A Biomarker Approach to Assessing Xenobiotic Exposure in Atlantic Tomcod from the North American Atlantic Coast

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We determined levels of hepatic cytochrome P4501A (CYP1A) mRNA, hepatic DNA adducts, and fluorescent aromatic compounds (FACs) in bile, a measure of exposure to polyaromatic hydrocarbons, in Atlantic tomcod from six river systems ranging from highly polluted to relatively pristine on the northeast North American coast (the Hudson River, New York; the St. Lawrence River, Quebec; the Miramichi River, New Brunswick; the Saco and Royal rivers, Maine; and the Margaree River, Nova Scotia). Hudson River tomcod showed the greatest response for all parameters, and tomcod from the Margaree River exhibited the least response. Tomcod from the Miramichi River exhibited marked induction of CYP1A mRNA but low levels of hepatic DNA adducts and biliary FACs, whereas fish from the St. Lawrence River showed no induction of CYP1A mRNA and moderately elevated levels of DNA adducts and biliary FACs. In tomcod from the Hudson and Miramichi rivers, the levels of CYP1A mRNA were 28 times and 14 times, respectively, as great as the levels in fish from the St. Lawrence, Saco/Royal, and Margaree rivers. Mean levels of DNA adducts varied from 120 nmol adducts/mol bases in Hudson River tomcod to < 3 nmol adducts/mol bases in fish from the Miramichi and Margaree rivers. Concentrations of FACs in the bile of tomcod from the Hudson and St. Lawrence rivers were 8 and 1.8 times, respectively, as great as the concentrations in tomcod from the Miramichi River and Margaree River. In tomcod from the Hudson River, all three biomarkers were markedly elevated; in the St. Lawrence River two biomarkers were elevated, in the Miramichi River one was elevated, but no biomarker was substantially elevated in fish from the Saco/Royal and Margaree rivers. Elevated levels of hepatic DNA adducts and biliary FACs in tomcod from the Hudson River suggest increased exposure to PAHs, consistent with previous studies. *Key words*: Atlantic tomcod, CYP1A mRNA, DNA adducts, FACs, hepatocellular carcinomas, Hudson River, PAHs, ^{32}P-postlabeling analysis. *Environ Health Perspect* 102:764–770 (1994).

The Atlantic tomcod (*Microgadus tomcod*) is a bottom-dwelling, anadromous fish species of the northeast coast of North America, and its overall distribution extends from Labrador to Virginia (*1*). Tomcod are a dominant fish species in many of the river systems within this range and as such have been used as an indicator species of environmental quality (*2*). The Hudson River supports their southernmost spawning population, and as a result they may be thermally stressed during the warmer months (*3*). Tomcod have extremely high hepatic lipid levels (*4*), are confined to estuaries, and undergo annual winter spawning migrations. In the Hudson River, they migrate from the New York City area at the mouth of the estuary to 50–100 miles upriver (*2*). As a result of these factors, Hudson River tomcod may be particularly sensitive to environmental insult from sediment-bound lipophilic environmental agents, and their exposure histories may integrate contaminant levels throughout the lower Hudson River estuary.

Previous studies have also demonstrated that tomcod from the Hudson River exhibit a remarkably high prevalence of hepatocellular carcinomas that exceeds 90% in older fish (*5*), compared to a cancer prevalence of <5% in tomcod from more pristine rivers in Rhode Island, Connecticut, and Maine (*6*). Additionally, the age structure of the Hudson River tomcod population is truncated and characterized by a small number of 2-year-old fish and absence of older age classes (>2 years olds), whereas tomcod from other populations frequently attain 3–7 years of age (*5,7*). An environmental etiology for the elevated prevalence of hepatomas in Hudson River tomcod is supported by activated K-*ras* oncogenes in a high percentage of tomcod liver tumor DNAs (*8*).

These studies suggest that Atlantic tomcod are a sensitive indicator species of the environmental quality of the estuaries of the Atlantic coast of North America. However, few studies have assessed conta-

minant exposure in this species from multiple rivers or used biological markers to document exposure and biochemical and physiological responses to xenobiotic compounds. Furthermore, toxicological studies on the biota of the Hudson River have focused primarily on exposure to halogenated aromatic hydrocarbons (HAHs), such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins and -furans (PCDDs and PCDFs), and not on exposure to polycyclic aromatic hydrocarbons (PAHs). Previous studies in fish show that PAHs are putative etiologic agents in hepatic neoplasia in several wild fish species from contaminated areas (*9*). Accordingly, the objective of the present study was to assess the response of a suite of biomarkers associated with exposure to HAHs and PAHs in tomcod from river systems along the Atlantic coast from New York, USA, to New Brunswick, Canada. These river systems show a range in chemical contamination from highly polluted (Hudson River) to moderately polluted (St. Lawrence River and Miramichi River) and relatively pristine (Saco/Royal Rivers and Margaree River). The biomarkers measured were levels of hepatic cytochrome P4501A (CYP1A) mRNA, levels of DNA adducts in liver detected by ^{32}P-postlabeling analysis, and fluorescent aromatic compounds (FACs) in bile, a measure of exposure to PAHs.

