Pure Appl. Chem., Vol. 72, No. 6, pp. 985–994, 2000.

Cytochrome P4501A and associated mixed-function oxidase induction in fish as a biomarker for toxic carcinogenic pollutants in the aquatic environment*

Emel Arinç, Alaattin Sen, and Azra Bozcaarmutlu

Joint Graduate Program in Biochemistry, Department of Biological Sciences, Middle East Technical University, 06531 Ankara, Turkey

Abstract: Polycyclic aromatic hydrocarbons (PAHs), dioxins, dibenzofurans, and polychlorinated biphenyls (PCBs) present in polluted environment induce cytochrome P4501A (CYP1A) isozyme in fish, which in turn results in a marked increased production of carcinogenic metabolites from PAHs. The induction of hepatic CYP1A in fish by certain classes of chemicals has been suggested as an early warning system, a "most sensitive biological response" for assessing environmental contamination conditions. This has implications for human fish consumption, as well as for the health status of aquatic organisms. Correlation between elevated CYP1A and altered steroid metabolism and decreased reproductive success has been pointed out. The induction of CYP1A and associated enzyme activities has now been confirmed in a number of field studies. Cases where these biomarkers have been studied in field conditions will be presented. Special emphasis will be given to field studies in which the induction of CYP1A activity, 7-ethoxyresorufin O-deethylase (EROD) activities and immunochemical detection of CYP1A in leaping mullet and common sole are used as a biomarker for PAH- and/or PCB-type pollutants along the Izmir Bay on the Aegean Sea.

INTRODUCTION

With the growth of civilization, an increasing number of chemicals are being introduced to our environment. These chemicals are hazardous to living organisms, to humans, and to our ecosystems. The aquatic environment is particularly sensitive to the toxic effects of contaminants since a considerable amount of the chemicals used in industry, urbanization, and in agriculture enter marine and other aquatic environments.

Organisms are often exposed to complex mixtures of pollutants, including polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polycyclic aromatic hydrocarbons (PAHs), alkyltin compounds, and metals. Pollutants that bioaccumulate in the organism first cause effects at the molecular and cellular levels. This may lead to adverse effects in the organism, which in turn may cause changes at the population and the community level in the years to come. Fish populations living in highly polluted areas often have high incidences of gross pathological lesions and neoplasms that may be associated with the elevated levels of toxic chemicals in the sediments [1]. The high levels of neoplasms in fish collected from a creosote (mixture of petroleum products) polluted site in Puget Sound, WA, USA were reported [2].

Even though chemical analyses are able to measure a wide range of pollutants quantitatively and accurately, the complex mixture of chemical pollutants cannot be fully assessed. Furthermore, it does not reveal the impact of chemical pollution on the aquatic environment. The use of biochemical markers fulfills this purpose. Biomarker responses are, broadly speaking, of two kinds: those that measure only

*Lecture presented at the 4th Congress of Toxicology in Developing Countries (4th CTOX-DC), Antalya, Turkey, 6–10 November, 1999. Other presentations are published in this issue, pp. 973–1066.

exposure to a pollutant and those that measure both exposure and toxic effect of environmental chemicals [3,4]. The best characterized and used biochemical marker so far is the induction of cytochrome P4501A (CYP1A) dependent mixed-function oxidases (MFO) or monooxygenases [5]. Organic contaminants such as PCB, PCDD, PCDF and PAH specifically induce liver CYP1A in fish and in other vertebrates, and CYP1A is used as a biomarker of exposure to these types of organic pollutants often serving as an early warning signal of possibly more serious pathologies [1].

CYTOCHROME P4501A (CYP1A)

Cytochrome P450 (CYP), the terminal oxidase of monooxygenases, is mainly localized in the endoplasmic reticulum and mitochondria of liver and other tissues in fish and other vertebrates. It catalyzes oxidation of a number of organic chemicals to more soluble metabolites that can be further conjugated by Phase II enzymes and excreted. More than 500 different CYP genes have been cloned and sequenced so far [6]. CYP-dependent monooxygenases of fish possess many properties similar to the well-studied systems of mammals [7–11].

Two genes (CYP1A1 and CYP1A2) in mammals characterize the CYP1A family. Both genes are coordinately regulated by the same aromatic hydrocarbon receptor (Ah) [12]. CYP1A1 can activate PAHs such as benzo(a)pyrene to mutagenic compounds, thus its increased synthesis may ultimately result in carcinogenicity. In fish, CYP1A seems to exist as a hybrid protein coded by a gene ancestral to both mammalian CYP1A1 and CYP1A2 forms, and the use of the name CYP1A (unless otherwise sequenced) rather than CYP1A1 has been suggested [13].

