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Histopathological alterations and induction of cytochrome P-450 1A in the liver and gills of the gilthead seabream (*Sparus aurata*) exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

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Summary

The toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been demonstrated in the seabream *Sparus aurata* specimens. Liver presented hepatocytic alterations, with an increase of lipid droplets and glycogen granules. Ultrastructural modifications of hepatocytes included RER fractionation, glycogen augmentation, as well as a rise in the number of lipid droplets, vacuoles and secondary lysosomes. In the gills, secondary lamellar epithelium showed hyperplasia, hypertrophy and lamellar fusion on the edge of the filaments. At the end of the exposure period (1 pg l^{-1} TCDD for 20 days), some organelles in epithelial cells of the secondary lamellae and the tubular system of the chloride cells appeared altered. In the liver of TCDD-exposed specimens, immunoreactive cytochrome P-450 1A was concentrated close to the cytoplasmic and nuclear membranes, and positive granules were also evident throughout cytoplasm of the hepatocytes. Significant cytochrome P-450 staining was especially evident in endothelium of the hepatic vascular system. At the beginning of the exposure (1 pg l^{-1} TCDD, for 5 and 10 days), cytochrome P-450 immunostaining was observed in the cytoplasm of scarce hepatic cells and after 20 days of treatment, specific immunostained cytoplasmic granules were detected in most hepatocytes. In gills of TCDD-treated specimens, pillar-endothelial cells showed a cytochrome P-450 1A immunostaining concentrated close to the base of gill filaments and dispersed through the gill lamellae. There was also significant cytochrome staining of the endothelium of the branchial vascular system. However, no cytochrome immunoreactivity was observed in epithelial-respiratory cells.

Introduction

Strong indications for a relationship between water pollution and fish diseases/histopathologies (pre- and neoplasic liver lesions, tumours, ulcerative skin lesions, virus prevalence) have been reported by several authors (Myers *et al*. 1994, Vethaak & Jol 1996, Vethaak *et al*. 1996, Ortiz *et al*. 2002a). Interestingly, the localization of cytochrome P-450 1A (CYP1A) calls attention to specific cell types useful in environmental monitoring studies, and specific differences in distribution and responses to xenobiotics can be demonstrated by immunohistochemical approaches (reviewed by Cajaraville *et al*. 2000, Sarasquete & Segner 2000). In this paper, two known ecotoxicological biomarkers of lipophilic organic contaminants (histology and immunohistochemical CYP1A induction) have been studied in a commercial fish species, under an experimental assay.

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofuranes (PCDFs) are important environmental pollutants. They are highly persistent and lipophilic and have been shown to cause several toxic effects in mammalian and non-mammalian species at relatively low concentrations (Grinwis *et al*. 2000). For example, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the by-product of industrial processes and pyrolitic reactions, has been shown to accumulate in various fish tissues (Safe 1990, Tietge *et al*. 1998, Walter *et al*. 2000). The extreme toxicity of TCDD is related to the highly lipophilic character of this compound. For this reason, it may more readily accumulate in the triglyceride fraction than in the phospholipid fraction, as has been demonstrated *in vitro* for other lipophilic chemicals (Helder 1981).

The hepatocellular effects of TCDD exposure on fish have been documented in several studies. Hepatocellular swelling and necrosis, nuclear chromatin margination, changes in glycogen and lipid contents, pericholangitis, increased mitotic figures, cytoplasmic inclusions and sinusoidal dilatation have been observed following exposure to TCDD at several concentrations in several fish species (Helder 1981, Spitsbergen *et al*. 1988, Van der Weiden *et al*. 1992, 1993,

Walter *et al*. 2000). Blunting and fusion of secondary lamellae and lymphocytic infiltrates were also observed in gills from fish exposed to TCDD (Walter *et al*. 2000).

In mammals it has been reported that exposure to 2,3,7,8-chlorine substituted PCDDs and PCDFs results in a combination of biological effects, such as strong and prolonged induction of cytochrome P450s, hepatotoxic effects, porphryria and body weight loss, mediated by the cytosolic Ah-receptor (Kennedy & Fox 1990, Safe 1990). The cytochrome P-450 (CYP) system has an essential function in the biotransformation of endogenous (fatty acids, steroids, prostaglandins, drugs) and exogenous compounds such as polyaromatic hydrocarbons (PAHs), dioxins, polychlorinated biphenyls (PCBs) and halogenated aromatic hydrocarbons (HAHs). In mammals and fish, TCDD is one of the most potent inductors of CYPs (Huang *et al*. 2000, Sarasquete & Segner 2000).

Much attention has been paid to the cytochrome P-450 1A (CYP1A) subfamily because its substrates include important environmental toxicants such as the ones already mentioned. A large number of investigations has been published over the last 20 years (reviewed by Cajaraville et al. 2000, Sarasquete & Segner 1999, 2000), which indicate its usefulness as a biomarker of aquatic pollution. Several studies have demonstrated, by means of immunohistochemistry, the expression of CYP1A in hepatocytes (Lorenzana *et al*. 1988, Smolowitz *et al*. 1989, 1992, Stegeman *et al*. 1991, Collier *et al*. 1993, Husøy *et al*. 1994, Lindström-Seppa *et al*. 1994, Van Veld *et al*. 1997, Reinecke & Segner 1998, Sarasquete *et al*. 1999, 2001). Although mostly studied in the liver, CYP1A induction has also been found in extrahepatic organs, including brain and pituitary, by enzymatic catalytic analysis (EROD) and CYP1A-protein-mRNA quantification (ELISA, Western Blot, PCR) (Ortiz *et al.* 2001a,b, 2002b).

The degree of alteration in organs/tissues (liver, gills, digestive tract and vascular endothelium) may be related to different pollutants, their concentration and the route of incorporation of contaminants. Xenobiotics can be metabolized at significant rates during their passage through the gill epithelium. Moreover, they may pass the gills essentially as parent compound and undergo metabolism in the liver (Van Veld *et al*. 1997). The strong CYP1A induction in the liver is consistent with the role of the hepatocytes in biotransformation of xenobiotics (Lorenzana *et al*. 1988, Stegeman *et al*. 1982, Van Veld *et al*. 1990, Andersson *et al*. 1993, Goksøyr & Husoy 1998, Reinecke & Segner 1998, Grinwis *et al*. 2000, Sarasquete & Segner 1999, 2000, Sarasquete *et al*. 1999, 2001).

Histopathological alterations and changes in mixed function oxygenase enzymes (MFO) are frequently used as indicators of effect and exposure to anthropogenic contaminants (Mondon *et al.* 2001). In order to investigate the role of a particular compound that induces the effects observed in the field, it is essential to carry out experiments under controlled conditions. In this paper, an important commercial species of the South Atlantic coasts, the seabream (*Sparus aurata*), was exposed to TCDD at sublethal concentrations, with the aim of studying the histopathological alterations and the induction of CYP1A in target organs.