The Hudson River estuary has been polluted with a variety of both HAHs and PAHs (*10*). Sediments from sites in the Hudson River exhibit among the highest concentrations of PAHs (7,100–34,000 ng PAHs/g dry weight) of any estuary sampled in the United States (*11*). In addition, point sources for PCB contamination have been identified in the upper Hudson River north of Albany, New York, and elevated sediment-borne levels of PCBs have also

been detected in the lower estuary off New York City resulting from transport from the Upper Hudson River or nonpoint sources of urban discharges, while PCDD and PCDF contamination have been identified in Newark Bay (12). Previous studies have shown that tomcod from the Hudson River have elevated concentrations of hepatic PCBs (5); however, there is no information on exposure to PAHs and little biochemical evidence of exposure to environmental xenobiotics, particularly PAHs. In estuaries where tomcod exhibit low prevalences of hepatic neoplasms, sediment concentrations of PAHs are considerably lower than in the Hudson River estuary. The sediment concentrations of PAHs in estuaries in southern Maine are reported to range from 800 to 6700 ng/g dry weight (11). In the St. Lawrence River, tomcod were sampled from spawning grounds on the Batiscan and St. Anne rivers, which had PAH concentrations in the sediments of 900–1300 ng/g dry weight. These fish also make feeding migrations into the St. Lawrence River downstream of Quebec City, where concentrations of PAHs in sediments range from 600 to 2200 ng/g dryweight (13). PCB levels in sediment of the St. Lawrence estuary vary from 10 to 330 ng/g dry weight (14). The concentrations of PAHs in sediment from the Miramichi River are reported to be moderate (200–600 ng/g dry weight) (15), and PCB levels are low (0–75 ng/g dry weight) (16), but dioxins and furans are discharged by a pulp and paper mill at Newcastle. Along the Margaree River, there is no industrialization or urbanization, and as a result sediment PAH concentrations are relatively low (5–120 ng/g dry weight) (15).

The suite of biomarkers used in the present study, specifically hepatic CYP1A mRNA, biliary FACs, and DNA adducts, has been shown in previous studies to be a sensitive indicator of exposure to a range of polycyclic aromatic compounds. Assessment of exposure to PAHs by measuring concentrations of parent compounds in tissue is impractical because of their rapid metabolism by fish (16). However, determining the concentration of FACs in bile is a semiquantitative means to assess exposure of fishes to environmental PAHs (17). The induction of CYP1A gene expression serves as a sensitive, dose responsive, and quantifiable measure of exposure and early biological effect of exposure to PAHs and HAHs in feral fishes (18), including induction of CYP1A mRNA in Atlantic tomcod (19). Additionally, induction of CYP1A mRNA in fishes is believed to be mediated by the Ah receptor pathway (20), whose activation is also hypothesized to be necessary for the carcinogenicity, teratogenicity, and toxicity resulting from exposure to PAHs (21). Hepatic DNA adducts, detect-

ed by ^{32}P-postlabeling, have been shown to arise in fishes from exposure to individual PAHs (22,23) and from exposure to complex mixtures of polycyclic aromatic compounds present in sediments from natural environments (22,24). It has also been shown that these DNA adducts are persistent and thus appear to accumulate to relatively high levels in the livers of fish exposed to polycyclic aromatic compounds (23,25). The concerted use of multiple biomarkers should improve the assessment of contaminant exposure in tomcod and potentially provide more specific information on the identity of environmental inducers of these biomarker responses. Previous studies (25,26) have shown that when interpreted collectively, a suite of biomarkers gave more consistent information on the level of contaminant exposure and biochemical response of wild fish than did any single parameter.

Methods

Sample collections and treatments. We collected tomcod from six rivers (Fig. 1),

including the Miramichi River, New Brunswick; Margaree River, Nova Scotia; St. Lawrence River, Quebec; Saco and Royal rivers, Maine; and the Hudson River, New York (Table 1). Results from the Saco and Royal rivers were pooled for data analysis because of small samples sizes. Fish were immediately sacrificed in the field, their livers excised, and gall bladders removed. We placed livers in plastic vials and immediately immersed them in liquid nitrogen. The livers were subsequently stored at -80°C until processing. Bile was removed from gall bladders with 1-ml syringes and stored in amber vials at -20°C until analysis.

Because tomcod in the Hudson River have high concentrations of hepatic PCBs and are potentially exposed to dioxins, we conducted a preliminary study to determine whether exposure to a planar PCB, 3,3′,4,4′-tetrachlorobiphenyl (TCB), or the toxic PCDD 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), induced or altered levels of hepatic DNA adducts. Tomcod

Figure 1. Collection sites of Atlantic tomcod analyzed for hepatic CYP1A mRNA, hepatic DNA adducts, and fluorescent aromatic compounds in bile.

