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Metabolism of Complex Mixtures of Oil Spill Surfactant Compounds by a Representative Teleost (*Salmo gairdneri*), Crustacean (*Cancer irroratus*), and Mollusc (*Chlamys islandicus*)

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Use of dispersants to combat the effects of petroleum spills has been a subject of intense debate in many countries for the past several years (e.g. OIL SPILL CONFERENCE PROCEEDINGS 1969-1981; NORTON & FRANKLIN 1980). This debate has stimulated a host of laboratory, as well as a few, "real world" studies (e.g. McAULIFFE et al. 1981), and a reasonable appreciation of the acute toxicity of both dispersant and dispersant/oil mixtures to many forms of aquatic life has now been obtained.

In reference to sublethal toxicity, one important criterion for the ecotoxicological assessment of any compound is its susceptibility to metabolism by target and non-target organisms. There is presently little information to indicate that aquatic organisms can degrade the active surfactant ingredients found in commercial oil dispersant formulations (WILDISH & BEATTY 1973). A colorimetric method for the detection of free fatty acids was adapted to assay esterase activity with polyethoxylate fatty acid ester substrates. It was possible with this method to demonstrate that a representative teleost, crustacean and mollusc have the capacity for enzymatic hydrolysis of the complex fatty acid ester mixtures found as surfactants in the 'new' generation oil spill dispersants.

MATERIALS AND METHODS

Liver or hepatopancreas extracts were prepared from rainbow trout (Salmo gairdneri), crab (Cancer irroratus) and scallop (Chlamys islandicus). Samples of tissue were homogenized in ice-cold Tris-Cl (0.05 M) - sucrose (0.25 M) -KCl (0.13 M) buffer, pH 7.4, and 9000 g supernatant fractions were precipitated with cold acetone (-15°C). Precipitates were placed on filters in Buchner funnels, rewashed with several volumes of acetone and stored at -20°C as dried powders. These powders were resuspended in 8-10 volumes of the same buffer on the day of assay. Reaction mixtures containing 1 mL of Tris buffer, 200-400 µL of homogenate and 20 µL of a 1:4 dilution (in propanol) of surfactant were incubated at 27°C for 1 hr. Enzyme activity was first established with Tween (sorbitan esterified with oleic acid and having an ethoxylate chain length of about C_{10}) and this was initially employed as the standard for establishing incubation protocol and extraction procedures. Dispersants then assayed, included Corexit 7664 (Esso Chemicals), Synperonic OSD 20 (Canadian Industries Ltd.), BP 1100

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X (BP Trading) and Oilsperse 43 (Diachem of B.C. Ltd.). A method reported to be effective for measuring free fatty acids in human blood (FALHOLT et al. 1973) was found to be suitable for assessing hydrolytic release (putatively via esterase activity) of fatty acids from the surfactant compounds. This method involves conversion of free fatty acids to copper soaps which can then be determined colorimetrically in the presence of diphenylcarbazide. Lauric acid was used as the standard fatty acid for establishing extraction procedures.

The potential for esterase induction by Oilsperse 43 was also assessed in rainbow trout. Fish (50-80 g) were exposed for a week in a flow through aquarium to a water concentration of 10 ppm dispersant. Enzyme activity was determined in 9000 g supernatant fractions prepared from liver homogenates. Reaction mixtures containing 5 mL Tris-Cl (0.05 M) - sucrose (0.25 M) - KCl (0.13 M) buffer, pH 8.0, homogenate and 50 µL nitrophenyllaurate (4 mg/mL dissolved in methanol) were incubated at 27°C for 1 hour. Nitrophenol release from the ester substrate was monitored directly at 420 nm (KRISCH, 1966).

RESULTS AND DISCUSSION

A micromethod for the detection of copper soaps of free fatty acids was adapted for assaying enzyme esterase activity towards fatty acid esters. Two similar methods of fatty acid detection were also assessed but "signal to noise" ratios were generally more variable with these procedures (MIKAC-DEVII et al. 1973; PINELLI 1973). Besides its use in demonstrating the metabolism of pure sources of fatty acid esters duch as Tweens, the method was also found to be suitable for measuring enzymatic hydrolysis of complex mixtures of polyethoxylate fatty acid esters, such as those commonly found in oil spill dispersants (Table 1). Copper soap formation and

TABLE 1

Aquatic animal esterase activity towards surfactants found in commercial oil spill dispersant formulations

Dispersant	Specific activity[a]		
	Trout (liver)	Crab (hepatopancreas)	Scallop (digestive gland)
Corexit-7664	0.05[b]	0.12 ± 0.14	0.08 ± 0.06
Synperonic 20	N.D.[c]	0.07 ± 0.01	N.D.[c]
BP 1100 X	0.08 ± 0.05	0.12 ± 0.02	0.13 ± 0.04
Oilsperse 43 (A)	0.05[b]	0.07 ± 0.02	0.04 ± 0.02
Oilsperse 43 (B)	0.05[b]	0.06 ± 0.02	0.05 ± 0.02
Oilsperse 43 (C)	0.06[b]	0.08 ± 0.03	0.04 ± 0.01

[a]Refers to optical density units/mg protein/hr. Values are reported as the mean of three determinations ± S.D. Blank values have been subtracted and one unit is equivalent to 10 µg of lauric acid.
[b]One determination only.
[c]Activity not detected.

extraction procedures with dispersants alone generally gave high blank values, probably due in part to contaminating fatty acids, but tissue mediated generation of fatty acids was easily resolvable. Tissue extracts alone gave low background values. Representative animals from three different phyla were assessed and the surfactants indicated to be amenable to hydrolysis varied in complexity as to fatty acid type, ethylene oxide chain length and type of solvent (PENROSE & DAWE 1976). In order to account for as many confounding factors as possible to metabolism, the dispersants were assessed neat. Also, three different samples of one formulation, Oilsperse 43, were assayed to check for possible variability between batches.

There was no significant increase in esterase activity in trout exposed to Oilsperse 43 (Table 2). Although dispersants are lipophilic, previous studies also indicated that Oilsperse 43 was ineffective in inducing mixed function oxygenase detoxifying enzymes (PAYNE & MAY 1979).

TABLE 2

Induction of esterase activity in trout liver by Oilsperse 43

	No. fish	Specific activity[a]
Control	5	0.34 ± 0.07
Experimental	5	0.40 ± 0.14

[a]Refers to optical density units/mg protein/hr. Values are reported as the mean of 3 determinations ± S.D. One unit is equivalent to 5 µg nitrophenol.

Hydrolysis cleaves surfactant molecules into hydrophilic and lipophilic components, thus effectively destroying the active surfactant moieties. One interesting response which has been observed in fish with oil spill dispersants and shown to be due to intact surfactant is the induction of bradycardia (KICENIUK et al. 1978). Representatives from three different animal phyla have now been shown to possess the capacity to hydrolyze heterogeneous mixtures of commercial surfactants. This observation will help to aid in the overall assessment of the long term fate and effects of oil spill dispersants in the marine environment.

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