Chapter 14
Effects of Organotins on the Drug Metabolizing Enzymes in Fish

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14.1 Introduction

Drug metabolizing enzymes play important roles in terrestrial and aquatic organisms for the synthesis, metabolism and excretion of various kinds of chemicals, especially lipophilic compounds including natural and synthetic chemicals. Drug metabolizing systems consist of two phases. In the first phase reaction, oxidative, reductive and hydrolytic reactions are dominant, and water solubility of chemicals increase through these processes. The toxicity of chemicals, however, are not necessarily decreased in the first phase and may be toxified in some cases through metabolic activation. In the second phase, conjugate reactions are dominant, and chemicals modified in the first phase are biotransformed to more water soluble compounds, and are readily excreted. These two-phase biotransformation processes are therefore important in the metabolism of lipophilic compounds such as endogenous steroids in the bodies of all animals, and also in the fates and effects of medicines in the medical and pharmaceutical fields. Since the 1970s, drug metabolizing enzymes have been found to be important in evaluating the toxicities of various lipophilic environmental pollutants, including pesticides and industrial chemicals. Although much knowledge has been accumulated on xenobiotic metabolism using experimental and wild animals, that obtained from aquatic organisms is insufficient for the complete understanding of fates of environmental chemicals incorporated in the body compared with mammals such as rats and mice. Many polycyclic aromatic hydrocarbons (PAHs) are known to induce cytochrome P450(CYP) and the metabolites act as potent carcinogens and/or mutagens and are, therefore, considered as important risk factors in epidemiological and epizootiological cancer. The P450 system is universally distributed in all organisms and plays a key role in the metabolism of xenobiotic compounds such as PAHs,

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dioxins, pesticides etc., leading to their detoxification or bioactivation. However, the system is intrinsically important in the metabolism of endogenous substrates including steroids, arachidonic acid, prostaglandins and others.

Thus, drug metabolizing enzyme systems are important for the understanding of both detoxification and bioactivation.

In this chapter, recent reports concerning the relationship between organotins exposure and xenobiotics metabolism by aquatic organisms, mainly fish, are reviewed and discussed.

14.2 Drug Metabolizing Enzymes in Fish

The ability to metabolize xenobiotics such as lipophilic persistent organic pollutants (POPs) is generally considered to be weak in fish compared with mammals. Therefore, various fat-soluble man-made organics, including DDTs and PCBs, readily accumulate in fish tissues. However, there are many reports describing drug metabolizing enzyme activities that are induced by exposure to, or injection of, various kinds of environmental chemicals as shown in Table 14.1 (Ishizuka 1999).

Table 14.1 Environmental relevant inducers and inhibitors of xenobiotic metabolisms

Induction
Aromatic hydrocarbons
PAHs (benzo[a]pyrene, crysene, benzo[k]fluoranthene, benzo[j]fluoranthene), benzen, xylene, buthylated hydroxytoluene, tert-butyl hydroxyquinone, ethoxyquin
Halogenated hydrocarbons
Polychlorinated naphthalene, trichloroethylene, PCBs, PCDDs, PCDFs, hexabromobenzene, buthylated monochlorodiphenylethers
Ethers
Diethyether
Pesticides
Hlogenated pesticides
DDT, alpha-hexachlorocyclohexane, hhlordane, TCPOBOP, mirex, endrin, lindane, dieldrin, toxaphene, trichloro-237, heptachlor, endsulphan
Organophospate pesticides
Malathion
Carbamate pesticides
Carbaryl
Herbicides
CNP, monuron 1967, diuron, tridiphane
Inhibition
Metals
Cadmium, cobalt, manganese, nickel, copper, mercury, organotin
Others
Acrylamide, 3-amino-1.2.3-triazol, paraquat, naphthalene

CYP comprises a family of haemo-proteins that are the dominant catalysts for mono-oxygenase reactions with a large number of drugs, carcinogens and pollutants in terrestrial and aquatic environments.

14.2.1 Cytochrome P450 in Fish

Various kinds of CYPs including CYP1, CYP2, CYP3, CYP4, CYP11 and CYP19-aromatase are known to be distributed in liver, kidney, reproductive gland and brain of fish. As to CYP1 families in fish: CYP1A subfamily (CYP1A1, 1A2, 1A3) CYP1B family and CYP1C family (CYP1C1, 1C2) have been reported. Among these CYP1 families CYP1A are especially important, and DNA responsible for CYP1A has been cloned in several fish species. CYP1A families in fish are induced by the exposure to several PAHs and dioxins. Wild fish have also been investigated regarding the relationship between the levels of several kinds of CYP, including CYP1A, distributing in more than ten species of fish, and water pollution.

β –Naphthoflavone (β –NF) is known to bind to arylhydrocarbon receptor (AhR), and to induce CYP1A activity, and β –NF is popularly used as a CYP1A inducer. Hepatic CYP1A (Ethoxyresorfin-*O*-deethylase:EROD) activity in rainbow trout (*Onchorynchus mykiss*, mean body weight, 80 g) was clearly induced by β –NF exposure at 0.5 µg/l after 24 h, and increased concentration-dependently (Fig. 14.1). EROD activities were induced after 6 h of exposure to β –NF at 1 mg/l, and increased time-dependently (Fig. 14.2) (Nakayama and Kawai 2006).

