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Benzo[a]pyrene Toxicokinetics in Rainbow Trout (*Oncorhynchus mykiss*) Acclimated to Different Salinities

J. M. Seubert,[1] C. J. Kennedy[2]

[1] Department of Pharmacology and Toxicology, University of Western Ontario, London, Ontario, Canada N6A 5C1
[2] Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada, V5A 1S6

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Abstract. The effects of environmental salinity on the distribution, metabolism, and elimination of benzo[a]pyrene (B[a]P) were examined in mature rainbow trout. Trout acclimated to either fresh water (0 ppt, FW) or sea water (20 ppt, SW) for 3 weeks received a single 10 mg/kg intra-arterial injection of [^{3}H]-benzo[a]pyrene (B[a]P) at their acclimation salinity or when subjected to an acute salinity change. Statistically significant differences in the percent body burden of B[a]P-derived radioactivity in various tissues were seen between fish in FW versus SW. Significant differences in the distribution of B[a]P and its metabolites were also noted when fish were subjected to an acute salinity change after chemical injection. Modulation of B[a]P metabolism by environmental salinity included: (1) significant differences in the proportions of Phase I metabolites in the bile of FW- (2.3%) versus SW-acclimated (14.1%) fish, and (2) alterations in the accumulations of specific metabolites (predominantly t-9, 10-dihydrodiol-B[a]P in FW fish, and 3-hydroxy-B[a]P in SW fish). The percentages of the [^{3}H]-B[a]P dose eliminated by 48 h was similar in FW and SW fish, but decreased in fish subjected to an acute salinity change (FW 98.8% eliminated, FW:SW 90.4%, SW 98.1%, and SW:FW 93.1%). Pharmacokinetic modeling confirmed that acute salinity changes can result in longer terminal half-lives and slower total body clearances of B[a]P.

Organisms must often deal with natural stressors, which may affect their responses to impingements of anthropogenic origin. Changes in environmental salinity may be such a stressor as they require organisms, such as fish, to significantly alter their biochemistry and physiology (Evans 1993; Heath 1995). Such alterations occur during smoltification in salmonids as they prepare for a subsequent marine existence. It has been shown that smoltification increases the sensitivity of salmonids to various xenobiotics, possibly through changes in chemical uptake, distribution, and metabolism (Seubert and Kennedy 1997; Lemke and Kennedy 1997). It has also been shown that there are differences in the toxicity of organic compounds to fish exposed to these chemicals in different osmotic environments (Thomas and Rice 1981). Outmigrants of pink salmon, sockeye salmon, and dolly varden acclimated to sea water were approximately twice as sensitive to aromatic hydrocarbons and the water-soluble fraction of Prudhoe Bay crude oil as outmigrants tested in fresh water (Moles *et al.* 1979), which may be related to an altered disposition of these compounds and a greater accumulation in target tissues (Thomas and Rice 1981, 1986).

There is a growing body of evidence showing that environmental and physiological factors can effect the toxicokinetics (absorption, distribution, metabolism, and excretion) of xenobiotics in fish (Koivussaari 1981; Kennedy *et al.* 1989a; Kennedy 1995). Such alterations can have major effects on tissue levels of toxicants, metabolite patterns, and xenobiotic half-life in an organism, culminating in very different responses to a given toxicant exposure. The pervasive effects of environmental salinity on metabolic processes suggest that the metabolism of xenobiotics and patterns of metabolites produced may be modified. In addition, alterations in fish physiology associated with osmoregulatory adjustments may alter the uptake, disposition, and excretion of xenobiotics.

The present study investigated the effects of acclimation salinity and acute salinity change on the kinetics of the model xenobiotic benzo[a]pyrene, a polycyclic aromatic hydrocarbon, in mature rainbow trout.

Materials and Methods

Chemicals

Unlabeled benzo[a]pyrene (>99% purity) was purchased from Sigma Chemicals (St. Louis, MO) and [1,3,6-^{3}H]-benzo[a]pyrene (52 Ci/mmol) was purchased from NEN Research Products (DuPont, Canada Ltd.). HPLC analysis of labeled and unlabeled B[a]P according to the method of Elnenaey and Schoor (1981) revealed no detectable metabolites. B[a]P metabolite standards were obtained from the National Cancer Institute Chemical Repository, Midwest Research Institute (Kansas City, MO). All other chemicals (reagent grade) and biochemicals were purchased from Sigma Chemicals.

Correspondence to: C. J. Kennedy

Fish and Salinity Regimes

Rainbow trout, *Oncorhynchus mykiss*, weighing approximately 300 g, were obtained from West Creek Trout Farm in Aldergrove, BC. Fish were maintained at seasonal temperatures (4–8°C) and under a natural photo period in 500-L fiberglass tanks supplied with flowing dechlorinated water at pH 6.4, O_2 saturation >95% and hardness 5.2–6.0 mg/L $CaCO_3$ for several days until an experiment was started. Fish were fed *ad libitum* until 48 h prior to an experiment.

