum and Odum, 1955).

Recently, concern has focused on the effects of oil terminals, tanker traffic and refineries on reefs in the Caribbean, Persian Gulf and Red Sea (Loya and Rinkevich, 1980). Shipping routes often lie close to coral reef areas and hazardous spills have occurred and will undoubtedly continue.

The fate of xenobiotics in coral tissues can provide insight into the effect of pollution on a reef ecosystem. Long-term accumulation in coral tissue may act as a consistent source of contamination, not least due to release of lipids from corals during mucus production (Ducklow and Mitchell, 1979). Thus, lipid-soluble xenobiotics in coral tissues may affect, over extended periods of time, other marine organisms in the reef community.

In order to determine immediate and long-term effects of oil hydrocarbons on corals (Knap et al., 1983), we have developed a method for assessing uptake and depuration of the radiolabelled hydrocarbon ^{14}C-phenanthrene by the scleractinian coral *Diploria strigosa* (Knap et al., 1982). This study indicated rapid uptake of this hydrocarbon and relatively slow depuration, compared with other subtropical marine organisms (Solbakken et al., 1982a, b). In order to test the intercomparability of our results on uptake and depuration (Knap et al., 1982) with other corals from Bermuda, we used a similar technique with an improved normalization method to determine the fate of selected xenobiotics on 1 hydrozoan and 19 anthozoans collected from the Bermuda platform (Fig. 1, Table 1). In this investigation 2 aromatic hydrocarbons (naphthalene, phenanthrene), 1 PCB (2,4,5,2′,4′,5′-hexachlorobiphenyl, IUPAC no. 153) and octachlorostyrene were chosen to represent components from different sources of pollution. Naphthalene and phenanthrene are found in oil. Chlorinated biphenyls are very persis-

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tent in the environment and are globally distributed. Octachlorostyrene is a byproduct of magnesium metal production.

MATERIALS AND METHODS

Coral colonies (Table 1) were collected in August 1982 from various reef sites around Bermuda (Fig. 1) and transferred to a clean flowing seawater system (36 ‰, flowrate 2 l min^{-1}) in the laboratory. Corals were divided into groups for experiments following acclimation (5 d). Brain corals *Diploria strigosa* (n = 16) and green cactus corals *Madracis decactis* (n = 8) were transferred into 4 aerated tanks containing ^{14}C-labelled naphthalene, phenanthrene, 2,4,5,2′,4′,5′-hexachlorobiphenyl, and octachlorostyrene, respectively, for 24 h exposure (Table 2). One colony of each of the other coral species (Table 1) was transferred into 2 separate aerated tanks (12 l) and dosed for 24 h with ^{14}C-labelled phenanthrene and octachlorostyrene, respectively (Table 2).

Labelled components, dissolved in 200 µl ethanol, were thoroughly mixed with 100 ml seawater, diluted to a larger volume of seawater (12 to 15 l), and then mixed well before addition of experimental organisms. After exposure, corals were transferred to aquaria in the flow-through seawater system (2 l min^{-1} in each tank) in the laboratory for a depuration period of 37 d.

Tissue samples (1 to 2 cm^2) were taken immediately after exposure and after 2, 4, 10, 21, and 37 d using a small chisel and hammer. Different areas of the colony were sampled in order to ensure healthy, representative specimens. The tissues were digested in 1 ml of 2 N NaOH. The protein content of each sample was determined by removing a small aliquot (0.1 ml) of the NaOH solution, neutralizing with 2 N HCl, and

