TEMPORAL TRENDS IN ETHOXYRESORUFIN-O-DEETHYLASE ACTIVITY OF BROOK TROUT (SALVELINUS FONTINALIS) FED 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

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Abstract—Changes in ethoxyresorufin-O-deethylase (EROD) activity were monitored through an extended 6-month dietary exposure to determine the relationship between EROD activity and uptake of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in brook trout, Salvelinus fontinalis. Brook trout were fed labeled TCDD during a 4-week loading phase and an 11-week maintenance phase to achieve whole-body concentrations of 0, 75, 150, 300, 600, and 1,200 pg TCDD/g fish. A spawning phase followed during which no TCDD was introduced. The TCDD had an extended half-life, with maximal levels detected in the late loading–early maintenance phases and 81 d after TCDD had been removed from the diet. Accumulation in liver increased as whole-body target concentration increased but was generally less than half of anticipated whole-body target concentrations. The EROD activity demonstrated a dose-dependent increase. Positive correlations were observed between EROD activity and TCDD body burdens for both males and females. For males, maximal induction was attained early in the maintenance phase and maintained during latter phases. For females, induction was characterized by a biphasic pattern. Maximal induction was attained during late loading–early maintenance, with an attenuated response observed just before spawning. In addition, the induction response was modulated by sex, as induction was lower in females when compared with males. If sexual biases are considered, increased EROD activity may serve as an indicator of level of TCDD exposure and a sublethal predictor of effects of exposure.

Keywords—Ethoxyresorufin-O-deethylase Induction 2,3,7,8-Tetrachlorodibenzo-p-dioxin Brook trout Salvelinus fontinalis

INTRODUCTION

Polychlorinated dibenzo-p-dioxins are planar chlorinated hydrocarbons that are formed as byproducts during chloro-organic manufacturing processes, as combustion byproducts, and, to a lesser extent, by natural processes. Planar chlorinated hydrocarbons are ubiquitous, resist chemical and biological degradation, and many forms bioaccumulate. The 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic of the aromatic heterocyclic polychlorinated dibenzo-p-dioxins and is a potent animal carcinogen [1–3]. Because TCDD has been widely investigated, it serves as a prototype for studying toxic effects of related planar chlorinated hydrocarbons, particularly the dioxin family.

The TCDD and other polychlorinated dibenzo-p-dioxins are released into the aquatic environment by atmospheric deposition of dry and wet aerosols formed during municipal waste incineration [4]. They can enter the aquatic environment directly from paper and pulp mill discharges, during production and/or use of chlorophenol and chloro-organic pesticides, and as leachates from hazardous waste landfills [5]. The TCDD present in fish and shellfish can, through consumption, be a leading contributor to human exposure [6]. Piscivorous fish and aquatic wildlife are jeopardized during exposure through aquatic food chains or contaminated sediments, and residues are evident in all aquatic species that have been examined [6]. Uptake of contaminants can occur via the gills or the gastrointestinal tract [7]. The predominant route, i.e., 75% of total uptake, for pelagic fish such as lake trout is through diet [8]. Previous determinations of TCDD residues in fish tissues have established that concentrations are low in the United States [9,10]. However, site-specific toxicologically disturbing concentrations have been identified, such as those in lake trout (Salvelinus namaycush) of Lake Ontario, where in 1978, whole-body 2,3,7,8-TCDD concentrations were 78 pg/g [11]. Conditions since then, however, have improved as evidenced by increased lake trout recruitment and decreasing TCDD concentrations in sediments and fish [11].