Material and methods

Immature male specimens of sea bream, *Sparus aurata* (average weight, 250–300 g), from a commercial fish farm (CUPIMAR, SA. San Fernando. Cádiz, Spain) were kept in tanks for two weeks to allow adaptation. The tanks were supplied with continuously flowing sea water at constant temperature ($19 \pm 1\,^{\circ}C$).

Xenobiotic exposure

After the acclimatisation period, fish were randomly distributed into the experimental tanks for 20 days and submitted to the following treatments: (a) control (only vehicle added, toluene), (b) exposure to $1\,pg\,l^{-1}$ TCDD (3.1 nM). Treatment was applied in duplicate, with 12 fish per each tank. A stock solution of TCCD was prepared in toluene and added to the water in suitable quantities to give a nominal concentration of $1\,pg\,l^{-1}$ TCCD. The maximum solvent concentration in the tanks was $0.05\,\mu l\,l^{-1}$ toluene.

The fish were exposed to TCDD for 20 days under semi-static conditions. Daily, the specimens were fed freely with *Loligo* spp. and the excess of food removed from the tanks. Immediately after feeding, the water in the experimental tanks was renewed every 24 h, which was followed by the addition of TCDD. Before the experiment were initiated, tanks were filled with TCDD solution for 24 h in order to guarantee complete adsorption of the compounds on the walls. Water temperature ($18.8 \pm 0.2\,^{\circ}C$), pH (7.5 ± 0.2), NO_2^- ($<0.1\,mg\,l^{-1}$), NO_3^- ($<8\,mg\,l^{-1}$), NH_4^+ ($<3\,\mu g\,l^{-1}$), dissolved oxygen ($8.3 \pm 0.3\,mg\,l^{-1}$) and salinity (32‰) were measured daily during the experimental period. Throughout no mortality was recorded. Samples of liver and gills of control and exposed fish were then taken at different days (5, 10, 15 and 20).

Histopathological studies

For light microscopical studies, small samples of gills and liver were fixed in Bouin's fluid and/or formaldehyde buffered with 0.1 M phosphate (pH 7.2). After dehydration in graded concentrations of ethanol, samples were embedded in paraffin wax. Sections, 6–7 μm thick, were stained with either Haematoxylin and Eosin or Haematoxylin followed by Light Green-Orange G-Fuchsin trichome (Gutiérrez 1990) for histomorphological studies.

For transmission electron microscopy (TEM), small pieces of gills and liver were fixed for 2 h in cold 2.5% glutaraldehyde – 0.1 M cacodylate buffer (pH 7.2), rinsed several times in cacodylate buffer and postfixed with 1% OsO_4 in 0.1 M cacodylate buffer. Samples were dehydrated in a graded series of acetone and embedded in Spur's medium. Ultrathin sections of 60–80 nm thickness (Reichert-Jung ultramicrotome)

were stained with uranyl acetate and lead citrate prior to observation in transmission microscope (Zeiss EM 9S2).

Immunohistochemical CYP1A analysis

For immunohistochemical studies of CYP1A, 5-μm-thick sections were prepared from paraffin-embedded tissues. Endogenous peroxidase activity was inhibited by treating the sections with methanol–H_2O_2 (6 ml of 3.3% H_2O_2 in 10 ml methanol) for 5 min at room temperature. The sections were then transferred for 5 min to phosphate-buffered saline (PBS) containing 0.1% Triton X-100 (PBS-T) followed by PBS-T containing 0.5% casein for 30 min to block non-specific binding sites. The sections were subsequently incubated overnight at room temperature with a primary monoclonal CYP1A antibody (Biosense AS, Bergen, Norway). The primary antibody (C10-7) was diluted in PBS at 1 : 250. For further staining, the Vectastain ABC System (Vector Laboratories, Canada, USA) including a biotinylated anti-mouse IgG secondary antibody was applied. To demonstrate the specificity of the CYP1A antibody, some sections were incubated with normal fish serum instead of the primary antibody.

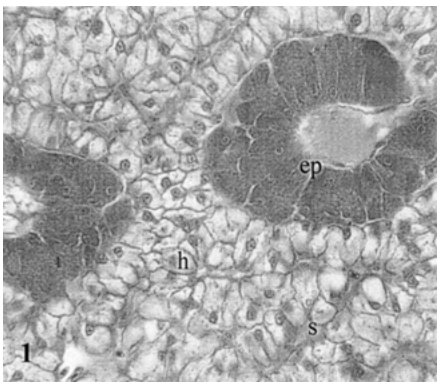


Figure 1. Histological section of liver of a control seabream, *Sparus aurata* specimen. Hepatocytes (h), sinusoid (s) and intrahepatic exocrine pancreas (ep) are shown. Haematoxylin–Eosin, 200×.

Results

Histopathology

The liver of the seabream, *Sparus aurata*, showed the same histological characteristics as those described for other fish species. The hepatocytes are distributed in cordons concentrically placed around the sinusoids and intrahepatic exocrine pancreas is also observed (Figure 1). At ultrastructural level, the hepatocytes show ovoid nucleus containing a distinct nucleolus (Figure 2). Extensive stacks of rough endoplasmic reticulum (RER) cisternae interspersed with few mitochondria (Figure 3) were detected although the smooth endoplasmic reticulum (SER) was restricted. The cytoplasm of hepatocytes contained lipid droplets without membrane (Figure 2) and glycogen granules. The biliary canaliculi were formed by the plasmalemma of adjacent hepatocytes, with microvilli being extended into the canaliculi (Figure 4). The sinusoids were lined by endothelial cells and space of Disse between these and the microvilli (Figure 5) can be observed.

Following exposure to TCDD (1 pg l^{-1} for 20 days), the microscopical features of the parenchyma of hepatocytes

