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ENVIRONMENTAL RESEARCH BRIEF

Hazardous Chemicals in Fish
Wisconsin Power Plant Impact Study

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Introduction

From the operation of a coal-fired power plant, a variety of organics present in coal might reach the aquatic environment via leaching from such sources as stored coal, stack emissions, and ash ponds. The use of petroleum-derived fuels for the transportation of coal could provide a source for additional hydrocarbons, and the use of chlorination procedures on cooling water to retard algal growth could give rise to chlorinated hydrocarbons. Chemicals which reach the aquatic environment but which have relatively low water solubility tend to be taken up by aquatic species including fish. Thus, the uptake and accumulation by fish of organics arising from the operation of a coal-fired power plant might serve as a vector for human exposure to such organics through the consumption of the contaminated fish. The present report summarizes studies of the uptake, disposition, metabolism, and elimination of selected chemicals by fish.

Findings and Conclusions

I. Uptake, Distribution and Elimination of Naphthalene, 2-Methylnaphthalene and 1,2,4-trichlorobenzene by Fish

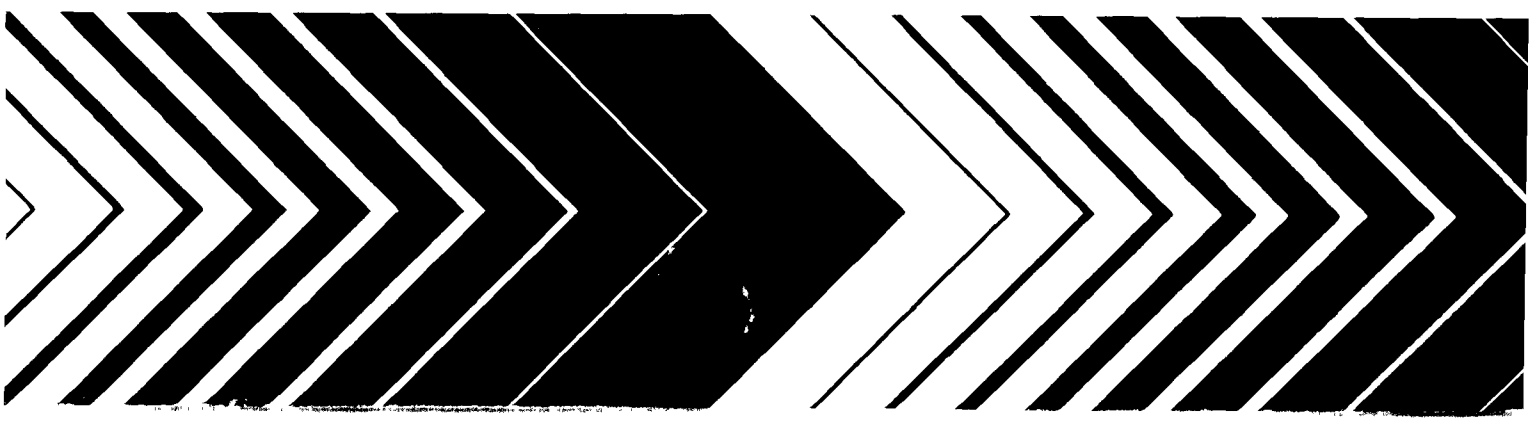
Naphthalene and 2-methylnaphthalene were selected for study because both are among the most water-soluble components of coal and petroleum and both chemicals are likely to reach the aquatic environment. 1,2,4-trichlorobenzene was selected as a representative compound for chlorination-caused chloro-organics.

The uptake and elimination of these three compounds by fingerling rainbow trout was studied using a continuous-flow delivery system in which groups of trout were exposed to the appropriate ^{14}C-labeled chemical for 4-5 weeks, followed by a depuration period. The results with naphthalene, 2-methylnaphthalene and trichlorobenzene are presented in Figures 1, 2, and 3, respectively. The maximum accumulations attained (muscle ^{14}C/ average water ^{14}C) were approximately 40 for naphthalene, 160 for 2-methylnaphthalene and 156 for trichlorobenzene. In additional experiments with 2-methylnaphthalene, the values were 123 for carp muscle and 403 for bluegill sunfish (whole body). The t-1/2 of elimination following each of these exposures is shown in Table 1.