CYP1A1 has been studied extensively in fish. Its biocatalytical and immunological properties and gene regulation appear to be similar to those of mammalian CYP1A1 [13–15]. Induction of CYP1A1 in fish has been observed with various PAH, PCB, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other PCDD, other halogenated compounds [11,16–19], crude oils [18–20], sediment extracts, and bleached paper effluents [21–24].

FIELD STUDIES

Liver CYP1A induction in fish by certain classes of chemicals described above has been applied extensively as a biomarker in field studies. In 1975 Payne and Penrose [25] showed that brown trout taken from a small urban lake in Newfoundland, Canada contaminated with petroleum hydrocarbons had increased arylhydrocarbon benzo(a)pyrene hydroxylase (BPH) activity. Payne then in 1976 [26], suggested that the use of arylhydrocarbon hydroxylase activity of fish liver as an environmental monitor for the first time. Subsequent studies showed that fish caught in waters contaminated with petroleum oil hydrocarbons, paper-pulp effluents, and industrial and municipal wastes exhibited elevated levels of CYP1A and associated enzyme activities. (For reviews, see refs. 1,5,11,13,24,27).

Examples of recent field studies employing CYP1A and/or associated enzyme activities in fish liver as a biomarker are given in Table 1. Most studies compare CYP1A concentrations and enzyme activities in fish from suspected sites with those in fish from reference sites. Most of the earlier field studies employed the induction of liver BPH activity in biomonitoring. The use of this assay has been declining because of the carcinogenic property of the substrate, benzo(a)pyrene as well as the possibility of substrate cross reactions with other CYP isozymes. CYP1A associated enzyme activity has been determined by using 7-ethoxyresorufin as a substrate. The measurement of 7-ethoxyresorufin O-deethylase (EROD) activity appears to be the most sensitive and the most widely used catalytic probe for determining induction response of CYP1A in fish. The advantages of using EROD activity as a biomarker are the specificity for CYP1A in fish, high sensitivity, feasibility and simplicity of its measurement [5,19,28].

With the development of immunochemical techniques and with the availability of poly- and monoclonal antibodies for fish CYP1A, the use of both EROD activity and immunoquantification of CYP1A

protein amount is recommended in field studies [10,13,15,27]. With the advances in the molecular biology techniques, determination of CYP1A mRNA by the Northern Blot analysis has been recently added to the biomonitoring studies [13,15,27]. These approaches complement one another, and all have value in detecting induction as a marker of exposure.

Table 1 Examples of recent field studies employing induction of liver CYP 1A (and/or associated activities) as a biomarker

Site	Contaminant fish	(Possible inducer)	Response	Ref.
Southern North Sea, German Bight Area	Dab		↑ × 6 EROD and ↑ × 3 CYP1A mRNA between sites.	(30)
Puget Sound, WA, USA, National Benthic Surveillance Project	11 species	Exxon Valdez Oil Spill	Induction EROD, BPH, and CYP1A Protein in all 11 Species.	(29)
Lake Vänern, Sweden	Pike Winter flounder	TCDDs	Relative Correlation between liver EROD and CYP1A protein and muscle TCDD.	(31)
Buyou Meto, Arkansas, USA	Channel catfish	Dioxins	↑ × 6.2 EROD, ↑ × 8 CYP1A protein.	(32)
Newark, New Jersey, USA	Killfish	TCDDs	↑ × 3 EROD, ↑ × 3 P4501A mRNA and higher CYP1A protein.	(33)
Puget Sound, WA, USA	English sole Rock sole Starry flounder	PCBs, PAHs	Induction EROD, BPH, CYP1A protein, DNA adducts. Species differed in range of response.	(34)
Skagerrak, Kettegatt, Baltic Sea, Sweden	Perch Dab Blenny		In all, elevated EROD and CYP1A protein. Highest levels close to industrial and municipal discharges.	(35)
German Bight, Germany	Dab		Significantly increased EROD in coastal areas and in the offshore region.	(36)
Bilbao Estuary, Spain	Sardine	PAHs, PCBs, pp'DDE	Highest EROD activity.	(37)
Boston Harbor, MA, USA	Winter flounder	Arochlor 1254 3, 3',4,4'-TCDD	Positive correlation CYP1A protein. EROD with Arochlor 1254, 3,3',4,4'-TCDD.	(38)

