Pergamon

Environmental Toxicology and Chemistry, Vol. 14, No. 4, pp. 589–595, 1995
Copyright © 1995 SETAC
Printed in the USA
0730-7268/95 $9.50 + .00

0730-7268(94)00236-3

COMPARISONS OF THE EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN ON CHEMICALLY IMPACTED AND NONIMPACTED SUBPOPULATIONS OF *FUNDULUS HETEROCLITUS*: II. METABOLIC CONSIDERATIONS

RUTH PRINCE* and KEITH R. COOPER
Joint Graduate Program in Toxicology, Environmental and Occupational Health Sciences Institute, Rutgers University, 681 Frelinghuysen Rd., P.O. Box 1179, Piscataway, New Jersey 08855-1179

(*Received* 22 *July* 1993; *Accepted* 12 *July* 1994)

Abstract—A previous paper reported resistance to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity in a subpopulation of *Fundulus heteroclitus* inhabiting the chemically impacted estuary of Newark Bay, New Jersey, as compared to a chemically nonimpacted subpopulation of *F. heteroclitus* in Tuckerton, New Jersey. This paper reports the results of toxicokinetic and metabolic investigations. The rates of [^{3}H]TCDD elimination following a single dermal treatment of [^{3}H]TCDD were similar between the Newark and Tuckerton *F. heteroclitus* subpopulations, as were the capabilities to metabolize TCDD. The Newark *F. heteroclitus* subpopulation consistently exhibited elevated hepatic P4501A catalytic activity (two- to fivefold), as measured by ethoxyresorufin *O*-deethylase (EROD), compared to the Tuckerton subpopulation. EROD activity in Newark *F. heteroclitus* could not be induced by dermally administered [^{3}H]TCDD, ranging from 0.020 to 8.0 ng/g [^{3}H]TCDD. The Tuckerton fish exhibited a linear increase in EROD activity at 96 h following dermal administration of [^{3}H]TCDD, ranging from 0.80 to 8.0 ng/g [^{3}H]TCDD. The maximum mean induction in the Tuckerton fish was 30-fold greater than the Tuckerton vehicle control mean. These data indicate that the Newark fish were resistant to both the toxicity of TCDD and the ability of TCDD to induce P4501A activity, which may imply an alteration in the Ah receptor complex of Newark *F. heteroclitus*.

Keywords—P4501A activity Chemical resistance Environmental biomonitoring *Fundulus heteroclitus* TCDD

INTRODUCTION

A subpopulation of *Fundulus heteroclitus* inhabiting the chemically impacted estuary of Newark Bay, New Jersey, was shown to be resistant to the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), as compared to a nonimpacted subpopulation of *F. heteroclitus* in Tuckerton, New Jersey [1]. The studies reported below were designed to investigate this resistance to TCDD toxicity, which was observed in the chemically impacted killifish subpopulation.

Studies were carried out to compare the elimination rates of TCDD and the ability to metabolize TCDD between the Newark and Tuckerton *F. heteroclitus* subpopulations. It was hypothesized that the Newark fish (the resistant subpopulation) would be capable of more rapid TCDD metabolism and elimination than the Tuckerton fish. There is some mammalian evidence that an altered capability to both eliminate and metabolize TCDD is associated with an altered sensitivity to TCDD. The guinea pig is the most sensitive mammal to acute TCDD toxicity, exhibiting an LD50 value of approximately 1 ng/g TCDD [2]. The guinea pig exhibits a relatively long TCDD elimination half-life of approximately 13 weeks, as compared to the 2- to 5-week elimination half-lives of the less sensitive rat, mouse, and hamster [3,4]. It has also been demonstrated that 70 to 90% of the radioactivity in guinea pig fecal samples represented unmetabolized TCDD, following a single [^{3}H]TCDD administration [3]. This is in contrast to rat, mouse, and hamster feces, in which 15 to 35% of the radioactivity represented unmetabolized TCDD [4,5]. This type of information is not available for fish.

The monooxygenase system in fish has been shown to exhibit an induction response to many classes of organic contaminants [6]. This was the basis for proposals that this biochemical response be used as a biomarker in monitoring the exposure of fish to such aquatic pollutants [7]. Many inducers of fish P4501A activity are also aquatic pollutants of major concern, including PAHs, PCBs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans, and other halogenated organic compounds [6]. A number of studies have demonstrated that P4501A activity, as measured by ethoxyresorufin *O*-deethylase (EROD) and arylhydrocarbon hydroxylase (AHH) activities, is elevated in fish collected from polluted waters, as compared to fish collected from reference sites [6,8]. This is a biomarker of exposure and is not necessarily considered a toxic effect.