The TCDD is highly toxic to both juvenile and adult fish. Responses such as wasting, mortality, histopathological effects (e.g., edema, hemorrhages, and epithelial lesions), reproductive toxicity, and developmental toxicity via the transfer of TCDD from adult to offspring have been observed [6]. Studies have determined that fish fry are more susceptible to toxic effects of TCDD than are adult fish. Sac fry mortality in salmonids due to low concentrations of TCDD in eggs has been associated with the stress syndrome known as blue-sac disease.
EROD response of TCDD-fed brook trout

[12]. Research has shown no significant effects in adult fish below 500 pg/g 2,3,7,8-TCDD. In contrast, the no observable adverse effects level for lake trout egg concentration was 34 pg/g 2,3,7,8-TCDD, while 104 pg/g caused complete hatched fry mortality [6]. However, these studies were limited to growth and survival responses. Investigations using other species have revealed that TCDD can affect several physiological systems resulting in a variety of symptoms [6]. These studies have prompted the search for sublethal measures of TCDD effects that may afford more predictive capability than survival and growth endpoints.

A consensus has evolved that the majority of the toxic effects of TCDD are mediated by binding to the aryl hydrocarbon (Ah) receptor [13]. Therefore, Ah receptor-mediated induction of the cytochrome P4501A (CYP1A) biotransformation enzyme system has emerged as an appropriate sublethal assay of TCDD effects [14]. The induction of the CYP1A enzyme system, characterized by increased enzyme activity following exposure to Ah-active chemicals such as TCDD, is well studied [15–17]. For instance, rainbow trout, mirror carp, and cod demonstrated induction when exposed to TCDD [18–20].

Increased catalytic activity of CYP1A-dependent ethoxyresorufin-O-deethylase is commonly used as a measure of CYP1A induction [21]. The use of ethoxyresorufin-O-deethylase (EROD) activity as an exposure biomarker is potentially a very effective tool to assess the exposure of native populations by Ah receptor-active chemicals like TCDD and the polycyclic aromatic hydrocarbons 3-methylcholanthrene and benzo(a)pyrene [22]. Fish can be induced by many classes of organic contaminants. For instance, 78- and 18-fold increases in induction were noted in mountain whitefish and in the pea-mouth chub, respectively, collected downstream of urban centers and pulp mills contaminated with dioxin, furan, and polychlorinated biphenyls [23]. Numerous studies have shown strong differences between male and female induction patterns, often typified by reduced EROD activity in reproducitively active females [24–29]. Such sexual differences and reproductive suppression of EROD activity should be clearly defined and considered in any instance where exposure is estimated by level of EROD activity. However, most of the data regarding EROD induction are derived from short-term studies. The profile of EROD activity with a chronic dietary exposure is undefined.

Previous investigations have shown that EROD activity was highly correlated with Ah receptor-active chemicals such as TCDD [18,30] and that TCDD was the most potent CYP1A inducer known [31]. The utility of EROD activity as an exposure biomarker would be enhanced by defining the activity profile with the prototypical inducer 2,3,7,8-TCDD and correlating hepatic EROD activity to whole-body or liver TCDD residues in the fish. The focus of this study was to characterize the relationship between EROD activity and TCDD body burdens in brook trout (Salvelinus fontinalis). Changes in EROD activity were monitored through time during an extended 6-month dietary exposure designed to mimic natural conditions. The goal of the dietary exposure was to achieve whole-body steady-state levels. We anticipated that increased TCDD body burden would result in increased levels of hepatic EROD activity.

**MATERIALS AND METHODS**

**Test organisms**

Brook trout are easily reared in the laboratory and spawn within two years at body weights between 100 and 400 g. In

**Fig. 1.** Experimental design for 6-month dietary 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) exposure study. An initial 4-week dioxin-loading phase (days 0–29) was used to bring trout to the target whole-body concentrations. An 11-week dioxin-maintenance phase (days 30–106) was used to sustain whole-body target concentrations over time. The TCDD was withdrawn from the diet for the final 12-week no-dioxin phase, which spanned the spawning and postspawning periods (days 107–187). For examination of effects of reproductive state on TCDD accumulation and EROD response, data from samples on days −17, 9, 23, 30, 44, and 64 were grouped as prespawn, days 85 and 99 as near-spawn, and day 187 as postspawn.
rodibenzo-464

The F1 generation is examined in Johnson et al. [32]. Pharamare presented in Tietge et al. [33]. Maternally derived dose to concerning tissue distribution and effects on adult brook trout larger cooperative study designed to determine the sensitivity conditions. Temperature was adjusted to 12°C to facilitate spawning. The photoperiod conformed to ambient which time ¯ow rates to each tank were reduced to 1 L/min.