14.2.2 CYP1A As an Indicator of PAH Pollution in the Aquatic Environments

Aquatic environments including lakes, rivers, estuaries, and coastal areas receive various kinds of anthropogenic inputs, and aquatic organisms are exposed to pollutants such as metals, man-made organic surfactants etc. Several biomarkers have been used to determine sublethal effects of pollutants in fish, for example, metallothionein for metals, plasma vitellogenin for estrogenic substances, brain acetylcholine esterase activities for organophosphorous pesticides and CYP1A1 activities for PAHs and some PCBs. CYP1A1 is one component of the mixed function oxidases (MFO) system, and is important in the detoxification and/or toxification of many PAHs and some PCBs. EROD activity is CYP1A1 dependent and is generally used as a marker of MFO induction. Hepatic EROD activity in flounder (*Platichtthys flesus*) has been measured as an indicator of contaminant exposure in seven English estuaries. Significant induction of EROD activity was clearly observed in flounder from several industrialized estuaries compared to a relatively unpolluted site (Fig. 14.3) (Kirby et al. 1999). Furthermore, close links between EROD induction and hepatic PAH/PCBs concentrations was noticed, as shown in Table 14.2.

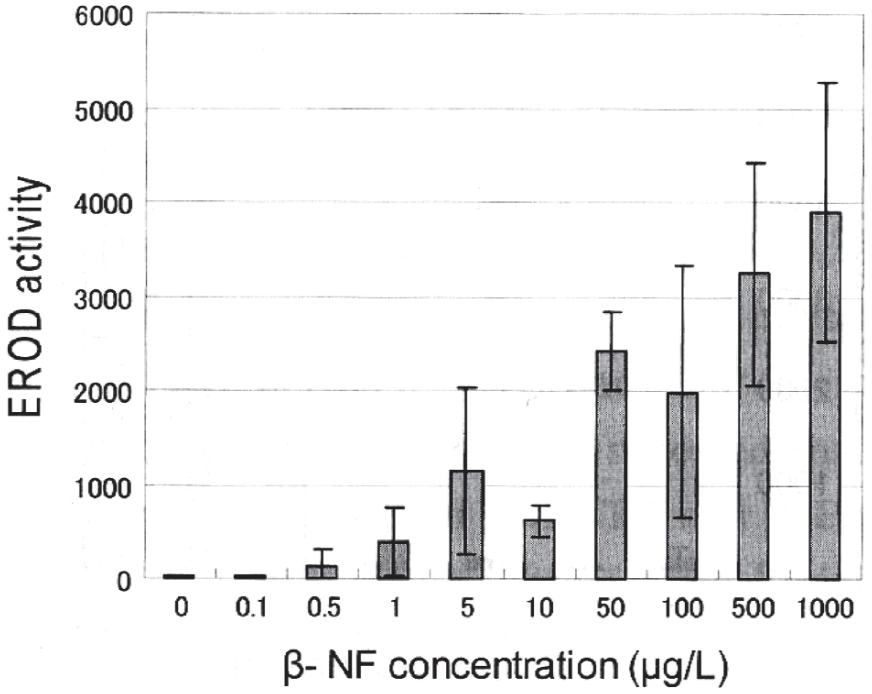


Fig. 14.1 Ethoxyresorufin *O*-deethylase (EROD) activity induced by different concentration of β-naphthoflavone (BNF) ranging from 0.1 to 1,000 μg/l in rainbow trout. EROD activities were expressed as unit/min/individual and means ± standard error (n = 5 for each group, except to control and 0.1 μg/l exposure group (n = 1))

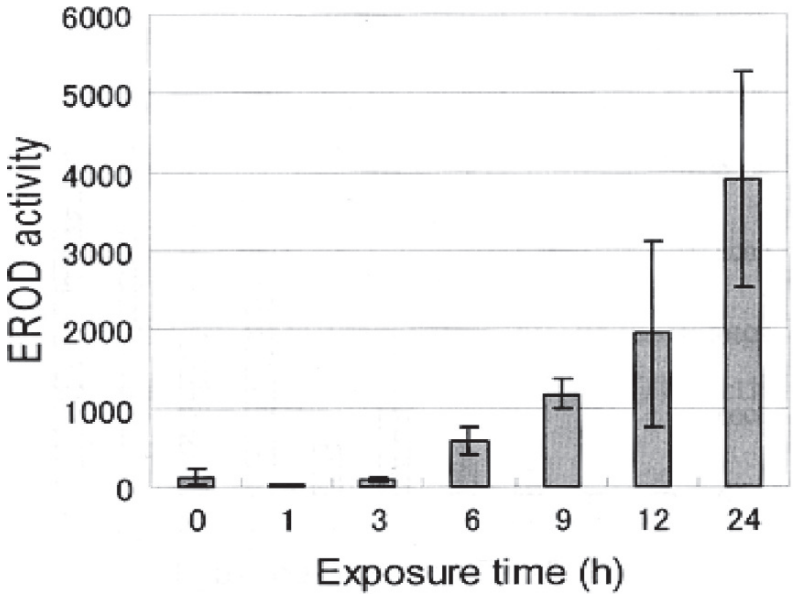


Fig. 14.2 Ethoxyresorufin *O*-deethylase (EROD) activity induced by different exposure time ranging from 0 to 24 h using 1 mg/l of β-naphthoflavone (BNF) in rainbow trout. EROD activities were expressed as unit/min/individual and means ± standard error (n = 5 for each group)

