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Detection of Waterborne Mutagens and Characterization of Chemicals in Selected Galveston Sites after an Oil Spill

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In our previous study, we proposed a unique sampling technique for mutagens in marine environment by suspending an adsorbent, blue rayon, selective to polycyclic mutagens with three or more fused rings (Hayatsu et al. 1983, Hayatsu 1992). By using this technique, we were able to bring back a small amount of adsorbent weighing less than 10 g, from remote sampling sites, rather than large volumes of water. In the summer of 1990, a collision of barge tankers occurred in Galveston Bay and approximately 500,000 gal of oil were spilled into the Bay. Several sites in Galveston Bay were sampled 5-7 d after the oil spill. We characterized the pollutants chemically and detected the mutagenicity. We designed the present study to examine the applicability of our technique from two points of view. One was to determine if there was a correlation between mutagenicity of blue rayon-adsorbed compounds and the level of known mutagens detected in water samples from the same site. The other was to certify if the sampling technique provided a convenient method for handling water samples collected at remote sites. The chemical analysis was carried out in Texas (U.S.A) and the mutagenicity testing was done in Okayama (Japan).

MATERIALS AND METHODS

Eight sites in Galveston Bay as shown in Figure 1 were selected for sampling. Sampling was conducted 5-7 d following the release of 500,000 gal of crude oil into Galveston Bay as the result of a tanker collision on July 28, 1990. The sites varied in their distance from the spill and included a "control site", i.e., at a remote (not obviously affected) site from the spill. Blue Rayon (BR) was purchased from Funakoshi Chemicals

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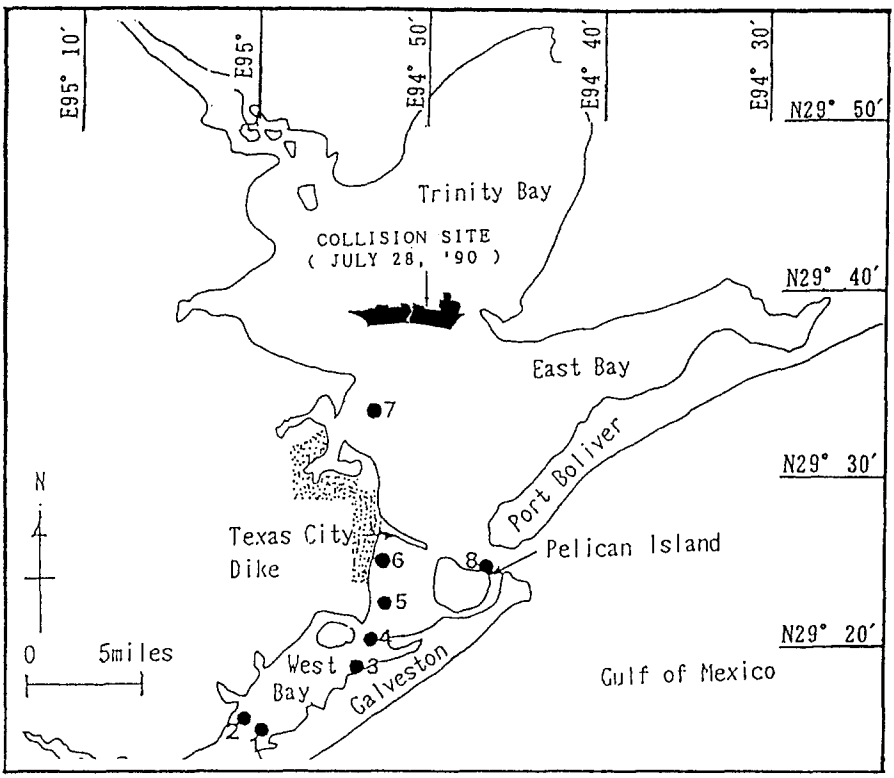

Figure 1. Sampling Sites of Water- and Oyster-samples in Galveston Bay. Dots indicate industrialized areas.

(Hongo, 2-9-7, Bunkyo-ku, Tokyo 113). One g of blue rayon in a net was placed at depth of 30-50 cm below the water surface; the rayon was collected 24 hr after being placed in the water.

The rayon was removed from the water and placed in a sealed container for transport to the laboratory. In the laboratory, the rayon was rinsed three times with 50 mL of reverse osmosis deionized water, the water was removed by aspiration and "blotting" with an absorbent towel, and the rayon was placed in a precleaned vial until time of extraction.

In addition to determining the mutagenic responses of the extract from the blue rayon, conventional sampling of water was carried out for chemical characterization. Water samples were taken at the sites immediately prior to placing the blue rayon in the water. Water samples were taken in precleaned glass bottles. Bottles were sealed and transported on ice to the laboratory. Water samples were refrigerated at 4°C for 1-2 d until time of

analysis. Oyster samples were also collected at sites 3 to 7. The extraction procedure for the oyster samples and preparation for the mutagenicity test were carried out using our previously published procedure (Kira et al. 1989). In this study, we used methylene chloride instead of 100% acetone (as in our original procedure) according to the procedure reported by Giam and Atlas (1986) for homogenization and extraction of oyster samples. Polycyclic compounds adsorbed to the blue rayon were extracted by shaking the blue rayon with 150 mL methanol/concentrated ammonia (50:1) solution for 30 min at room temperature. The extract was evaporated to dryness under reduced pressure and stored in a freezer until time of mutagenicity determination. Histidine, which can interfere with the assay, was removed by this blue rayon extraction (Hayatsu 1992).

