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Influence of dispersants on the bioavailability and trophic transfer of phenanthrene to algae and rotifers

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Abstract

Use of chemical dispersants as oil spill clean-up agents alters normal behavior of petroleum hydrocarbons (PH) by increasing functional water solubility. Different PHs may respond differently to dispersant based on their individual physical properties and altering the composition of the bioaccessible fraction of the oil. The objective of this research was to determine the impact of dispersing agents on the bioavailability and trophic transfer of phenanthrene, a model for a class of compounds in oil characterized by limited water solubility and the potential to bioaccumulate. Uptake, bioaccumulation, and depuration of [^{14}C]phenanthrene, were compared for Prudhoe Bay crude oil (PBCO) dispersed with Corexit® 9527 (dispersed oil or DO) and undispersed preparations of the water-accommodated fraction (WAF) of PBCO. The model food chain consisted of *Isochrysis galbana*, a primary producer, and *Brachionus plicatilis*, a primary consumer. Direct aqueous (AQ) exposure was compared with combined aqueous and dietary (AQ&D) exposure. Results showed phenanthrene uptake by algae increased significantly ($P < 0.05$) from 102 to 172 nmol eq phenanthrene/g algae in DO exposures. No significant difference ($P > 0.05$) in phenanthrene uptake was observed in rotifers in DO, however, phenanthrene depuration significantly ($P < 0.01$) decreased following DO exposures. Phenanthrene uptake increased significantly ($P < 0.05$) with trophic transfer in WAF, from 51 to 87 nmol eq phenanthrene/g rotifers, and similarly, from 57 to 130 nmol eq phenanthrene/g rotifers, in DO exposures. Depuration kinetics from both AQ and AQ&D treatments indicated that although phenanthrene was present at higher concentrations when dispersant was used, the rate of clearance from all compartments was more rapid. Phenanthrene bioaccumulation in DO samples was consistently less than comparable WAF samples supporting the kinetics findings and indicating a non-concentration dependent mechanism involved in altering the behavior of phenanthrene in both algae and rotifers with the use of dispersants.

Keywords: Marine oil spills; Phenanthrene; Dispersant; Bioavailability; Trophic transfer; Rotifer

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1. Introduction

Use of chemical dispersants (or surfactants) in oil spill clean-up offers the benefit of reducing the threat of oiling shorelines and surface inhabitants such as marine mammals and seabirds. In addition, degradation may be enhanced by increasing aqueous concentrations of petroleum hydrocarbons (PH) through solubilization or emulsification (Tiehm, 1994). The bioaccessible fraction may also be increased resulting in increased bioaccumulation and/or changes in metabolic disposition, and consequently increased toxicity either directly or through the food chain.

Phyto- and zooplankton are widely distributed in the marine environment and, because of their limited mobility, have little ability to escape stresses such as an oil spill. Contaminated algae and rotifers may subsequently be eaten by other grazing zooplankton and larval fish or their dead cells may fall to the ocean floor where they either decompose or enter the food chain via detritavores, thus playing an important role in the fate of hydrophobic organic compounds in aquatic systems.

Previous studies examined the fate of naphthalene, a bicyclic, relatively water soluble component of crude oil which was equally miscible in WAF and DO. Because the concentrations of naphthalene in WAF and DO were not significantly different, results of food chain studies with naphthalene were considered non-concentration dependent (Wolfe et al., 1998a–d). Results indicated that under a variety of temperature and salinity conditions, naphthalene uptake by the golden-brown algae, *Isochrysis galbana*, increased significantly with the use of the chemical dispersant Corexit 9527®. However, no significant effect on uptake and biotransformation by the rotifer, *Brachionus plicatilis*, was observed. The importance of trophic transfer of naphthalene was notable, accounting for nearly half of uptake and significantly increasing exposure. Conversely, naphthalene depuration from rotifers increased significantly with dispersant, manifesting as increased rates of depuration and a net decrease in bioaccumulation.

The goal of the current investigation was to determine the influence of a chemical dispersant on the bioavailability and trophic transfer of another class of PHs to primary levels of a marine food chain. Phenanthrene was selected to represent a class of more persistent, polyaromatic hydrocarbons (PAHs), which are relatively insoluble in water and have the potential to bioaccumulate in organisms. Because the solubility of phenanthrene is facilitated significantly by dispersant it will help determine the extent of dispersant altered bioavailability with increasing aqueous concentrations of PH.

2. Materials and methods

A detailed description of the materials and methods developed for this project are available in Wolfe et al. (1998a). An abbreviated description follows.

2.1. Chemicals

The model crude oil used was Prudhoe Bay crude oil (PBCO; EPA Standard, RT, Laramie, WY). The model dispersant was Corexit 9527® (Exxon Chemical, Houston, TX) which is composed of 48% nonionic surfactants (ethoxylated sorbitan monooleate, ethoxylated sorbitan trioleate, and sorbitan monooleate), 35% anionic surfactant (sodium dioctyl sulfosuccinate), and 17% solvent (ethylene glycol monobutyl ether). The radiolabeled model PH [^{14}C]phenanthrene, and standards of selected degradation products were obtained from Sigma Chemical (St. Louis, MO). Filtered (0.2 μm) seawater (34‰), used for culturing of organisms and preparation of exposure media, was obtained from the University of California, Santa Cruz (UCSC) Long Marine Laboratory (LML) and stored at 4°C.

