The Biodegradation of Dispersed Oil Does Not Induce Toxicity

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ABSTRACT

The acute toxicity of dispersed oil is well understood, and oil dilutes to levels below those of acute concern within hours of dispersion whether oil is dispersed by waves or by lower energy turbulence in the presence of chemical dispersants. Once dispersed, the hydrocarbon components of the spilled oil are degraded promptly by the native microbes in seawater, typically with an apparent half-life of 7-30 days even under Arctic conditions. Nevertheless, concern has been raised that this biodegradation might increase the oil's acute toxicity by generating and releasing toxic by-products. We show here, using *Americamysis bahia* as the test species, that this does not occur when dispersed oil is present at environmentally-relevant concentrations (initially 3 ppm oil dispersed with Corexit 9500 at a dispersant to oil ratio of 1:20). The guidelines for this toxicity test mandate a temperature of 26 ± 1C, rather warmer than the temperature of collection of the seawater from the New Jersey shore that we used as our experimental medium, so it is not surprising that biodegradation was especially rapid with a half-life for the loss of detectable hydrocarbons of approximately 4 days. We conducted sequential 4-day acute toxicity tests for 20 days, by which time the indigenous microorganisms had removed almost 80% of the detectable hydrocarbons in the lightly weathered crude oil. We saw no mortality in any of the five sequential tests.

INTRODUCTION

Dispersants are important, if contentious, tools in oil spill response (Prince, 2015; Lewis and Prince, 2018). Developed to minimize the oiling of birds and shorelines by dispersing floating oil as tiny droplets that remain below the sea surface and diffuse apart (Canevari, 1974; Lessard and DeMarco, 2000), it has become clear that this process dramatically enhances oil biodegradation (McFarlin and Prince, 2021).

Dispersed crude oil is likely toxic at the concentrations found immediately after dispersion. For example, Barron et al. (2013) reported LC50 values for 48-96 hour exposure of 1-30 ppm for several marine animal species, and similar ranges were reported in a review of plant species by Lewis and Pryor (2013). Fortunately such levels do not persist because the dispersed plume dilutes to the sub ppm level within hours (Lee et al., 2013; Bejarano et al., 2014). It is significant that although most samples collected following the *Deepwater Horizon* tragedy were collected in locations expected to be contaminated by oil, the majority (84%) of the >13,000 water samples had oil concentrations below 1ppb (Wade et al., 2016). Nevertheless, there has been concern that the process of biodegradation might increase the toxicity of spilled oil by generating partially oxidized intermediates released to the sea (Shelton et al., 1999; Middaugh et al., 2002; Hansen et al., 2018).

We show here that 3 ppm dispersed oil is not acutely toxic to mysid shrimps, a particularly sensitive species routinely used for environmental toxicity testing (Nimmo and Hamaker, 1982; Barron et al., 2017), and that no acute toxicity becomes apparent as extensive biodegradation proceeds at those concentrations.

MATERIALS AND METHODS

Seawater (30 practical salinity units, psu) was collected in June 2016 from Sandy Hook, New Jersey in large polyethylene carboys, and gently aerated with an aquarium bubbler in the laboratory overnight. Nitrate and phosphate levels were below detection limits with simple laboratory colorimetric tests, but are likely to have been near 7 and 0.5 μM, respectively (Louanchi and Najjar, 2001). The biodegradation experiments were initiated the next day (Day 0) in twelve 1 L glass bottles, almost filling them with seawater, and adding 3 μl of lightly weathered Black Sea oil (initial API gravity 32.7) amended with Corexit 9500 at a dispersant to oil ratio of 1:20. The bottles were shaken to fully disperse the oil after they were transiently sealed with Teflon tape. The sealing tape was removed, a Teflon stir bar added, and the bottles stirred slowly (20 rpm) throughout the experiment. The incubations were carried out in an environmental chamber at the temperature (26 ± 1C) and light conditions (16h light: 8h dark) mandated for the USEPA-defined test (USEPA 2002a,b).

Mysid shrimp (*Americamysis bahia*) were purchased from Aquatic Research Organisms, (Hampton, NH 03843) so that all organisms were 5-7 days old at the initiation of each test. Routine

toxicity testing by the supplier ensured organisms were typical of the species. Animals were held in 20 L of artificial seawater (30 psu) prepared from Instant Ocean Sea Salts (Blacksburg, VA 24060) and deionized water.

