(1995a)

Pergamon

Environmental Toxicology and Chemistry, Vol. 14, No. 4, pp. 579–587, 1995
Copyright © 1995 SETAC
Printed in the USA
0730-7268/95 $9.50 + .00

0730-7268(94)00237-1

Environmental Toxicology

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

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 subpopulation of *Fundulus heteroclitus* inhabiting the chemically impacted estuary of Newark Bay, New Jersey, was found to be resistant to the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as compared to a chemically nonimpacted subpopulation of *F. heteroclitus* in Tuckerton, New Jersey. The Newark/Raritan Bay estuary is a major site of TCDD contamination. The Newark *F. heteroclitus* embryos, when exposed to 12 to 100 parts per trillion (pptr) [^{3}H]TCDD/acetone (nominal water concentrations) did not exhibit TCDD-dependent lesions or death. In contrast, the Tuckerton embryos exhibited both TCDD concentration-related lesions and death that was associated with the lesions. The Newark embryonic resistance to TCDD toxicity could not be attributed to a lesser absorption of [^{3}H]TCDD as compared to the Tuckerton embryos. The Newark *F. heteroclitus* adult fish, when dermally treated with 0.30 to 60 ng/g [^{3}H]TCDD/DMSO, did not exhibit TCDD treatment-related increases in death, lesions, or liver-to-body-weight ratios, whereas the Tuckerton adult fish did. The Newark fish did exhibit greater parasitic infestations of the liver, greater hepatic neoplasias, and greater liver-to-body-weight ratios, all of which were independent of laboratory TCDD treatment, as compared to the Tuckerton fish. These hepatic effects in the Newark fish may be indicative of the chronic chemical exposure of this *F. heteroclitus* subpopulation.

Keywords—TCDD *Fundulus heteroclitus* Chemical resistance Newark Bay

INTRODUCTION

Newark/Raritan Bay is one of the most chemically polluted estuaries in the United States [1]. Sediments within the estuary contain elevated levels of numerous heavy metals, chlorinated pesticides, PCBs, and PAHs [2]. Populations of organisms that have been chronically exposed to chemical contaminants, such as the biota of Newark Bay, may exhibit increased resistance to those contaminants [3]. Pollution has been described as a selective agent causing more resistant genotypes to predominate in polluted areas, assuming the population contains enough initial genetic variation to respond to the environmental stress [4].

In 1985, the New Jersey Department of Environmental Protection reported levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in fish collected from Newark Bay and adjacent waters that exceeded the 50 parts per trillion (pptr) level of concern established by the Food and Drug Administration, and subsequently prohibited the sale or consumption of striped bass and blue crabs from the area [5]. Bopp et al. [6] estimated that the total deposition of TCDD into the Newark/Raritan Bay sediments was between 4 to 8 kg TCDD. In Newark Bay, TCDD concentrations in suspended particulate samples dated to 1985 ± 1 year ranged from 260 to 430 pptr [6,7].

TCDD was selected for these studies based on both the extensive TCDD contamination of the Newark/Raritan Bay estuary and the toxic potency of the compound [6,8]. Additionally, the toxicity of TCDD has been characterized in multiple fish species [9–14].

Fundulus heteroclitus, also known as the killifish or mummichog, is a hardy species that is routinely found in both contaminated and more pristine estuarine waters, and therefore has the potential for development of chemical resistance. *F. heteroclitus* is widely distributed in coastal eastern North America, from southwestern Newfoundland to northeastern Florida [15,16]. Few teleost species maintain as restricted a home range as *F. heteroclitus*, making this species potentially useful as a bioindicator of environmental contamination. Although *F. heteroclitus* is not a commercially important fish, this species is a key ecological component of tidal marshes, and thus may be useful in assessing the ecological health of an estuary [17].

The studies described below tested the hypothesis that an *F. heteroclitus* population inhabiting the heavily contaminated Newark Bay environment will exhibit resistance to TCDD toxicity. Chemical resistance present in an impacted fish population must be assessed in comparison to the same species of fish collected from a relatively nonimpacted site.

*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.

Newark Bay Study Area
NBSA-00101

Tuckerton, New Jersey, was chosen as the control site; the Tuckerton estuary is partially protected as a wildlife refuge and is a relatively pristine site.

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). Acetone (HPLC grade) and dimethyl sulfoxide (spectra-analyzed) were purchased from Fisher Scientific (Springfield, NJ). Liquiscint® was purchased from National Diagnostics (Somerville, NJ), and 3-aminobenzoic acid ethyl ester was purchased from Sigma Chemical Co. (St. Louis, MO).

Animals

Adult *F. heteroclitus* were collected in baited minnow traps placed within tidal pools and streams located in both Newark Bay, New Jersey, and Tuckerton, New Jersey. For the *F. heteroclitus* embryonic studies, eggs and sperm were stripped from the adults on site via abdominal pressure, and fish were returned to their respective sites. For the *F. heteroclitus* adult studies, 2 to 5 g fish were transported back to the laboratory in sealed plastic bags containing fish and water which were aerated and placed on ice. On arrival in the laboratory, fish were given a 10 s formalin dip (250 ppm) prior to entering aquaria in order to reduce disease. The fish were housed in 10 or 15 gallon glass aquaria under static renewal conditions, in salt solutions made up from Instant Ocean® (Mentor, OH) and activated carbon-filtered (Continental Water Co., Ann Arbor, MI) tap water. The salt solutions were made up according to the respective site salinities (Newark Bay: 10 ppt, Tuckerton: 20 ppt). Aquaria were equipped with aerating filters (Lustar, Springfield, NJ) containing activated carbon and filter floss, which were cleaned and changed several times weekly. Aquarium salt water was changed (one-quarter tank volume) every 5 to 7 d. The fish were maintained on a 9:15-h light:dark photoperiod at approximately room temperature (20°C), and were fed Tetramin® Tropical Fish Food (TetraWerke, Melke, Germany) twice daily.

