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Characterization of Polycyclic Aromatic Hydrocarbons (PAHs) by the Kinetics of Depuration in Bivalve Molluscs, Mercenaria Mercenaria

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CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) BY THE KINETICS OF DEPURATION IN BIVALVE MOLLUSCS, *MERCENARIA MERCENARIA*

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ABSTRACT

The objectives of this study were to examine depuration aspects of polycyclic aromatic hydrocarbons (PAHs) in a hard-shell clam *Mercenaria mercenaria*, and to characterize PAHs by the depuration kinetics. In this investigation, clams were exposed to artificial sea water containing a mixture of eight PAHs (i.e., naphthalene, fluorene, phenanthrene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[a]pyrene) for 48 hours. The clams were then transferred into clean (PAH-free) artificial seawater for release, and sampled at predetermined intervals. The target PAHs were extracted from the clam tissue and quantified by a gas chromatograph equipped with a capillary glass column and FID.

The results revealed single- and multi-component release mechanisms which were described by single- and multi-stage first-order kinetics, respectively. Benz[a]anthracene and benzo[a]pyrene exhibited the single-stage depuration, while naphthalene, fluorene, phenanthrene, fluoranthene, pyrene, and chrysene showed the two-stage depuration.

KEYWORDS

Clams; mollusc, *Mercenaria mercenaria*; polycyclic aromatic hydrocarbons; depuration; kinetics.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment. The major routes of entry of PAHs into marine waters include discharges of domestic/industrial wastes, fall/rainout from air, urban runoff, creosoted wharfs and pilings, and spillage/seepage of fossil fuels. In the ocean, some PAH compounds are quickly volatilized and degraded, but others are likely to be taken-up, assimilated and concentrated by marine species. Many aquatic organisms tend to absorb PAHs from water as a result of equilibrium partitioning between organism lipids and water.

Increased levels of PAHs in molluscs are of significant importance in the light of public health as well as economic concerns. The consumption of molluscs that have been exposed to PAHs at/or above ambient

concentrations in urban estuaries, may expose the consumer to PAHs potentially above normal health risk levels (Tanacredi, 1988). Lee *et al.* (1972), after 4 hours of exposure to mineral oil in seawater, transferred marine mussels *Mytilus edulis* to fresh seawater. They observed significant amounts of hydrocarbons remained in *Mytilus edulis* after 76 hours of depuration. Neff and Anderson (1975) reported that release of C14-benzo[a]pyrene from a clam *Rangia cuneata* to nondetectable level (>0.01 ppm) required 30 to 58 days. Based on the results with *Mercenaria mercenaria* in controlled laboratory aquaria, Tanacredi (1988) reported that a 45-day depuration period for clams may not be sufficient for cleansing PAHs in view of safe consumption of clams by man. Understanding of PAHs in clams with respect to depuration kinetics is of importance in reducing human health risks and managing marine resources. Although a number of depuration studies have been performed, only limited kinetic information is currently available.

The objectives of this study were to examine and characterize elimination of PAHs in *Mercenaria mercenaria*, and to evaluate depuration rates for various PAHs. In this study, eight PAH compounds were investigated including naphthalene, fluorene, phenanthrene, fluoranthene, pyrene, benz[a]anthracene, chrysene, and benzo[a]pyrene. The experiment was conducted in laboratory under controlled environmental conditions.

MATERIALS AND METHODS

Hard-shell clams *Mercenaria mercenaria* were used in this study. *Mercenaria mercenaria* is a filter-feeding bivalve mollusc, subtidal species and always dwells underwater during tidal exchanges. *Mercenaria mercenaria* represents a large portion of the shellfish catch for human consumption purposes in northeast America. For this project, approximately the same weight group (30 grams) of *Mercenaria mercenaria* were purchased from a local oyster hatchery. Seawater was prepared with artificial sea salts, Instant Ocean (Aquarium Systems, Inc., Eastlake, Ohio) dissolved in distilled water. The clams were fed supplemental invertebrate diet (Marine Invertebrate Diet, Hawaiian Marine Imports, Inc.). None of the target PAH compounds was detected in the clam food nor in the artificial seawater.

