Biochemical Pharmacology, Vol. 31, No. 4, pp. 561–566, 1982.
Printed in Great Britain.

0006-2952/82/040561-06 $03.00/0
Pergamon Press Ltd.

EFFECTS OF POLYCHLORINATED BIPHENYLS AND ENVIRONMENTAL TEMPERATURE ON *IN VITRO* FORMATION OF BENZO[*a*]PYRENE METABOLITES BY LIVER OF TROUT (*SALMO GAIRDNERI*)

ELIANN EGAAS* and USHA VARANASI†

Environmental Conservation Division, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, WA 98112, U.S.A.

(*Received* 27 *March* 1981; *accepted* 7 *July* 1981)

Abstract—Rainbow trout (*Salmo gairdneri*) held at 7° and 16° were given Aroclor 1254 (PCB) (10 mg/kg body wt) via intraperitoneal injections. The binding of [^{3}H]benzo[*a*]pyrene (BaP) to deproteinized salmon sperm DNA was assayed (pmoles BaP equivalents per mg DNA per mg protein) using the post-mitochondrial supernatant (S 10) fractions from livers of fish at 24–168 hr after the PCB exposure. Liver enzymes from the untreated fish acclimated at 7° yielded an average binding value (0.37 ± 0.17) which was significantly greater ($P < 0.05$) than the value (0.07 ± 0.03) for untreated fish at 16°. Liver supernatant fractions from PCB-induced fish acclimated at 16° and sampled at 24–120 hr showed a substantial increase ($P < 0.05$) in the binding (average value 2.4 ± 1.8) compared to the value obtained with untreated fish at 16°. At 24, 48 and 120 hr after the PCB treatment of fish held at 7°, there was no significant increase in the binding value or extent of metabolism of BaP compared to that obtained with the untreated fish at 7°. However, at 168 hr, three of four fish at 7° responded to the PCB treatment with significantly ($P < 0.05$) increased binding values (3.3 ± 1.6). Chromatographic analyses of the ethyl acetate-soluble metabolites revealed that 3-hydroxy BaP and 7,8- and 9,10-dihydrodiols were the major metabolites; K-region metabolites were formed in trace amounts in untreated and PCB-treated fish at both temperatures. No marked qualitative differences were observed in metabolite profiles after the PCB treatment; however, overall metabolism of BaP and production of reactive metabolites by liver enzymes were considerably ($P < 0.05$) enhanced in the PCB-induced fish at both 7° and 16°.

In the aquatic environment, fish encounter a myriad of chemical contaminants such as polynuclear aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB). PAH [e.g. 3-methylcholanthrene (3-MC), benzo[*a*]pyrene (BaP) and benzanthracene] induce hepatic mixed function oxygenase (MFO) activity in fish [1–6]. Furthermore, exposure of pleuronectid and salmonid fish to PAH increases the capacity of the liver enzymes to convert BaP into reactive metabolites that bind to DNA [6–9]. Similar to PAH, PCB also influence hepatic MFO in fish [10–15]. However, little information [16] is available on the effect of PCB exposure of fish on the formation of reactive metabolites of BaP by the liver.

A number of factors affect the ability of fish liver to respond to chemical inducers. Environmental temperature is known to influence a variety of biochemical functions in fish [17–19]; however, conflicting results [20, 21] are reported on the influence of habitat temperature on hepatic MFO activity in fish. Dewaide [20] reported that cold-acclimation of rainbow trout (*Salmo gairdneri*) and roach (*Leuciscus rutilus*) resulted in increased hepatic aminopyrine demethylase activity, whereas Stegeman [21] noted no such increase in cold-acclimated *Fundulus heteroclitus*. Moreover, *F. heteroclitus* held at 6.5° showed no change in hepatic BaP hydroxylase activity at 48 or 96 hr after an intraperitoneal (i.p.) injection of BaP, whereas liver microsomes from the fish held at 16.5° showed a significant increase in hepatic BaP hydroxylase activity after 48 hr [21]. Such a temperature-related response of fish to inducers could adversely influence the validity of using the hepatic MFO activity as an indicator of chemical pollution [2, 22, 23].

