UPTAKE, DISTRIBUTION, AND DEPURATION OF ^{14}C-BENZENE IN NORTHERN ANCHOVY, *ENGRAULIS MORDAX*, AND STRIPED BASS, *MORONE SAXATILIS*

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ABSTRACT

The uptake, distribution, and depuration of water-soluble, monocyclic hydrocarbon contained in petroleum and refined products was studied in two species of marine fish. Mature northern anchovy, *Engraulis mordax*, and juvenile striped bass, *Morone saxatilis*, were exposed to sublethal concentrations of ^{14}C-benzene for 48 h. Residues in tissues exhibiting a high lipid content or representing apparent major metabolic sites were measured during the exposure and afterwards when the fish were transferred to clean seawater. Fish exhibited a rapid uptake over a wide range of benzene concentrations in the water column. Accumulation in anchovy was considerably greater than in striped bass. Results indicate that the pathway of hydrocarbons through the liver, gallbladder, intestines, and colon is a major depuration route. Residues were depurated rapidly after cessation of exposure; in striped bass tissues most residues were undetectable by 7 days.

Increased drilling, transportation, and refining of crude oils near or on coastal waters has led to the need for research on the effects of oil on estuarine biota. Considerable public concern has evolved from such occurrences as tanker spills and the Santa Barbara well blowout. However, long-term sublethal effects of low levels of oil in inshore areas may be of greater importance to marine populations than short-term lethal effects of high levels resulting from catastrophic events such as tanker spills and drilling blowouts. It is important to study the effects of chronic oil exposure on marine organisms.

Benzene is a principal aromatic oil component (up to 6.75 ppm in the water-soluble extract [Anderson et al. 1974]) that is relatively water soluble (1,993 µl/liter [Benville and Korn 1974]) and has significant effects on fishes (Brocksen and Bailey 1973; Korn et al. in press). The preceding studies demonstrated the effects of benzene on the nervous system, respiration, and growth of fish. Brocksen and Bailey showed latent effects of benzene on respiratory response lasting up to 6 days after fish were placed in clean water.

Concentrations of highly volatile monocyclic aromatics such as benzene are not thought to be very high in areas subject to chronic exposure to oil. However, measurements of monocyclic aromatics in such situations are scarce. Our preliminary measurements in San Francisco Bay indicate a maximum range from 1 to 10 µl/liter benzene in relatively unpolluted bay areas. Although the chronic levels are low, if fish accumulate benzene over field concentrations and if energy is required to metabolize, detoxify, and depurate accumulated aromatics, detrimental long-term physiological effects are possible.

Investigators such as Lee, Sauerheber, and Benson (1972); Lee, Sauerheber, and Dobbs (1972); Anderson et al. (1974); and Lee (1975) examined uptake of higher aromatics in invertebrates and fish, but no work has been done with benzene.

The fish we studied were San Francisco Bay species but also occur widely in other areas where chronic oil pollution may pose a problem. Striped bass, *Morone saxatilis*, is an important recreational species on the west and east coasts, while northern anchovy, *Engraulis mordax*, is not only a major forage fish for striped bass but also constitutes the greatest biomass of any fishery in the California Current.

The objective of this study was to determine the uptake, distribution, and depuration of benzene in these two species of fishes.

METHODS

Adult northern anchovies were obtained from a local bait dealer and acclimated under controlled

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environmental conditions comparable to those used in experiments. Juvenile striped bass were obtained from the water diversion facilities of the Bureau of Reclamation at Tracy, Calif. Fish were acclimated in 2,000-liter circular tanks for at least 2 wk before testing and fed ground squid once daily to satiation.