Adult *F. heteroclitus* were killed by the following procedure: Fish were anesthetized with 3-aminobenzoic acid ethyl ester dissolved into aquarium water, and the fish spinal cord was severed prior to dissection.

Liquid scintillation counting

All samples were counted on a Tracor Mark III liquid scintillation counter (Tracor Analytic, Elk Grove Village, IL). Disintegrations per minute were converted to concentration equivalents after background subtraction. The detection limit ranged from 0.2 to 1.0 pptr TCDD-derived [^{3}H].

All tissue samples were prepared for liquid scintillation counting by digestion in 1 N NaOH (1 to 2 ml), followed by neutralization in glacial acetic acid (50 µl per 1 ml of 1 N NaOH). The tissue samples were counted in Liquiscint (9 ml per 1 ml of 1 N NaOH).

Histology

In preparation for light microscopy, fish livers were fixed in 10% phosphate-buffered formalin. Livers were dehydrated in 70% alcohol and embedded in paraffin using a Histomatic® tissue processor, model 166, equipped with a Histo-Center II embedding apparatus (Allied Co.–Fisher Scientific, Pittsburgh, PA). The paraffin blocks were cut into 6 micron sections with a Spencer 820 microtome (American Optical Co., Buffalo, NY). Sections were stained with hematoxylin and eosin, using standard histological techniques. The sections were examined on a Zeiss light microscope (Carl Zeiss Inc., Germany).

Statistical analysis

All parametric (ANOVA, Student's *t* test) and nonparametric (Mann–Whitney) 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. The statistical significance for all tests was set at $p \leq 0.05$. The EC50 values with 95% confidence intervals were determined using a probit analysis program.

Procedure

Groups of 20 to 30 embryos each were exposed individually to no treatment (control); 0.05% acetone (vehicle control); and 12, 25, 50, and 100 pptr [^{3}H]TCDD. The fertilized *F. heteroclitus* eggs were individually loaded into 2 ml glass vials containing various concentrations of [^{3}H]TCDD in a 1 ml salt solution (Instant Ocean®, carbon-filtered tap water). The [^{3}H]TCDD was first dissolved in acetone, followed by mixing into the salt solution at a ratio of 0.5:1,000 (v/v).

Embryos were exposed from early cell division (day of fertilization) to either hatch or approximately 30 d postfertilization. A static nonrenewal protocol was used to minimize personnel exposure. Embryos were maintained at 25°C in a low-temperature incubator (Fisher Scientific, Fair Lawn, NJ). All embryos were examined daily under a dissecting microscope (through the wall of the exposure vial) for developmental stage, lesion occurrence, and death. The stages of development used in these studies were similar to those described by Armstrong and Child [18]. A subset of embryos from each group was prepared for liquid scintillation counting on day 16 postfertilization.

Adult *F. heteroclitus* were treated with a [^{3}H]TCDD/DMSO drop directly onto the fish abdomen. The [^{3}H]TCDD was delivered in DMSO by Eppendorf® pipette. The dose volume was 5 µl/g fish, adjusted according to a weight range (0.5 g–1.4 g = 5 µl; 1.5 g–2.4 g = 10 µl, etc.). Fish abdomens were wiped with Kimwipes® prior to TCDD application to remove the mucous layer, which would act as a barrier to absorption. Following the [^{3}H]TCDD/DMSO drop application, the fish abdomens were dried under a warm stream of air.

In study I, 25 fish per group were housed in 15 gal aquaria and maintained at room temperature (approximately 20°C). The treatment groups consisted of no treatment (control);

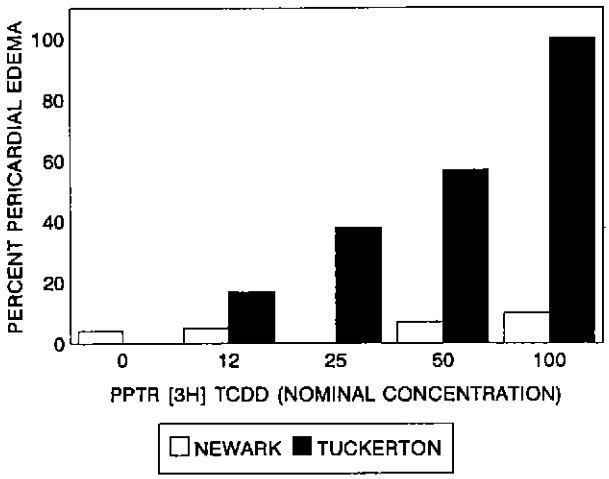

Fig. 1. Total percentages of pericardial edema in Newark and Tuckerton *F. heteroclitus* embryos exposed to 0.05% acetone (vehicle), 12, 25, 50, and 100 pptr [^{3}H]TCDD (nominal concentration), from day of fertilization to day 27 postfertilization. $N = 30$ embryos per treatment group.

DMSO (vehicle control); and 0.30, 2.4, 4.4, and 9.9 ng [^{3}H]TCDD/g fish. At 24 h following [^{3}H]TCDD treatment, three fish per group were killed and prepared for liquid scintillation counting to determine [^{3}H]TCDD absorption. The fish were maintained for 35 d and monitored daily for lethality. All dead fish were examined for gross lesions. All fish were weighed on a weekly basis. At 10 d following [^{3}H]TCDD treatment, three fish per group were killed and fixed in buffered formalin for later histological processing and analysis. This was repeated at 35 d with six fish per group. Liver-to-body-weight ratios were determined for all remaining fish at 35 d.