Biodegradation began as the bottles were assembled at Day 0. Duplicate mysid toxicity tests in general agreement with EPA-821-R-02-014 (USEPA 2002b) were begun at day 0, 4, 8, and 16 in the ongoing incubations. Ten randomly selected mysids were placed into replicate bottles on the appropriate day, fed approximately 1500 brine shrimp (*Artemia* sp.) nauplii, and observed for mortality or morbidity every 24 h. After assessment at 96 h, tests were terminated by the addition of dichloromethane, and the oil was extracted, dried and analyzed by GC/MS to establish the extent of biodegradation (Prince et al., 2013) using hopane as a conserved internal standard (Prince et al., 1994). Duplicate experimental bottles were also extracted immediately after initial assembly to provide t=0 samples.

RESULTS

Oil biodegradation

Figure 1 shows GC/MS total ion chromatograms of the initial oil used in the experiment, and a sample extracted from an incubation at t=0 days. In order to mimic an oil that had been at sea for less than a day and thus a likely target for aerial dispersant application, we used a partially weathered oil that had lost only the most volatile components – the first significant peak in the initial oil chromatogram is decane. Extraction of this oil from the experimental bottles soon after assembly, and then concentrating the extract by evaporation (but not to dryness), led to the loss of further material and the first prominent peak in the t=0 sample is pentadecane. Thus, although molecules smaller than pentadecane are likely biodegraded along with the larger molecules we will discuss below, they might also be lost during the extraction and subsequent analysis, and we have used this extracted oil at t=0 as the benchmark for quantifying hydrocarbon biodegradation.

Figure 2 shows GC/MS total ion chromatograms of oils extracted at various times during the experiment. As expected (McFarlin and Prince, 2021), hydrocarbon biodegradation was rapid, and essentially all the *n*-alkanes and most of the resolvable *iso*-alkanes, and parent two and three-ring aromatics had been consumed within the first four days (Figure 3). More alkylated

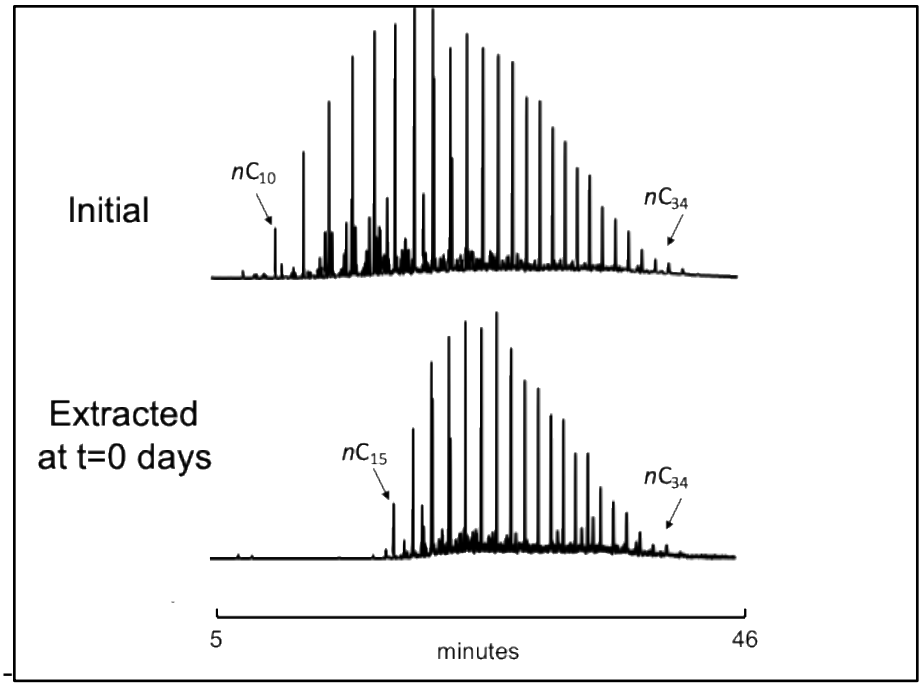

Figure 1. GC/MS total ion chromatograms of the lightly weathered oil used here, and the oils extracted at t=0. The traces are normalized to hopane content.