Table 1. Taxa tested. For collection sites consult Fig. 1

Class: Hydrozoa	
Order: Hydrocorallina	
Family: Milleporidae	
1. *Millipora alcicornis*	Fire coral
Class: Anthozoa	
Sub-class: Octocorallia	
Order: Gorgonacea	
2. *Plexaura flexuosa*	
Sub-class: Zoantharia	
Order: Zoantinidae	
3. *Palythoa mamillosa*	
Sub-class: Hexacorallia	
Order: Scleractinia	
Sub-order: Astrocoeniida	
Family: Astrocoeniidae	
4. *Stephanocoenia michelini*	Blushing star coral
Family: Seriatoporidae	
5. *Madracis decactis*	Green cactus coral
6. *M. mirabilis*	Yellow pencil coral
Sub-order: Fungiida	
Family: Agariciidae	
7. *Agaricia fragilis*	Hat coral
Family: Siderastreidae	
8. *Siderastrea* sp.	Starlet coral
Family: Poritidae	
9. *Porites asteroides*	Mustard hill coral
10. *P. porites*	Clubbed finger coral
Sub-order: Faviida	
Family: Faviidae	
11. *Favia fragum*	Golf ball coral
12. *Diploria labyrinthiformis*	Brain coral
13. *D. strigosa*	Common brain coral
14. *Monastrea annularis*	Common star coral
15. *M. cavernosa*	Large star coral
Family: Oculinida	
16. *Oculina* sp.	Ivory tree coral
Family: Trochosmiliidae	
17. *Meandrina meandrites*	Rose coral
18. *Dichocoenia stokesii*	Elliptical star coral
Family: Mussidae	
19. *Scolymia lacera*	Mushrom coral
20. *Isophyllia sinuosa*	Rose coral

Table 2. Components used and initial concentration of radioactivity in exposure tanks

Component	Specific activities (MBq $mmol^{-1}$)	Initial concentration of radioactivity in seawater, Dpm ml^{-1} and [µg l^{-1}]	
		D. strigosa *M. decactis*	All other corals
[1(4, 5, 8)-^{14}C]Naphthalene	185 (Amersham)	1369 [16]• (1353, 1385)••	
[9-^{14}C]Phenanthrene	714 (Amersham)	745 [3] (733, 757)	3881 [16] (3; 88)[a]
[2, 4, 5, 2′, 4′, 5′-^{14}C]Hexachlorobiphenyl (PCB)	766 (New England Nuclear)	2023 [15] (2002, 2043)	
[^{14}C]Octachlorostyrene	321 (New England Nuclear)	7164 [141] (3; 3744)[a]	754 [15] (3; 42)

• Mean value; •• Observed values; [a] n, SEM

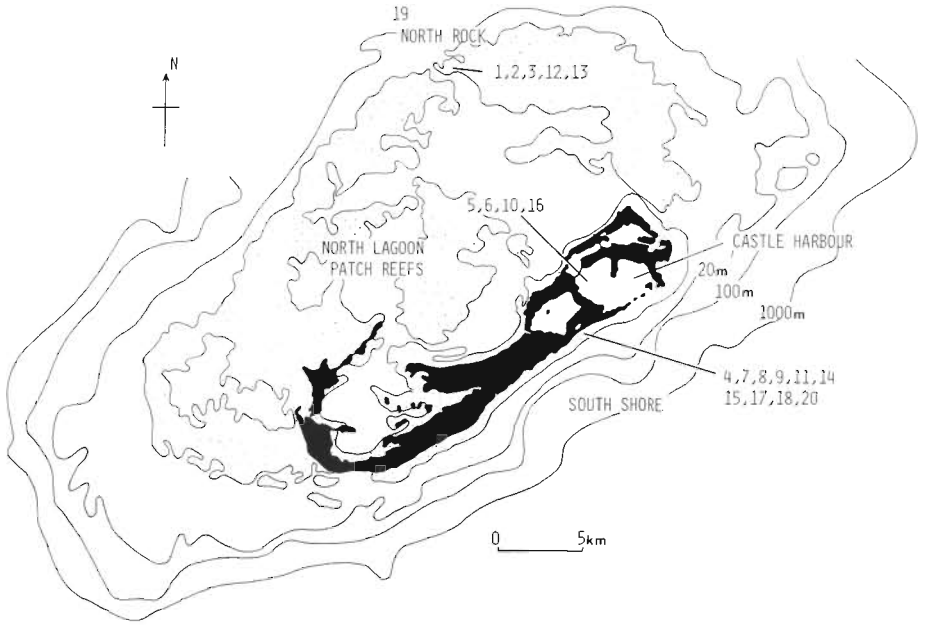

Fig. 1. Map of Bermuda showing collection sites. Numbers refer to species names listed in Table 1