A clearer understanding of the influence of habitat temperature on the ability of fish to metabolize carcinogenic PAH is necessary especially because fish have been suggested as experimental animals to study mechanisms of carcinogenesis and other toxic effects [24–26]. The objective of the present work was to assess in detail the influence of environmental temperature on the metabolism of BaP and the binding of metabolites to DNA by the liver enzymes of untreated and PCB-treated rainbow trout. The results show that cold-acclimation of fish increased both the basal hydrocarbon metabolism and time needed for the inductive effect of PCB to be evident. Induction with PCB resulted in a significant increase in the *in vitro* formation of bound and free metabolites of BaP by liver.

* Visiting scholar. Present address: The Governmental Vitamin Institute of Norway, p.b. 4285, N-5013 Nygardstangen, Norway.

† To whom all correspondence should be addressed.

MATERIALS AND METHODS

Chemicals. Deproteinized salmon sperm DNA, NADPH, sucrose, EDTA and BaP were obtained from the Sigma Chemical Co., St. Louis, MO,* and [^{3}H]BaP was purchased from the New England Nuclear Corp., Boston, MA. BaP was purified prior to use [7]. The standards for the oxygenated metabolites of BaP were provided by the courtesy of Dr. David G. Longfellow, NCI Carcinogenesis Research Program, Bethesda, MD. Aroclor 1254 (PCB) was a gift from Dr. E. H. Gruger, Jr.

Fish. Rainbow trout (200 ± 40 g) were obtained from Springbrook Trout Farm, Renton, WA. Fish were acclimated in flowing dechlorinated fresh water either at 7° for 3 weeks, or at a gradually increased temperature (1°/day) from 7 to 16°, with a final 14 days of acclimation at 16°. The fish were fed Oregon Moist Pellets twice a week, and the feeding was stopped 3 days before initiation of the experiment. No significant alterations are reported in hepatic MFO activity in rainbow trout for several weeks after feeding is stopped [20, 27]. Fish were injected intraperitoneally with a dose (10 mg/kg body wt) of Aroclor 1254 (dissolved in corn oil) after mild treatment with tricaine methanesulfonate (Crescent Research Chemicals, Inc., Scottsdale, AZ). Some control fish received injections of corn oil; however, no significant difference was observed in binding values for fish treated with corn oil and for untreated fish. Therefore, in subsequent experiments untreated fish were used as controls.

The fish were sampled at 24, 48, 72, 120, and 168 hr after the PCB exposure. The post-mitochondrial supernatant fractions, S 10 (10,000 *g* for 15 min), of individual liver homogenates were prepared according to Pedersen *et al.* [28] except that 0.25 M sucrose was used instead of 1.15% KCl [7]. Protein concentrations were determined by the method of Lowry *et al.* [29] using bovine serum albumin as the standard.

Determination of covalent binding of activated BP to DNA. The basic incubation procedure is described in detail by Varanasi and Gmur [7]. Optimum incubation temperature for trout liver enzymes was found to be 29°. The reaction mixture [0.02 M phosphate buffer (pH 7.4), 2 mg DNA, 0.75 mg NADPH, 0.1 ml of 0.1 M EDTA, 5 nmoles BaP and 0.2 ml of S 10 fraction (5 mg protein) in a total volume of 2.8 ml] was incubated at 29° for 15 min. The reaction mixture was then treated as described previously [7] with the following modifications: only one phenol extraction and one redissolution of DNA in water were performed. Radioactivity in each sample was determined by liquid scintillation spectrometry and the concentration of DNA was measured by the diphenylamine reaction [30]. Reaction mixtures without NADPH were used as blanks in each experiment and blank values were subtracted from each test value. Each supernatant fraction was analyzed in duplicate.

Table 1. Metabolism and binding of BaP to DNA catalyzed by extracts of liver from trout acclimated to 16°*

