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A comparison of the dose and time response of CYP1A1 mRNA induction in chemically treated Atlantic tomcod from two populations

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Aims and Scope
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A comparison of the dose and time response of CYP1A1 mRNA induction in chemically treated Atlantic tomcod from two populations

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Abstract

Quantification of cytochrome P4501A1 (CYP1A1) mRNA levels in environmentally exposed Atlantic tomcod (*Microgadus tomcod*) has revealed significantly induced gene expression in fish from contaminated locales including the Hudson River, New York, and the Miramichi River, New Brunswick. In order to calibrate this response, determine its sensitivity and dose-responsiveness, levels of hepatic CYP1A1 mRNA were quantified in depurated Atlantic tomcod intraperitoneally (i.p.) injected with various concentrations of: β-naphthoflavone (β-NF), the PAH benzo[*a*]pyrene (B[*a*]P), the non-*ortho* coplanar PCB congener-3,3′,4,4′- tetrachlorobiphenyl (IUPAC: PCB-77), and the dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). Additionally, the rates of CYP1A1 mRNA induction and disappearance were quantified in depurated Atlantic tomcod i.p. injected with single doses of these chemicals and sacrificed at times ranging up to 72 days. Levels of CYP1A1 mRNA were dose-responsive for all four chemicals with maximum induction ranging from 50- to 460-fold and first significant induction being observed in the low mg per kg fish (wet weight) range for β-NF and B[*a*]P, μg/kg range for PCB-77 and ng/kg range for 2,3,7,8-TCDD. However, while tomcod from the Miramichi River responded to both PAHs and halogenated aromatic hydrocarbons (HAHs), Hudson River tomcod responded only to PAHs indicating population level differences in CYP1A1 mRNA inducibility in tomcod. Furthermore, differences in the responsiveness to PAHs and HAHs suggest that more than one molecular mechanism mediates CYP1A1 transcription in Atlantic tomcod. Kinetic profiles of CYP1A1 mRNA induction differed greatly between tomcod treated with HAHs and PAHs. Initial induction occurred within hours of treatment with PAHs and peaked after 1–3 days, compared to initial induction 4–7 days after treatment with HAHs,

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and maximum induction not occuring for up to 72 days after exposure. Quantification of halogenated aromatic hydrocarbons (HAH) in the livers of tomcod caught in the Hudson and Miramichi Rivers confirmed exposure and accuumlation of known CYP1A1 inducing chemicals including 2,3,7,8–TCDD at concentrations as high as 1.5 μg/kg lipid (554 ng/kg w.w.) and PCB-77 at concentrations as high as 108 μg/kg lipid (15 μg/kg w.w.). These results suggest that hepatic CYP1A1 mRNA concentration can be a useful bioindicator of exposure to some aromatic hydrocarbon compounds in the aquatic environment and that profiles of gene induction and disappearance may help identify environmental inducers provided that gene responsiveness is also evaluated under controlled laboratory conditions.

Keywords: Atlantic tomcod; CYP1A1 mRNA; β-NF; B[*a*]P; Dose–response; PCB-77; 2,3,7,8-TCDD; Time–response

1. Introduction

Cytochrome P450 is a superfamily of enzymes which function in the metabolism of many endogenous and exogenous substrates including toxicants of environmental concern (Stegeman and Hahn, 1994). The subfamily cytochrome P4501A (CYP1A) contains three known genes (Nelson et al., 1996) which are responsive to some of the more common and pernicious of the anthropogenic organic contaminants including polycyclic aromatic hydrocarbons (PAHs), co-planar polychlorinated biphenyl (PCB) congeners and polychlorinated dioxins and furans. At least two CYP1A genes have been identified in fish: CYP1A1 in rainbow trout (*Oncorhynchus mykiss*) (Heilmann et al., 1988; Berndston and Chen, 1994), plaice (*Pleuronectes platessa*) (Leaver et al., 1993), red sea bream (*Pragus major*) (Mizukami et al., 1994), scup (*Stenotomus chrysops*), toadfish (*Opsanus tau*) (Morrison et al., 1995), killifish (*Fundulus heteroclitus*) (Morrison et al., 1998), European sea bass (*Dicentrarchus labrax*) (Stien et al., 1998), and Atlantic tomcod (*Microgadus tomcod*) (Roy et al., 1995), and CYP1A3 in rainbow trout (Berndston and Chen, 1994).

CYP1A encodes monooxygenases which metabolize environmental procarcinogens to reactive metabolites which adduct to cellular DNA and proteins or alternatively are conjugated with carrier molecules such as glutathione and glucuronic acid which expedite their elimination from the body. It has been suggested that induction of CYP1A in fishes may serve as a sensitive marker of xenobiotic exposure and early biological response (Addison, 1984; Payne et al., 1987; Goksoyr and Forlin, 1992). Correlations have been found between CYP1A enzyme activity and body burdens of contaminants such as coplanar PCBs (Monosson and Stegeman, 1994). A mounting body of evidence points to a concurrence of elevated CYP1A in fish and a variety of toxicological effects including: mortality, growth inhibition, liver damage and lymphocyte depletion in rainbow trout (van der Weiden et al., 1992a), oxidative stress in lake trout (*Salvelinus namaycush*) (Palace et al., 1996), reduced innate immune responses in channel catfish (*Ictalurus punctatus*) (Rice and Schlenk, 1995), reproductive impairment in starry flounder (*Platichthys stellatus*) (Spies et al., 1984, 1988a,b) and white sucker (*Catostomus commersoni*) (McMaster et al., 1991; Munkittrick et al., 1991), increased overall DNA damage, K-*ras* oncogene activation in winter flounder and tomcod (Wirgin et al., 1989; McMahon et al., 1990), and neoplasia in English sole (*Pleuronectes vetulus*) (Stein et al., 1993) and Atlantic tomcod (Wirgin et al., 1994).