Fish were separated into two groups and acclimated for 3 weeks in water of 0 ppt (FW) or 20 ppt (SW) sea water (source: Burrard Inlet, BC). A dorsal aortic cannula and urinogenital catheter were implanted into each fish using modified procedures of Smith and Bell (1964) and Wood *et al.* (1994), respectively. Fish were then allowed a minimum of 24 h to recover from the surgery in dark Plexiglas chambers.

Fish from each of the two acclimation groups, FW and SW, were exposed to B[a]P at both test salinities. Thus, there were four experimental exposure groups (acclimation salinity:test salinity): FW:FW, FW:SW, SW:SW, SW:FW.

Intra-Arterial Administration

The B[a]P injection solution was prepared by dissolving B[a]P (10 mg (1 µCi)/kg of [^{3}H]-B[a]P) in a few drops of Mulgofen EL 719 (GAF Ltd., Wythenshawe, Manchester, UK) and mixed into a homogenous solution with 0.9% saline heparin (5 IU/ml) (Law *et al.* 1994). This solution (0.1 ml) was injected as a bolus through the cannula into the dorsal aorta and was followed by an injection of 0.2 ml of 0.9% saline with heparin through the cannula to ensure that the entire dose of B[a]P entered the circulatory system. In fish subjected to an acute salinity change, transfer was done immediately after dosing.

Distribution, Metabolism, and Elimination of B[a]P in Trout

Fish from each salinity group were sampled 48 h following the administration of B[a]P. The liver, gallbladder, spleen, kidney, visceral fat, intestine, stomach, gill muscle, urinary bladder, and skin were dissected, weighed, homogenized in 0.9% saline and then oxidized using an R. J. Harvey OX-100 biological oxidizer (R. J. Harvey Instruments Corp., Hillside, NJ). The oxidized samples in liquid scintillation cocktail (Amersham, Oakville, ON, Canada) were dark adapted for 24 h and counted on a Beckman LS 3801 liquid scintillation counter (Beckman Instruments, Irvine, CA). Bile was released from excised gallbladders and rinsed with 1.0 ml sodium citrate buffer (pH 7.0). An aliquot of this solution was removed and counted for total radioactivity by LSC, and the remainder analyzed for B[a]P metabolites as described below. Urine was collected for 48 h from each fish.

The method used by Kennedy *et al.* (1991) was followed for the quantitation of B[a]P and its metabolites. Briefly, bile and urine were extracted with ethyl acetate to separate organic-soluble Phase I metabolites. The remaining aqueous layer then underwent a series of incubations and extractions; it was first adjusted to pH 5.0 and incubated for 24 h at 37°C with 60 U of glucuronidase to hydrolyze glucuronic acid conjugates; it was then adjusted to pH 7.0 and incubated for 24 h at 37°C with 20 U of sulfatase to hydrolyze sulfate conjugates; and finally it was adjusted to pH 2.0 and incubated for 24 h at 80°C with 1 N H_2SO_4 to acid hydrolyze any remaining conjugated metabolites. After each step, the aqueous layer was extracted with ethyl acetate. Aliquots of each ethyl acetate extraction were removed, dried with nitrogen, resuspended in 1 ml of methanol and added to liquid scintillation cocktail (Amersham) and counted for radioactivity by LSC.

B[a]P and Phase I metabolites in the first ethyl acetate extracts of bile and urine were separated by HPLC using a Hewlett Packard 1050 series liquid chromatograph equipped with a Phenomenex Primesphere 5 mm C18 reverse phase column (250 × 4.6 mm), an HP 1046A fluorescence detector and an HP 3396 Series II Integrator (excitation 340–380 nm and emission >430 nm). The methods of Varanasi *et al.* (1986) were followed using a nonlinear solvent gradient of Solvent A (0.005% glacial acetic acid in water, v/v) and Solvent B (methanol). The flow rate was 1.0 ml/min. Fractions were collected every 30 s, and each fraction was counted for radioactivity by LSC. B[a]P and its metabolites were tentatively identified by comparison of retention times with known standard metabolites (Seubert and Kennedy 1997).

The total elimination of [^{3}H]-B[a]P was calculated by subtracting the amount radioactivity remaining in the organism (sum of tissues) after 48 h from the amount injected. Urine was collected into graduated cylinders and sampled 6, 12, 24, and 48 h following the administration of B[a]P. Urine volumes were noted and the urine frozen at −80°C until analyzed for metabolites and parent compound by the above methods used for bile analysis.