Sampling

Flow rates were maintained at 2 L/min through the completion of the exposure (loading and maintenance) period, at which time flow rates to each tank were reduced to 1 L/min to facilitate spawning. The photoperiod conformed to ambient conditions. Temperature was adjusted to 12°C preexposure, 10°C during exposure (loading phase and maintenance phase, August 10–October 25, 1993), and 8°C postexposure. Water temperature, dissolved oxygen, alkalinity, hardness, and pH were monitored throughout the test.

Experimental parameters

Six treatment groups in four replicate tanks were defined by the following whole-body TCDD concentrations: 0.0, 75, 150, 300, 600, and 1,200 pg/g tissue. These whole-body target concentrations, based on an egg:whole-body ratio assumed to be 0.5, were chosen as bracketing the LCegg50 concentrations of lake trout and brook trout [33]. Seventeen fish, in the sex ratio of 10 female, 6 male, and 1 unknown, were initially installed in each tank. Nominal concentrations were determined during the loading phase by using the target residue in adult fish, daily feeding rate, and an assumed assimilation rate of 50% and by estimating the time required to reach target residues. Nominal concentrations in the maintenance phase were determined using the target residue in adults, the daily feeding rate, the expected growth during the exposure, and the depuration of original residue.

Tissue residue analyses

Lever tissue was oxidized and counted on a liquid scintillation counter. Equipment performance was monitored before each use using a tritium standard. Scintillation counting operation was maintained by routinely counting a set of quench standards. Blanks were used to monitor oxidizer efficiency. Tissues were analyzed in ascending order of nominal concentration to minimize cross-contamination.

EROD assay

The EROD activity was determined from the rate of formation of the fluorescent product resoru®n according to Lin et al. [35] with some modification. Microsomes for the catalytic assay were obtained from frozen fish liver samples. A 60-µl subsample was used for protein determination using a BCA Protein Reagent Kit (Pierce Chemical, Rockford, IL, USA) and bovine serum albumin provided as a standard. Two hundred and fifty microliters in tubes were stored for no more than 6 weeks at −80°C for EROD analysis. The deethylation reaction was performed in a shaking water bath at 20°C rather than 37°C. The reaction was stopped after a 10-min incubation. Resoru®n formation was measured directly using a Shimadzu RF 5000U Spectro®uorometer (Shimadzu, Kyoto, Japan). The fluorometer was calibrated with a 10 µl of 0.01 mM resoru®n in ethanol solution. Activity was expressed as picomoles resoru®n per 10 min per milligram protein.

Statistics

Since EROD induction, or increase of activity, was of interest, Dunnet’s one-tailed test was used to test for differences in EROD activity between controls and each level of TCDD treatment. Linear regression analyses were used to determine the relationship between liver TCDD concentrations and EROD activity. The general linear model was employed to test for the equality of regression slopes between male and female fish by reproductive stage and between reproductive stages by sex. A partial F statistic was calculated to determine the signi®cance of the interaction between slope and fish sex. A significant interaction would cause slopes to be signi®cantly different between sexes. The same statistic was calculated to determine the signi®cance of the interaction between slope and reproductive stage. A significant interaction would cause slopes to be signi®cantly different between reproductive stages within a sex. Statistical analyses were performed using SAS® software [36]. Statistical signi®cance was set at p < 0.05.