Further studies were carried out to test the hypotheses that (a) the chemically impacted Newark *F. heteroclitus* subpopulation would exhibit elevated levels of P4501A activity as compared to the nonimpacted Tuckerton subpopulation, and (b) the P4501A activity of the Newark fish would be

*To whom correspondence may be addressed.
The current address of Ruth Prince is Division of Science and Research, New Jersey Department of Environmental Protection and Energy, CN 409, Trenton, NJ 08625-0409.

resistant to induction by TCDD treatment, as compared to the Tuckerton fish. These hypotheses were based on the observations of resistance to TCDD toxicity in the Newark embryos and adults, as well as the elevated liver-to-body-weight ratios observed in the Newark adults, as previously reported [1].

MATERIALS AND METHODS

Chemicals

Both [U-^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin (20.5 Ci/mmol, purity of 94%) and [1,6-^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin (40.0 Ci/mmol, purity of >97%) were purchased from Chemsyn Science Laboratories (Lenexa, KS). Resorufin, 7-ethoxyresorufin, NADPH, Tris-HCl, and 3-aminobenzoic acid ethyl ester were purchased from Sigma Chemical Company (St. Louis, MO). Biorad protein assay dye reagent was purchased from Biorad Laboratories (Richmond, CA). Acetone (HPLC grade), dimethyl sulfoxide (spectra-analyzed), and methanol were purchased from Fisher Scientific (Springfield, NJ). Liquiscint® was purchased from National Diagnostics (Somerville, NJ).

Animals

The collection and maintenance of *F. heteroclitus* was conducted as previously described [1].

Liquid scintillation counting

Procedures were as previously described [1].

Statistical analysis

Kruskal–Wallis and Newman–Keuls multiple comparison tests were carried out using the Kwikstat® statistical data analysis program (Texasoft, Cedar Hill, TX). Data sets were first assessed for normality of distribution using the Kwikstat program to determine which type of test was appropriate. The statistical significance for all tests was set at $p \leq 0.05$. For the EROD assay, slopes were calculated by linear regression analysis. The exponential models for the toxicokinetics data were generated by the DeltaGraph professional program (DeltaPoint, Inc., Monterey, CA).

[^{3}H]TCDD treatment

For all the studies described below, the TCDD dermal treatment consisted of a [^{3}H]TCDD/DMSO drop, delivered by Eppendorf® pipette, directly onto the fish abdomen. The dose volume was 5 μl/g fish, adjusted according to a weight range (0.5–1.4 g = 5 μl; 1.5–2.4 g = 10 μl, etc.). The fish abdomens were wiped with Kimwipes® prior to TCDD application to remove the mucous layer; following application, they were dried under a warm stream of air.

[^{3}H]TCDD elimination studies

Results of a preliminary 28-d toxicokinetic study indicated that the most rapid changes in tissue concentrations of [^{3}H]TCDD occurred in the 96-h period following treatment (data not presented). In the 96-h study, all fish were treated with 5 ng/g [^{3}H]TCDD. Eight Newark and eight Tuckerton fish each were killed at the following times post-TCDD treatment: 0, 1, 8, 24, and 96 h. Each fish was then dissected to the following tissues: belly flap (site of application), liver, gallbladder, feces, remaining viscera (including intestine, spleen, pancreas, heart), gonads, fillets, carcass, and blood. The blood was collected by severing the tail, and inserting an Eppendorf® pipette into the caudal vein to collect 1 μl of blood. All tissues were weighed and prepared for liquid scintillation counting to determine the amount of [^{3}H]TCDD present in each tissue. Approximately 1 h was required to dissect the fish; therefore, each time point is ±1 h.

[^{3}H]TCDD metabolism studies

All fish were treated with 30 ng/g [^{3}H]TCDD. Fifteen Newark and 15 Tuckerton fish each were killed at 1 and 8 h post-TCDD treatment, and all gallbladders and livers were removed. Three pools of gallbladders (5 each) and three 100 mg aliquots of individual livers were each placed in 1 ml methanol, for each group. These were prepared for HPLC analysis by homogenization, centrifugation at 10,000 *g* in 0.45 μm Microfilterfuge® tubes (Rainin Instrument Company, Inc., Woburn, MA), and 0.2 μm filtration. The gallbladder and liver samples (two per group) were analyzed by reverse-phase HPLC (Kratos, Spectroflow 400) on a Beckman Ultrasphere-ODS (4.6 mm × 25 cm) column with a 25-min linear-gradient elution of 100% water to 100% methanol, followed by a 20-min isocratic elution of 100% methanol, at a flow rate of 1 ml/min. Fractions of the column effluent were collected at 1-min intervals for 45 min, and the radioactivity was determined by liquid scintillation counting.

