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Effect of Environmental Temperature on Naphthalene Metabolism by Juvenile Starry Flounder (*Platichthys stellatus*)

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Abstract. Juvenile starry flounder (*Platichthys stellatus*) maintained at 4° or 12°C were forced-fed ^{3}H-1-naphthalene. At 24 hr, after the initiation of exposure, significantly ($p < 0.05$) higher concentrations (2 to 15 times) of naphthalene were present in tissues of starry flounder at 4°C than those present in fish held at 12°C. The influence of lowering of water temperature on naphthalene retention was even more marked after one week. At this time, muscle and liver of fish at 4°C contained 26 and 34 times, respectively, more naphthalene than did muscle and liver of fish at 12°C. Concentrations of total metabolites, in most tissues were not substantially higher at the lower temperature either 24 or 168 hr after the naphthalene-exposure.

Thin-layer chromatographic separation of the metabolites revealed that at 24 hr, 1,2-dihydro-1,2-dihydroxynaphthalene (dihydrodiol) was the major component in liver (40 to 50% of extracted metabolites) and muscle (~80% of extracted metabolites) regardless of the temperature. Bile contained, primarily, conjugates (*e.g.*, glucuronides), which yielded the dihydrodiol as the principal metabolite on enzymatic hydrolysis. From 24 to 168 hr, the concentrations of each metabolite class did not vary directly with the concentrations of total metabolites. Accordingly, at 168 hr, the ratio of total metabolite concentrations in liver of fish at 4°C compared to 12°C was 1.6, whereas the ratios for the dihydrodiol, sulfate/glucoside conjugates and glucuronide conjugates were 4.5, 0.6 and 3.8 respectively.

Generally, lowered water temperature increased tissue concentrations of the parent hydrocarbon and its metabolites. However, the magnitude of the increase was dependent upon the compound, the tissue, and the time after the initiation of the exposure. The results emphasize the importance of determining concentrations of individual metabolites together with parent hydrocarbons in tissues of fish when assessing effects of environmental parameters on xenobiotic toxicity.

The ability to biotransform and dispose of potentially toxic compounds, such as polycyclic aromatic hydrocarbons (PAH), presumably contributes to the long-term survival of an organism threatened by petroleum pollution. A mul-

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titude of endogeneous and exogeneous factors may affect this ability. For poikilotherms, the influence of environmental temperature should be of vital importance. At low temperatures, rates of evaporation and biodegradation of oil will be greatly reduced compared to those in a more temperate environment (Atlas *et al.* 1978). If an oil spill should occur in the colder regions, a greater possibility may exist for extended exposure of an organism to water-soluble and volatile components, such as naphthalenes. Naphthalenes are believed to be one of the primary components of oil causing mortality (Anderson *et al.* 1973; Moore and Dwyer 1974). Studies with both marine invertebrates (Fucik *et al.* 1977; Harris *et al.* 1977) and fish (Collier *et al.* 1978) demonstrate that after a short term exposure (≤24 hr) to water-borne or dietary naphthalene an inverse relation existed between water temperature and retention of naphthalene. In these studies, the effect of temperature on the extent of naphthalene metabolism or on retention of individual metabolites was not examined.

Pleuronectids, which contribute substantially to the demersal biomass of the continental shelf, are found in all regions of the northern Pacific Ocean and, therefore, can experience very low temperatures (Hodgins *et al.* 1977). For example, starry flounder (*Platichthys stellatus*) residing near the mouth of the Columbia River can be exposed to water temperatures ranging from 3° to 18°C within a twelve-month period (Durkin *et al.* 1977). In a two-part study, we examined the influence of time, mode of exposure, and temperature of biotransformation of naphthalene by juvenile starry flounder. The first part of this study (Varanasi *et al.* 1979) demonstrated that starry flounder extensively metabolized dietary naphthalene at 12°C. Moreover, both the time-lapse after the naphthalene-exposure and the mode of naphthalene exposure significantly influenced the retention of naphthalene and its metabolic products in tissues of fish. In the present report, we describe the influence of lowered water temperature (12°C vs 4°C) on retention and metabolism of dietary naphthalene by juvenile starry flounder. We also determined the influence of environmental temperature on types and concentrations of metabolites accumulated in tissues of naphthalene-fed fish.

It was found that the lowering of the water temperature resulted in increases in both concentrations and residence times of naphthalene in starry flounder. Increases in concentrations at the lower temperature were much greater for naphthalene than for its metabolites. Lowering of the temperature also altered relative proportions of the individual classes of metabolites accumulated in flounder tissues. Moreover, the results show that starry flounder biotransform naphthalene primarily into the dihydrodiol and its conjugates.

Methods

Starry flounder (82 ± 30 g) were obtained from the mouth of the Columbia River and acclimated to the experimental temperature (4° or 12°C) for one week prior to naphthalene-exposure. The fish were not fed three days prior to and during the one week-exposure. Fish were force-fed 56 μCi (sp. act. 198 mCi/mMole) of ^{3}H-1-naphthalene (>99.8% purity) (Amersham-Searle, Arlington Heights, IL)[1] dissolved in 25 μl of salmon oil. Four to six fish were analyzed at 24 hr and another group was analyzed at 168 hr after naphthalene exposure.

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[1] Mention of commercial products is for information only and does not constitute endorsement by the U.S. Department of Commerce.