2.2. Organisms and culture conditions

Inoculating cultures of the primary producer, *I. galbana*, a unicellular, photosynthetic, golden brown marine flagellate (class Prymnesiophyceae, division Chrysophyta) were obtained from Long

Marine Laboratory, University of California, Santa Cruz (LML) and batch cultured in double-filtered (0.2 μm), autoclaved 34‰ sea water. F/2 nutrient (Fritz Aquaculture, Dallas, TX) was added to all cultures and exposure media at manufacturer's prescribed concentrations. The primary consumer, *B. plicatilis*, a euryhaline rotifer (class Monogonota, order Plioma) was obtained as live culture from the Los Angeles County Natural History Museum, Halibut Hatching Project and cultured under the same conditions as the algae.

2.3. Characterization and analysis

HPLC fractionation and co-chromatography was done on a Hewlett Packard Model 1090 HPLC (Palo Alto, CA) system outfitted with a 25-cm C_{18} column (Alltech, Deerfield, IL), diode-array (Sig = 254 nm) and fluorescence (Ex = 260 nm, Em = 380 nm) detection and a Gilson FC 203 automated fraction collector (Middleton, WI). [^{14}C] incorporation was quantified with a Beckman LS6500 (Palo Alto, CA) liquid scintillation counter (LSC).

2.4. Method development

2.4.1. Toxicity testing

The amount of oil and dispersant used in the preparation of exposure media was originally selected to deliver the maximum concentration of hydrocarbon without causing phytotoxicity as defined by significant differences in cell density between exposed and control populations (Wolfe et al., 1998b). Using concentrations determined to be non-toxic to algae (2 g/l of PBCO and dispersant at 10 μg/g of oil), rotifers (10 rotifers/ml) also showed no significant differences in population density between exposed and control populations (Wolfe et al., 1998a).

2.4.2. Preparation of exposure media

The water-accommodated fraction (WAF) exposure medium preparation consisted of weighing 2000 g of water into 2 l glass aspirator bottles customized with glass spigots. PBCO (2 g/1000 g seawater) was added to the top of the water. Dispersed oil (DO) was prepared in the same fashion with the addition of Corexit 9527® at a concentration of 10 μg/g of crude oil. Bottles were covered with aluminum foil to inhibit photodegradation, and stirred at a minimal speed (200 rpm, 20°C, 24 h) to eliminate the transfer of visible oil droplets to the water column. Results of GC-FID characterization studies (Wolfe et al., 1998b) showed the concentrations of native (the aqueous concentration of phenanthrene in exposure media from PBCO) phenanthrene in 24 h WAF and DO preparations were significantly different ($P > 0.10$), 7.4 ± 0.35 and 11 ± 0.44 μg/l, respectively ($\pm$ S.D., $n = 9$). Because Corexit 9527 increased the aqueous solubility of native phenanthrene by $\approx$ 50%, DO was spiked with 150% of the [^{14}C]phenanthrene with which WAF was spiked to maintain the same relative concentrations in the two exposure media. A concentrated stock solution of [^{14}C]phenanthrene in methanol was used to spike exposure media to a final labeled concentration of 1000 or 1500 dpm/ml and a final total (native and labeled) phenanthrene concentration of 13.50 ± 0.35 and 20.14 ± 0.44 μg/l in WAF and DO, respectively. The volume of the spike varied depending on the concentration of the stock, but never exceeded 70 μl/l.

2.5. Analytical techniques

HPLC co-chromatography techniques were optimized for the separation and fractionation of phenanthrene and its metabolites based on the methods of Wolfe et al. (1998b). Retention times of the individual standards were determined by reverse-phase separation using a 0.1% acidified water:acetonitrile mobile phase gradient and fluorescence detection.

Analytical confirmation of parent and metabolite standards was done using HPLC fractionation and co-chromatography with a 25-cm C_{18} column and diode-array detection. Retention times of the standards were determined by reverse-phase separation with a 0.1% acidified water:methanol mobile phase gradient.

2.6. Algae exposure

2.6.1. Chamber studies

Exposure studies were run with a total of five temperature controlled, flow-through chambers. Each study consisted of only one exposure medium, WAF or DO. Three replicate exposure chambers contained algae (750 ml), exposure medium (250 ml), and F/2. The fourth chamber was used as an algal control, consisting of algae, F/2, and culture medium to dilute to the 1 l volume. The fifth chamber was a radioactivity mass-balance control, consisting of exposure medium, culture medium, F/2, and [^{14}C]phenanthrene.

2.6.2. Uptake

Duplicate samples (11 ml) were drawn at 1, 2, 4, 8, 12, and 24 h and centrifuged using a tabletop IEC Clinical centrifuge (Fisher Scientific, Fairlawn, NJ). Supernatant radioactivity was determined by LSC. The pellet was digested with Scintigest (Fisher Scientific) in a shaking water bath at 50°C for 6 h. Algae associated [^{14}C] was quantified by LSC.

2.6.3. Metabolite profile

At 24 h, chamber contents were spun for 15 min at $7500 \times g$ at 20°C on a Sorvall RC-5B Centrifuge (DuPont, Wilmington, DE) with a GSA rotor. Supernatant was extracted on a conditioned 6-cc, 1-g, C_{18} SPE cartridge. Sample was eluted with 20 ml of methanol, followed by 20 ml of ethyl acetate and eluates were individually injected and analyzed via HPLC co-chromatography. All fractions were collected and subjected to quantitation by LSC. The remaining pellet was sonicated in methanol to disrupt cells, followed by centrifugation. The supernatant was also analyzed via HPLC co-chromatography and LSC.

2.7. Rotifer exposure

2.7.1. Chamber studies

Rotifers were exposed to WAF or DO preparations using the same chamber set-up as described for algae. Exposure medium (250 ml) was added to each of four chambers. Nonaxenic rotifer culture (750 ml; for a final population density of approximately 50 rotifers/ml)) was added to each of four chambers which were then covered and sealed. For aqueous (AQ) exposures, a reservoir of exposure medium was added to the system and medium was pumped in and out of the chamber using a peristaltic pump at a rate of 1 ml/min. For the combined aqueous and dietary (AQ&D) studies, algae (pre-exposed to WAF or DO medium for 24 h) were added to the reservoir and flowed through the chamber with exposure medium.