P4501A activity studies

The *F. heteroclitus* adults were collected from the Newark and Tuckerton sites in October (study I) and December (study II), 1990; April (study III) and December (study IV), 1991; and August (study V), 1992.

Sixty fish per group were utilized in studies I through III. The treatment groups consisted of no treatment (control), DMSO (vehicle control), and 0.020 (study I), 0.10 (study II), and 0.80 (study III) ng [^{3}H]TCDD/g fish. At 24 and 96 h following [^{3}H]TCDD treatment, 30 fish per group were killed, and the livers were removed and pooled for microsome preparation. Eight and six fish per group, respectively, were utilized in studies IV and V. In study IV, the treatment groups consisted of no treatment (control), DMSO (vehicle control), 5.7 and 8.1 ng [^{3}H]TCDD/g fish. Study V was a repetition of studies I and II, with treatment groups consisting of no treatment (control), DMSO (vehicle control), 0.020 and 0.10 ng [^{3}H]TCDD/g fish. However, the actual absorbed mean TCDD dose at 24 h was 0.160 and 0.290 ng/g TCDD. At 96 h following TCDD treatment, all fish in each group were killed. Microsomes were prepared from individual livers.

Microsomes were prepared by differential centrifugation as previously described [9], and stored at −80°C until the EROD assay could be performed. At the same time points, five fish per group were killed and prepared for liquid scintillation counting to determine [^{3}H]TCDD absorption. The protein content of the microsomes was determined using the Bio-Rad protein reagent [10] with bovine serum albumin as the standard. The 7-ethoxyresorufin *O*-deethylase (EROD) activity [11] was measured, following incubation to 25°C, by

a direct fluorometric technique on either a Shimadzu (model RF-540) spectrofluorometer or a Perkin-Elmer LS-5B luminescence spectrometer, using either 2.5 mg microsomal protein (pooled microsome samples) or 0.03 to 0.09 mg microsomal protein (individual microsome samples) per reaction mixture. The detection limit ranged from 12 to 30 pmol resorufin.

RESULTS

Following a preliminary 28-d study (data not shown), a 96-h study was carried out to compare the rates of elimination of [^{3}H]TCDD from Newark and Tuckerton *F. heteroclitus* tissue compartments, following a single dermal treatment of 5 ng/g [^{3}H]TCDD. The apparent half-lives ($t_{1/2}$) of elimination of [^{3}H]TCDD from the liver, gallbladder, carcass, and blood of Newark and Tuckerton *F. heteroclitus* are presented in Table 1. These were estimated by a first-order exponential model and calculated from values that were normalized on a weight basis (ng [^{3}H]TCDD/g organ). The half-lives were similar in Newark and Tuckerton *F. heteroclitus*, with the exception of the blood $t_{1/2}$s. The liver, gallbladder, and carcass half-lives were 12, 9, and 8 h shorter, respectively, in the Newark fish, than in the Tuckerton fish. The blood $t_{1/2}$ was significantly shorter in the Newark fish, by 42 h, than in the Tuckerton fish.

Following the toxicokinetic studies, a study was carried out to compare the percentages of unmetabolized TCDD in the gallbladders and livers of Newark and Tuckerton *F. heteroclitus*, at 1 and 8 h following a single dermal treatment of 30 ng/g [^{3}H]TCDD. Results are presented in Table 2. Two samples from each subpopulation were analyzed for each time point. No difference existed between Newark and Tuckerton *F. heteroclitus* in the amounts of unmetabolized TCDD present in either the gallbladders or the livers at either 1 or 8 h.

Studies were then carried out to compare hepatic EROD activity in Newark and Tuckerton *F. heteroclitus* following dermal [^{3}H]TCDD treatment. Three preliminary studies (I–III) utilized pooled microsome preparations, and these initially involved TCDD concentrations that have been reported under field conditions (tissue and sediment concentrations), including those in Newark Bay. Later studies (IV–V) utilized individual microsome preparations and involved TCDD concentrations that were toxic to the Tuckerton fish, or repeated preliminary studies. Biologically significant EROD induction was defined as at least a twofold increase in activity over that of control.