Many studies have demonstrated high CYP1A expression in environmentally exposed fish from contaminated waterways (Payne and Penrose, 1975; Stegeman et al., 1987; Van Veld et al., 1990). In almost all of these studies, levels of CYP1A gene expression were quantified at the protein level, either by immunodetection of CYP1A protein using monoclonal (Stegeman et al., 1987) and polyclonal antibodies (Goksoyr, 1985) or by measurement of catalytic activity of CYP1A encoded enzymes such as ethoxyre-

sorufin-*O*-deethylase (EROD) and benzo[*a*]pyrene hydroxylase (Payne and Penrose, 1975; Stegeman et al., 1987; Van Veld et al., 1990). However, recent studies have demonstrated that CYP1A protein levels and enzyme activities in fishes can be: (1) inhibited at high substrate concentrations (Gooch et al., 1989; Monosson and Stegeman, 1991; Hahn et al., 1993); (2) degraded by other contaminants such as tributyltin (Haasch et al., 1992; Fent and Bucheli, 1994; Fent, 1996); and (3) modulated by intrinsic biological factors such as sex and reproductive state (Elskus and Stegeman, 1991). It has therefore been suggested that measurement of induction at the transcriptional level (i.e. CYP1A mRNA) may minimize some of the potential interferences that may occur at later stages in induced gene expression (Haasch et al., 1993).

The Atlantic tomcod is a common estuarine species along the northwestern Atlantic coast of North America with spawning populations extending from Labrador to the Hudson River, New York (Bigelow and Schroeder, 1953). Characteristics that render the tomcod an excellent sentinel species include a bottom-dwelling existence and benthic diet, movements limited to the area of its natal estuary, abundance, and a large, extremely lipid-rich liver (Courtenay et al., 1995). Furthermore, tomcod appear to show greater CYP1A1 mRNA inducibility than do several other species of Atlantic coast estuarine fishes (Wirgin et al., 1996). Evidence of the sensitivity of tomcod to xenobiotic exposure is the high prevalence of hepatocellular carcinomas found in tomcod from the Hudson River, at one time exceeding 90% in 2-year-old fish (Dey et al., 1993), whereas hepatic lesions are either absent or in significantly lower frequency in tomcod from less-impacted systems (Cormier and Racine, 1990). Evidence for chemical etiology to the high prevalence of hepatic neoplasms in the Hudson River population is the presence in tomcod of the continuum of hepatic lesions; basophilic foci, neoplastic nodules, and hepatocellular carcinomas generally found in rodents exposed to chemical carcinogens (Dey et al., 1993).

We have used CYP1A1 mRNA expression in tomcod as a biomarker of exposure to organic xenobiotics. We reported that levels of hepatic CYP1A1 mRNA in tomcod from five rivers along the Atlantic coast of North America were correlated with the relative levels of pollution, bioavailability of high-molecular weight PAH compounds as revealed by their metabolites in bile, and hepatic DNA damage revealed by ^{32}P postlabelling (Wirgin et al., 1994). Within the Miramichi River, New Brunswick, exposure of caged tomcod to bleached kraft mill effluent significantly induced levels of CYP1A1 mRNA and a gradient in levels of gene expression was seen in fish caged downstream of the mill (Courtenay et al., 1993). Based on differential rates of CYP1A1 mRNA reduction in tomcod taken from the Hudson River (Kreamer et al., 1991) and Miramichi River (Courtenay et al., 1993) and depurated in clean water in the laboratory, we hypothesized that characterization of the rates of CYP1A1 mRNA induction and reduction in environmentally exposed fish would prove informative in identifying classes of environmental inducing agents. Thus, in this study the rates of CYP1A1 mRNA induction and disappearance were determined in tomcod treated under controlled laboratory conditions with single doses of the commonly used model inducer β-naphthoflavone (β-NF) and three chemicals of environmental concern: the PAH benzo[*a*]pyrene (B[*a*]P), the non-*ortho* coplanar PCB congener 3,3′,4,4′-tetrachlorobiphenyl (IUPAC: PCB-77), and the dioxin congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). Additionally, dose–response curves were determined for each of these chemicals to evaluate the sensitivity of the response, to determine if CYP1A1 mRNA levels were dose responsive at low concentrations of inducers, and to calibrate the magnitude of CYP1A1 mRNA induction in environmentally exposed tomcod. Finally, to confirm that tomcod living in the wild are exposed to, and accumulate, known CYP1A1 inducers including those tested in this study, contaminant burdens were quantified in livers of tomcod sampled from the highly industrialized Hudson River, moderately industrialized Miramichi River and relatively undeveloped Margaree River (Nova Scotia, Canada). PAH burdens in these populations have been quantified previously

Table 2
Relative mean (±1 SEM) levels of hepatic CYP1A1 mRNA in Miramichi River tomcod treated with a single i.p. injection of β-naphthoflavone (β-NF) (100 mg/kg fish) dissolved in corn oil and sacrificed 72 h after treatment[a]

Experiment	Treatment	n	CYP1A1 mRNA	Fold induction
1	Pooled controls	15	0.727 ± 0.168	–
	β-NF 72 h	22	3.528 ± 0.560	4.9*
2	Pooled controls	14	0.971 ± 0.139	–
	β-NF 72 h	8	18.136 ± 4.311	18.7*

[a] Values are in integrated optical density (I.O.D.) units. In Experiment 1, Control values include fish which were uninjected or given a single i.p. injection of corn oil and sacrificed at the beginning (five uninjected) or end (five injected, five uninjected) of the experiment. No significant difference in CYP1A1 mRNA levels was seen among the three control groups so they were pooled ($n = 15$) for comparison with β-NF-treated fish. In Experiment 2, seven uninjected controls and seven injected controls sacrificed at the end of the experiment were not significantly different and so were pooled ($n = 14$) for comparison with β-NF treated fish.
* Significantly different than control at $P < 0.05$.

The decline in response was not monotonic but linear extrapolation of the decline in CYP1A1 mRNA levels between 80 and 168 h suggests a return to control levels around day 10. Equivalent experiments were not carried out with Miramichi River tomcod.