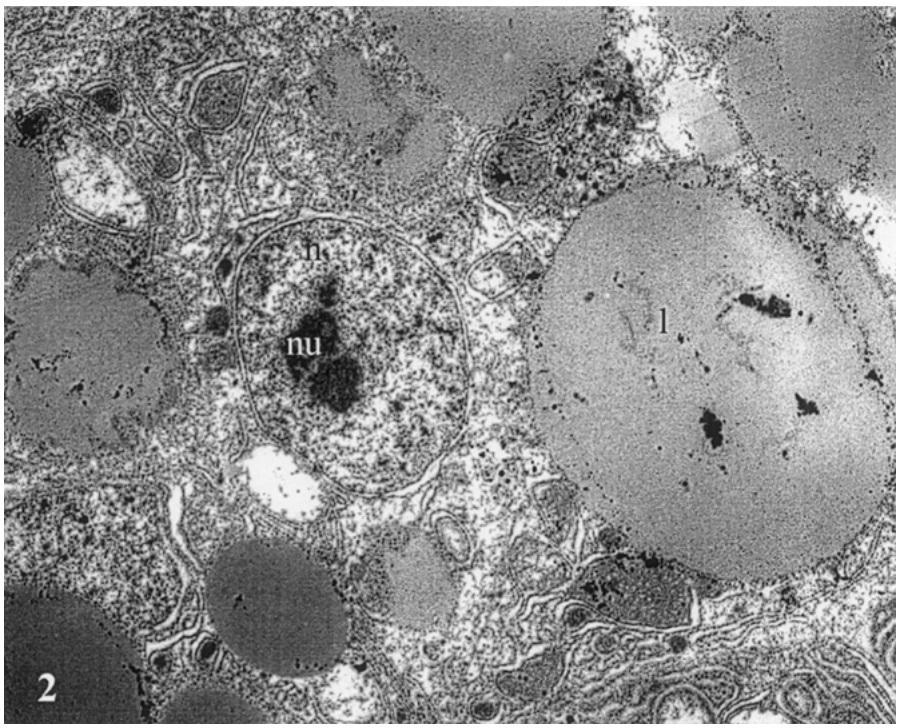
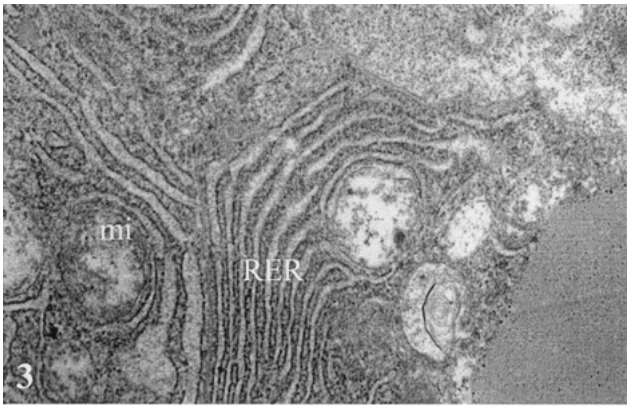
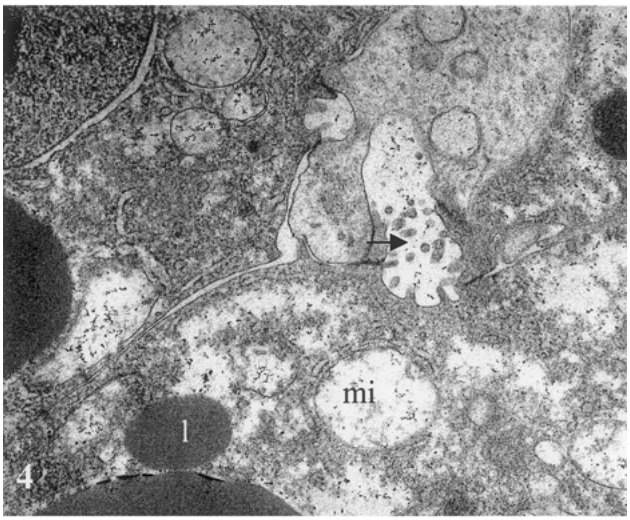
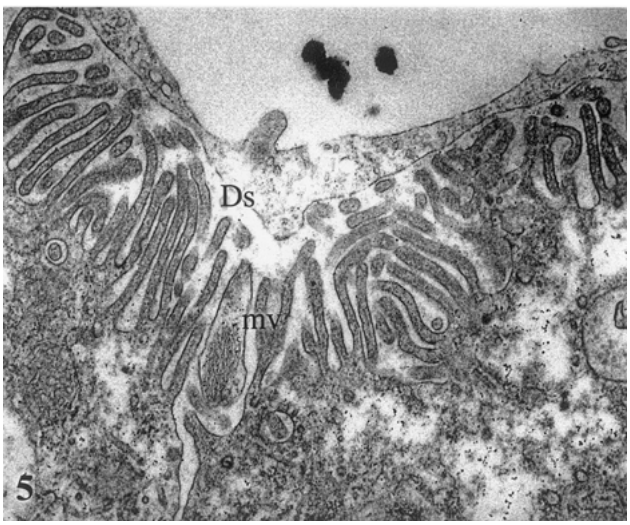


Figure 2. Ultrastructural section of hepatocytes of an untreated specimen liver. Nucleus (n), nucleolus (nu) and lipid droplets (l) are observed, 4000×.







Figures 3–5. (3) Ultrastructural section of hepatocytes of control seabream liver. Extensive stacks of long continuous cisternae of rough endoplasmic reticulum (RER) and few mitochondria (mi) are observed, 10,000×. (4) Hepatocytes of unexposed fish. Bile canaliculus (→), lipid droplets (l) and mitochondria (mi) are shown ultrastructurally, 10,000×. (5) Histological liver section of a control specimen, showing sinusoids lined by endothelial cells and the space of Disse (Ds) between these and microvilli (mv) of the hepatocytes is observed, 15,000×.

appeared altered: they showed cellular disorganization, variation in nuclear size, picnosis and an increase in lipid droplets (Figure 6). Furthermore, ultrastructural alterations such as modifications of hepatocytes including fractionation and depletion of RER (Figure 7), glycogen augmentation (Figure 8), a rise in the number of lipid droplets, vacuoles and secondary lysosomes (Figure 9) were also detected.

In addition, the histology of seabream gills showed similar aspects to other teleostean fish species. In particular, the hemibranchs contained a row of long thin filaments, the primary lamellae with the surface area of these forming regular and parallel folds across its dorsal and ventral surface – the secondary lamellae – (Figure 10). The respiratory lamellae were covered by an epithelial layer or respiratory epithelium (Figure 11) and internally, the lamellar blood sinuses were lined and spanned by pillar cells (endothelial cells) (Figure 12). In the filament and through the interlamellar regions, chloride and goblet/mucous cells were easily observed (Figures 10 and 11).