Table 1. Sample characterizations

River	Site	Number of fish	When collected
St. Lawrence, Quebec	Batiscan River	48	January 1991
Margaree, Nova Scotia	Mouth	45	October 1990
Saco-Royal, Maine	Biddeford	16	December 1987
Hudson, New York	New York City Yonkers-Irvington Garrison	88	March 1990, 1993 January 1992 January 1989, 1990
Miramichi, New Brunswick	Loggieville Chatham Newcastle	85	May 1992 October 1990, May 1992 May 1992

from the Miramichi River were transported to the laboratory and maintained in clean water at 4°C at a salinity of 15 ppt for >60 days. These fish were then treated with single intraperitoneal injections of TCDD (500 ng/kg fish; n = 5) in corn oil (0.1–0.3 ml); TCB (10 mg/kg fish; n = 6) in corn oil (0.1–0.3 ml); or corn oil vehicle (0.1–0.3 ml; n = 6). The TCDD- and corn oil-treated fish were sacrificed after 10 days and the TCB-treated fish after 7 days.

RNA isolation and CYP1A mRNA analysis. We isolated total RNA from liver tissue from each fish using the method of Chomczynski and Sacchi (*27*). Approximately 20–80 mg of hepatic tissue was homogenized in RNAzol reagent (Biotecx Laboratories, Houston, Texas) in microcentrifuge tubes with Teflon pestles following the manufacturer's recommendations. RNA pellets were resuspended in diethylpyrocarbonate treated-water, and concentrations and purity of RNA were determined spectrophotometrically at 260 and 280 nm. We denatured 5 mg of total RNA from each sample with formaldehyde and electrophoresed it in 1.0% agarose gels (*28*). Gels were stained in ethidium bromide solutions (5 μg/ml) for 30 min, destained, and photographed under UV illumination to evaluate the integrity of ribosomal RNAs in each lane. Degraded RNAs were omitted from statistical analysis. Samples to be compared were electrophoresed on the same gels to minimize any differences that may be associated with intergel or interhybridization variability.

We transferred RNA from gels to Nytran nylon membranes (Schleicher and Schuell, Inc.) by capillary action overnight (*29*) and immobilized RNAs by baking at 80°C for 2 hr under vacuum. Prehybridization and hybridizations were performed at 65°C as described by Wahl et al. (*30*) using nick-translated (*31*), ^{32}P-radiolabeled Atlantic tomcod CYP1A cDNA probes. The Atlantic tomcod CYP1A cDNA probe is full length (2.6 kb) and developed from a ß-naphthoflavone-treated fish. Final wash stringencies were 1.0 × SSPE at 65°C for 60 min. Autoradiography was performed with intensifying screens at -70°C for 1 hr to 2 days to visualize CYP1A mRNA bands. We quantified levels of CYP1A mRNA by using the Whole Band Analysis software package available in the Millipore Bioimage Analysis System (Ann Arbor, Michigan).

To ensure equal loading of total RNA in each lane, filters were stripped of the CYP1A cDNA probe by immersion in boiling water, and filters were rehybridized exactly as described above to a rat rRNA probe, pHRR118 (*32*). Autoradiographs were visually inspected to evaluate mRNA loading levels in each lane.
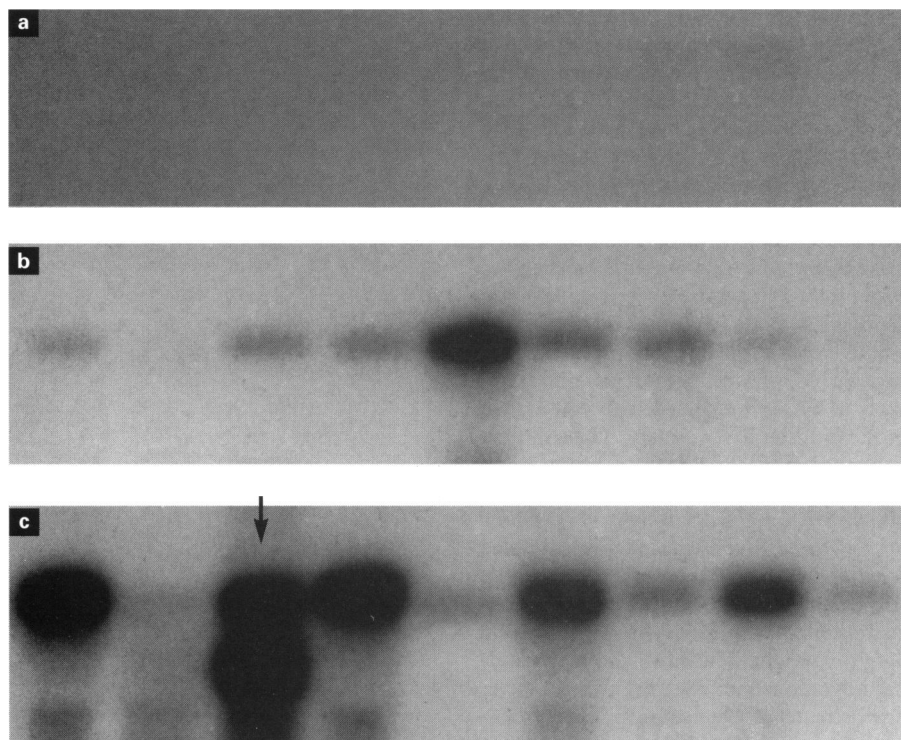

