THE UPTAKE, DISTRIBUTION, AND DEPURATION OF ^{14}C BENZENE AND ^{14}C TOLUENE IN PACIFIC HERRING, *CLUPEA HARENGUS PALLASI*

This note is a sequel to Korn et al. (1976), where uptake, distribution, and depuration of ^{14}C benzene were examined in striped bass, *Morone saxatilis*, and northern anchovy, *Engraulis mordax*. Like benzene, toluene is a prevalent, water-soluble, and toxic monoaromatic component of petroleum and associated products. According to Anderson et al. (1974a), toluene is second only to benzene as the most abundant aromatic oil component in the water-soluble extracts of southern Louisiana and Kuwait crude oils (6.75–3.36 μl/liter benzine; 4.13–3.62 μl/liter toluene, respectively).

Although levels of the volatile aromatics are thought to be low in areas subject to chronic oil exposure, few actual measurements have been made. Further, if fish can accumulate benzene and if energy is required to metabolize, detoxify, and depurate these aromatics, long-term physiological and population effects are possible.

In this study, a comparison of the uptake, distribution, and depuration of ^{14}C benzene and ^{14}C toluene, at a low sublethal concentration [100 parts per billion (ppb)], was undertaken to determine which of these prevalent aromatics may pose the greatest problem. It was hypothesized that, although toluene is less soluble in seawater (Anderson et al. 1974a), it may be more toxic and exhibit greater accumulation levels and persistence. Our previous work with striped bass and northern anchovy indicated other tissues that should be examined, such as kidney, pyloric caeca, gonad, and intestine, and in the present comparison, residues in the additional tissues were measured. Pacific herring, *Clupea harengus pallasi*, were selected as test animals because of their importance as estuarine and nearshore forage fish for many important recreational and commercial species, including striped bass and chinook salmon.

Methods

Pacific herring were obtained from a San Francisco Bay bait dealer and were transported directly to the Tiburon Laboratory dock. The fish were acclimated under test conditions for at least 2

wk in 2,000-liter tanks. Fish were not in spawning condition.

In each of two separate studies, 10 fish were placed into each of six 660-liter fiber glass tanks and further acclimated for 1 wk before exposure. Salinity and temperature were 24‰ and 9°–11°C, respectively, during the acclimation and test periods. In the first study, fish were exposed to 100 nl/liter (ppb) ^{14}C benzene (4.2 dpm/ng specific activity). In the second study, fish were exposed to 100 nl/liter (ppb) ^{14}C toluene (3.2 dpm/ng specific activity). In both studies, one of the six tanks was a control, with no exposure. Exposures were static (single dose with delining concentration) for 48 h, preceded and followed by a continuous water flow of 2 liters/min.

Water samples for radiometric aromatic analyses were taken from all tanks at 0, 6, 24, and 48 h after initial dosage. Gallbladder, intestine, pyloric caeca, gill, brain, liver, muscle, kidney, and immature male and female gonad tissues were sampled for radiometric analyses at 6 h, then daily for 7 days.

Methods of exposure and radiometric analyses are identical to Korn et al. (1976), except that the tissues from fish exposed to toluene were digested at 50°C for 24 h.

Since accumulation levels in the gallbladder were based solely on radiometric analysis of the ^{14}C present and could include metabolites of the monoaromatics as well as unchanged benzene or toluene, an additional study was made to interpret the residue. Two groups of fish, with six fish per tank, were exposed to 100 nl/liter ^{14}C benzene (1 tank), and 100 nl/liter ^{14}C toluene (1 tank) for 48 h. Exposure was the same as in the above experiments. At the end of the 2-day exposure, the gall bladders were removed, weighed, and extracted with 0.2 ml trifluorotrichloroethane-Freon.[1] The extracts were analyzed for benzene and toluene by gas chromatography (Benville and Korn 1974). Efficiency of extraction was not determined and therefore the gas chromatography analyses were more qualitative than quantitative.