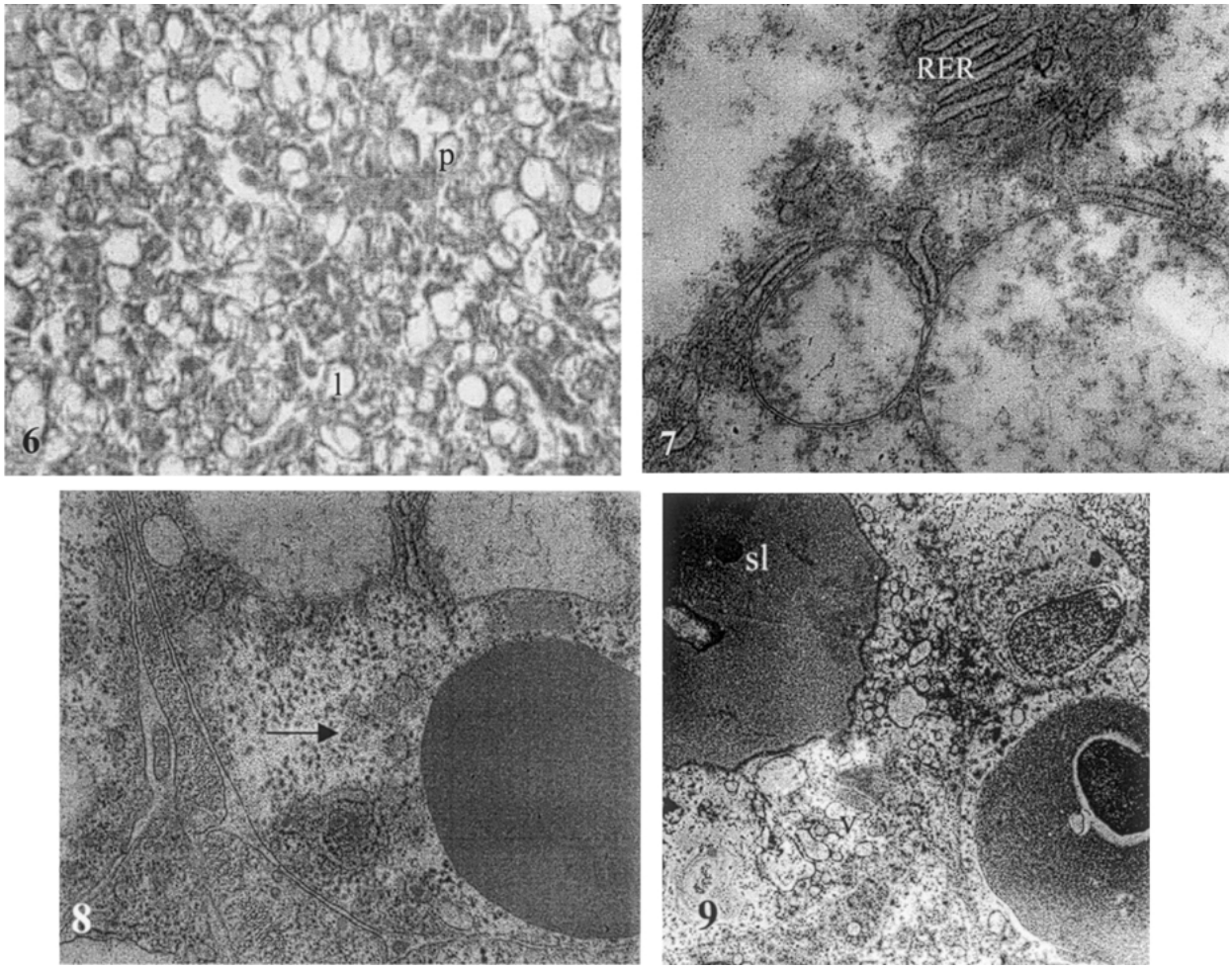
Hyperplasia and hypertrophy of lamellar epithelium and fusion of adjacent secondary lamellae were the main alterations observed in gills of TCDD-exposed seabream specimens (Figure 13). Epithelial cells of the secondary lamellae showed altered shapes, with an increased volume with respect to control cells and larger nuclei slightly rounded. The rise in the primary lamellar epithelium resulted in the obliteration of the interlamellar spaces between secondary lamellae. The rupture of pillar cells and capillaries led to an accumulation of erythrocytes in the distal portion of the secondary lamellae (telangiectasis/aneurism). Fusion of adjacent secondary lamellae was produced by adhesion of the plasmatic membrane of the respiratory epithelium (Figure 13). Finally, the tubular system of the chloride cells located in the interlamellar regions appeared altered (Figure 14) since this system did not show a clear ramified and elaborated structure, as that observed in control sections.

In general, the histological alterations observed in gills and liver from TCDD-exposed seabream were highest at the end of the TCDD experimental period.

Immunohistochemistry of CYP1A

In the liver of control seabream specimens, moderate to strong CYP1A staining located at the endothelium of the vascular system was observed but no cytoplasmic staining was detected in the cytoplasm of hepatocytes (Figures 15 and 16).

In the liver of TCDD-exposed specimens, a mild staining of bile duct epithelium was also evident and a specially strong CYP1A staining within endothelial cells of vascular system enclosing the sinusoids, arteries and veins (Figure 17) was observed. CYP1A staining was concentrated close to the cytoplasmic and nuclear membranes, and positive granules were distributed throughout cytoplasm of the hepatocytes. During the initial TCDD exposure period (5–10 days), CYP1A staining was only observed in the cytoplasm of certain



Figures 6–9. (6) Hepatic histological section of TCDD-seabream specimen showing a cellular disorganization, picnosis (p) and lipid droplets (l) increases. Haematoxylin and Eosin, 200×. (7) Ultrastructural section of liver of a treated specimen (TCDD exposure, 20 days) showing fractionation and depletion of RER, 20,000×. (8) Ultrastructural section of liver of a TCDD-exposed fish (20 days) showing increased hepatocellular glycogen cytoplasmic granules, 20,000×. (9) TCDD-exposed seabream liver (20 days) showing increased numbers of vacuoles (v) and secondary lysosomes (sl) in the hepatocytes, 6000×.

cells (Figure 18) but after 20 days of treatment, CYP1A specificity stained cytoplasmic granules in most hepatocytes (Figure 19).

Furthermore, mild to moderate staining was present in ducts of pancreatic tissue and in exocrine pancreatic acinar cells, although of treated specimens no staining was detected in adipocytes (Figures 20 and 21). The presence of such staining was not related to the degree of CYP1A induction in other cells.

In control seabream gills, a spotty staining of the endothelium of the branchial arteries and veins and a mild immunostaining in goblet cells were observed. In contrast, there was no staining of the epithelial (respiratory) and pillar (endothelial) cells (Figure 22).