Samples of skin, muscle, liver, kidney, brain, gills, stomach, intestine, and bile were homogenized and subsequently analyzed for concentrations of naphthalene and total metabolites by a solvent partitioning method using hexane and sodium hydroxide; radioactivity in each sample was determined by liquid scintillation spectrometry (Roubal *et al.* 1977; Varanasi *et al.* 1978, 1979). Differences in values for naphthalene and metabolite concentrations in various tissues of fish at 4° and 12°C were compared using the Student's t test (Norwich 1977).

Details of extraction procedure for metabolites are given elsewhere (Varanasi *et al.* 1979). The metabolite classes in liver, muscle, and bile were characterized by thin-layer chromatography (TLC). Standards of non-conjugates (1- and 2-naphthol, 1,2-dihydro-1,2-dihydroxynaphthalene) and conjuates (1-naphthyl glucuronide, 1-naphthyl sulfate, 1-naphthyl glucoside) were co-chromatographed with the metabolite extracts as described earlier (Varanasi *et al.* 1979). The nonconjugates were separated by TLC with solvent system A (*p*-dioxane:benzene:glacial acetic acid, 25:90:4, v/v/v), and the conjugates were separated by TLC with solvent system B consisting of the upper phase of concentrated NH_4OH: H_2O:*n*-butanol (10:50:40, v/v/v). After determination of the position of various metabolites, the adsorbent was scraped from the plates in 5 mm bands and radioactivity in each band was measured. 1-Naphthyl sulfate and 1-naphthyl glucoside had similar R_f values when solvent system B was used; therefore, this fraction was described as sulfate/glucoside. Residual radioactivity in each sample was determined. The representative values for non-extractable radioactivity in liver, muscle and bile are given in Table 2, footnote b.

Conjugated metabolites present in bile were further characterized by enzymatic hydrolysis (Dodgson *et al.* 1953). Sulfate/glucoside and glucuronide fractions were scraped off the plates and extracted 3× with 5 ml of H_2O. Methanol (30 ml) was added to aqueous extracts to prevent foaming and to facilitate evaporation. The samples were concentrated (1 to 2 ml) under reduced pressure. The aqueous extracts containing either glucuronide or sulfate/glucoside fractions were incubated for 48 hr at 37°C with β-glucuronidase from *Helix pomatia* (Sigma G 0751), which contained significant aryl sulfatase activity. The reaction medium consisted of 4 ml of 0.5 M sodium acetate (pH = 5.0), the metabolite fraction in 4 ml of water and 1.0 ml of β-glucuronidase (1 mg/ml). After hydrolysis, the solution was extracted 3× with 5 ml of ethyl acetate (EtAc). The EtAc extract was concentrated and spotted on a silica gel 60 (E. Merck) plate. The plate was first developed up to about 6 cm from the origin with EtAc and then allowed to dry. Then, the plate was redeveloped with solvent system C (toluene:EtAc, 100:3, v/v). This solvent system, which was originally developed by J. Schnell and M. Uyeda (personal communication), yielded a much better resolution of 1-naphthol, 2-naphthol, and the dihydrodiol derivative than did solvent system A (Figures 1 and 2). For example, when ^{14}C-1-naphthol was chromatographed by this procedure, less than 3% of total radioactivity was associated with the 2-naphthol band.

Results

At 24 hr, after the initiation of exposure, naphthalene was widely distributed in tissues and body fluids of fish held at 4°C (Table 1). Values for naphthalene and metabolite concentrations in tissues of fish at 12°C from this study were reported previously (Varanasi *et al.* 1979), and are used here only for comparison. At 24 hr, tissue concentrations of naphthalene in fish held at 4°C were 1.6 to 15 times greater than those in corresponding tissues of fish at 12°C. The largest differences were observed for liver, stomach, and intestine. At 24 hr, large amounts of naphthalene (>30% of the administered dose) were present in the gastrointestinal (G.I.) tracts of the fish at 4°C, whereas less than 3% of the dose was retained in the G.I. tracts of fish at 12°C. As with fish at 12°C, tissue concentrations of naphthalene at 4°C were much lower at 168 hr than at 24 hr; however, the decline in naphthalene concentrations from 24 to 168 hr was much greater at 12°C than that at 4°C resulting in even larger differences between naphthalene concentrations in most tissues of the two groups of fish at 168 hr (Table 1).