Mathematical Analysis

All percent data were arcsine transformed before statistics were performed. One-factor ANOVA and the Student-Newman-Keuls multiple comparison test was used to establish differences between tissue body burdens, metabolite classes, and B[a]P elimination between the different treatment groups at a significance level of $p < 0.05$ (Zar 1984).

Exposure, Blood Sampling, and Toxicokinetic Analysis

A control blood sample (0.2 ml) was withdrawn from the dorsal aortic cannula prior to chemical administration. All blood samples were subsequently replaced with an equal volume of heparinized saline. The B[a]P injection solution was prepared and administered as previously described except that no radiolabeled B[a]P was used. Blood samples (0.2 ml) were withdrawn from the cannula at various time points following chemical administration.

A modified method of Kennedy and Law (1990) was used to extract the blood samples for B[a]P analysis. Distilled water (0.5 ml) and 0.1 N sulfuric acid (0.5 ml) were added to deproteinize the blood to enhance B[a]P extraction. The mixture was vortexed and extracted three times with ethyl acetate. The organic extracts were combined and evaporated to dryness under nitrogen and the residue redissolved in 1 ml of methanol containing benzanthracene (1 µg/ml) as an internal standard. The sample was analyzed by HPLC using the column previously described and eluting B[a]P from the column isocratically with methanol:water (80%:20%) according to Varanasi *et al.* (1986).

The time course of B[a]P in the blood of trout after intra-arterial administration was analyzed by a nonlinear least squares regression program (Metzler and Weiner 1986). The statistical weighting factor in the least-square procedure was the inverse of the observed blood concentrations (Ottaway and Sato 1973). The overall goodness of fit was determined by comparing the sum of the squared deviations and by scatter of the actual points around the fitted function. Akaike's information criterion was used to select the most appropriate model (Yamaoka *et al.* 1978). Calculation of the model dependent parameters were derived from estimates of parameters from the nonlinear regression analysis (Gibaldi and Perrier 1975).

Results

Distribution of B[a]P in Trout

Radioactivity was found in all tissues examined after a bolus injection of [^{3}H]-B[a]P via the dorsal aorta. The percent body burden in each tissue (radioactivity in a tissue as a percent of the whole body radioactivity) is shown in Table 1. The liver had the highest burden of radioactivity in all treatment groups, followed by the gallbladder and kidney. The urinary tract and skin showed the lowest body burden of radioactivity in all treatment groups. Statistically significant differences in the percent body burden for specific tissues between salinity treatment groups were found. Fish exposed to B[a]P in the SW-acclimated treatment had higher tissue burdens of radioactivity in the intestine, stomach, and visceral fat than fish exposed in the FW-acclimated treatment group. Fish that were exposed to an acute salinity change showed an increase in B[a]P-derived radioactivity in the spleen, kidney, and muscle and a decrease in radioactivity in the gallbladder compared to their respective acclimation treatment groups.

Table 1. The percent body burden distribution of [^{3}H]-B(a)P-derived radioactivity in mature rainbow trout

Organs and Tissues	Salinity Regimes			
	FW:FW	FW:SW	SW:SW	SW:FW
	Radioactivity (% body burden)			
Liver	46.0 ± 7.7^{a}	48.7 ± 8.8^{a}	52.1 ± 0.9^{a}	42.4 ± 3.3^{a}
Gallbladder	15.0 ± 4.6^{b}	$5.8 \pm 2.9^{c,d}$	11.4 ± 0.9^{c}	$7.6 \pm 2.6^{b,c}$
Spleen	$1.4 \pm 0.2^{?d}$	$1.8 \pm 0.2^{?c,d}$	$1.2 \pm 0.08^{?}$	$6.4 \pm 3.7*^{d}$
Kidney	9.2 ± 2.7^{c}	$21.5 \pm 7.5*^{,b}$	9.2 ± 0.8^{c}	$14.8 \pm 3.7*^{,b}$
Visceral fat	5.9 ± 2.6^{c}	$2.5 \pm 1.1^{c,d}$	8.7 ± 0.9^{c}	$8.8 \pm 1.9^{c,d}$
Intestine	2.1 ± 1.4^{d}	$3.1 \pm 1.4^{c,d}$	$10.1 \pm 1.4*^{c}$	$6.9 \pm 2.8*^{c,d}$
Stomach	0.8 ± 0.07^{d}	1.1 ± 0.5^{d}	$3.7 \pm 0.27*^{d}$	0.7 ± 0.2
Gill	$3.7 \pm 0.2^{c,d}$	$3.7 \pm 1.5^{c,d}$	2.6 ± 0.4^{d}	$2.1 \pm 0.5*$
Muscle	$2.4 \pm 0.7^{c,d}$	9.6 ± 5.0^{c}	1.5 ± 1.1	$2.3 \pm 0.9^{d,8}$
Urinary tract	0.2 ± 0.1^{d}	0.2 ± 0.03^{d}	0.6 ± 0.4	0.5 ± 0.2
Skin	0.5 ± 0.1^{d}	$2.0 \pm 1.1^{c,d}$	0.7 ± 0.4	0.7 ± 0.1