2.7.2. Uptake

An 8-h exposure period was used to ensure maximal uptake of [^{14}C]phenanthrene in a time frame that would not cause undue stress to fasting rotifers during AQ studies. Duplicate samples (11 ml) of exposure medium and rotifers were drawn at 1, 2, 4, and 8-h timepoints. A 1 ml portion was fixed in methanol to determine population density and the remaining 10 ml were centrifuged for 10 min using an IEC Clinical centrifuge (Fisher Scientific). The pellet was digested with Scintigest (Fisher Scientific) in a Precision 25 (Chicago, IL) shaking water bath at 50°C for 6 h. Supernatant and rotifer associated [^{14}C] were quantified by LSC.

2.7.3. Metabolite profile

After 8 h, exposure medium was separated from rotifers by filtering. Filtrate was extracted on a conditioned 6-cc, 1-g, C_{18} SPE cartridge. Sample was eluted with 20 ml of methanol, followed by 20 ml of ethyl acetate and eluates were individually injected and analyzed via HPLC co-chromatography. All fractions were collected and subjected to quantitation by LSC.

Rotifers were transferred to a glass test tube, diluted to 3 ml with methanol, and sonicated for 10 min to disrupt cells. After 10 min of centrifugation, the supernatant was also analyzed via HPLC co-chromatography.

2.7.4. Depuration

Depuration experiments were identical to uptake studies during the first 8 h with the exception that rotifers were placed within a glass sleeve inside the chamber. The opening at the bottom of

the cylinder was covered with stainless steel mesh (51 μm), allowing the free movement of algae and media and aiding in rotifer transfer to clean water for depuration. At the 8-h time point, the sleeve was carefully lifted from the exposure chamber, inverted, and rotifers were gently rinsed into an identical chamber with clean sea water. Depuration samples and population densities were taken at 9, 10, 12, and 16 h of the experiment. Sample preparation and analysis were as described above.

2.7.5. Statistics

All data at all time points was normalized to reflect the population density and eliminate 'population dilution' errors. Exposure studies consisted of a minimum of six replications per treatment, with duplicate determinations per replicate. Significant differences between treatments were determined by student's *t* test or Mann–Whitney methods (Zar, 1974).

3. Results

Uptake and depuration results are reported as nmol eq of phenanthrene (mean ± the S.D.), accounting for all [^{14}C] recovered and native phenanthrene contribution from WAF or DO, and assuming a 1:1 conversion between parent phenanthrene and transformation products. Quantitative data was determined by LSC, thus all values may deviate ± 5% of the reported value with a minimum value of 2.5 times background (100 dpms). Population density data was collected with all algae uptake and rotifer uptake and depuration data and results have been normalized to nmol eq of phenanthrene/g of algae to eliminate any biomass dilution error introduced by a replicating culture. Phenanthrene uptake was the sum of both adsorption and absorption. For uptake and depuration studies, an adjusted DO is included in the graphed results. The adjusted DO is a theoretical value which reflects a DO preparation with the same concentration of phenanthrene as the WAF as calculated by the equation:

$$\frac{[\text{phenanthrene in WAF}]}{[\text{phenanthrene in DO}]} * \text{DO}_{\text{exp}}$$

where DO_{exp} equals the uptake or depuration of phenanthrene from DO observed experimentally. The adjusted DO was reported to help identify concentration dependent results but were not confirmed experimentally.

Results of the algae exposure studies (Fig. 1) indicated the uptake of phenanthrene was significantly increased in the presence of dispersant ($P < 0.05$). Uptake at 24 h was 102 ± 33 and 172 ± 11 nmol eq phenanthrene/g algae for WAF and DO, respectively.

Phenanthrene uptake by rotifers via AQ exposure was not significantly increased in the presence of dispersant during the course of the 8-h exposure ($P > 0.05$; Fig. 2). Maximum uptake for rotifers exposed to WAF was 51 ± 7 nmol eq phenanthrene/g rotifers ($n = 12$) compared with 57 ± 15 nmol eq phenanthrene/g rotifers from DO ($n = 12$). Phenanthrene uptake by rotifers via AQ&D exposure (with algae) was also not increased in the presence of dispersant during the exposure ($P > 0.05$; Fig. 3). Maximum uptake from combined aqueous and dietary exposure by

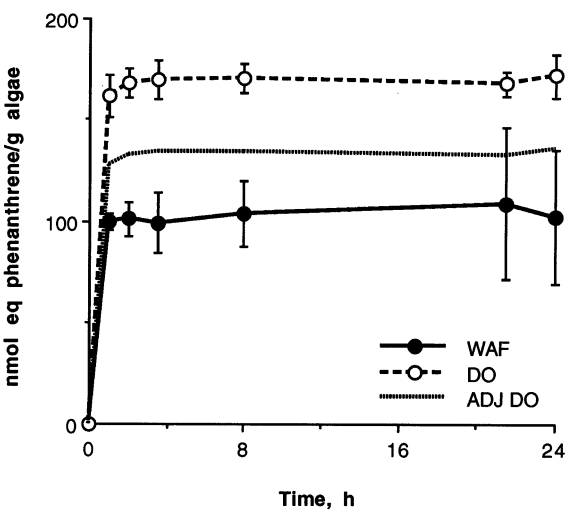

Fig. 1. Phenanthrene uptake (nmol equivalents/g algae) from water-accommodated fraction (WAF) and dispersed oil (DO) by *Isochrysis galbana* over a 24-h exposure period. The adjusted DO is a theoretical value which reflects a DO preparation with the same concentration of phenanthrene as the WAF (see Section 3). Results show a significant increase in uptake in the presence of dispersant ($P < 0.05$). Error bars indicate S.D., $n \geq 6$.