Although these chemicals were accumulated by fish muscle at 40-160 times the exposure level, they were generally eliminated rapidly upon termination of the exposure, with the exception of naphthalene. In earlier experiments, the elimination of ^{14}C from trout tissues following short-term (8 hr) exposures to any of these ^{14}C-chemicals was rapid. Subsequent studies suggested that the slow elimination of naphthalene metabolites from muscle tissue following the longer term exposure was probably responsible for the slower ^{14}C elimination rate.

II. Metabolism of Naphthalene, 2-methylnaphthalene and 1,2,4-trichlorobenzene by Fish

Exposure of fish to these chemicals containing ^{14}C-label resulted in the appearance of ^{14}C-labeled materials in the bile of the exposed fish. Thin-layer chromatography indicated that in each case most of the bile ^{14}C resided in high polarity compounds, suggestive of conjugated metabolites. By the use of β-glucuronidase, an enzyme which hydrolyzes glucuronide conjugates, it was demonstrated that glucuronide conjugates made up a major fraction of these bile metabolites. Additional studies suggested that other metabolites may have arisen from glutathione conjugates.



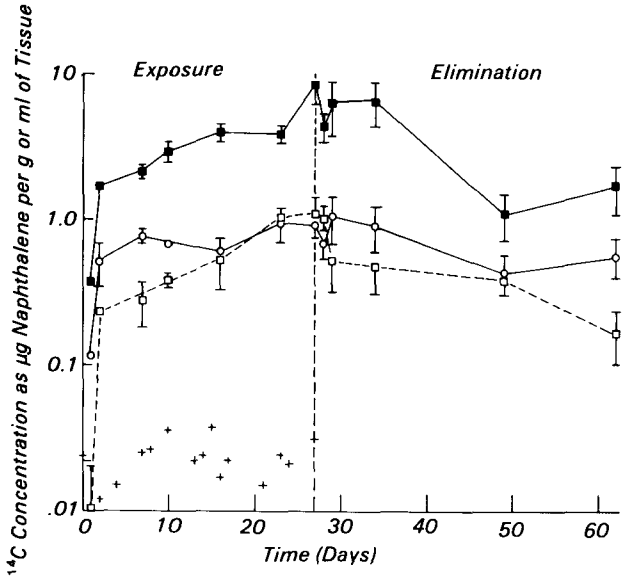


Figure 1. Tissue levels of ^{14}C in trout during a 27-day exposure to ^{14}C-naphthalene and subsequent elimination. Each point represents the average of values from live trout, and the verticl lines represent the standard error. The average concentration of ^{14}C-naphthalene in water during the exposure was 0.023 mg/liter.
■ = liver, o = muscle, □ = blood, and + = exposure water.

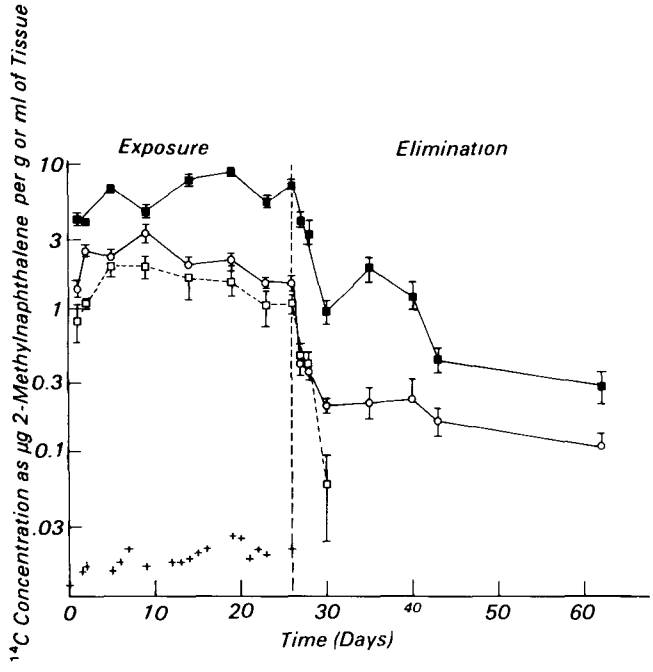


Figure 2. Tissue levels of ^{14}C in trout during a 26-day exposure to ^{14}C-2-methylnaphthalene and subsequent elimination. Each point represents the average of values from five trout, and the vertical lines represent the standard error. The average concentration of ^{14}C-2-methylnaphthalene in water during the exposure was 0.017 mg/liter.
■ = liver, o = muscle, □ = blood, and + = exposure water.