Time elapsed after PCB-treatment	Fish	BaP† (pmoles metabolized/mg protein)	BaP equivalents† (pmoles/mg DNA/mg protein)	Unmetabolized BaP	Quinones	Phenols‡	7,8-Diol§	9,10-Diol	Polar	Unclassified	Aqueous phase
				% Total radioactivity							
24 hr	Induced‖ (4)¶	870 ± 120**	1.9 ± 2.3	13 ± 12	3.5 ± 1.9	14 ± 8.7	6.8 ± 4.3	11 ± 6.2	6.3 ± 4.0	5.3 ± 1.3	41 ± 27
	Not induced (1)	180	0.07	82	1	2	1	2	tr††	2	11
72 hr	Induced (5)	970 ± 13	2.5 ± 1.7	2.8 ± 1.3	5.6 ± 3.8	10 ± 7.3	5.0 ± 3.7	13 ± 7.4	9.4 ± 3.0	6.0 ± 2.6	48 ± 21
	Not induced (1)	600	0.18	40	5	10	9	9	1	5	20
120 hr	Induced (4)	870 ± 150	2.9 ± 1.7	13 ± 15	3.5 ± 2.6	8.3 ± 8.5	5.0 ± 4.1	7.8 ± 7.6	7.3 ± 6.8	3.8 ± 1.7	52 ± 30
	Not induced (1)	120	0.04	88	tr	tr	tr	tr	tr	2	9
Control	(13)	210 ± 100	0.07 ± 0.03	79 ± 10	tr	3 ± 2	2 ± 2	3 ± 2	tr	3 ± 2	9 ± 3

* See Fig. 1 for explanation of abbreviations and the test for experimental details.
† For 15 min incubation time.
‡ High performance liquid chromatographic data indicate 3-hydroxy BaP as the major phenol present.
§ Less than 1% of BaP 4,5-diol was detected.
‖ Liver extracts yielding a binding value more than three times the mean control value were considered as induced [31].
¶ Number of individual fish.
** Mean ± S.D.
†† Less than 1%.

* Mention of trade names is for information only and does not constitute endorsement by the U.S. Department of Commerce.

Table 2. Metabolism and binding of BaP to DNA catalyzed by liver extracts from trout acclimated at 7°*

Time elapsed after PCB-treatment	Fish	BaP† (pmolcs metabolized/ mg protein)	BaP equivalents† (pmoles/mg DNA/mg protein)	Unmetabolized BaP	Quinones	Phenols‡	7,8-Diol§	9,10-Diol	Polar	Unclassified	Aqueous phase
				% Total radioactivity							
24 hr	Induced‖ (0)¶										
	Not induced (4)	650 ± 270**	0.28 ± 0.13	36 ± 27	6.5 ± 4.2	12 ± 4.8	8.5 ± 3.4	9.3 ± 5.6	3.5 ± 2.4	6.8 ± 3.1	19 ± 6.4
48 hr	Induced (0)										
	Not induced (4)	370 ± 100	0.43 ± 0.13	63 ± 10	2.3 ± 0.5	7.8 ± 3.6	5.0 ± 2.2	4.8 ± 2.2	1.0 ± 0.1	4.5 ± 1.9	15 ± 4.4
120 hr	Induced (0)										
	Not induced (4)	310 ± 140	0.38 ± 0.23	70 ± 14	2.5 ± 2.4	4.0 ± 2.9	2.5 ± 1.9	3.3 ± 2.6	1.5 ± 1.0	3.8 ± 1.7	14 ± 1.4
168 hr	Induced (3)	810 ± 150	3.3 ± 1.6	19 ± 15	5.7 ± 1.5	16 ± 1.2	9.3 ± 2.9	14 ± 2.0	5.3 ± 2.3	5.3 ± 1.5	26 ± 12
	Not induced (1)	500	0.07	50	4	11	9	9	2	6	11
Control	(13)	680 ± 220	0.37 ± 0.17	32 ± 22	5 ± 2	14 ± 7	7 ± 3	11 ± 14	4 ± 1	6 ± 2	21 ± 7

* See Fig. 1 for abbreviations and the text for experimental details.
† For 15-min incubation time.
‡ High performance liquid chromatographic data indicate 3-hydroxy BaP as the major phenol present.
§ Less than 1% of 4,5-diol was detected.
‖ Liver extracts giving a binding value more than three times the mean control value were considered as induced [31].
¶ Number of individual fish.
** Mean ± S.D.

Metabolism of BaP and characterization of ethyl acetate-soluble metabolites. For preparation of BaP metabolites, the reaction mixture without DNA was incubated for 15 min at 29° and metabolites were extracted with ethyl acetate (2 × 6 ml). The extracts were co-chromatographed with standards of BaP and its metabolites in toluene–ethanol (9:1, v/v) on high-efficiency thin-layer chromatographic (t.l.c.) plates (Analtech, Inc., Newark, DE). Several duplicate samples were analyzed to determine reproducibility. All operations were carried out under dim light to reduce possible photooxidation. Certain samples were also analyzed by high performance liquid chromatography (h.p.l.c.) under conditions described earlier [7, 8].