employing the Bio-Rad protein assay technique. Human blood serum (3 C Coulter chemistry calibrator) was used as a standard. The precision of the protein assay technique was checked by running replicates of several coral samples (S.D. < 5 %). The remaining portion of the sample was bleached using 0.1 ml H_2O_2. Ten ml of Dimilume-30 (Packard Instrument Co.) were added to each sample and the pieces of skeleton were removed. The coral tissue analyzed also included the associated parts of a coral colony including symbiotic algae (zooxanthellae) tissues. Scintillation counting was performed on a Packard 300 CD liquid scintillation counter. During sampling, behaviour and physical appearance of the corals were checked regularly and unhealthy specimens were removed. Following the 21 d sampling, *Diploria strigosa* colonies dosed with PCB, phenanthrene or octachlorostyrene were returned to their natural reef environment for long-term depuration studies.

RESULTS AND DISCUSSION

Concentrations of radioactivity in corals (Tables 3 to 5) show that the lipid-soluble xenobiotics used in these experiments were readily taken up by the corals. Radioactivity accumulated was not only a function of species, but also of the specific component. In *Diploria strigosa* and *Madracis decactis* uptake of aromatic hydrocarbons (naphthalene, phenanthrene) was much greater than that of chlorinated components (PCB, octachlorostyrene). Most of the other corals exhibited a similar trend when dosed with phenanthrene and octachlorostyrene.

Of the species tested, the octocoral *Plexaura flexuosa* and the zoanthid *Palythoa mamillosa* appeared to take up less ^{14}C-labelled phenanthrene than did the scleractinians, with the exception of *Diploria strigosa*. There was a similar trend in the octachlorostyrene experiment. We cannot explain these results other than to attribute them to taxonomic differences. For *Diploria strigosa* the amount of uptake was far lower than in our previous experiments (Knap et al., 1982). In this study we used one tenth of the concentration of radioactivity in the water compared to the previous study. This resulted in lower concentrations in the coral tissue. However, when normalized to water concentration, uptake was similar in both studies.

Depuration of phenanthrene and octachlorostyrene occurs irrespective of the class of organism. There also appears to be no specific trend of depuration within sub-order or family. In *Diploria strigosa* depuration rate was slower than in most of the other scleractinians. However, this may be an artifact due to the very low uptake of labelled components.

The most rapid elimination of the ^{14}C-labelled components occurred during the first 10 d. There was a much higher concentration of phenanthrene than octachlorostyrene throughout the entire experiment. A more efficient elimination of phenanthrene, compared to octachlorostyrene, therefore does not occur in corals, unlike in other marine organisms such as flounder *Platichthys flesus* (Solbakken, unpubl.). The chlorinated biphenyl does not appear to be depurated rapidly in the 2 corals tested; this is similar to the findings of others using chlorinated biphenyls with other marine organisms (Bend et al., 1974).

Naphthalene was the most efficiently eliminated

Table 3. Concentration of radioactivity in corals at various time intervals following exposure to [9−^{14}C] phenanthrene. Values given as (dpm mg^{-1} protein)/(dpm ml^{-1} of initial concentration in seawater)