3.1.3. 3,3′,4,4′-Tetrachlorobiphenyl (PCB-77)

No evidence of CYP1A1 mRNA induction was observed in Hudson River tomcod i.p. injected with 1 mg/kg PCB-77 in three different experiments, the first of which examined fish 4 days and the latter two 7 days after treatment (Table 4). To reduce the potential of refractory hepatic HAHs inhibiting transcription, Hudson River tomcod used in these three experiments had been first depurated in clean water for 19, 100 and 215 days, respectively. The third experiment was carried out in October—the pre-spawning period during which tomcod may show low levels of CYP1A inducibility—but the lack of response observed in experiments 1 and 2, carried out in April and July, respectively, cannot be explained by reproductive physiology. Nor did lack of induction in Hudson River tomcod appear to be dose-related; a fourth experiment, carried out in May with a depuration period of 30 days, failed to detect significant induction at any of 0.1-, 1- or 10-mg/kg doses (Table 4).

In contrast, Miramichi River tomcod exhibited significantly elevated CYP1A1 mRNA levels (7-fold) 4 days, but not 3 days after i.p. injection with 1 mg/kg PCB-77 (Table 5, Experiment 1). Significant induction was observed in this first experiment despite it having been carried out during the pre-spawning period in December. On the strength of this result a more extensive experiment, with additional time points, was carried out with Miramichi River tomcod in April, after the spawning period. Fish showed initial response (9-fold) 7 days after injection which was maintained to the end of the experiment at day 10 (Table 5, Experiment 2). A third experiment with Miramichi River tomcod treated during the spawning season (January) examined levels of

Table 3
Relative mean (±1 SEM) levels of hepatic CYP1A1 mRNA in Hudson River tomcod i.p. injected with benzo[*a*]pyrene (B[*a*]P) (10 mg/kg fish) dissolved in 1:1 emulphor:acetone and sacrificed 8, 24, 48, 80, 120 or 168 h after treatment[a]

Treatment	n	CYP1A1 mRNA	Fold induction
Pooled controls	8	0.049 ± 0.008	–
B[*a*]P 8 h	4	1.838 ± 0.441	38*
B[*a*]P 24 h	4	9.694 ± 1.391	198*
B[*a*]P 48 h	4	9.133 ± 5.296	186*
B[*a*]P 80 h	4	8.460 ± 3.669	173*
B[*a*]P 120 h	4	2.247 ± 0.906	46*
B[*a*]P 168 h	4	3.863 ± 2.035	79*

[a] Values are in integrated optical density (I.O.D.) units. Control groups of four fish injected with 1:1 emulphor:acetone and sacrificed at 24 and 48 h did not differ significantly in CYP1A1 mRNA levels and were pooled ($n = 8$) for comparison with B[*a*]P-injected fish.
* Significantly different than control at $P < 0.05$.

Table 4
Relative mean (± 1 SEM) levels of hepatic CYP1A1 mRNA in Hudson River tomcod i.p. injected with 3,3',4,4'-tetrachlorobiphenyl (PCB-77) dissolved in corn oil and sacrificed 4 or 7 days later[a]

Experiment	PCB-77 Dose (mg/kg fish)	Treatment	*n*	CYP1A1 mRNA	Fold induction
1	–	Control 1 day	3	0.440 ± 0.183	–
	1	PCB-77 4 days	3	0.251 ± 0.193	0.6
2	–	Control 7 days	3	0.405 ± 0.072	–
	1	PCB-77 7 days	3	1.405 ± 0.128	3.5
3	–	Control 7 days	2	0.495 ± 0.313	–
	1	PCB-77 7 days	3	0.302 ± 0.221	0.6
4	–	control 7 days	8	2.333 ± 0.547	–
	0.1	PCB-77 7 days	6	4.827 ± 2.909	2.1
	1	PCB-77 7 days	7	1.141 ± 0.292	0.5
	10	PCB-77 7 days	7	5.449 ± 1.878	2.3

[a] Values are in integrated optical density (I.O.D.) units. Controls were injected with corn oil and sacrificed 1 or 7 days later (Experiments 1–3) or were uninjected (Experiment 4). In no experiment did CYP1A1 mRNA levels differ significantly between experiments and controls.

CYP1A1 mRNA in mature males 25 and 72 days after treatment (Table 5, Experiment 3). While no significant induction was observed at day 25—still within the spawning period—strong induction (37-fold) was observed subsequently at day 72 indicating that treatment with PCB-77 can result in extremely persistent induction of CYP1A1 mRNA in tomcod.

3.1.4. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD)

Hudson River tomcod treated with 2,3,7,8-TCDD (0.5 μg/kg) exhibited no significant elevation of CYP1A1 mRNA 5 or 7 days after treatment (Table 6). In contrast, Miramichi River tomcod showed significant induction of CYP1A1 mRNA in response to 2,3,7,8-TCDD treatment in each of three experiments (Table 7). In the first experiment, initial induction (11-fold) was observed 7 days after treatment, but not before. Induction was apparent in this first experiment despite it being carried out in the pre-spawning period (December) with a mix of mature male and female fish. The second experiment, carried out in April after spawning and examining a longer time period, showed initial induction (5-fold) 5 days after treatment and increasing levels of gene expression to 7-fold through the end of the experiment at day 25. A decline in levels of CYP1A1 mRNA and subsequent recovery appeared to occur between days 14 and 25. A repeat of this period of the time course in Experiment 3 (May) confirmed induction at days 10, 18 and 24 and still increasing CYP1A1 mRNA levels 24 days after treatment (32-fold) but showed no indication of a decline and recovery within this period.

3.2. Dose–response experiments

Hudson River tomcod were treated in the following experiments with β-NF and B[*a*]P and Miramichi River tomcod with the HAHs because, as demonstrated previously, Hudson River tomcod showed no response to either PCB-77 or 2,3,7,8-TCDD. Also based on the results of the kinetic experiments, the interval between treatment and sacrifice was chosen to maximize the induction response. All experiments were carried out between March and May, outside of the pre-spawning and spawning periods.