Table 1 *Continued*

Site	Contaminant fish	(Possible inducer)	Response	Ref.
Hempsted Harbor, NY, USA	Winter flounder		Strong induction of CYP1A in the industrialized east coast.	(38)
Lake Coleman, VIC, Australia	Carp	Treated pulp and paper mill effluents	Significantly increased EROD in fish exposed to effluents.	(39)
Sydney, Nova Scotia, Canada	Winter flounder	Coal Tar PAHs	↑ × 7 EROD, BPH highest near the coal tar source, but more variable and less sensitive.	(40)
Sydney, Nova Scotia, Canada	Winter flounder	PAHs in sediment	↑× 6 EROD ↑ × 9 BPH × 5 CYP1A protein.	(41)
British Columbia, near coastal pulp mills, Canada	English sole	PCDDs, PCDFs	↑ × 8 EROD ↑ × 3 CYP1A protein.	(42)
Freshwater sites, Amsterdam, Netherlands	Eel	PAHs, PCBs, PCDDs, PCDFs	Significant induction in EROD, CYP1A protein, DNA adducts in polluted sites.	(43)
Ponds, Czech Republic	Common carp	PAHs, PCBs (in sediment)	Highly elevated hepatopancreatic EROD when exposed to PAH. Less induction in EROD when exposed to PCBs.	(44)
Rhone Watershed, France	Barbel Chub Gudgeon	PCBs, Lindane HCBs	Highly variable EROD. Species differences. Gudgeon < sensitive than Chub.	(45)
The Frazer River, British Columbia, Canada	Juvenile chinook salmon	BKME	Higher EROD activity at the pulp mill site; activity poorly correlated with PCDD/F. Not statistically significant.	(46)
Izmir Bay, Turkey	Leaping mullet	PAHs and others	↑ × 62 EROD, app. ↑ × 14 CYP1A protein in the most polluted site.	(47)
	Common sole		↑× 17.7 EROD between sites.	
Izmir Bay, Turkey	Leaping mullet	PAHs and others	↑x 41 EROD vs reference site.	This paper
	Gray mullet		↑ × 56 EROD vs reference site.	

As seen in Table 1, in the field studies carried out after 1991 at various sites in North America (USA and Canada), Europe, Australia, and Turkey contaminated with industrial, municipal, oil or pulp mill effluents, CYP1A induction has been observed at the level of enzyme activity, protein amount and mRNA. Moreover, various direct positive correlations between CYP1A content and/or catalytic activity

and contaminant levels (PCB, PAH, and TCDD) in fish and in environment were evident. Dose-response relationships of CYP1A induction were not noted in all cases (Table 1). The CYP1A response measured as EROD or BPH activities has been incorporated into some major monitoring programs, e.g., the National Status and Trends Program in the United States [29] and the North Sea Task Force Monitoring Master Plan of the North Sea Nations in Europe [11].

Factors influencing CYP1A induction and biomonitoring

Species, sex, reproductive stage, age, season, temperature, dietary factors, and inhibitors have been found to effect total inductive response of CYP1A. Some of these will be discussed in the following.

Species differences

Species variations in the induction of liver CYP1A and enzyme activities are observed in response to organic contaminants [34]. Benthic fish, English sole (*Parophrys vetulus*), rock sole (*Lepidopsette bilineata*), and starry flounder (*Platichthys stellatus*) were sampled from up to five sites in Puget Sound, WA, USA that were contaminated with PAH, PCB and other organic chemicals. English sole and rock sole caught from the most polluted site had the highest EROD activity while the EROD activity in starry flounder from the same area was only one-half of the others [34]. Similar results were obtained in the field studies in Rhone watershed, France. The benthic fish, gudgeon (*Gobio gobio*) was found to be less sensitive than chub (*Leuciscus cephalus*) to the contaminants containing PCB, lindane, and hexachlorobenzene (HCB) [45].

Inhibitors

Inhibition of catalytic activity by certain pollutants was observed. *In vivo* treatment of benzene was found to reduce both cytochrome P450 content and EROD activity of gilthead sea bream [48]. Similar reductive effects were observed in fish treated with cadmium [49,50]. When the combined effects of both an inducer, benzo(a)pyrene and an inhibitor (cadmium) on MFO activities were examined, it was found that pre-exposure to cadmium increased the apparent induction effects of benzo(a)pyrene [51]. In addition, CYP1A-associated enzyme activity was found to be inhibited at the higher concentrations of some inducers, such as certain PCBs and β-naphthoflavone [5,11,27,52,53].