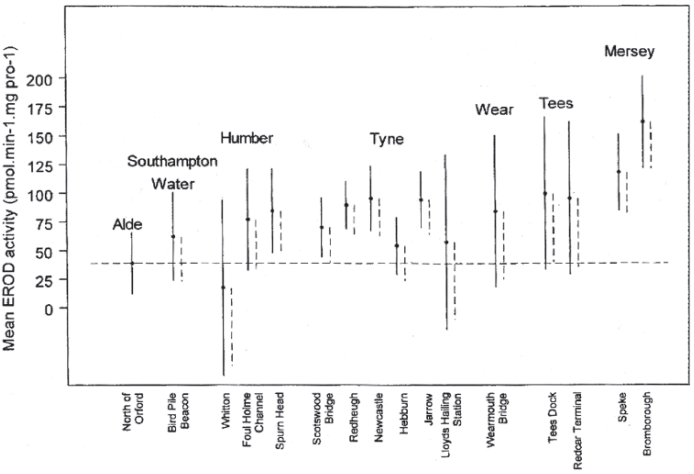


Fig. 14.3 Mean hepatic EROD activity in flounder form English estuarine sites (● is the mean EROD level; – the 95% confidence interval for mean; *horizontal* --- the mean EROD level at reference (Alde) site; *vertical* --- the graphical representation on one-sided *t*-test against reference site)

Table 14.2 Mean total PAH and PCB in bulked samples of flounder liver from selected estuaries

Location	PAH (μg kg^{-1} wet wt)	PCB (μg kg^{-1} wet wt)
River Mersey	363	1,082
River Tees	365	n/a
River Humber	71.2–100.6	424
River alde	109.7	32

n/a – not analysed.

Fuel oil spilled in 1969 and distributing in sediment of Buzzards Bay, Massachusetts was monitored by the induction of EROD activity in marsh fish (*Fundulus heteroclitus*) in 1989. Although residual concentrations of oil or biodegraded metabolites were in trace concentrations, these hydrocarbons appear to induce EROD activity as shown in Fig. 14.4. The fish collected from the spilled area were held in clean water to test whether the induced EROD activity decreased, or not. EROD activity fell to 10% of initial activity after 13 weeks, indicating that initial activity was not fixed and represented recent induction. (Teal et al. 1992).

Rainbow trout injected intraperitoneally with PCBs and polybrominated biphenyls (PBBs) showed a ten-fold increase in aryl hydrocarbon hydoxylase (AHH) activity within 7 days compared with a control group, and the high activity remained constant after 2–3 weeks (Elcombe and Lech 1978).

CYP1A (EROD) activity of gobioid fish (Gobio gobio) living close to a sewage treatment plant (STP) was significantly higher than fish living upstream of the STP (Faller et al., 2003). This suggests that the treated effluent still contained chemicals inducing CYP1A activity. From these findings CYP1A (EROD) activity is an excellent biomarker of pollution by some PAHs or organochlorines.

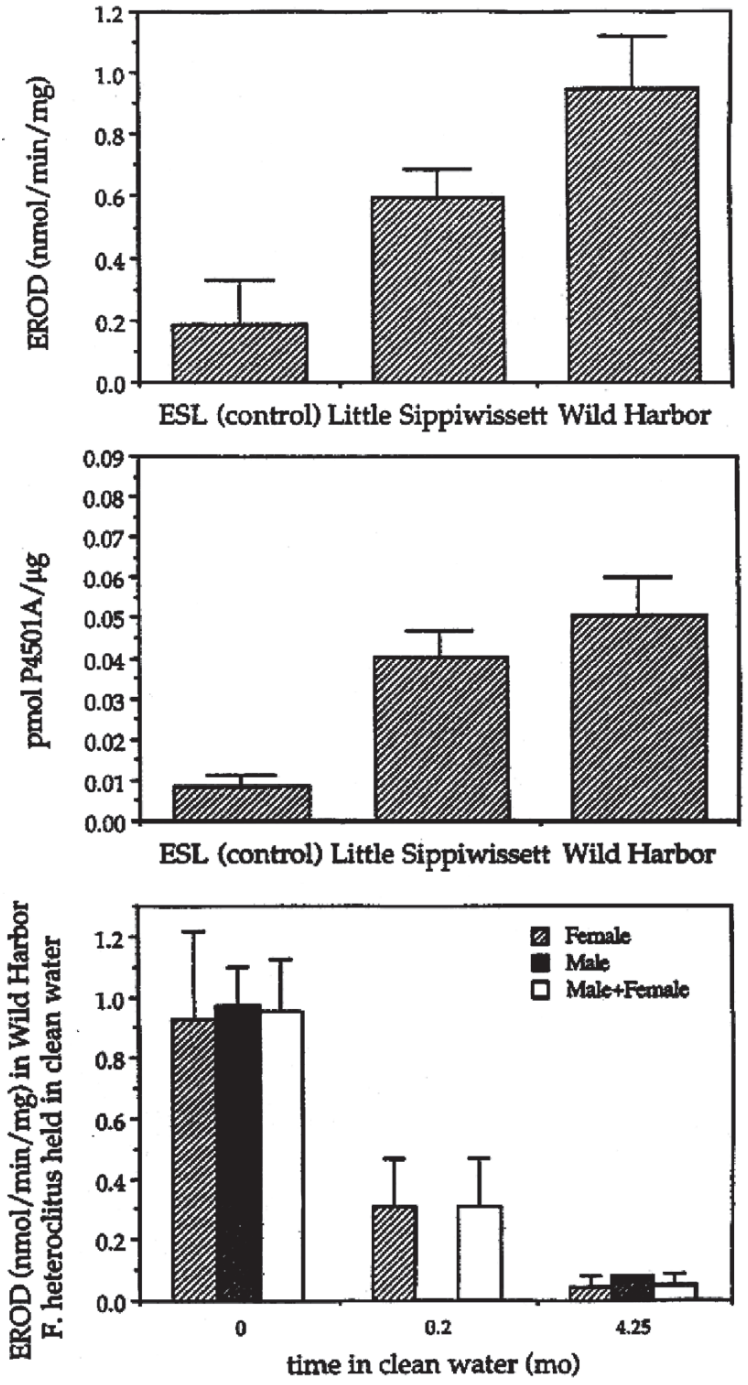
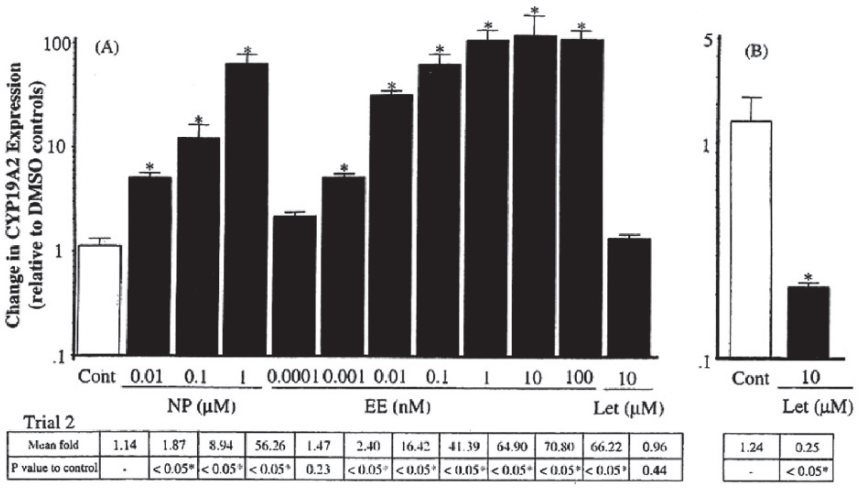


