Use of the fish cytochrome P-450-dependent 7-ethylresofurin O-deethylase activity as a biochemical indicator of water pollution, study of the liver and the kidney of male and female nase (Chondrostoma nasus) from the river Rhône

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USE OF THE FISH CYTOCHROME P-450-DEPENDENT 7-ETHYLRESORUFIN *O*-DEETHYLASE ACTIVITY AS A BIOCHEMICAL INDICATOR OF WATER POLLUTION. STUDY OF THE LIVER AND THE KIDNEY OF MALE AND FEMALE NASE (*CHONDROSTOMA NASUS*) FROM THE RIVER RHÔNE

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ABSTRACT

7-Ethylresorufin *O*-deethylase (EROD) activity and the cytochrome P-450 content of liver and kidney microsomes were measured in male and female nase (*Chondrostoma nasus*) from the River Rhône (France) caught downstream and upstream of a PCB incineration plant. Concurrently, PCB concentrations in the flesh of nase were also measured. The results showed that sex affected hepatic, but not renal, EROD activity during gonadal maturation. The male nase demonstrated higher activity than the female. Upstream/downstream comparisons clearly revealed a more elevated hepatic EROD activity in fish contaminated by PCB pollution than in fish captured in the reference area throughout the reproductive cycle, demonstrating the reliability of this enzymatic activity as a biochemical indicator. Renal EROD activity did not seem to be sensitive to PCB pollution, since neither male nor female nase showed significant upstream/downstream differences.

INTRODUCTION

First described in mammals, xenobiotic metabolizing enzymes have more recently been studied in fish (Chambers and Yarbrough, 1976; Lech and Bend, 1980), with particular emphasis on the cytochrome P-450-dependent monooxygenases (MO). The reactions catalyzed by MO result in the insertion or addition of one oxygen atom from dioxygen to a substrate molecule, increasing its hydrosolubility and facilitating its elimination from the body.

Several studies have demonstrated the inducibility of MO activity by chemicals such as polyaromatic hydrocarbons (Payne and Fancey, 1982) and polychlorobiphenyls (PCBs) (Elcombe et al., 1979). Because of this property, MO were proposed as biomonitors, since their activities could be related to fish contamination by environmental pollutants (Payne et al., 1987). The usefulness of MO induction in biomonitoring studies has been confirmed in a number of field studies covering various species and pollutants (Spies et al., 1982; Kezic et al., 1983; Monod et al., 1988). Moreover, it has been shown that MO are involved in the activation of chemicals to reactive intermediates, and the

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formation of mutagens and carcinogens (Ioannides and Parke, 1987). As MO are also involved in steroid metabolism, it has also been proposed that the induction of MO could lead to hormonal disturbances (Förlin and Haux, 1985). Therefore, MO should be a useful indicator of chemical quality of the aquatic environment as well as of toxicological risks to fish.

Cytochrome P-450-dependent monooxygenases have mainly been studied in liver, but several authors have characterized the reactions in extrahepatic tissues (especially in kidney). In some cases, they observed higher induction in fish kidney than in liver (Truscott et al., 1983; Payne et al., 1984). In addition, several studies have shown the influence of factors such as sex or hormonal levels on MO activity (Förlin et al., 1984) and inducibility (Walton et al., 1983).

We recently observed the induction of various hepatic MO activities in several fish species from the River Rhône, captured downstream from a PCB incineration plant (Monod et al., 1988). A particular MO, 7-ethylresorufin *O*-deethylase (EROD), was shown to be very responsive to pollution. The present study was performed in the same areas of the River Rhône on a profuse fish species, the nase (*Chondrostoma nasus*). The aim was to investigate the influence of sex and sexual maturation on the response of EROD activity to chemical pollution, and to compare the rate of induction in liver and kidney.