Results and Discussion

There were no mortalities in either exposed or control fish. Unlike herring exposed during spawning condition (Struhsaker 1977), no abnormal behavior was noted, thus immature herring appear less sensitive to exposures than mature herring in spawning condition.

The concentration of benzene and toluene in seawater in all tanks declined linearly ($\hat{Y} = a + bX$ where $\hat{Y}$ = concentration in microliters per liter, a = initial concentration in microliters per liter, b = rate of decline in microliter per liter per hour, and X = time in hours), during the 48-h static exposure, as follows:

Item	*Benzene*	*Toluene*
Total no. samples	20	20
a ($\hat{Y}$-intercept)	0.094997	0.09195
b	−0.0006075	−0.0007587
Percentage of initial concentration remaining:		
24 h	85	80
48 h	69	60

The equation for decline in benzene and toluene is probably a function of the volume of seawater. In earlier studies, at smaller volumes, decline was exponential over the 48-h static exposure. At the volume in these experiments it was linear, but probably would have been exponential over a longer time period. The rate of decline appears to decrease with increasing volume.

In all herring tissues, toluene accumulated to higher levels than did benzene (Table 1), despite the faster loss of toluene compared with benzene from the test solution. Certain trends were common to both aromatic components. The tissue exhibiting the highest accumulation was the gallbladder (3.1 nl/g benzene, 34 nl/g toluene, maximum level). The lowest level of maximum accumulation was found in the immature gonad (0.24 nl/g benzene, 0.44 nl/g toluene). Pyloric caeca and intestine contained varying amounts of bile and therefore had a wide range of ^{14}C activity and a resulting wide variance in calculated concentrations.

Benzene was accumulated up to 31 times the initial water concentration (gallbladder) and toluene reached 340 times the initial water concentration (gallbladder).

In most tissues, and for most components, maximum accumulation levels were reached rapidly. Within 24 h, maximum residues were obtained in all tissues except the gallbladder and pyloric caeca. Toluene accumulated to the maximum level (0.25 days) before benzene peaked (1–2 days) in all tissues except the gallbladder and intestine.

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

TABLE 1.—Residues of benzene and toluene and/or metabolites (mean nl/g±SE) accumulated during and after a 48-h exposure to 100 nl/liter (ppb) ^{14}C benzene or 100 nl/liter (ppb) ^{14}C toluene in the tissues of *Clupea harengus pallasi*. Number of samples in parentheses.