In study II, 25 fish per group were housed in two 10 gal aquaria, in replicate, and maintained at room temperature (approximately 20°C). These fish received two [^{3}H]TCDD treatments at an interval of 14 d. The final treatment groups consisted of no treatment (control); DMSO (vehicle control); and 2, 14, 30, and 60 ng [^{3}H]TCDD/g fish. At 24 h following the first [^{3}H]TCDD treatment, five fish per group were killed and prepared for liquid scintillation counting to determine [^{3}H]TCDD absorption. The remaining fish were maintained for 132 d and monitored daily for lethality. All dead fish were examined for gross lesions. Liver-to-body-weight ratios were determined for all remaining fish at 132 d.

RESULTS

Several studies were carried out to compare the occurrence of lesions and death in response to TCDD exposure in the Tuckerton and Newark *F. heteroclitus* embryos. Additionally, [^{3}H]TCDD absorption was compared between the Tuckerton and Newark embryos. The observed lesions were pericardial edema and hemorrhages (caudal, vitelline veins, head region, pectoral fins, liver). There were no alterations in the normal sequence of developmental stages in either the Newark or Tuckerton TCDD-treated embryos.

The results illustrated in Figure 1 are representative of a series of embryo studies. The Tuckerton *F. heteroclitus* embryos exhibited a dose-related occurrence of pericardial edema (0% in control to 100% at 100 pptr TCDD). The Newark embryos did not exhibit a dose response in the occurrence of pericardial edema, and exhibited a maximum percentage of 10% pericardial edema (at 100 pptr TCDD), which is not considered significant above control.

Tables 1 and 2 summarize the percentages of death, survival, and hatching in the Tuckerton and Newark *F. heteroclitus* embryos. The numbers of dead embryos on a percent basis are broken down into lesioned and nonlesioned embryos.

The Tuckerton embryo death was associated primarily with pericardial edema and/or hemorrhage in the 25 to 100 pptr TCDD groups (Table 1). In the 12 pptr TCDD Tuckerton group, 9% died with pericardial edema and 9% died

Table 1. Death, survival, and hatching in Tuckerton *F. heteroclitus* embryos exposed to TCDD from day of fertilization to day 27 postfertilization

Nominal TCDD concentration	Of the dead[a] embryos (% of total)				Of the surviving embryos (% of total)		Hatch (% of total)
			No visible lesions				
	PE[b]	H[c]	Before day 14	After day 14	PE	No PE	
No treatment control (19)	0	0	0	5	0	95	95
Acetone control[d] (20)	0	0	5	10	0	85	85
12 pptr (23)	9	0	9	0	9	74	61
25 pptr (19)	10	5	0	10	21	53	63
50 pptr (21)	48	0	5	5	19	24	24
100 pptr (23)	100	0	0	0	0	0	0

[a]Living embryos taken for liquid scintillation counting are subtracted from total numbers of 29 to 30 embryos per group; exact group number is in parentheses following each concentration.
[b]Pericardial edema (may be accompanied by hemorrhage).
[c]Hemorrhage only.
[d]Maximum vehicle concentration was 0.05%.

Table 2. Death, survival, and hatching in Newark *F. heteroclitus* embryos exposed to TCDD from day of fertilization to day 27 postfertilization

Nominal TCDD concentration	Of the dead[a] embryos (% of total)				Of the surviving embryos (% of total)		Hatch (% of total)
			No visible lesions				
	PE[b]	H[c]	Before day 14	After day 14	PE	No PE	
No treatment control (20)	5	0	0	30	0	65	55
Acetone control[d] (25)	0	4	48	4	4	40	28
12 pptr (20)	5	0	0	20	0	75	70
25 pptr (22)	0	0	14	18	0	68	50
50 pptr (22)	0	0	23	32	9	36	18
100 pptr (24)	8	4	33	29	0	25	17

[a]Living embryos taken for liquid scintillation counting are subtracted from total numbers of 29 to 30 embryos per group; exact group number is in parentheses following each concentration.
[b]Pericardial edema (may be accompanied by hemorrhage).
[c]Hemorrhage only.
[d]Maximum vehicle concentration was 0.05%.

with no visible lesions. The Tuckerton control groups had a maximum embryo death of 15%. These control embryos died with no visible lesions, primarily after day 14. The majority of the Newark embryos died with no visible lesions (Table 2). Results similar to these were obtained in the other studies.

The Tuckerton *F. heteroclitus* embryo survival showed an inverse relationship to the TCDD concentration (Table 1). The Newark embryo survival did not show a relationship to the TCDD concentration (Table 2). The percent hatch was generally less than the percent survival for both Tuckerton and Newark *F. heteroclitus* embryos (Tables 1 and 2), which was most likely due to the individual vial rearing conditions employed in these studies. Survival included 3 d posthatch.

Table 3 summarizes the whole embryo absorption of [^{3}H]TCDD for the study described above. Whole embryo absorption was not significantly different between Newark and Tuckerton *F. heteroclitus*, with the exception of the 12 pptr nominal concentration. The Newark embryos absorbed a significantly greater amount of [^{3}H]TCDD at this concentration. Results similar to these were obtained in the other studies. Embryonic and chorionic absorption of [^{3}H]TCDD were also compared in both Newark and Tuckerton *F. heteroclitus* (Table 4). In both groups, the embryos (including yolk sac) absorbed significantly greater amounts of [^{3}H]TCDD than did the chorions. Additionally, whole embryo absorption of [^{3}H]TCDD was significantly greater in Newark as compared to Tuckerton *F. heteroclitus*.

The ED50 and 95% confidence limit for pericardial edema in the Tuckerton *F. heteroclitus* embryos was 2,025 (1,626–2,515) pg/g egg.

Two studies were carried out to compare the occurrence of lesions and death in response to TCDD treatment in the Newark and Tuckerton *F. heteroclitus* adults.