Trout were sacrificed 48 h after receiving a single intra-arterial injection of 10 mg (1 mCi)/kg ^{3}[H]B(a)P. Values are mean $\pm$ SEM for three trout. Tissue values with common letters are not significantly different from each other within a given salinity treatment at $p < 0.05$. Values with common symbols are not significantly different for the same tissue between salinity treatments at $p < 0.05$

Metabolism of B[a]P in Trout

The separation of B[a]P and its metabolites by organic solvent extraction, hydrolytic enzyme incubations, and subsequent HPLC analysis, revealed that the vast majority of the radioactivity excreted into the bile or urine of trout, regardless of salinity treatment, was as metabolites of B[a]P in either a conjugated or unconjugated form. The proportion of unmetabolized B[a]P in either bile or urine by 48 h was always less than 2% of the recovered radioactivity.

The percentages of total B[a]P-derived radioactivity recovered in the bile as organic soluble, glucuronide, sulfate, or unknown conjugates, and other aqueous-soluble metabolites are listed in Table 2. There was a significant difference in the proportion of organic soluble metabolites between the fish that were acclimated to either FW or SW. The levels in FW fish were significantly lower (2.3%) than in SW fish (14.1%). However, the proportion of organic soluble metabolites did not change when fish were subjected to acute salinity changes (FW:SW 2.54%; SW:FW 11.0%). There were no significant differences between different salinity treatments in the proportions of either glucuronic acid or sulfate conjugates. The proportion of unidentified conjugated metabolites in FW fish (28.6%) was significantly higher than in SW fish (11.6%). This proportion increased in SW fish subjected to an acute salinity decrease (SW:SW 11.6% versus SW:FW 13.9%), and reversed during a salinity increase (FW:FW 28.6% versus FW:SW 17.2%). This trend was the opposite when describing the proportions of aqueous soluble metabolites.

HPLC analysis of bile showed that trout can metabolize B[a]P to a variety of Phase I metabolites including tetrols, diols, quinones and phenols (Table 3). There were no statistically significant differences in the proportions of these specific metabolites between any treatment group. The identified Phase I metabolites which accumulated to the greatest degree were 7,8,9,10-tetrahydro-B[a]P, 3-hydroxy-B[a]P, and t-9,10-dihydrodiol. If these identified metabolites were grouped according to major type and expressed as a percent of radioactivity in the organic soluble fraction of the bile, significant effects of salinity treatment were noted. FW fish bile contained lower proportions of phenols and higher proportions of quinones than SW fish. An acute salinity increase resulted in an increase in phenols and decrease in quinone production, whereas acute salinity decreases showed the reverse trend.

The percentage of total B[a]P excreted into the urine at 48 h was 4.82 $\pm$ 2.28% (FW:FW), 6.05 $\pm$ 3.8% (FW:SW), 8.68 $\pm$ 2.57% (SW:SW), and 12.2 $\pm$ 3.2% (SW:FW). Urine contained less than 2% unmetabolized B[a]P at any time point. The percentages of total B[a]P-derived radioactivity recovered in the urine at any time point as organic soluble, glucuronide, sulfate, unknown conjugates, and other aqueous soluble metabolites are listed in Tables 4 and 5. The results obtained from the urinary excretion data are very different from that obtained from the biliary data. Significant differences between the two routes of excretion include: (1) proportions of organic soluble, unknown and aqueous metabolites in FW versus SW fish, (2) acute salinity changes affected the proportions of organic-soluble metabolites excreted, and (3) more complex effects of salinity acclimation and acute changes on individual metabolite patterns (Tables 4 and 5).

Elimination of B[a]P in Trout

Fish injected in the FW:FW treatment eliminated similar amounts of [^{3}H]-B[a]P (and its metabolites) as fish in the SW:SW treatment group (FW:FW 98.8% versus SW:SW 98.1%) by 48 h. B[a]P was eliminated marginally slower in fish which underwent an acute salinity change (FW:SW 90.4% and SW:FW 93.1%) when compared to fish exposed at their acclimation salinity.