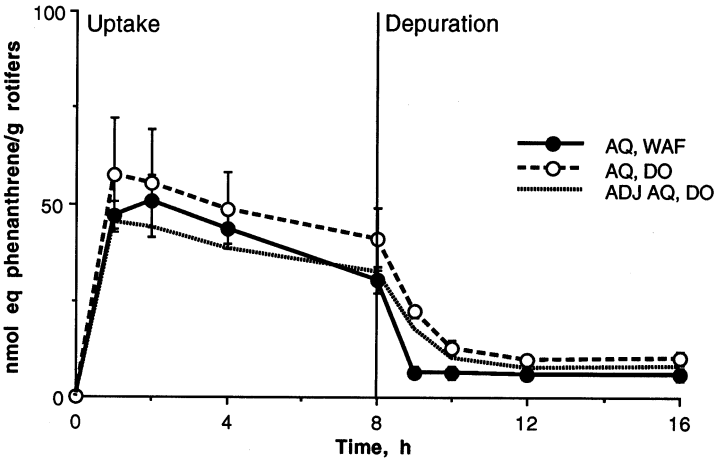

Fig. 2. Aqueous (AQ) uptake (8 h) and depuration (8 h) of phenanthrene (nmol equivalents/g rotifers) from water-accommodated fraction (WAF) and dispersed oil (DO) by *Brachionus plicatilis*. Error bars indicate S.D., $n \geq 6$.

rotifers exposed to WAF was 87 ± 7 nmol eq phenanthrene/g rotifers ($n = 12$) compared with 130 ± 29 nmol eq phenanthrene/g rotifers from DO ($n = 12$). Comparing AQ and AQ&D exposures of the same medium, uptake of phenanthrene was significantly increased ($P < 0.01$) during AQ&D exposures from both WAF and DO.

Phenanthrene depuration from AQ exposures was significantly different with the use of dispersants ($P < 0.05$; Fig. 2). At the 16-h sample (8-h depuration), WAF-exposed rotifers retained 6 ± 2 nmol eq phenanthrene/g rotifers ($n = 6$) compared with 10 ± 2 nmol eq phenanthrene/g rotifers ($n = 6$) in DO treatments. Depuration of phenanthrene by rotifers from AQ&D exposures was also significantly different ($P < 0.01$; Fig. 3) with retention increasing from 13 ± 2 nmol eq phenanthrene/g rotifers in WAF to 23 ± 5 nmol eq phenanthrene/g rotifers in DO by 16-h. Comparing AQ and AQ&D exposures of the same medium, retention of phenanthrene by rotifers was significantly higher throughout the 8-h of depuration in WAF samples from AQ&D exposures ($P < 0.01$; Figs. 2 and 3).

Due to experimental design and the protracted sample preparation period, it was impossible to obtain multiple data points during the first hour of uptake and subsequent depuration phases. Non-linear distribution of depuration data points on a semilog scale suggested a two-compartment bioaccumulation model, but analysis of data using the methods of Spacie and Hamelink (1985) indicated depuration rates of food- and water-derived residues were significantly different, thus ruling out the applicability of the bioaccumulation model. Consequently, a biphasic, two-compartment body model (O'Flaherty, 1981), as visualized in Fig. 4. and graphically depicted in Figs. 5–8, was used to describe the depuration kinetics according to the following equations:

$$k_2 = \frac{Ab + Ba}{A + B}$$

$$k_d = \frac{ab}{k_2}$$

$$k_1 = a + B - k_2 - k_d$$

$$t_{1/2a}; t_{1/2ab} = \frac{0.693}{a;\ b}$$

where:

A = tissue concentration ($t = 0$) intercept for 'fast' depuration phase
B = tissue concentration ($t = 0$) intercept for 'slow' depuration phase
a = first-order rate constant for 'fast' depuration phase
b = first-order rate constant for 'slow' depuration phase

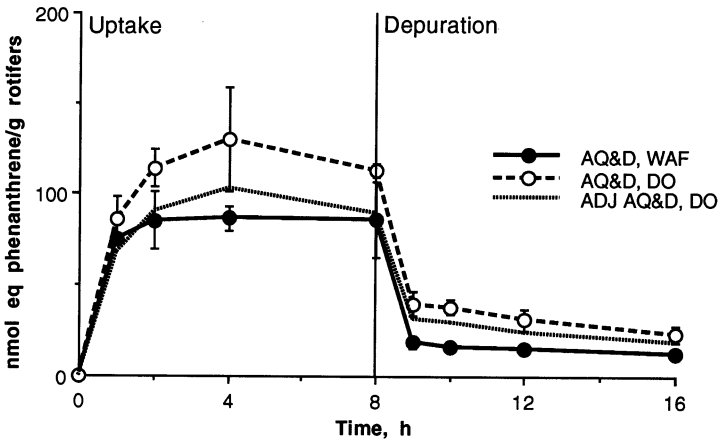

Fig. 3. Combined aqueous and dietary (AQ&D) uptake (8 h) and depuration (8 h) of phenanthrene (nmol equivalents/g rotifers) from water-accommodated fraction (WAF) and dispersed oil (DO) by *Brachionus plicatilis*. Depuration was significantly enhanced ($P < 0.01$) with dispersant. Error bars indicate S.D., $n \geq 6$.