MATERIALS AND METHODS

Fish collection

Nase (*Chondrostoma nasus*) of both sexes were collected by professional fishermen with the aid of a net (mesh size 50 mm). About 10 fish of similar size were captured for each sex. During 1988, three sampling periods were chosen covering the critical periods of the sexual cycle of the nase: prior to spawning (March), sexual quiescence (June) and gonadal maturation (November). The fish were caught at two stations on the River Rhône, Pont de Lucey (reference area) and Miribel (polluted area; Monod et al., 1988). The nase were kept alive in flowing river water until sacrifice, which occurred no more than 3 h after capture. After sacrifice by a blow on the head, fish were weighed and measured, the liver and kidney were removed (kidney was rinsed with 0.15 *M* KCl, 50 m*M* phosphate buffer, pH 7.4, in order to eliminate haemoglobin), wrapped in aluminium foil and frozen in liquid nitrogen until preparation of microsomes the following day. Gonads were collected and weighed.

Chemicals

7-Ethylresorufin, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, nicotinamide adenine dinucleotide phosphate, dithiothreitol (DTT) and phenylmethyl sulfonylfluoride (PMSF) were purchased from Boehringer-Mannheim, France. Other analytical grade chemicals were purchased commercially.

Preparation of microsomes and enzyme assays

Hepatic microsomes were prepared as previously described (Monod et al., 1987). Since preliminary studies have shown a high lability for the renal xenobiotic metabolizing enzymes, PMSF (protease inhibitor) was added (0.2 m*M* final concentration) to the homogenization buffer. All microsomal pellets were resuspended in 50m*M* phosphate buffer, pH 7.4, containing 0.15 *M* KCl, 1 m*M* EDTA, 1 m*M* DTT and 20% glycerol. All microsomal fractions were stored at − 80°C until use (the following month). 7-Ethylresorufin *O*-deethylase activity was assayed according to Monod et al. (1987) at pH 7.4. All reactions were performed at ambient temperature: 6.5 and 8°C, respectively, for Pont de Lucey and Miribel samples in March; 17°C for both samples in June; 10 and 11.5°C, respectively, for Miribel and Pont de Lucey samples in November. The extinction coefficient of resorufin was 73 mM^{-1} cm^{-1} (Klotz et al., 1984) and EROD activity was expressed as picomoles per minute per milligram microsomal protein. All analyses were carried out in duplicate. All reactions were linear with respect to time and protein concentration. Cytochrome P-450 content was measured according to Matsubara et al. (1976) and protein concentration was determined by the method of Hartree (1972) with bovine serum albumin as a standard.

Dosage of PCBs

Polychlorinated biphenyl analyses were performed as previously described (Devaux and Monod, 1987) on pooled edible portions of male and female individuals from Pont de Lucey and Miribel captured in June and November.

Statistical analyses

All results are expressed as mean ± SEM and statistical analyses were performed using the two-tailed (intersexual comparisons) or one-tailed (upstream/downstream comparisons) Mann–Whitney U-test. Levels of significance are indicated in the legends of the figures.

RESULTS

In June, PCB concentrations in the nase were 0.75 and 0.84 $\mu g\ g^{-1}$, respectively, for males and females captured at Pont de Lucey. The nase caught at Miribel were much more contaminated: 3.97 $\mu g\ g^{-1}$ in males and 3.52 $\mu g\ g^{-1}$ in females. In November, contamination levels were 0.47 and 0.50 $\mu g\ g^{-1}$, respectively, for males and females from Pont de Lucey; 2.33 and 2.18 $\mu g\ g^{-1}$, respectively, were found in males and females from Miribel. No analysis was carried out in March.

Sexual maturity of nase was evaluated by measuring the gonadosomatic

TABLE 1

Seasonal variations in the gonadosomatic index (GSI) of male and female nase calculated as [gonad weight/(body weight)] × 100

	Male		Female	
	Miribel	Pont de Lucey	Miribel	Pont de Lucey
March	6.6 ± 1.8[a]	7.8 ± 1.9	16.9 ± 2.8	18.9 ± 4.7
June	< 0.5	< 0.5	< 2.0	< 2.0
November	3.4 ± 0.3	3.3 ± 0.4	13.8 ± 1.7	13.0 ± 2.4

[a]Mean ± SEM; each mean was calculated from 10 fish.

