Bull. Environm. Contam. Toxicol. 21,719-726 (1979)

Uptake and Depuration of Petroleum Hydrocarbons in the Manila Clam, *Tapes semidecussata* Reeve

Pepsi Nunes[1] and Pete E. Benville, Jr.[2]

[1]*Bodega Marine Laboratory, University of California, Bodega Bay, Calif. 94923 and* [2]*National Marine Fisheries Service, Southwest Laboratory, 3150 Paradise Drive, Tiburon, Calif. 94920*

Increased offshore production and transoceanic shipment of petroleum and petroleum-derived products place a burden of oil upon the oceans of the world and pose potential problems for marine and estuarine organisms. Their commercial importance, wide distribution, and the major role they play in intertidal ecosystems led to the utilization of marine bivalve molluscs in oil pollution studies. The Manila clam, Tapes semidecussata, is a commercially important shellfish occurring on the North American Pacific Coast from Alaska to California. To date, there have been no attempts to examine the sensitivity of this bivalve to petroleum hydrocarbons and their ability to accumulate and store these materials in their tissues.

Being sessile filter-feeders, clams are readily exposed to pollutants adsorbed on suspended particulate matter. Little is known of the extent to which petroleum hydrocarbons taken up by marine bivalves are retained and concentrated in their tissues. Such assimilation and concentration could pose a health threat to human consumers of edible marine shellfish as some of the polycyclic aromatic hydrocarbons have been reported to be carcinogenic.

Aromatic hydrocarbons are more water soluble than paraffinic and naphthenic hydrocarbons (MCAULLIFFE 1966) with the paraffins reported to be preferentially attached by micro-organisms (BLUMER et al. 1970) resulting in an increase in the aromaticity of the residue. Evidence presented by STRAUGHAN (1972) indicated that the more highly refined oils are more toxic to the biota than heavier oils, perhaps due to their greater percentage of aromatic hydrocarbons which are known to be toxic to a wide variety of marine organisms. In this study we selected to look at six specific monocyclic aromatics of the water-soluble fraction (WSF) of Cook Inlet crude oil and the extent to which they are accumulated and retained by the Manila clam.

0007-4861/79/0021-0719 $01.60

MATERIALS AND METHODS

Test specimens were collected from South San Francisco Bay and immediately transferred to the Tiburon Laboratory where they were placed in holding tanks with flowing bay water of 30 ppt salinity and 14 ± 1°C for an acclimation period of one week. Bay water entering the laboratory is filtered and sterilized by ultraviolet light to reduce the ambient levels of petroleum components to approximate near-zero level (KORN 1975). Periodic analysis of water samples revealed levels below our limits of detectability.

In addition to the differences in the solubilities of the many chemicals found in crude oil, there are many other physical and chemical variations which make it difficult to produce and maintain a consistent concentration of the water-soluble fraction over an extended period of time (LEINONEN and MCKAY 1973, CRADDOCK 1977). A continuous-flow apparatus designed to deliver uniform solutions of the water-soluble components of Cook Inlet crude oil without the loss of the more volatile compounds and without the formation of emulsions had to be developed for this study (Fig. 1).

Seventy clams of one size class (mean volume = 20 ml) were placed in a 30-gallon glass aquarium and exposed for eight days to a mean concentration of 3.1 ppm of the six monocyclics analyzed for. Control clams were kept in holding tanks whose ambient levels of petroleum components were held to near-zero. The hydrocarbon content of the test medium was monitored three times daily throughout the exposure period. Photoperiod, intensity, and wavelength distribution of light approximated that in the natural environment as closely as possible. Subsamples of 10 test specimens were taken every 48 hr for analysis of their aromatic content. At the end of the exposure period, the remaining clams were transferred to uncontaminated holding tanks with flowing seawater and allowed to depurate.