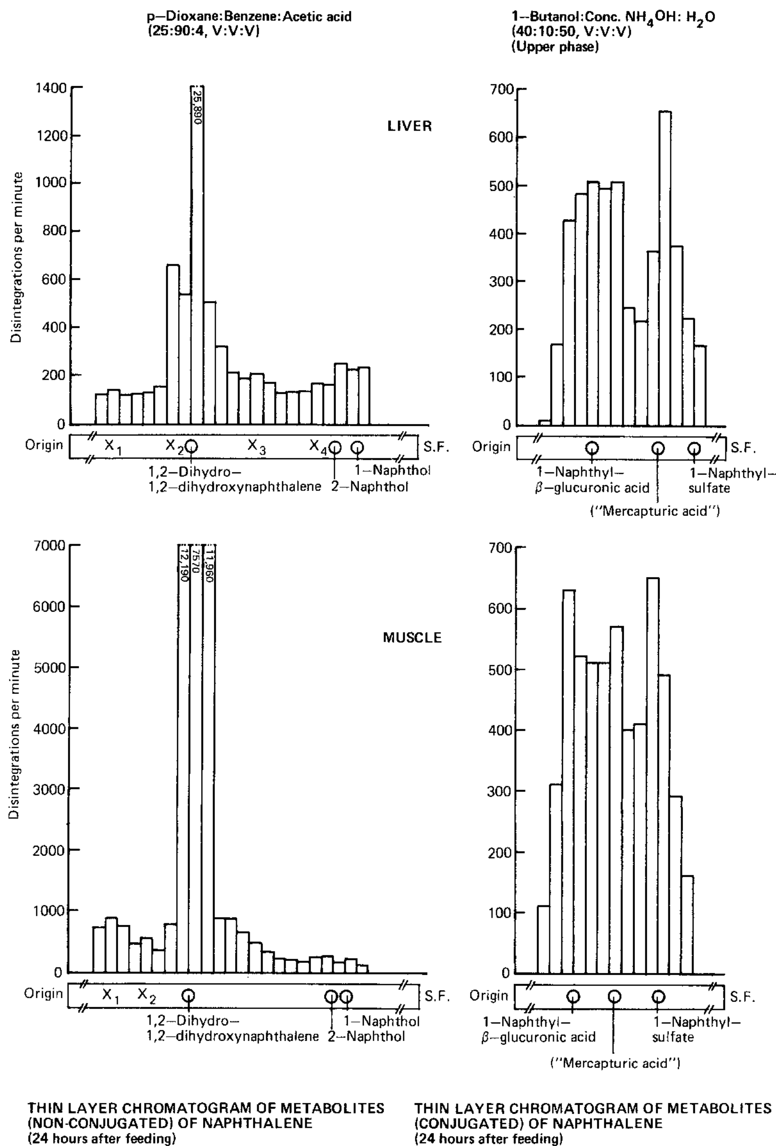

Fig. 1. Metabolite profiles in liver and muscle of naphthalene-fed starry flounder at 4°C

Concentrations of metabolites in tissues of fish after 24 hr were not substantially larger at the lower temperature (Table 1). Whereas ratios of values for naphthalene concentrations in most tissues of fish at 4° vs 12°C increased markedly from 24 to 168 hr, such a marked increase was not observed for the metabolite concentrations. After one week, metabolite concentrations in most tissues of fish at 4°C were not much greater than those at 12°C (Table 1); exceptions were the blood and muscle of fish at 4°C which contained substantially higher concentrations of metabolites than blood and muscle of fish at 12°C.

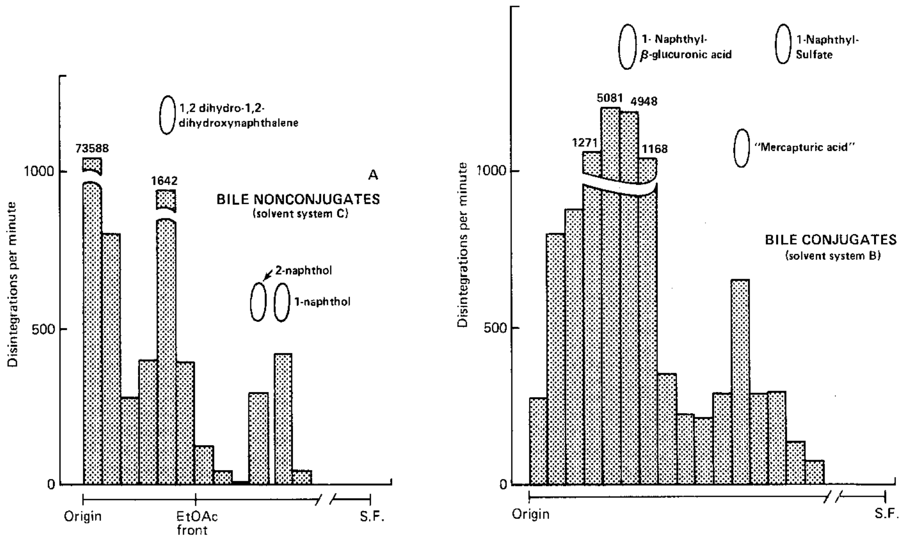

Fig. 2A. Thin-layer chromatograms of biliary metabolites from naphthalene-fed starry flounder at 12°C (168 hr after feeding)

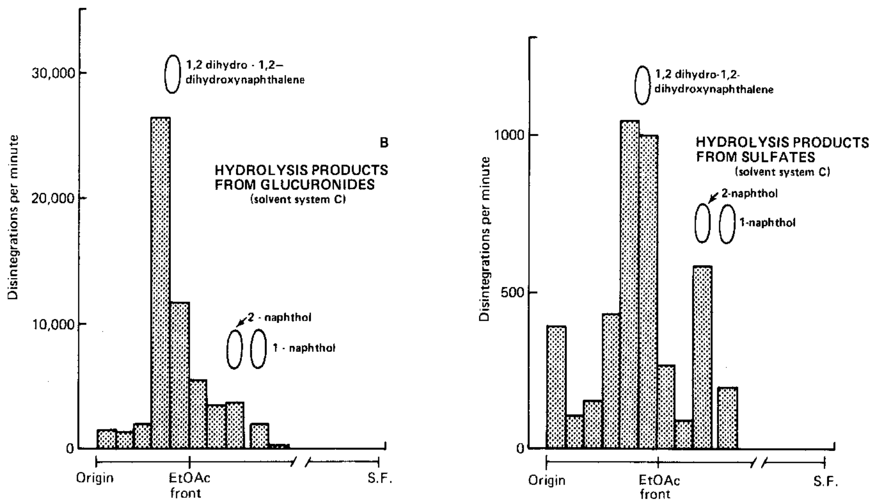

Fig. 2B. Thin-layer chromatograms of the hydrolysis products of glucuronide and sulfate/glucoside fractions isolated from bile of naphthalene-fed starry flounder