Species	Days after exposure								
	0	2	4	10	21	37	123	275	365
Millipora alcicornis	6.5	5.2	2.6	0.8	0.8	0.6			
Plexaura flexuosa	3.3	6.1	1.9	1.6	1.1	0.4			
Palythoa mamillosa	0.8	0.3	0.2	0.2	0.4	0.2			
Stephanocoenia michelinii	10.2	5.8	3.0	0.6	0.3	0.1			
Madracis decactis	4.7••	2.7••	2.4••	2.4••	0.8••[a]	−			
M. mirabilis	17.2	9.5	2.7	0.6	0.3	0.2			
Agaricia fragilis	16.6	12.3	7.2	2.0	1.0	0.3			
Siderastrea sp.	11.2	8.3	3.2	1.0	0.3	0.3			
Porites asteroides	7.6	5.4	3.2	2.4	1.6	0.7			
P. porites	8.9	16.4	5.6	6.6	1.8	0.8			
Favia fragum	21.0	25.8	12.6	−	1.5	+			
D. labyrinthiformis	12.2	9.2	2.1	1.4	0.8	0.5			
Diploria strigosa	2.6•	2.5•	2.1•	1.3•	0.6•[a]	−	0.04	0.01	0.01
	(0.3)	(0.1)	(0.2)	(0.1)	(0.2)		(0.01)	(0.003)	(0.003)
Monastrea annularis	22.9	19.9	6.1	3.7	1.4	1.1			
M. cavernosa	15.9	12.6	13.2	4.8	2.8	2.0			
Oculina sp.	12.7	13.7	5.2	2.0	0.8	0.4			
Meandrina meandrites	6.8	12.4	6.2	3.3	1.7	0.8			
Dichocoenia stokesi	7.3	8.5	4.9	0.8	+	+			
Scolymia cubensis	12.2	11.1	13.3	7.0	1.5	+			
Isophyllia sinuous	10.0	17.1	5.5	2.8	2.5	2.9			

• Mean of four samples (standard error of the mean, SEM)
•• Mean of two samples
[a] Returned to reef environment for long-term studies
\+ Died
− Not measured

component of the 4 tested. By Day 10, ca. 2 % of the initial concentration remained in *Diploria strigosa* and *Madracis decactis,* whereas the levels of the more lipid soluble components (hexachlorobiphenyl, octachlorostyrene, phenanthrene) were much higher. Efficient elimination of naphthalene, compared to phenanthrene, has been reported for other marine organisms (Solbakken et al., 1983b).

In subtropical marine molluscs *(Macrocallista maculata, Arca zebra)* and the teleost *Haemulon sciurus,* the ability to depurate phenanthrene is greater than in corals (Solbakken et al., 1982a, b; Solbakken et al., 1983a). In Bermudian sponges (*Verongia* sp., *Haliclona* sp.), however, elimination of phenanthrene is more related to that found for the organisms used in the present study (Solbakken, unpubl.).

Data obtained after 123, 275 and 365 d indicate that depuration of phenanthrene from corals is slow compared to other marine organisms (Solbakken et al., 1979, 1982a, b, 1983a, b; Solbakken and Palmork, 1980; Palmork and Solbakken, 1981). Four months after dosing, less than 2 % of the original ^{14}C-phenanthrene radioactivity and 3 % of the ^{14}C-octachlorostyrene remained in the tissue. In contrast, 2,4,5,2′,4′,5′-hexachlorobiphenyl was depurated very slowly in *Diploria strigosa*; even after 1 yr, one third of the original radioactivity was present.

Previous results on aspects of the biology of corals indicate a very high degree of variability in regard to behaviour, growth, and response to pollutants (Knap et al., 1983). This appears to be a common phenomenon when working with corals, as discussed by Barnes and Crossland (1982) and with regard to radioactive tracer studies (Neff and Anderson, 1981; Knap et al., 1982). In the present work there were a few cases where the concentration of radioactivity 2 d after dosing was higher than immediately after dosing. We assume that this is not due to the degree of analytical accuracy but rather to variations between different parts of the coral colony. All our results have been normalized to protein in order to eliminate variation caused by inhomogeneity of coral tissue which could lead to a variation in accumulation of radioactivity. Patchiness in the distribution of zooxanthellae may also contribute to some of the variability recorded. As the compounds used in these experiments are lipophilic, the

Table 4. Concentration of radioactivity in corals at various time intervals following exposure to ^{14}C-labelled octachlorostyrene. Values given as (dpm mg^{-1} protein)/(dpm ml^{-1} of initial concentration in seawater)

