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Toxicity and Metabolism of Naphthalene: A Study with Marine Larval Invertebrates (39625)

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Introduction. The toxic effects of waterborne hydrocarbons on marine larvae are presently of great interest in relation to petroleum contamination of marine waters. Several studies have examined the acute toxicity of crude petroleum and water-soluble fractions (WSF) on several marine larval forms (1–5, 14); however, virtually nothing is known about the toxicity of individual hydrocarbons to marine larvae or about the degree of accumulation of petroleum hydrocarbons in larval tissues. Moreover, no detailed information is available on the capability of early life stages of marine organisms to discharge accumulated hydrocarbons.

In the present work, marine larval invertebrates were exposed to 8–12 parts per billion (ppb) concentrations of [1-^{14}C]naphthalene or [1-^{14}C]naphthalene complexed with bovine serum albumin (BSA) in seawater. This aromatic hydrocarbon is a prominent component of WSF (6) and is known to be toxic to marine organisms (7–9). The findings showed that substantial differences occurred in the retention and metabolism of naphthalene between the two chemical forms. In both experiments the larvae were shown to readily depurate naphthalene; however, metabolic products to naphthalene were strongly resistant to depuration. In each case, 100% mortality occurred when larvae were exposed for 24–36 hr.

Materials and methods. Spot shrimp (*Pandalus platyceros*) and Dugeness crabs (*Cancer magister*) were hatched in the laboratory from ovigerous females. The shrimp larvae hatched each day were kept separately in holding tanks and fed brine shrimp. Animals used in the challenge experiments were newly metamorphosed larval stages.

[1-^{14}C]Naphthalene (5.10 mCi/mmole) was obtained from Amersham–Searle, Arlington Heights, Ill. Solutions of [1-^{14}C]naphthalene (A), [1-^{14}C]naphthalene-BSA (B), and BSA free of naphthalene (C) were prepared. [1-^{14}C]Naphthalene (1.8×10^7 dpm) was added to a 3-liter portion of a 6-liter solution of salt water buffered with phosphate (pH = 7.5) to form solution A. Solution B was obtained by adding 1.50 g of BSA and [1-^{14}C]naphthalene (1.8×10^7 dpm) to the remaining 3 liter. Then, the naphthalene-BSA complex was formed according to the procedures of Sahyun (10). Solution C was treated in a manner similar to solution B, excluding the [1-^{14}C]naphthalene to provide water for a control experiment.

Solutions were obtained for challenge experiments by mixing the above refrigerated (1°C) stock solutions (A, B, and C) with filtered sea water (5 μm). Nine-liter test aquaria were filled by simultaneously adding 7.80 liter of sea water and 1.65 liter of the appropriate stock solution. A peristaltic pump maintained constant flow rates of 1.1 ml/min for the stock solutions and 5.2 ml/min for the sea water during the exposure period. The resulting solutions were delivered to test aquaria which were maintained at $10 \pm 1°$. Duplicate water samples were taken at the initiation of the experiments and concurrently with larval samples. These were analyzed for carbon-14 by liquid scintillation spectrometry. The concentrations of [1-^{14}C]naphthalene in aquaria waters ranged from 8–12 ppb on the basis of measured carbon-14 (^{14}C) and the specific activity of [1-^{14}C]naphthalene. The larvae were netted from the holding tanks, counted, and placed in the test aquaria. Specimens were removed for analysis at various time intervals.

Larval tissues were examined for both [1-^{14}C]naphthalene and ^{14}C-labeled metabolites. The total ^{14}C in the animals was determined as follows. Two larvae were sampled