k_1, k_2 = first-order disposition microconstants
k_d = first-order disposition whole-body depuration microconstant
$t_{1/2a}$ = half-life in the central compartment ('fast' depuration phase)
$t_{1/2b}$ = half-life in the peripheral compartment ('slow' depuration phase

Depuration kinetics in all four treatments followed the same relative trends with rate constants (a, b, k_1, k_2, and k_d) decreasing in the order WAF, AQ < DO, AQ < WAF, AQ&D < DO, AQ&D, coincident with increasing tissue residues of phenanthrene and metabolites. During the 'fast' depuration phase, transfer of phenanthrene and metabolites between the central and peripheral compartments was extremely rapid in both WAF and DO as indicated by high a values (22.9–70.5h^{-1}) and short half-lives, $t_{1/2a}$ (0.01–0.03 h; Figs. 5–8). No significant differences ($P > 0.05$) in depuration kinetics were observed during the 'fast' phase as a result of dispersant use from either AQ or AQ&D exposures. However, the inclusion of exposure via trophic transfer significantly increased ($P < 0.01$) transfer between compartments in both WAF and DO, reducing half-lives in the central compartment by up to 66%. A significant decrease in whole-body depuration during the 'slow' phase was indicated by low b values (0.08–2.3h^{-1}) coupled with longer half-lives (0.29–8.6 h). Further, results suggested distribution from the central compartment to the peripheral compartment was greater than the reverse or whole-body depuration based on k_1 rates greater than either k_2 or k_d rates in all treatments.

In the absence of equilibrium, an 8-h bioaccumulation factor (BAF) was calculated using the mass of [^{14}C]phenanthrene from the 8-h sample associated with an experimentally determined 1 g wet mass sample of rotifer divided by the [^{14}C]phenanthrene of 1 g of exposure medium in the 8-h sample. BAF results were significantly lower with dispersant for both AQ exposures ($P < 0.01$; WAF = 1593, DO = 794) and AQ&D exposures ($P < 0.05$; WAF = 4942, DO = 3040).

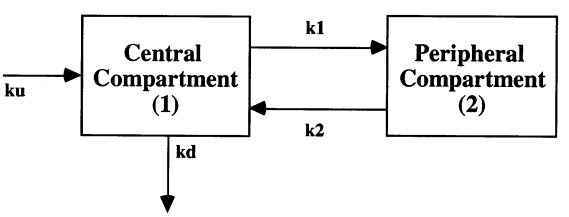

Fig. 4. Representation of the two-compartment body model for phenanthrene uptake and depuration by the rotifer, *Brachionus plicatilis*. Uptake (k_u) and depuration (k_d) may only occur through the main compartment, C1.

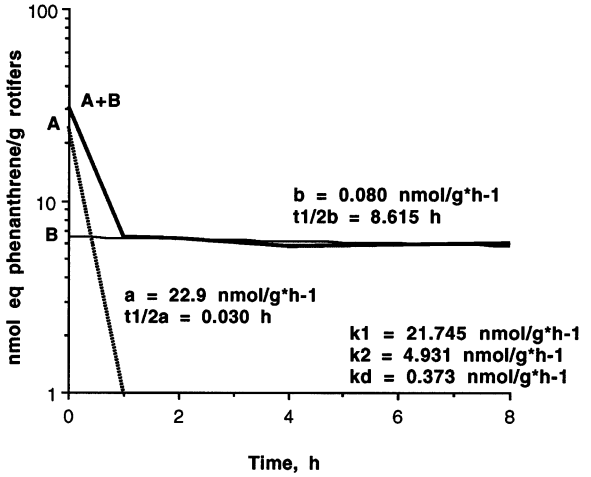

Fig. 5. Two-compartment model for phenanthrene and metabolite (nmol eq/g rotifers) depuration following AQ exposure to WAF. 'Fast' depuration phase accounts for elimination of 79% of residues with 20% remaining associated with tissues after 8 h of depuration.

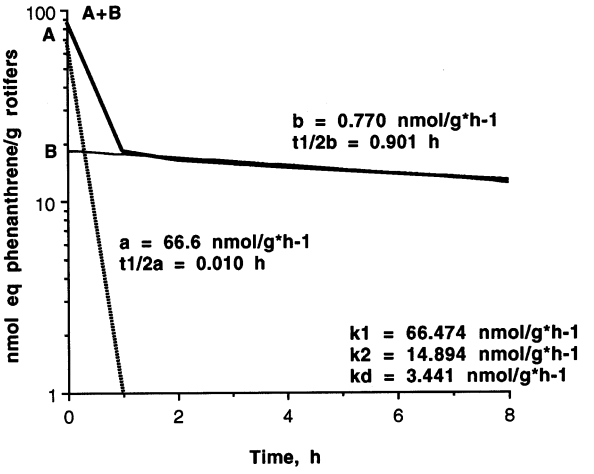

Fig. 7. Two-compartment model for phenanthrene and metabolite (nmol eq/g rotifers) depuration following AQ&D exposure to WAF. 'Fast' depuration phase accounts for elimination of 79% of residues with 15% remaining associated with tissues after 8 h of depuration.

BAF significantly increased ($P < 0.01$) for both WAF and DO with AQ&D exposure. A BAF for [^{14}C]phenanthrene in *I. galbana* was calculated similarly and the same trend was observed. The maximum BAF for algae exposed to the WAF medium was 5420 ($n = 6$), significantly higher ($P < 0.01$) than the maximum observed from DO medium, 3569 ($n = 12$).