RESULTS

TCDD uptake

Mean TCDD concentrations for liver of female and male trout are shown in Figure 2A and B. The TCDD concentration in livers of control fish sampled were <10 pg/g for both male and female fish (Table 1). No differences between TCDD concentrations in female and male control fish were noted (mean for all sampling days was <1 pg/g for both female and male fish). For female fish, some treatments showed biphasic accumulation with near-spawn decline followed by a postspawn course of the experiment to monitor exposure-related effects. Liver samples were analyzed for EROD activity and TCDD levels once before the loading phase began (day −17), on two occasions during the loading phase (days 9 and 23), five times during the maintenance phase (days 30, 44, 64, 85, and 99), and on one occasion during the no-TCDD phase (day 187).

EROD assay

The EROD activity was determined from the rate of formation of the fluorescent product resoru®n according to Lin et al. [35] with some modification. Microsomes for the catalytic assay were obtained from frozen fish liver samples. A 60-µl subsample was used for protein determination using a BCA Protein Reagent Kit (Pierce Chemical, Rockford, IL, USA) and bovine serum albumin provided as a standard. Two hundred and fifty microliters in tubes were stored for no more than 6 weeks at −80°C for EROD analysis. The deethylation reaction was performed in a shaking water bath at 20°C rather than 37°C. The reaction was stopped after a 10-min incubation. Resoru®n formation was measured directly using a Shimadzu RF 5000U Spectro®uorometer (Shimadzu, Kyoto, Japan). The fluorometer was calibrated with a 10 µl of 0.01 mM resoru®n in ethanol solution. Activity was expressed as picomoles resoru®n per 10 min per milligram protein.

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EROD response of TCDD-fed brook trout

Fig. 2. The 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) accumulation in liver of female (A) and male (B) trout over the time course of the experiment. Bars represent the mean (±1 standard error) TCDD liver concentration by date for each whole-body target treatment group (0.0, 75, 150, 300, 600, and 1,200 pg/g). A. (n = 1–4 per treatment for all sample days except day 187, when n = 3–6). B. (n = 1–3 per treatment for all sample days except day 187, when n = 2–5). Loading, maintenance, and no-TCDD feeding phases are delineated by dashed vertical lines. Corresponding reproductive stage is indicated below X axis.

increase. Male fish of most treatments showed concentration increases up to days 23 to 30 and a plateau between days 23 to 30 and 187. Maximal levels of TCDD were measured in liver of all fish after 23 to 30 d of dietary exposure. The appearance of TCDD in liver as early as 9 d indicates a rapid uptake and distribution. Elevated concentrations were maintained throughout the exposure (loading and maintenance phases) and throughout the no-TCDD phase even though TCDD was removed from the diet on day 106. The TCDD residues equivalent to day 23 to 30 loading-phase levels were measured for both sexes on day 187 of the no-TCDD (postspawn) phase. Generally, TCDD liver concentrations were less than half of targeted whole-body concentrations, although measured TCDD liver concentrations increased in relatively similar increments as whole-body target concentrations (Table 1).

EROD responses

Temporal changes in EROD activity for female and male brook trout are shown in Figure 3A and B. The EROD activity for both females and males exhibited a dose-dependent increase. For female fish, statistically significant (p < 0.05) EROD induction differences between treatment and control fish were noted when whole-body target concentrations were ≥600 pg/g TCDD and corresponding mean liver TCDD residues were ≥147 pg/g. Statistically significant (p < 0.05) EROD induction was also noted in male fish when whole-body target concentrations were ≥600 pg/g TCDD and mean liver TCDD was ≥170 pg/g (Table 1).