For chemical analysis, 1 L of water was extracted with 3 volumes of pesticide-grade methylene chloride, purchased from Burdock and Jackson (Muskegon, Michigan). Extracts were combined and concentrated with a K-D concentrator to a volume of 1 mL. 2 µL of concentrated extract was injected for GC-MS and GC-FID analysis. The equipment used here were Hewlett Packard 5890 series II model with 5971A mass selective detector for GC-MS analysis; 5880 model gas chromatograph with flame ionization detector for GC-FID analysis. The GC conditions were: initial oven temperature, 35°C for 4 min; final temperature 270°C for 20 min; temperature program rate, 8°C/min; injector temperature, 200°C; detector temperature, 280°C. The GC column was a glass capillary column, PTE-5, 0.25 mm id, 30 m length and 0.25 µm film thickness, bonded 5% phenyl/95% dimethyl polysiloxane, purchased from Supelco (Bellefonte, Pennsylvania). Recoveries for 21 chemicals detected here were 75 ± 5 % (mean ± SD), but all data are reported uncorrected for recovery. Process blanks indicated no interfering contamination.

The tester strain used for mutagenicity test was *Salmonella typhimurium* TA98 (McCann et al. 1975), which is a kind gift of Dr. B.N. Ames of the University of California, Berkeley. The S9 mix was prepared from livers of SD rats treated with polychlorinated biphenyl (KC-54, the chlorine content of which was 54%). The mutation assay was done according to the preincubation technique; the bacteria were preincubated with the test sample and S9 mix for 20 min at 37°C, as reported by Yahagi et al.(1977).

In order to test the applicability of the blue rayon adsorption technique to water sampling at remote sites, a special method of handling samples were required. The method used is given in Scheme 1.

Scheme 1. Handling of Samples from Galveston Sites to the Laboratory

Location	Water sample for mutagenicity test	Water sample for chemical analysis	Oyster sample
Sampling site, Galveston Bay Texas, USA	BR suspended in water for 24 hr and recovered. ↓	Water sample (ca. 4 L) taken into clean bottle and kept on ice. ↓	Oyster samples taken, wrapped in aluminum foil and kept on ice. ↓
TAMUG CZL lab., Texas, USA	Recovered BR rinsed with distilled water. ↓ Residual water removed with clean paper towel. ↓ Packed in clean container and covered with aluminum foil. ↓ Kept at -40°C until transported to Japan ↓	Samples kept at 4°C until analysis started. ↓ Chemical analysis ↓ Extracted with methylene chloride. ↓ GC-FID or GC-MS analysis	Shell removed and edible part weighed. ↓ Packed in clean container covered with alminum foil. ↓ Kept at -40°C until extraction started. ↓ Thawed, homogenized and extracted with methylene chloride. ↓ Extract residue dissolved in methanol, transferred to clean container and methanol evaporated to dryness. ↓ Dried residue was kept at -40°C until shipping to Japan ↓
Transported to Japan	Packed in a container with dry ice and flown. ↓		Packed in container with dry ice and mailed. ↓
Okayama Univ. lab., Okayama, Japan	Thawed and mutagens extracted from BR. ↓ Condensation ↓ Mutagenicity test		Thawed and residue dissolved in methanol. ↓ Suspended in distilled water and treated with blue rayon.

All solvents used in Okayama University Laboratory were pesticide grade, purchased from Wako Pure Chemicals (Osaka, Japan).

RESULTS AND DISCUSSION

Table 1 shows the characterization of organics and mutagenic activities in Galveston water- and oyster-samples. Ten organics were detected at measurable amounts in samples from all sites by GC-FID and GC-MS. Although several polycyclic aromatic hydrocarbons (PAHs), such as fluoranthene, pyrene and chrysene, were detected in the samples, no PAHs with potent mutagenicity were found under the present analysis conditions. However, blue rayon from areas in close proximity to the spill site (site 7), which were also more industrialized areas (sites 5, 6 and 7), had a higher mutagenic response than blue rayon from more distant sites (sites 1 and 4). No obvious correlation was observed between the mutagenic activity and the levels of petroleum hydrocarbons detected in the water samples. Although the blue rayon extract showed high mutagenic activity, the oyster samples collected at all sites showed only weak activity. The activity detected in the oyster samples was similar to the levels in oysters reported previously (Hayatsu and Hayatsu, 1988) and also to those in mussels (Kira et al., 1989), both harvested in the Seto Inland Sea of Japan.