Analysis of algae (24-h samples) and rotifer (8-h samples) residues showed $> 96\%$ of the recovered [^{14}C] was in the form of parent phenanthrene in all treatments. The remainder of [^{14}C]

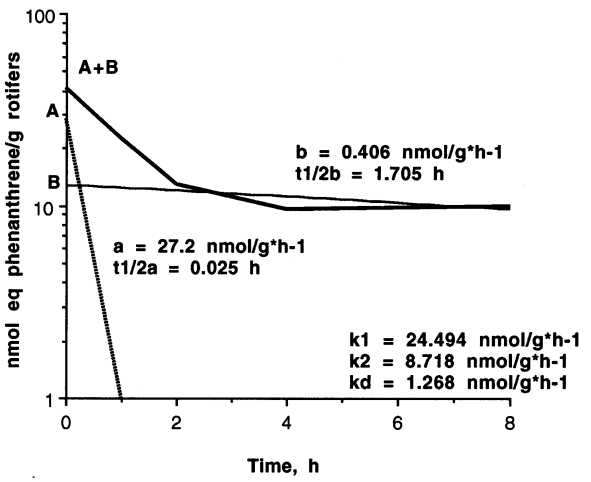

Fig. 6. Two-compartment model for depuration of phenanthrene and metabolites (nmol eq/g rotifers) following AQ exposure to DO. 'Fast' depuration phase accounts for elimination of 74% of residues with 24% remaining associated with tissues after 8 h of depuration.

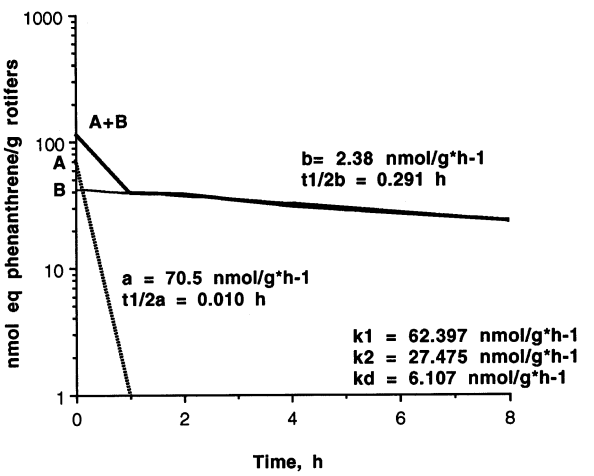

Fig. 8. Two-compartment model for phenanthrene and metabolite (nmol eq/g rotifers) depuration following AQ&D exposure to DO. 'Fast' depuration phase accounts for elimination of 65% of residues with 21% remaining associated with tissues after 8 h of depuration.

recovered from uptake samples was in the form of unresolved metabolites. Similarly, in rotifers following 8 h of depuration, $>96\%$ of recovered [^{14}C] was in the form of parent phenanthrene.

4. Discussion

Results of this study are part of a project examining the fate of different classes of model petroleum hydrocarbons in response to chemical dispersion. From earlier work it was determined that naphthalene concentrations in WAF and DO were not significantly different (205 and 238 μg/l, respectively), consequently results of the previous food chain studies were considered non-concentration dependent (Wolfe et al., 1998a,d). Phenanthrene represents a class of more persistent PAHs, whose aqueous miscibility is significantly enhanced through the use of chemical dispersant (Grimberg et al., 1995; Guha and Jaffe, 1996). Thus results of phenanthrene exposure studies reflect a net effect of both concentration and non-concentration dependent mechanisms simulating what might be observed in the environment.

The uptake trends observed in exposure studies showed an initial rapid association of phenanthrene with algae with maximum uptake occurring within the first hour similar to that previously observed in naphthalene studies (Wolfe et al., 1998a). Phenanthrene uptake from the WAF of PBCO was significantly enhanced with the addition of dispersant in this study in both the experimental and adjusted DO data, suggesting both concentration and non-concentration dependent mechanisms at work. Increasing aqueous concentrations facilitated by dispersant would drive increasing uptake to a maximum at thermodynamic equilibrium, while non-concentration dependent increases must be attributed to other factors.

The uptake trends observed in both AQ and AQ&D exposure studies showed an initial rapid association of phenanthrene with rotifers enhanced with the use of dispersant. The increase in experimental uptake from DO by rotifers was considerably less evident than in algae exposures, possibly the result in differences in tissue lipid concentration (Kayal and Connell, 1995). However, examining adjusted DO data suggests a strong correlation between concentration and uptake. Maximum uptake occurred within the first several hours of exposure regardless of medium or route of exposure, consistent with previous findings (Wolfe et al., 1998c) and characteristic of PH in aquatic organisms where steady state or maximum uptake may be obtained within hours (Neff et al., 1976; Harris et al., 1977; Varanasi and Malins, 1977; Anderson, 1979). Typically, hydrocarbon and polar metabolite release upon return to clean water eliminates up to 90% of accumulated residues within the first 24 h of depuration (Rossi and Anderson, 1977; Neff, 1979). Loss of phenanthrene and metabolites from rotifers during the initial depuration phase accounted for 74–79% of the total residue present compared with 73–93% of naphthalene in an earlier study. This would be expected based on decreased aqueous solubility of phenanthrene (0.075 mg/l) compared with 34 mg/l for naphthalene, and the resulting increased lipid solubility (Mackay et al., 1992). The increased rate of phenanthrene release observed in feeding populations following both WAF and DO exposures was consistent with the naphthalene studies in topsmelt and noted by Corner et al. (1976), possibly the result of increased metabolic activity in non-fasting organisms (Jobling, 1981; Spigarelli et al., 1983)

Exposure data is commonly limited to direct interaction between organism and individual xenobiotics via direct aqueous exposure, neglecting the contribution of trophic transfer to overall exposure. As with naphthalene, the significance of trophic transfer was obvious when comparing similar media (i.e. WAF, AQ with WAF, AQ&D). Calculating the percent phenanthrene of [^{14}C] recovered times the nmol eq taken up in AQ&D exposures shows $\leq 100\%$ increase in phenanthrene uptake via trophic transfer. This would account for as much as half of the total exposure potentially being overlooked.