The total percent death in Newark and Tuckerton *F. heteroclitus* at 35 d post-TCDD treatment (study I) is presented in Figure 2. Death in the Newark fish was independent of

Table 3. Whole embryo absorption of [^{3}H]TCDD in Newark and Tuckerton *F. heteroclitus* embryos exposed from day of fertilization to day 16 postfertilization

Nominal TCDD concentration	[^{3}H]TCDD absorbed[a] (pg/g egg)	
	Newark	Tuckerton
12 pptr[b]	825 ± 90	635 ± 147
25 pptr	1,415 ± 271	1,459 ± 130
50 pptr	2,919 ± 496	2,248 ± 531
100 pptr	6,352 ± 431	6,797 ± 1,040

[a]Each value represents the mean ± SD of 5 whole embryo determinations.
[b]Whole embryo absorption was significantly different between Newark and Tuckerton *F. heteroclitus* at $p < 0.05$ (Mann–Whitney).

Table 4. Comparison of embryonic and chorionic absorption of [^{3}H]TCDD in Newark and Tuckerton *F. heteroclitus* embryos exposed to 100 pptr [^{3}H]TCDD from day of fertilization to day 13 postfertilization

[^{3}H]TCDD absorbed (pg)			
Newark		Tuckerton	
Embryo[a]	Chorion	Embryo	Chorion
9.36 (56)[b]	7.48 (44)	2.71 (55)	2.20 (45)
13.0 (90)	1.38 (9)	6.68 (65)	3.61 (35)
10.1 (65)	5.43 (35)	6.48 (80)	1.60 (20)
12.1 (64)	6.71 (36)	8.00 (74)	2.87 (26)
6.93 (46)	8.13 (54)	8.64 (65)	4.61 (35)
Means ± SD:			
10.3 ± 2.39	5.83 ± 2.68[c]	6.50 ± 2.30	2.98 ± 1.18[c]
Combined (embryo and chorion) means ± SD:			
16.13 ± 1.75[d]		9.48 ± 3.15[d]	

[a]Embryos include yolk sac.
[b]Percentage of total [^{3}H]TCDD per egg in parentheses.
[c]Embryo and chorion values were significantly different from each other at $p < 0.05$ (Mann–Whitney).
[d]Whole embryo absorption was significantly different between Tuckerton and Newark at $p < 0.05$ (Mann–Whitney).

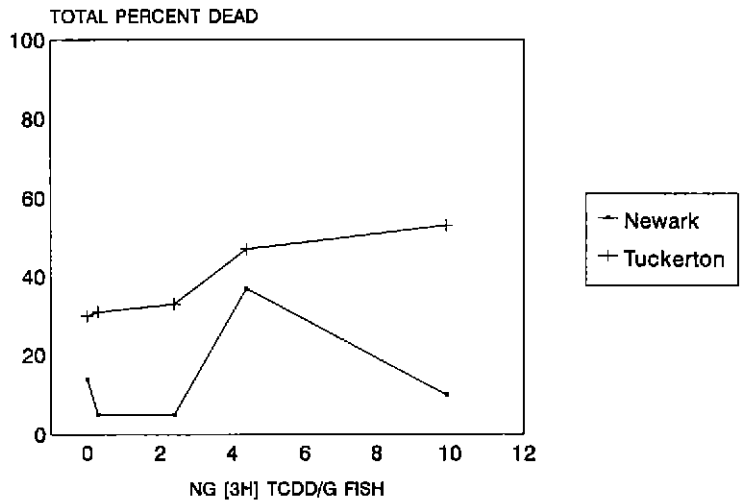

Fig. 2. Total percent death in Newark and Tuckerton *F. heteroclitus* at 35 d post-[^{3}H]TCDD treatment (study I). Treatment groups were vehicle (DMSO) control; 0.30, 2.4, 4.4, and 9.9 ng/g [^{3}H]TCDD ($N = 22$).

TCDD treatment. Only one Newark TCDD dose group (4.4 ng/g TCDD) exhibited increased death over that of control (37% compared to 23% in no treatment control). In the Tuckerton fish, death was equivalent to vehicle control (30%) at the 0.30 and 2.4 ng/g TCDD doses. In the 4.4 and 9.9 ng/g TCDD groups, there was a 17% and 23% increase over vehicle control in death in the Tuckerton fish, respectively. The [^{3}H]TCDD absorption was approximately equivalent between the Newark and Tuckerton fish in study I (not shown).

Final mean liver-to-body-weight ratios for Newark and Tuckerton *F. heteroclitus* (study I) are presented in Figure 3.

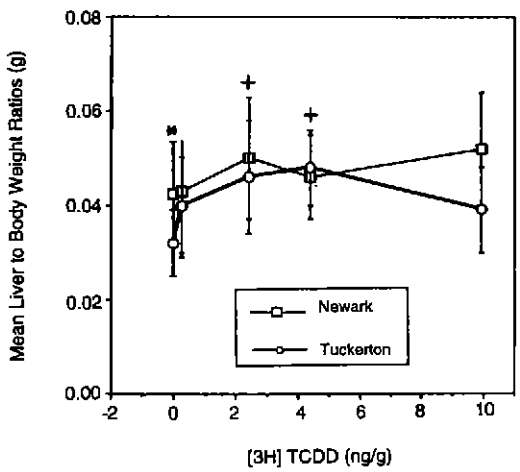

Fig. 3. Final mean liver-to-body-weight ratios for Newark and Tuckerton *F. heteroclitus* at 35 d post-[^{3}H]TCDD treatment (study I). Treatment groups were no treatment (control); 0.30, 2.4, 4.4, and 9.9 ng/g [^{3}H]TCDD (N varied with survivorship: range of 9–19). (*) The Newark no treatment control mean was significantly greater than the Tuckerton no treatment control mean at $p < 0.05$ (Student's t test). (+) The Tuckerton 2.4 and 4.4 ng/g TCDD means were significantly greater than the Tuckerton no treatment control mean at $p < 0.05$ (ANOVA, Newman–Keuls multiple comparisons).