Figure 2. Representative autoradiogram of northern blot of hepatic CYP1A mRNA in Atlantic tomcod from three North American rivers. (a) Margaree River, (b) Miramichi River, (c) Hudson River. Total RNAs were separated in a 1.0% denaturing agarose gel, transferred to membrane, and hybridized to a ^{32}P-radiolabeled, full-length, Atlantic tomcod CYP1A cDNA probe. One sample from the Hudson River (arrow) exhibited a genetic polymorphism in CYP1A mRNA.

Fluorescent aromatic compounds in bile. Bile from a subset of tomcod (n = 5–18) collected from four of the river systems was analyzed by the HPLC/UVF method of Krahn et al. (*33*) using a wavelength pair of 380 nm (excitation) and 430 nm (emission), which is appropriate for high molecular weight (4–6 aromatic ring) PAHs. Bile was not available from fish from Maine. The levels of biliary FACs are reported on the basis of milligrams biliary protein. Studies have shown that normalization to levels of biliary protein can, in part, account for differences in feeding status of fish (*34*). The concentrations of biliary protein were measured by the method of Lowry et al. (*35*) using bovine serum albumin as the standard.

^{32}P-postlabeling analysis of hepatic DNA adducts. We conducted ^{32}P-postlabeling analysis on a subset of the tomcod samples from each river system. Hepatic DNA was isolated by a phenol extraction method (*22*), and the nuclease P1 version of ^{32}P-postlabeling assay was conducted essentially according to Gupta and Randerath (*36*). We used salmon sperm DNA as a negative control, and the [γ^{32}P]ATP was prepared according to Gupta and Randerath (*36*).

Polyethyleneimine-cellulose thin-layer chromatography (TLC) of the ^{32}P-labeled adducts was done on laboratory-prepared TLC sheets (*36*). The solvent systems used were as follows: D1, 1.0 M sodium phosphate, pH 6.0; D2 was omitted; D3, 7.65 M urea and 4.32 M lithium formate, pH 3.5; and D4, 7.65 M urea, 1.44 M lithium chloride, and 0.45 M Tris, pH 8.0. The ^{32}P-labeled adducts were located by autoradiography and quantitated by liquid scintillation counting [Saco/Royal rivers, only for storage phosphor imaging (*37*)]. Total nucleotides were determined from one-dimensional TLC of 3′, (^{32}P)5′-labeled mononucleotides using 0.24 M ammonium sulfate in 8 mM sodium phosphate, pH 7.4, as a solvent and quantitation of the deoxyguanosine 3′,(^{32}P)5′-bisphosphate spot. Adduct levels are expressed as nanomoles adducts/mole bases.

Statistical analyses. The data on levels of CYP1A mRNA are expressed as means ± 95% CI. Heterogeneity of variance in the mean CYP1A mRNA levels was detected by Bartlett's test for both untransformed data and log (x + 0.001)-transformed data. Therefore, we analyzed the data using the Kruskal-Wallis nonparametric analysis of variance test (SYSTAT) (*38*) followed by the nonparametric multiple range test (*39*). Differences among means were considered significant at $p \leq 0.05$.

The data for biliary FACs and hepatic DNA adducts are expressed as means ± SEM. The data were log transformed to reduce heteroscedasticity and then analyzed by one-way analysis of variance and Fisher's protected least significant differ-
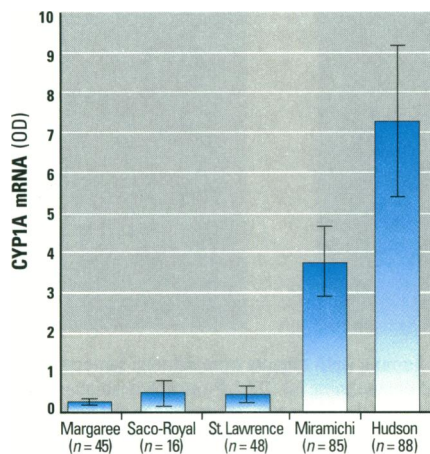
Figure 3. Levels of hepatic CYP1A mRNA in Atlantic tomcod collected from five river systems along the Atlantic coast of North America. Levels of CYP1A mRNA are shown as optical density (OD) units. The numbers in parentheses indicate the number of samples analyzed from that river. Vertical lines represent 95% confidence intervals around the arithmetic means of the raw data.
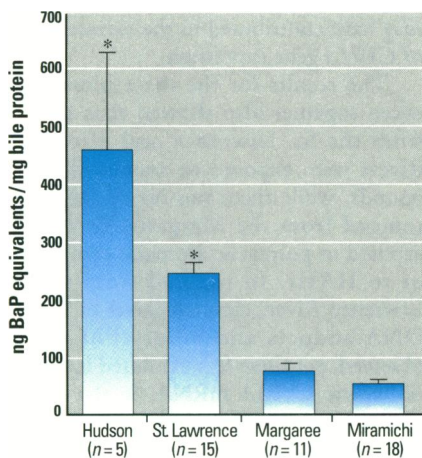