A standard PAH solution (Supelco Co, Inc.) was used to prepare a PAH exposure solution. The standard solution contained 0.5 g of naphthalene, 0.5 g of fluorene, 0.5 g of phenanthrene, 0.5 g of fluoranthene, 0.5 g of pyrene, 0.05 g of benz[a]anthracene, 0.05 g of chrysene, and 0.05 g of benzo[a]pyrene in 100 mL acetone.

Exposure/Depuration The experiment was carried out in triplicate using 18 clams (6 per run) and six 5-gallon glass aquarium tanks (1 exposure tank and 1 depuration tank per run). The tanks were equipped with flow recirculation units and filters with granular activated charcoal. All tanks were placed in one large incubator at 13 ^{o}C. All tanks were equilibrated for 60 days to stabilize operating conditions: dissolved oxygen, 7.5 mg/L; pH 8.0; salinity, 27.0 per mil ‰.

Prior to clam exposure to PAHs, the recirculation systems of the exposure tanks were halted, and the PAH solution was added to each exposure tank. The solution was mixed thoroughly and acetone was volatilized using diffused air. The organisms were exposed to PAHs in the dark for 48 hours. The clams were not fed during the exposure period. After 48 hours, the clams were removed from the exposure tanks, and transferred into the equilibrated depuration tanks. Sea water was continuously recirculated through activated charcoal filters during a 50-day depuration period. The clams were fed supplemental invertebrate diet

during the depuration phase of the experiment. Clams were removed from the depuration tanks after 2, 10, 20, 30, 40, and 50 days for PAH analysis. (Total of three individuals were sampled on each sampling occasion).

Sample Preparation and GC Analysis The clam specimens were carefully sampled, rinsed several times in distilled water to remove surface-adsorbed PAHs, blotted dry and weighed in a tared aluminum foil pan. Clam tissue was wrapped in aluminium foil and stored in a freezer at -20°C until analysis. The frozen tissue was ground with anhydrous sodium sulfate and extracted with hexane in a Soxhlet extractor for 6 hours. The sample was cleaned using a glass column containing copper powder, alumina and silica gel. The extract was concentrated to 1 to 3 mL. The concentrated sample was analyzed for PAHs by a gas chromatograph (Perkin-Elmer Sigma 3B) equipped with a capillary glass column (Wide Bore 60 m x 0.75 mm ID, Supelco Co., Inc.) according to the EPA Method (Method 610). The target PAH compounds were detected by a flame ionization detector (FID).

RESULTS

The depuration rates were evaluated based on mean concentrations at each sampling time. Figure 1(a) shows the mean and standard deviation of pyrene concentrations (ng/g whole wet tissue) which remained in *Mercenaria mercenaria* at each sampling occasion. Figure 1(b) shows semilog plots (i.e., logarithmic concentrations vs. time) of the mean concentrations. As indicated by two linear lines, the depuration kinetic was first order possessing two different rate constants. Pyrene was released steadily from *Mercenaria mercenaria* at a rate of 0.09/day in the first 2 to 30 days, and at a reduced rate of 0.02/day between 30 and 50 days.

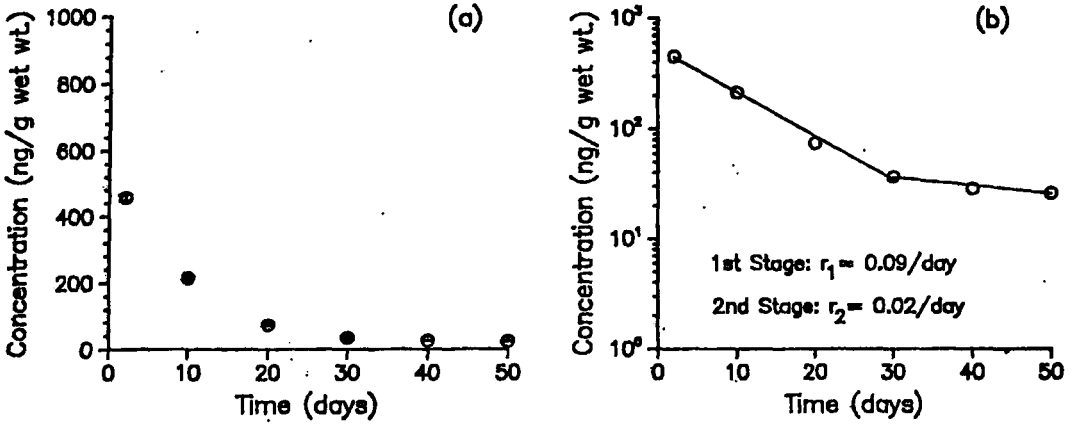

Fig. 1. Depuration of pyrene in *Mercenaria mercenaria*.