The hepatobiliary system (liver, gallbladder, and bile) contained the majority of the radioactivity recovered 48 h follow-

Table 2. The percent of total [^{3}H]-B(a)P-derived radioactivity as organic soluble metabolites, glucuronide, sulfate, and other aqueous soluble metabolites in the bile of rainbow trout, 48 h following intra-arterial injections of 10 mg (1 mCi)/kg of B[a]P

Salinity Treatment	Organic Soluble	Glucuronide	Sulfate	Unknown Conjugates	Unknown Aqueous Soluble
FW:FW	2.33 ± 0.37†[a]	23.89 ± 4.78	32.23 ± 1.67	28.59 ± 2.55[c]	12.97 ± 0.88*[f]
FW:SW	2.54 ± 0.56[a]	24.43 ± 1.55	37.13 ± 0.82	17.23 ± 0.54†[d]	18.69 ± 0.37†[f,g]
SW:SW	14.1 ± 5.9*[b]	18.91 ± 0.5*	29.17 ± 5.6*	11.58 ± 3.9*	26.25 ± 3.6*[e,g]
SW:FW	11.04 ± 3.0*[b]	27.37 ± 4.55†	28.49 ± 5.14†	13.85 ± 2.82*[d]	19.25 ± 2.79*[f,g]

The values are mean ± SEM for three fish. Metabolite groups with common symbols are not significantly different from each other in a given salinity treatment at $p < 0.05$. Values with common letters are not significantly different for the same metabolite group between salinity treatments at $p < 0.05$

Table 3. The percent of individual metabolites of the identified organic soluble metabolites in the bile of rainbow trout exposed for 48 h via intra-arterial injection of 10 mg (1 mCi)/kg [^{3}H]B(a)P (values are means ± SEM for three trout)

	FW:FW	FW:SW	SW:SW	SW:FW
Biliary Metabolites	Percent Organic Soluble			
7,8,9,10 tetrahydroB[a]P	10.2 ± 6.2	16.6 ± 0.1	13.7 ± 4.0	31.5 ± 9.6
t 9,10 dihydrodiol	25.2 ± 17.7	12.4 ± 2.3	12.4 ± 6.3	10.6 ± 1.0
t 7,8 dihydrodiol	6.3 ± 2.8	10.7 ± 1.3	6.6 ± 2.8	3.1 ± 1.3
1,3 dione	10.9 ± 8.6	11.2 ± 1.2	7.4 ± 1.0	7.8 ± 2.6
3,6 dione	6.7 ± 2.1	8.6	3.3 ± 0.5	10.7 ± 1.9
6,12 dione	10.7 ± 5.8	5.1	4.7 ± 1.2	12.1 ± 3.6
9-hydroxy	6.7 ± 1.9	10.9 ± 3.0	8.4 ± 6.3	4.6 ± 0.6
1-hydroxy	8.3 ± 2.3	19.9 ± 3.0	4.5 ± 1.6	7.4 ± 1.4
3-hydroxy	12.7 ± 6.7	17.0 ± 7.7	32.1 ± 19.4	7.7 ± 1.9
B[a]P	2.4 ± 0.9	2.8 ± 0.2	6.9 ± 2.3	4.8 ± 1.2

Table 4. The percent of total [^{3}H]B(a)P-derived radioactivity as organic soluble metabolites, glucuronide, sulfate, and other aqueous soluble metabolites in the urine of rainbow trout, 48 h following an intra-arterial injection of 10 mg (1 mCi)/kg of B[a]P

Salinity Treatment	Organic Soluble	Glucuronide	Sulfate	Unknown Conjugates	Unknown Aqueous Soluble
FW:FW	32.2 ± 6.9	24.3 ± 5.3	23.3 ± 7.9	12.4 ± 4.6*	7.8 ± 2.6*
FW:SW	22.2 ± 1.7	21.2 ± 1.3	9.8 ± 7.1	34.6 ± 10.8	12.2 ± 6.8
SW:SW	15.1 ± 6.9	21.2 ± 3.3	18.5 ± 0.8	27.5 ± 8.6	17.8 ± 0.8
SW:FW	21.1 ± 4.5	25.5 ± 4.1	21.8 ± 5.9	15.7 ± 8.1	15.9 ± 3.5

Values are mean ± SEM values of urine sampled at 6, 12, 24, and 48 h for three fish. Metabolite groups with common symbols are not significantly different from each other in a given salinity treatment at $p < 0.05$

Table 5. The percent of individual metabolites of the total identified organic soluble metabolites in the urine of rainbow trout, 48 h following an intra-arterial injection of 10 mg (1 mCi)/kg [^{3}H] B(a)P (values are means ± SEM for metabolites in urine sampled at 6, 12, 24, and 48 h for three trout)

