ACCUMULATION, RELEASE, AND DISTRIBUTION OF BENZO[A]PYRENE-C^{14} IN THE CLAM *RANGIA CUNEATA*

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ABSTRACT

*Groups of estuarine clams, **Rangia cuneata**, were exposed for 24 hours to synthetic seawater containing in solution 0.0305 ppm benzo[a]pyrene-C^{14}. In two experimental exposures, average total concentrations of benzo[a]pyrene (BAP) in the clam tissues were 7.2 ppm and 5.7 ppm, approximately 200 times above the ambient level. The majority of the radioactivity was localized in the viscera which contains the digestive system, gonads, and heart. The other tissues analyzed, the mantle, gills, adductor muscle, and foot, each contained 3-16% of the radioactivity. When returned to isotope-free seawater, the clams immediately began to release the accumulated BAP. After 30 days only 0.07 ppm BAP remained in the tissues. BAP could not be detected (limits of detection, 0.01 ppm) in clams maintained in isotope-free seawater for 58 days. During depuration, the distribution of radioactivity in the tissues remained relatively constant. The viscera contained most of the activity at all sampling times.*

INTRODUCTION

Crude and refined petroleums are extremely complex mixtures of thousands of different hydrocarbons and related substances, some of which are known to be highly toxic to marine animals and toxic or carcinogenic to consumers of marine fisheries products. The high-boiling fractions of petroleum as well as pyrolysis products resulting from incomplete combustion of oil contain significant amounts of polycyclic aromatic hydrocarbons (PAH). Some PAH are known to be potent carcinogens. Graf and Winter [1] found 0.4–1.6 ppm (milligrams/kilogram) of BAP, the most widely studied of the carcinogenic PAH, in three crude oils. They found that spent crank case oil contained 5.8 ppm BAP.

Charter et al. [2] have estimated that the annual input of petroleum to the oceans from all sources is in excess of 3 million tons. Therefore, significant amounts of PAH enter the marine environment via this route. When exposed to oil-contaminated seawater, marine animals rapidly accumulate a wide variety of different petroleum hydrocarbons in their tissues [3]. However, very little information is available concerning the extent to which oil-derived PAH are accumulated. Lee et al. [4,5] showed that the marine mussel, *Mytilus edulis*, and several species of marine fish were capable of accumulating BAP from dilute seawater solution. However, they provided little information about the distribution of BAP in the tissues of the molluscs or on the rate of BAP release when the mussels were returned to clean seawater. To better understand the health hazards that might arise from the consumption by man of oil-contaminated shellfish, we have investigated the accumulation during exposure and release following exposure of the polycyclic aromatic hydrocarbon benzo[a]pyrene by the estuarine clam *Rangia cuneata*.

Materials and Methods

Clams *Rangia cuneata* were collected from San Antonio and Trinity Bay, Texas. They were maintained in the laboratory in large aquaria containing artificial seawater (Instant Ocean, Aquarium Systems, Inc.) at 20 ± 2°C and 20 o/oo salinity. For exposure, groups of 30-48 clams were placed in large circular all-Pyrex tanks with 12 liters of 20 o/oo salinity seawater containing in solution 0.0305 ppm 3,4 (benz-3,6-C^{14}) pyrene (Amersham/Searle, specific activity 21 millicuries/millimole). The seawater was stirred gently with a magnetic stirrer but was not artificially aerated during the exposure period. After 24-hour exposure, the clams were removed and placed in large aquaria containing isotope-free seawater which was continuously recirculated through activated charcoal filters. At the end of the exposure period, and at different times after return to hydrocarbon-free seawater, 3-8 clams were sacrificed and dissected into gills, adductor muscles, foot, mantle, and visceral mass. The body regions of each clam were weighed and then homogenized in n-hexane. The homogenates were centrifuged and the supernatant hexane layers were saved. Aliquots of the hexane extracts were evaporated to dryness, the residue dissolved in Aquasol (Nuclear Chicago, Inc.), and the radioactivity counted on a Beckman model DPM-200 liquid scintillation counter. The total radioactivity accumulated by each clam was computed as the sum of the radioactivity counted in each body region. This value was used to compute the total concentration of BAP in each clam based on the specific activity of the BAP-C^{14} in the exposure water.

Results

In the first experiment (table 1), the clams were exposed to 0.0305 ppm BAP-C^{14} for 24 hours and then allowed to depurate for up to 480 hours (20 days). After 24-hour exposure, the clams had a mean of 7.2 ppm (micrograms/gram wet weight of tissue) BAP in their tissues. Nearly 75% of the radioactivity was localized in the viscera which includes the digestive system, gonads, and heart. The mantle, gills, adductor muscles, and foot each had from 3.5-10% of the total radioactivity.

When returned to isotope-free seawater, the clams immediately began to release the accumulated BAP from their tissues. More than 70% of the BAP was released in 10 days. After 20 days in isotope-free seawater, only 0.1 ppm BAP remained in the clam tissues. During depuration, the distribution of radioactivity in the tissues remained relatively constant with the viscera containing the majority of the radioactivity at all sampling times.