Statistical comparisons were made using Student's *t*-test, Pearson's correlation analysis and the treatment of Reed *et al.* [31]. The level of probability for all analyses was 95%.

RESULTS

The ratio of liver weight to body weight for the untreated fish at 7° was significantly ($P < 0.05$) different (0.0135 ± 0.0023 for thirteen fish) from that (0.0118 ± 0.0011 for thirteen fish) for fish at 16°. Treatment with Aroclor 1254 did not influence significantly the ratios of liver weight to body weight for fish held at either temperature.

Binding of activated BaP to DNA by liver extracts from untreated and PCB-treated fish. The average binding value obtained with liver extracts (S 10) of thirteen untreated fish at 16° was 0.07 ± 0.03 pmole of BaP equivalents per mg DNA per mg protein (Table 1). The binding value with S 10 fractions from fish acclimated at 7° was 5-fold greater ($P < 0.05$) than the value for untreated fish at 16° (Tables 1 and 2).

Based on the statistical treatment of Reed *et al.* [31], liver enzymes of PCB-treated fish yielding binding values three times the value (control) for untreated fish at the same temperature were considered as induced; binding values greater than three times the mean control value were significantly ($P < 0.05$) outside the normal range. Of the sixteen PCB-treated fish held at 16°, the liver supernatant fractions of thirteen fish yielded binding values which were significantly ($P < 0.05$) greater than the control value. The mean binding values obtained with liver extracts from PCB-induced fish at 24, 72 or 120 hr were not significantly different from each other (Table 1). The three uninduced, PCB-treated fish at 16° yielded an average binding value (0.10 ± 0.07) similar to the control value.

The mean binding value (0.36 ± 0.17) obtained with liver extracts from fish treated with PCB at 7° and sampled at 24, 48 and 120 hr after the treatment were not significantly different from the corresponding (0.37 ± 0.17) control value (Table 2). However, at 168 hr, three of four fish gave rise to binding values which were significantly ($P < 0.05$) larger (3.3 ± 1.6) than the control value.

Metabolism of BaP by liver extracts of untreated and PCB-treated fish. The ethyl acetate-soluble metabolites of BaP produced by fish liver extracts were characterized by t.l.c. (Fig. 1) as quinones, phenols, 7,8-dihydro 7,8-dihydroxybenzo[*a*]pyrene

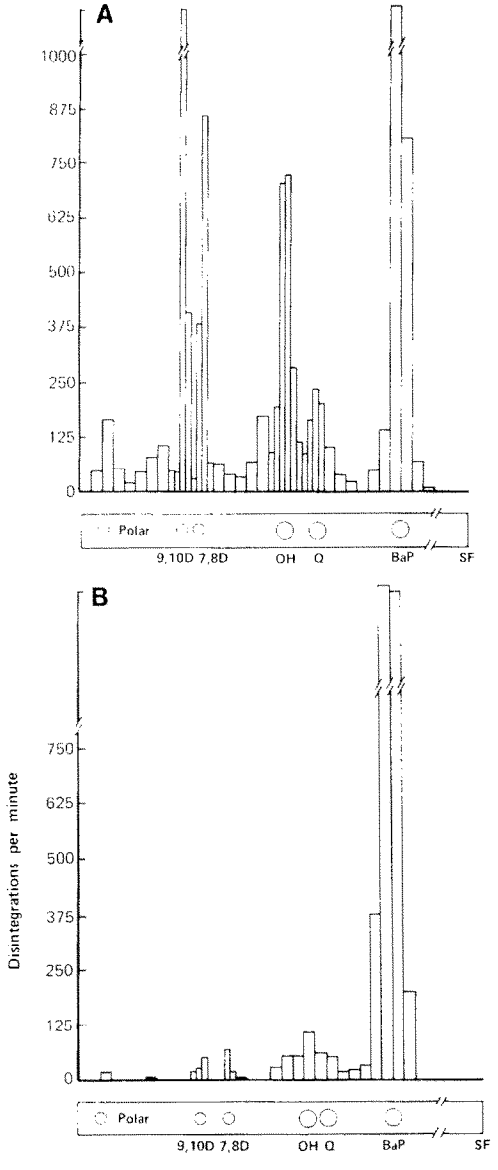

Fig. 1. Thin-layer chromatographic separation of metabolites formed by incubating 5 nmoles [^{3}H]BaP with liver supernatant fractions (S 10) of untreated rainbow trout acclimated at 7° (A) and 16° (B). The ethyl acetate extracts of the incubating mixture were spotted on high-efficiency t.l.c. plates and developed in toluene–ethanol (9:1, v/v) together with standards of BaP and its metabolites. Abbreviations: polar (metabolites migrating between origin and 9,10D); 9,10D, BaP 9,10-dihydrodiol; 7,8D, BaP 7,8-dihydriodol; OH, phenols; Q, quinones; BaP, benzo[*a*]pyrene; and SF, solvent front. (4,5-Dihydrodiol migrated between 7,8D and OH.)