Tissue and compound	Time (days) from start of exposure[1]							
	Uptake			Depuration				
	0.25 (6 h)	1	2	3	4	5	6	7
Gallbladder:								
Benzene	0.37±0.075 (4)	2.1±0.71 (5)	3.1±0.48 (5)	2.7±1.5 (3)	0.56±0.30 (3)	0.92±0.79 (4)	0.60±0.14 (4)	0.61 (1)
Toluene	4.6±3.4 (5)	30±11 (5)	27±15 (5)	34±17 (5)	19±9.0 (5)	1.7±0.95 (5)	0.24±0.49 (3)	6.0±4.9 (2)
Intestine:								
Benzene	0.83±0.78 (4)	0.42±0.28 (5)	0.61±0.55 (5)	0.16 (1)	—[2] —	0.087 (1)	0.081 (1)	— —
Toluene	3.9±2.4 (5)	2.3±2.1 (4)	2.1±1.7 (5)	0.70±0.7 (5)	0.09±0.014 (2)	0.092±0.013 (3)	0.11±0.025 (2)	0.13±0.70 (3)
Pyloric caeca:								
Benzene	0.058±0.34 (5)	0.63±0.38 (5)	0.64±0.38 (5)	0.095±0.039 (3)	— —	0.056 (1)	— —	— —
Toluene	3.6±3.6 (5)	1.8±0.32 (5)	2.4±1.4 (5)	0.77±0.46 (5)	0.23±0.94 (5)	0.13±0.03 (5)	0.11±0.037 (5)	0.16±0.081 (4)
Gill:								
Benzene	0.51±0.12 (5)	0.61±0.33 (5)	0.73±0.46 (5)	0.073 (2)	0.068 (1)	— —	— —	— —
Toluene	1.8±0.58 (5)	1.2±1.2 (5)	1.0±0.96 (5)	0.20±0.12 (5)	— —	— —	— —	— —
Brain:								
Benzene	0.74±0.11 (5)	0.75±0.14 (5)	0.62±0.052 (5)	0.59 (2)	— —	— —	— —	— —
Toluene	2.1±0.19 (5)	2.0±0.28 (5)	1.5±0.18 (5)	0.13±0.073 (3)	— —	— —	— —	— —
Liver:								
Benzene	0.45±0.070 (5)	0.53±0.096 (5)	0.50±0.067 (4)	— —	— —	— —	— —	— —
Toluene	1.5±0.44 (5)	1.4±0.44 (5)	1.2±0.13 (5)	0.36±0.15 (5)	0.23±0.05 (4)	— —	— —	— —
Muscle:								
Benzene	0.41±0.22 (5)	0.63±0.36 (5)	0.44±0.33 (4)	0.035 (1)	0.066 (1)	— —	— —	— —
Toluene	1.3±0.80 (5)	0.52±0.28 (5)	0.66±0.71 (5)	0.33 (2)	— —	— —	— —	— —
Kidney:								
Benzene	0.32±0.066 (5)	0.32±0.066 (5)	0.40±0.12 (5)	— —	— —	— —	— —	— —
Toluene	1.3±0.50 (5)	1.1±0.40 (5)	0.75±0.33 (5)	0.18±0.099 (4)	— —	— —	— —	— —
Gonad:								
Benzene	0.15±0.021 (5)	0.24±0.062 (5)	0.21±0.10 (5)	— —	— —	— —	— —	— —
Toluene	0.43±0.24 (5)	0.44±0.21 (5)	0.44±0.28 (4)	0.16 (1)	— —	— —	— —	— —

[1]Exposure terminated after 2 days; then fish remained in flowing seawater for 5 days.
[2]— = nondetectable levels.

Residues were depurated rapidly, with most tissues having nondetectable amounts after 3–4 days (1–2 days after termination of exposure). The gallbladder, intestine, and pyloric caeca retained residues through the duration of the study (7 days).

In the experiment in which gas chromatographic analyses were performed on the gallbladder, no detectable benzene (<0.1 nl/g) was measured. Gas chromatography analysis resulted in only 0.56–1.5 nl/g toluene. This indicates that most or all of the radioactivity measured by liquid scintillation in the gallbladders of fish exposed to benzene is not the parent compound, but one or more metabolites. Fish exposed to toluene had a small amount of the parent compound as opposed to metabolites (1.5 nl/g toluene maximum, compared with 27 nl/g expected [Table 1]).

The above result and the occurrence of delayed depuration in the gallbladder, intestine, and pyloric caeca supports the contention that benzene and toluene are metabolized in the liver, stored in the gallbladder, then passed into the intestine and are excreted with the feces. This agrees with Roubal et al. (in press) who found high levels of benzene metabolites in the liver and gallbladder of salmon which had previously received intraperitoneal benzene injections. This also agrees with our previous results with benzene in other fishes (Korn et al. 1976), results of Neff (1975), and with work by Lee et al. (1972) who demonstrated metabolism of polycyclic aromatics in the liver

and subsequent storage in the gallbladder. Studies with polycyclic aromatics (naphthalene, benzpyrene) by other investigators (Lee et al. 1972; Anderson et al. 1974b; Neff 1975; Roubal et al. in press) indicate higher accumulation levels and slower depuration than we have found with benzene and toluene. However, different species are involved, and these higher aromatics are also less prevalent in the water-soluble extract of crude oil.