The Newark no treatment control mean was significantly greater than the Tuckerton no treatment control mean. There were no significant effects following TCDD treatment in the Newark liver-to-body-weight ratio means. The means of both the 2.4 and 4.4 ng/g TCDD Tuckerton groups were significantly greater than the Tuckerton no treatment control liver-to-body-weight ratio mean.

Summaries of histologic analysis by light microscopy of Newark and Tuckerton *F. heteroclitus* livers at 10 d following TCDD treatment (study I) are presented in Tables 5 and 6. There were no dose-related effects in either of the *F. heteroclitus* subpopulations. More extensive parasitism, necrosis, and macrophage centers were observed in the Newark livers than in the Tuckerton livers. In addition, neoplastic lesions were observed only in the Newark livers. Similar trends were observed in the *F. heteroclitus* livers at 35 d following TCDD treatment.

The total percent death in Newark and Tuckerton *F. heteroclitus* replicates at 132 d post-TCDD treatment (study II) is presented in Table 7. The Newark vehicle control replicates exhibited similar percentages of death (70%, 80%). In the TCDD treatment groups, the Newark replicates exhibited opposite trends in death, excepting the 14 ng/g TCDD replicates. Death in the Tuckerton vehicle control replicates did not exceed 30%. Death was increased over control by 30% (replicate I) and 60% (replicate II) in the Tuckerton 30 ng/g TCDD group, and was increased over control by 20% (replicate I) and 30% (replicate II) in the Tuckerton 60 ng/g TCDD group. The Tuckerton 2 and 14 ng/g TCDD groups did not exhibit increased death above control, except the 14 ng/g TCDD group (replicate II), by 10%. The [^{3}H]TCDD absorption was approximately equivalent between the Newark and Tuckerton fish in study II (not shown).

DISCUSSION

In the *F. heteroclitus* embryo studies, the occurrence of pericardial edema was independent of the TCDD concentra-

Table 5. Histologic analysis of Newark *F. heteroclitus* livers at 10 d post-TCDD treatment (study I)

Treatment group[a]	Trematode parasites	Sporocyte parasites	Fungal infection	Necrosis	Macrophage centers	Altered staining	Neoplastic lesions
No treatment control	67	0	0	0	0	0	33[b]
Vehicle control	100	33	0	33	33	67	33[c]
0.30 ng/g TCDD	100	0	0	33	33	0	0
2.4 ng/g TCDD	100	0	0	33	33	33	0
4.4 ng/g TCDD	67	0	0	33	33	67	33[d]
9.9 ng/g TCDD	67	0	0	67	100	67	33[e]

[a]Three fish livers per group were analyzed. Results are reported as percentages.
[b]Focal mixed tumor; moderate lipoma.
[c]Bile duct proliferation; moderate mixed tumor.
[d]Widespread lipoma.
[e]Focal bile duct proliferation.

tion in the Newark *F. heteroclitus* embryos, and was TCDD concentration-related in the Tuckerton *F. heteroclitus* embryos. Death in the Tuckerton embryos was associated primarily with pericardial edema and/or hemorrhage in the 25 to 100 pptr TCDD groups. The majority of the Newark embryo deaths were not associated with any visible lesions. Based on these studies, the Newark *F. heteroclitus* embryos were resistant to TCDD toxicity as compared to the Tuckerton *F. heteroclitus* embryos.

In the category of death with no visible lesions, the majority of embryos died after day 14 in both Tuckerton and Newark *F. heteroclitus*. The *F. heteroclitus* eggs generally hatch in the range of days 12 to 15 postfertilization [18]. Embryo deaths after day 14 (with no visible lesions) were assumed to be due to a failure to hatch. The failure to hatch was attributed to the rearing conditions employed in these studies.

The Newark embryonic resistance to TCDD toxicity could not be attributed to a lesser absorption of [^{3}H]TCDD when compared to the Tuckerton embryos. The [^{3}H]TCDD absorption was either (a) not significantly different between the Newark and Tuckerton embryos, or (b) the Newark embryos tended to absorb significantly greater amounts of [^{3}H]TCDD. If absorption played a role in the Newark embryonic resistance to TCDD, then it would be expected that the Tuckerton embryos would have absorbed greater amounts of TCDD since they exhibited concentration-dependent lesions. It should also be noted that the Newark embryos most likely had some pre-existent TCDD concentration in the yolk sac, based on tissue residue analysis of Newark/Raritan Bay *F. heteroclitus* (29 pptr in a pooled gonad sample). This indicates a greater Newark embryonic resistance to TCDD toxicity than is evident from the data, based on the sum of a pre-existent TCDD concentration and an administered TCDD dose.

These results support the hypothesis that populations which are chronically exposed to chemical pollutants can exhibit toxicant resistance. Resistance to methylmercury-induced craniofacial defects has also been observed in *F. heteroclitus* embryos collected from the Newark/Raritan Bay estuary [19]. Absorption studies demonstrated that the resistant embryos had a decreased chorionic permeability to methylmercury, absorbing more onto the chorion and less into the embryo [3]. The same did not hold true in our studies; both the Newark and Tuckerton *F. heteroclitus* embryos absorbed significantly greater amounts of [^{3}H]TCDD into the embryo (including yolk sac) as compared to the chorion.

The Tuckerton *F. heteroclitus* embryos exhibited a characteristic response to TCDD exposure that has also been observed in the early life stages of Japanese medaka (*Oryzias latipes*), pike (*Esox lucius*), rainbow trout (*Salmo gairdneri*, Richardson), and lake trout (*Salvelinus namaycush*) [9–11, 14,20]. This response consists of edema, hemorrhages, and arrested development culminating in death, and has been shown to be essentially identical to blue-sac disease, which is a naturally occurring edematous syndrome observed in hatchery-reared salmonids [20,21]. This response is not pathognomonic for TCDD in fish embryos, since the same syn-

Table 6. Histologic analysis of Tuckerton *F. heteroclitus* livers at 10 d post-TCDD treatment (study I)

Treatment group[a]	Trematode parasites	Sporocyte parasites	Fungal infection	Necrosis	Macrophage centers	Altered staining	Neoplastic lesions
No treatment control	33	33	0	0	0	33	0
Vehicle control	0	0	67	0	0	0	0
0.30 ng/g TCDD	33	0	67	0	33	67	0
2.4 ng/g TCDD	33	0	0	0	0	33	0
4.4 ng/g TCDD	33	0	0	33	67	33	0
9.9 ng/g TCDD	0	0	33	0	0	67	0

[a]Three fish livers per group were analyzed. Results are reported as percentages.