Table 1. Apparent $t_{1/2}$ for elimination of [^{3}H]TCDD from Newark and Tuckerton *F. heteroclitus*, 96-h study

Organ	Apparent $t_{1/2}$ (h)[a]	
	Newark	Tuckerton
Liver[b]	34	46
Gallbladder[c]	19	28
Carcass	53	61
Blood	8	50

[a]Apparent half-lives of elimination of [^{3}H]TCDD were estimated by a first-order single exponential model ($t_{1/2} = 0.693/K_{elim}$). Half-lives were calculated from ng [^{3}H]TCDD/g organ (N = 5–8 per time point).
[b]In a separate study involving HPLC analysis, a small percentage (0.3–15%) of liver TCDD-derived ^{3}H was associated with a more polar compound than the parent TCDD.
[c]In a separate study involving HPLC analysis, the majority (69–94%) of gallbladder TCDD-derived ^{3}H was associated with a more polar compound than the parent TCDD.

Table 2. Percent unmetabolized TCDD in Newark and Tuckerton *F. heteroclitus* liver and gallbladder

Time post-TCDD treatment (30 ng/g)	% Unmetabolized TCDD	
	Newark	Tuckerton
Gallbladder[a]		
1 h	20	20
	31	12
8 h	15	7
	7	6
Liver[b]		
1 h	85	98
	87	93
8 h	96	99
	97	99

[a]Each gallbladder sample was a pool of 5 individual gallbladders. Two samples were analyzed by HPLC for each time point. Percentages were calculated from total dpm above background. The retention time of the parent compound was 42 min ([^{3}H]TCDD-spiked control gallbladder).
[b]Each liver sample was a 0.10 g aliquot from an individual liver. Two samples were analyzed by HPLC for each time point. Percentages were calculated from total dpm above background. The retention time of the parent compound was 42 min ([^{3}H]TCDD-spiked control liver).

Results of studies I through III are summarized in Table 3. The Newark *F. heteroclitus* no treatment and vehicle control EROD activity values were consistently elevated (two- to fivefold) as compared to the same Tuckerton values, with the exception of one value (96-h no treatment control, study I). The EROD control activity in the Newark fish is also illustrated (Fig. 1) as a percent of the EROD control activity in the Tuckerton fish.

The EROD activity in the Tuckerton fish was induced (2.7-fold) at 96 h following the 0.80 ng/g [^{3}H]TCDD treatment only, in study III (Table 3). There was no induction of EROD activity in the Newark fish in studies I through III. The EROD activity values were depressed in the Newark fish at both 0.020 and 0.10 ng/g [^{3}H]TCDD (studies I–II). The [^{3}H]TCDD absorption was approximately equivalent between Newark and Tuckerton *F. heteroclitus* in studies I through III, with approximately 50% of the administered dose present in the livers at 24 h (not shown).

Results of studies IV and V are summarized in Table 4. The Newark *F. heteroclitus* no treatment and vehicle control EROD activity means were significantly greater than the same Tuckerton means. This is illustrated in Figure 1. The EROD activity in the Tuckerton fish was significantly induced at 96 h following 5.7 and 8.1 ng/g [^{3}H]TCDD treat-

Table 3. Hepatic EROD activities (pmol resorufin/min/mg protein) in pooled Newark and Tuckerton *F. heteroclitus* treated with [^{3}H]TCDD

	EROD activities			
	Newark		Tuckerton	
	24 h	96 h	24 h	96 h
Study I (Oct. 1990)				
No treatment control	660[a]	140	200	110
Vehicle control	360	460	ND[b]	120
0.020 ng/g [^{3}H]TCDD	120	ND	140	90
Study II (Dec. 1990)				
No treatment control	390	420	180	ND
Vehicle control	360	390	160	ND
0.10 ng/g [^{3}H]TCDD	240	ND	120	160
Study III (April 1991)				
No treatment control	480	500	240	260
Vehicle control	530	750	140	140
0.80 ng/g [^{3}H]TCDD	600	680	450	700

[a]Each value was determined from the microsomal fraction of pooled livers ($N = 25$ livers per value).
[b]Nondetectable.