Although 8-h phenanthrene uptake by rotifers from the WAF of PBCO was not significantly enhanced with the addition of dispersant via either route of exposure in this study, DO samples consistently had higher phenanthrene residues, at times significantly greater, suggesting greater ex-

posure overall. Surfactants, being surface active agents have been shown to induce leakiness and lysis of algal cells (Brown, 1973; Fedulova, 1976), thus increased cell permeability may play a role in the increased 'slow phase' depuration observed in DO samples. Following 8 h depuration, absolute values began to converge. However, DO samples retained a small, but significantly higher, level of phenanthrene residue.

Bioavailability and bioaccumulation of PHs may be influenced by numerous variables, including the physical properties of the individual compounds, the physical characteristics (i.e. salinity, temperature) of the water, and route of exposure (Livingstone, 1991; Knezovich, 1994; Wolfe et al., 1998b) and vary with the species of organism based on factors such as lipid content and surface area for uni-cellular algae and additionally, metabolic capacity and grazing rates for consumer organisms. In the case of phenanthrene, the additional aromatic ring in the structure, compared with naphthalene, gave rise to BAFs that increased over an order of magnitude. As with naphthalene, other factors in combination with dispersant may play a role in altering metabolism (Malins et al., 1979; Malins and Hodgins, 1981). It is notable that up to 50% of bioaccumulation was via the food chain. This would account for the increase in BAFs observed in this study and would be consistent with BCFs that have previously been reported for phenanthrene in a variety of marine species (Mackay et al., 1992).

Recovered tissue associated [^{14}C] was almost exclusively ($>96\%$) in the parent form for all treatments with both algae and rotifers. Although lower organisms, including algae, have been shown to degrade certain aromatic hydrocarbons (Walker et al., 1975; Cerniglia et al., 1979; Cerniglia, 1981; Warshawsky et al., 1995), including phenanthrene, no evidence of metabolism along established pathways was evident with either algae or rotifers in this study. This may in part be the result of inadequate time to activation or absence of detoxication pathways for more complex PHs (D'Adamo et al., 1997). Also, the presence of [^{14}C] metabolites in the medium was not investigated, thus excreted metabolites were not detected, as were previously observed in the naphthalene studies (Wolfe et al., 1998a–c).

5. Conclusions

Under experimental conditions, the use of the chemical dispersant Corexit 9527® significantly affected the bioavailability and trophic transfer of phenanthrene to the algae, *I. galbana*, and the rotifer, *B. plicatilis*. The importance of trophic transfer of phenanthrene from algae to rotifer from both WAF and DO was notable, doubling the uptake, thus significantly increasing exposure. 'Slow phase' depuration rates for the elimination of phenanthrene from both AQ and AQ&D DO treatments significantly increased with dispersant although absolute tissue residues of phenanthrene were higher than in corresponding WAF samples. The significantly higher aqueous concentrations of phenanthrene in DO samples coupled with only slightly increased tissue residues resulted in lower bioaccumulation of phenanthrene in both AQ and AQ&D exposures. The biotransformation of phenanthrene was negligible during the uptake and depuration phases in both algae and rotifers. Whether the result of altered cell membranes or metabolic processes these results may be of concern as they may also indicate the potential for loss of important biomolecules, including nutrients.

Overall, results suggest the potential for trophic transfer of phenanthrene from rotifers to the next trophic level in the marine food chain would not be enhanced with the use of dispersants. However, understanding the mechanisms by which alterations in rotifer physiological or metabolic functions net these results warrants further investigation.

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References

Anderson, J.W., 1979. An assessment of knowledge concerning the fate and effects of PHs in the marine environment. In: Verberg, W.B., Calabrese, A., Thurberg, F.P., Vernberg, F.J. (Eds.), Marine Pollution: Functional Responses. Academic Press, New York, pp. 76–89.

Brown, D.H., 1973. Toxicity studies on the components of an oil-spill emulsifier using *Lichina pygmae* and *Xanthoria parietina*. Mar. Biol. 18, 291–297.

Cerniglia, C.E., Gibson, D.T., Van Baalen, C., 1979. Algal oxidation of aromatic hydrocarbons: formation of 1-naphthol from naphthalene by *Agmenellum quadruplicatum*, strain PR–6. Biochem. Biophys. Res. Commun. 88, 50–58.

Cerniglia, C.E., 1981. Aromatic hydrocarbons: metabolism by bacteria, fungi, and algae. Rev. Biochem. Toxicol. 3, 321–361.

Corner, E.D.S., Harris, R.P., Kilvington, C.C., O'Hara, S.C.M., 1976. Petroleum compounds in the marine food web: short-term experiments on the fate of naphthalene in *Calanus*. J. Mar. Bio. Assn. U.K. 56, 121–123.

D'Adamo, R., Pelosi, S., Trotta, P., Sansone, G., 1997. Bioaccumulation and biomagnification of polycyclic aromatic hydrocarbons in aquatic organisms. Mar. Chem. 56, 45–49.

Fedulova, A.N., 1976. Effects of petroleum dispersing agents on bioelectric characteristics of the alga *Nitella flexilis*. Biol. Nauki. 19, 31–35.

Grimberg, S.J., Nagel, J., Aitken, M.D., 1995. Kinetics of phenanthrene dissolution into water in the presence of nonionic surfactants. Environ. Sci. Technol. 29, 1480–1503.

Guha, S., Jaffe, P.R., 1996. Bioavailability of hydrophobic compounds partitioned into the micellar phase of nonionic surfactants. Environ. Sci. Technol. 30, 1382–1391.