Table 7. Percent death in Tuckerton and Newark *F. heteroclitus* 132 d post-[^{3}H]TCDD treatment (study II)

Treatment group[a]	Death (%)	
	Tuckerton	Newark
No treatment control (I)	30	40
No treatment control (II)	0	40
Vehicle control (I)	30	70
Vehicle control (II)	30	80
2 ng/g TCDD (I)	10	50
2 ng/g TCDD (II)	10	0
14 ng/g TCDD (I)	20	70
14 ng/g TCDD (II)	40	60
30 ng/g TCDD(I)	60	10
30 ng/g TCDD (II)	90	60
60 ng/g TCDD (I)	50	0
60 ng/g TCDD (II)	60	70

[a]Each replicate numbered 10 fish.

drome is observed in blue-sac disease and in response to other chemical exposures [22]. In our studies, both Tuckerton and Newark *F. heteroclitus* embryos exhibited background levels of pericardial edema and/or hemorrhage. In *F. heteroclitus* and other fish embryos, this edematous syndrome may represent a generalized response to any number of endogenous and exogenous stressors. In the Newark *F. heteroclitus* embryos, TCDD did not elicit this edematous syndrome.

In comparison with reports of TCDD-exposed embryos of other fish species, the Tuckerton *F. heteroclitus* embryos are the least TCDD-sensitive. The ED50 for lesions in the Japanese medaka was reported to be 240 pg/g dechorionated egg [14]. The LD50 for sac fry mortality in lake trout and rainbow trout was reported to be 65 pg/g egg and 300 pg/g egg, respectively [11]. In comparison, the ED50 for pericardial edema in the Tuckerton *F. heteroclitus* embryos was 1,934 pg/g egg (mean of three separate studies). However, this relative insensitivity would be expected in a species well known for its hardiness and ability to adapt to varied environments, both pristine and contaminated [3].

In the *F. heteroclitus* adult studies, death in the Newark adults was independent of TCDD treatment. In study I, only the Newark 4.4 ng/g TCDD group (second highest dose) exhibited increased death over control. In study II, both Newark vehicle control replicates exhibited similarly high percentages of death, which were most likely due to pre-existing parasitic infestations and/or infectious agents. The Newark TCDD treatment group replicates exhibited opposite percentages of death (except the 14 ng/g TCDD group), which makes it unlikely that death in these groups was associated with TCDD treatment. It should also be noted that the Newark adults most likely had some pre-existent TCDD body burden, based on tissue residue analysis of Newark/Raritan Bay *F. heteroclitus* (>100 pptr whole body burden). This indicates a greater Newark adult resistance to TCDD toxicity than is evident from the data, based on the sum of a pre-existent TCDD concentration and an administered TCDD dose.

The Tuckerton *F. heteroclitus* adults exhibited increased death over control at the two highest TCDD doses employed in both studies I and II. However, the Tuckerton fish were not responsive in a dose-dependent manner to TCDD treatment. For example, in study II, the greatest number of fish died in the second highest dose group; 75% of the 30 ng/g TCDD group (combined replicates) died, while 55% of the 60 ng/g TCDD group (combined replicates) died. Nonetheless, TCDD treatment (at the higher doses) did increase Tuckerton *F. heteroclitus* death above control, which did not occur in Newark *F. heteroclitus*.

The Tuckerton adults were sensitive to lower TCDD doses in study I as compared to study II, and also exhibited effects over a shorter period of time in study I. Because there was a year's interval between studies I and II, and approximately the same weight ranges were collected for each study, the fish utilized in studies I and II represent two different spawnings of a large and outbred population. In this situation, variation in response to a toxicant would be expected. Additionally, the Tuckerton fish exhibited more parasitism in study I, which may have increased their sensitivity to TCDD toxicity.

The chemically impacted Newark *F. heteroclitus* adults did not exhibit a TCDD treatment effect, as compared to the nonimpacted Tuckerton adults. Similar examples of resistance have also been observed in other fish species. Resistance to DDT was reported in mosquito fish (*Gambusia affinis*) living in areas treated with DDT; this resistance persisted into the F1 generation bred in the laboratory [23,24]. Angus [25] observed the distribution of phenol tolerance in mosquito fish collected from polluted and nonpolluted waters. Tolerant individuals comprised 25% of the population from the clean site, with a shift to 75% tolerant individuals in the polluted site. This difference was considered evidence for selective pressure for phenol tolerance on the impacted population.

The reported LD50s for other species of fish [rainbow trout (*Salmo gairdneri*), yellow perch (*Perca flavescens*), bluegill (*Lepomis macrochirus*), carp (*Cyprinus carpio*), largemouth bass (*Micropterus salmoides*), bullhead (*Ictalurus melas*)] treated by intraperitoneal TCDD injection range from 3 to 16 ng/g TCDD [12,13,26]. These LD50s were determined at 80 d. In comparison, based on the range of TCDD doses in studies I and II, Tuckerton *F. heteroclitus* would appear to be one of the least TCDD-sensitive fish species tested to date.

Gross and microscopic lesions of epithelial and lymphomyeloid tissues have been observed in other fish species following TCDD treatment [12,13,26]. Similarly, Tuckerton *F. heteroclitus* exhibited petechial hemorrhages and fin necrosis (20 to 50% occurrence) in both replicates of the 30 and 60 ng/g TCDD groups in study II (not shown). These were the treatment groups that also exhibited increased death above control. The Newark fish did not exhibit TCDD-dependent lesions.