Table 4. Mean 96-h hepatic EROD activities (pmol resorufin/min/mg protein) in individual Newark and Tuckerton *F. heteroclitus* treated with [^{3}H]TCDD

	EROD activities	
	Newark	Tuckerton
Study IV (Dec. 1991)		
No treatment control	306 ± 158[a]	138 ± 84.9[b]
Vehicle control	371 ± 148	117 ± 72.5[b]
5.7 ng/g TCDD	498 ± 255	2,284 ± 719[b,c]
8.1 ng/g TCDD	180 ± 128[d]	3,536 ± 1,246[b,c]
Study V (Aug. 1992)		
No treatment control	804 ± 260[d]	205 ± 136[b]
Vehicle control	1,460 ± 598	296 ± 156[b]
0.160 ng/g TCDD	1,298 ± 569	502 ± 323[b,c]
0.290 ng/g TCDD	1,753 ± 370[d]	386 ± 194[b]

[a]Each value is the mean ± SD of 6 to 8 individual determinations.
[b]Newark and Tuckerton means are significantly different at $p < 0.05$ (Kruskal–Wallis, Newman–Keuls multiple comparisons).
[c]Mean is significantly different from all other Tuckerton means within the study at $p < 0.05$ (Kruskal–Wallis, Newman–Keuls multiple comparisons).
[d]Mean is significantly different from all other Newark means within the study at $p < 0.05$ (Kruskal–Wallis, Newman–Keuls multiple comparisons).

ments, by 16-fold and 26-fold, respectively. There was no significant induction of EROD activity in the Newark fish in studies IV and V. The [^{3}H]TCDD absorption was approximately equivalent between Newark and Tuckerton *F. heteroclitus* in studies IV and V, with approximately 30 to 60% of the administered dose present in the livers at 24 h (not shown).

A dose–response curve of 96-h EROD activity (as a percent of vehicle control) versus the 24-h absorbed TCDD dose (ng/g) in Newark and Tuckerton *F. heteroclitus* is presented in Figure 2. This is a summary of studies III through V (pooled microsome values are used only for the 0.80 ng/g TCDD dose). There was no effect in EROD activity in Newark *F. heteroclitus* at any TCDD dose. The EROD activity in the Tuckerton fish exhibited a significant, linear increase with dose ($r^2 = 0.982$) beginning at approximately 0.80 ng/g TCDD, with 3,000% of vehicle control activity at approximately 8 ng/g TCDD (Fig. 2).

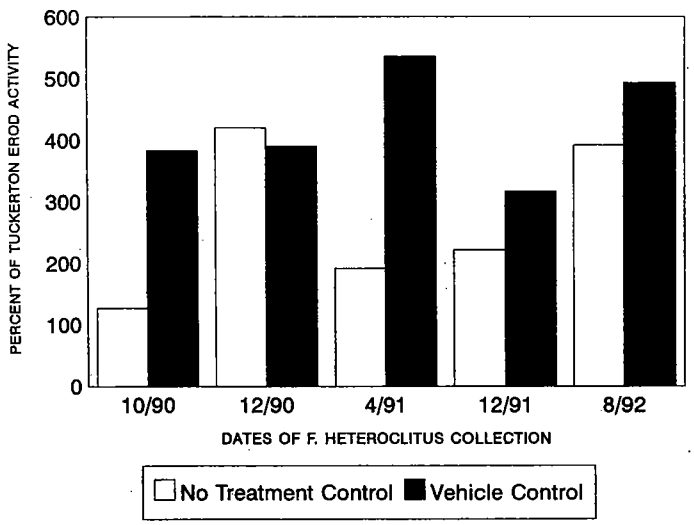
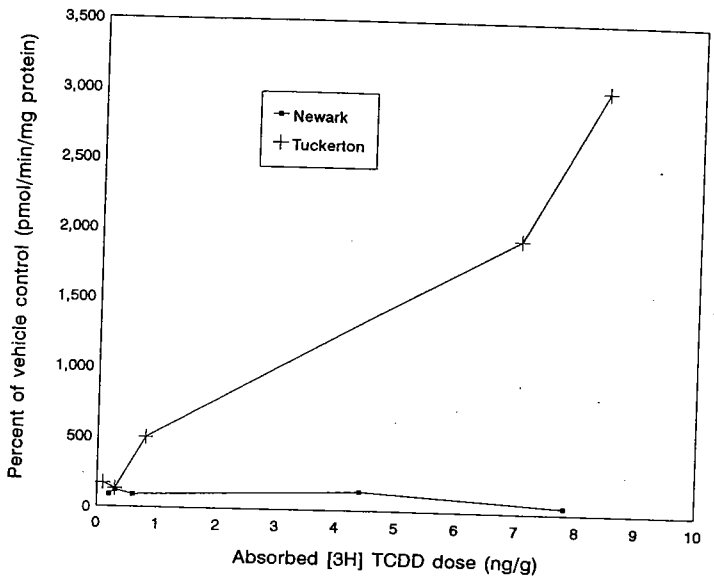