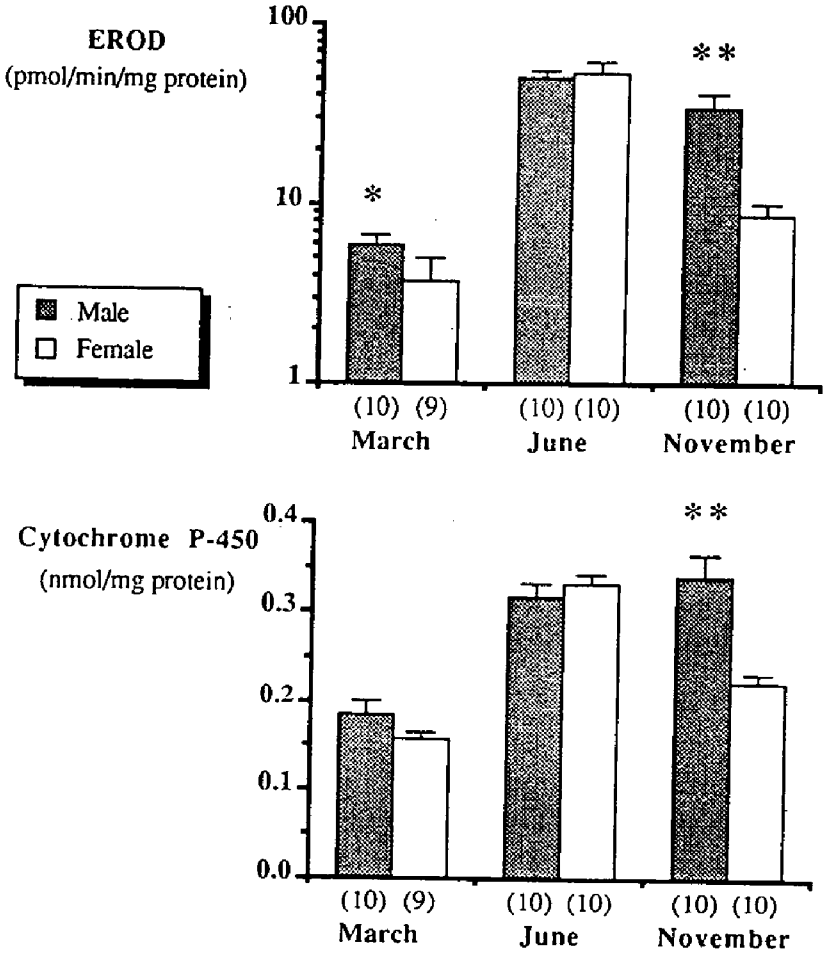

Fig. 1. Sexual differences affecting EROD activity (log scale) and cytochrome P-450 concentration determined in hepatic microsomal fractions obtained from nase caught in Pont de Lucey in March, June and November. Data are presented as the mean ± SEM (number of fish). Levels of significance of the two-tailed Mann–Whitney U-test: 5% (*) and 1% (**).