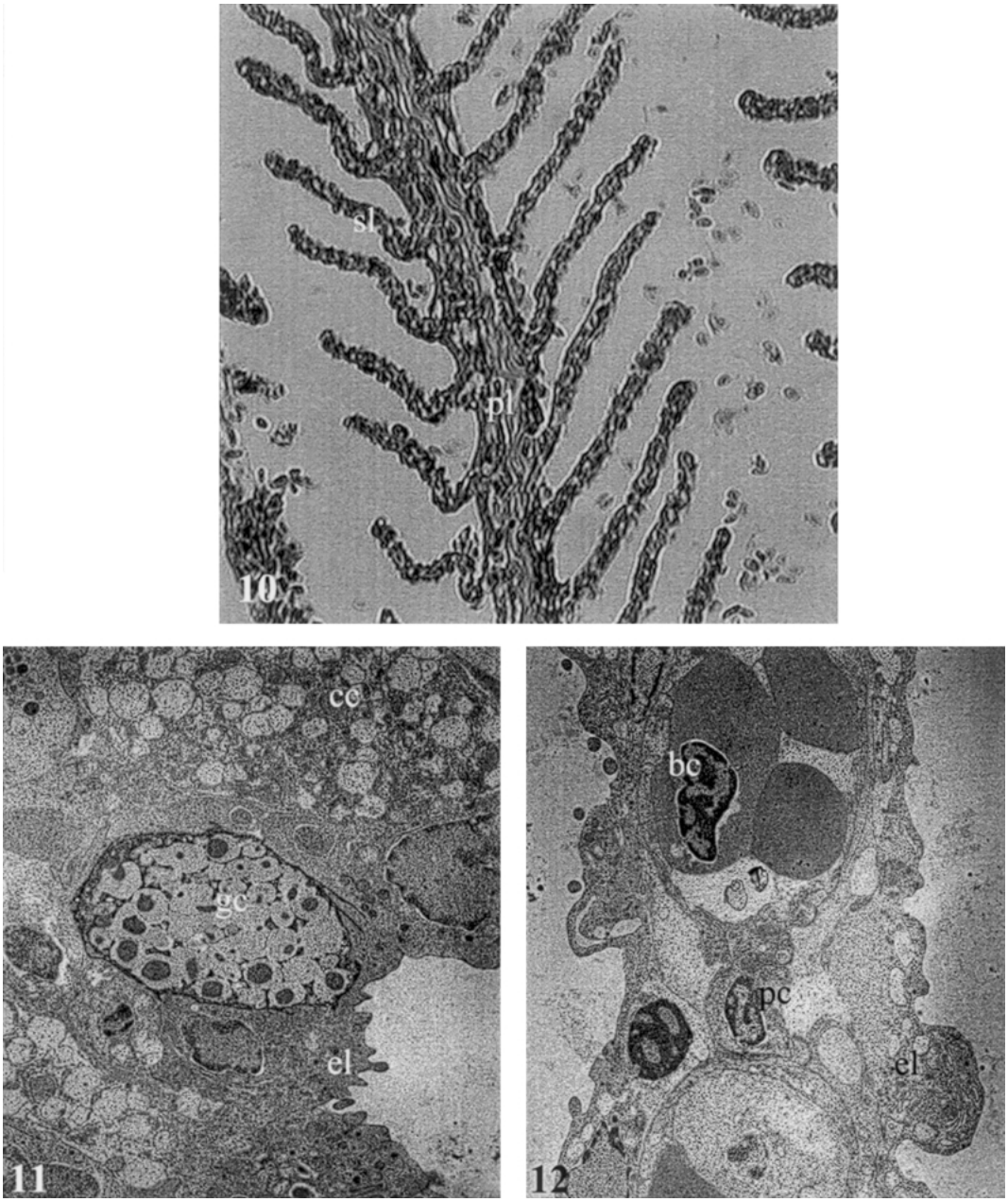
In TCDD-treated specimens, gills showed a CYP1A immunostaining of pillar cells and negative staining of epithelial cells. The first one was concentrated close to the base of gill filaments at the beginning of TCDD exposure (Figure 23) whereas at the end of the treatment it was dispersed throughout the gill lamellae (Figure 24). There was also a significant CYP1A staining of the endothelium of the vascular system (Figures 25 and 26).

Discussion

Histopathology

In contaminated fish, histopathological alterations of different organs and tissues show changes related to toxicant-type, species sensitivity, age, sex, concentration and route of administration of contaminants. In order to investigate the particular role that a substance has in the induction of the effects observed, experiments under controlled conditions are essential.

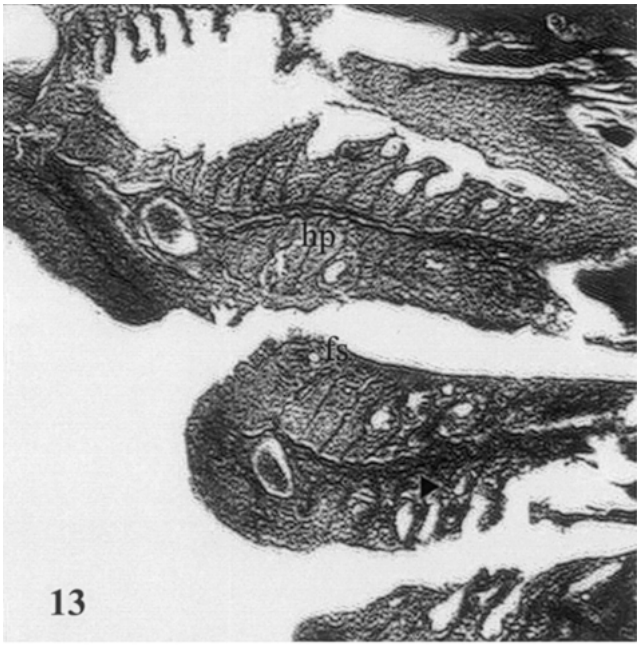
Our results show that exposure to TCDD in seabream causes significant alterations in the liver. Basically, an augmentation in the number of glycogen and lipid droplets, a RER fractionation and a higher number of secondary lysosomes are observed. However, Van der Weiden *et al*. (1992)

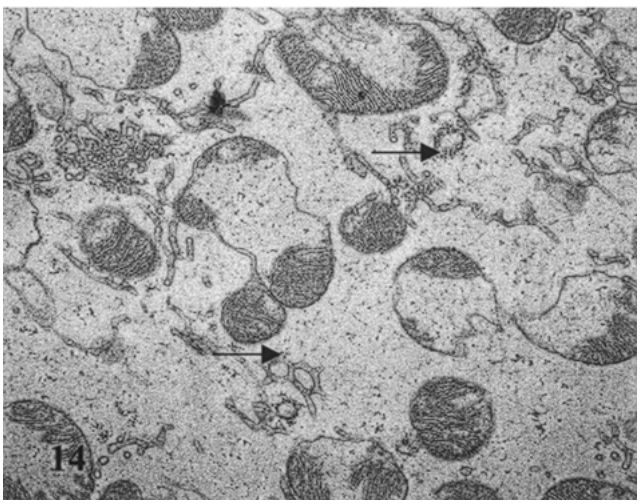


Figures 10–12. (10) Gills of control seabream specimens showing basic features of primary (pl) and secondary (sl) lamellae. Haematoxylin and Eosin, 400×. (11) Ultrastructural section of a control specimen gill. Primary lamellae containing goblet (gc) and chloride (cc) cells, 3000×. (12) Ultrastructural section of control seabream gills. Epithelial layer (el), blood (bc) and pillar (pc) cells are observed in a secondary lamellae, 4000×.