Figure 5. Representative autoradiogram of ^{32}P-labeled hepatic DNA digests from Atlantic tomcod collected from five river systems of the North American Atlantic coast. (a) St. Lawrence River, (b) Margaree River, (c) Miramichi River, (d) Saco River, and (e) Hudson River. The origin is located at the bottom-left corner. The hepatic DNA was analyzed by the nuclease P1 version of the ^{32}P-postlabeling assay.

Figure 4. Concentrations of fluorescent aromatic compounds in bile of Atlantic tomcod collected from four river systems of the North American Atlantic coast. Values are means ± SE. (*)Significantly different from values for tomcod from the Margaree and Miramichi Rivers.

ence test using SuperANOVA 1.11 software (Abacus Concepts, Berkeley, California). Values were considered significantly different if p and the F ratio had a significance of ≤ 0.05.

Results

Levels of CYP1A mRNA were significantly higher in tomcod from the Hudson River than in fish from all other rivers except the Miramichi (Fig. 2). The levels of CYP1A mRNA in tomcod from the Hudson River were 28.3-fold higher than in fish from the Margaree River and 16-fold higher than in fish from the Saco/Royal rivers, Maine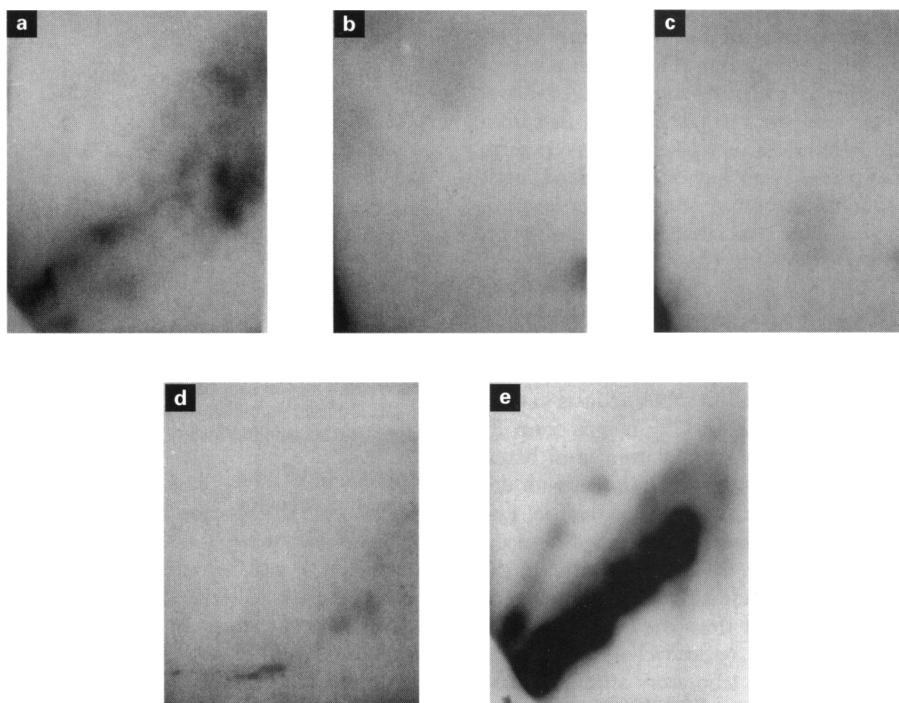, or the St. Lawrence River (Fig. 3). The levels of CYP1A mRNA in tomcod from the Miramichi River were significantly higher than in fish from the St. Lawrence (8.3-fold), Saco/Royal (8.5-fold), and Margaree (14.4-fold) rivers. A noteworthy feature of *CYP1A* gene expression was the high variability in CYP1A mRNA levels among individuals within all populations. This included fish from the Hudson and Miramichi, in which mean levels of *CYP1A* gene expression were very high, and the Margaree, in which overall levels of CYP1A mRNA were extremely low. For example, in the Hudson River fish, 3% of the fish exhibited CYP1A mRNA levels that were less than the mean value observed in tomcod from the Margaree River. The coefficient of variation for the Hudson River population was 118%, whereas it was 138% for the Margaree River collection.

To estimate exposure of tomcod to polycyclic aromatic compounds, concentrations of FACs in bile were determined by HPLC with fluorometric detection at a wavelength pair appropriate for high molecular weight (3–5 benzenoid rings) polycyclic aromatic compounds, such as benzo[a]pyrene. The concentrations of biliary FACs in tomcod from the Hudson River and the St. Lawrence River were significantly greater than the concentrations in tomcod from either the Miramichi or Margaree rivers (Fig. 4). No significant dif-

ferences in concentrations were observed between tomcod from the Hudson and St. Lawrence Rivers or between fish from the Miramichi and Margaree rivers.