Reproductive failure and sex steroids

The seasonal reproductive cycle of salmonoids involves changes in plasma hormonal levels and it is likely that these hormones are involved in the sex related differences in CYP activities [54]. Elksus *et al*. [51,55] found that estradiol suppresses CYP1A suggesting that it regulates monooxygenase activity. Reversibly, the induction of CYP1A by xenobiotics resulted in decreased levels of the sex steroids, estradiol and testosterone [56–58]. Johnson *et al* [59] found that female English sole from contaminated areas had depressed levels of plasma estradiol and showed reproductive impairment and elevated arylhydrocarbon hydroxylase activity. These results suggest that an inverse relationship between CYP1A induction and estradiol synthesis exists. Some compounds that induce CYP1A1 are found to be antiestrogenic in mammalian bioassay, and this effect is linked to Ah receptor and/or increased catabolism of 17-β-estradiol [60].

Thus, reproductive state and sex, as well as the other parameters discussed above, require consideration in CYP1A induction response. But these generally should not provide any major obstacle to its field application [27].

CYP1A induction and chemical carcinogenesis

CYP1A mostly activates certain classes of PAH pro-carcinogens and other chemicals by forming oxygenated compounds [61]. Oxygenation of benzo(a)pyrene by CYP1A1 in the presence of epoxide

hydrolase results in the formation of the ultimate carcinogen, benzo(a)pyrene 7,8 dihydrodiol 9,10-epoxide (BPDE), which forms DNA-adducts [61]. Greater CYP1A induction may result in high levels of activated carcinogens, and consequently to higher degree of persistent DNA-adduct formation or to an enhanced oxidative DNA damage [13]. Induction of CYP1A1 has been correlated with the development of PAH-associated cancers and other disorders in mammals [62].

Positive correlations have been found between the levels of sediment and tissue contaminants, fish liver CYP1A, bile metabolites, liver DNA-adducts, and liver neoplasms and related lesions [27,63,64]. Malins *et al.* [2] observed high levels of neoplasms in fish collected from creosote- polluted Puget Sound USA. Kocan *et al.* [65] reported that much of the cellular toxicity associated with the extracts of sediments from Puget Sound, requires metabolic activation. DNA isolated from neoplastic nodules of hepatic tissues of English sole exposed to creosote pollution in Puget Sound was shown to contain modified guanine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy Gua) [66].

BIOMONITORING STUDIES ALONG THE IZMIR BAY, TURKEY

Izmir Bay is located on the Aegean Sea of the Mediterranean west of Turkey. It is the one of the most polluted areas of Turkey. The port of Izmir City and several industries are located at the Inner Bay. Domestic and industrial wastes, urban and agricultural run off, discharges from ships, sediments and contaminated waters of rivers have cumulatively had significant adverse effects on the water quality of the Bay. Industrial activities cover a large range of industries including food processing, tanneries, paint, paper and pulp factories, chemical and textile factories, vegetable oil and soap production, and a petroleum refinery. Industrial and domestic wastes as well as contaminated waters of several small rivers heavily pollute the Inner Bay. The Middle Bay is a transition zone with pollutant concentrations intermediate between those in Outer and Inner Bays, and the pollution in the Outer Bay is considered not significant [67].

We carried out our first biomonitoring studies in May 1995 and February and June 1996 [47]. Two different fish species—leaping mullet (*Liza saliens*), a pelagic fish, and common sole (*Solea vulgaris*), a benthic fish—were sampled from the six different sites along the Izmir Bay. Site 10, a relatively clean site in the Outer Bay was used as a reference site. The sampling sites are given in Fig. 1. Antibodies raised against purified leaping mullet CYP1A were used to detect the degree of induction of liver CYP1A protein response to pollutants. Cross-reactivity of CYP1A in liver microsomes of leaping mullet caught from sites 1–4 and 10 were analyzed by Western blotting. EROD activities of leaping mullet and common sole sampled from the different sites of the Bay in that study are shown in Fig. 1.