Fig. 1. Shown are 96-h hepatic EROD activities (pmol resorufin/min/mg protein) in no treatment control and vehicle control Newark *F. heteroclitus*, expressed as a percent of no treatment control and vehicle control 96-h hepatic EROD activity in Tuckerton *F. heteroclitus*. Percentages are shown for five collection dates. 10/90 to 4/91 are single pooled values ($N = 25$ per value); 12/91 to 8/92 are means of individual values ($N = 6$–8).



g. 2. Dose–response curve of 96-h hepatic EROD activity (pmol resorufin/min/mg protein) as percent of vehicle control versus the 24-h ›sorbed [^{3}H]TCDD dose (ng/g) in Newark and Tuckerton *F. heteroclitus*. Data from P4501A activity studies III, IV, and V (means of individual values are used for four doses; single pooled values are used for the 0.80 ng/g TCDD dose).

DISCUSSION

Mammalian distribution studies have demonstrated that CDD is cleared rapidly from the blood following adminis-ıtion of a single dose, distributing to the liver, muscle, skin, ipose, and other tissues [12,13]. This is followed by redis-bution to the liver and adipose tissues, which exhibit in-:asing tissue concentrations over several days following)osure. The *F. heteroclitus* tissues utilized in the $t_{1/2}$ de-minations were chosen for the following reasons. The liver d gallbladder are tissues that are most actively involved in : metabolism and excretion of TCDD. The distribution of :DD to other tissues will be governed by perfusion rates l lipid content [5]. The elimination trends of other tissues)uld be dissimilar from the liver only in those tissues that y serve as deep storage sites, due to high lipid content. The cass was chosen as potentially representative of a deep rage site tissue. Clearance from the blood should reflect)le body elimination phases.

The elimination half-life from the blood, or central com-tment, was 42 h shorter in the Newark fish than in the kerton fish. The other tissue compartment half-lives were ilar between the Newark and Tuckerton fish, although all e somewhat shorter in the Newark fish. These data indi-that Newark *F. heteroclitus* are capable of slightly more d elimination of [^{3}H]TCDD as compared to the Tuck-n fish. However, it is unclear as to whether this slight tox-inetic difference contributes to the resistance to TCDD city observed in Newark *F. heteroclitus*.

A wide range of mammalian and aquatic species have ı shown to be capable of biotransforming TCDD to po-netabolites [4,14]. In most mammalian species, excre-occurs primarily through the feces. The metabolism of)D appears to be required for urinary and biliary elimination, and therefore plays a major role in regulation of the rate of excretion. The metabolism of TCDD is generally considered to be a detoxification process.

There was no difference between Newark and Tuckerton *F. heteroclitus* in the amounts of unmetabolized TCDD present in either the gallbladders or the livers at either 1 or 8 h. This would indicate that the two *F. heteroclitus* subpopulations have similar capabilities to metabolize TCDD. The amounts of unmetabolized TCDD in the Newark and Tuckerton *F. heteroclitus* livers were substantially greater than the amounts present in the Newark and Tuckerton gallbladders, at both time points. Therefore, the majority of the TCDD that was excreted through the bile was metabolized to more polar compounds. It also appears that, once the metabolites were generated, these more polar compounds were rapidly removed from the liver through the bile.

The chemically impacted Newark *F. heteroclitus* subpopulation consistently exhibited basally elevated levels of P4501A catalytic (EROD) activity, in comparison to the Tuckerton subpopulation. The elevation of P4501A activity in the Newark fish was constant, regardless of season or different spawnings of the Newark population tested. There was no induction in EROD activity following any TCDD treatment in the Newark fish, at doses ranging from 0.020 to 8.0 ng/g TCDD. In comparison, TCDD treatment did elicit EROD induction in the chemically nonimpacted Tuckerton *F. heteroclitus* subpopulation. The EROD activity in the Tuckerton fish was increased over control at 0.80 ng/g TCDD, and exhibited a 3,000% increase over vehicle control at 8.0 ng/g TCDD. In the Tuckerton fish, EROD activity exhibited a linear increase with increasing TCDD dose. A similar TCDD dose response in EROD induction has been demonstrated in rainbow trout [15].

Seasonal variation was evident in the EROD activities

of Newark *F. heteroclitus*, in comparing studies IV (winter 1991 collection) and V (summer 1992 collection). The summer EROD activities in the Newark fish were elevated (at least 2.6-fold) as compared to the winter EROD activities in the Newark fish. In the Newark/Raritan Bay estuary, *F. heteroclitus* actively feed during the summer, but are dormant in the winter. The higher summer EROD activity could reflect greater exposure to inducing PAH contaminants through the diet.