In all uptake studies, an appropriate number of fish (Table 1) were transferred into oval 200-liter test tanks and further acclimated for 1 wk. The number of fish per tank was limited to the number (≤1 g/liter) that could be maintained during a 48-h static exposure period when oxygen is a limiting factor. The 48-h static exposure period instead of an open-system constant exposure was necessitated by the expense of the ^{14}C-benzene required for a relatively large volume of water. Except for the 48-h static exposure period, a flow of 1 liter/min of filtered seawater was maintained throughout. During flow periods the salinity and temperature of the water were monitored and controlled by the seawater system components (Korn 1975), whereas temperature was not controlled during the static exposure period.

Stock benzene solutions used for dosing the exposure tanks were prepared as follows: A saturated benzene solution (1 ml benzene in 250 ml seawater) was prepared in a separatory funnel by vigorous shaking and then allowed to settle for 1 h. The resulting solution was analyzed by the gas chromatography method of Benville and Korn (1974). Next, ^{14}C (99.9% ring-labeled benzene, specific activity, 85 μCi/mmol) was mixed with another 200 ml of seawater to make a stock solution and was kept frozen until used. The saturated benzene solution was then mixed with ^{14}C stock solution to the proper specific activity, and the appropriate volume was poured into each tank and mixed by gentle stirring. After mixing, 1-ml water samples were added to a scintillator (10-ml Packard Instagel)[3] and the benzene concentration was measured. Carbon 14 counting was done on a Packard Model 2008 Tri-Carb liquid scintillation spectrometer system. Internal standardization yielded 85% counting efficiency, and all water values were corrected accordingly.

Uptake, distribution, and depuration were determined by sampling fish, rinsing them externally with methanol to remove adsorbed benzene, dissecting out tissues, weighing tissue samples (<200 mg), placing each tissue in a vial with tissue digester solution (1 ml/100 mg tissue Packard Soluene-100), and allowing 48-h digestion at room temperature. Scintillator (10-ml Packard Dimilume) was added to these samples and ^{14}C radioactivity measured. Approximate mean counting efficiencies of 60% and 67% were calculated from spiked samples and used to correct anchovy and striped bass tissue residue values respectively. Water and tissue samples yielding below 40 counts per minute were considered below the detectable limits of our system.

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

TABLE 1.—Summary of experimental conditions for ^{14}C-benzene uptake and depuration tests with northern anchovy and striped bass. Salinity was 24-26‰

Species and test number	Specific activity (cpm/nl)	Initial mean benzene concentration[1] (μl/liter)	Tanks per concentration	Fish per tank	Time of tissue sampling[2] (days)	Total wet wt (g) Mean	Total wet wt (g) SD	Type of tissue examined
Northern anchovy:								
1	0.11	3.7	3	8	0.042, 0.125, 0.25, 1, 2	17.03	6.55	Liver, brain, gill, muscle
2a	5	0.11	4	8	0.042, 0.25, 1, 2, 4	12.93	5.04	Liver, brain, gill, muscle, gallbladder, intestine
2b	5	0.0097	4	8	0.042, 0.25, 1, 2, 4	11.70	4.74	Liver, brain, gill, muscle, gallbladder, intestine
3a	40	0.0048	4	8	0.25, 1, 2, 3, 4, 7	12.74	3.35	Liver, brain, gill, muscle, gallbladder, intestine
3b	320	0.00069	4	8	0.25, 1, 2, 3, 4, 7	13.94	4.46	Liver, brain, gill, muscle, gallbladder, intestine
Striped bass:								
4	5	0.088	9	5	0.25, 1, 2, 3, 4, 5, 6, 7, 8, 9	76.87	34.60	Liver, brain, gill, muscle, gallbladder, intestine, mesenteric fat, colon, heart, stomach

[1]Exposure to ^{14}C-benzene was static for 48 h followed by resumption of water flow for the duration. Recent analyses by gas-liquid chromatography yielded 0.00015-0.0010 μl/liter background benzene concentration in the seawater at this facility which is not included in these values.

[2]One fish per tank at each sampling time.

Water samples were taken first; then tissues were sampled until open flow was reestablished. Tissues were sampled as noted for each of the four experiments included in this report (Table 1). It is recognized that the residues reported may contain metabolites and degradation products in addition to benzene.