At both 4° and 12°C, the dihydrodiol derivative (same R_f value as 1,2-dihydro-1,2-dihydroxynaphthalene) was the major metabolite (39.7-80.9% of total extractable metabolites) present in liver and muscle of starry flounder at 24 hr after feeding of naphthalene (Table 2). At 24 hr, naphthols represented from 1.5 to 10.7% of the total extracted metabolites in liver, muscle or bile of fish at 4° and 12°C (Table 2). The 1- and 2-naphthols were not clearly resolved when solvent system A was used (Figure 1). The peak X_4 in chromatograms (Figure 1) was tentatively identified as 1,2-naphthoquinone. Glucuronides, sulfates/glucosides and mercapturic acids were also present in liver, muscle,

Table 1. Naphthalene and its metabolites in naphthalene-fed starry flounder at 4°C

Tissue	Naphthalene: pmoles/mg dry wt.	Naphthalene: $[C_{4°}/C_{12°}]$[a]	Naphthalene: % admin. dose[b]	Metabolites: pmoles/mg dry wt.	Metabolites: $[C_{4°}/C_{12°}]$[c]	Metabolites: % admin. dose
	24 hr after feeding ^{3}H-naphthalene[d]					
Liver	35.7 ± 10.8[e]	[7.8][f]	6.7 ± 1.6	1.2 ± 0.4	[1.4]	0.22 ± 0.04
Muscle	1.9 ± 0.6	[5.6][f]	5.1 ± 1.3	0.4 ± 0.1	[2.1]	1.05 ± 0.02
Bile	6.2 ± 2.0	[3.3]	0.09 ± 0.01	24.7 ± 6.8	[2.2]	0.39 ± 0.05
Stomach	17.7 ± 8.9	[9.8][f]	1.30 ± 0.60	2.9 ± 1.4	[2.9]	0.22 ± 0.09
Intestine	86.1 ± 31.2	[15][f]	30.80 ± 12.7	9.5 ± 1.4	[2.7]	3.07 ± 0.48
Skin	1.2 ± 0.4	[3.6][f]	1.02 ± 0.3	0.2 ± 0.1	[1.6]	0.16 ± 0.03
Brain	6.5 ± 2.0	[2.2]	0.08 ± 0.03	0.4 ± 0.1	[1.1]	0.005 ± 0.001
Blood	1.1 ± 0.4	[1.9]	—	0.9 ± 0.2	[2.5]	—
Kidney	2.5 ± 0.6	[1.6]	—	1.7 ± 0.5	[1.3]	—
Gills	2.5 ± 0.6	[3.7][f]	—	0.7 ± 0.2	[3.0][f]	—
Mucus	0.4 ± 0.1	[5.1]	—	0.3 ± 0.1	[2.1]	—
	168 hr after feeding ^{3}H-naphthalene					
Liver	2.75 ± 0.82	[34][f]	0.57 ± 0.16	0.52 ± 0.18	[1.6]	0.09 ± 0.03
Muscle	0.34 ± 0.11	[26][f]	1.30 ± 0.58	0.12 ± 0.04	[3.6][f]	0.33 ± 0.10
Bile	2.42 ± 0.97	[1.3]	0.04 ± 0.01	74.5 ± 35.3	[0.7]	1.21 ± 0.47
Stomach	0.42 ± 0.11	[4.2][f]	0.03 ± 0.004	0.28 ± 0.08	[0.1]	0.02 ± 0.003
Intestine	0.57 ± 0.16	[3.4][f]	0.14 ± 0.03	0.53 ± 0.17	[0.3]	0.14 ± 0.04
Skin	0.12 ± 0.02	[10][f]	1.30 ± 0.03	0.06 ± 0.02	[2.0]	0.06 ± 0.01
Brain	0.77 ± 0.29	[5.6][f]	0.010 ± 0.004	0.11 ± 0.04	[1.1]	0.0014 ± 0.0006
Blood	0.10 ± 0.03	[9.6][f]	—	0.36 ± 0.09	[5.6][f]	—
Kidney	0.29 ± 0.07	[4.8][f]	—	0.55 ± 0.18	[1.6]	—
Gills	0.28 ± 0.09	[7.0][f]	—	0.21 ± 0.06	[1.9]	—

[a] Ratio of concentration of naphthalene in tissues of fish exposed at 4° and 12°C

[b] % administered dose was calculated using individual concentration value and total weight of each organ; each value for % admin. dose is mean ± S.E. (6 fish)

[c] Ratio of concentration of metabolites in tissues of fish exposed at 4° and 12°C

[d] Fish were fed 56 μCi (198 mCi/mMole) of ^{3}H-1-naphthalene

[e] Average value for six fish ± S.E.