Leaping mullet from the highly urbanized and industrial section of the Bay, Pasaport (site 1) showed highly elevated enzyme activities (1293 ± 292 pmol/min/mg protein, n = 208), which were about 62 times higher than the value at the reference site (25 ± 9, pmol/min/mg protein, n = 4). Leaping mullet caught along a pollutant gradient at three other sites, Karsiyaka (site 2), Inciralti (site 3), and Tuzla (site 4), also had highly elevated EROD activities, namely 761 ± 139 (n = 13), 417 ± 39 (n = 12), and 334 ± 40 (n = 12) pmol/min/mg protein, respectively. These were 36, 18, and 15-fold higher than those obtained from the reference point, site 10 [47].

Leaping mullet sampled from site 1, Pasaport, containing the highest concentrations of petroleum hydrocarbons (12.45 mg/L), showed the highest liver EROD activity. In addition, these livers had highest CYP1A protein levels as determined by Western blotting. Next to site 1, mullet captured from sites 2 and 3 also had very high EROD activities, and there was a good correlation between EROD activity and CYP1A protein content measured immunochemically [47].

Thus, two biochemical indices, highly induced EROD activities and CYP1A protein levels, in the liver of leaping mullet caught from sites 1, 2, 3, and 4 suggest that these sites are highly contaminated with PAH and/or PCB and possibly other toxic compounds.

In addition, the benthic fish, common sole, captured in site 3 (Inciralti) had EROD activity of 2000 ± 115 pmol/min/mg protein (n = 13), indicating that sediments from that area were highly con-

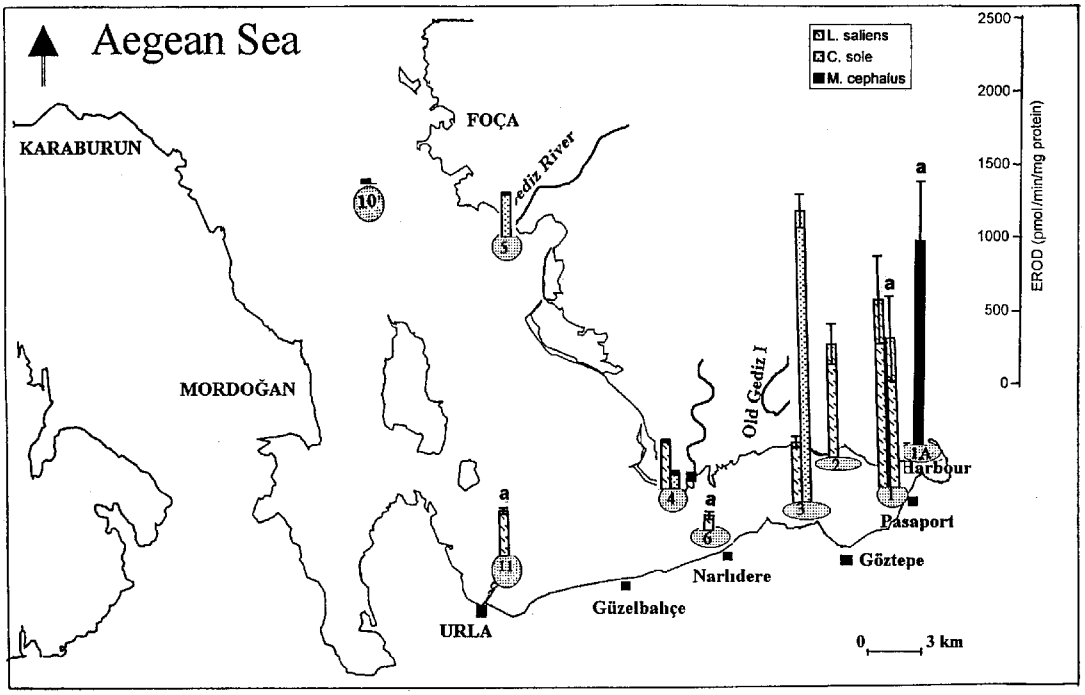

Fig. 1 EROD activities of liver microsomes of common sole (*Solea vulgaris*) leaping mullet (*Liza saliens*) and gray mullet (*Mugil caphalus*) captured in Izmir Bay at eight different sampling sites. The activity bars with "a" represent the field studies carried out in 1999. The others are taken from Arinç and Sen [47].

taminated with CYP1A-inducing chemicals. As can be seen from Fig. 1 an inverse relationship was found between the distances to the charge point of Inner Harbour (site 1) and EROD activity of fish caught along the Izmir Bay (sites 2, 3, 4, and 10). For common sole, these findings differed somewhat from those obtained for leaping mullet. Common sole captured from site 5 (mouth of the Gediz river) in Outer Bay had higher EROD activity (300 ± 10 pmol/min/mg protein, n = 5) than that of the fish caught from site 4, Tuzla in the Middle Bay (113 ± 14 pmol/min/mg protein, n = 49) (Fig. 1). The Gediz River runs from the fertile agricultural area treated with herbicides and other pesticides. Higher EROD activity of common sole captured from this site demonstrated that sediments at the mouth of the Gediz River were also polluted with CYP1A-inducing chemicals [47].