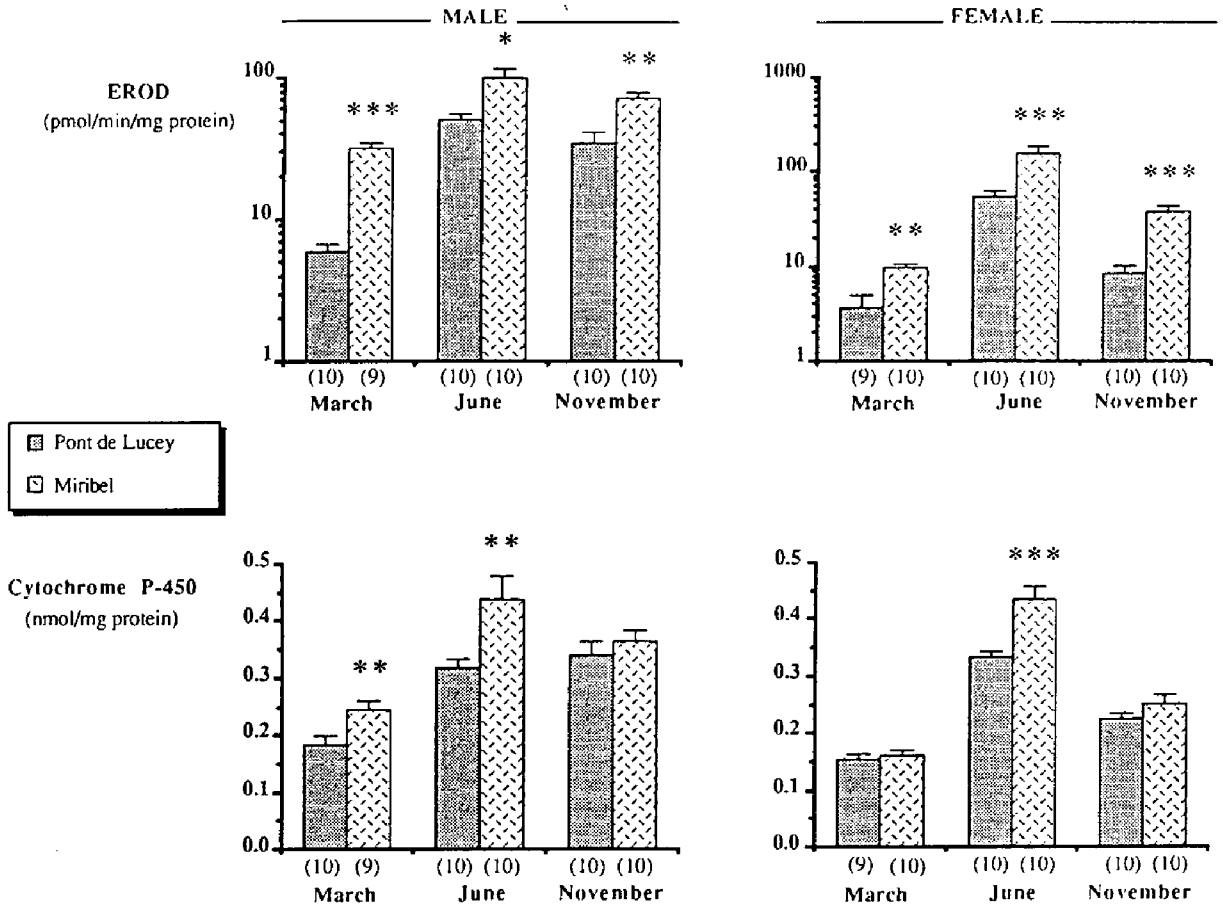

Fig. 2. Inter-site comparisons of the hepatic microsomal EROD activity (log scale) and cytochrome P-450 concentration in male and female nase captured in March, June and November. Data are presented as mean ± SEM (number of fish). Levels of significance of the one-tailed Mann–Whitney U-test: 5% (*), 1% (**) and 0.1% (***).

index (GSI). As shown in Table 1, GSI was maximum in March just prior to spawning (April). In June, after spawning, GSI was very low and again reached quite a high level in November.

Results of intersexual comparisons of hepatic EROD activity and cytochrome P-450 concentration carried out for Pont de Lucey samples obtained in March, June and November are presented in Fig. 1 (results from Miribel were similar and consequently are not shown). Sexual differences affecting hepatic EROD activity were significant in March and November (male > female in both cases), but not in June. The hepatic microsomal cytochrome P-450 concentration showed significant sexual dimorphism only in November (male > female). For the kidney (data included in Fig. 3), significant sexual differences were noted at Pont de Lucey in June for EROD activity (female > male), and in March for cytochrome P-450 content (male > female); no sexual differences were found at Miribel for either variable. According to these results, it appeared necessary to distinguish between sexes when studying upstream/downstream differences in hepatic and renal EROD activities and cytochrome P-450 contents.