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from each challenge experiment; each was placed in a cone of Whatman 42 filter paper and was washed 10 times with 10-ml aliquots of filtered (5 μm) seawater. The final washings were shown to be free of measurable ^{14}C. Larvae were weighed, and each animal was placed in a scintillation vial with 1.0 ml of soluene 350 (Packard Inst. Co., Downers Grove, Ill.). The tissues were digested for 1 hr at 25°, then, 15 ml of InstaGel (Packard Inst. Co.) was added, and ^{14}C was determined by liquid scintillation spectrometry. The total metabolites of [1-^{14}C]naphthalene were determined by employing methods described previously (11). Two larvae were washed as above, frozen, and held at −10°. Each frozen larvae was crushed in a 5-ml glass-stoppered graduated centrifuge tube containing 0.7 ml of formic acid and 1.5 ml of hexane. The tissues were held at room temperature in stoppered tubes until digestion was complete (12–24 hr). Upon completion of digestion, 1.0 ml of distilled water was added. Then 1.0 ml of a saturated aqueous solution of sodium hydroxide was added slowly with rapid stirring at 0°. An additional 0.5 ml of hexane was added, and the resultant mixture was agitated vigorously and allowed to stand for 5 min. The ^{14}C in the aqueous phase was determined by analysis of a 1.0-ml aliquot using liquid scintillation spectrometry. Background ^{14}C levels were measured by following the above procedure exclusive of animal tissue. The amount of [1-^{14}C]naphthalene was obtained by subtracting the ^{14}C associated with the aqueous phase of the formic acid digest from the total ^{14}C in the larvae. Analysis of test aquaria water by the above formic acid procedure revealed that virtually all of the ^{14}C (determined by liquid scintillation spectrometry) was in hexane washings. Hence, radioactivity was in the form of naphthalene rather than as products of biodegradation.

The naphthalene uptake and depuration data were treated statistically by fitting regression equations to the uptake data for the naphthalene and BSA-naphthalene exposures. Regression equations were also fitted to the depuration data for both exposures. Correlation coefficients for the naphthalene and BSA-naphthalene uptakes were 0.650 and 0.509, respectively, and, for depuration, the coefficients were 0.740 and 0.620, respectively. A statistical treatment of the data indicated that the slopes of the regression equations for naphthalene and the protein complex were the same during uptake as during depuration. However, by analysis of covariance, it was shown that the regression does not coincide during either uptake or depuration: $F = 2.374$ ($F_{05[1,38]} = 4.08$) and $F = 0.699$ ($F_{05[1,66]} = 4.00$). Graphs of the regression equations are shown in Figs. 1A and 2A. In the graphs of metabolite data (Figs. 1B and 2B) curves of mean values and ranges are given.

Results and discussion. [1-^{14}C]Naphthalene and the [1-^{14}C]naphthalene-BSA complex were acutely toxic to stage I and V spot shrimp (*P. platyceros*) and to newly hatched Dungeness crab (*C. magister*) zoea at naphthalene concentrations of 8–12 ppb ($8–12 \times 10^{-9}$ mg/ml). All organisms died within 24–36 hr. In each case, narcosis was observed in 18–24 hr. No differences

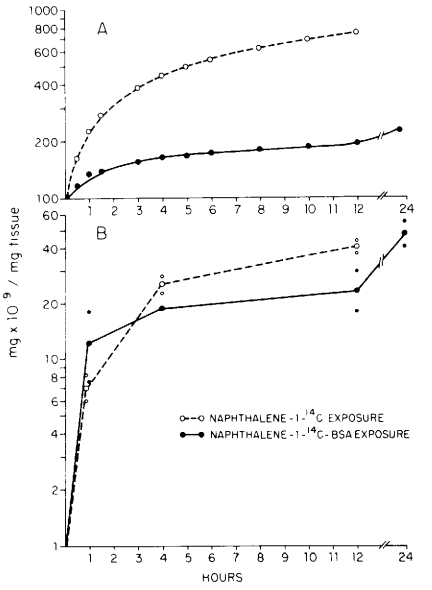

FIG. 1. Accumulation of naphthalene and metabolic products (expressed as naphthol) on exposure of stage V spot shrimp (*Pandalus platyceros*) to 8–12 ppb of water-borne [1-^{14}C]naphthalene and [1-^{14}C]naphthalene complexed with BSA. (A) Regression lines of concentrations of [1-^{14}C]naphthalene with sampling points indicated. (B) Median values of metabolic products with data ranges.

