A Preliminary Study of the Toxic Effects of Irradiated vs. Non-Irradiated Water Soluble Fractions of #2 Fuel Oil

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As part of an on-going study of the biological effects upon aquatic life of petroleum and petroleum by-products upon the Delaware Estuary (Trenton to the Delaware Bay) we have measured the toxic effects of the water soluble fraction of #2 fuel oil upon a number of test organisms. In the course of this study it was determined that exposure to ultra violet light as provided by a 275 watt Sylvania sunlamp, increased the toxicity of the water soluble fraction of #2 fuel oil.

Oil (bioassay) studies are abundant and many of these involve specifically fuel oils (MICHAEL, et al, 1975; STAINKEN, 1975; BLUMER, 1969; HAMPSON and SANDERS, 1969; BLUMER, et al, 1970; BLUMER and SASS, 1972). Spilled oil can be altered by many factors, however, the photochemical change which apparently occurs in #2 fuel oil is of interest because it may be one of the most important of all modifying factors and at this point one of the least studied. Number 2 fuel oil is commonly used in the industrial and residential areas surrounding the Delaware Estuary and spills of #2 fuel oil in the estuary and other areas are not uncommon. The #2 fuel oil used in this study was commercial grade #2 fuel oil supplied by the Cundiff Oil Company, a local distributor which is supplied by the Mobil Oil Company.

The water soluble fraction was prepared by stirring a 10:1 mix (water to oil) of natural river or bay water with #2 fuel oil for 72 hours. This was accomplished in five gallon wide mouth glass jars using a magnetic stirrer. After 72 hours the water soluble portion was removed from beneath the #2 fuel oil layer. The irradiated portion was prepared under continuous light source from a Sylvania 275 watt sunlamp located 36" from the top of the glass jar. The non-irradiated water soluble fractions were prepared

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in an area of the laboratory which was shielded by large partitions from all light.

Acute continuous flow bioassay studies according to the methods of SCHEIER and BURTON (1973) were performed on five aquatic species. These species were chosen for testing for a number of reasons. They are commonly found in the estuary and are important members of the supporting food web of the estuary. They are representative organisms as regards the varying salinities to be found throughout the estuary from fresh to ocean water. They can be cultured in the laboratory and are therefore valuable for the life cycle studies with which we are also concerned. The test species used were the grass shrimp, Palaemonetes pugio and the sheephead minnow, Cyprinodon varigatus both brackish to salt water species; the mummichog, Fundulus heteroclitis which is found in brackish water as well as water of low salinity; the channel catfish, Ictalurus punctatus and the bluegill sunfish, Lepomis macrochirus which are both fresh water species.

The bioassay studies were 96 hours in duration and an LC_{50} was derived for each species. In all cases natural Delaware Bay or Delaware River water was used as the dilution water. The test temperature was 25°C. Table 1 lists the results of these tests.

A further set of experiments was performed in an attempt to more closely simulate the natural conditions which might be found in the estuary. To this end a large vat was constructed measuring eight feet by four feet by eight inches deep. Three hundred and sixty liters of natural Delaware Bay or River water were added to the vat with a three liter per hour flow through of fresh dilution water into and out of the vat. One liter of #2 fuel oil was floated on top of the water and the oil was not removed for the entire course of the experiment. Two Sylvania sunlamps were suspended three feet above the vat. At 24 hours, 72 hours and six days water was drawn off from under the #2 fuel oil slick and used for bioassay tests. The same five test species were used as in the first water soluble fraction experiments. Table 2 describes the results of these experiments.

Infrared analyses were used to analyse for phenols, aromatics and aliphatics during the course of the bioassay tests. Table 3 presents comparisons of typical

TABLE 1

96 hour LC_{50} for five test species exposed to irradiated and non-irradiated water soluble fraction of #2 fuel oil, 72 hour mix.

Test Organism	96 Hr. LC_{50} and 95% confidence intervals with U. V. Lite	Effects without U. V. Lite
1. *Palaemonetes pugio* (Grass Shrimp)	34.4% by Volume (32.7 - 36.5)	100% by Volume 96 Hr. LC_{50}
2. *Fundulus heteroclitus* (Mummichog)	47.53% by Volume (45.7 - 49.2)	100% Survival at 100% by Volume for 96 Hrs.
3. *Cyprinodon varigatus* (Sheephead Minnow)	45.5% by Volume (43.4 - 47.6)	100% Survival at 100% by Volume for 96 Hrs.
4. *Ictalurus punctatus* (Channel Catfish)	75% by Volume (71.2 - 78.7)	90% Survival at 100% by Volume for 96 Hrs.
5. *Lepomis macrochirus* (Bluegill Sunfish)	39.6% by Volume (36.8 - 43.4)	80% Survival at 100% by Volume for 96 Hrs.

TABLE 2

Bioassay results for 24 hour, 72 hour and 6 day water removed from under a #2 fuel oil slick, irradiated vs. non-irradiated, test temperature 25°C.

Test Organisms	24 hrs. with U. V. Lite	24 hrs. without U. V. Lite	72 hrs. with U. V. Lite
Palaemonetes pugio 1. Grass Shrimp	50% Surv.-3hrs.10m. 0% Surv.-5hrs.40m.	100% Survival at 100% by Vol. for 96 hrs.	50% Surv.-1hr. 0% Surv.-1hr. 25m.