	FW:FW	FW:SW	SW:SW	SW:FW
Urinary Metabolites	Percent of Organic Soluble			
7,8,9,10 tetrahydroxyB[a]P	16.8 ± 4.0	24.1 ± 1.6*	9.6 ± 1.9	23.1 ± 4.2*
t 9,10 dihydrodiol	7.4 ± 2.6	9.3 ± 2.3	15.7 ± 4.6	17.0 ± 4.9
t 7,8 dihydrodiol	6.8 ± 1.1	11.4 ± 1.5	12.4 ± 4.5	13.7 ± 4.7
1,3 dione	18.7 ± 7.9	12.2 ± 3.7	5.0 ± 1.1	5.2 ± 1.6
3,6 dione	6.5 ± 1.8	9.1 ± 2.4	3.7 ± 0.7	5.3 ± 0.8
6,12 dione	13.3 ± 5.9	12.2 ± 0.7	4.6 ± 1.2	4.9 ± 1.2
9-hydroxy	14.9 ± 3.9	6.9 ± 4.3	7.4 ± 1.9	5.9 ± 1.1
1-hydroxy	6.7 ± 0.6	9.6 ± 2.6	5.3 ± 1.1	5.6 ± 1.3
3-hydroxy	13.9 ± 3.9	6.2 ± 1.6	25.0 ± 10.4	14.7 ± 4.8
B[a]P	4.2 ± 0.6	4.5 ± 1.3	3.5 ± 0.9	4.5 ± 0.7

Individual metabolites with common symbols are not significantly different between salinity treatments at $p < 0.05$

ing an intra-arterial administration of B[a]P (Table 1). Greater than 50% of the remaining radioactivity in the tissues after 48 h was recovered in the liver and gallbladder in all salinity treatments.

Fish acclimated to FW produced more urine (32.8 ml) than fish acclimated to SW (6.4 ml) over the experimental period. Fish subjected to an acute salinity increase reduced the amount of urine produced (7.9 ml), and SW fish transferred to FW increased urine production (25.0 ml). No significant differences in the percent of radioactivity recovered in urine between any treatment group was found.

Toxicokinetics of B[a]P in the Blood of Trout

The time course of B[a]P in the blood of trout following an intraarterial administration is shown in Figure 1. The concentration of B[a]P in the blood declined biexponentially with time. The mean data from three trout were best fit to a two-compartment open toxicokinetic model using nonlinear least-squares regression analysis.

The parameter estimates of the exponential equation fitted to these data are shown in Table 6. The terminal half-lives of decay from the blood ($b2_{HL}$) for FW fish were similar to that of SW fish. Fish that underwent an acute salinity increase or decrease had longer terminal half-lives compared to fish exposed at their respective acclimation salinity. The total body clearance (Qb) of B[a]P from trout acclimated and exposed in the FW had the same value as fish acclimated and exposed in SW. Acute salinity changes resulted in slower Qb compared to fish exposed at their acclimation salinity.

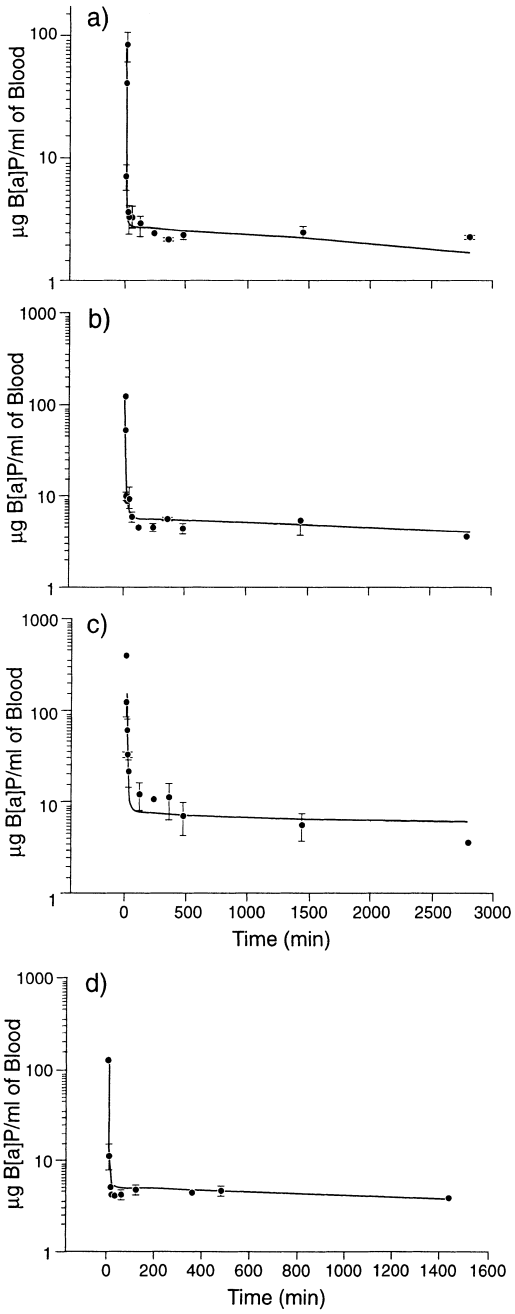

Fig. 1. Time course of unchanged B[a]P in the blood of trout following a single intra-arterial administration of 10 mg/kg under different salinity regimes (a) FW:FW, (b) FW:SW, (c) SW:SW, and (d) SW:FW. The curves represent the two-compartment model predictions of the data. Values are the means ± SEM of five fish