The level of hydrophobic DNA adducts in livers of a subset of tomcod was determined by the nuclease P1 version of the ^{32}P-postlabeling assay. The chromatograms (Fig. 5) of ^{32}P-labeled hepatic DNA digests from fish from the Hudson and St. Lawrence rivers exhibited diagonal radioactive zones consisting of multiple, overlapping spots. In fish from the Hudson River there was also evidence of a faster-migrating (upper) diagonal radioactive zone. The upper zone was less intense and represented 20% of total DNA adducts. The upper zone was rarely detected in fish from the St. Lawrence River. Diagonal radioactive zones were not observed in fish from the Saco/Royal, Margaree, or the Miramichi rivers.

The levels of total DNA adducts in tomcod from the Hudson River were significantly greater than the levels in tomcod from all other sites (Fig. 6) and the levels of adducts in fish from the St. Lawrence River were significantly elevated in comparison to fish from the Saco/Royal, Miramichi and Margaree rivers. The levels of total adducts in tomcod from the Saco/Royal rivers (13 nmol/mol bases) were significantly greater than in fish from the Miramichi (6 nmol/mol bases) and Margaree rivers (<3 nmol/mol bases),

while the adduct levels in fish from the latter two rivers were not significantly different from each other. The higher levels of adducts in tomcod from the Saco/Royal rivers compared to the levels in fish from the Miramichi and Margaree rivers were due primarily to higher background levels of radioactivity and not to higher levels of radioactivity associated with distinct spots on the chromatograms.

The extent of variability in levels of DNA adducts in tomcod from the Hudson River, which included fish collected from the same site on the same day, was very high. For example, levels of DNA adducts in tomcod from New York City ranged from 27 nmol/mol bases to 370 nmol/mol bases. This variation did not correspond with differences in either size or sex of the fish. Less variability was observed in tomcod from the less-contaminated sites; for example, the ranges in DNA adduct levels was 10–14 and <3–3 in tomcod from the Saco/Royal and Miramichi rivers, respectively.

In an initial laboratory study, tomcod from the Miramichi River exposed to either TCDD or TCB showed no evidence of elevated adduct levels compared to untreated fish or fish treated with the solvent vehicle, corn oil (Fig. 7). The mean levels of adducts was <13 nmol/mol in all groups, and there were no significant differences in adduct levels between any of the treatment groups. None of the fish in these treatment groups showed levels of DNA adducts significantly elevated above those of tomcod collected from the Margaree River, where tomcod are exposed to bleached kraft mill effluent.

Discussion

The results of the present study show that of the populations of tomcod sampled along the northeast North American coast,
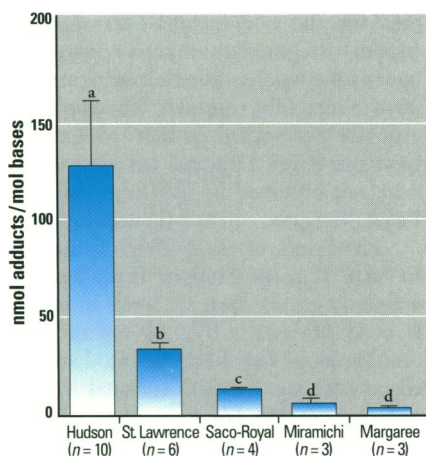

Figure 6. Levels of hepatic DNA adducts in Atlantic tomcod collected from five river systems along the North American Atlantic coast. Values are means ± SE. Common letters indicates means that were not significantly different.
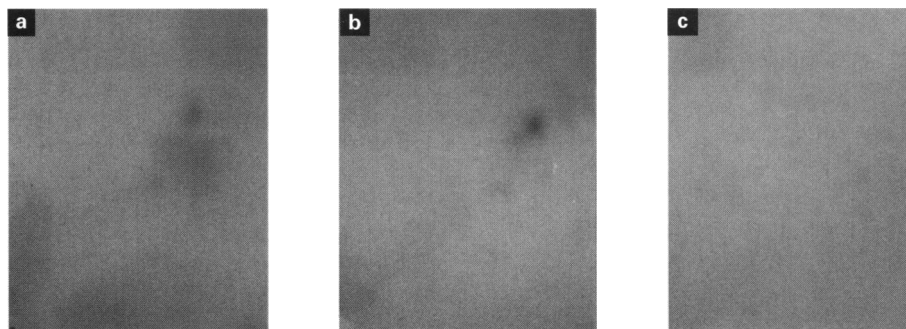

Figure 7. Representative autoradiograms of ^{32}P- labeled hepatic DNA digests from Atlantic tomcod injected intraperitoneally with single doses of two chlorinated compounds: (a) 3,3',4,4'-tetrachlorobiphenyl and (b) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or (c) with corn oil vehicle. The origin is located at the bottom-left corner. The hepatic DNA was analyzed by the nuclease P1 version of the ^{32}P-postlabeling assay.