The original data on declining seawater concentrations of benzene during tests and on decreasing concentrations of residues in fish tissues during depuration were first analyzed with a least-squares curve-fitting computer program to determine if the hypothesized function was the best fit. Linear regression analyses were then performed on logarithmically transformed data, and regression coefficients were tested for significance of differences between slopes and a pooled regression coefficient (Snedecor and Cochran 1968).

TABLE 2.–Benzene concentration during 48-h exposure period. Exponential decline ($Y = ae^{-bX}$); coefficients for each experiment from least-squares curve fitting.

Test no.	Benzene-seawater actual initial concentration		n	a	b	Percentage remaining after	
	µl/liter	nl/liter				24 h	48 h
1	3.7	3,700	15	3.53	−0.1997	54	30
2a	0.110	110	16	0.104	−0.02381*	54	31
2b	0.097	9.7	16	0.094	−0.01262*	65	—
3a	0.0048	4.8	12	0.00457	−0.01983	48	42
3b	0.00069	0.69	12	0.000692	−0.01847	62	42
4	0.088	88	27	0.0991	−0.01813	65	43

*No significant difference between slopes (at $\alpha = 0.05$) except between tests 2a and 2b.
The equation $Y = ae^{-0.0183X}$ describes the exponential decline in benzene, using a pooled regression coefficent.

RESULTS

There were no deaths during the tests. The benzene concentration in the seawater declined exponentially ($\hat{Y} = ae^{-0.0183X}$, where $\hat{Y}$ is concentration and X is time) during all tests. After 24-h exposure, 48-65% remained; after 48 h, 30-43% remained, at which point the water flow was renewed (Table 2).

In general, accumulation in striped bass was greatest in the gallbladder, followed by mesenteric fat, colon, intestine, liver, brain, gill, heart, stomach, and muscle (Table 3). Anchovy exhibited similar results minus the mesenteric fat, colon, heart, and stomach tissues, which were not sampled. The order of decreasing accumulation varied slightly according to experiment. The gallbladder accumulated 53.4-8,450 times the initial water concentration, while muscle accumulated 1.11-135 times the initial water concentration. Maximum concentrations were obtained in the tissues from 0.25 to 4 days after starting exposure. Mesenteric fat, gallbladder, liver, and intestine usually reached a maximum accumulation later than the other tissues.

Accumulation in anchovies was considerably greater than in striped bass in the tissues measured in both species (Figures 1, 2; Table 3). The pattern of uptake in the gill and gallbladder was

TABLE 3.–Mean maximum concentration factors[1] in various tissues and days elapsed (numbers in parentheses) from beginning of exposure for northern anchovy and striped bass.

Species and test number	Initial mean benzene-seawater concentration (µl/liter)	Concentration factor in tissue of									
		Gill	Brain	Muscle	Fat	Heart	Stomach	Liver	Gall-bladder	Intestine	Colon
Northern anchovy:											
1	3.7	34.3 (2)	30.0 (2)	22.7 (1)	—	—	—	45.1 (1)	—	—	—
2a	0.11	41.8 (1)	41.8 (1)	10 (1)	—	—	—	54.6 (0.25)	4,360 (2)	209 (2)	—
2b	0.0097	113 (1)	113 (1)	29.9 (0.25)	—	—	—	309 (2)	8,450 (2)	505 (2)	—
3a	0.0048	7.92 (0.25)	7.5 (0.25)	5.42 (4)	—	—	—	66.7 (4)	229 (2)	60.4 (2, 3)	—
3b	0.00069	7.1 (0.25)	9.13 (0.25)	135 (4)	—	—	—	31.9 (2)	116 (3)	34.8 (2)	—
Striped bass:											
4	0.088	5.57 (1)	7.16 (0.25)	1.11 (0.25)	1.14 (0.25)	2.95 (0.25)	2.72 (0.25)	9.77 (0.25)	53.4 (2)	5.45 (0.25)	14.8 (0.25)

[1]Factor X (benzene-seawater concentration in microliter per liter) = actual nanoliters per gram mean tissue value or (benzene in nanoliters) / (tissue wet weight in grams).