aromatics, such as C_4-naphthalenes and phenanthrenes, and C_2-chrysenes were degraded more slowly (Prince et al., 2013; 2017) and their biodegradation was proceeding throughout the experiment. By 20 days, some 78% of the total detectable hydrocarbons had been consumed by the indigenous seawater microbiota, including 50-80% of the initial 4-ring aromatics, C_4-3-ring aromatics, and C_2-4-ring aromatics (see legend of Figure 3 for the chemical species included in these groups).

hr	Initiated day 0	Initiated day 4	Initiated day 8	Initiated day 12	Initiated day 16
0	0%	0%	0%	0%	0%
24	0%	0%	0%	0%	0%
48	0%	0%	0%	0%	0%
72	0%	0%	0%	0%	0%
96	0%	0%	0%	0%	0%

Table 1. Mysid mortality in the five tests. Each test was performed in duplicate. There were no signs of any morbidity during the tests.

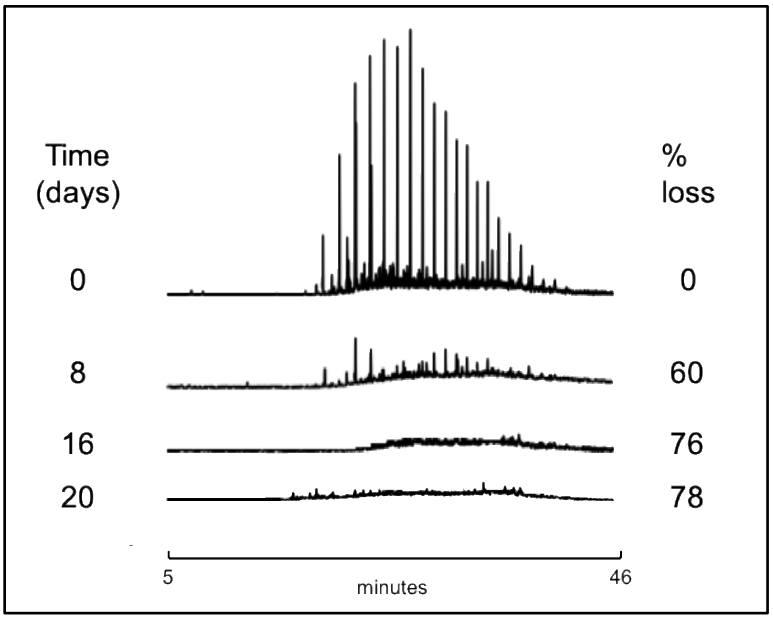

Figure 2. GC/MS total ion chromatograms of the oils extracted at various times from the experiment. % loss is calculated from the extracted oil at t=0, using hopane as a conserved internal marker, and the traces are normalized to hopane content.

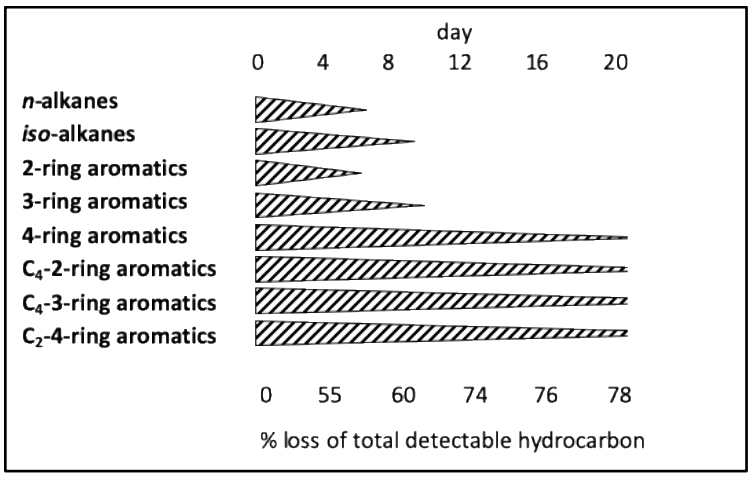

Figure 3. Schematic representation of when the biodegradation of different components was occurring, as monitored by the disappearance of the components relative to hopane as a conserved internal marker. *n*-alkanes = *n*C15-*n*C36; *iso*-alkanes = norpristane, pristane and phytane; 2-ring aromatics = naphthalene and fluorene; 3-ring aromatics = phenanthrene and dibenzothiophene; 4-ring aromatics = chrysene and benz[*a*]anthracene. The C_4 notation indicates those components with four methyl-equivalent additions (tetramethyl-, dimethyl,ethyl-, diethyl-, methyl,propyl-, methyl,isopropyl-, butyl-, etc.); the C_2 notation indicates those components with two methyl-equivalent additions (dimethyl- and ethyl-). Some 20-50% of the initial C_4-3-ring aromatics, chrysene and C_2-chrysenes remained at the end of the experiment.