Fig. 14.4 Top – Erod (ethoxy resorufin O-deethylase) activity in *Fundulus heteroclitus* from the oiled Wild Harbor marsh, a reference marsh in Buzzards Bay, Little Sippewissett, and fish held under clean conditions at ESL. Middle – concentrations of P4501A in the same fish. Bottom – the time course of decrease in EROD activity in fish held in clean water



Mean fold	1.14	1.87	8.94	56.26	1.47	2.40	16.42	41.39	64.90	70.80	66.22	0.96
P value to control	-	< 0.05*	< 0.05*	< 0.05*	0.23	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	0.44

1.24	0.25
-	< 0.05*

Fig. 14.5 Response of CYP19A2 transcription to the estrogenic chemicals, NP and EE, and an aromatase inhibitor, letrazole. CYP19A2 transcript abundance in zebrafish juveniles exposed to xenobiotics for 3 days (a) and 30 days (b). Transcript abundance is expressed relative to that of the vehicle control group. The results represent the mean ± SEM on six samples. The asterisk indicates statistically significant differences ($P < 0.05$)

Cytochrome P450 aromatase (CYP19) is the key steroidgenic enzyme responsible for conversion of androgens to estrogens, which play a critical role in developmental sex differentiation and the adult reproductive cycle in vertebrates. The influence of multiple classes of endocrine disrupting chemicals on the transcript abundance of two CYP19A1 and A2 were investigated in juvenile zebrafish. Estrogenic compounds such as nonylphenol and a pharmaceutical estrogen (ethinylestradiol) strongly enhanced the expression of the CYP19A2 gene in a dose-dependent manner. Exposure to benzo[a]pyrene (BaP) also significantly increased CYP19A2 transcript abundance (Fig. 14.5) (Kazeto et al. 2004)

14.3 Inhibition of CYP Activities by Organotins

Organotins such as TBT and triphenyltin (TPT) have been widely used as antifouling biocides where it is highly toxic to aquatic organisms. It has also been reported to inhibit cytochrome P450.

14.3.1 CYP1A Activity and Organotins

Hepatic microsomes were incubated in vitro with TBT and TPT, and various components analyzed. EROD activity was strongly inhibited by TBT and TPT in a