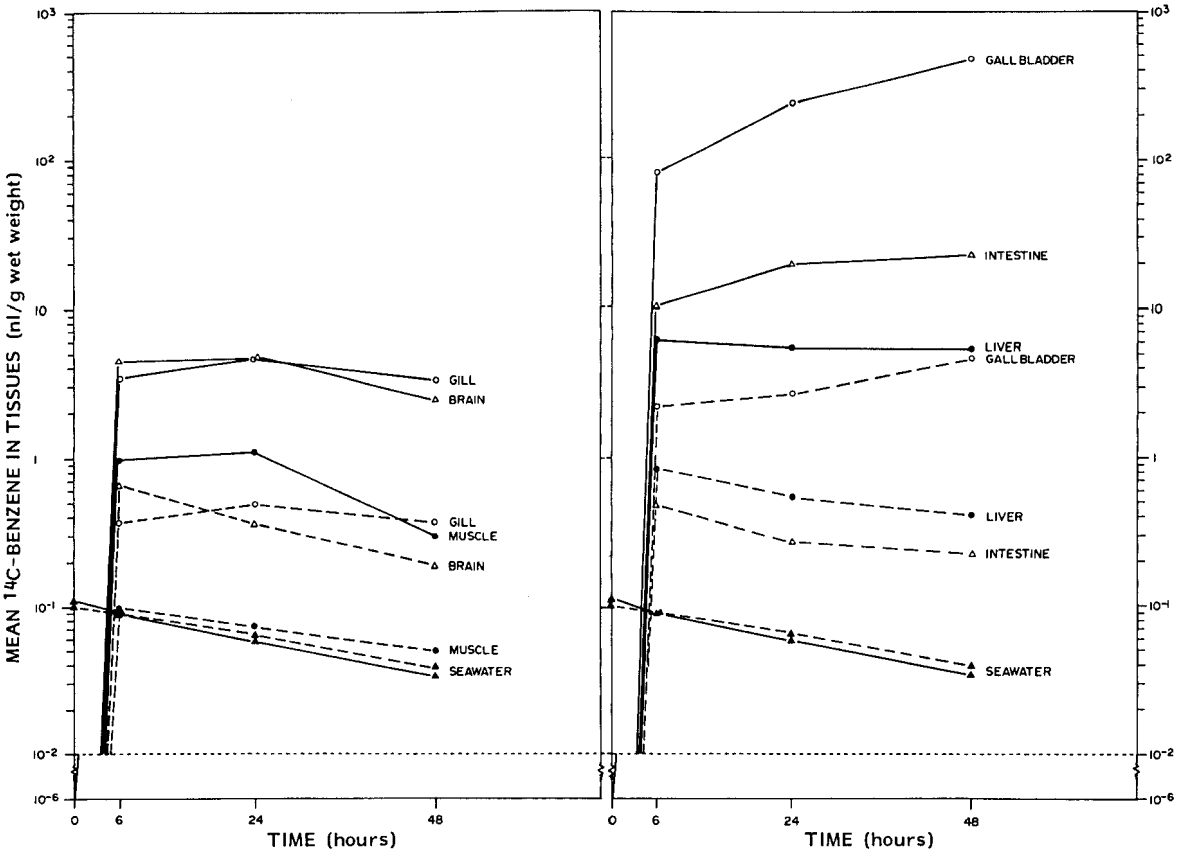

FIGURE 1.—Mean ^{14}C-benzene uptake in tissues (nl/g wet weight) in anchovy (solid lines) and striped bass (dashed lines); sample number: three or four fish. Also shown are mean ^{14}C-benzene concentrations in seawater in anchovy tanks (solid lines) and in striped bass tanks (dashed lines); sample number in Table 2. The concentrations on the Y-axis are calculated from total ^{14}C radioactivity and may include metabolites of benzene.

similar between species, while in the brain, liver, muscle, and intestine, a maximum level was maintained longer in the anchovy. In both species, the greatest rate of uptake occurred in the first 6 h.