Mysid Toxicity

All mysids survived their 96 h incubations (Table 1), from those exposed to the freshest (albeit partially weathered) dispersion to those exposed to the biodegraded oil that contained almost no resolvable components except the more alkylated aromatics. They appeared healthy and fully motile throughout.

DISCUSSION

Untreated oil slicks may persist for some time, killing birds and other animals, and potentially stranding on shorelines. Dispersants were originally developed to protect birds and other charismatic wildlife from oiling by floating slicks (National Research Council, 2005), and it took some time for the remarkably rapid biodegradation of dispersed oil that follows successful dispersion to be appreciated (McFarlin and Prince, 2021). The toxicity of dispersed crude oil is relatively well understood (Barron et al., 2013, 2017; Lewis and Pryor, 2013; Redman et al., 2012; Adams et al., 2012), and dispersed oil soon dilutes to levels below acute concern (Bejarano et al., 2014). But a nagging disquiet remained that perhaps the biodegradation process released intermediates that might be more toxic than the initial hydrocarbons in the spill (Shelton et al., 1999; Middaugh et al., 2002; Hansen et al., 2018). On the one hand this is not an unreasonable concern, since many highly toxic anticancer drugs are activated from almost benign prodrugs by intracellular enzymes (Rooseboom et al., 2004), and the metabolic conversion of many compounds by cellular (hepatic) extracts substantially increases their mutagenicity in the Ames test (Tejs, 2008). Interspecies interactions can be very important in the microbial food webs that degrade spilled oil, and this synergism is probbly facilitated by metabolite transfer between organisms (McGenity et al., 2012). Certainly some organisms release partial metabolites into the extracellular medium (e.g. Kazunga and Aitken, 2000), and other organisms can consume them (e.g. Boonchan et al., 2000). Fourier transform ion cyclotron resonance mass spectrometry is characterizing a variety of partially oxidized hydrocarbons that may be metabolic, or possibly photochemically-generated, intermediates generated after oil release (Ruddy et al., 2014; Chen et al., 2016). But on the other hand we know that all known aerobic hydrocarbon metabolism begins with oxidation requiring the reduction of one of the two atoms of diatomic oxygen to water at the expense of an NADH (Prince and Walters, 2016), so any organism that promiscuously oxidized hydrocarbons to dead-end metabolites would put itself at a potentially significant energetic disadvantage.

In any case, we set out to test the potential toxicity of biodegrading oil under conditions that are as close to 'environmental' as possible. In fact dispersed oil typically dilutes to much lower than 1 ppm (Lee et al., 2013; Bejarano et al., 2014; Wade et al., 2016), but such concentrations cannot provide enough oil for the analysis of biodegradation in experimentally tractable volumes. We therefore did our experiments at 3 ppm oil, which is higher than the vast majority of samples measured in the field (Wade et al., 2016). It is important to recognize that the goal of environmentally-relevant toxicity tests is NOT to be sure that we protect the tested species; rather a very sensitive species is chosen in the expectation that protecting IT will thereby protect the vast majority of less sensitive species. Mysids are indeed amongst the most sensitive organisms to oil toxicity (Barron et al., 2013, 2017; Bejerano et al., 2017). Our demonstration of no acute toxicity to a sensitive species, even at concentrations above those likely to be experienced in the sea, indicates that the biodegradation process, which is essentially complete in a few weeks following oil release, is very unlikely to cause acute toxicity to other organisms at environmentally-relevant concentrations. Since oil hydrocarbons are biodegraded so promptly, chronic toxicity from dispersed oil (Langdon et al., 2016) also seems very unlikely.

We have recently published this work elsewhere (Prince et al., 2019).

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