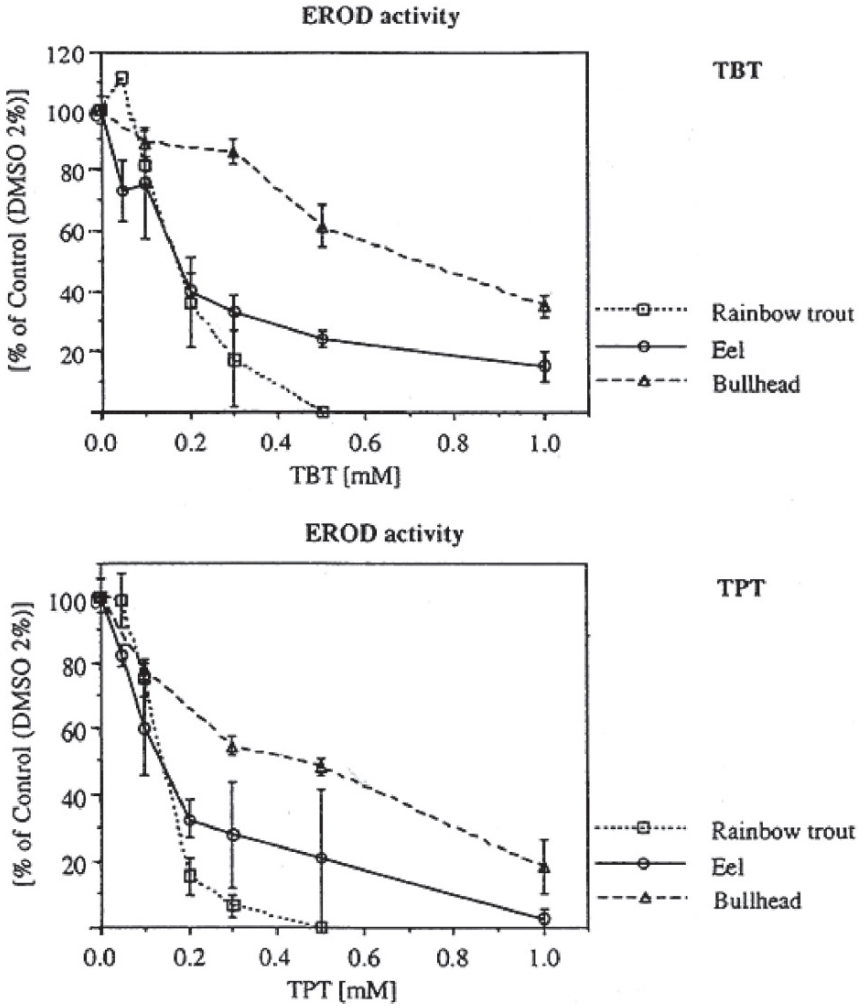


Fig. 14.6 Concentration dependence of the inhibition of ethoxyresorufin *O*-deethylase (EROD) activity after 5 min incubation at 30°C in presence of TBT (*top*) and TPT (*below*) in DMSO in rainbow trout, eel, and bullhead. Averages ± SEM of at least three separate determinations

concentration-dependent manner in fish, including rainbow trout (*Oncorhynchus mykiss*), European eel (*Anguilla anguilla*) and bullhead (*Cottus gobio*) (Fent and Bucheli 1994). Rainbow trout microsomes were more sensitive than were eel or bullhead microsomes (Fig. 14.6). In all fish, both organotins led to a time- and concentration-dependent decrease in spectral total microsomal P-450 content, together with formation of cytochrome p420. As shown in Fig. 14.7, the TPT-induced decrease in absorbance at 450 nm was accompanied by an increase of absorbance at 420 nm. TPT led to a greater inactivation of P450 enzyme than TBT, and caused

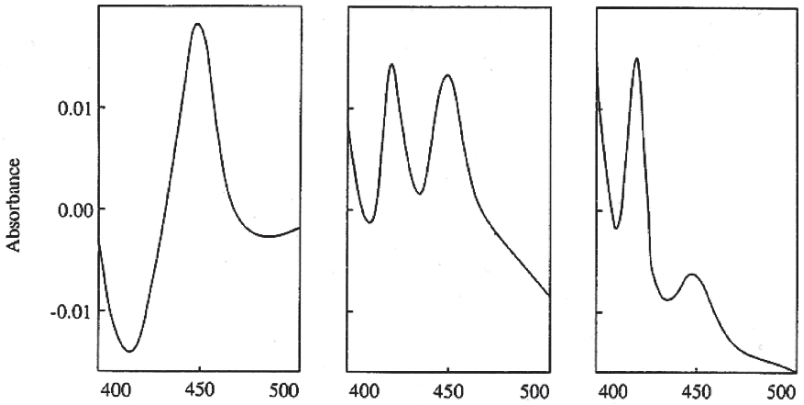


Fig. 14.7 Decrease of absorbance at 450 nm (cytochrome P450) and increase of absorbance at 420 nm (formation of cytochrome P420) in rainbow trout microsomes. The difference spectra were recorded after incubation of microsomal suspension with 2% DMSO (*left*), 0.2 mM TPT (*middle*), and 0.5 mM TPT (*right*) in DMSO for 5 min at 30°C. With increasing concentrations of TPT, suspensions became turbid and the baseline shifted

a 50% loss in all fish at 0.08 mM TPT, whereas in the case of TBT, a 50% loss occurred at 0.18 mM in rainbow trout, 0.30 mM in bullhead, and 0.83 mM in eel.

After hepatic EROD activity in rainbow trout was induced by 0.5 mg/l of β–NF exposure for 24 h, fish were exposed to 10 µg/l of TBT for 5 days. EROD activity was clearly inhibited compared with the group exposed to β–NF only (Fig. 14.8) (Nakayama and Kawai 2006).

EROD activity and the content of microsomal cytochrome P-450 in liver of flounder (*Platichthys flesus*) from Langesudfjord, Norway, were reported to be positively correlated with the field pollution gradient (Stegeman et al. 1988).

Different doses of TBT (0, 3.3, 8.1 and 16.3 mg/kg body weight) were injected intraperitoneally to scup (*Stenotomus chrysops*), and P450 and cytochrome b_5 content in the microsome fraction of liver homogenate were measured after 24 h (Fent and Stegeman 1993). Cytochrome b_5 content was not changed, however, P450 content was markedly decreased in the fish administered 16.3 mg/kg of TBT and moreover, P420 content clearly increased in all TBT injected groups (Table 14.3). This demonstrates that TBT induces the inactivation of P450 and inhibits CYP1A activity (Fent and Stegeman 1993).

Clear relationships between TBT concentration in the liver of reared olive flounder (*Paralichthys olivaceus*) and P450 content has been recorded (Fig. 14.9), and very low levels of TBT, as low as 3.65 ng/l, being the actual TBT level in the coastal area, affected the P450 content (Shim et al. 2003). In vitro CYP1A-EROD activity of Mullet (*Mullus barbatus*) and flounder (*Platichthys flesus*) was reduced at the TBT level of 0.1 mM (30 mg/l) (Morcillo et al. 2004).