Harris, R.P., Berdugo, V., O'Hara, S.C.M., Corner, E.D.S., 1977. Accumulation of ^{14}C-1-naphthalene by an oceanic and an estuarine copepod during long-term exposure to low-level concentrations. Mar. Biol. 42, 187–195.

Jobling, M., 1981. Influence of feeding on the metabolic rate of fishes: a short review. J. Fish Biol. 18, 385.

Kayal, S., Connell, D.W., 1995. Polycyclic aromatic hydrocarbons in biota from the Brisbane River Estuary, Australia. Estuar. Coast. Shelf Sci. 40, 475–493.

Knezovich, J.P., 1994. Chemical and biological factors affecting bioavailability of contaminants in seawater. In: Hamelink, J.L., Landrum, P.F., Bergman, H.L., Benson, W.H. (Eds.), Bioavailability: Physical, Chemical, and Biological Interactions. Lewis, Boca Raton, pp. 23–30.

Livingstone, D.R., 1991. Organic xenobiotic metabolism in marine invertebrates. Adv. Comp. Environ. Physiol. 7, 79–89.

Mackay, D., Shiu, W.Y., Ma, K.C., 1992. Illustrated Handbook of Physical–Chemical Properties and Environmental Fate for Organic Chemicals, vol. 2. Lewis, Chelsea, MI, pp. 62–71.

Malins, D.C., Collier, T.K., Sanborn, H.B., 1979. Disposition and Metabolism of Aromatic Hydrocarbons in Marine Organisms. In: Kahn, M.A.Q., Lech, J.J., Menn, J.J. (Eds.), Pesticide and Xenobiotic Metabolism in Aquatic Organisms. ACS Symposium Series No. 99, Chap. 4, pp. 57–75.

Malins, D.C., Hodgins, H.O., 1981. Petroleum and marine fishes: a review of uptake, disposition, and effects. Environ. Sci. Technol. 15, 1272–1280.

Neff, J.M., Cox, B.A., Dixit, D., Anderson, J.W., 1976. Accumulation and release of petroleum-derived aromatic hydrocarbons by four species of marine animals. Mar. Biol. 38, 279–289.

Neff, J.M., 1979. Accumulation and release of PAH by aquatic organisms. In: Polycyclic Aromatic Hydrocarbons in the Aquatic Environment: Sources, Fates, and Biological Effects. Applied Science Publishers, London, pp. 152–195

O'Flaherty, E.J., 1981. Toxicants and drugs: kinetics and dynamics. Wiley Interscience, New York, NY.

Rossi, S.S., Anderson, J.W., 1977. Accumulation and release of fuel oil-derived diaromatic hydrocarbons by the polychaete *Neanthes arenaceodentata*. Mar. Biol. 39, 51–55.

Spacie, A., Hamelink, J.L., 1985. Bioaccumulation. In: Rand, G.M., Petrocelli, S.R. (Eds.), Fundamentals of Aquatic Toxicology. Hemisphere, New York, pp. 495–525.

Spigarelli, S.A., Thommes, M.M., Prepejchal, W., 1983. Thermal and metabolic factors affecting PCB uptake by adult brown trout. Environ. Sci. Technol. 17, 88–94.

Tiehm, A., 1994. Degradation of polycyclic aromatic hydrocarbons in the presence of synthetic surfactants. Appl. Environ. Microbiol. 60, 258–263.

Varanasi, U., Malins, D.C., 1977. Metabolism of petroleum hydrocarbons: Accumulation and biotransformation in marine organisms. In: Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms, vol. II. Biological Effects. Academic Press, pp. 175–270.

Walker, J.D., Colwell, R.R., Vaituzis, Z., Meyer, S.A., 1975. Petroleum-degrading achlorophyllous alga *Prototheca zopfii*. Nature 254, 423–424.

Warshawsky, D., Cody, T., Radike, M., Reilman, R., Schumann, B., Ladow, K., Schneider, J., 1995. Biotransformation of benzo(a)pyrene and other polycyclic aromatic hydrocarbons and heterocyclic analogs by several green algae and other algal species under gold and white light. Chemico-Biol. Interact. 97, 131–148.

Wolfe, M.F., Schwartz, G., Singaram, S., Mielbrecht, E.E., Tjeerdema, R.S., Sowby, M.L., 1998a. Influence of dispersants on the bioavailability of petroleum hydrocarbons to golden-brown algae, *Isochrysis galbana*. Arch. Environ. Contam. Toxicol. 35, 268–273.

Wolfe, M.F., Schwartz, G., Singaram, S., Mielbrecht, E.E., Tjeerdema, R.S., Sowby, M.L., 1998b. Effects of salinity and temperature on the bioavailability of dispersed petroleum hydrocarbons to the golden brown algae, *Isochrysis galbana*. Arch. Environ. Contam. Toxicol. 35, 274–280.

Wolfe, M.F., Schlosser, J., Schwartz, G., Singaram, S., Mielbrecht, E.E., Tjeerdema, R.S., Sowby, M.L., 1998c. Influence of dispersants on the bioavailability and trophic transfer of petroleum hydrocarbons to primary levels of a marine food chain. Aquat. Toxicol. 42, 211–227.

Wolfe, M.F., Schwartz, G., Singaram, S., Mielbrecht, E.E., Tjeerdema, R.S., Sowby, M.L., 1998d. Influence of dispersants on the bioavailability and trophic transfer of petroleum hydrocarbons to larval topsmelt (*Atherinops affinis*). Aquat. Toxicol. Submitted.

Zar, J.H., 1974. Biostatistical Analysis. Prentice Hall, Englewood Cliffs, NJ, pp. 620.