All four chemicals tested produced dose-dependent induction of CYP1A1 mRNA in tomcod (Figs. 2–5). Lowest concentrations of chemicals to induce significant response were in the low mg/kg range for β-NF and B[*a*]P, μg/kg range for PCB-77, and ng/kg range for 2,3,7,8-TCDD. Despite very high levels of induction, (50- to over 450-fold), there was no indication of maximal

Table 5
Relative mean (±1 SEM) levels of hepatic CYP1A1 mRNA in Miramichi River tomcod i.p. injected with 3,3′,4,4′-tetrachlorobiphenyl (PCB-77) (1 mg/kg fish) dissolved in corn oil[a]

Experiment	Treatment	*n*	CYP1A1 mRNA	Fold induction
1	Control 0 days	5	0.205+0.029	–
	PCB-77 3 days	9	0.380+0.070	1.9
	PCB-77 4 days	5	1.394+0.335	6.8*
2	Pooled controls	8	0.453+0.123	–
	PCB-77 1 days	7	2.592+1.098	5.7
	PCB-77 2 days	7	1.565+0.674	3.5
	PCB-77 3 days	7	1.081+0.391	2.4
	PCB-77 5 days	7	0.822+0.116	1.8
	PCB-77 7 days	7	4.049+0.735	8.9*
	PCB-77 10 days	8	3.837+0.633	8.5*
3	Control 25 days	10	0.228+0.070	–
	PCB-77 25 days	9	0.363+0.233	1.6
	Control 72 days	10	0.487+0.188	–
	PCB-77 72 days	10	8.331+2.054	37*

[a] Values are in integrated optical density (I.O.D.) units. Controls were uninjected (Experiment 1) or injected with corn oil (Experiments 2 and 3). In Experiment 2 Controls ($n=4$ per group) sacrificed at days 0 and 10 did not differ significantly in CYP1A1 mRNA level and were pooled ($n=8$) for comparison with PCB-77-treated fish. PCB-77-treated fish in Experiment 3 were compared with controls sacrificed on the same day.

* Significantly different than control at $P<0.05$.

responses having been achieved with the exception of β-NF treatments in which the response produced by 500 mg/kg was lower than that produced by 50 mg/kg β-NF (Fig. 2).

3.3. Exposure of Atlantic tomcod to CYP1A-active aromatic hydrocarbons in the wild

Analyses of livers sampled from tomcod living in the highly industrialized and urbanized lower Hudson River estuary, moderately industrialized Miramichi River and unindustrialized Margaree River confirmed a broad range of exposure to, and bioaccumulation of dioxins, furans and PCBs known to induce CYP1A in fish, including 2,3,7,8-TCDD and PCB-77 shown to induce CYP1A1 mRNA in tomcod in the present study (Table 8). Expressed on a lipid-normalized basis, livers from tomcod sampled from the least industrialized site (Margaree) were virtually free of dioxins and furans and showed low levels of CYP1A active PCBs (360 ng/kg lipid). Other sites ranged from 27 to 626 times higher than Margaree in dioxins and furans and 2–330 times higher in PCBs. Additionally, lipid-normalized concentrations of 2,3,7,8-TCDD were 19–26-fold higher in tomcod from the Hackensack River than in fish from the Miramichi River.

Tomcod sampled from the two different sites within the Hudson River system showed different patterns of exposure to CYP1A inducing chemi-

Table 6
Relative mean (±1 SEM) levels of hepatic CYP1A1 mRNA in Hudson River tomcod i.p. injected with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (0.5 μg/kg fish) dissolved in corn oil[a]

Treatment	*n*	CYP1A1 mRNA	Fold induction
Control 5 days	3	0.218+0.037	–
TCDD 5 days	4	0.560+0.076	2.6
Control 7 days	3	0.437+0.111	–
TCDD 7 days	4	0.713+0.296	1.6

[a] Values are in integrated optical density (I.O.D.) units. Controls were injected with corn oil and compared with 2,3,7,8-TCDD-injected fish sacrificed on the same day. CYP1A1 mRNA levels did not differ significantly between controls and 2,3,7,8-TCDD-treated fish 5 or 7 days after treatment.

Table 7
Relative mean (±1 SEM) levels of hepatic CYP1A1 mRNA in Miramichi River tomcod treated with a single i.p. injection of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (0.5 μg/kg fish) dissolved in corn oil[a]

Experiment	Treatment	*n*	CYP1A1 mRNA	Fold induction
1	pooled controls	10	2.039+0.444	–
	TCDD 9 h	7	0.896+0.104	0.4
	TCDD 16 h	7	1.278+0.150	0.6
	TCDD 1 days	7	0.815+0.256	0.4
	TCDD 3 days	7	5.171+1.437	2.5
	TCDD 5 days	7	10.381+2.757	5.1
	TCDD 7 days	8	21.703+3.765	10.6*
2	pooled controls	15	0.151+0.010	–
	TCDD 2 days	7	0.228+0.021	1.5
	TCDD 3 days	7	0.152+0.017	1.0
	TCDD 5 days	7	0.687+0.110	4.6*
	TCDD 7 days	7	0.661+0.172	4.4*
	TCDD 10 days	7	0.822+0.079	5.4*
	TCDD 14 days	7	0.821+0.091	5.4*
	TCDD 18 days	7	0.574+0.095	3.8*
	TCDD 25 days	6	1.114+0.090	7.4*
3	Control 10 days	8	0.348+0.080	–
	TCDD 10 days	8	8.828+1.949	25*
	TCDD 18 days	8	10.192+1.781	29*
	TCDD 24 days	8	11.163+0.891	32*

[a] Values are in integrated optical density (I.O.D.) units. In Experiment 1, control groups of corn oil-injected fish and uninjected fish ($n=5$ per group) did not differ in level of CYP1A1 mRNA 7 days after treatment and were pooled for comparison with 2,3,7,8-TCDD-treated fish. In Experiment 2, control groups of five fish injected with corn oil and sacrificed at 0, 10, and 25 days did not differ in level of CYP1A1 mRNA and were pooled ($n=15$) for comparison with 2,3,7,8-TCDD-treated fish. In Experiment 3, controls were injected with corn oil and sacrificed 10 days later.