In mammals, the induction response of the P4501A family occurs via binding of the inducer to a soluble, intracellular receptor, which is associated with other proteins, termed the Ah (aryl hydrocarbon) receptor complex [16–19]. This is followed by transcriptional activation of the P4501A gene via the Ah receptor–inducer complex binding to dioxin-responsive genomic elements [20]. Although the induction process in fish is not fully characterized, the available evidence is consistent with the mammalian data [21]. Also, recent studies clearly indicate that the Ah receptor is present in fish livers, including those of *F. heteroclitus* [22]. In *F. heteroclitus* collected from Newark Bay, Haasch et al. [23] demonstrated a threefold increase in P4501A mRNA and EROD activity, as well as similarly increased immunoreactive (P4501A) protein. This increase was in comparison to the Tuckerton subpopulation.

The EROD activity in Newark *F. heteroclitus* was consistently elevated as compared to the Tuckerton subpopulation. However, this basal elevation could not be increased further by TCDD treatment, which is the most potent P4501A inducer known [24]. In addition, a 90-d period of clean water depuration of the Newark fish did not alter either the non-inducibility of EROD activity following TCDD treatment, or the basal elevation of EROD activity in the Newark fish (data not shown). These results suggest that the P4501A non-responsiveness to TCDD in the Newark fish may be due to a biochemical alteration.

A similar lack of induction of P4501A activity following treatment with TCDD and other PAHs has been studied in the "nonresponsive" DBA/2 inbred mouse strain, as compared to the "responsive" C57BL/6 inbred mouse strain [25,26]. The nonresponsiveness of the DBA/2 mouse strain was associated with a genetically defective Ah receptor complex, which was manifested in less biological responsiveness to TCDD. Poland and Glover [27] demonstrated that the ED50 for TCDD induction of AHH activity was approximately 10-fold greater in the DBA/2 mouse strain as compared to the C57BL/6 strain.

Because of the multistep process thought to be associated with the mechanism of TCDD action [20], an alteration in any of the major steps involving the Ah receptor complex would have a profound effect on an organism's response to TCDD. A potential mechanism for both the P4501A non-responsiveness to TCDD and the resistance to TCDD toxicity (reported previously) in Newark *F. heteroclitus* could be an alteration in the function of the Ah receptor complex. The basal P4501A elevation in the Newark fish could be constitutive and thus completely independent of the Ah receptor mechanism. This elevated metabolic activity might be expected in an animal that inhabits a chronically contaminated environment [6].

A necessary prerequisite for this theoretical mechanism of resistance to TCDD would be the existence of an altered Ah receptor complex genotype in the Newark *F. heteroclitus* gene pool. Chronic exposure of the Newark population to PAH contaminants could then have acted as a selective pressure, ultimately narrowing the population to the non-responsive phenotype. This implies that only those populations having the necessary initial genetic variability would be likely to survive in a highly polluted environment.

Acknowledgement—The authors would like to thank Gisela Witz and Fred Kauffman for their technical advice and support. This research was funded in part by the New Jersey Department of Environmental Protection, Division of Science and Research, and Rutgers University D-0140702-95.