The EROD activity for female fish demonstrated a biphasic pattern somewhat reflective of TCDD concentrations in the liver. An attenuated induction response was noted in late maintenance (near-spawn) female fish, followed by a return to loading-induction levels during the last sampling event on day 187 of the no-TCDD (postspawn) phase. A similar near-spawn decline was not observed in male fish. For treated males, maximal loading-phase levels of EROD induction were generally maintained throughout the maintenance no-TCDD phases. As noted for TCDD liver residues, EROD activity levels at day 187 during the no-TCDD (postspawn) phase were comparable to
loading-phase activity levels despite the removal of TCDD from the diet 81 d earlier. The relationship between EROD levels and TCDD concentrations in the liver of female brook trout is shown in the dose–response curve of Figure 4. A significant ($p < 0.001$) regression equation was calculated for this relationship ($Y = 4.703X - 64.929$, $r^2 = 0.675$, $r = 0.822$). Variation surrounding the best fit regression line increased as TCDD liver concentrations increased. The relationship between EROD levels and TCDD concentrations in the liver of male brook trout is illustrated in the dose–response curve of Figure 5. A significant ($p < 0.001$) regression equation was also calculated for this relationship ($Y = 5.956X - 66.471$, $r^2 = 0.715$, $r = 0.846$). Variation surrounding the best fit regression line increased as TCDD liver concentrations increased and was greater than that observed in female fish. Generally, TCDD liver concentrations versus EROD activity in males showed the same pattern as females, although activity levels in males were higher than those observed in females.

Figure 6 depicts the possible influence of sex and/or reproductive stage on EROD response and TCDD level. Both females and males were exposed to a constant dose of TCDD through the food. Prespawn samples were collected up to day 64, near-spawn samples were collected on days 85 and 99, and postspawn samples were collected on day 187. For female trout, shown in Figure 6, the near-spawn linear curve for EROD versus TCDD was less than the prespawn, which was, in turn, less than the postspawn. The female near-spawn slope was significantly lower ($p < 0.001$) than the postspawning slope, indicating a reproductively influenced attenuation of EROD activity. In addition, TCDD levels in near-spawn females were reduced when compared with TCDD levels attained in pre- and postspawn female trout. For male trout, the prespawn linear curve was less than the near-spawn curve, which was less than the postspawn. Near-, pre-, and postspawn EROD and TCDD levels showed fewer differences than those of females. Slopes for male near- and postspawning stages were not significantly different, confirming the absence of a near-spawn decline of EROD activity in this sex. When males and females were compared, pre- and postspawning regression lines were not significantly different. Regression lines for near-spawn male and female fish were significantly different ($p < 0.001$), further evidencing near-spawn suppression of EROD activity in females and the influence of sex as well as reproductive state on EROD response.

### Table 1. Comparison of mean liver 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) concentration and ethoxyresorufin-O-deethylase (EROD) activity by sex and whole-body target concentrations

<table>
<thead>
<tr>
<th>Sex</th>
<th>Treatment (pg/g)</th>
<th>Liver TCDD concentration (pg/g)</th>
<th>EROD activity (pmol RS/mg protein/10 min)</th>
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<tbody>
<tr>
<td>Female</td>
<td>0</td>
<td>0.82</td>
<td>23.28</td>
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<tr>
<td></td>
<td>(2.37)</td>
<td></td>
<td>(29.62)</td>
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<tr>
<td></td>
<td>75</td>
<td>17.66</td>
<td>54.25</td>
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<tr>
<td></td>
<td>(9.32)</td>
<td></td>
<td>(86.78)</td>
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<tr>
<td></td>
<td>150</td>
<td>32.02</td>
<td>104.83</td>
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<tr>
<td></td>
<td>(16.40)</td>
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<td>(148.65)</td>
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<td>300</td>
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<tr>
<td></td>
<td>(35.68)</td>
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<td>(220.39)</td>
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<td></td>
<td>600</td>
<td>147.34</td>
<td>608.76*</td>
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<tr>
<td></td>
<td>(76.19)</td>
<td></td>
<td>(772.36)</td>
</tr>
<tr>
<td></td>
<td>1,200</td>
<td>310.22</td>
<td>1,060.17*</td>
</tr>
<tr>
<td></td>
<td>(172.24)</td>
<td></td>
<td>(1,251.80)</td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>0.45</td>
<td>73.89</td>
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<tr>
<td></td>
<td>(0.77)</td>
<td></td>
<td>(73.20)</td>
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<tr>
<td></td>
<td>75</td>
<td>23.42</td>
<td>107.06</td>
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<td>150</td>
<td>37.92</td>
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<td></td>
<td>(18.25)</td>
<td></td>
<td>(120.78)</td>
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<td>300</td>
<td>108.50</td>
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<td>(25.24)</td>
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<td>600</td>
<td>169.78</td>
<td>1,088.51*</td>
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<tr>
<td></td>
<td>(63.44)</td>
<td></td>
<td>(897.37)</td>
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<tr>
<td></td>
<td>1,200</td>
<td>400.04</td>
<td>2,165.21*</td>
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<tr>
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<td>(194.67)</td>
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<td>(1,724.11)</td>
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</table>