* Significantly different than controls at $P<0.05$

cals, suggesting that they inhabit different areas of the lower Hudson River estuary not only during the spawning run when they were caught, but during much or all of their lives. Lipid-normalized concentrations of dioxins and furans were 4–7 times higher in Hackensack River tomcod than tomcod sampled at River Mile 50 on the main Hudson River. This difference was not a function of increasing size or age; the more contaminated Hackensack River tomcod were in fact significantly smaller than tomcod from the main stem of the Hudson River (19.5 vs. 27.0 cm TL, respectively, for females) and younger (all 1-year-olds vs. all 2-year-olds). Additionally, the ratio of 2,3,7,8-TCDD to 2,3,7,8-TCDF differed between fish from the main stem of the Hudson River at Garrison and the Hackensack River. Concentrations of 2,3,7,8-TCDD were 4–15-fold higher than 2,3,7,8-TCDF in fish from the Hackensack River, whereas 2,3,7,8-TCDF concentrations were higher in fish from Garrison (data not shown). PCB concentrations, on the other hand, were similar in tomcod sampled at the two sites in the Hudson River estuary. In total these results suggest multiple sources of PCB exposure to fish in the lower Hudson River estuary. Interestingly, PCB levels were twice as high in females as males, perhaps related to the very low hepatic lipid concentrations in females around the time of spawning (7–14% by weight, cf. 36–37% in males; Table 8). It is evident from data in Table 8 and also from other studies of the weight of the liver relative to the body (liver somatic index) (Williams et al., 1998) that hepatic lipid concentrations vary a great deal seasonally in Atlantic tomcod and fall precipitously around the time of reproduction.

Table 8
Concentrations of chlorinated hydrocarbons that have been shown to induce CYP1A in fish (Stegeman and Hahn 1994) found in livers of Atlantic tomcod from the relatively undeveloped Margaree River (Nova Scotia, Canada; 13 October 1993), the moderately industrialized Miramichi River (New Brunswick, Canada; 8 September 1993) and from two sites in the heavily industrialized Hudson River estuary (New York, USA): Garrison, NY, on the main Hudson River at River Mile 50 (22 December 1997), and the Hackensack River approximately 2–5 km upstream from Newark Bay (17 December 1996)[a]

Sample (*n* in pool)	Water (% w.w.)	Lipid (%w.w.)	2,3,7,8-TCDD (ng/kg)		Total dioxins and furans (2,3,7,8-TCDD TEQ)		PCB-77 (ng/kg)		Total coplanar PCBs[b] (ng/kg)	
			Wet	Lipid	Wet	Lipid	Wet	Lipid	Wet	Lipid
Margaree females (10)	46	51	1	1	1	3	87	172	182	360
Miramichi males (8)	34	58	48	83	67	116	280	483	498	859
Miramichi females (15)	31	66	38	58	53	80	260	396	409	623
Hudson males (10)	44	37	47	128	99	270	17 395	47 436	21 153	57 683
Hudson females (10)	72	7	11	166	31	476	6341	96 665	7365	112 267
Hackensack males (6)	47	36	554	1536	673	1867	17 366	48 172	21 119	58 583
Hackensack females (11)	68	14	208	1525	256	1878	14 713	107 949	16 182	118 720

[a] Concentrations are expressed on a wet-weight basis (wet) as well as lipid-normalised basis (lipid) (g lipid/g wet liver). Dry weights may be calculated from percent moisture (water).

[b] Total coplanar PCBs, sum of concentrations of 3,3′,4,4′-tetrachlorobiphenyl (# 77), 3,3′,4,4′,5-pentachlorobiphenyl (# 126) and 3,3′, 4,4′,5,5′-hexachlorobiphenyl (# 169).

4. Discussion

4.1. Kinetic responses

The kinetics of hepatic CYP1A1 mRNA induction in tomcod, in response to PAHs and HAHs, were markedly different. Treatment with either β-NF or B[*a*]P resulted in significant induction within hours compared to a 4–7-day delay for PCB-77 or 2,3,7,8-TCDD. Duration of induction also differed between chemicals. Responses to PAHs peaked in 1–3 days and declined thereafter, almost certainly disappearing within 2 weeks. In contrast, HAHs induced increasing levels of CYP1A1 mRNA for at least 25–72 days after treatment.

Rapid and brief induction of CYP1A in response to PAHs and the model inducer β-NF have been reported in other in vivo studies with fishes. For example, in blennies (*Zoarces viviparus*) treated with β-NF, CYP1A mRNA levels peaked after 6 h and returned to basal levels within 3 days (Celander et al., 1994). In β-NF treated rainbow trout, CYP1A1 mRNA was maximally induced at 18 h and decreased to near basal levels by 48 h (Haasch et al., 1989). In mummichogs (*Fundulus heteroclitus*) CYP1A mRNA peaked at 1 day and declined rapidly thereafter (Kloepper-Sams and Stegeman, 1989). In comparison, induction of CYP1A was far more persistent in fish treated with HAHs. For example, in the marine species, scup, CYP1A1 mRNA was sustained for at least 14 days following treatment with 2,3,7,8-TCDF (Hahn and Stegeman, 1994) and induced EROD activity was observed in Atlantic cod (*Gadus morhua*) for at least 17 days after treatment with 2,3,7,8-TCDD (Hektoen et al., 1994). In scup, however, initial induction of CYP1A1 mRNA with TCDF was observed 1 day after i.p. injection—a much more rapid response than we observed in tomcod treated with either 2,3,7,8-TCDD or PCB-77. The delayed and more persistent induction response in tomcod may be due to their unusually high hepatic lipid content (Cormier and Racine, 1990). In the only other

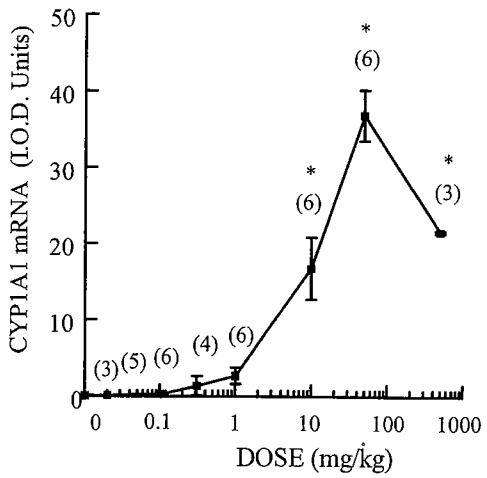

Fig. 2. Relative mean (±1 SEM) levels of hepatic CYP1A1 mRNA (integrated optical density units) in Hudson River tomcod i.p. injected with different doses of β-naphthoflavone (β-NF) dissolved in corn oil and sacrificed 70 h later. Doses are represented on a log scale. Controls (0 mg/kg fish) were injected with corn oil and sacrificed 7 days later. *Significantly different than control at $P < 0.05$. Numbers above bars represent sample size.