Residues were depurated rapidly after cessation of exposure (Table 4). Gallbladder, mesenteric fat, liver, and gill maintained residues the longest. Depuration appeared to occur more rapidly in striped bass than in anchovies in some tissues. In striped bass, depuration is generally described by the logarithmoc form of a power function ($\ln \hat{Y} = \ln a + b \ln X$) after cessation of exposure on day 2 until day 4 or 5 (Figure 3). Subsequently, several of the tissues showed a secondary increase and decrease in concentration. In muscle tissue, residues were undetectable 24 h after exposure ended.

DISCUSSION

Accumulation levels are based solely on radiometric analysis. This analytical technique does not distinguish between ^{14}C-labeled benzene and derived ring metabolites. Complementary analysis by thin-layered chromatography or gas chromatography could have determined some of the actual compounds present, but it was not performed during these experiments. It is hypothesized that fish are capable of excreting and metabolizing benzene. Although there is no direct evidence, the residues reported in selected tissues may be representative of the unchanged parent benzene or associated metabolites and degradation products. Any or all of these may be toxic to fish.

Benzene and/or metabolites accumulate

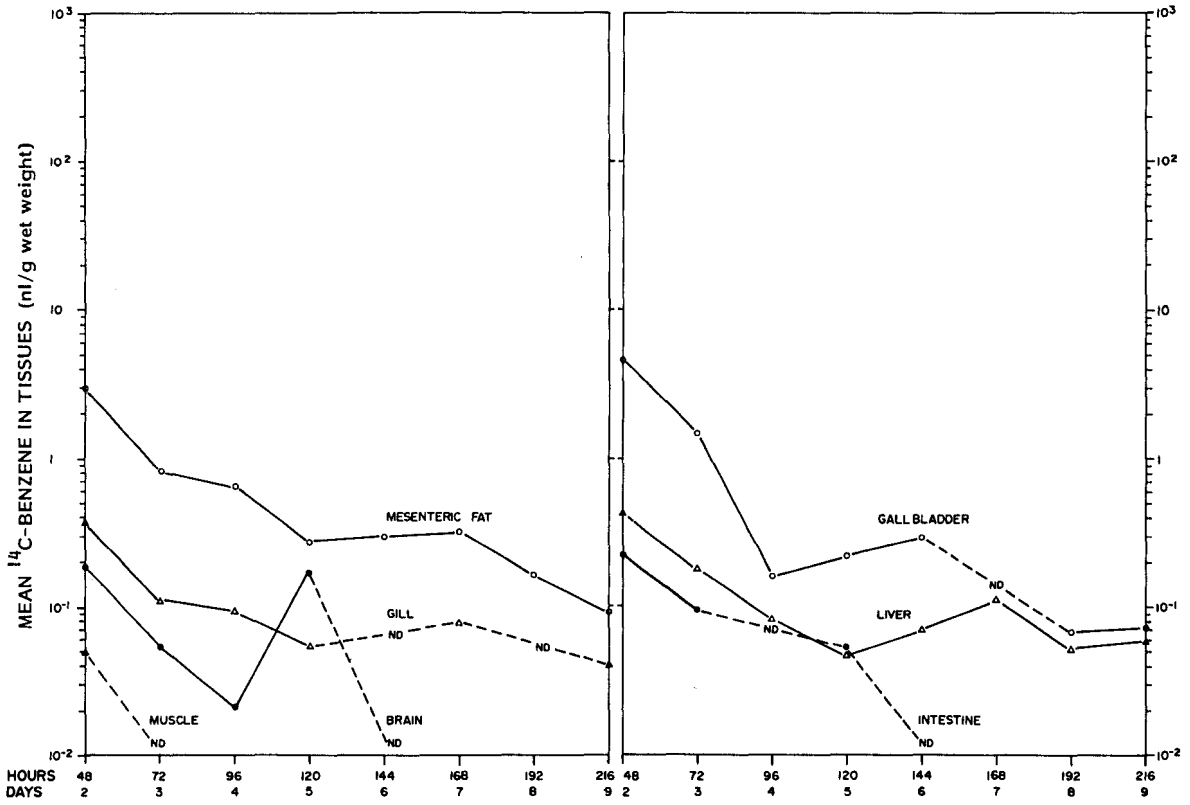

FIGURE 2.—Mean ^{14}C-benzene depuration from tissues (nl/g wet weight) of striped bass; sample number in Table 4. ND = nondetectable level (see Methods). The concentrations on the Y-axis are calculated from total ^{14}C radioactivity and may include metabolites of benzene.