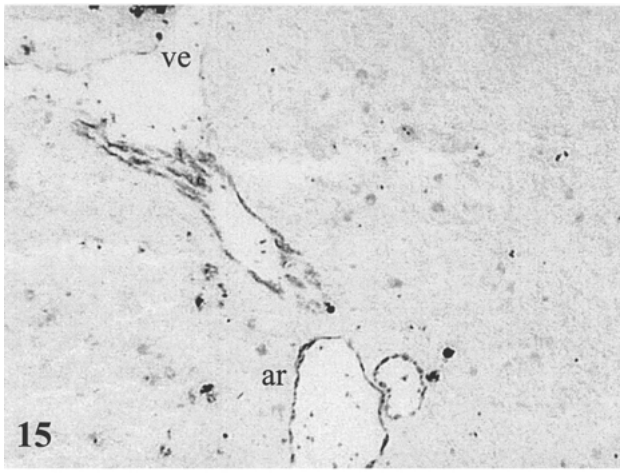
reporterd that in rainbow trout following a single injection of TCDD (3.06 μg kg^{-1}) the liver contained less glycogen compared to unexposed fish. This discrepancy could be related to interspecific metabolic/physiological differences between both species. Hepatic alterations, such as variation in nuclear size and chromatin pattern, mild variation in hepatocytes size and/or increased number of mitotic figures and lipid vacuoles have been detected in rainbow trout exposed to dietary TCDD (90 ng kg^{-1} food) (Walter *et al*. 2000). Fractionation, vesiculation and dilation of RER cisternae have been frequently observed in liver from specimens exposed to herbicides (Oulmi *et al*. 1995). In our study, fractionation and depletion of RER in hepatocytes were detected. Similar alterations have been documented in mammals after exposure to PCBs and PAHs, and these changes are generally related to induction of detoxification processes both in mammals (Phillips *et al*. 1987, Ghadially 1988) and fish (Braunbeck et al. 1990, Braunbeck & Völkl 1991).

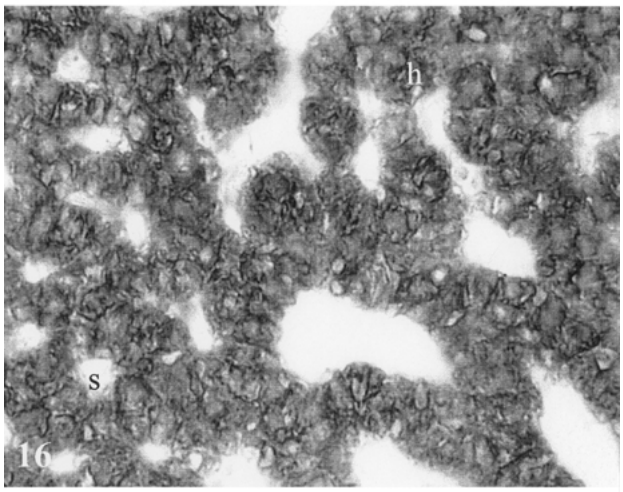
Interestingly, hepatic lipid accumulation originally described as 'steatosis' by Baglio and Farber (1965), may result from either a blockade in VLDL (Very Low Density Lipoprotein) secretion or from an imbalance between protein and lipid components involved in lipoprotein metabolism. These alterations have repeatedly been reported in fish liver under adverse conditions, such as ambiental stress, toxicants, inadequate artificial feeding, etc. (Deplano *et al*. 1989, Braunbeck & Segner 1992). The considerable increase in hepatocellular lipid deposits, in TCDD-exposed

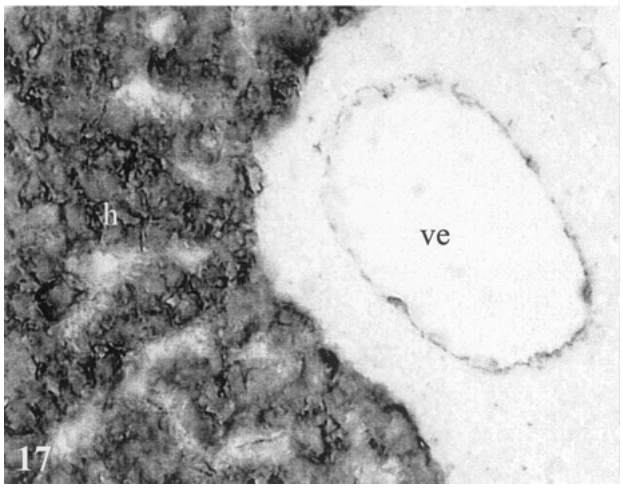




Figures 13–14. (13) Histological section of gills of treated specimens. Hypertrophy of lamellar epithelium (hp), fusion of adjacent secondary lamellae (fs) and primary lamellar epithelium showing an increased number in cellular layers (▶). Haematoxylin–Light Green/Orange G/Fuchsin trichrome, 50×. (14) Ultrastructural section of gills of treated specimens (TCDD exposure, 20 days) showing the tubular system (→) of the chloride cells altered, 4000×.