[f] Concentrations at 4° and 12°C were significantly ($P < 0.05$) different from each other

Table 2. Comparison of metabolite profiles in starry flounder exposed to dietary naphthalene at 4° and 12°C

	Liver			Muscle			Bile	
	% of total metabolites[b]			% of total metabolites[b]			% of total metabolites[b]	
Metabolites[a]	4°C	12°C[d]	$[C_{4°}/C_{12°}]$[c]	4°C	12°C	$[C_{4°}/C_{12°}]$[c]	4°C	12°C[d,e]
	24 hr after feeding of ^{3}H-naphthalene							
Total conjugates	27.1 ± 1.8	43.6 ± 9.9	[0.5]	11.5 ± 0.3	9.4 ± 0.04	[2.7]	86.5 ± 1.6	91.8 ± 0.3
Total non-conjugates	72.9 ± 1.8[e]	56.4 ± 9.9[e]	[1.1]	88.5 ± 0.3[e]	90.6 ± 0.04[e]	[2.1]	13.5 ± 1.6	8.2 ± 0.3
Glucuronides	15.9 ± 0.4[f]	32.2 ± 7.2	[0.4]	4.6 ± 0.4[f]	5.4 ± 0.9[f]	[1.9]	73.9 ± 0.1[f]	81.6 ± 0.1
Mercapturic acids	11.2 ± 0.4	9.5 ± 3.6	[0.9]	3.2 ± 0.5	2.6 ± 1.1	[2.8]	10.9 ± 0.2	8.9 ± 0.1
Sulfate/glucosides	N.D.	1.9 ± 0.1[f]	—	3.6 ± 0.9[f]	1.5 ± 0.2[f]	[5.4]	1.8 ± 0.3	1.4 ± 0.1
Dihydrodiol (1,2-isomer)	48.4 ± 0.2[f]	39.7 ± 13.9[f]	[1.1]	77.3 ± 0.7[f]	80.9 ± 0.5[f]	[2.1]	6.5 ± 1.4	4.0 ± 0.1
Naphthols (1- & 2-)	8.8 ± 0.9	10.7 ± 3.9	[0.6]	1.8 ± 0.1	2.4 ± 0.01[f]	[1.6]	2.8 ± 0.6	1.5 ± 0.1
Uncharacterized ($X_1 - X_4$)	15.7 ± 0.6	6.0 ± 0.7	[2.1]	9.4 ± 0.2	7.2 ± 0.9	[2.8]	4.2 ± 0.5	2.6 ± 0.3
	168 hr after feeding of ^{3}H-naphthalene							
Total conjugates	54.1 ± 1.3	62.3 ± 8.8	[1.7]	28.9 ± 0.5	46.1 ± 0.3	[2.6]	94.6 ± 0.2	91.5 ± 0.3
Total non-conjugates	45.9 ± 1.3	37.7 ± 8.8	[2.1]	71.1 ± 0.5	53.9 ± 0.3	[5.5]	5.5 ± 0.2	8.5 ± 0.3
Glucuronides	32.3 ± 0.3	16.6 ± 1.6	[3.8]	13.1 ± 1.3	23.7 ± 1.4	[2.3]	85.1 ± 0.2	81.7 ± 0.3
Mercapturic acids	11.7 ± 0.2	10.9 ± 6.6	[1.8]	7.2 ± 0.2	8.0 ± 0.9	[3.8]	7.7 ± 0.2	8.8 ± 0.1
Sulfate/glucosides	10.1 ± 0.1	34.8 ± 14.8	[0.6]	8.7 ± 2.1	14.4 ± 0.5	[2.5]	1.8 ± 0.1	0.9 ± 0.1
Dihydrodiol (1,2-isomer)	29.7 ± 0.5	12.4 ± 11.7	[4.5]	47.4 ± 0.4	23.9 ± 0.9	[8.3]	2.5 ± 0.1	3.0 ± 0.1
Naphthols (1- & 2-)	6.2 ± 0.2	11.5 ± 3.9	[0.9]	6.9 ± 1.2	21.6 ± 0.6	[1.3]	0.6 ± 0.1	2.9 ± 0.2
Uncharacterized ($X_1 - X_4$)	10.0 ± 1.1	14.0 ± 7.1	[5.1]	16.7 ± 1.1	8.4 ± 0.4	[8.2]	2.3 ± 0.3	2.6 ± 0.1

[a] Characterized by R_f values of TLC standards. Each value is mean ± S.D. of three samples of pooled homogenates prepared from 4 to 6 fish

[b] Based on total extracted metabolites; an average of 8, 13, and <0.5% of total radioactivity respectively, remained in liver, muscle, and bile from fish taken at 24 hr

[c] Ratio of concentration (pmole/mg dry wt) of each class of metabolite at 4°C to that at 12°C.

[d] Taken from Varanasi *et al.* (1979)

[e] Biliary metabolites were also analyzed at 8 hr after the exposure; major component (>75%) was glucuronide fraction. Free dihydrodiol and naphthols constituted less than 10% of the total extracted metabolites

[f] Significantly ($p < 0.05$) different from the corresponding value at 168 hr

and bile from the naphthalene-exposed starry flounder (Table 2) at both 4° and 12°C. At 24 hr, no marked differences were observed in the proportions of most metabolite classes in liver of fish at 4° and 12°C.