Multiple biological effects of TBT on juvenile salmon (*Salmo salar*) exposed for 7 days to TBT at 50 and 250 µg/l in water were investigated. Hepatic samples were

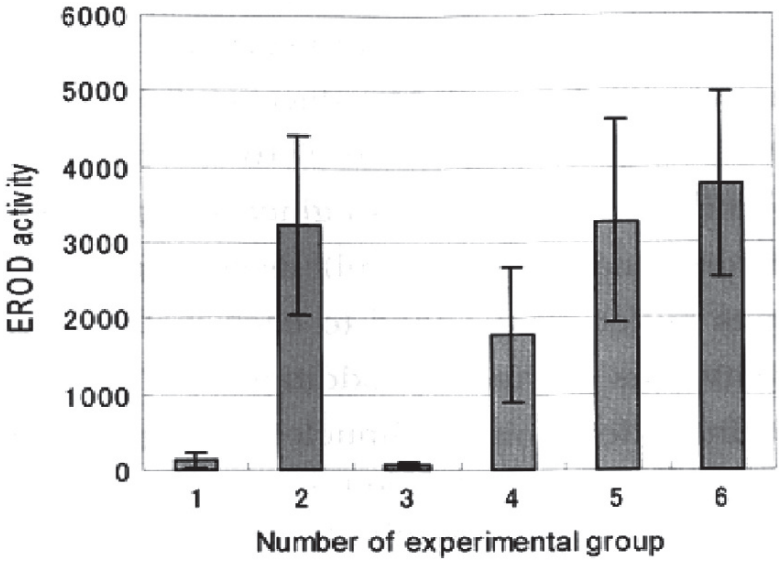


Fig. 14.8 Ethoxyresorufin *O*-deethylase (EROD) activity induced by 0.5 mg/l of β-Naphthoflavone (BNF) and/or 10 μg/l of tributyltin (TBT) under different experimental groups in rainbow trout. The number in this figure shows the experimental groups; (1) control, (2) BNF 0.5 mg/l for 24 h exposure, (3) TBT 10 μg/l for 5 days exposure, (4) BNF 0.5 mg/l for 24 h exposure after fish were exposed to 10 μg/l of TBT for 5 days, (5) BNF 0.5 mg/l for 5 days exposure, and (6) TBT 10 μg/l and BNF 0.5 mg/l for 5 days at the same time exposure. EROD activities were expressed by unit/min/individual and means ± standard error (n = 5 for each group)

Table 14.3 Effect of TBT in vivo on total cytochrome P450 and cytochrome b5

TBT dose (mg/kg)	Animals (n)	Total cytochrome P450 (pmol mg^{-1} protein)[a]	Cytochrome P420 (pmol mg^{-1} protein)[a]	Cytochrome b5 (pmol mg^{-1} protein)[a]
0 (1% ethanol)	5	83.4 ± 10.5	0	38.5 ± 5.0
3.3	4	112.3 ± 11.6	5.1 ± 5.9	49.8 ± 7.8
8.1	4	91.9 ± 11.6	3.5 ± 3.4	48.9 ± 7.6
16.3	5	61.9 ± 11.6	113.5 ± 38.1	44.2 ± 6.0

All values represent averages of separate determinations ± SEM.
[a]Average of 2–3 determinations per animal.

analyzed for gene expression patterns in the hormonal and xenobiotic biotransformation pathways using real-time PCR methods. TBT produced concentration-specific decreases in estrogen receptor-α (ER-α), vitellogenin (Vtg), zona radiate protein (Zr-protein) and increase of ER-β and androgen receptor-β (AR-β) in the hormonal pathway (Fig. 14.10). In the xenobiotic biotransformation pathway, TBT produced apparent increases and decreases at respective low and high concentration, on AhR α, AhR nuclear translocator (ARNT) and AhR repressor (AhRR) mRNA. The expression of CYP1A1 and glutathione *S*-transferase (GST) which

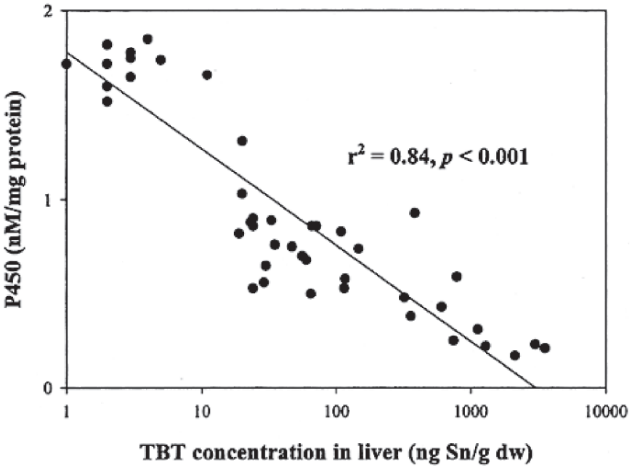


Fig. 14.9 Relationship between hepatic cytochrome P450 content and tributyltin (TBT) concentrations in liver of olive flounder exposed to TBT chloride, including control and five different exposure groups at all the sampling periods

plays an important role in the conjugation process of chemicals, showed a TBT concentration-dependent decrease. Immunochemical analysis of CYP1A1 protein levels confirmed the TBT effects observed at the transcriptional levels. These findings suggest endocrine effects of TBT, in addition to effects on hepatic CYP1A isoenzymes at the transcriptional level that causes the increase of protein and enzymatic levels (Mortensen and Arukwe 2007).