tomcod from the Hudson River exhibit the highest levels of hepatic CYP1A mRNA, biliary FACs, and hepatic DNA adducts. Previous studies have shown elevated hepatic concentrations of total PCBs (5) and individual PCB congeners (40) in tissues of tomcod from the Hudson River. Although hepatic concentrations of dibenzodioxins and dibenzofurans have not been measured in tomcod tissues, elevated concentrations of these HAHs are detected in bottom-dwelling invertebrates, such as crabs and lobsters (12), and in the mid-water-dwelling striped bass (41) from the lower Hudson River estuary. In addition, Hudson River tomcod are exposed to high levels of nonhalogenated aromatic hydrocarbons during their life cycle. From late winter to mid-fall, densities of adult tomcod are high in the lower Hudson River estuary off New York City (42) and in Newark Bay (43), at which time fish are exposed to the highest sediment-borne concentrations of PAHs and dibenzodioxins and furans in the estuary (11). These observations are consistent with the present results showing substantially elevated hepatic CYP1A mRNA, which is induced by certain HAHs and by PAHs in tomcod (40), and the increased levels of biliary FACs and hepatic DNA adducts as measured by ^{32}P-postlabeling, which are indicative of exposure to polycyclic aromatic compounds. Previous studies (17,44) with several fish species have shown that many of the FACs in bile of fish from contaminated areas are metabolites of PAHs. The identity of the individual DNA adducts detected in tomcod is unknown; however, studies (22,23) with other fish species have shown that carcinogenic PAHs and extracts of sediments from sites contaminated with polycyclic aromatic compounds yield adducts with chromatographic characteristics similar to those observed in tomcod. Thus, the results with the three biomarkers measured in the present study showed that tomcod from the

Hudson River were exposed to polycyclic aromatic compounds and suggest that while the highly induced level of CYP1A mRNA expression in Hudson River tomcod may have resulted partially from increased exposure to HAHs, which are common xenobotics in the Hudson River estuary, increased exposure to PAHs also may have contributed to the elevated levels of *CYP1A* gene expression.

The results for the three biomarkers taken together also showed that tomcod from the St. Lawrence and Miramichi Rivers were exposed to xenobiotic compounds, while there was no evidence that tomcod from the Margaree River were exposed to polycyclic aromatic compounds or to HAHs. In tomcod from the St. Lawrence River, elevated levels of hepatic DNA adducts and biliary FACs were observed, yet these fish exhibited low levels of hepatic CYP1A mRNA. In the present study, tomcod were sampled in January near their spawning grounds in the Batiscan River, a tributary of the St. Lawrence River, and in other studies (45; Wirgin et al., unpublished data) we have demonstrated that CYP1A mRNA inducibility in tomcod depends on reproductive state. Inducibility of CYP1A mRNA in both male and female tomcod is reduced during spawning, although the exact timing of the inhibition of inducibility differs between the two sexes. This may account for the relatively low levels of CYP1A mRNA in St. Lawrence River tomcod. Recently, however, we have demonstrated in controlled laboratory experiments that levels of CYP1A mRNA were rapidly induced in tomcod treated with single intraperitoneal injections of ß-napthoflavone or benzo[*a*]pyrene and returned to near basal levels within 72 hr of treatment, reflecting the rapid metabolism of these compounds in fish hepatic tissues (Wirgin et al., unpublished data). In the St. Lawrence River, adult tomcod make feeding migrations from these river-

ine breeding grounds into the estuary, at which time they would be exposed to xenobiotics. Results from previous studies with other fish species suggest that both hepatic DNA adducts (23) and biliary FACs (34) decline less rapidly than do hepatic CYP1A mRNA levels in fish treated with PAHs, suggesting that if St. Lawrence River tomcod were exposed intermittently to PAHs just before sampling, they could exhibit the pattern of induction of the biomarkers that we observed.