* Concentration values are means (standard deviation in parentheses).
* Significant ($p < 0.05$) difference from control treatment.

**DISCUSSION**

Accumulation, distribution, retention, and toxicity of TCDD vary among different organisms exposed through an aquatic system. Invertebrates are relatively insensitive and can potentially accumulate high concentrations, which could be passed up the food chain to vertebrates [37]. In fish, ingested toxicants may be excreted or, as can be the case for TCDD, may be translocated from the gastrointestinal tract via the circulatory system to be stored or metabolized in the liver, adipose tissue, kidney, bone and support tissues, brain, and central nervous system [38]. Fish as a class tend to be the most sensitive to TCDD of the vertebrates, though toxicological sensitivity varies by species [39]. Studies have indicated that TCDD half-life may also vary by species and by exposure history. For killifish (*Fundulus heteroclitus*) following a single dermal injection of TCDD, half-lives were 34, 19, 53, and 8 h for liver, gall bladder, carcass, and blood of resistant fish, respectively, and 46, 28, 61, and 50 h for the same organs of sensitive fish [40]. For rainbow trout and mirror carp, TCDD half-lives were 98 and 325 d, respectively [41]. Tietge et al. [33] estimated a whole-body TCDD half-life of 84 d for the brook trout used in this study. This was reflected in the current study by liver TCDD concentrations that showed an extended half-life with maximal levels detected in the late loading–early maintenance phases and even 81 d after TCDD had been removed from the diet. Accumulation in liver increased as whole-body target concentration increased but was generally less than half of whole-body target concentrations.

The EROD induction in our study generally followed expected trends, increasing with dose. For females, induction was biphasic, as maximal induction was attained during loading and spawning phases but a decrease was observed just before spawning. For males, maximal induction attained early in the maintenance phase was maintained during later phases. Mean induction levels in males were generally higher than those observed in female fish in this study. Bioaccumulation of TCDD was generally higher in male fish, basal EROD activity was higher in males as compared with females in the control treatment (Table 1), and some research indicates that, generally, enzyme levels and EROD activity levels are elevated in males when compared with females of the same species [42]. However, when normalized to baseline, EROD response to incremental increase of TCDD dose was proportionally greater for females than for males. For males, activity levels increased 1.4-, 1.8-, 5.4-, 15-, and 29-fold for each increase in whole-body target concentrations, while for female fish, induction normalized to basal activity increased 2.3-, 4.6-, 6.3-, 26-, and 46-fold (Table 1). When sexes were combined and normalized in this manner, 1.7-, 2.7-, 5.3-, 19-, and 45-fold
Fig. 3. The ethoxyresorufin-O-deethylase (EROD) activity levels in female (A) and male (B) trout. Bars represent mean (±1 standard error) EROD induction level. Other details as in Figure 2.

Fig. 4. Dose–response curve of EROD versus TCDD for female brook trout. Values of all treatment groups (0.0, 75, 150, 300, 600, and 1,200 pg/g TCDD whole-body target residues) for nine sampling events were combined. A significant ($p < 0.05$) regression equation, $Y = 4.703X - 64.929$, was calculated, $r^2 = 0.675$. The cluster of points near zero activity and below 200 pg/g TCDD are generally prespawning females.