Decreases in P450 content and the activities of EROD and penthoxyresorufin *O*-dependentylase (PROD, catalyzed by CYP2B subfamily) by TBT were measured in vitro using hepatic microsomes of a Steller sea lion (*Eumetopias jubatus*) and a Dall's porpoise (*Phocoenoides dalli*). EROD activity (Fig. 14.11) was more sensitive to TBT than P450 content and PROD activity in both species. TBT concentrations that affected P450 content and activity were over ten times higher than the values found in the liver of various marine mammals (Kim et al. 1998).

14.3.2 Interaction of Organotins and Benzo[a]pyrene on CYP1A Activity

Benzo[a]pyrene (BaP), a widespread carcinogenic polycyclic aromatic hydrocarbon, is metabolized and bioactivated to carcinogenic BaP diol-epoxide metabolite primarily by hepatic CYP1A. Interaction between BaP and TBT was investigated in male brook trout (*Salvelinus fontinalis*). Short-term (48 h), single exposure to a high dose (10 mg/kg) of TBT inhibited both the in vivo metabolism and bioactivation of BaP at least by inhibiting the BaP-mediated induction of CYP1A-mediated EROD

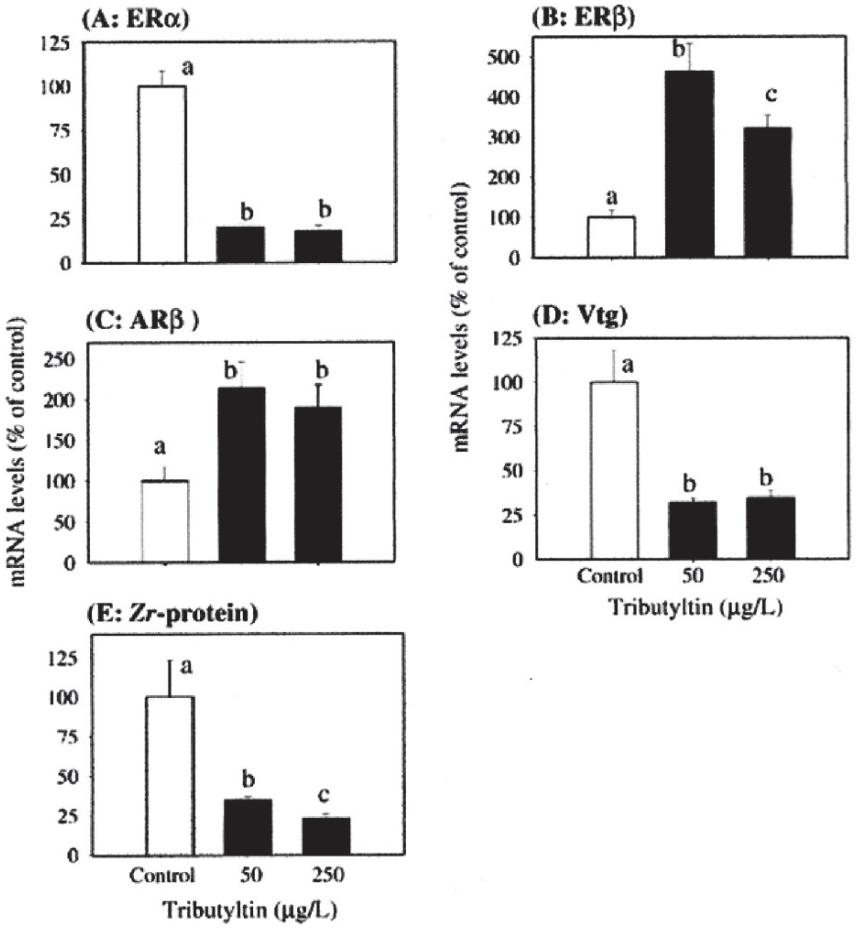


Fig. 14.10 Modulation of hepatic estrogen receptors (ERα and ERβ: a and b, respectively), androgen receptor-β (ARβ: c), vitellogenin (Vtg: d) and eggshell *zona radiata* protein (*Zr*-protein: e) mRNA levels of juvenile Atlantic salmon after exposure to nominal waterborne tributyltin (TBT) concentrations. Real-time PCR of mRNA expression levels with gene-sequence primer pairs of control, 50 and 250 μg TBT/l after 7 days exposure. All values represent the mean ($n = 6$) ± standard error of the mean (SEM). Different letters denote exposure groups that are significantly different ($p < 0.05$), analyzed using multi-parametric analysis of variance (ANOVA)

activity (Padrós et al. 2000). Further mechanistic evidence of mutual metabolic interactions between BaP and TBT in response to long term (56 days), repeated exposures (every 6 days) to low doses (BaP 3 mg/kg, TBT 0.3 mg/kg) was investigated in juvenile arctic charr (*Salvelinus alpinus*). Blood, bile and liver were collected and analyzed for biomarkers associated with P450 activity, BaP metabolism and bioactivation, and TBT metabolism (Padrós et al. 2003). TBT significantly inhibited the induction of hepatic CYP1A-mediated EROD activity at 8 days after