predominantly in tissues that exhibit a high lipid content or represent apparent major metabolic sites. Thus, lipid-rich mesenteric fat and brain tissues had high accumulations; while liver, gallbladder, intestine, and colon (which are tissues associated with the metabolic breakdown and excretion of benzene) also accumulated benzene to higher levels (Table 3).

TABLE 4.—Percent residues[1] remaining in northern anchovy and striped bass after termination of 48-h exposure to benzene. (Sample sizes in parentheses.)

	Northern anchovy[2]		Striped bass[2]						
	Test 2a	Test 2b	Test 4						
			Days from termination of exposure						
Tissue	2	2	1	2	3	4	5	6	7
Gill	61(3)	26(2)	30(3)	25(3)	14(2)	ND[3]	21(3)	ND	11(1)
Brain	22(4)	42(1)	29(2)	11(1)	95(2)	ND	ND	ND	ND
Muscle	93(3)	ND	ND	ND	ND	ND	ND	ND	ND
Fat	—	—	28(2)	21(2)	9.0(4)	9.7(4)	11(4)	5.3(4)	3.0(2)
Heart	—	—	38(1)	ND	ND	ND	ND	ND	ND
Stomach	—	—	ND	ND	35(1)	ND	ND	ND	106(1)
Liver	70(4)	13(2)	43(3)	20(3)	11(3)	16(3)	26(3)	12(2)	14(1)
Gallbladder	69(4)	63(3)	32(4)	3.4(2)	4.7(3)	6.2(3)	ND	1.4(2)	1.3(1)
Intestine	70(4)	17(3)	42(4)	ND	24(1)	ND	ND	ND	ND
Colon	—	—	44(3)	26(2)	ND	ND	ND	ND	ND

[1]Actual nanoliters per gram tissue residues = (benzene in nanoliters) / (tissue wet weight in grams) (mean tissue residue) / 48-h mean tissue residue) (100% residue).
[2]The initial mean benzene seawater concentrations (ul/liter) were 0.11 for test 2a, 0.0097 for test 2b, and 0.088 for test 4.
[3]ND = nondetectable.

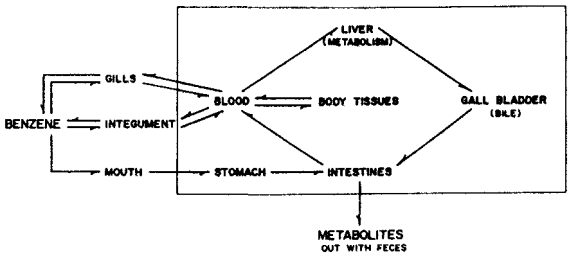

FIGURE 3.—Hypothetical pathway and distribution of benzene in fish.