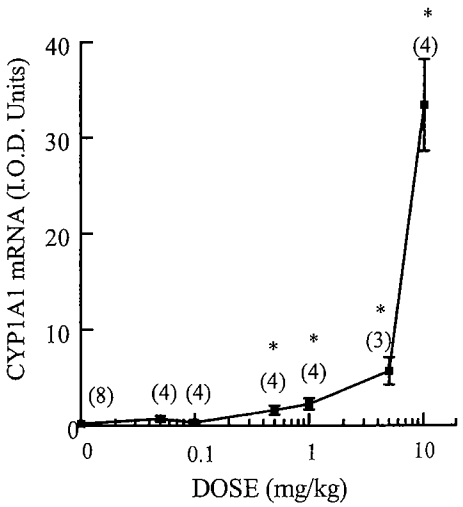

Fig. 3. Relative mean (±1 SEM) levels of hepatic CYP1A1 mRNA (integrated optical density units) in Hudson River tomcod i.p. injected with different doses of benzo[*a*]pyrene (B[*a*]P) dissolved in 1:1 emulphor:acetone and sacrificed 24 h later. Doses are represented on a log scale. Controls (0 mg/kg fish) injected with 1:1 emulphor:acetone and sacrificed 24 or 48 h later (four per group) did not differ significantly and were pooled for comparison with B[*a*]P exposed fish. *Significantly different than control at $P < 0.05$. Numbers above bars represent sample size.

study, in which the kinetics of CYP1A1 mRNA induction were compared for PAHs and HAHs in the same species (rainbow trout), i.p. injection of the commercial PCB mixture, Clophen A50, resulted in increasing levels of CYP1A1 mRNA for a 15-week period, whereas response to the PAH, 3-methylcholanthrene, peaked 5 days after injection and declined to control levels within 5 weeks (Celander and Forlin, 1995).

Two reasons may be advanced to explain the differences between PAHs and HAHs in the kinetics of the induction of CYP1A mRNA. First, these differences are consistent with the rapid metabolism of PAHs compared to the relatively long-term retention of HAHs, particularly in lipid-rich tissues such as the liver (Niimi, 1987; DeVito and Birnbaum, 1995). For example, many studies have demonstrated that it is difficult or impossible to quantify PAH levels in fish livers because of their rapid metabolism (Varanasi and Stein, 1991). In contrast, the half-life of 2,3,7,8-TCDF ranged between 6 and 11 weeks in rainbow trout liver (Muir et al., 1992), the half-life of total

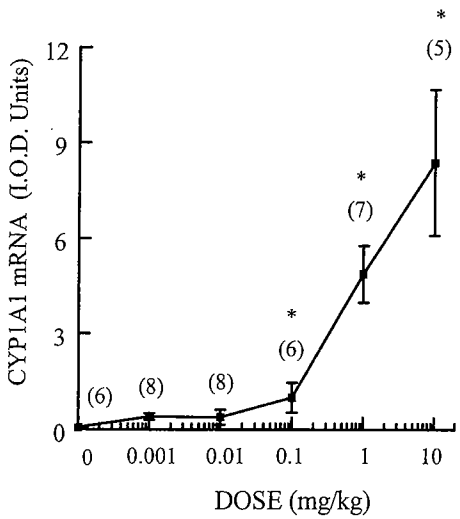

Fig. 4. Relative mean (±1 SEM) levels of hepatic CYP1A1 mRNA (integrated optical density units) in Miramichi River tomcod i.p. injected with different doses of 3,3',4,4'- tetrachlorobiphenyl (PCB-77) dissolved in corn oil and sacrificed 7 days later. Doses are represented on a log scale. Controls (0 mg/kg fish) were injected with corn oil and sacrificed 7 days later. *Significantly different than control at $P < 0.05$. Numbers above bars represent sample size.

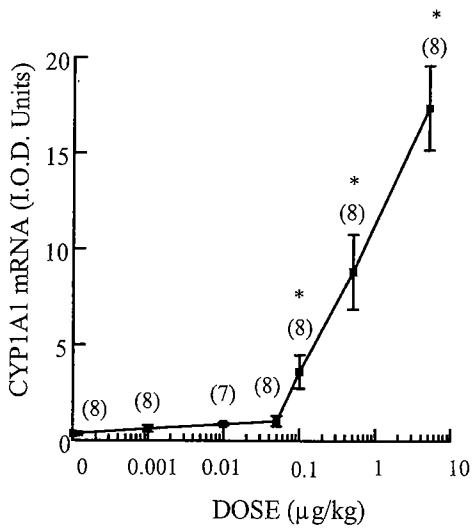

Fig. 5. Relative mean (±1 SEM) levels of hepatic CYP1A1 mRNA (integrated optical density units) in Miramichi River tomcod i.p. injected with different doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) dissolved in corn oil and sacrificed 10 days later. Doses are represented on a log scale. Controls (0 mg/kg fish) were injected with corn oil and sacrificed 10 days later. *Significantly different than control at $P < 0.05$. Numbers above bars represent sample size.

PCBs was 11–16 weeks in mouse carcass and slightly shorter in mouse lung (Beebe et al., 1992), and the half-life of 2,3,7,8-TCDD in plasma and sera was estimated to range between 7.1 and 9.0 years in two highly exposed human populations (Michalek et al., 1996; Landi et al., 1998).

It is also possible that variation in the kinetic profiles of the induction response may reflect underlying mechanistic differences whereby CYP1A1 transcription is activated by HAHs and PAHs. While it is generally assumed that CYP1A1 transcription in response to 2,3,7,8-TCDD, PCBs and PAHs is mediated exclusively through activation of the aromatic hydrocarbon receptor (AhR) pathway, that may not always be the case. Recent in vitro studies have shown that CYP1A1 transcription can be mediated by an AhR-independent pathway in mouse hepatoma cells treated with PAHs (Sterling et al., 1994). Thus, it is possible that kinetic differences between AhR-dependent and AhR-independent pathways in ligand binding, nuclear translocation,

or receptor complex binding to DNA enhancer elements 5′ to CYP1A1 may contribute to differences between PAHs and HAHs in the regulation and kinetics of CYP1A1 transcription. Perhaps the transient decrease that we observed in CYP1A1 levels in tomcod 18 days after treatment with 2,3,7,8-TCDD, and that was seen in rainbow trout 35 days after treatment with the Clophen A50 mix (Celander and Forlin, 1995), are indicative of regulation of CYP1A1 transcription by multiple molecular pathways in fishes.