Figure 2(a) shows the mean and standard deviation of benzo[a]pyrene concentrations. Figure 2(b) is semilog plots of the mean concentrations of benzo[a]pyrene vs time. It should be noted that the concentrations of

benzo[a]pyrene in the exposure seawater was ten-fold lower than pyrene. Benzo[a]pyrene was released at a rate of 0.03/day throughout the 50-day depuration period.

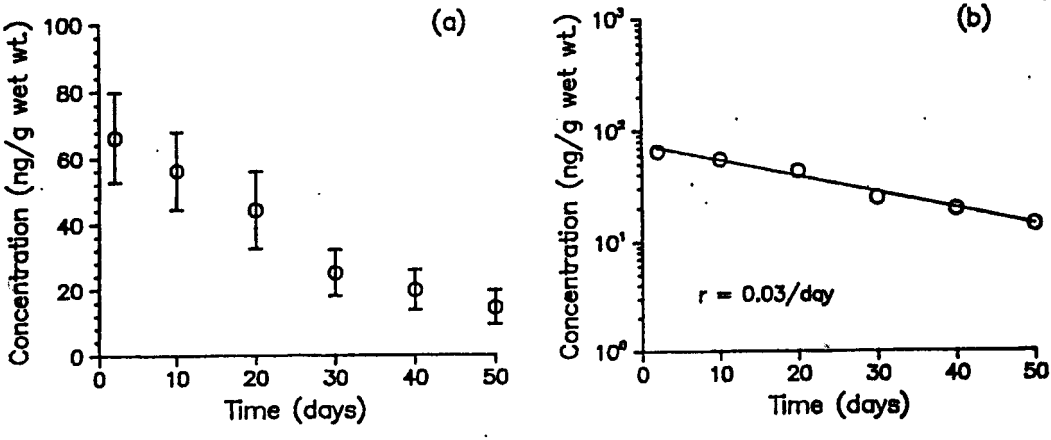

Fig. 2. Depuration of benzo[a]pyrene in *Mercenaria mercenaria*.

Similarly, kinetics of remaining PAHs were evaluated. TABLE 1 lists the depuration rate constants (first-order) of eight PAHs, along with their chemical properties (i.e., molecular weight, solubility in sea water).

TABLE 1. First-Order Depuration Rate Constants For PAHs

PAH	Molecular Weight (gm/mole)	Solubility in Sea Water (mg/L)	Depuration Rate Constants (per day)* r1	r2
Naphthalene	128.19	31.7	0.08	0.03
Fluorene	166.23	1.98	0.05	0.02
Phenanthrene	178.24	1.29	0.12	0.04
Fluoranthene	202.26	0.26	0.09	0.02
Pyrene	202.26	0.135	0.09	0.02
Benz[a]anthracene	228.30	0.14	0.06	----
Chrysene	228.30	0.002	0.05	0.02
Benzo[a]pyrene	252.31	0.0003	0.03	----

* r1, First-stage depuration rate; r2, Second-stage depuration rate.
----, First-stage only (single-stage kinetics).

Naphthalene was released steadily from *Mercenaria mercenaria* at a rate of 0.08/day in the first 2 to 20 days, and at a reduced rate of 0.03/day between 20 and 50 days. Fluorene was steadily discharged at a rate about 0.05/day up to 30 days, then the rate decreased to 0.02/day after 30 days. A sharp decrease in phenanthrene concentration (0.12/day) occurred between 2 and 10 days, then the concentration decreased slowly at a rate of 0.04/day. The depuration pattern of fluoranthene was similar to

pyrene. The fluoranthene concentration reduced at rates of 0.09/day in the first 30 days and 0.02/day after 30 days. Similar to the depuration of benzo[a]pyrene, benz[a]anthracene was released at a constant rate of 0.06/day throughout the 50 day depuration period. Chrysene was released at two different depuration rates: 0.05/day before 30 days, and 0.02/day after 30 days.