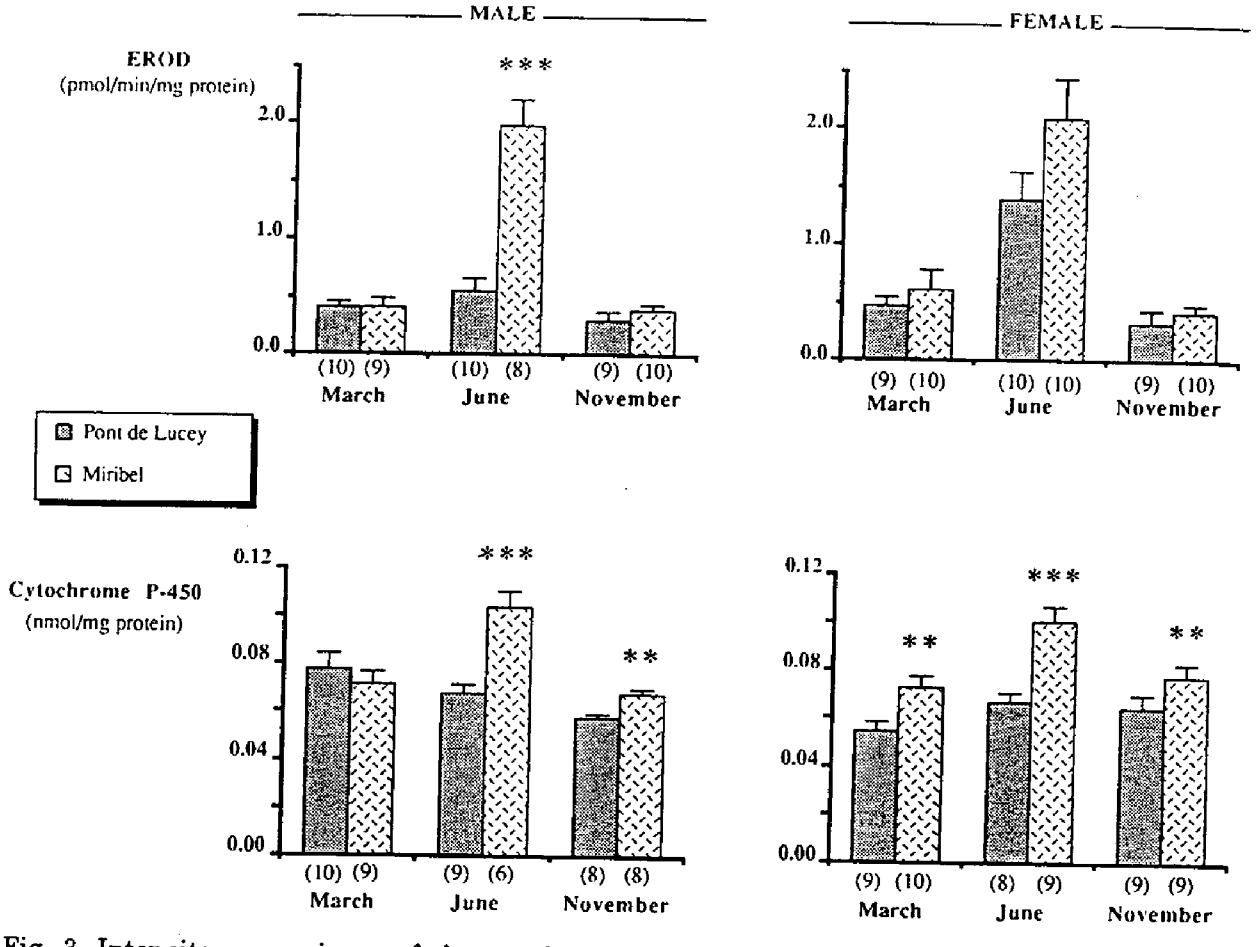

Fig. 3. Inter-site comparisons of the renal microsomal EROD activity and cytochrome P-450 concentration in male and female nase captured in March, June and November. Data are presented as mean ± SEM (number of fish). Levels of significance of the one-tailed Mann–Whitney U-test: 1% (**) and 0.1% (***).

The hepatic EROD activity was significantly higher in nase from Miribel than in those from Pont de Lucey, for both sexes and for the three sampling dates (ratio varied approximately from 2 to 5; Fig. 2). Results concerning hepatic cytochrome P-450 concentration were not so clear-cut, since significant differences were only obtained in March and June for males or only in June for females (Fig. 2).

The renal EROD activity was not influenced by water quality, since statistical comparisons led to only one significant result (males captured at Miribel in June showed a higher activity than those caught at Pont de Lucey) (Fig. 3). On the contrary, renal cytochrome P-450 concentration exhibited significant site differences for both male (June and November) and female (March, June and November) nase (Fig. 3).

DISCUSSION

In a previous study (Monod et al., 1988) we observed the influence of chemical pollution of the River Rhône on hepatic xenobiotic metabolizing

enzymes of three fish species, roach (*Rutilus rutilus*), nase (*Chondrostoma nasus*) and grayling (*Thymallus thymallus*). Monooxygenases and conjugating activities were significantly higher in the liver of fish exposed to the effluents of a PCB incineration plant than in those from reference areas. Additionally, PCB concentrations were shown to be much higher in fish from polluted areas, suggesting a close relationship between PCB contamination and enzymatic induction. Unfortunately, the number of fish was not sufficient to study the influence of sex on enzymatic activity level and on the response of these activities to PCB pollution. Moreover, we did not measure the influence of water pollution on extrahepatic enzymes.