From 24 to 168 hr, there was a significant ($p < 0.05$) decrease in the proportion of the dihydrodiol fraction and an increase in sulfate/glucoside fraction in both liver and muscle of fish at 4° and 12°C (Table 2). Moreover, the proportion of the glucuronide fraction also increased with time in liver, muscle, and bile of fish at 4°C. Individual metabolite classes in liver and muscle of fish at 4° and 12°C did not vary directly with the concentrations of total metabolites; ratios of concentrations of total metabolites (Table 1) at 4° and 12°C for the liver and muscle at 168 hr were 1.6 and 3.6, respectively, whereas ratios for the dihydrodiol at this time were 4.5 and 8.3, respectively (Table 2).

Metabolites in bile from both groups of fish (4° or 12°C) at 24 and 168 hr were characterized by high percentages (>85%) of the conjugates, of which glucuronides were the major components (Table 2). Analyses of bile from fish at 8 hr after exposure at 12°C also showed the glucuronides to be the major components (Table 2, footnote e). Very small proportions (<10%) of the non-conjugates (dihydrodiol and naphthols) were present in bile of starry flounder from 8 to 168 hr.

Enzymatic hydrolysis of glucuronide fractions isolated from the bile revealed that these derivatives were formed primarily by conjugation with the dihydrodiol of naphthalene (Table 3, Figure 2). Smaller amounts of naphthols (1- and 2-isomers) were also present in the hydrolysis products from the glucuronide fraction. At 24 hr, patterns of hydrolysis products from biliary glucuronides from fish at 4° and 12°C were similar (Table 3), even though the proportions of unhydrolyzed fraction were 28 and 56%, respectively.

The sulfate/glucoside fraction was a minor component of biliary metabolites; therefore, there was not sufficient radioactivity in most samples to permit analysis of hydrolysis products. Only one fraction isolated from the bile of fish held at 12°C was treated with aryl sulfatase; greater than 50% of the total radioactivity was released into the hydrolysis products. Hydrolysis of the sulfate fraction yielded primarily the dihydrodiol derivative together with a small

Table 3. Ethyl acetate-soluble hydrolysis products of conjugated metabolites[a] isolated from bile of naphthalene-fed starry flounder

Temp (°C)	Time after feeding (hr)	Conjugates	Hydrolysis products[a] dihydrodiol[b] (%)	1-naphthol[b] (%)	2-naphthol[b] (%)	% of conjugate fraction unhydrolyzed
12	8	glucuronides	62	8	30	45
12	24	glucuronides	71	7	22	28
12	168	glucuronides	89	4	7	28
4	24	glucuronides	74	6	20	56
12	168	sulfates/ glucosides	78	5	17	45

[a] Metabolite fractions were incubated with β-glucuronidase from *Helix pomatia* which contained significant amounts of aryl sulfatase for 48 hr at 37°C. (pH = 5.0)
[b] characterized by R_f values of TLC standards

amount of naphthols, of which 2-naphthol was once again the major isomer (Table 3).

Discussion

The present results show that the decrease in water temperature resulted in retention of substantially higher concentrations of naphthalene in tissues of the naphthalene-exposed starry flounder. These results agree with the findings with clams, *Rangia cuneata* (Fucik *et al.* 1977), copepods, *Calanus helgolandicus* (Harris *et al.* 1977) and coho salmon, *Oncorhynchus kisutch* (Collier *et al.* 1978). The differences observed in naphthalene concentrations in tissues of starry flounder at 4° and 12°C could be due to several factors: Differences in rates of absorption of the ingested dose, differences in rates of excretion of naphthalene from tissues, and differential rates of biotransformation of naphthalene at these two temperatures.

Environmental temperature is known to have a marked effect on the activity of the alimentary canal of fish; both the rate of passage of food and the rate of its absorption are much slower in fish at lower temperatures (Barrington 1957). The present results indicate that both absorption and elimination of the ingested dose from the alimentary canal were slower for fish at 4°C than at 12°C. Thus, during the period of 24 to 168 hr, as much as one-third of the ingested dose was still in the digestive tract and potentially available to fish at 4°C compared to less than 3% of the dose in the tracts of fish at 12°C. This may explain the slower decline in naphthalene concentrations from 24 to 168 hr in tissues of starry flounder at the lower temperature.

It is also likely that the lowering of water temperature significantly influenced the rate of elimination of naphthalene from tissues of fish. For example, Collier *et al.* (1978) did not observe any effect of lowered water temperature on the retention of ingested naphthalene in the gut of coho salmon, but did report significantly higher concentrations of naphthalene in tissues of fish at 4°C compared to those at 10°C.

The effect of decreased temperature on metabolite concentrations was not as marked as that observed for the naphthalene concentrations, thereby suggesting that a much smaller proportion of naphthalene was biotransformed by liver of starry flounder held at the lower temperature. However, such a result may also be due to altered rates of excretion of naphthalene and its metabolites at the lower temperature. Nevertheless, when considering consequences (*e.g.*, toxicity) of increased concentrations and increased residence times of naphthalene in cold-acclimated fish, the decreased bioconversion of naphthalene to its metabolic products should be taken into account.