Unlike reports of other TCDD-exposed fish species, histological analysis of Newark and Tuckerton *F. heteroclitus* livers (study I) did not reveal any dose-related lesions at either 10 or 35 d. In both the Newark and Tuckerton fish, the occurrences of nondose-dependent necrosis, macrophage centers, and altered staining regions in the livers could be par-

tially attributed to the parasitic infestations that were observed. Newark *F. heteroclitus* exhibited more extensive parasitic infestation, accompanied by more extensive necrosis and macrophage centers, as compared to the Tuckerton fish. Chronic exposure to pollutants can indirectly affect animals through physiological stress, thus increasing their susceptibility to parasites or other disease agents [27]. This may partially explain the increased parasitism observed in the chemically impacted Newark fish.

Newark *F. heteroclitus* also exhibited hepatic neoplastic lesions (independent of TCDD treatment), which were not observed in the Tuckerton fish. Similarly, there have been numerous reports of higher incidences or epizootics of neoplastic lesions in other fish species collected from chemically contaminated sites. These include documentation of high incidences of hepatocellular carcinomas in winter flounder from Boston Harbor [28], epithelial carcinomas and hepatomas in brown bullhead from the Great Lakes region [29], and hepatomas in English sole collected from Puget Sound [30].

Liver-to-body-weight ratio increases in chemically exposed fish are often associated with induction of the hepatic microsomal monooxygenase (MO) system [31,32]. In 3-methylcholanthrene-treated fish, the changes in relative liver weight were shown to be due to proliferation of the smooth and rough endoplasmic reticulum of hepatocytes [33]. Increased MO activity and corresponding increases in liver-to-body-weight ratios have been measured repeatedly in Canadian fish exposed to bleached kraft mill effluent [34].

In our studies, the liver-to-body-weight ratios of Newark *F. heteroclitus* were basally elevated, as compared to the liver-to-body-weight ratios of the Tuckerton fish. This may be partially attributed to the chronic exposure of the Newark *F. heteroclitus* subpopulation to numerous chemicals, including TCDD, which is a potent MO inducer [1,8]. Additionally, the TCDD-treated Tuckerton fish had significantly increased liver-to-body-weight ratios in study I, at 35 d, while the same effect was not observed in the TCDD-treated Newark fish. This may indicate an induction of the hepatic MO system in the TCDD-treated Tuckerton fish, which did not occur in the TCDD-treated Newark fish.

In conclusion, several general statements can be made concerning these studies, which compared the responses of Newark and Tuckerton *F. heteroclitus* embryos and adults to TCDD treatment.

1. *Fundulus heteroclitus* is the least TCDD-sensitive fish species, in both embryonic and adult life stages, studied to date.
2. The chemically impacted Newark *F. heteroclitus* embryos did not exhibit TCDD-dependent lesions or death, as did the Tuckerton *F. heteroclitus* embryos, and, therefore, they were resistant to TCDD toxicity. The Newark embryonic resistance to TCDD toxicity could not be attributed to a lesser absorption of [^{3}H]TCDD, as compared to the Tuckerton embryos.
3. The TCDD-treated Tuckerton fish exhibited increased death over that of controls; this was not dose-dependent, however. The increased death over controls occurred only at the higher doses employed in studies I and II. The TCDD-treated Tuckerton fish also exhibited TCDD-related lesions and significant increases in liver-to-body-weight ratios, which were TCDD-dependent.
4. The chemically impacted Newark fish did not respond to TCDD treatment in any of the following categories: death, liver-to-body-weight ratios, and lesion occurrence. The Newark fish did exhibit greater parasitic infestations of the liver, greater hepatic neoplasias, and greater liver-to-body-weight ratios, all of which were independent of laboratory TCDD treatment, as compared to the Tuckerton fish. These all represent potential biomarkers of chemical exposure. Resistance to a toxicant, such as TCDD, can also be considered a potential biomarker of exposure.

Acknowledgement—This research was funded in part by the New Jersey Department of Environmental Protection, Division of Science and Research, and Rutgers University D-0140701-95.

REFERENCES

1. **Squibb, K.S., J.M. O'Connor** and **T.J. Kneip.** 1991. New York/New Jersey Harbor estuary program, Module 3.1: Toxics characterization report. Institute of Environmental Medicine, New York University Medical Center, Tuxedo, NY.
2. **National Oceanic and Atmospheric Administration.** 1988. A summary of selected data on chemical contaminants in sediments collected during 1984, 1985, 1986, and 1987. Technical Memorandum. NOS OMA 44. Rockville, MD.
3. **Weis, J.S.** and **P. Weis.** 1989. Tolerance and stress in a polluted environment—the case of the mummichog. *BioScience* **39**:89–95.
4. **Levinton, J.S.** 1980. Genetic divergence in estuaries. In V.S. Kennedy, ed., *Estuarine Perspectives*. Academic, New York, NY, pp. 509–520.
5. **Belton, T.J., R. Hazen, B.E. Ruppel, K. Lockwood, R. Mueller, E. Stevenson** and **J.J. Post.** 1985. A study of dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) contamination in select finfish, crustaceans, and sediments of New Jersey waterways. Final Report. New Jersey Department of Environmental Protection and Energy, Trenton, NJ.
6. **Bopp, R.F., M.L. Gross, H. Tong, H.J. Simpson, S.J. Monson, B.L. Deck** and **F.C. Moser.** 1991. A major incident of dioxin contamination: Sediments of New Jersey estuaries. *Environ. Sci. Technol.* **25**:951–956.
7. **Bopp, R.F.** 1988. Dioxins in Newark Bay. Lamont Doherty Geological Observatory Annual Report. Columbia University, Palisades, NY.
8. **Safe, S.H.** 1986. Comparative toxicology and mechanism of action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. *Annu. Rev. Pharmacol. Toxicol.* **26**:371–399.
9. **Helder, T.** 1980. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on early life stages of the pike *Esox lucius*. *Sci. Total Environ.* **14**:255–264.
10. **Helder, T.** 1981. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on early life stages of rainbow trout (*Salmo gairdneri*, Richardson). *Toxicology* **19**:101–112.
11. **Walker, M.K., J.M. Spitsbergen, J.R. Olson** and **R.E. Peterson.** 1991. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin toxicity during early life-stage development of lake trout (*Salvelinus namaycush*). *Can. J. Fish. Aquat. Sci.* **48**:875–883.
12. **Spitsbergen, J.M., J.M. Kleeman** and **R.E. Peterson.** 1988. Morphologic lesions and acute toxicity in rainbow trout (*Salmo gairdneri*) treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Toxicol. Environ. Health* **23**:333–358.
13. **Spitsbergen, J.M., J.M. Kleeman** and **R.E. Peterson.** 1988. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin toxicity in yellow perch (*Perca flavescens*). *J. Toxicol. Environ. Health* **23**:359–383.
14. **Wisk, J.D.** and **K.R. Cooper.** 1990. Comparison of the toxicity of several polychlorinated dibenzo-*p*-dioxins and 2,3,7,8-