The biomonitoring studies were also carried out in January 1999. As seen in Fig. 1, EROD activities of leaping mullet from site 1 again showed highly elevated enzyme activities (1028 ± 287 pmol/min/mg protein, n = 4). Gray mullet (*Mugil cephalus*) caught from the near site 1A also had highly increased enzyme activities (1398 ± 410 pmol/min/mg protein). Leaping mullets caught from site 6 and site 11 displayed moderately elevated EROD activities that were 105 ± 26 (n = 4) and 310 ± 21 (n = 4) pmol/min/mg protein respectively (Fig. 1).

Quantitative metal analyses in Izmir Bay have demonstrated that concentrations of heavy metals such as chromium, copper, lead, cadmium and mercury in sediments and surface waters of Inner Bay were 6–15 times higher than those of the Outer Bay, reflecting the intensity of anthropogenic inputs in the Inner Bay [68]. Metals are known to inhibit CYP1A-dependent monooxygenase activities [49,50]. The concentrations of metals in the Outer Bay were found to be rather low. The concentration of metals present in the Middle and Inner Bays of Izmir (sites 1–6) were significantly less than the concentration required to cause a significant reduction of EROD activities in fish.

Some conclusions concerning the suitability and selection of fish species can be drawn from the results of field studies. The mullet are found to be suitable for environmental monitoring. Mullet be-

longs to the family of Mugilidae of the class of osteichthyes. It is an economically important marine fish due to marketing of their meats and eggs, inhabiting usually inshores, entering lagoons and estuaries along the Atlantic coast northward of Bay of Biscay, also the whole of Mediterranean, Black Sea and Sea of Azov. Mullet provide advantages for environmental biomonitoring because they can be easily trapped and because of their ability to withstand the conditions of highly polluted areas such as Izmir Bay, and their demonstrable CYP1A induction in response to chemical contamination.

REFERENCES

1. J. F. Payne, L. L. Fancey, A. D. Rahimtula, E. L. Porter. *Comp. Biochem. Physiol*. **86C**, 233–245 (1987).
2. D. C. Malins, M. M. Krahn, M. S. Myers, L. D. Rhodes, D. W. Brown, C. A. Krone, B. B. McCaine, S-L. Chan. C*arcinogenesis* **6**, 1463–1469 (1985).
3. D. B. Peakall. *Animal Biomarkers as Pollution Indicators*, Chapman & Hall, London/New York (1992).
4. C. H. Walker. *Ecotox. Environ. Safety*. **40**, 65–70 1998.
5. T. D. Bucheli and K. Fent. *Critical Reviews in Environmental Science and Technology* **25**, 201–268 (1995).
6. D. R. Nelson, L. Loymans, T. Kamataki, J. J. Stegeman, R. Feyereisen, D. J. Waxman, M. R. Waterman, O. Gotoh, M. J. Coon, R. W. Estabrook, I. C. Gunsalus, D. W. Nebert. *Pharmacogenetics* **6**, 1–42 (1996).
7. E. Arinç, R. M. Philpot, J. R. Fouts. *Fed. Proc*. **35**, 666 (1976).
8. E. Arinç and O. Adali. *Comp. Biochem. Physiol*. **76B**, 653–662 (1983).
9. J. J. Stegeman, H. B. Kloepper-Sams. *Environ. Health Persp*. **71**, 87–95 (1987).
10. R. D. Buhler. In *Molecular Aspects of Oxidative Drug Metabolizing Enzymes: Their Significance in Environmental Toxicology, Chemical Carcinogenesis and Health*, E. Arinç, J. B. Schenkman, E. Hodgson (Eds.), pp. 159–180, Springer-Verlag, Heidelberg (1995).
11. A. Goksøyr, L. Förlin. *Aquat. Toxicol*. **22**, 287–312 (1992).
12. F. J. Gonzalez. *Pharmacol. Rev.* **40**, 243–288 (1989).
13. J. J. Stegeman. In *Molecular Aspects of Oxidative Drug Metabolizing Enzymes: Their Significance in Environmental Toxicology, Chemical Carcinogenesis and Health*, E. Arinç, J. B. Schenkman, E. Hodgson (Eds.), pp. 135–158 , Springer-Verlag, Heigdelberg, NATO ASI Ser. (1995).
14. A. Sen and E. Arinç. *Comp. Biochem. Physiol.* **121C**, 249–265 (1998).
15. D. R. Buhler and J. L. Wang-Buhler. *Comp. Biochem. Physiol*. **121C**, 107–137 (1998).
16. E. Arinç, R. J. Bend, J. R. Bend, R. M. Philpot. *IV^es Jourées Etud. Pollutions, Antalya C. I. E. S. M.* 273–276 (1978).
17. F. P. C. Law and R. F. Addison. *Bull. Environ. Contam. Toxic*. **27**, 5–10 (1981).
18. R. B. Spies, J. S. Felton, L. Dillard. *Mar. Biol.* **70**, 117–127 (1982).
19. E. Arinç, A. Sen. *Comp. Biochem. Physiol*. **107C**, 405–414 (1994).
20. J. H. Vandermeulen. *Comp. Biochem. Physiol.*, **95C**, 169–175 (1990).
21. L. Förlin, T. Andersson, B. E. Bengtsson, J. Härding, A. Larson. *Mar. Environ. Res.* **17**, 109–112 (1985).
22. A. Andersson, B. E. Bengtsson, L. Förlin, J. Härding, A. Larsson. *Ecotoxicol. Environ. Safety.* **13**, 53–60 (1987).
23. T. K. Collier, U. Varanasi. *Arch. Environ. Contam. Toxicol*. **20**, 462–473 (1991).
24. R. F: Addison. *Environ. Rev*. **4**, 225–237 (1996).
25. J. F. Payne and W. R. Penrose. *Bull. Environ. Contam. Toxic*. **14**, 112–116 (1975).