(BaP 7,8-dihydrodiol), 9,10-dihydro 9,10-dihydroxybenzo[*a*]pyrene (BaP 9,10-dihydrodiol) and polar metabolites (R_f value lower than that of BaP 9,10-dihydrodiol).

Comparison of t.l.c. and h.p.l.c. data revealed that the total amounts of dihydrodiols, phenols, and quinones were similar in any one sample analyzed by both methods. 3-Hydroxy BaP was the major ethyl acetate-soluble metabolite formed in all samples; however, comparable proportions of BaP 9,10-dihydrodiol and 7,8-dihydrodiol and smaller amounts of quinones (Tables 1–3) were also formed. Less than 1% of 4,5-dihydro-4,5-dihydroxybenzo[*a*]pyrene (BaP 4,5-dihydrodiol), BaP 4,5-oxide, 1-hydroxy BaP, and 9-hydroxy BaP were detected in the incubation mixtures from untreated and PCB-treated fish at both 7 and 16°.

Substantially more BaP was metabolized by the liver extracts from untreated fish at 7° than at 16°. This was reflected primarily in the formation of a larger proportion of ethyl acetate-soluble metabolites in the former group (Tables 1 and 2). The ratios of percentage of radioactivity in the ethyl acetate phase vs in the aqueous phase obtained with liver extracts of both PCB-treated and untreated fish at 7° were significantly ($P < 0.05$) greater than the ratios for fish at 16°, regardless of the extent of conversion of BaP (Table 3).

Liver extracts from the PCB-induced fish at 16° metabolized substantially ($P < 0.05$) more BaP than those from untreated or uninduced fish held at the same temperature. Although larger amounts of 3-hydroxy BaP, BaP 7,8-dihydrodiol and BaP 9,10-dihydrodiol were produced by liver extracts from PCB-induced than uninduced fish at 16°, relative proportions of these metabolites were not markedly different in these groups (Table 3).

In this study, reaction conditions were optimized for the binding assay and not for the BaP hydroxylase (pmoles BaP metabolized per mg protein) assay. Therefore, in those incubations where BaP was extensively metabolized, the rate of BaP conversion may not be linear for 15 min. However, Pearson's correlation analysis ($P < 0.05$) of extent of BaP metabolism and binding values revealed that for the untreated fish at 7° and for the induced fish at 7 and 16° (Table 3) there was a positive linear relationship between the binding value and the radioactivity in the aqueous phase, and a negative linear relationship between binding value and ethyl acetate-soluble metabolites. With increased binding value, there was also significantly increased BaP conversion. No such relation was observed between binding and metabolism of BaP for untreated fish at 16° and uninduced fish at 7° and 16°. No consistent correlation was evident between binding values and proportion of individual metabolites in any group of fish.

DISCUSSION

The present results indicate a strong relationship between the habitat temperature and the *in vitro* hepatic metabolism of BaP in rainbow trout. Dewaide [20] reported that rainbow trout and roach kept at 5° exhibited higher hepatic MFO activities than did those at 18° and that the fish kept at the lower temperature possessed significantly more liver tissue per unit body weight. We also obtained a significantly higher ratio of liver weight to body weight in rainbow trout maintained at the lower temperature. Stegeman [21] noted that cold-acclimation of *F. heteroclitus* resulted in a small increase in hepatic BaP hydroxylase activity, when normalized to microsomal protein, but not when based on body weight. This was due to the finding that *F.*

Table 3. Comparisons of metabolism of BaP by livers of uninduced and PCB-induced trout*