REFERENCES

1. **Prince, R.** and **K.R. Cooper.** 1995. Comparisons of the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on chemically impacted and nonimpacted subpopulations of *Fundulus heteroclitus*: I. TCDD toxicity. *Environ. Toxicol. Chem.* **14**:579–587.
2. **Poland, A.** and **J.C. Knutson.** 1982. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: Examinations of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* **22**:517–554.
3. **Olson, J.R.** 1986. Metabolism and disposition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in guinea pigs. *Toxicol. Appl. Pharmacol.* **85**:263–273.
4. **Neal, R.A., J.R. Olson, T.A. Gasiewicz** and **L.E. Geiger.** 1982. The toxicokinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in mammalian systems. *Drug Metab. Rev.* **13**:355–385.
5. **Gasiewicz, T.A., J.R. Olson, L.E. Geiger** and **R.A. Neal.** 1983. Absorption, distribution, and metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in experimental animals. In R.E. Tucker, A.L. Young and A.P. Gray, eds., *Human and Environmental Risks of Chlorinated Dioxins and Related Compounds.* Plenum, New York, NY, pp. 495–525.
6. **Goksøyr, A.** and **L. Förlin.** 1992. The cytochrome P450 system in fish, aquatic toxicology and environmental monitoring. *Aquat. Toxicol.* **22**:287–311.
7. **Payne, J.F.** 1976. Field evaluation of benzopyrene hydroxylase induction as a monitor for marine pollution. *Science* **191**:945–946.
8. **Vindimian, E.** and **J. Garric.** 1989. Freshwater fish cytochrome P450-dependent enzymatic activities: A chemical pollution indicator. *Ecotoxicol. Environ. Saf.* **18**:277–290.
9. **Wisk, J.D.** and **K.R. Cooper.** 1992. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on benzo(*a*)pyrene hydroxylase activity in embryos of the Japanese medaka (*Oryzias latipes*). *Arch. Toxicol.* **66**:245–255.
10. **Bradford, M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal. Biochem.* **72**:248–254.
11. **Burke, M.D.** and **R.T. Mayer.** 1974. Ethoxyresorufin: Direct fluorometric assay of a microsomal *O*-deethylation which is preferentially inducible by 3-methylcholanthrene. *Drug Metab. Dispos.* **2**:583–588.
12. **Abraham, K., R. Krowke** and **D. Neubert.** 1988. Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: I. Dose-dependent tissue distribution and induction of hepatic ethoxyresorufin *O*-deethylase in rats following a single injection. *Arch. Toxicol.* **62**:359–368.
13. **Abraham, K., R. Krowke** and **D. Neubert.** 1989. Absorption of TCDD following parenteral application in rats using various vehicles. *Chemosphere* **19**:893–898.

14. **Kleeman, J.M., J.R. Olson** and **R.E. Peterson.** 1988. Species differences in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity and biotransformation in fish. *Fundam. Appl. Toxicol.* **10**:206–213.
15. **van der Weiden, M.E.J., J. van der Kolk, R. Bleumink, W. Seinen** and **M. van den Berg.** 1992. Concurrence of P450IA1 induction and toxic effects after administration of a low dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* **24**:123–142.
16. **Nebert, D.W.** and **J.E. Jones.** 1989. Regulation of the mammalian cytochrome P450 (CYPIA1) gene. *Int. J. Biochem.* **21**: 243–259.
17. **Perdew, G.H.** 1988. Association of the Ah receptor with the 90-kDa heat shock protein. *J. Biol. Chem.* **263**:13802–13808.
18. **Henry, E.C., G. Rucci** and **T.A. Gasiewicz.** 1989. Characterization of multiple forms of the Ah receptor: Comparison of species and tissues. *Biochemistry* **28**:6430–6440.
19. **Hoffman, E.C., H. Reyes, F.-F. Chu, F. Sanders, B.A. Conley, B.A. Brooks** and **O. Hankinson.** 1991. Cloning of the factor required for activity of the Ah (dioxin) receptor. *Science* **252**: 954–958.
20. **Whitlock, J.P., Jr.** 1990. Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin action. *Annu. Rev. Pharmacol. Toxicol.* **30**:251–277.
21. **Andersson, T.** and **L. Forlin.** 1992. Regulation of the cytochrome P450 enzyme system in fish. *Aquat. Toxicol.* **24**:1–20.
22. **Hahn, M.E., A. Poland, E. Glover** and **J.J. Stegeman.** 1992. The Ah receptor in marine animals: Phylogenetic distribution and relationship to cytochrome P450IA inducibility. *Mar. Environ. Res.* **34**:87–92.
23. **Haasch, M.L., E.M. Quardokus, L.A. Sutherland, M.S. Goodrich, R. Prince, K.R. Cooper** and **J.J. Lech.** 1992. CYPIA1 protein and mRNA in teleosts as an environmental bioindicator: Laboratory and environmental studies. *Mar. Environ. Res.* **34**: 139–145.
24. **Safe, S.H.** 1986. Comparative toxicology and mechanism of action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. *Annu. Rev. Pharmacol. Toxicol.* **26**:371–399.
25. **Nebert, D.W., F.M. Goujon** and **J.E. Gielen.** 1972. Aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons: Simple autosomal dominant trait in the mouse. *Nat. New Biol.* **236**:107–115.
26. **Thomas, P.E., R.E. Kouri** and **J.J. Hutton.** 1972. The genetics of aryl hydrocarbon hydroxylase induction in mice: A single gene difference between C57BL/6J and DBA/2J. *Biochem. Genet.* **6**:157–168.
27. **Poland, A.** and **E. Glover.** 1975. Genetic expression of aryl hydrocarbon hydroxylase by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: Evidence for a receptor mutation in genetically non-responsive mice. *Mol. Pharmacol.* **11**:399–405.