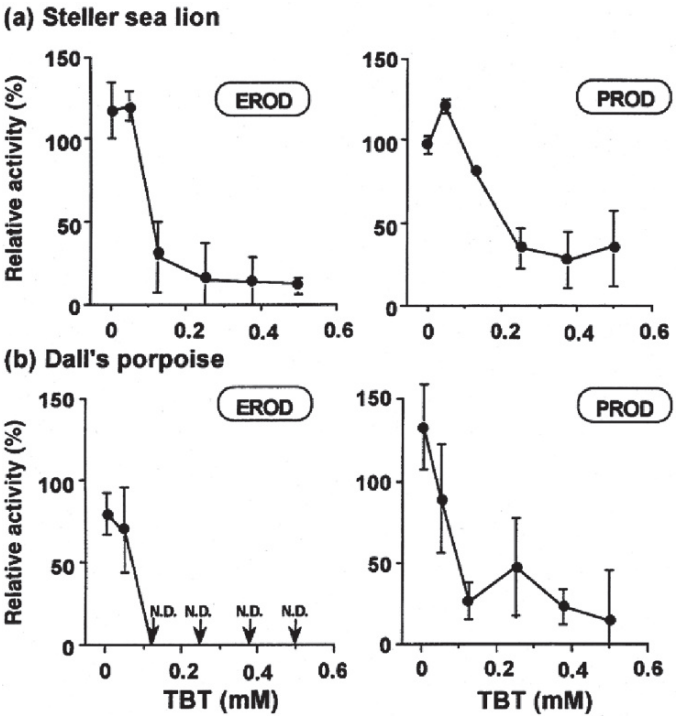


Fig. 14.11 Variations in EROD and PROD activities with different TBT concentrations. Average ± standard deviation of three separate determinations are given. Y-axis revealed a relative activity (%) to control (spiked with ethanol alone). (N.D.: not detected)

two intraperitoneal injections (Fig. 14.12). The formation of biliary BaP metabolites was also inhibited by exposure to TBT. A single high dose of TBT might antagonize the metabolism and bioactivation of BaP at least by inhibiting the induction of CYP1A. Histopathological examinations of liver, kidney and pseudobranch tissue samples originating from these same fish revealed higher lesion incidences at all sampling time points (days 8, 32 and 56) among BaP-exposed fish compared with fish exposed to either TBT alone or combined with BaP. The severity of lesions, like necrosis was also higher in BaP-exposed fish. These results suggest that TBT can antagonize BaP toxicity in fish exposed to both pollutants under controlled laboratory conditions. In contrast, BaP does not appear to provide protection against TBT toxicity (Ribeiro et al. 2007).

Metabolic interaction between TBT and benzo[a]pyrene (BaP) was also reported in scorpion fish (*Sebasticus marmoratus*) which were given a single intraperitoneal injection of TBT (0.5, 1, 5, and 10 mg/kg), BaP (0.5, 1, 5 and 10 mg/kg), or both in combination (0.5, 1, 5, and 10 mg/kg) (Wang et al. 2006). Samples were collected for biochemical analysis 7 days after injection. Co-treatment with TBT and BaP at the highest dose (10 mg/kg) resulted in inhibition of the glutathione S-transferase activity.

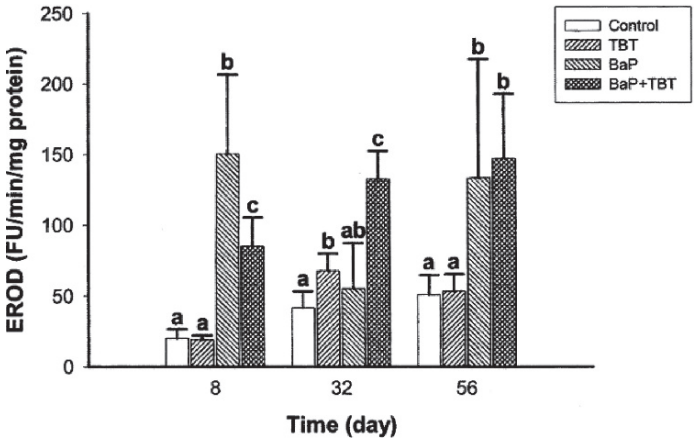


Fig. 14.12 Interactive effects of BaP and TBT on hepatic microsomal EROD activity (FU/min/mg protein). Fish were repeatedly exposed to BaP (3 mg/kg), TBT (0.3 mg/kg), or both in combination; control fish received corn oil vehicle alone. Data are presented as means ± SEM ($n = 6$ individual fish per treatment per time point). Treatments at a given time point not sharing a common letter are significantly different at $p < 0.005$ as assessed by one-way ANOVA with Student–Newman–Keuls test

14.4 CYP19 Aromatase Activity and Organotins

The effects of organotin compounds on human placental aromatase activity has been examined, and TBT was found to be a partial competitive inhibitor of aromatase to androstenedione, but did not affect electron transfer from NADPH to aromatase by inhibiting NADPH reductase (Heidrich et al. 2001). Imposex in neogastropods is well known to be caused by organotins, and organotins inhibit aromatase activity. However, whether aromatase inhibition is directly responsible for imposex or not is now uncertain, and other mechanism such as the involvement of the retinoid X receptor have recently been suggested (Nishikawa et al. 2004).

14.5 Conclusions

Among CYP families, CYP1A is an excellent biomarker for PAH pollution in aquatic environments, and is also responsible for the fate and behavior of various lipophilic chemicals. CYP1A-mediated EROD activity is inhibited by organotins such as TBT and TPT in vivo and in vitro, suggesting that detoxification and bioactivation are affected by the exposure of fish to organotins. Interaction between TBT and PAHs such as BaP, being a strong inducer of CYP1A, are also important for the evaluation of toxicities of both chemicals.

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