	72 hrs. without U. V. Lite	6 days with U. V. Lite	6 days without U. V. Lite
	100% Surv. at 100% by Vol. for 96 hrs.	50% Surv.-50m. 0% Surv.-1hr. 10m.	100% Survival at 100% by Vol. for 96 hrs.

Test Organisms	24 hrs. with U. V. Lite	24 hrs. without U. V. Lite	72 hrs. with U. V. Lite
Fundulus heteroclitus 2. Mummichog	50% Surv.-3hrs. 0% Surv.-4hrs.55m.	100% Survival at 100% by Vol. for 96 hrs.	50% Surv.-1hr.5m. 0% Surv.-1hr. 40m.

TABLE 2 (Continued)

Bioassay results for 24 hour, 72 hour and 6 day water removed from under a #2 fuel oil slick, irradiated vs. non-irradiated, test temperature 25°C.

	72 hrs. without U. V. Lite	6 days with U. V. Lite	6 days without U. V. Lite
Fundulus heteroclitus (Continued)	100% Surv. at 100% by Vol. for 96 hrs.	50% Surv.-55m. 0% Surv.-1hr. 20m.	100% Surv. at 100 % by Vol. for 96 hrs.

Test Organisms	24 hrs. with U. V. Lite	24 hrs. without U. V. Lite	72 hrs. with U. V. Lite
Cyprinodon varigatus 3. Sheephead Minnow	50% Surv.-2 1/2hrs. 0% Surv.-5hrs.15m.	100% Surv. at 100% by Vol. for 96 hrs.	50% Surv.-25m. 0% Surv.-1hr. 50m.

	72 hrs. without U. V. Lite	6 days with U. V. Lite	6 days without U. V. Lite
	90% Surv. at 100% by Vol. for 96 hrs.	50% Surv.-25m. 0% Surv.-45m.	100% Surv. at 100% by Vol. for 96 hrs.

TABLE 2 (Continued)

Bioassay results for 24 hour, 72 hour and 6 day water removed from under a #2 fuel oil slick, irradiated vs. non-irradiated, test temperature 25°C.

Test Organisms	24 hrs. with U. V. Lite	24 hrs. without U. V. Lite	72 hrs. with U. V. Lite
Ictalurus punctatus 4. Channel Catfish	Complete death in 55 mins.	100% Surv. at 100% by Vol. for 96 hrs.	Complete death in 30 mins.
	72 hrs. without U. V. Lite	6 days with U. V. Lite	6 days without U. V. Lite
	90% Surv. at 100% by Vol. for 96 hrs.	Complete death in 25 mins.	90% Surv. at 100% by Vol. for 96 hrs.
Test Organisms	24 hrs. with U. V. Lite	24 hrs. without U. V. Lite	72 hrs. with U. V. Lite
Lepomis macrochirus 5. Bluegill Sunfish	50% Surv.-45m. 0% Surv.-1hr. 45m.	100% Surv. at 100% by Vol. for 96 hrs.	50% Surv.-30m. 0% Surv.-2hr.5m.

TABLE 2 (Continued)

Bioassay results for 24 hour, 72 hour and 6 day water removed from under a #2 fuel oil slick, irradiated vs. non-irradiated, test temperature 25°C.

	72 hrs. without U. V. Lite	6 days with U. V. Lite	6 days without U. V. Lite
Lepomis macrochirus (Continued)	100% Surv. at 100% by Vol. for 96 hrs.	50% Surv.-40m. 0% Surv.-1hr. 35m.	100% Surv. at 100% by Vol. for 96 hrs.

results of infrared analyses of stock solutions (100% by volume of either mixed or vat produced water soluble fraction) used in the bioassay tests. It should be noted that the 10:1 water soluble mix bioassays were only performed on 72 hour water soluble mix, although chemical analyses were performed on 24 hour and six day samples, while the vat tests were performed on 24 hour, 72 hour and six day vat water soluble material.

TABLE 3

Comparisons of typical results of infrared analyses of stock solutions used in the bioassay tests. Given as mg/l.

	Phenols	Aromatics	Aliphatics
L 24 hr. mix	.35	2.7	3.0
L 24 hr. vat	.32	1.6	2.2
D 24 hr. mix	.24	1.6	2.0
D 24 hr. vat	<.03	.42	.41
L 72 hr. mix	.55	3.8	6.0
L 72 hr. vat	.70	2.2	3.4
D 72 hr. mix	.33	1.9	2.2
D 72 hr. vat	.23	.35	1.4
L 6 day mix	2.3	13.0	15.0
L 6 day vat	.75	2.7	4.3
D 6 day mix	.30	2.0	2.7
D 6 day vat	.23	.35	1.4

L light used in preparation of test sample
D test sample prepared in darkness

An attempt was made to relate the rate of photochemical reaction caused by the Sylvania sunlamp to that of actual sunlight. The method used was the conversion of anthracene to dianthracene (DORE, 1958). The comparison was between the sunlamp at 36" from the anthracene for 24 hours and a bright cloudless September day, September 29, 1975. The standards were exposed to sunlight for one, two, three, four and five hours. Comparisons of the samples showed that 24 hours of exposure to the sunlamp was equivalent to about one-and-one-half hours of actual sunlight.

Chromatographic studies are being performed to be followed by attempts to isolate and test the toxic components of the irradiated oil. We also intend to repeat these studies under natural conditions in the Delaware Estuary.

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