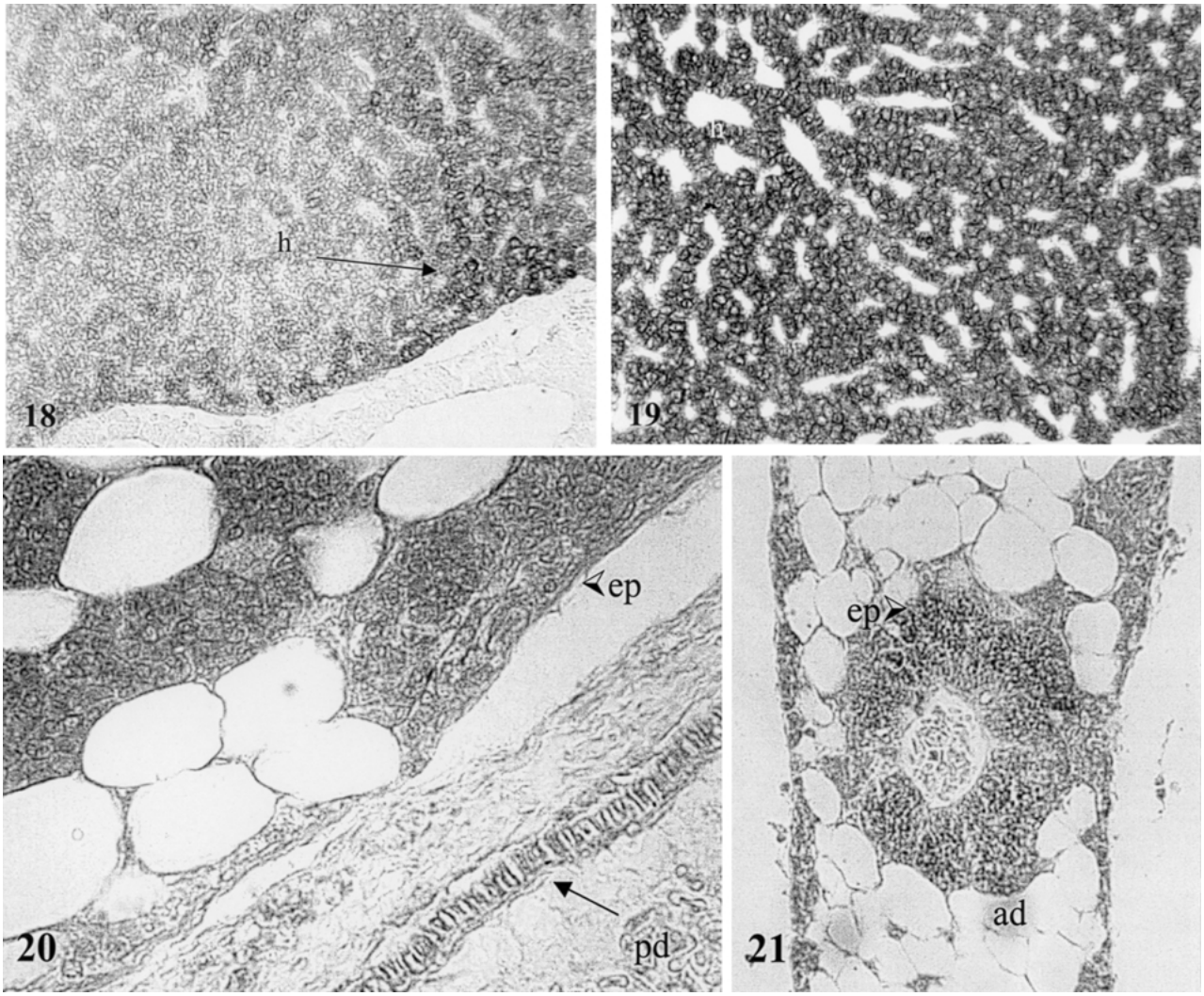




Figures 15–17. (15) CYP1A staining in endothelium of vascular system (ve = veins, ar = arteries) of liver of control seabream specimens. Note the absence of immunoreactivity in hepatocytes, 200×. (16) Strong CYP1A immunoreactivity in hepatocytes (h) and sinusoids (s) in liver from TCDD-exposed seabream (20 days), 200×. (17) CYP1A immunoreactivity in hepatocytes (h) and veins endothelium (ve) in liver of TCDD-exposed seabream (20 days), 400×.

seabream specimens, could be due to a stimulation of lipid metabolism, and since there is also a fractionation and depletion of RER, disturbances in protein synthesis appear more likely as a reason for hepatic steatosis than a blockage in lipoprotein secretion. In fact, a higher number of secondary lysosomes could indicate a decomposition of membrane structures containing an excess of phospholipid compounds (Oulmi *et al*. 1995).

The degree of alteration in organs/tissues (liver, gills, digestive tract, vascular endothelium) may be related to different toxicants, their concentration and route of incorporation of contaminants. In rainbow trout exposed to dietary toxicant (90 ng kg^{-1} TCDD), lesions most frequently noted were: focal chronic granulomas, occasional blunting, fusion of secondary lamellae and lymphocytic infiltrates (Walter *et al*. 2000). Nonetheless, no histological alteration was detected in gills, liver and digestive tract of the European flounder exposed to 500 μg kg^{-1} TCDD (Grinwis *et al*. 2000). In TCDD-exposed



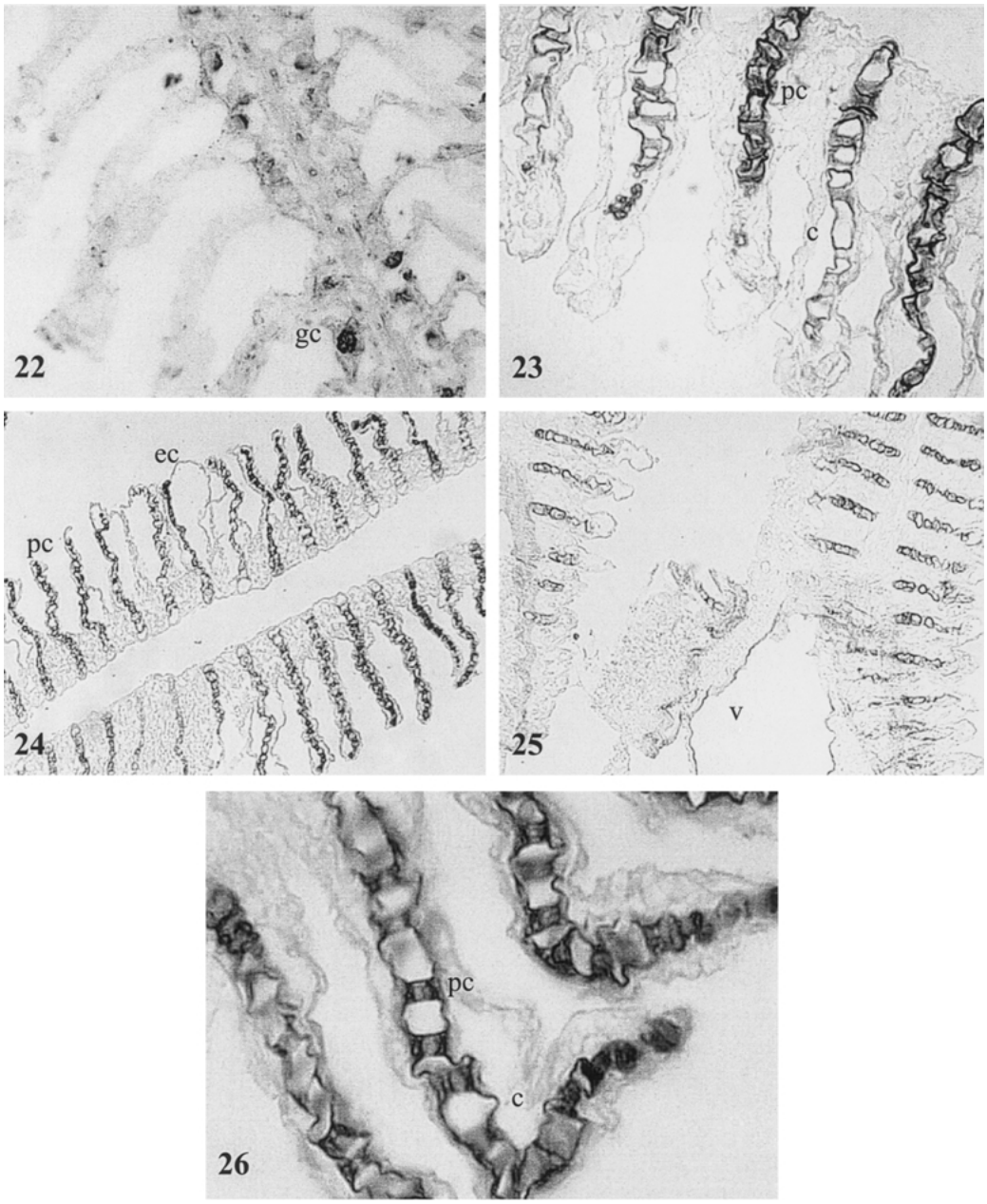
Figures 18–21. (18) Moderate CYP1A immunoreactivity in hepatocytes (h) at the beginning of TCDD exposure (5 and 10 days). Note the CYP1A staining is concentrated only in the cytoplasm of certain cells in liver of exposed fish, 200×. (19) Strong CYP1A immunoreactivity in hepatocytes (h) of seabream exposed specimens (20 days). CYP1A specific stained granules are spread in most hepatocytes, 200×. (20) Moderate staining in ducts of pancreatic (pd) tissue and in exocrine pancreatic acinar cells (ep) in TCDD-exposed seabream specimens (20 days), 400×. (21) Moderate to strong CYP1A staining zymogen granules of exocrine pancreatic acinar cells (ep). Note the absence of CYP1A induction in adipocytes (ad), 200×.