A change in environmental temperature is known to alter activities of certain enzymes in fish (Hochachka and Somero 1971). Stegeman (1979) reported that *in vitro* activities of hepatic benzo[a]pyrene hydroxylase and NADPH cytochrome C reductase measured at 25°C were significantly greater for *Fundulus heteroclitus* maintained at 6.5°C than those at 16.5°C. Moreover, a change in the environmental temperature of a fish also brings about changes in structural (membrane) and depot lipids (Hazel 1979a, 1979b), which may result in altered rates of absorption and excretion of lipophilic PAH and their metabolites. Thus, environmental temperature can alter both types and concentrations

of individual metabolites retained in tissues of fish exposed to PAH. Our results show that a decrease in water temperature brought about marked changes in individual metabolite classes, which would have gone unsuspected if only total metabolite concentrations were determined. Virtually no information is available on the toxicity of different metabolites of naphthalene on fish. However, it is known that phenols and dihydrodiols of certain PAH (*e.g.* benzo[a]pyrene) are more toxic to mammals than the parent compound, and some of these metabolites also interact with cellular macromolecules (DNA, RNA protein) (Sims and Grover 1974; Brookes 1977; Burke *et al.* 1977; Kapitulnik *et al.* 1977). Certain hydroxylated metabolites of naphthalene also bind irreversibly to cellular protein (Hesse and Mezger 1979). Accordingly, the results showing a significant increase in the concentrations of the dihydrodiol derivative of naphthalene in liver and muscle of cold-maintained starry flounder is important. Moreover, it should be noted that one week after naphthalene-exposure, fish held at 12°C contained very low concentrations of naphthalene, whereas tissues (liver and muscle) of fish at 4°C still retained substantial concentrations of naphthalene, a potential source of metabolic products.

Our results, together with previous findings (Varanasi *et al.* 1979), demonstrate the tendency of starry flounder to biotransform naphthalene primarily into 1,2-dihydro-1,2-dihydroxynaphthalene and its conjugates. The presence of a significant proportion of the free dihydrodiol in biotransformation products of naphthalene by other fish species (Lee *et al.* 1972; Roubal *et al.* 1977; Collier *et al.* 1978; Collier *et al.* 1980) has been reported, but until now, only the conjugates of naphthol were characterized. Krahn *et al.* (1980) characterized, with high-performance liquid chromatography/ultraviolet fluorescence detection, 1-naphthyl glucuronide and 1-naphthyl sulfate in bile of rainbow trout (*Salmo gairdneri*) exposed to dietary naphthalene. The present results show, for the first time, that glucuronides and sulfates isolated from bile of naphthalene-exposed starry flounder were conjugated primarily with the dihydrodiol and to a much smaller extent with 1-naphthol. In mammals, the dihydrodiol of naphthalene is shown to conjugate with both glucuronic acid and sulfuric acid (Sims and Grover 1974). Furthermore, 1,2-dihydro-1,2-dihydroxynaphthalene may be dehydrogenated *in vivo* to 1,2-dihydroxynaphthalene which can then be conjugated with either glucuronic or sulfuric acid (Sims and Grover 1974). Because 1,2-dihydroxynaphthalene is unstable, its presence in the hydrolysis products was not detected by the methods employed in this study. Small amounts of 1,2-naphthoquinone, a product of 1,2-dihydroxynaphthalene, were present in biliary metabolites. The presence of 2-naphthol in the hydrolysis products from sulfates and glucuronides may arise from the diol conjugates (Sims and Grover 1974).

In conclusion, the lowering of water temperature altered residence times and concentrations of both naphthalene and its metabolites in tissues of starry flounder. The effect of temperature on the naphthalene concentrations was more pronounced after one week than after one day. It is evident that environmental temperature may strongly influence both the nature and severity of toxic effects of naphthalene in flatfish. Furthermore, the results support the contention made in earlier reviews (Malins 1977; Varanasi and Malins 1977) that it is vital to include information on the types and concentrations of metabolites, together with the concentrations of parent PAH, when defining the

body-burdens of these xenobiotics and their possible biological effects in marine organisms.

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References

Atlas, R. M., A. Horowitz, and M. Busdosh: Prudhoe crude oil in arctic marine ice, water, and sediment ecosystems: degradation and interactions with microbial and benthic communities. J. Fish. Res. Board Can. **35**, 585 (1978).

Anderson, J. W., J. Neff, B. Cox, H. Tatan, and G. M. Hightower: Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity on estuarine crustaceans and fish. Mar. Biol. **27**, 75 (1973).

Barrington, E. J. W.: The alimentary canal and digestion. In M. E. Brown (ed.): The physiology of fishes. Vol. 1, p. 109. New York: Academic Press (1957).

Brookes, P.: Mutagenicity of polycyclic aromatic hydrocarbons. Mutation Res. **39**, 257 (1977).

Burke, M. D., H. Vadi, B. Jernstrom, and S. Orrenius: Metabolism of benzo[α]pyrene with isolated hepatocytes and the formation and degradation of DNA-binding derivatives. J. Biol. Chem. **252**, 6424 (1977).

Collier, T. K., L. C. Thomas, and D. C. Malins: Influence of environmental temperature on disposition of dietary naphthalene in coho salmon (*Oncorhynchus kisutch*): Isolation and identification of individual metabolites. Comp. Biochem. Physiol. **61c**, 23 (1978).