Variation in the kinetics of the induced CYP1A1 response may offer the potential to characterize environmental inducers as PAH- or HAH-like compounds when high levels of CYP1A1 expression are observed in wild populations. For example, we suggested that elevated levels of CYP1A1 mRNA in tomcod from the Hudson River resulted largely from exposure to PAHs based on the rapid decline of CYP1A1 mRNA in environmentally exposed fish which were depurated in clean laboratory water (Kreamer et al., 1991). This assertion was supported by the high levels of hepatic, bulky DNA adducts and bile metabolites of PAHs detected in tomcod from the Hudson River (Wirgin et al., 1994), and the non-inducibility of CYP1A1 in Hudson River tomcod treated with HAHs (Wirgin et al., 1992). In contrast, we concluded that CYP1A1 mRNA induction in tomcod from the Miramichi River did not result from exposure to PAH compounds, but instead from more persistent compounds found in bleached kraft mill effluent (Courtenay et al., 1993). However, a reservation to the use of this approach is the possible effects of dose on the kinetics of the induction response. There is some evidence that depuration of organochlorines in fish (van der Oost et al., 1991; van der Weiden et al., 1992b) and mammalian tissues (Beebe et al., 1990) is dose dependent with no clear relationship apparent. For example, little clearance of PCB-congener 126 was observed over a 30-week period in lake trout that were injected with high doses, whereas clearances of low doses were observed within 5 weeks (Palace et al., 1996). Concurrently, significant EROD induction was observed for the high doses throughout the 30-week period, but only at week 5 for the low dose. In contrast, the depuration of a high dietary dose of 2,3,7,8-TCDF in rainbow trout liver was significantly more rapid than that of a lower dose (Muir et al., 1992). Similarly, dose-dependent differences in the persistence of induced hepatic EROD activity were observed in rainbow trout and mirror carp (*Cyprinus carpio*) which were i.p. injected with 2,3,7,8-TCDD (van der Weiden et al., 1992a, 1994, respectively).

The persistent response to HAHs suggests a number of precautions which should be exercised in the interpretation of results from biomarker studies in natural populations using CYP1A mRNA induction. First, elevated CYP1A expression does not necessarily indicate recent exposure; exposure to HAHs may have occurred months previous. Second, unless the species being examined is sedentary, CYP1A expression may not reflect contamination of the immediate environment in which the fish was collected. Third, levels of CYP1A gene expression in reproductively active fish may not accurately reflect exposure to xenobiotics. In our study i.p. injection of tomcod with PCB-77 during the spawning season resulted in strong hepatic CYP1A1 induction 72 days after treatment, but not after 25 days. These fish were treated with PCB-77 in January and when sampled 25 days later were still undergoing a period of extended reproductive activity. We previously reported that environmentally exposed female and male tomcod from the Miramichi River showed reduced expression of CYP1A1 mRNA when in a reproductively active state, but high expression thereafter (Courtenay et al., 1994; Williams et al., 1998). A number of studies with other fish species have also reported suppression of CYP1A expression at the protein level during periods of heightened reproductive activity (Stegeman and Hahn, 1994; Addison, 1995). Thus quantification of CYP1A gene expression during periods of reproductive activity may result in underestimations of exposure to aromatic hydrocarbon compounds.

4.2. Dose experiments

The lowest concentration at which statistically significant CYP1A1 mRNA induction was observed in Atlantic tomcod—low mg/kg range for

PAHs and ng/kg–μg/kg range for HAHs—are similar to levels at which CYP1A protein induction was reported in other fish species treated by i.p. injection. Induction was observed in response to 0.01–1 mg/kg for co-planar PCBs in scup (Gooch et al., 1989), winter flounder (*Pleuronectes americanus*) (Monosson and Stegeman (1991) and channel catfish (Rice and Schlenk, 1995). Intraperitoneal injections of 2,3,7,8-TCDD have resulted in significant CYP1A induction at concentrations of 0.03 and 0.3 μg/kg in mirror carp (*Cyprinus carpio*) (van der Weiden et al., 1994) and rainbow trout (van der Weiden et al., 1992a), respectively. Inductions reported here for Atlantic tomcod should be considered minimal sensitivities due to the relatively low power of statistical tests in the face of large inter-individual variances in CYP1A1 mRNA levels and relatively small sample sizes (Courtenay et al., 1994). For example, the lowest concentration of β-NF to significantly elevate CYP1A1 mRNA levels above controls was 10 mg/kg even though the lower doses of 0.3 and 1.0 mg/kg resulted in 17- and 34-fold inductions respectively—levels that should prove statistically significant with sample sizes larger than the three to six individuals used in this experiment. Indeed, in subsequent experiments with sample sizes of ten individuals, we observed significant, 15-fold induction in Miramichi tomcod i.p. injected with 0.1 and 1.0 mg/kg β-NF (Williams and Courtenay, unpublished data).