Fish	No. of fish	Temp. (°)	Range of binding value	Unmetabolized BaP	Ethyl acetate-soluble metabolites	Aqueous phase	Phenols	7,8-Diol	9,10-Diol
				(% of total radioactivity)			(% of metabolites in ethyl acetate)		
Untreated	13	7	(0.08–0.51)	32 ± 22†‡	46 ± 16‡	21 ± 7‡	28 ± 8	16 ± 4	22 ± 4
PCB-treated,§ not induced	13	7	(0.07–0.63)	56 ± 22	36 ± 16	15 ± 5	28 ± 6	6 ± 3	20 ± 3
PCB-treated,‖ induced	3	7	(2.3–5.1)	19 ± 15	55 ± 5	26 ± 12	28 ± 2	17 ± 6	25 ± 3
Untreated	13	16	(0.03–0.11)	79 ± 10	12 ± 8	9 ± 3	25 ± 6	21 ± 8	18 ± 5
PCB-treated,¶ not induced	3	16	(0.04–0.18)	70 ± 26	12 ± 11	13 ± 6	17 ± 15	12 ± 10	16 ± 13
PCB-treated,‖ induced	13	16	(0.30–5.2)	9 ± 11¶	44 ± 18¶	47 ± 24¶	19 ± 13	11 ± 5	21 ± 10

* Statistical comparisons were made using Pearson's correlation analysis on binding values (pmoles BaP per mg DNA per mg protein) and relative proportions of BaP and its metabolites in each sample.

† Mean ± S.D.

‡ Significantly ($P < 0.05$) different from the corresponding value at 16°.

§ Liver extracts giving binding values less than three times the corresponding control value.

‖ Liver extracts giving binding values greater than three times the corresponding control value.

¶ Significantly ($P < 0.05$) different from the corresponding values for uninduced fish at 16°.

heteroclitus which were fed to satiation during acclimation responded to the cold-acclimation with lower liver weight per unit body weight. It was suggested [21] that a restricted diet may have caused higher liver weight to body weight ratios in the cold-acclimated fish in Dewaide's study; however, recent results of Hazel [19] demonstrate that, even when rainbow trout were fed to satiation, the cold-acclimated (5°) fish possessed more liver tissue per unit body weight than warm-acclimated (20°) fish. Thus, it appears that species specific differences markedly influence the response of fish to changes in habitat temperature.

The greater conversion of BaP by the liver extracts prepared from rainbow trout maintained at the lower temperature in our study may be due to a higher proportion of polyunsaturated fatty acids (PUFA) in microsomal membranes. Hazel [19] reported a higher proportion of PUFA in the phospholipids associated with membranes in liver of cold-acclimated trout compared to that in warm-acclimated fish. Wills [32] reported a linear relation between rate of BaP oxidation and percentage of PUFA in endoplasmic reticulum of rat. As with aryl hydrocarbon monooxygenase (AHM) activity, epoxide hydrase (EH) activity can also be influenced by both the amount and the type of lipid present in endoplasmic reticulum [33]. Preliminary results (E. Egaas and U. Varanasi, unpublished data) show a significantly larger hepatic EH activity in the untreated trout at the lower temperature. The present results show that the ratios of 3-hydroxy BaP to the dihydrodiols formed by liver enzymes of fish at both 7° and at 16° were similar. Thus, environmental temperature did not appear to alter the balance between the EH and AHM systems in trout. However, environmental temperature seemed to affect the balance between primary and secondary metabolism of BaP as evidenced by the finding that the ratio of ethyl acetate-soluble metabolites to aqueous-soluble metabolites was significantly greater for liver extracts of fish at 7° than at 16°. Because conjugation of primary metabolites of BaP should result in detoxification, any alteration in relative activities of AHM, EH and the conjugating enzymes should alter resultant toxicity of BaP. Thus studies to investigate effects of chemical inducers and environmental variables on conjugating enzymes are warranted. Poikilothermic organisms, such as fish, provide a unique opportunity to study factors controlling xenobiotic metabolism and carcinogenesis.

It is likely that, when rainbow trout were held at the lower temperature, the time required to accumulate minimum concentrations of Aroclor 1254 in liver for induction was longer, thereby yielding a delayed response to the PCB treatment at 7°. James and Bend [34] reported that the rate of absorption of 3-MC from muscle after an intramuscular injection was considerably greater in fish held at 28° than in those held at 22°. Varanasi *et al.* [35] have shown that the lowering of water temperature significantly reduces the rate of uptake of ingested aromatic hydrocarbons in liver of flatfish. The results showing delayed response of fish liver enzymes to the effect of a chemical inducer should be considered preliminary because such a response was observed at only one time period. However, these findings, together with our results on the effect of temperature on basal hydrocarbon metabolism, serve to make an important point; the thermal history of fish must be considered when xenobiotic metabolism is studied and when hepatic MFO activity is used as an indicator of environmental pollution. Further, delayed response of cold-acclimated fish to chemical inducers may explain why in some short-term laboratory experiments no induction is observed. Nevertheless, the possibility that certain fish species do not respond to inducers at low temperatures cannot be excluded.