26. J. F. Payne. *Science* **191**, 945–946 (1976).
27. D. R. Livingstone. *J. Chem. Tech. Biotechnol.* **57**, 195–211 (1993).
28. M. D. Burke, S. Thompson, C. R. Elcombe, J. Halpert, T. Haaparanta, R. T. Mayer. *Biochem. Pharmacol.* **34**, 3337–3345 (1985).
29. T. K. Collier, D. Conor, B. –T.L. Eberthart, B. F. Anulacion, A. Goksøyr, U. Varanasi. *Mar. Environ. Res*. **34**, 193–198 (1992).
30. K. W. Renton, R. F. Addison. *Mar. Ecol. Prog. Ser*. **91**, 65–69 (1992).
31. F. Förlin, L. Balk, M. Celander, S. Bergek, M. Hjelt, C. Rappe, C. de Witt, B. Jansson. *Mar. Environ. Res*. **34**, 169–173 (1992).
32. M. J. J. Ronis, M. Celander, L. Förlin, T. M. Badger. *Mar. Environ. Res*. **34**, 181–188 (1992).
33. M. L. Haasch, E. M. Quardokus, L. A. Sutherland, M. C. Goodrich, R. P. Prince, K. R. Cooper, J. J. Lech. *Mar. Environ. Res*., 139–145 (1992).
34. J. E. Stein, T. K. Collier, W. L. Reichert, E. Casillas, T. Hom, U. Varanasi. *Mar. Environ. Res*. **35**, 95–100 (1993).
35. L. Förlin, M. Celander. *Aquat. Toxicol.* **26**, 41–56 (1993).
36. U. Lange, D. Danischewski, D. Siebers. *Fish: Toxicology and Ecophysiology*, T. Braunbeck, H. Senger (Eds.), pp. 37, VCH Publishers, Weinhein, West Germany (1993).
37. L. D. Peters, C. Porte, J. Albaigés, D. R. Livingstone. *Mar. Pollut. Bull*. **28**, 299 (1994).
38. E. Monosson and J. J. Stegeman. *Can. J. Fish Aquat. Sci*. **51**, 933 (1994).
39. J. T. Ahokas, D. A. Holdway, S. E. Brennan, R. W. Goudey, H. B. Bibrowska. *Environ. Toxicol. Chem*. **13**, 41 (1994).
40. V. Vignier, J. H. Vandermeulen, J. Singh, D. Mossman. *Can. J. Aquat. Sci*. **51**, 1368–1375 (1994).
41. R. F. Addison, D. E. Willis, M. E. Zinck. *Mar. Environ. Res*. **37**, 283–296 (1994).
42. R. F. Addison and T. L. Fraser. *Mar. Environ. Res*. **42**, 273 (1996).
43. R. Van Der Oost, A. Goksøyr, M. Celander, H. Heida, N. P. E. Vermeulen. *Aquat. Toxicol.* **36**, 189–222 (1996).
44. M. Machala, M. Petrivalský, K. Nezveda, R. Ulrich, L. Dušek, V. Piacka, Z. Svobodova. *Environ. Toxicol. Chem.* **16**, 1410–1416 (1997).
45. P. Flammarion, B. Migeon, J. Garric. *Ecotoxicol. Environ. Safety* **40**, 144–153 (1998).
46. R. F. Addison and J. Y. Wilson. *Molecular and Applied Aspects of Oxidative Drug Metabolizing Enzymes*, E. Arinç, J. B. Schenkman, E. Hodgson (Eds.), pp. 259–270, Kluwer Academic/ Plenum Publishers, New York (1999).
47. E. Arinç and A. Sen. *Mar. Environ. Res*. **48**, 147–160 (1999).
48. E. Arinç and A. Sen. *Comp. Biochem. Physiol*. **104C**, 61–65 (1993).
49. L. Förlin, C. Haux, L. Karlson-Norrgren, P. Runn, A. Larson. *Aquat. Toxicol.* **8**, 51–64 (1986).
50. S. G. George. *Aquat, Toxicol.* **15**, 303–310 (1989).
51. S. Lemaire-Goni, P. Lemaire. *Aquat. Toxicol*. **22**, 145–150 (1992).
52. A. A. Elksus, J. J. Stegeman, L. C. Susani, D. Black, R. J. Pruell, S. J. Fluck. *Mar. Environ. Res*. **28**, 25–30 (1989).
53. E. Monosson and J. J. Stegeman. *Environ. Toxicol. Chem*. **10**, 765–774 (1991).
54. T. Andersson and L. Förlin. *Aquat. Toxicol*. **24**, 1–20 (1992).
55. A. A. Elksus, R. J. Pruell, J. J. Stegeman. *Mar. Environ. Res*. **34**, 97–101 (1992).
56. K. R. Munkittrick, M. E. McMaster, C. B. Portt, G. J. Van Der Kraak, I. R. Smith, D. G. Dioxin. *Can. J. Fish Aquat. Sci*. **49**, 1560–1567 (1992).
57. K. R. Munkittrich, G. L. Van Der Kraak, M. E. McMaster, C. B. Portt, M. R. Van Der Heutel, M. R. Serves. *Environ. Toxicol. Chem*. **13**, 1089–1101 (1994).