Tissues from ten animals from each sampling interval were pooled and the 10 g wet weight composites mascerated to fine homogenates which were then digested with 6 ml of 4N NaOH and 4 ml of TF Freon (trichlorotrifluoroethane) and left in an oven at 30°C for 18 hr. After the samples had cooled off, they were centrifuged at 3000 RPM for 10 min. A 3.6 µl of the Freon extract was injected into a Micro-Tek 220 Gas Chromatograph equipped with a flame ionization

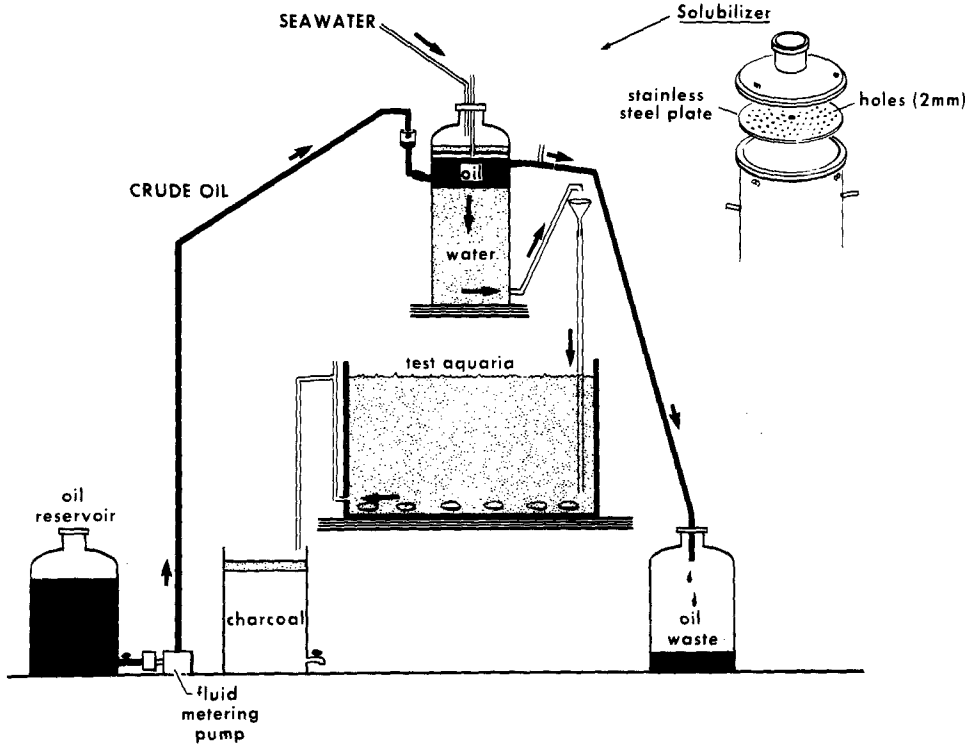

Fig. 1. A Continuous Flow System for Exposing Marine Organisms to the Water-Soluble Fraction of Crude Oil.

		Concentrations of WSF-MA in Water Phase (ppm)							Tissue Concentration (ppm)						
	Day	Benzene	Toluene	Ethyl-benzene	Para-xylene	Meta-xylene	Ortho-xylene	Total	Benzene	Toluene	Ethyl-benzene	Para-xylene	Meta-xylene	Ortho-xylene	Total
	1	2.2	1.2	.07	ND	.14	.10	3.67							
	2	1.7	1.3	.09	ND	.13	.15	3.33	ND	2.3	.34	ND	.68	.69	4.04
	3	2.7	1.7	.09	ND	.23	.32	4.87							
	4	1.7	1.4	.10	ND	.16	.12	3.46	ND	2.2	.32	ND	.89	.87	4.24
	5	1.4	1.2	.06	ND	.15	.12	2.85							
	6	0.9	0.9	.06	ND	.11	.09	1.98	ND	.87	.17	ND	.64	.61	2.29
	7	1.1	1.0	.06	ND	.15	.08	2.34							
	8	1.1	1.1	.07	ND	.14	.12	2.47	ND	2.0	.37	ND	.90	.87	4.16
	$\bar{X}$	1.6	1.2	.08		.15	.14	3.12							
	S.D.	0.61	0.26	.02		.04	.08	0.92							
Depuration	9								ND	3.30	.50	ND	.79	.68	5.10
Depuration	15								ND	0.80	ND	ND	.30	.22	1.31
Depuration	22								ND	1.10	ND	ND	ND	ND	1.10
Recovery	%	90	93	82	78	74	78		85	87	89	89	89	89	
Detection Limit	ppm	0.10	0.025	0.021	0.009	0.042	0.012		0.60	0.15	0.13	0.10	0.24	0.08	

ND - Not detected

Table 1. Uptake and Depuration of Six Water-Soluble Components of Cook Inlet Crude Oil in Clams.

detector and a 1.8 m x 4 mm ID column of 5% Bentone 34/5% SP-1200 on Supelcoport. Air, helium, and hydrogen flowed at 1.2 SCFH, 40 cc/min, and 60 cc/min, respectively. Column, detector, and inlet temperatures were 105, 230, and 130 °C, respectively. The chromatographic curves were integrated with a Hewlett-Packard 3380A Integrator. Recoveries were determined by fortifying a control tissue sample with a standard of each monocyclic and the mixture incubated as previously described.