Studies with rodents [36–40] show that PCB which contain many chlorobiphenyl isomers and congeners

exert the inductive effects of both PAH and phenobarbital. However, several investigators [14, 27, 41, 42] have reported that fish are not responsive to the barbiturates. Thus, the net effect of PCB on fish should be similar to that of PAH, resulting in increased production of the non-K-region metabolites of BaP (mediated by cytochromes P_1-450) by liver enzymes. Although a number of studies [10–15] have reported induction of hepatic BaP hydroxylase activity in fish treated with PCB, no information on its effect on formation of BaP metabolites has been available. Our results show that significant increases in amounts of non-K-region metabolites were obtained with liver extracts of PCB-induced rainbow trout at both temperatures and that liver enzymes of both untreated and PCB-induced fish produced only trace amounts of the K-region metabolites. Thus, similar to PAH inducers [6–8], Aroclor 1254 induced increased hepatic production of the non-K-region metabolites of BaP. In contrast to the effect of environmental temperature, PCB induction did not alter the proportion of ethyl acetate-soluble metabolites in relation to that present in the aqueous phase.

In conclusion, the results show that the exposure of rainbow trout to Aroclor 1254 substantially increased the ability of liver enzymes to convert BaP into carcinogenic and mutagenic intermediates that bind to DNA. Further, the results show that the environmental temperature influenced both the initial hydrocarbon metabolism in fish and the time needed for the effect of a chemical inducer to be evident. The present results *in vitro* serve primarily as an indication that a number of variables, such as chemical inducers and habitat temperature, markedly influence the ability of fish liver to metabolize a carcinogen. These findings should be taken into account when assessing xenobiotic metabolism and formation of toxic metabolites that interact with critical cellular constituents *in vivo*.

Acknowledgements—We thank Tom Hom for excellent technical assistance, Frank J. Ossiander for statistical treatment of data, Victor D. Henry for h.p.l.c. analyses, Øyvind Bøhren for assistance in the fish handling, and Marianne Tomita for typing of the manuscript. This work was funded, in part, by the Environmental Protection Agency. Eliann Egaas thanks the Fulbright Foundation and the Inger Haldorsen's Fund for financial support.