Discussion

The patterns of distribution of B[a]P-derived radioactivity in the tissues of trout in this study were consistent with those in other teleost species, including salmonids (Kennedy *et al.* 1991; Steward *et al.* 1991; Seubert and Kennedy 1997). The distribution of B[a]P or its metabolites was similar between fish acclimated to either FW or SW, however, an acute salinity change resulted in alterations in chemical distribution in several tissues. This may in part be explained by blood flow and circulatory pattern alterations, which occur in fish faced with osmoregulatory challenges (Maxime *et al.* 1991; Larsen and Jensen 1993; Madsen *et al.* 1996).

Following an intra-arterial administration, rainbow trout metabolized B[a]P to both Phase I and II metabolites. Major differences between FW and SW fish were seen in the amounts of organic-soluble metabolites and Phase II conjugates excreted. The proportion of radioactivity recovered as organic-soluble metabolites (Phase I) in fish acclimated to FW was similar to other teleost species (Varanasi *et al.* 1986; Kennedy *et al.* 1989b; Steward *et al.* 1991), whereas fish acclimated to SW excreted a significantly higher proportion of Phase I metabolites in the bile. The relatively high levels of aqueous soluble fractions recovered in fish under all salinity regimes is consistent with other marine and freshwater species (Varanasi *et al.* 1989a; Kennedy *et al.* 1989b; Kennedy and Law 1990). The higher amount of sulfate over glucuronide conjugation in the bile differed from values reported for other teleosts, such as gulf toadfish (Kennedy *et al.* 1989b), English sole (Gmur and Varanasi 1982), and starry flounder (Varanasi *et al.* 1989b) in which glucuronidation was the more predominate pathway. The aqueous soluble radioactivity in the bile that was not conjugated to sulfate or glucuronic acid may be a glutathione conjugate (Varanasi *et al.* 1986; Kennedy *et al.* 1989b).

The major identified Phase I metabolites of B[a]P in rainbow trout acclimated to FW was t-9, 10-dihydrodiol-B[a]P, which is

Table 6. Model parameters describing the blood concentration-time data for B[a]P in trout following a single intra-arterial administration of 10 mg/kg. These parameters were derived from a two-compartment model

Parameters	FW:FW	FW:SW	SW:SW	SW:FW
A1 (μg/ml)	253.9	218.3	226.8	110.7
A2 (μg/ml)	5	7.7	5	7.7
b1 (min^{-1})	0.292	0.305	0.305	0.1
b2 (min^{-1})	0.00017	0.0012	0.000237	0.0001
b1 HL (min)	2.37	2.3	2.27	9
b2 HL (min)	4,105.9	5,708.6	3,015.19	6,804.3
K12 (min^{-1})	0.278	0.263	0.288	0.100
K21 (min^{-1})	0.006	0.012	0.006	0.010
K10 (min^{-1})	0.010	0.032	0.011	0.002
AUC (μg ∗ min/ml)	30,378.2	63,945	21,291.342	77,174.1
Vc (ml)	22.8	24.3	17.51	29.5
Qb (ml/min)	0.2	0.09	0.2	0.05
Dose (μg)	5,900	5,487	4,060	3,487

consistent with other freshwater species of fish (Gmur and Varanasi 1982; Kennedy *et al.* 1989b; Varanasi *et al.* 1989a, 1989b). The major identified Phase I metabolite of B[a]P in SW-acclimated trout was 3-hydroxy-B[a]P, which differs from other saltwater species, such as the English sole and gulf toadfish, in which t-7,8-dihydrodiol was the major metabolite identified (Gmur and Varanasi 1982; Kennedy *et al.* 1989b; Varanasi *et al.* 1989a, 1989b). In fish subjected to an acute salinity decrease, 7,8,9,10-tetrahydro-B[a]P was the predominant Phase I metabolite found in bile.