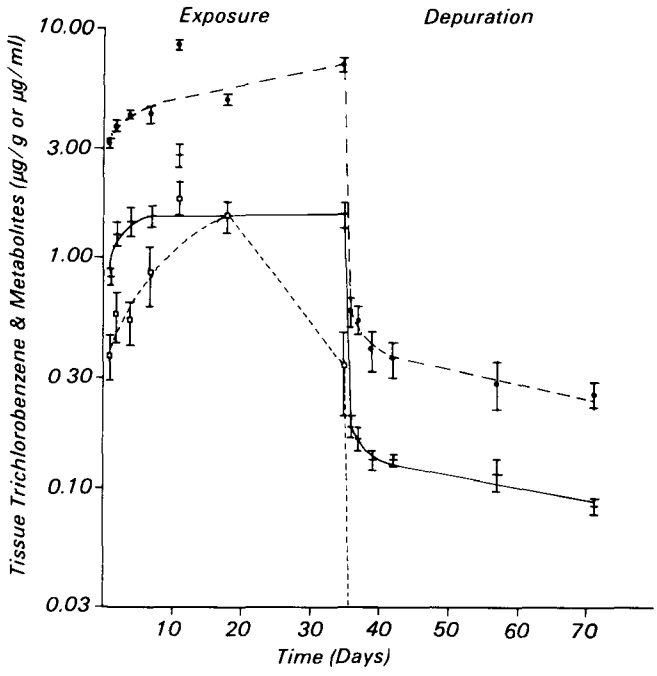


Figure 3. Tissue levels of ^{14}C in trout during a 35-day exposure to ^{14}C-1,2,4-trichlorobenzene and subsequent elimination. Each point represents the average of values from five trout, and the vertical lines represent the standard error. The average concentration of ^{14}C-1,2,4-trichlorobenzene in water during the exposure was 0.018 mg/liter.
■ = liver, + = muscle, and o = blood.

III. Studies of the Hepatic Microsomal Monooxygenase System in Rainbow Trout and Carp

As evidence began to accumulate on the *in vivo* metabolism of xenobiotics by fish, studies were initiated to compare the metabolizing systems in fish to those characterized in mammalian species. Our studies with liver, a major locus of xenobiotic metabolism, showed that trout liver responded to homogenization, differential centrifugation and various enzyme assays in much the same way as did rat liver. Because a number of aquatic pollutants such as PAHs and PCBs are known to induce (increase) hepatic microsomal monooxygenase (xenobiotic metabolizing) activity in mammalian species, the effect of these aquatic pollutants in fish was examined. The data in Table 2 show the effects of administration of a PAH, a PCB and phenobarbital to rainbow trout on two hepatic microsomal enzyme activities. The data in Table 3 show similar information for carp. Because chemical aquatic pollutants such as PAHs and PCBs can increase hepatic microsomal metabolism, such chemicals might affect the metabolism and disposition of foreign chemicals by fish *in vivo*.

IV. Studies on the Effects of Modifiers of Hepatic Metabolism in Fish on Metabolism and Disposition of Xenobiotics in Fish

The effect of preadministration of β-naphthoflavone to rainbow trout on the metabolism of naphthalene, 2-methylnaphthalene, and trichlorobenzene was examined. The

Table 1. Elimination Half-Lives of ^{14}C from Fish Exposed to Aqueous ^{14}C-Naphthalene, ^{14}C-2-Methylnaphthalene or ^{14}C-Trichlorobenzene for Several Weeks

Exposure	Half-Lives, h			
	Muscle	Liver	Blood	Whole Fish
Trout	*909*	*343*	*379*	
Naphthalene				
0.017 mg/liter				
Trout	*13*[a]	*211*	*23*	
2-Methylnaphthalene	*711*			
0.023 mg/liter				
Carp	*31*[b]	*59*[b]		
2-Methylnaphthalene	*1942*	*781*		
0.013 mg/liter				
Bluegill sunfish				*<24*[c]
2-Methylnaphthalene				*353*
0.013 mg/liter				
Trout	*8*[d]	*7*[d]		
1,2,4-Trichlorobenzene	*36*	*32*		
0.018 mg/liter				

[a]*When two values are given, the upper value is the early rapid phase of elimination and the lower value is the later slower phase. The slope and intercept for the slow phase of elimination were calculated using the data for days 4-36. The data from days 0-2 were corrected for this, and the resulting values were used to calculate slope for the rapid phase of elimination from days 0-2. Muscle ^{14}C decreased by approximately 75% during the first 3 days of depuration.*

[b]*Data for slow phase, days 8-73 and for rapid phase, days 0-3. Whole fish ^{14}C decreased by approximately 61% during the first three days of depuration.*

[c]*Data for slow phase, days 1-26. Muscle ^{14}C decreased by approximately 60% during the first day of depuration.*

[d]*Data for slow phase, days 1-36. Muscle ^{14}C decreased by approximately 88% during the first day of depuration.*

results presented in Table 4 show that pretreatment resulted in substantial increases in metabolites of each chemical appearing in bile, and a decrease in the amount of each chemical remaining in muscle.