Species	Days after exposure								
	0	2	4	10	21	37	123	275	365
Millipora alcicornis	3.2	1.4	1.2	0.05	0.06	0.02			
Plexaura flexuosa	0.6	+	+	+	+	+			
Palythoa mamillosa	−	0.3	0.9	0.3	0.5	+			
Stephanocoenia michelinii	2.6	1.2	1.2	0.4	0.3	0.2			
Madracis decactis	1.0••	0.1••	0.6••	0.1••	0.02••[a]	−			
M. mirabilis	4.8	2.6	1.0	0.1	0.09	0.06			
Agaricia fragilis	10.3	5.9	2.9	−	1.0	0.4			
Siderastrea sp.	0.6	0.7	0.4	0.2	0.1	0.04			
Porites asteroides	2.6	6.1	4.8	0.2	0.3	0.2			
P. porites	6.9	−	3.0	2.3	2.4	1.1			
Favia fragum	6.7	12.0	4.3	2.1	1.6	+			
D. labyrinthiformis	2.5	4.9	0.4	0.6	0.3	+			
Diploria strigosa	0.3• (0.01)	0.2• (0.03)	0.2• (0.02)	0.1• (0.02)	0.1•[a] (0.01)	−	0.01 (0.01)	0.01 (0.01)	0.005 (0.002)
Monastrea annularis	2.2	4.7	3.1	1.0	0.7	0.7			
M. cavernosa	12.0	3.9	1.4	0.4	0.2	0.2			
Oculina sp.	5.4	+	+	+	+	+			
Meandrina meandrites	1.2	3.7	0.7	0.4	0.1	0.1			
Dichocoenia stokesi	1.9	−	1.0	0.2	0.1	+			
Scolymia cubensis	26.7	22.6	9.4	5.9	4.8	3.0			
Isophyllia sinuous	11.4	13.5	5.0	3.4	+	+			

• Mean of four samples (SEM)
•• Mean of two samples
[a] Returned to reef environment for long term studies
+ Died
− Not measured

Table 5. Concentration of radioactivity (dpm mg^{-1} protein)/(dpm ml^{-1} of initial concentration in seawater) in *Diploria strigosa* and *Madracis decactis* following 24 h exposure to PCB and naphthalene

Species	Days after exposure						
	0	2	4	10	21	123	275
			2, 4, 5, 2′, 4′, 5′-Hexachlorobiphenyl				
D. strigosa	0.3• (0.01)	0.3 (0.01)	0.3 (0.04)	0.3 (0.04)	0.2[a] (0.03)	0.2••[a]	0.2
M. decactis	0.8••	1.1	0.7	2.3	0.3[a]		
			Naphthalene				
D. strigosa	1.3• (0.3)	0.1 (0.04)	0.05 (0.01)	0.02 (0.001)	0.01 (0.001)		
M. decactis	2.3	0.4	0.1	0.05	0.03		

• Mean of four samples (SEM)
•• Mean of two samples
[a] Organisms returned to reef environment for long-term elimination studies

normalization of the results to lipid might have resulted in less variation. However, based on more detailed work on the biology of these corals in their response to pollutants (Knap et al., 1983; Cook and Knap, pers. comm.), high variability appears to be an important aspect of coral lipid biology.

CONCLUSIONS

Bermuda reef building corals take up xenobiotics from seawater. Elimination of naphthalene from the corals was most rapid; elimination of phenanthrene, octachlorostyrene, and 2,4,5,2′,4′,5′-hexachlorobi-

phenyl was slow, and significant amounts of radioactivity were present 21 to 37 d following exposure. Long-term depuration studies of *Diploria strigosa* returned to the field after dosing have thus far shown that radioactivity was still present in the tissues even after 1 yr. Individual corals appear to be extremely variable in their ability to take up and to depurate these xenobiotics. The use of radio-labelled tracers is an important tool in assessing the possible long-term impact of pollutants on coral ecosystems, since individuals can be sub-sampled without sacrificing the whole colony at each sampling.

Persistence of xenobiotics is important when evaluating pollutants' effects on coral reefs. Long-term accumulation increases the possibility of mobilization of chemicals throughout these important communities of tropical and sub-tropical marine areas long after the pollutant source has been removed.

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