The metabolite accumulation patterns found in the urine and bile of trout in the various salinity regimes indicate that environmental salinity can alter xenobiotic biotransformation, possibly through the physiological and biochemical adjustments required for maintaining osmoregulatory function. For example, to maintain optimal membrane function, a shift from saturated fatty acids to polyunsaturated fatty acids occurs in aquatic organisms transferred from a freshwater to saltwater environment (Sheridan 1989), as well as warm and cold water environments (Hazel 1995). Lipid and fatty acid compositional changes have also been associated with smoltification in salmonids (Tocher *et al.* 1995). These membrane-associated changes may alter the activities of enzymes in the mixed-function oxidase system. In this regard, it has been reported that a higher rate of B[a]P oxidation is associated with a higher proportion of polyunsaturated fatty acids in microsomal membranes (Wills 1980).

The present study suggests that an alteration in salinity will produce a different metabolite profile indicating the involvement of different biotransformation enzyme(s). The regulation of osmoregulatory processes in teleosts are under hormonal control (Bern and Madsen 1992; Heath 1995), for example both cortisol and growth hormone are involved in hypo-osmoregulation. The MFO system has a very broad substrate base due to the presence of multiple isozymes (Andersson and Forlin 1992), and like osmoregulation, can be modified by the endocrine system (Heath 1995). For example, cortisol has been found to elevate the activity of the MFO system (Devaux *et al.* 1992) and growth hormone has been shown to be involved in the regulation of hepatic cytochrome P450–dependent monooxygenases and UDP glucuronyl transferases in rainbow trout (Cravedi *et al.* 1995). It is possible that the endocrine control of osmoregulation affects the biotransformation of xenobiotics such as B[a]P, although the mechanisms at this point are unclear, and the literature does not yet provide conclusive connections supporting this hypothesis.

The large amount of B[a]P-derived radioactivity recovered in the bile indicates that the hepatobiliary system remains the most important route for excretion regardless of environmental salinity. This finding is consistent with other studies in which B[a]P was primarily excreted via the biliary route in both marine and freshwater aquatic species (Kennedy *et al.* 1991; Steward *et al.* 1991; Seubert and Kennedy 1997). B[a]P and its metabolites were excreted into the urine, but to a much lesser extent than through the bile. It is suspected that for compounds that are mainly excreted via routes such as the kidney, osmoregulatory adjustments would have more of an impact on their excretion.

Although the vast majority of the injected dose of B[a]P was excreted from fish by 48 h postinjection, a statistically significant reduction in biliary excretion occurred in fish subjected to an acute salinity change. Among other explanations, this reduction in elimination may be a reflection of an altered blood circulation resulting in a slower distribution of the chemical to detoxification and excretory organs (Maxime *et al.* 1991; Madsen *et al.* 1996). Moreover, although little information is available on transport protein structure-function relationships and the physiological and pharmacological regulation of transport mechanisms (Pritchard and Miller 1993), environmental salinity may also affect excretion by such mechanisms.

Toxicokinetic modeling was used to determine the kinetics of B[a]P in fish acclimated to fresh water, salt water, or following an acute salinity change. The toxicokinetics of intra-arterially administered B[a]P can be adequately described by a two-compartment open toxicokinetic model that consists of a central and peripheral compartment. The central compartment represents the vascular system of the fish since the calculated volume of the central compartment (Vc) was similar to the estimated blood volume in these fish (Gibaldi and Perrier 1975). The blood volume of rainbow trout in this study was estimated to be approximately 5.58% of body weight or 24.1 ml (Milligan and Wood 1982).

The large rate constants describing the distribution of B[a]P from the central into the peripheral compartment (k12) and

small rate constants describing the redistribution back from the peripheral to central compartment (k21) indicate, an overall fast disposition of B[a]P to the tissues and a slow return to the blood. Other studies done with freshwater rainbow trout and PAHs, such as 2-methylnaphthalene, fluorene, and pyrene, also showed the fast disposition into the tissues and slow movement back into the blood for elimination (Kennedy and Law 1990). The long terminal half-lives and small body clearances for B[a]P in all the salinity regimes are consistent with the lipophilic characteristics of PAHs. Fish subjected to an acute salinity change had longer terminal half-lives and lower body clearances than the respective acclimation groups. Again, previously mentioned alterations in physiology and biochemistry or stress may be responsible.

This study has shown that environmental salinity can be an important modulator of xenobiotic toxicokinetics in fish. Significant differences were seen in the toxicokinetics of B[a]P between fish in FW and SW and when fish were subjected to an acute salinity change. These results suggest that the susceptibility of anadromous fishes to xenobiotics may be affected by different or changing osmotic environments. This research illustrates the complex modulatory effects of environmental parameters on chemical toxicokinetics in aquatic organisms, factors that must be taken into account if accurate predictions or assessments regarding chemical fate and effects in aquatic organisms are to be made.

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