Collier, T. K., M. M. Krahn, and D. C. Malins: The disposition of naphthalene and its metabolites in the brain of rainbow trout (*Salmo gairdneri*). Environ. Res. In press (1980).

Dodgson, K. S., J. I. M. Lewis, and B. Spencer: The arylsulfatase and glucuronidase of marine molluscs. Biochemical Journal **55**, 253 (1953).

Durkin, J. T., S. J. Lipovsky, G. R. Snyder, and J. M. Shelton: Changes in epibenthic estuarine fish and invertebrates from propeller agitation dredging. Report to North Pacific Division for Corps of Engineers. (DACW 57-76-C-0123) (1977).

Fucik, K. W., J. M. Neff, and M. L. Byington: Effects of temperature and salinity on naphthalenes uptake in the temperate clam, *Rangia cuneata* and the boreal clam *Prototheca staminea*. In D. A. Wolfe (ed.): Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms. New York: Pergamon Press (1977).

Harris, R. P., V. Berdugo, E. D. S. Corner, C. C. Kilvington, and S. C. M. O'Hara: Factors affecting the retention of a petroleum hydrocarbon by marine planktonic copepods. In D. A. Wolfe (ed.): Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms. New York: Pergamon Press (1977).

Hazel, J. R.: Influence of thermal acclimation on membrane lipid composition of rainbow trout liver. Am. J. Physiol. **236**, R91 (1979a).

Hazel, J. R.: Influence of temperature adaptation on composition of the neutral lipid fraction of rainbow trout (*Salmo gairdneri*) liver. J. Exp. Zool. **207**, 33 (1979b).

Hesse, S., and M. Mezger: Involvement of phenolic metabolites in the irreversible protein-binding of aromatic hydrocarbons: Reactive metabolites of [^{14}C] naphthalene and [^{14}C] 1-naphthol formed by rat liver microsomes. Mol. Pharmacol. **16**, 667 (1979).

Hochachka, P. W., and G. N. Somero: Biochemical adaptation to the environment. In W. S. Hoar and P. J. Randall (eds): Fish physiology. Vol. VI, p. 99. New York: Academic Press (1971).

Hodgins, H. O., B. B. McCain, and J. W. Hawkes: Marine fish and invertebrate diseases, host disease resistance, and pathological effects of petroleum. In D. C. Malins (ed.): Effects of petroleum on arctic and subarctic marine environments and organisms. Vol. II, p. 95. New York: Academic Press (1977).

Kapitulnik, J., W. Levin, A. H. Conney, H. Yagi, and D. M. Jerina: Benzo[α]pyrene 7,8-dihydrodiol is more carcinogenic than benzo[α]pyrene in newborn mice. Nature **266**, 378 (1977).

Krahn, M. M., D. W. Brown, T. K. Collier, A. J. Friedman, R. G. Jenkins, and D. C. Malins: Rapid analysis of naphthalene and its metabolites in biological systems: Determination by high performance liquid chromatography/fluorescence detection and by plasma desorption chemical ionization mass spectrometry. J. Biochem. Biophys. Methods **2**, 233 (1980).

Lee, R. F., R. Sauerheber, and G. H. Dobbs: Uptake, metabolism and discharge of polycyclic aromatic hydrocarbons by marine fish. Mar. Biol. (Berl.) **17**, 201 (1972).

Malins, D. C.: Metabolism of aromatic hydrocarbons in marine organisms. Ann. N.Y. Acad. Sci. **298**, 482 (1977).

Moore, S. F., and R. L. Dwyer: Effects of oil on marine organisms: A critical assessment of published data. Water Res. **8**, 819 (1974).

Norwich, K. H.: Molecular dynamics in biosystems. New York: Pergamon Press (1977).

Roubal, W. T., T. K. Collier, and D. C. Malins: Accumulation and metabolism of carbon-14 labeled benzene, naphthalene, and anthracene by young coho salmon (*Oncorhynchus kisutch*). Arch. Environ. Contam. Toxicol. **5**, 513 (1977).

Sims, P., and P. L. Grover: Epoxides in polycyclic aromatic hydrocarbon metabolism and carcinogenesis. In G. Klein, S. Weinhouse, and A. Hadden (eds.): Advances in cancer research. Vol. 20, p. 165. New York: Academic Press (1974).

Stegeman, J. J.: Temperature influence on basal activity and induction of mixed function oxygenase activity in *Fundulus heteroclitus*, J. Fish. Res. Board Can. **36**, 1400 (1979).

Varanasi, U., D. J. Gmur, and P. A. Treseler: Influence of time and mode of exposure on biotransformation of naphthalene by juvenile starry flounder (*Platichthys stellatus*) and rock sole (*Lepidopsetta bilineata*). Arch. Environ. Contam. Toxicol. **8**, 673 (1979).

Varanasi, U., and D. C. Malins: Metabolism of petroleum hydrocarbons: Accumulation and biotransformation in marine organisms. In D. C. Malins (ed): Effects of petroleum on arctic and subarctic marine environments and organisms. Vol. II, p. 175. New York: Academic Press (1977).

Varanasi, U., M. Uhler, and S. I. Stranahan: Uptake and release of naphthalene and its metabolites in skin and epidermal mucus of salmonids. Toxicol. Appl. Pharmacol. **44**, 277 (1978).

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