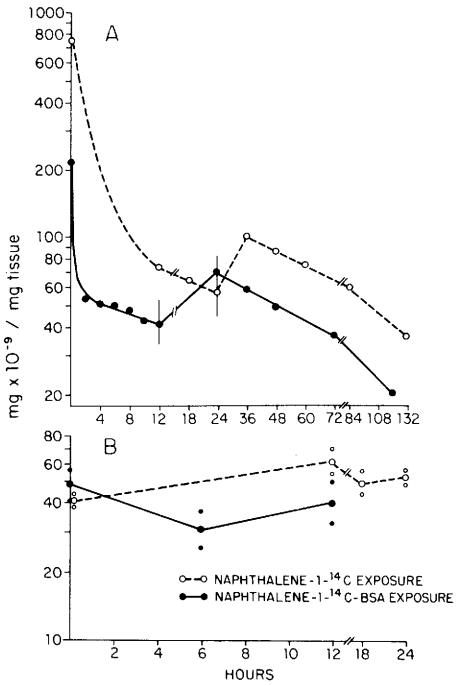

FIG. 2. Depuration of naphthalene and metabolic products (expressed as naphthol) after exposure of stage V spot shrimp (*Pandalus platyceros*) to 8–12 ppb of water-borne [1-^{14}C]naphthalene (12 hr) and [1-^{14}C]-naphthalene complexed to BSA (24 hr). (A) Regression lines of concentrations of [1-^{14}C]naphthalene with sampling points indicated by vertical lines; thereafter, primarily metabolic products containing small amounts of [1-^{14}C]naphthalene. (B) Median values of metabolic products with data ranges.

were noted between larvae exposed to [1-^{14}C]naphthalene or the [1-^{14}C]naphthalene protein complex. Less than 1% mortality occurred in the control groups, thus indicating that BSA did not contribute to the toxic effects observed.

The results are not consistent with findings cited by McAuliffe *et al.* (12), suggesting that larval stages of marine organisms are quite resistant to petroleum hydrocarbons (13–15). Under our conditions, naphthalene appeared to be considerably more toxic to invertebrate larvae than WSF of crude petroleum administered via static bioassay (16). These workers found that mortalities did not occur among stage I Dungeness crab (*C. magister*) and tanner crab (*Chionoecetes bairdi*) in 48-hr and 96-hr exposures to 8 ppm of the WSF of crude petroleum. This level corresponds to a naphthalene concentration approximately five times greater than concentrations of naphthalene used in the present work (17).

It is not possible to compare directly our findings with those obtained using the static bioassay technique, notably because low molecular weight hydrocarbons, such as naphthalene, are volatilized under static conditions. Static bioassay techniques, thus, are largely restricted to providing a rough first-order approximation of the toxicity of water-soluble hydrocarbon fractions to marine larval forms in view of the present data. In considering this question, it is interesting to note that laboratory preparations of WSF of four crude oils were shown to contain from 20–840 ppb of naphthalene (6). This range is well in excess of the acutely toxic concentration of 8–12 ppb found in the present work.

Stage V spot shrimp larvae (*P. platyceros*) were studied with respect to the accumulation of [1-^{14}C]naphthalene and metabolic products. Also studied was the capacity of larvae to depurate these compounds when placed in clean water. The larvae showed significant differences in accumulations of naphthalene when it was presented in the free state rather than as the naphthalene-BSA complex (Fig. 1A). Maximum levels of 820 ppb (818×10^{-9} mg/mg of tissue, 10.0 hr) and 220 ppb (221×10^{-9} mg/mg of tissue, 5.0 hr) were found in larvae exposed to naphthalene and naphthalene-BSA, respectively. These values represent an accumulation of naphthalene in larvae equal to 25–100 times the concentration in the water.