A number of in vivo studies with fish have reported ED50s for CYP1A inducers, the effective dose producing a half-maximal response, at the protein level though not at the mRNA level (Janz and Metcalfe, 1991a,b; van der Weiden et al., 1992a, 1994; Newsted and Giesy, 1993). ED50s are useful in comparing sensitivities of different species and toxic equivalency factors (TEF) of different chemicals (Zabel et al., 1996). In the present study, maximal induction of CYP1A1 mRNA in tomcod was observed at 50 mg/kg β-NF, similar to the dose (100 mg/kg) at which maximal AHH activity was detected in rainbow trout i.p. injected with β-NF (Elcombe and Lech, 1979; Janz and Metcalfe, 1991b). Interestingly, Janz and Metcalfe (1991b) observed a decrease in CYP1A activity at a dose higher than 100 mg/kg as we did in the present study. Based on their dose–response curve, an ED50 of 20 mg/kg β-NF was reported for rainbow trout (Janz and Metcalfe, 1991b). The comparable ED50 for tomcod, based on the dose–response curve in Fig. 2, is slightly lower at approximately 12 mg/kg. ED50s cannot be calculated for the other chemicals tested in the present study because maximal responses were not achieved. However, using the largest responses that were realized, we can say that ED50s for B[*a*]P, PCB-77 and 2,3,7,8-TCDD, respectively, must be greater than 7 mg/kg, 0.6 mg/kg and 0.4 μg/kg. These estimates are higher than has been reported for rainbow trout exposed to PCB-77 (0.1–0.6 mg/kg; Janz and Metcalfe, 1991a,b) and mirror carp exposed to 2,3,7,8-TCDD (0.048 μg/kg; van der Weiden et al., 1994) but not rainbow trout exposed to 2,3,7,8-TCDD (0.64–1.61 μg/kg; Janz and Metcalfe, 1991a,b; van der Weiden et al., 1992a; Newsted and Giesy, 1993). Some of these studies (e.g., Janz and Metcalfe, 1991a,b) derived ED50s for aromatic hydrocarbons indirectly by relating induction by low concentrations of the toxic AH under question to a dose–response curve obtained with a more innocuous inducer such as β-NF. This approach assumes that different CYP1A inducers produce similarly shaped dose–response curves and maximal levels of induction which appears not to be the case, at least for CYP1A catalyzed enzyme induction (Zabel et al., 1996). Furthermore, if the mechanistic bases of regulation of CYP1A transcription differ among classes of inducing compounds, it is likely that the shape of dose–response curves will vary among chemicals.

Maximal levels of CYP1A1 induction observed in the present study ranged between 50- and 467-fold which are high, but not unprecedented. Controlled laboratory experiments in which fish were treated with CYP1A inducers via i.p. injection, food, sediments or water have observed CYP1A induction levels of 100-fold (van der Weiden et al., 1994) or even much higher. Haasch et al. (1992) reported induction of CYP1A1 mRNA in rainbow trout, exposed for 1 day to 50 or 100 μg/kg β-NF, of 2600- and 5000-fold, respectively. Field studies of hepatic EROD activities in fish

from contaminated sites occasionally report levels over 100-fold higher than fish from reference sites, but inductions in the 10-fold range are more usual (Addison, 1996). For example, we reported that levels of CYP1A1 mRNA were 28-fold higher in tomcod from the Hudson River than in fish from the more pristine Margaree River, Nova Scotia (Wirgin et al., 1994). It has been shown that species differ in CYP1A responsiveness to environmental or hepatic concentrations of inducers (Hektoen et al., 1994). High levels of CYP1A inducibility have been interpreted as one indication of sensitivity to particular xenobiotics (van der Weiden et al., 1992a, 1994) and in this regard, the Atlantic tomcod appears to be a more sensitive biosentinel species than several other anadromous species from the Atlantic coast of North America (Wirgin et al., 1996).

4.3. Non-inducibility of CYP1A1 expression in Hudson River tomcod

Our results indicate that the extent of CYP1A1 gene inducibility can vary among populations within a species, perhaps resulting from prior exposure history. This phenomenon has also been observed in other species of estuarine and marine fish including the mummichog, from several highly contaminated North American Atlantic coast sites, including Newark Bay, an arm of the Hudson River estuary (Prince and Cooper, 1995a,b), New Bedford Harbor, MA (Nacci et al., 1996), and the Elizabeth River, a tributary of the Chesapeake Bay (Van Veld et al., 1996) and in cancer-prone (Vethaak et al., 1996) flounder (*Platichthys flesus*) from the North Sea (Besselink et al., 1998). New Bedford Harbor is highly contaminated with PCBs, the Elizabeth River with creosote-derived PAHs, and Newark Bay with a myriad of AH compounds including unusually high concentrations of 2,3,7,8-TCDD.

In European flounder from the Western Wadden Sea, The Netherlands, inhibition of CYP1A catalyzed EROD activity was observed in fish treated with various PCB congeners (including PCB-77), but not with 2,3,7,8-TCDD (Besselink et al., 1998). These workers observed that a variety of coplanar PCB congeners (including PCB-77) and the commercial PCB mixture, Clophen A50, competitively inhibited hepatic microsomal CYP1A catalytic activity in flounder. Furthermore, they observed that hepatic AhR levels in flounder were low compared to those in rats based on adsorption analyses and velocity sedimentation and they observed an absence of AhR complex binding to rodent-derived dioxin responsive elements (DRE) with cytosols prepared from flounder hepatic cells exposed in vitro to 2,3,7,8-TCDD. These results suggest that the mechanisms of inhibition of CYP1A inducibility may differ between tomcod and European flounder. However, it is difficult to compare the mechanistic bases of non-inducibility of CYP1A in flounder to what we report in tomcod because these studies; (1) failed to evaluate CYP1A mRNA inducibility in these fish, and (2) did not compare CYP1A inducibility in fish from clean and contaminated sites. We do know that unlike European flounder, AhR complex binding to DREs is observed with hepatic proteins prepared from the livers of PCB-77 (only Miramichi River) and B[*a*]P-treated tomcod (both Hudson and Miramichi Rivers).

In our study, CYP1A1 mRNA was inducible in tomcod from the Miramichi River by treatment with both PAHs and HAHs, but tomcod from the Hudson River responded only to PAHs. Hudson River tomcod also failed to show a CYP1A1 response to i.p. injections of Aroclor® 1254 (Wirgin et al., unpublished data). This is the first study with fish to report a difference in CYP1A1 inducibility by halogenated versus non-halogenated aromatic compounds and that non-inducibility of CYP1A1 in this model results from inhibition at the transcriptional, not post-transcriptional level.

Both physiological acclimation and genetic adaptation can be advanced to explain the non-inducibility of hepatic CYP1A1 mRNA with HAHs in tomcod from the Hudson River. Distinguishing between these two alternatives is important in determining the persistence of this non-response in organisms from a remediating environment. Farber and colleagues (Farber, 1990; Farber and Rubin, 1991) have proposed the 'resistant hepatocyte model' in which rare hepatocytes initiated by exposure to xenobiotics eventually clonally expand into resistant hepatocyte nodules and neo-