RESULTS

Clams were exposed to a continuous-flow of the water-soluble fraction of Cook Inlet cruide oil for eight days and the water and clam tissues analyzed for six monocyclic aromatics, namely, benzene, toluene, ethylbenzene, p-xylene, m-xylene, and o-xylene. Five of the monocyclics were found in the water and four of the monocyclics were found to have been accumulated by the clams. Detectability for water samples was below 0.10, 0.025, 0.021, 0.016, 0.042 and 0.012 ppm (V/V), respectively. Our analytical procedure for tissue samples was not as sensitive as with the water samples with detectability being 0.6, 0.15, 0.13, 0.10, 0.24 and 0.08 ppm (V/W), respectively. None of the six monocyclics were detected in the control samples.

Benzene concentration was the highest in the water phase followed by toluene, m-xylene, o-xylene and ethylbenzene. The same order of concentration was observed in clam tissues except for benzene which our analytical method was unable to detect during uptake and depuration. p-Xylene was not detected in either water or tissue samples. p-Xylene is approximately 10% of the m-xylene concentration in the water-soluble fraction generated by our solubilizing apparatus. Thus, p-xylene would be below the level of detectability for the analytical method we used which was modified from that by WARNER (1976).

The greatest accumulation of the monocyclic hydrocarbons occurred on day 4 with a decrease in tissue concentration noted on day 6 followed by an increase on day 8 (Table 1). Thus, a constant increase in the hydrocarbon content of tissues was not observed. The levels accumulated in the tissues appeared to be related to the concentration of the hydrocarbon fractions in the water phase. An increase in mucus production coupled with an apparent reduction in feces production was observed with increasing time in the exposure medium. No attempt was made to quantify the amounts of mucus and feces produced

during exposure to petroleum hydrocarbons.

A rapid discharge of accumulated petroleum fractions was observed in the first seven days following termination of the test period and subsequent transfer to uncontaminated holding tanks (Table 1). Thereafter loss of accumulated hydrocarbons was more gradual exhibiting a tendency toward protracted retention of a certain portion of the accumulated aromatics.

DISCUSSION

Historically, oil studies of marine organisms reported calculated hydrocarbon levels in the exposure media which were determined at the beginning and end of the test period. ANDERSON et al. (1974) and ANDERSON (1975) made the observation that calculated values based on proportions of oil and water in unstable dispersion mixtures are misleading and pointed to the need for accurate analysis of actual hydrocarbon concentrations to which the organisms are exposed in the test medium. The development of an apparatus designed to deliver a continuous-flow of uniform solutions of the water-soluble components of Cook Inlet crude oil allowed frequent monitoring of the actual hydrocarbon concentrations of the test medium throughout the exposure period in this study.

The observed rapid accumulation of petroleum hydrocarbons by this bivalve is similar to uptake rates reported for other marine bivalve molluscs (BLUMER et al. 1970, LEE et al. 1972, STEGEMAN and TEAL 1973, ANDERSON 1975, CLARK and FINLEY 1975, COX et al. 1975, FOSSATO 1975, NEFF and ANDERSON 1975, FOSSATO and CANZONIER 1976, LAKE and HERSHNER 1977, VANDERMEULEN et al. 1977). Accumulation rates in the clam tissues generally approximated the water-soluble fraction concentrations in the water phase. Similar equilibrium states in oysters exposed to petroleum hydrocarbons have been reported by STEGEMAN and TEAL (1973) and ANDERSON (1975).

Although microbial populations were not studied, a steady change in the color of the water-soluble fraction with increasing time of exposure indicated their growing presence. Alterations in the hydrocarbon content of the water phase during the test period from possible uptake of petroleum hydrocarbons by microbial organisms would explain the decrease in tissue content that occurred on day 4. Microbial degradation and subsequent solubilization of hydrocarbons could account for the increase in tissue content that occurred on day 8.