A second experiment (table 2) was designed to determine the amount of time required for complete release of BAP to undetectable levels (less than 0.01 ppm). After 24-hour exposure, the clams had a mean of 5.7 ppm BAP in their tissues. In general, the tissue

Table 1. Uptake, tissue distribution, and release of benzo[a]pyrene-C^{14} in *Rangia cuneata* exposed to 0.0305 ppm BAP-C^{14} for 24 hours

Sampling time	Mean total ppm BAP per animal ± S.D.	% of 24-hour concentration remaining	Mean percentage total radioactivity per tissue fraction ± S.D.				
			Viscera	Mantle	Gill	Adductor	Foot
24-hour uptake (1 day)	7.2 ± 12.8 (5)*	–	74.7 ± 15.1	10.1 ± 6.5	6.8 ± 4.3	4.9 ± 2.7	3.5 ± 2.0
144-hour depuration (6 days)	6.5 ± 5 (4)	90	81.2 ± 14.9	7.1 ± 6.2	2.9 ± 2.5	5.0 ± 3.5	3.9 ± 3.0
244-hour depuration (10 days)	2.1 ± .97 (5)	29	82.2 ± 12.3	5.8 ± 3.3	4.1 ± 4.4	4.5 ± 3.2	3.4 ± 1.6
312-hour depuration (13 days)	1.4 ± .94 (4)	19	82.7 ± 8.8	7.4 ± 3.2	2.8 ± 2.8	4.4 ± 1.8	2.8 ± 2.6
480-hour depuration (20 days)	0.10 (1)†	1.4	58.8	14.3	7.1	9.4	10.5

*Number of clams sampled
†Only one sample taken at this time

distribution of the radioactivity was similar to that observed in the first experiment. The viscera contained approximately 65% of the radioactivity and the remaining tissues each contained 3-16% of the radioactivity. After 30 days in isotope-free seawater, only 0.07 ppm BAP remained in the tissues. No radioactivity above background levels could be detected in the tissues of clams sacrificed following 58 days in clean seawater.

In both experiments, there was considerable individual variation in the total concentration of BAP in the clams and in the distribution of radioactivity in their tissues as indicated by the large standard deviations. This variability is typical in bivalve molluscs which are able to close their valves and remain isolated from the external environment for variable periods of time.

Discussion

The present investigation has shown that the estuarine clam *Rangia cuneata* is capable of rapidly accumulating and concentrating the polycyclic aromatic hydrocarbon benzo[a]pyrene from dilute solution in seawater. In the two experiments reported here, the clams accumulated average totals of 7.2 ppm and 5.7 ppm BAP in their tissues during 24-hour exposure to 0.03 ppm BAP. This represents average magnification factors (BAP in tissues/BAP in exposure water) of 236 and 187 respectively. These magnification factors are higher than those obtained for naphthalene and alkylnaphthalenes in clams exposed to water-soluble fractions of oil for 24 hours [6]. Petrocelli et al. [7] showed the *R. cuneata* accumulated the organochlorine insecticide dieldrin to levels approximately 200 times above ambient, a value similar to those reported here for BAP.

When the clams were returned to isotope-free seawater, they released the accumulated BAP from their tissues. Complete depuration to nondetectable levels (> 0.01 ppm) required from 30 to 58 days. This rate of depuration is similar to or slightly lower than the rate of depuration of other petroleum hydrocarbons by *Rangia* [8].

At all time intervals, the viscera contained more than half of the total accumulated radioactivity. Lee et al. [4] obtained similar results with C^{14}-heptadecane and C^{14}-naphthalene in the mussel *Mytilus edulis* and suggested that the hepatopancreas (contained in the visceral mass) was the site of hydrocarbon storage in these molluscs. Hydrocarbon storage in this tissue is probably due to its high lipid content.

Although the present investigation and the work of Lee et al. [4] have demonstrated that marine molluscs are capable of accumulating BAP to levels well above ambient, it has not yet been conclusively demonstrated that they accumulate this compound from oil in the natural environment. BAP and other PAH have been detected in the tissues of marine invertebrates and fish from many different locations. Tissue concentrations of BAP from nil to more than 2.0 ppm have been recorded [9,10,11,12,13,14]. The higher levels of contamination were usually associated with polluted habitats. It should be recognized, however, that petroleum is not the

Table 2. Uptake, tissue distribution, and release of benzo[a]pyrene-C^{14} in *Rangia cuneata* exposed to 0.0305 ppm BAP-C^{14} for 24 hours

Sampling time	Mean total ppm BAP per animal ± S.D.	% of 24-hour concentration remaining	Mean percentage total radioactivity per tissue fraction ± S.D.				
			Viscera	Mantle	Gill	Adductor	Foot
24-hour uptake (1 day)	5.7 ± 4.0 (8)*	–	64.5 ± 10.0	16.2 ± 11.6	12.0 ± 3.4	4.3 ± 1.7	2.94 ± 0.6
144-hour depuration (6 days)	0.34 ± 0.26 (8)	6.0	95.3 ± 6.4	2.8 ± 3.4	1.8 ± 3.2	>0.05	>0.05
720-hour depuration (30 days)	0.07 ± 0.07 (8)	1.2	58.5 ± 32.0	19.3 ± 16.0	12.6 ± 19.0	5.4 ± 6.0	8.7 ± 16.0
1392-hour depuration (58 days)	>0.01 (10)	>0.2	–	–	–	–	–

*Number of clams sampled

sole source of PAH entering the marine environment. Some types of PAH are synthesized by microorganisms, algae, and higher plants [13,14,15]. Significant amounts of PAH are produced by refuse burning and coke production, and some of this airborne PAH may reach the marine environment in rain. Therefore, there is a strong need for more information concerning the sources, distribution, and persistance of carcinogenic polycyclic aromatic hydrocarbons in the marine environment.

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