The time trend of the GSI was in accordance with the study of Philippart (1980) on the reproductive biology of nase from a Belgian river. For hepatic EROD activity, we noted significantly higher values in males than in females when collected with developed gonads (in March and November). No such difference was seen after spawning (June). Higher MO activities in mature males than in mature females were also observed in Californian flatfish (Spies et al., 1982) and in vendace from a Finnish lake (Lindström-Seppä, 1985). In agreement with our results, Stegeman et al. (1987) did not find any sexual dimorphism in winter flounder outside the gonadal maturation season. Hormonal control may be responsible for the sex differences in MO activity in gonadally developed fish; the low level of enzymatic activity in females could be ascribed, at least in part, to the suppressive effect of high levels of plasma 17-β-estradiol during the development of the female gonads (Förlin et al., 1984). Since neither cytochrome P-450 nor sexual differences in PCB accumulation could explain the sexual dimorphism affecting the hepatic EROD activity, it is therefore possible that hormonal control may be directed towards particular forms of cytochrome P-450 involved in EROD activity. Hormonal analyses and more frequent sampling could confirm this hypothesis. Moreover, we did not observe any difference between male and female renal EROD activity, suggesting independent regulation of hepatic and extrahepatic xenobiotic metabolizing enzymes. Also, no significant statistical correlation was found between hepatic and renal EROD activities in the nase for any sampling date or for either sex (data not shown).

Sexual dimorphism in hepatic EROD activity during gonadal development implies a practical consequence when using EROD as a biochemical indicator of pollution; if, for instance, on the sampling dates corresponding to gonadal development (March or November) we had caught only females at Miribel and males at Pont de Lucey, we would not have observed any difference between these stations. Consequently, caution must be taken to compare fish of the same sex when monitoring water quality by analyzing MO activities.