An important factor in considering the utilization of these organisms for human consumption is their capacity for eliminating accumulated pollutants. Ready loss of hydrocarbons adhering to exposed surfaces of such structures as the gills and mantles could account for the initial rapid discharge observed while tissue-bound hydrocarbons would explain the later and more gradual decline in the rate of discharge. This study indicates that complete clearance would probably require a long time. The oyster, Crassostrea virginica (BLUMER et al. 1970, STEGEMAN and TEAL 1973) and the mussel, Mytilus edulis (CLARK and FINLEY 1975, FOSSATO 1975, FOSSATO and CANZONIER 1976, VANDERMEULEN et al. 1977) were reported to be unable to achieve total elimination upon transfer to clean water following exposure to petroleum hydrocarbons. The absence of enzyme mechanisms for the detoxification of aromatic hydrocarbons is indicated by the apparent lack of complete elimination of accumulated hydrocarbon pollutants. Thus, the organism's only defense is slow depuration.

The observed persistence of toluene in tissue samples suggests that the persistence of this monocyclic may be a possible indicator of chronic oil pollution. Other physical and chemical characteristics of toluene would make it a good component of crude oil to quantitate for oil pollution. Its solubility in seawater is relatively high for the aromatics (BENVILLE and KORN 1977) and it is easy to quantitate by gas chromatography. Toluene was also high in concentration in the water-soluble fraction of Cook Inlet crude oil.

ACKNOWLEDGEMENTS

This study was partially supported by the Bureau of Land Management through interagency agreement with the National Oceanic and Atmospheric Administration, under which a multi-year program responding to needs of petroleum development of the Alaskan Continental Shelf is managed by the Outer Continental Shelf Environmental Assessment Program (OCSEAP) office.

Our thanks to Maxwell Eldridge and John R. Clayton, Jr. for reviewing the manuscript and for their suggestions. We are greatly indebted to John Karinen, Stanley Rice, Sid Korn and Loren Cheatham for obtaining our Cook Inlet crude oil. Special thanks to Alice Jellett and Alicia Rhodes for typing the manuscript.

REFERENCES

ANDERSON, J.W., J.M. NEFF, B.A. COX, H.E. TATEM, and G.M. HIGHTOWER: Mar. Biol. 27, 75 (1974).

ANDERSON, R.D.: Conference on Prevention and Control of Oil Spills, Proceedings, 541 (1975).

BENVILLE, P.E., JR. and S. KORN: Calif. Fish and Game (In Press).

BLUMER, M., G. SOUZA, and J. SASS: Mar. Biol. 5, 196 (1970).

CLARK, R.C., JR. and J.S. FINLEY: Fish. Bull. U.S. 73, 508 (1975).

COX, B.A., J.W. ANDERSON, and J.C. PARKER: Conference on Prevention and Control of Oil Spills, Proceedings, 607 (1975).

CRADDOCK, D.R.: Ms. in review.

FOSSATO, V.U.: Mar. Pollut. Bull. 6, 7 (1975).

FOSSATO, V.U. and W.J. CANZONIER: Mar. Biol. 36, 243 (1976).

KORN, S.: NOAA Tech. Rpt. NMFS SSRF-694 (1975).

LAKE, J.L. and C. HERSHNER: Conference on Prevention and Control of Oil Spills, Proceedings, 627 (1977).

LEE, R.F., R. SAUERHEBER, and A.A. BENSON: Science 177, 344 (1972).

LEINONE, P.J. and D. MCKAY: Can. J. Chem. Eng. 51, 230 (1973).

MCAULIFFE, C.D.: J. Phys. Chem. 70, 1267 (1966).

NEFF, J.M. and J.W. ANDERSON: Conference on Prevention and Control of Oil Spills, Proceedings, 469 (1975).

STEGEMAN, J.J. and J.M. TEAL: Mar. Biol. 22, 37 (1973).

STRAUGHAN, D.: J. Petroleum Technology March, 250 (1972).

VANDERMEULEN, J.H., P.D. KEIZER, and W.R. PENROSE: Conference on Prevention and Control of Oil Spills, Proceedings, 469 (1977).

WARNER, J.S.: Anal. Chem. 48, 578 (1976).