The effect of administration of an inhibitor, piperonyl butoxide, on metabolism and elimination of two organic compounds, pentachloroanisole and di-2-ethylhexylphthalate, was studied. Bile from the pretreated trout contained only one-third of the amount of pentachlorophenol-glucuronide, the major metabolite of pentachloroanisole, as did the control trout. In the case of di-2-ethylhexylphthalate, the pretreatment reduced the level of bile metabolites by one-half and increased muscle levels of the parent chemicals 3-fold.

V. Possible Use of Fish Bile as an Aid in Monitoring for Aquatic Pollutants

Some aquatic pollutants are of concern because they collect in fish to levels many times higher than the levels present in the water. For highly lipophilic pollutants such as PCBs, monitoring for these chemicals might be easier if fish flesh, rather than the water, were examined. For other pollutants which are more readily metabolized, the metabolites may appear in bile at much higher levels than in the water. The data in Table 5 demonstrates the bile-to-water ratio for a variety of chemicals. A tetrachlorobiphenyl which is not readily metabolized was present at only 11-fold concentration; phenols, which are readily glucuronidated, were concentrated 1,000-fold to 10,000-fold, with other chemicals falling between these two extremes. In the long-term exposures described earlier, the bile-to-water ratios were much higher for PAHs but not for trichlorobenzene as shown in Table 6.

Recommendations

1. The rapid elimination of certain organics after short-term exposure suggests that intermittent brief exposures should not lead to substantial bioaccumulation. Long-term exposure however will lead to significant bioaccumulation dependent upon the lipophilicity and metabolism of a particular chemical. Additional studies should be done on representative PAHs of greater lipophilicity and on mixtures of these chemicals.

Table 2. The Effect of Inducers on the Kinetics of Monooxygenation in Rainbow Trout Hepatic Microsomes Following Intraperitoneal Pretreatment

Pretreatment	Ethoxyresorufin-O-deethylase			Ethoxycoumarin-O-deethylase		
	Vmax nmol/min/mg	% control	Km, nM	Vmax nmol/min/mg	% control	Km, nM
Corn oil	*0.136±0.26*[a]	*100*	*144±6*	*0.101±0.010*	*100*	*129±9*
1 ml/kg						
Aroclor	*1.85±0.04*[b]	*1367*	*154±0*	*0.286±0.47*[a]	*283*	*51±1*[b]
150 mg/kg						
β-Naphthoflavone	*6.06±0.18*[b]	*4455*	*125±8*	*1.19±0.28*[a]	*1178*	*41±4*[b]
100 mg/kg						
Pentobarbital	*0.088±0.017*	*65*	*170±0*	*0.065±0.017*	*64*	*105±5*
65 mg/kg						

[a]*Values are mean ± SE; all values obtained 72 h after injection of fish.*

[b]*Significantly different from corn oil control group, $P < 0.05$.*

Table 3. Maximum Induction of Cytochrome P450 and Mixed-Function Oxidase Activities in Carp Liver and Kidney Microsomes

	Liver Microsomes			*Kidney Microsomes*		
	BNF	*A1254*	*TCB*	*BNF*	*A1254*	*TCB*
P450	*2.8*[a]	*1.9*	*0.8*			
ECOD[b]	*1.6*	*1.5*	*0.8*	*2.3*	*2.2*	*1.5*
EROD	*73.5*	*84.9*	*1.4*	*1.7*	*61*	*2.3*
BaPH	*23 4*	*29.6*				

[a]*Values represent the maximum ratios of P450 content or enzyme activity of treatment group to control (corn oil) group.*

[b]*ECOD = ethoxycoumarin-O-deethylase activity; EROD = ethoxy-resorufin-O-deethylase activity and BaPH = benzo(a)pyrene hydroxylase activity.*

2. The substantial bioconcentration of metabolites of PAHs which are carcinogens and of phenols in fish bile, compared to the levels in exposure water suggest that use of bile could prove useful in monitoring for certain pollutants in the aquatic environment.