Our study, considering male and female nase separately, demonstrated that both sexes on all sampling dates showed a similar increasing rate in hepatic EROD activity in the polluted area. As in our previous study (Monod et al., 1988), the data suggest induction by PCBs in the nase living downstream of the PCB incineration plant. For other species, conflicting results have been

obtained. Spies et al. (1988), studying the starry flounder from San Francisco Bay, noticed that, in both sexes, site differences affected hepatic aryl hydrocarbon hydroxylase (AHH) activity more during the period of gonadal maturation than during sexual quiescence. On the contrary, Walton et al. (1983) reported that inducibility of AHH in cunner was suppressed during the end of gonadal maturation, whereas it returned about 3 weeks after spawning. From our study, male and female nase seem to be of similar indicative value during the whole sexual cycle. Nevertheless, this assumption needs to be substantiated by more frequent sampling, particularly around spawning time.

7-Ethylresorufin *O*-deethylase activity and cytochrome P-450 content of nase kidney were respectively 10–200 times and 3–7 times lower than in the liver. Literature data indicates pronounced variations of the ratio between hepatic and renal MO in other species. In rainbow trout, cytochrome P-450 content (Stegeman and Chevion, 1980) and EROD activity (Pesonen and Andersson, 1987) were shown to be similar in liver and kidney. In carp, Pesonen and Andersson (1987) reported that hepatic EROD activity was 10 times higher than renal activity, and in our laboratory we noted a ratio of about 30 for the same activity (Rivière et al., 1990). In channel catfish, EROD activity and cytochrome P-450 content were about 3 times higher in liver than kidney (Tate, 1988).

Few studies deal with the use of renal MO activities as biochemical indicators of pollution, but some of them look very promising. For instance, in salmon and flounder exposed to oil pollution, Truscott et al. (1983) observed induction of AHH which was 2–3 times higher in kidney than in liver. Moreover, Payne et al. (1984) found significant induction of renal AHH, whereas they did not detect any change in hepatic variables in flounder collected in an oil-polluted area. We recently noticed a 4 times higher induction of EROD activity in the kidney than in the liver of carp experimentally exposed to a model inducer, β-naphthoflavone (Rivière et al., 1990). Conversely, neither EROD activity nor cytochrome P-450 content of the kidney were induced in channel catfish, while they were induced 3–5 times in the liver (Tate, 1988). Similarly, our results did not demonstrate the inducibility of renal EROD activity in nase (apart from males caught in June) and led us to regard renal MO of this species as an unsuitable biochemical indicator of pollution. It is noteworthy that the cytochrome P-450 content of nase kidney was generally significantly higher at Miribel than at Pont de Lucey, but the very low rate of induction (about 1.2-fold) makes this result questionable.

To date, AHH activity has certainly been the most studied MO activity in biomonitoring programs (for a review, see Payne et al., 1987). However, a close correlation was revealed between AHH and EROD activities (Monod et al., 1988), and recent studies have demonstrated the usefulness of EROD as a biochemical indicator of chemical pollution (Melancon et al., 1987; Andersson et al., 1988). Furthermore, the toxicological significance of MO induction (especially AHH and EROD) has recently been emphasized, particularly with regard to mutagenic and carcinogenic risks (Buhler and Williams, 1988;

Ioannides and Parke, 1987), as well as to reproductive disorders (Spies and Rice, 1988).

In conclusion, this study stresses the usefulness of hepatic cytochrome P-450 enzymatic activities in fish as biochemical indicators of chemical pollution of the aquatic environment. 7-Ethylresorufin *O*-deethylase activity seems to be of particular interest regarding its inducibility by major organic pollutants (PCBs, polyaromatic hydrocarbons, dioxins) and its toxicological significance. Furthermore, our results demonstrate the need to take into account the sexual dimorphism affecting MO activities when comparing the response of fish exposed to various water qualities. Finally, hepatic MO activities seem to be responsive to chemical pollution in all fish species studied, but the inducibility of renal MO appears to be species dependent.

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