DISCUSSION

Because of various factors (e.g., particulate deposition, storm runoff, accidental spills, tides,), levels of PAHs frequently fluctuate in estuaries and inshore marine waters. Therefore, it is difficult to obtain reproducible results and to interpret data in a repeatable, fundamental and meaningful manner. In this study, experiment was conducted in a laboratory so that various environmental variables were controlled. All aquarium tanks were stored in one large refrigerated incubator at a constant temperature of 13 $\pm$ 0.2 ^{o}C. Other parameters were also kept relatively constant: dissolved oxygen, 7.5 $\pm$ 0.2 mg/L; pH, 8.0 $\pm$ 0.1; salinity, 27.0 $\pm$ 0.2 ‰. The test clams were thoroughly decontaminated before use and acclimated to the experimental conditions. The organisms were fed clean (PAH-free) food to eliminate PAH-uptake effect during the depuration phase. We carried out three runs under the same conditions. A larger standard deviation was exhibited by lower molecular-weight PAH compounds. The largest standard deviation was shown by fluorene, while fluoranthene and pyrene gave minimal deviations.

Literature review by Varanasi *et al.* (1989) showed that extent and route of excretion of PAHs in fish were dependent on their molecular size. They found that lower-molecular-weight PAHs (e.g., naphthalene) were extensively excreted. Our results (Table 1), in general, support the molecular-weight dependency of the depuration rates, although the trend was limited to the first-depuration stage. It is interesting to note that fluoranthene and pyrene (having the same molecular weight of 202 gram/mole) showed the similar depuration pattern yielding the same depuration rate constants (i.e., r1 = 0.09/day, r2 = 0.02/day). The depuration rate also appeared to be a function of PAH's solubility in sea water.

Widdows *et al.* (1983) studied kinetics of elimination of C14-labeled naphthalene from digestive gland, gill, kidney, mantle, and remaining tissue of mussel *Mytilus edulis*. They hypothesized two component tissues (fast and slow components) by means of their kinetics of elimination. Widdows *et al.* observed the shortest biological half-time of C14-naphthalene in the gill and kidney tissues (fast component). They also observed that the biological half-time of C14-naphthalene in the digestive gland and remaining tissue (slow component) is longer than in the gills, kidney and mantle tissues. In the study with starry flounder and rock sole, Varanasi *et al.* (1989) demonstrated that the organisms acutely exposed to PAHs depurated them rapidly during the first few days, then proceeded at a much slower rate. The PAHs decreased sharply in 4 to 6 days in blood, muscle and urine, but showed very little decline in liver and bile of the flounder in 14 days. Our results support the hypothesis of two component by Widdows *et al.* and the observation by Varanasi *et al.* In the present study, for each PAH (with exceptions of benz[a]anthracene and benzo[a]pyrene), at least two release mechanisms were indicated by means of their distinguishable rates of depuration. The rapid release of PAHs in *Mercenaria mercenaria* was followed by slow elimination of PAHs. The transition occurred generally between 20 and 30 days.

In the present study, we used the depuration period of 50 days. At the end of the depuration period, the PAH compounds were still retained at detectable levels in *Mercenaria mercenaria* with the total PAHs of 300 ng/g of whole wet tissue. The evaluation of factors affecting elimination of PAHs are difficult because it is likely that both excretion and metabolism are affected by the extent of exposure (duration and concentration). Varanasi *et al.* (1989), in their study with starry flounder and rock sole, showed that the depuration of PAHs was slow when the organisms were removed from an area chronically contaminated with oil. In our experiment, clams were acutely exposed (for 48 hours) to PAHs, thus the determined depuration rates are likely underestimated.

CONCLUSIONS

The depuration rates of eight PAHs in *Mercenaria mercenaria* were determined. The single and two-stage kinetics were applied to characterize PAHs. Benz[a]anthracene and benzo[a]pyrene showed the single-stage depuration. Naphthalene, fluorene, phenanthrene, fluoranthene, pyrene, and chrysene exhibited the two-stage depuration during the 50-day cleansing period.

PAHs of higher molecular weights were eliminated from *Mercenaria mercenaria* at slower rates than those of lower molecular weights. Although an average of 90% of total PAHs was released from *Mercenaria mercenaria* between 2 and 50 days, approximately 300 ng of total PAHs per gram of whole wet tissue still remained at the end of 50-day depuration period.

ACKNOWLEDGEMENT

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