The results of this study are generally consistent with our previous work exposing striped bass and northern anchovy to ^{14}C benzene at the same initial concentration and exposure period (100 nl/liter for 48 h; Korn et al. 1976), except for the considerably higher accumulation in the anchovy than in the other species. This is probably primarily a result of the higher stress, activity level, and scale and mucus loss in anchovy while in captivity.

The gonads sampled in this study were immature and showed low accumulation levels. In another study exposing mature spawning herring to 100 nl/liter benzene for 48 h (Struhsaker 1977), higher accumulation occurred in the ovary, with associated deleterious effects on the ripe ovarian eggs and on development of larvae subsequent to exposure of the parental females.

Of the two components studied here, toluene would appear to be potentially a greater problem to fish. Toluene could be rapidly accumulated to high levels in fish after even a brief contact during an oil spill. Since toluene is one of the more prevalent water-soluble oil components, further research on the effects and uptake of this component are indicated. Further, chronic exposures are probably of more importance to the survival of fish populations than are spills, and studies of long-term exposure to chronic concentrations should be made.

Finally, the probability that benzene and toluene are rapidly metabolized or converted to metabolites (possibly phenol, which is also highly toxic) leads to the need for metabolite research. Uptake studies with phenolic metabolites would be of interest, as would be the determination of uptake over extended time intervals.

Acknowledgments

We acknowledge the considerable assistance of other members of the Physiology Investigation, Tiburon Laboratory, particularly Pete Benville for the gas chromatography analysis. We also thank Stanley Rice, Northwest and Alaska Fisheries Center, Auke Bay Laboratory, and Jerry M. Neff of Texas A&M University for their critical reviews of the manuscript.

Literature Cited

ANDERSON, J. W., J. M. NEFF, B. A. COX, H. E. TATEM, AND G. M. HIGHTOWER.
1974a. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. (Berl.) 27:75–88.
1974b. The effects of oil on estuarine animals: toxicity, uptake and depuration, respiration. *In* F. S. Vernberg and W. B. Vernberg (editors), Pollution and physiology of marine organisms, p. 285–310. Academic Press, N.Y.

BENVILLE, P. E., JR., AND S. KORN.
1974. A simple apparatus for metering volatile liquids into water. J. Fish. Res. Board Can. 31:367–368.

KORN, S., N. HIRSCH, AND J. W. STRUHSAKER.
1976. Uptake, distribution, and depuration of ^{14}C-benzene in northern anchovy, *Engraulis mordax*, and striped bass, *Morone saxatilis*. Fish. Bull., U.S. 74:545–551.

LEE, R. F., R. SAUERHEBER, AND G. H. DOBBS.
1972. Uptake, metabolism, and discharge of polycyclic aromatic hydrocarbons by marine fish. Mar. Biol. (Berl.) 17:201–208.

NEFF, J. M.
1975. Accumulation and release of petroleum-derived aromatic hydrocarbons by marine animals. *In* Proceedings, Symposium on Chemistry, Occurrence, and Measurement of Polynuclear Aromatic Hydrocarbons. Am. Chem. Soc., Chicago, 24–29 Aug. 1975, p. 839–849.

ROUBAL, W. T., T. K. COLLIER, AND D. C. MALINS.
In press. Accumulation and metabolism of carbon-14 labeled benzene, naphthalene, and anthracene by young coho salmon (*Oncorhynchus kisutch*). Arch. Environ. Contam. Toxicol.

STRUHSAKER, J. W.
1977. Effects of benzene (a toxic component of petroleum) on spawning Pacific herring, *Clupea harengus pallasi*. Fish. Bull., U.S. 75:43–49.

SID KORN

Northwest and Alaska Fisheries Center Auke Bay Laboratory
National Marine Fisheries Service, NOAA
P.O. Box 155, Auke Bay, AK 99821

NINA HIRSCH
JEANNETTE W. STRUHSAKER

Southwest Fisheries Center Tiburon Laboratory
National Marine Fisheries Service, NOAA
3150 Paradise Drive
Tiburon, CA 94920