The maximum concentration of naphthalene metabolites (based on the molecular weight of naphthol) in larvae after 12 hr of naphthalene exposure was 42 ppb (41.8×10^{-9} mg/mg of tissue); larvae exposed to naphthalene-BSA for 24 hr contained (based on the molecular weight of naphthol) 49 ppb (49.2×10^{-9} mg/mg of tissue) (Fig. 1B). The former and latter values represent 9 and 21% of the total radioactive compounds isolated from the larval tissues. At each interval of time, the ratio of naphthalene metabolites:naphthalene was substantially greater with larvae exposed to naph-

thalene-BSA than with larvae exposed to naphthalene alone. Thus, a substantially greater proportion of metabolic products occurs in larvae exposed to naphthalene complexed with protein. Although this finding is not readily explained, the data clearly lead to the conclusion that naphthalene metabolism is altered in relation to whether the hydrocarbon is in the free state or bound to protein. The fact that the larvae are capable of metabolizing naphthalene in either chemical form suggests the presence of degradative enzyme systems (aryl hydrocarbon hydroxylases) common to most animals (18). Increased lipid solubility has been shown to be directly related to adsorption through the intestinal mucosa in rats (19); thus, increased uptake of naphthalene over the naphthalene-BSA complex may be attributed to the higher lipid solubility of the former structure. The apparent increase in the proportion of metabolic products found with the naphthalene-BSA exposure cannot be explained at this time.

The question arose as to whether the accumulated levels of hydrocarbons and metabolites are readily depurated. It was shown that transfer of the larvae to clean salt water after exposure to [1-^{14}C]naphthalene or [1-^{14}C]naphthalene-BSA for 12 and 24 hr, respectively, resulted in substantial depuration. Moreover, mortalities that normally occurred on continued exposure for 24–36 hr were prevented. In naphthalene-exposed larvae, a rapid decline in the aromatic hydrocarbon levels over a 12-hr period was observed, followed by a gradual decrease for 132 hr (Fig. 2A). After 24 hr, the concentration of naphthalene decreased by 83% of the original values. A decline of 81% in accumulated naphthalene occurred in larvae exposed to naphthalene-BSA in a 12-hr period (Fig. 2A). Thus, regardless of the chemical form of naphthalene, substantial amounts of the hydrocarbon were depurated from larvae after termination of the exposure. However, metabolites were present in the larvae throughout the depuration experiments (Fig. 2B).

The present study provides details, for the first time, on the toxic and biochemical effects of water-borne naphthalene on larval invertebrates. The results, showing that death (preceded by narcosis) occurred from exposure to low parts per billion levels of naphthalene in 1 or 2 days, raises questions about the nature of long-term effects (e.g., alterations in cellular structure, molting behavior, etc.) at even lower hydrocarbon concentrations.

The finding that the proportion of metabolites to recovered aromatic hydrocarbon is greater with larvae exposed to the naphthalene-BSA complex than with those exposed to naphthalene itself is of interest with respect to the transport of aromatic hydrocarbons in ecosystems. That is, aromatic hydrocarbons acquired in food may be metabolized quite differently than such compounds acquired via other routes (e.g., through the water column), involving other chemical forms.

Summary. [1-^{14}C]Naphthalene and the [1-^{14}C]naphthalene complexed with BSA at 8–12 ppb in flowing sea water produced 100% mortality in 24–36 hr in newly hatched Dungeness crab (*Cancer magister*) zoea and stage I and V spot shrimp (*Pandalus platyceros*). Maximum accumulation of [1-^{14}C]naphthalene in the stage V spot shrimp exposed to [1-^{14}C]naphthalene was nearly four times greater than in shrimp exposed to the [1-^{14}C]naphthalene-BSA complex (820 and 220 ppb, respectively). These values represent a magnification of 25–100 times the exposure levels. Metabolic products of [1-^{14}C]naphthalene in larval tissues exposed to the complexed and noncomplexed naphthalene reached a maximum of 21 and 9%, respectively, of the total radioactive compounds (based on the molecular weight of naphthol). [1-^{14}C]Naphthalene was almost entirely depurated from the tissues in 24–36 hr, whereas metabolic products were strongly resistant to depuration.

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