The following concept of the measurement may explain the results obtained. Levels of pollutants in a "spot" sampled water represent the pollution at that moment only. These measurements are conventional, and frequent sampling is necessary to monitor the pollution. Mutagenicities detected by the blue rayon immersion represent a total amount of mutagens adsorbed during immersion for 24 hr. Levels of mutagens (i.e., polycyclic aromatic compounds) in marine biota (i.e., those in mussels and oysters) represent the accumulated amount of these compounds over a period of time during their presence in those particular sites. Therefore, these measurements may represent chronic pollution even if the levels of pollutants are extremely low in the ambient water. We reported (Kira et al. 1989) the measurements of mutagenicity in mussels and their ambient water in the Seto Inland Sea of Japan using the same technique as used in the present study. The results obtained there also showed discrepancies between the mutagenicities in the mussels and those in their ambient waters. Therefore, it is not surprising to find a similar discrepancy in this Galveston study. We suspect the presence of some mutagenic compounds in the Galveston Bay blue rayon samples, perhaps at trace

Table 1. Mutagenic activities and concentrations of organics in Galveston Bay water

	Sampling site[a]							
	1	2	3	4	5	6	7	8
Mutagenic activity[b]	No. of revertants/plate							
Water sample								
0.16 g BR extract	18	5	19	22	56	5656	3742	8
0.80 g BR extract	108	44	42	89	308	5888	5250	40
Oyster sample								
10 g extract	NS	NS	87	30	21	47	51	NC
Compound	Concentration of compound (ng/mL)							
Pentadecane	0.16	0.25	0.52	0.48	0.11	0.82	0.54	7.0[c]
DEP	0.07	0.70	0.09	0.54	0.99	0.25	0.60	NC
Heptadecane	0.03	0.04	0.14	0.39	0.16	0.09	0.43	28.9[c]
1,1'-Biphenyl, 2,2'-diethyl	ND	ND	0.01	ND	0.04	0.01	ND	NC
Triazine diamine[1]	0.02	0.22	0.07	0.08	0.04	0.05	0.08	NC
Phenanthrene	0.002	0.22	0.008	ND	0.02	0.04	ND	NC
Phenanthrene[2]	0.003	0.22	0.008	0.03	0.02	0.02	0.03	NC
DBP	0.09	0.06	0.15	1.14	0.13	0.23	1.23	NC
9,10-Anthracenedione	0.01	0.27	0.02	0.06	0.02	0.03	0.06	NC
Methanone[3]	0.02	0.06	0.06	0.02	0.03	0.01	0.03	NC
Hexadeca-3,15-diene[4]	ND	0.02	0.05	0.04	0.08	0.05	0.04	NC
Fluoranthene	0.009	0.92	0.01	0.05	0.03	0.01	0.06	NC
Pyrene	ND	0.40	0.01	0.09	0.01	0.02	0.11	NC
1-Methylpyrene	0.009	0.04	ND	ND	ND	ND	ND	NC
11H-Benzo(b)fluorene	ND	0.04	0.006	0.02	ND	ND	0.02	NC
BBP	0.01	0.09	0.01	0.84	0.01	ND	0.93	NC
Benzo(ghi)fluoranthene	0.006	0.01	0.04	ND	ND	ND	ND	NC
Phosphoric acid[5]	ND	ND	0.02	0.04	0.36	0.01	0.05	NC
Chrysene	0.07	0.01	0.01	0.04	0.005	0.02	0.05	NC
Benzo(a)anthracene	ND	0.09	0.03	ND	ND	ND	ND	NC
DEHP	0.18	0.01	0.27	0.46	0.005	0.06	0.51	NC

a): See Figure 1.

b): The assay was done with *Salmonella typhimurium* TA98 in the presence of S9-mix. Values represent net increase over the solvent (DMSO) control; which was 25±5.

c): Sixteen alkanes (C_{15-30}) were found in the water sample from site 8. The highest one was octadecane (C_{18}) at 33.7 ng/mL.

1-5 indicate derivatives of compounds. 1= 1,3,5-triazine-2,3-diamine, 6-chloro-N-ethyl-N'(1-methlethyl), 2= 4-H-cyclopenta(def)phenanthrene, 3= methanone, (2-hydroxy-4-methyl-phenyl)phenyl, 4= tricyclo(8,6,0,0E2,9)hexadeca-3,15-diene, 5= phosphoric acid, 2-ethylhexyl, diphenyl ester.

DEP= 1,2-benzene dicarboxylic acid diethyl ester, DBP= 1,2-benzene dicarboxylic acid di-butyl ester, BBP= 1,2-benzene dicarboxylic acid, butyl phenylmethyl ester, DEHP= 1,2-benzene dicarboxylic acid, bis(2-ethylhexyl)ester.

ND: not detected (less than 0.001 ng/mL). NS: no sample. NC: not confirmed by GC-MS.

concentrations, that were not detected by GC-FID and GC-MS analysis.

Our results suggested that the Blue Rayon adsorption technique is useful and convenient for monitoring mutagenicity in the marine environment, especially whenever the sampling sites are far from the laboratory.

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