REFERENCES

1. J. J. Payne and W. R. Penrose, *Bull. environ. Contam. Toxic.* **14**, 112 (1975).
2. C. N. Statham, C. R. Elcombe, S. P. Szyjka and J. J. Lech, *Xenobiotica* **8**, 65 (1978).
3. U. Varanasi and D. C. Malins, in *Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms*, (Ed. D. C. Malins), Vol. II, p. 175. Academic Press, New York (1977).
4. J. R. Bend and M. O. James, in *Biochemical and Biophysical Perspectives in Marine Biology* (Eds. D. C. Malins and J. R. Sargent), Vol. IV, p. 125. Academic Press, New York (1978).
5. J. R. Bend, L. M. Ball, T. H. Elmamlouk, M. O. James and R. M. Philpot, in *Pesticide and Xenobiotic Metabolism in Aquatic Organisms* (Eds. M. A. Q. Khan, J. J. Lech and J. J. Menn), p. 297. American Chemical Society, Washington, DC (1979).
6. J. T. Ahokas, H. Saarni, D. W. Nebert and O. Pelkonen, *Chem. Biol. Interact.* **25**, 103 (1979).
7. U. Varanasi and D. J. Gmur, *Biochem. Pharmac.* **29**, 753 (1980).
8. U. Varanasi, D. J. Gmur and P. K. Krahn, in *Polynuclear Aromatic Hydrocarbon Chemistry and Biological Effects* (Eds. A. Bjørseth and A. J. Dennis), p. 455. Battelle Press, Columbus, OH (1980).
9. U. Varanasi and D. J. Gmur, *Aquat. Toxic.* **1**, 49 (1981).
10. E. H. Gruger, Jr., M. M. Wekell, P. T. Numoto and D. R. Craddock, *Bull. environ. Contam. Toxic.* **17**, 512 (1977).
11. E. H. Gerhart and R. M. Carlson, *Environ. Res.* **17**, 284 (1978).
12. U. Lidman, L. Forlin, O. Molander and G. Axelson, *Acta pharmac. tox.* **39**, 262 (1976).
13. M. M. Lipsky, J. E. Klauming and D. E. Hinton, *J. Toxic. environ. Hlth* **4**, 107 (1978).
14. C. R. Elcombe and J. J. Lech, *Toxic. appl. Pharmac.* **49**, 437 (1979).
15. L. Forlin, *Toxic. appl. Pharmac.* **54**, 420 (1980).
16. E. Egaas and U. Varanasi, *Fedn Proc.* **39**, 1013 (1980).
17. P. W. Hochachka and G. N. Somero, in *Fish Physiology* (Eds. W. S. Hoar and P. J. Randall), Vol. IV, p. 99. Academic Press, New York (1971).
18. J. R. Hazel and C. L. Prosser, *Physiol. Rev.* **54**, 620 (1974).
19. J. R. Hazel, *Am. J. Physiol.* **236**, R91 (1979).
20. J. H. Dewaide, *Comp. gen. Pharmac.* **1**, 375 (1970).
21. J. J. Stegeman, *J. Fish. Res. Bd Can.* **36**, 1400 (1979).
22. J. T. Ahokas, N. T. Karki, A. Oikari and A. Soivio, *Bull. environ. Contam. Toxic.* **16**, 270 (1976).
23. D. W. Statham, M. J. Melancon and J. J. Lech, *Science* **193**, 680 (1976).
24. J. D. Hendricks, T. P. Putnam, D. D. Bills and R. O. Sinhuber, *J. natn. Cancer Inst.* **59**, 1545 (1977).
25. J. H. Wales, R. O. Sinhuber, J. D. Hendricks, J. E. Nixon and T. A. Eisele, *J. natn. Cancer Res.* **60**, 1133 (1978).
26. R. B. Franklin, C. R. Elcombe, M. J. Vodichick and J. L. Lech, *Fedn Proc.* **39**, 3144 (1980).
27. D. R. Buhler and M. E. Rasmusson, *Comp. Biochem. Physiol.* **25**, 223 (1968).
28. M. G. Pedersen, W. K. Hershberger, P. K. Zachariah and M. R. Juchau, *J. Fish. Res. Bd Can.* **33**, 666 (1976).
29. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
30. S. G. Buty, S. Thompson and T. J. Slaga, *Biochem. biophys. Res. Commun.* **70**, 1102 (1976).
31. A. H. Reed, R. S. Henry and W. B. Mason, *Clin. Chem.* **17**, 275 (1971).
32. E. D. Wills, in *Microsomes, Drug Oxidations, and Chemical Carcinogenesis* (Eds. M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette and P. J. O'Brien), p. 545. Academic Press, New York (1980).
33. A. Y. Lu and W. Levin, *Biochim. biophys. Acta* **344**, 205 (1974).
34. M. O. James and J. R. Bend, *Toxic. appl. Pharmac.* **54**, 117 (1980).
35. U. Varanasi, D. J. Gmur and W. L. Reichert, *Archs environ. Contam. Toxic.* **10**, 203 (1981).
36. C. O. Abernathy, R. M. Philpot, F. E. Guthrie and E. Hodgson, *Biochem. Pharmac.* **20**, 2395 (1971).
37. J. E. Kielen and D. W. Nebert, *Science* **172**, 167 (1971).
38. J. A. Glodstein, P. Hickman, J. Bergman, J. D. McKinney and M. P. Walker, *Chem. Biol. Interact.* **17**, 69 (1977).
39. A. Poland and E. Glover, *Molec. Pharmac.* **13**, 924 (1977).
40. A. R. Boobis, D. W. Nebert and O. Pelkonen, *Biochem. Pharmac.* **28**, 111 (1979).
41. R. F. Addison, M. E. Zinck and D. E. Willis, *Comp. Biochem. Physiol.* **576**, 39 (1977).
42. J. R. Bend, R. J. Pohl and J. R. Fouts, *Bull. Mt Desert Isl. biol. Lab.* **13**, 9 (1973).