seabream specimens, the important pathologies in gills such as hypertrophy, hyperplasia, telangiectasis in respiratory epithelium, fusion of secondary lamellae and alterations in chloride cells could be mainly due to the experimental design, since in our study, gills were directly in contact with water/contaminant. In several fish species, as occured in TCDD-exposed seabream specimens, the respiratory diffusion distance in gills increases after exposure to different inorganic and organic xenobiotics (Dalela *et al*. 1979, Nowak 1992, Randi *et al*. 1996). This fact could be a consequence of either a lifting in lamellar epithelium and/or hyperplasia of the branchial epithelium frequently observed in different contaminated fish (Arellano *et al*. 1999). As gills provide the most extensive interface with the aquatic environment, it is expectable that branchial structural changes are present in contaminated fish. A high prevalence of hyperplasia in chloride and epithelial cells and lamellar fusion in gills, as well as multifocal coagulative necrosis and inflammation of the seabream liver were also detected in the greenback flounder *Rhombosolea tapirina* exposed to several contaminated sediments (Mondon *et al*. 2001).

Hyperplasia and hypertrophy of lamellar epithelium and fusion of adjacent secondary lamellae were the principal alterations observed in gills from *Sparus aurata* exposed to TCDD. Similar results were observed by Mallatt (1985) in different fish species treated with organic compounds.

Immunohistochemistry

In fish, the primary organ of CYP1A expression is the liver, although extrahepatic occurrence of CYP1A has been demonstrated in a variety of tissues, including gill epithelia, heart endothelium, gut mucosa, olfactory epithelium, kidney tubules, spleen, different brain areas and pituitary



Figures 22–26. (22) CYP1A immunoreactivity in gills from control seabream specimens. Note the mild to moderate immunostaining located at goblet cells (gc), 200×. (23) CYP1A immunostaining of gills during the initial TCDD exposure period. The CYP1A immunostaining is located at pillar cells (pc) concentrated close to the base of gill filaments and in capillaries endothelium, 400×. (24) CYP1A immunostaining of gills the last day of TCDD exposure (20th day) showing CYP1A immunostaining in pillar cells (pc) dispersed throughout the gill lamellae. Note the absence of CYP1A staining in epithelial cells (ec), 200×. (25) CYP1A immunostaining of the endothelium of the branchial veins (v) of TCDD-exposed specimens, 200×. (26) Detail of secondary lamella from gills at the last day of TCDD exposure (20th day) showing CYP1A immunostaining in pillar cells (pc) and capillaries (c), 400×.

(Miller *et al*. 1988, Stegeman *et al*. 1989, Van Veld *et al*. 1997, Goksøyr & Husoy 1998, Sarasquete & Segner 1999, 2000, Sarasquete *et al*. 1999, 2001, Grinwis *et al*. 2000, Walter *et al*. 2000, Ortiz *et al*. 2002b). Information on tissue distribution of CYP1A is important since xenobiotic metabolism in extrahepatic sites is likely involved in the systemic effects of CYP1A-inducing toxicants (Stegeman & Hahn 1994).

In TCDD seabream specimens, a moderate or strong CYP1A induction was observed in the liver and gills, and specially in the vascular endothelium of both organs. The prominent expression and induction of CYP1A in the liver is consistent with the role of this organ in xenobiotic metabolism, detoxification and excretion. In fish, the liver is mainly composed of hepatocytes which account for up to 80% of the total liver volume (Hampton *et al*. 1989, Hinton 1994, Segner 1998). Contrary to mammals where CYP1A shows an heterogeneous distribution throughout the liver parenchyma, no zonation was observed in fish liver (Smolowitz *et al*. 1989, 1991). In the liver of TCDD-exposed seabream specimens,

CYP1A induction was detected in cytoplasmic and nuclear membranes, and positive granules were also evident throughout the cytoplasm of the hepatocytes, the number of these immunostained granules increasing with exposure time to TCDD.

Induction of CYP1A in treated seabream also occurred in the epithelial cells lining the biliary pass ways, in exocrine pancreatic cells and specially in vascular endothelia of all structures. The staining of biliary epithelium was probably a result of the deposition of inducers or their metabolites in the bile (Hinton 1994). In fish hepatocytes, CYP1A is located at the RER, the nuclear envelope, and in the plasma membrane of the microvilli of the bile canaculi (Lester *et al.* 1993). Typically, expression of biliary or endothelial CYP1A can be detected only after xenobiotic exposure (Stegeman & Hahn 1994; Goksøyr & Husøy 1998), as observed in TCDD-exposed seabream specimens.

Gills serve as a portal of entry of xenobiotics present in the aquatic environment. In TCDD-exposed seabream specimens, CYP1A induction was observed in pillar or endothelial cells but not in respiratory epithelium. In different fish species exposed to waterborne chemicals, CYP1A induction has been documented in the respiratory (epithelial) cells, the pillar (endothelial) cells of the secondary lamellae and in the vascular endothelia of filaments and arches (Miller *et al*. 1988, Stegeman *et al*. 1989, Smolowitz *et al.* 1992, Husøy *et al.* 1994, 1996, Van Veld *et al.* 1997).

CYP1A-induction was observed in the vascular system of different organs and tissues in TCDD-exposed seabream. A positive immunostaining was also detected, however, in the vascular system of control fish. The vascular endothelium is involved in a wide range of regulatory functions, including control of blood volume or the synthesis of vasoactive regulators, such as arachidonic acid metabolites and prostaglandins. Similarly, endothelial cells are responsible for the transfer of chemicals from the blood to the underlying cells and tissues. Due to their relatively high CYP1A levels, the endothelial cells may activate xenobiotics during their blood passage or transfer into tissues, which could negatively affect either the endothelium itself or the underlying cells (Andersson *et al*. 1993). Interestingly, from studies with freshwater species (Guiney *et al*. 1997), the vascular endothelium has been recognized to be a major target for lipophilic xenobiotics (biotransformation/detoxification and/or toxicity). This effect might be linked to the enhanced expression of CYP1A in the endothelia.

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