tetrachlorodibenzofuran in embryos of the Japanese medaka (*Oryzias latipes*). *Chemosphere* **20**:361–377.
15. **Leim, A.H.** and **W.B. Scott.** 1966. Fishes of the Atlantic coast of Canada. *Fish. Res. Board Can. Bull.* 155.
16. **Relyea, K.** 1983. A systematic study of two species complexes of the genus *Fundulus* (Pisces: Cyprinodontidae). *Bull. Fl. State Mus. Biol. Sci.* **29**:1–20.
17. **Meredith, W.H.** and **V.A. Lotrich.** 1979. Production dynamics of a tidal creek population of *Fundulus heteroclitus* (Linnaeus). *Estuarine Coastal Mar. Sci.* **8**:99–118.
18. **Armstrong, P.B.** and **J.S. Child.** 1965. Stages in the normal development of *Fundulus heteroclitus*. *Biol. Bull.* **128**:143–168.
19. **Weis, J.S., P. Weis, M. Heber** and **S. Vaidya.** 1981. Methylmercury tolerance of killifish (*Fundulus heteroclitus*) embryos from a polluted vs. nonpolluted environment. *Mar. Biol.* **65**:283–287.
20. **Spitsbergen, J.M., M.K. Walker, J.R. Olson** and **R.E. Peterson.** 1991. Pathologic alterations in early life stages of lake trout, *Salvelinus namaycush*, exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as fertilized eggs. *Aquat. Toxicol.* **19**:41–72.
21. **Roberts, R.J.** and **C.J. Shepherd.** 1986. Diseases of eggs and sac fry. In *Handbook of Trout and Salmon Diseases*. Fishing News Books Ltd., Farnham, Surrey, UK, pp. 94–105.
22. **Cooper, K.R.** 1989. Effects of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans on aquatic organisms. *CRC Crit. Rev. Aquat. Sci.* 1:227–242.
23. **Vinson, S.B., C. Boyd** and **D. Ferguson.** 1963. Resistance to DDT in the mosquitofish *Gambusia affinis*. *Science* **139**:217–218.
24. **Andreason, J.K.** 1985. Insecticide resistance in mosquitofish of the lower Rio Grande Valley of Texas—an ecological hazard? *Arch. Environ. Contam. Toxicol.* **14**:573–577.
25. **Angus, R.A.** 1983. Phenol tolerance in populations of mosquitofish from polluted and nonpolluted waters. *Trans. Am. Fish. Soc.* **112**:794–800.
26. **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.
27. **Overstreet, R.M.** and **H.D. Howse.** 1977. Parasites and diseases of estuarine fishes in polluted habitats of Mississippi. *Ann. N.Y. Acad. Sci.* **298**:427–463.
28. **Murchelano, R.A.** and **R.E. Wolke.** 1985. Epizootic carcinoma in the winter flounder, *Pseudopleuronectes americanus*. *Science* **228**:587–592.
29. **Baumann, P.C., W.D. Smith** and **W.K. Parland.** 1987. Tumor frequencies and contaminant concentrations in brown bullheads from an industrialized river and a recreational lake. *Trans. Am. Fish. Soc.* **116**:79–92.
30. **McCain, B.B., K.V. Pierce, S.R. Wellings** and **B.S. Miller.** 1977. Hepatomas in marine fish from an urban estuary. *Bull. Environ. Contam. Toxicol.* **18**:1–19.
31. **Neff, J.M.** 1985. Polycyclic aromatic hydrocarbons. In G.M. Rand and S.R. Petrocelli, eds., *Fundamentals of Aquatic Toxicology, Methods and Applications*. Hemisphere, New York, NY, pp. 416–454.
32. **Haasch, M.L., E.M. Quardokus, L.A. Sutherland, M.S. Goodrich** and **J.J. Lech.** 1993. Hepatic CYP1A1 induction in rainbow trout by continuous flowthrough exposure to B-naphthoflavone. *Fundam. Appl. Toxicol.* **20**:72–82.
33. **Schoor, W.P.** and **J. Couch.** 1979. Correlation of mixed-function oxidase activity with ultrastructural changes in the liver of a marine fish. *Cancer Biochem. Biophys.* 4:95–104.
34. **Hodson, P.V., M. McWhirter, K. Ralph, B. Gray, D. Thivierge, J.H. Carey, G. Van Der Kraak, D.M. Whittle** and **M.-C. Levesque.** 1992. Effects of bleached kraft mill effluent on fish in the St. Maurice River, Quebec. *Environ. Toxicol. Chem.* **11**: 1635–1651.