58. K. R. Munkittrich, M. E. McMaster, L. H. McCarthy, M. R. Serves, G. L. Van Der Kraak. *J. Toxicol. Environ. Health B Crit. Rev*. **1**, 347–371 (1998).
59. L. L. Johnson, E. Casillas, T. K. Collier, B. B. McCain, U. Varanasi. *Can. J. Fish Aquat. Toxicol*. **45**, 2133–2146 (1988).
60. M. J. Anderson, M. R. Miller, D. E. Hinton. *Aquat. Toxicol*. **34**, 327–350 (1996).
61. D. V. Parke, C. Ionnides, D. F. V. Levis. *Can. J. Physiol. Pharmacol*. **69**, 537–549 (1991).
62. D. W. Nebert. *Cur. Rev. Toxicol*. **20**, 153–174 (1989).
63. U. Varanasi, J. E. Stein, M. Nishimoto, W. L. Reichert, T. K. Collier. *Environ. Health Perspect*. **71**, 155–170 (1987).
64. J. E. Stein, W. L. Reichert, M. Nishimoto, U. Varanasi. *Sci. Total Environ*. **94**, 51–69 (1990).
65. R. M. Kocan, K. M. Sabo, M. L. Landilt. *Aquat. Toxicol*. **6**, 165–177 (1985).
66. D. C. Malins, G. K. Ostrander, R. Haimanot, P. Williams. *Carcinogenesis* **11**, 1045–1047 (1990).
67. I. T. Balkas, F. Juhasz, Ü. Yetis, G. Tuncel. *Water Sci. Technol*. **26**, 2613–2616 (1992).
68. A. Balci and M. Türkoglu. *Mar. Pollut. Bull*. **26**, 106–107 (1993).