The finding that tomcod from the Miramichi River exhibited high levels of CYP1A mRNA induction (16 times as great as in fish from the Margaree River), yet relatively low levels of hepatic DNA adducts and FACs in their bile, suggests significant exposure to xenobiotics other than PAHs. Levels of *CYP1A* gene expression may be induced by a variety of aquatic environmental toxicants, including some dibenzodioxins, dibenzofurans, coplanar PCB congeners, and PAHs. We have demonstrated under controlled laboratory conditions that compounds from all of these classes of xenobiotics are effective inducers of CYP1A mRNA at environmentally relevant concentrations (40,46; Wirgin et al., unpublished data). Furthermore, given the expected finding of no hepatic DNA adducts detected in Atlantic tomcod exposed to TCDD and TCB at the doses used in this study, it is likely that elevated levels of CYP1A mRNA in tomcod from the Miramichi River resulted from exposure to xenobiotics other than PAHs. In an earlier study (46), it was demonstrated that tomcod caged in the effluents of a bleached kraft mill on the Miramichi River exhibited significantly elevated levels of CYP1A mRNA compared to fish caged at an up-river site, and a gradient in levels of CYP1A mRNA expression was observed in tomcod caged at two sites downstream of the mill. Relatively high levels of HAHs have been detected in shellfish and finfish collected downstream of the mill. For example, levels of 22 pg/g body weight TCDD and 174 pg/g body weight TCDF have been recorded in lobster hepatopancreas and 16 pg/g body weight TCDD and 56 pg/g body weight TCDF in whole bodies of striped bass from the Miramichi estuary (47). Thus, elevated levels of CYP1A mRNA in tomcod from the Miramichi River probably resulted from exposure to HAHs or other constituents present in bleached-kraft mill effluent discharged into the river (46). Moreover, these results emphasize the advantage of using a suite of biomarkers in assessing contaminant exposure in species exposed to anthropogenic chemicals.

The elevated levels of DNA adducts in Hudson River tomcod compared to tomcod from other rivers and our earlier finding of an activated K-*ras* oncogene in tomcod liver tumor DNA (8) suggest that DNA damage at relevant loci by chemical carcinogens is occurring in Hudson River tomcod. Furthermore, tomcod from the Hudson River exhibit one of the highest prevalences of neoplasms of any outbred population; the prevalence of neoplasms exceeds 50% in one-year-old fish and 90% in two year olds (5). In comparison, tomcod from more pristine rivers show much lower prevalences of liver lesions, <5% in tomcod from the moderately polluted Pawcatuck River in Connecticut-Rhode Island and the Saco River, Maine (6). There are no reports of hepatic tumors in tomcod from the Canadian Maritime Provinces (S. Courtenay, personal observation) or the St. Lawrence River, although a low prevalence (1%) of epidermal masses or nodules has been observed in tomcod spawning in the St. Anne River, a tributary of the St. Lawrence River (Yves Mailhot, MLCP Quebec, personal communication). Additionally, the prevalence of hepatocellular carcinomas increases directly with age and size of the fish, providing further evidence for an environmental etiology to this disease (5).

Although the present results support the hypothesis that hepatic neoplasms in tomcod from the Hudson River are chemically induced, three other factors may also contribute to the observed high prevalence of hepatic neoplasia in the Hudson River population. First, the Hudson River supports the southernmost spawning population of Atlantic tomcod. This may result in thermal stress to tomcod during the warmer months and the possibility of an increased growth rate during the spring and fall, factors that may accelerate the neoplastic process as evidenced by the findings of a significantly increased incidence of hepatic neoplasms in rainbow trout (*Oncorynchus mykiss*) exposed to 7,12-dimethylbenzanthracene at elevated water temperature (48) and the increase in liver cell proliferation in *Poeciliopsis* exposed to heat stress (49). Second, the Hudson River population may support a genetically distinct population of tomcod that is predisposed to neoplasia as indicated by the finding of a transcribed genetic polymorphism in the structure of the *CYP1A* gene (500 bp deletion) in 10% of Hudson River tomcod, which is absent in tomcod from other populations (50). Third, tomcod from the Hudson River have elevated tissue concentrations of PCBs (40,51), which can serve as promoters of carcinogenesis (52); however, in a previous study no correlation was found between total hepatic PCB concentrations and the prevalence of hepatic

lesions in tomcod from the Hudson River population (5). The above factors show the need for further studies to delineate the possibility of increased genetic susceptibility of the Hudson River population to neoplasia, possible interactions between genotypes at critical loci, and interactive effects of the environmental xenobiotics (e.g., PAHs and HAHs) in the initiation and promotion of hepatic neoplasia in the Hudson River tomcod population. Recent studies have demonstrated a dose–response relationship of TCDD promotion on biochemical, cell proliferative, and histological endpoints associated with hepatocarcinogenesis in rats initiated with diethylnitrosamine (53).

In summary, we have demonstrated, using a suite of biomarkers, that there are substantial differences in exposure of tomcod from sites on the northeast North American coast to xenobiotics that induced CYP1A mRNA, gave rise to DNA adducts, as measured by ^{32}P-postlabeling, and increased levels of biliary FACS, a measure of exposure to PAHs. The results also demonstrate that the use of multiple biomarkers can provide a degree of specificity in assessing xenobiotic exposure, as evidenced by the marked difference in the response of the individual biomarkers, specifically induction of CYP1A mRNA and no increase in DNA adducts, or FACs, in tomcod exposed to bleached kraft mill effluent in the Miramichi River compared to tomcod from the other sites. In addition, tomcod from the Hudson River exhibited the highest levels of hepatic CYP1A mRNA, hepatic DNA adducts, and biliary FACs, which is consistent with previous studies (5,6) showing high prevalences of hepatic neoplasms in tomcod from this estuary, as well as consistent with the association between exposure to PAHs and prevalence of neoplasms observed in other wild fish species (9).

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