Figure 3 shows a hypothetical distribution and pathway of benzene in fish which is substantiated by our results. Benzene is absorbed across the gills into the blood where, being lipid soluble, it attaches to the erythrocytes and lipoproteins (Gerarde 1960). It is then translocated via the blood to the tissues where it either accumulates or is metabolized. Parke (1968), Meyers (1970), and Lee, Sauerheber, and Dobbs (1972) described metabolism of benzene to phenol in the liver of fish and mammals. The metabolites are excreted from the liver with the bile and stored in the gallbladder. From the gallbladder the bile is excreted into the intestine and finally eliminated through the colon with the feces. Our results show high accumulation in the liver and gallbladder. Lee, Sauerheber, and Dobbs (1972) also found the gallbladder of fish to be a storage site for polycyclic aromatic compounds. Our results indicate that the pathway through the liver, gallbladder, intestine, and colon is a major depuration route. These tissues take the longest to accumulate and depurate. This is probably due to the time needed for metabolism of benzene. The gill also was one of the tissues which depurated later. Some unchanged benzene metabolites are probably excreted across the gills.

The secondary increase in ^{14}C radioactivity (days 4-7) observed after initial depuration (days 2-4) in several striped bass tissues (Figure 3) is difficult to interpret. One explanation may be that the metabolism of benzene is limited to a certain rate and that until the initial metabolism is complete, some benzene accumulates in nonmetabolic tissues and is not totally metabolized until later. The secondary increase in residues in fat and brain tissues, however, suggests that perhaps metabolites such as phenol are accumulating in the tissues for a period before they too are depurated. Additional work with uptake and depuration in herring tissues shows a similar pattern. Further research must be done to clarify this point.

The low accumulation tissues such as heart, muscle, and stomach are also low in lipid content and apparently do not directly contribute to the metabolism of benzene. Lee, Sauerheber, and Dobbs (1972) found similar results with naphthalene and benzopyrene in fish. Later work at Tiburon has demonstrated that little benzene and/or metabolites accumulate in the kidney tissue of herring. Because of this and the fact that fish in salt water excrete little urine, we feel the kidneys are not a major depuration pathway in fish from saline waters. Further study of urinary depuration is needed.

Northern anchovies are schooling fish, and they swam constantly during the tests—striped bass were more sedentary. This difference in activity might explain the higher accumulation in anchovies.

The short duration of low-level water column exposures of benzene in these experiments did not reveal obvious detrimental effects on behavior or physiology of fish. However, equilibrium accumulation levels have not been obtained because of the static exposure with decreasing benzene-water concentration. During chronic exposures, higher accumulations of benzene and toxic metabolites (such as phenol) with deleterious effects are possible. Further, because of the rapid uptake rate over a wide range of concentrations, it is conceivable that both species could accumulate significant benzene levels after brief exposure during an oil spill. The severity of effects at chronic and acute levels will depend greatly on the energy requirements of the fish and the degree of stress to which they are already subjected. Fish in spawning condition are particularly susceptible to additional stress from pollutants (e.g., spawning Pacific herring [Struhsaker[4]]). Further study of uptake in the lipid-rich mature ovaries of fish should be done.

The rapid depuration of benzene the first day after exposure ended appears to be due to metabolism and excretion via the liver-intestine route. Because of this rapid depuration, the possibility of bio-amplification in fish does not appear

[4]Struhsaker, J. W. Effects of benzene (a toxic component of petroleum) on spawning Pacific herring. Manuscr. in prep. Southwest Fish. Cent. Tiburon Lab., Natl. Mar. Fish. Serv., NOAA, Tiburon, CA 94920.

likely, at least after exposure from the water. Exposure from the ingestion of food organisms may result in a different metabolic process, however, and this work should be done before further conclusions are made. Our results from uptake studies with a rotifer (*Brachionus plicatilus*) (Echeverria[5]) and those of Lee, Sauerheber, and Benson (1972) and Lee (1975) with mollusks and zooplankton indicate that some invertebrates may be unable to metabolize aromatic hydrocarbons—accumulating them to very high levels and depurating them slowly. Fish feeding on such organisms may be exposed to high and potentially damaging levels of aromatics.

Additional chronic uptake studies under continuous-flow conditions are needed. Analyses of metabolites are proceeding and will be reported later.

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