Fig. 5. Dose–response curve for ethoxyresorufin-O-deethylase (EROD) versus 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for male brook trout. Values of all treatment groups (0.0, 75, 150, 300, 600, and 1,200 pg/g TCDD whole-body target residues) for nine sampling events were combined. A significant ($p < 0.05$) regression equation, $Y = 5.956X - 66.471$, was calculated, $r^2 = 0.715$. 
increases in activity were noted for corresponding increases in TCDD dose.

Some studies have shown that withdrawal of the inducing agents similar in activity to TCDD results in a return to baseline induction levels within 48 h [42], whereas in the van der Weiden [41] study with mirror carp, significant induction levels were observed 12 weeks postexposure to TCDD. For our study, the return to loading-phase activity levels in treated female fish and the continuous EROD induction response pattern in treated male fish is reflective of the pattern of levels of TCDD detected in the liver. Tietge et al. [33] found that TCDD levels in target tissues generally followed lipid concentrations. However, while liver lipid percentage in females in that study did change cyclically in response to sexual maturity and spawning, the variation was less than 2% and the percentage increased, rather than decreased, to near-spawn. The mechanism for liver TCDD decline near-spawn is therefore not clear on this basis alone. The relatively large investment of lipids in ovaries and eggs may, however, be a factor since these researchers estimated 61 and 39% of the whole-body TCDD load to sequester in these tissues. The same study also noted a net increase in TCDD per gram body weight concentration resulting from spawning [33]. This may be reflected in the postspawn increase in liver TCDD concentrations.

The prespawn decline in EROD activity seen in fish, particularly in female fish, during the maintenance phase is consistent with current understanding that CYP1A levels vary seasonally, decline with the approach of spawning, and are influenced by the health, condition, sex, temperature, and reproductive and developmental status of the organism [24,28,42]. Where studies have shown strong differences between male and female responses, induction patterns suggest that gender differences are related to suppression of CYP1A gene expression in reproductively active females. This suppression in gravid females has been tied to the presence of estradiol, which is responsible for stimulation and maintenance of sex organs and for breeding behavior, in reproductively active female fish [43]. Andersson and Förlin [27] observed gender-related differences in fish, specifically diminished CYP1A activity in females, related to plasma hormone levels. Several reports are available that demonstrate sex differences in trout with respect to CYP1A induction [28]. They have shown that both EROD and CYP1A protein levels are lower in reproductively active mature female fish when compared with male fish. The EROD activity decreased in prespawning female dab from the North Sea [29], in reproductive female bluegill sunfish when compared with males sampled during the same season [24], and in spring sampling of white sucker females when compared with males [25,26]. The influence of these various factors may have in TCDD tissue distribution was not discussed.

Experimental results from our study confirm current understanding that EROD activity is lower in female fish generally (Table 1, Figs. 2–5) and particularly in gravid females (Fig. 6) when compared with male fish. Not only were EROD activity levels generally lower in female than in male fish, but a significant attenuation was noted in the reproductively mature brook trout. When data were grouped into pre-, near-, and postspawn reproductive stages and EROD was plotted against TCDD, male and female pre- and postspawn regression lines were not significantly different while regression lines for near-spawn male and female fish were different, reflective of a reproductively suppression in near-spawn females.

Hahn et al. [44], Melancon and Lech [45], Monosson and Stegeman [43], and Gooch et al. [46], among others, have reported induction patterns that were not always consistent and did not follow expected trends, with reduced induction in high concentrations when strong induction was observed at low concentrations and when linear dose–responses were predicted. Gooch et al. [46] found lower activity at high doses in vitro studies. The literature has attributed these attenuated events to hepatotoxicity, cytotoxicity, and, more recently, kinetic mechanisms (i.e., competitive inhibition whereby the inducer interferes with activity by competing with the substrate, in this case 7-ethoxyresorufin, for the active site on the enzyme) [47]. The increase in variation of EROD response with increased liver TCDD concentration noted in the present study could be attributed to these factors or to other modulating factors such as diet, sex, temperature, age, and body weight [48].