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Table 4. Effect of Pre-Administration of β-Naphthoflavone on the Disposition and Metabolism of ^{14}C-Labeled Chemicals in Rainbow Trout

	Corn Oil		*β-Naphthoflavone*	
Tissue	*Tissue level of parent chemical + metabolites (μg/g or μg/ml)*	*Metabolites %*	*Tissue level of parent chemical + metabolites (μg/g or μg/ml)*	*Metabolites %*
		Naphthalane		
Bile[a]	*67 2±5.1*	*98*	*308±21*	*99*
Muscle[b]	*2.25±0 23*	*5.1±0 4*	*1.25±0.16*	*12.3±0.9*
Liver[b]	*2.05±0.12*	*8.5±0.5*	*1.72±0 01*	*24.0±1.8*
Blood[a]	*1 83±0.23*	*0 97±0.08*		
		2-Methylnaphthalene		
Bile[a]	*150±24*	*96* *1233±201*	*100*	
Muscle[c]	*4.9*	*2*	*2 6*	*10*
Liver[c]	*10 8*	*10*	*5.0*	*40*
Blood[a]	*3 3±0 2*		*1.9±0 1*	
		1,2,4-Trichlorobenzene		
Bile[a]	*14.7±0.8*	*65*	*87.5±5 5*	*98*
Muscle[c]	*575*[d]	*0 8*	*299*[d]	*2 1*
Liver[c]	*22*[d]	*3.7*	*42*[d]	*6.2*
Blood[a]	*2.01±0.12*		*1 03±0 04*	

Groups of eight trout were injected intraperitoneally with corn oil or a solution of BNF in corn oil (100 mg/ml) at a rate of 1 ml/kg After 48 h, groups of fish were exposed to one of the above chemicals for 24 h The water levels of the chemicals for control and induced trout were naphthalene, 0.52 and 0.45 mg/liter; 2-methylnaphthalene, 0.28 and 0.36 mg/liter, and 1,2,4-trichlorobenzene, 0 20 and 0.20 mg/liter, respectively.

[a]*Aliquots of blood and bile from each fish were used to determine levels of ^{14}C. Values are the average ± S E. Metabolite determinations utilized pooled bile samples.*

[b]*Each sample consisted of pooled muscle or liver from two fish Thus four samples per group were used to determine tissue ^{14}C levels and percentage of metabolites Values are average ± S E*

[c]*Each sample consisted of pooled muscle or liver from all eight fish in the group*

[d]*Tissue weights were not calculated The total parent compound plus metabolites were extracted as given*

Table 5. Biliary Concentration of Xenobiotics by Rainbow Trout

	Concentration in H_2O (mg/liter)	Radioactivity (dpm/ml) H_2O (0 hours)	Radioactivity (dpm/ml) Bile[a] (24 hours)	Ratio (bile ^{14}C)/(H_2O ^{14}C)
2',5-Dichloro-4'-nitrosalicylanilide (Bayer 73; chlorosalicylic acid; ring-UL-^{14}C)	0.05	3,010	30,500,000	10,100
Di-2-ethylhexylphthalate (DEHP; carboxyl-^{14}C)	0.5	1,070	265,000	247
2-Methylnaphthalene (ring UL-^{14}C)	0.005	310	796,000	2,570
1-Naphthyl-N-methylcarbamate (carbaryl; naphthyl-1-^{14}C)	0 25	1,030	975,000	947
Naphthalene (ring-UL-^{14}C)	0.005	305	127,000	414
Pentachlorobiphenyl (PCP; ring-UL-^{14}C)	0.1	4,070	21,800,000	5,360
2,5,2',5'-Tetrachlorobiphenyl (TCB; ring-UL-^{14}C)	0.5	3,640	39,000	11
1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane (p,p'-DDT; ring-UL-^{14}C)	0.1	180	22,500	124
3-Trifluoromethyl-4-nitrophenyl (TFM; ring-UL-^{14}C)	0.5	2,020	2,150,000	1,064

[a]Exposures were made at 12°C for 24 hours.

Table 6. Maximum Concentration of Bile Metabolites Found in Fish During Long-Term Continuous Exposure

Chemical	Species	Water Level mg/l	Bile level, parent Chemical + Metabolites mg/ml	Bile Level / Water Level
Naphthalene	trout	0.017	0.327	19,200
2-Methylnaphthalene	trout	0.023	0.434	18,900
	carp	0.013	1.835	141,200
1,2,4-Trichlorobenzene	trout	0.018	0.024	1.3

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