In the current study, significant and positive correlations were observed when EROD was plotted against TCDD for both males and females. The induction response has been identified as a sensitive early warning indicator for assessing exposure and chemical effects of environmental pollutants in fish [49]. The inducibility of the EROD response demonstrates that TCDD has effects at the molecular level in fish. However, the toxicological and physiological significance of increased CYP1A induction is uncertain and the subject of much debate. Mammalian studies have found correlations between induction and toxic effects such as thymic atrophy, porphyria, weight loss, and immunological changes. In studies with rainbow trout and carp, correlations were observed between EROD and decreased growth and histopathological changes [41]. Poland and
Knutsen [50] speculate that inducers such as TCDD may bind to the Ah receptor and elicit toxic responses by disrupting other functions normally regulated by the receptor.

The CYP1A activities in mammals are important for detoxifying xenobiotics and in pathways that lead to cytotoxic, mutagenic, carcinogenic, and teratogenic metabolites [51–53]. It has been suggested that CYP1A induction is associated with an increased risk for environmentally induced mutagenesis and carcinogenesis [5]. The EROD activity may represent the active form of the CYP1A protein involved with the bioactivation of reactive procarcinogens that affect DNA and increase production of DNA-damaging intermediates [54,55]. Recently, a quantitative positive relationship between induction and mutation was demonstrated when fish with an integrated transgenic reporter bacteriophage gene were exposed to a potent carcinogen [56]. Physiological and biochemical changes in fish precede the onset of more serious population- and community-level effects [57,58]. In studies conducted by the U.S. Environmental Protection Agency, mummichogs were fed an artificial diet containing dioxin in a 4-month study [59]. A suite of endpoints was measured and used to develop a population model for ecological risk assessments. A negative relationship between dioxin dose administered through diet and population growth rate was observed. Differences between high and low treatments were considered ecologically significant. The CYP1A induction was not discussed. The current study, by correlating EROD induction level with TCDD level in a similar long-term dietary exposure, does, however, when coupled with F results [32], address the utility of induction as a biomarker and early indicator of such potential higher level effects. This concurrent study confirmed sac fry mortality caused by low TCDD egg concentrations to be one of the most sensitive indicators of TCDD effects. While none of the treatment levels affected survival, growth, or reproductive endpoints in adults [33], a LC₅₀ of 127 pg/g egg was determined for brook trout at a corresponding whole-body load of 326 pg/g tissue (300 pg/g target) [32]. This implies that adult females can accumulate whole-body burdens that, though not toxic to them, may be toxic to their offspring. A corresponding target whole-body concentration nearly twice this level was required to evoke a statistically significant EROD response in female brook trout in this study. In this respect, EROD activity levels in adult fish, if found to be significantly elevated above background in circumstances where TCDD contamination is indicated, could potentially forewarn hatch and recruitment difficulties in wild populations. The EROD activity measurement could, therefore, serve in a predictive and planning, as well as diagnostic, capacity at the population level.

CONCLUSIONS

The utility of induced EROD activity as an exposure biomarker was reinforced by defining the activity profile with the prototypical inducer 2,3,7,8-TCDD and correlating EROD induction to liver TCDD residues. The long-term dietary exposure mimicked natural conditions and not only provided information about the persistence of TCDD in exposed fish but also permitted time-course examination of EROD response to a chronic exposure. In brook trout fed TCDD, EROD response generally increased with dose. Differences in level of induction were noted between sexes, with males generally more induced than females by the same level of TCDD. A sex effect of particular note was that EROD activity was attenuated in prespawn females. Such gender biases need to be considered when EROD activity is used as an indicator of exposure if level of activity is intended to imply level of exposure. Induction of EROD activity suggests that TCDD has effects at the molecular level in fish. Increased EROD activity may, therefore, serve as a sublethal predictor of more serious population and community effects potential in TCDD exposure.

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