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Effects of experimental terbuthylazine exposure on the cells of *Dicentrarchus labrax* (L.)

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Abstract

The effects of acute exposure to the herbicide terbuthylazine (3.55, 5.01 and 7.08 mg l^{-1}) on the cells of farmed European sea bass, *Dicentrarchus labrax* L., were investigated by means of light and electron microscopy. In gills of treated fish, the number of chloride cells (CCs) and rodlet cells (RCs) increased significantly within 24 h and 48 h, respectively; the intestine showed the largest increase in RCs linked to treatment and exposure time. In kidney, 24 h exposure induced a significant increase in RCs and the number and global area of macrophage aggregates (MAs).

Treated fish displayed cellular and/or ultrastructural alterations in all the organs examined. In the gills necrosis, lamellar and cellular oedema, epithelial lifting, telangectasia, and fusion of secondary lamellae were encountered. The liver presented myelin-like figures, cytoplasmic rarefaction and acute cell swelling of hepatocytes. In both organs, the severity of damage was dose–dependent.

In RCs of gills, the intestine and kidney of exposed sea bass, high cytoplasmic vacuolization, myelin-like figures, cristolysis and varying degrees of rodlet degeneration were observed. Extensive rodlet expulsion occurred in the gut lumen. Exposure to terbuthylazine also affected the renal tubular epithelial cells, which exhibited 'blebs'. Damage to the intestinal epithelial cells was also observed.

Keywords: Rodlet cells; Chloride cells; Macrophage aggregates; *Dicentrarchus labrax*; Terbuthylazine

1. Introduction

Terbuthylazine (TBA, 2-chloro-4-tart-butylamino-6 ethylamino-s-triazine) a relatively widespread triazine herbicide with increasing agricultural significance, is replacing the better known and longer studied atrazine in several regions of the world (Steinberg et al., 1994). There are several studies on the toxicity of terbuthylazine to terrestrial animals: Lang et al. (1996, 1997) on mammals and Salminen et al. (1996) on soil fauna. Less is known about herbicide toxicity to aquatic organisms, including fish (Szarek et al., 2000). Marchini et al. (1988) and Steinberg et al. (1994) examined the effects of TBA on fish but their studies did not include acute effects.

Specific lesions in fish exposed to pollutants under laboratory conditions may be developed as biomarkers of exposure (Oliveira Ribeiro et al., 2002). Organs such as gills, liver and kidney are valuable as sensitive indicators of toxicant-induced injury. The gills are the first to be affected by many xenobiotics because they provide a very large interface between the external and internal environments of fish (Oliveira Ribeiro et al., 2002; Jiraungkoorskul et al., 2003). Fish liver is ascribed to play a pivotal role in xenobiotic metabolism and excretion; both primary modification of chemicals (e.g., oxidation, methylation, hydrolysis, etc.) and conjugation are reported to be involved in hepatic metabolic processes (Murty, 1986). Ultrastructural investigations on liver tissue provide valuable and simple measurements of degraded environmental conditions (Braunbeck et al., 1990; Biagianti-Risbourg and Bastide, 1995; Oulmi et al., 1995).

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Knowledge of sublethal effects of xenobiotic compounds on the fish kidney is very important for determination of animal health status (Jiraungkoorskul et al., 2003). Indeed, kidney is among the few organs that possess macrophage aggregates (MAs). MAs, also named as melano–macrophage centres, are focal accumulations of pigmented macrophages characteristically present in fish tissues, namely spleen, kidney, liver and ovary (Wolke, 1992). MAs are thought to be involved in foreign and effective material storage, iron recycling, cellular inflammatory reaction, antigen processing and exposure (Wolke, 1992). Their size and frequency increase in environmental stress conditions and have been suggested as reliable biomarkers for water quality (Couillard and Hodson, 1996; Manera et al., 2000; Agius and Roberts, 2003).

There are two types of cells exclusive to fish, chloride cells (CCs) and rodlet cells (RCs). Chloride cells, the main site of ion absorption and secretion, are found in adults only in gills and occupy a small fraction of the branchial epithelial surface area (Evans et al., 2005). The CCs are ovoid-shaped cells, and are characterized by their high densities of mitochondria and an extensive intracellular tubular system (Wilson and Laurent, 2002). The effect of dissolved cadmium on chloride cells was reported by Oronsaye and Brafield (1984), but, there is lack of data on the relationship between CCs and TBA. Rodlet cells occur in the tissues, particularly the epithelia, of many organs of virtually every fish species; the RCs have a distinctive cell cortex, round–oval nuclei and conspicuous inclusions called rodlets (Manera and Dezfuli, 2004). These cells presumably contribute to fish defences against pathogenic organisms (Leino, 1996; Dezfuli et al., 1998; Manera and Dezfuli, 2004).

The aim of the present study was to investigate the effects of acute exposure of TBA on gill, liver, kidney and intestine of farmed European sea bass, *Dicentrarchus labrax* (L.), by means of light and electron microscopy, with particular reference to the biometry and morphology of RCs, CCs and MAs.

2. Materials and methods

2.1. Experimental fish

Twenty-nine European sea bass (mean ± S.E., total length, LT, 135.17 ± 1.47 mm; mean ± S.E., mass, M, 24.24 ± 0.84 g) were obtained from a commercial fish farm "Valle Ca' Zulian" (Pila di Porto Tolle, RO, Italy) and were acclimated for 2 weeks in well-aerated 200 l aquaria, containing saltwater 22 at 19.4 ± 0.1 °C under a standard

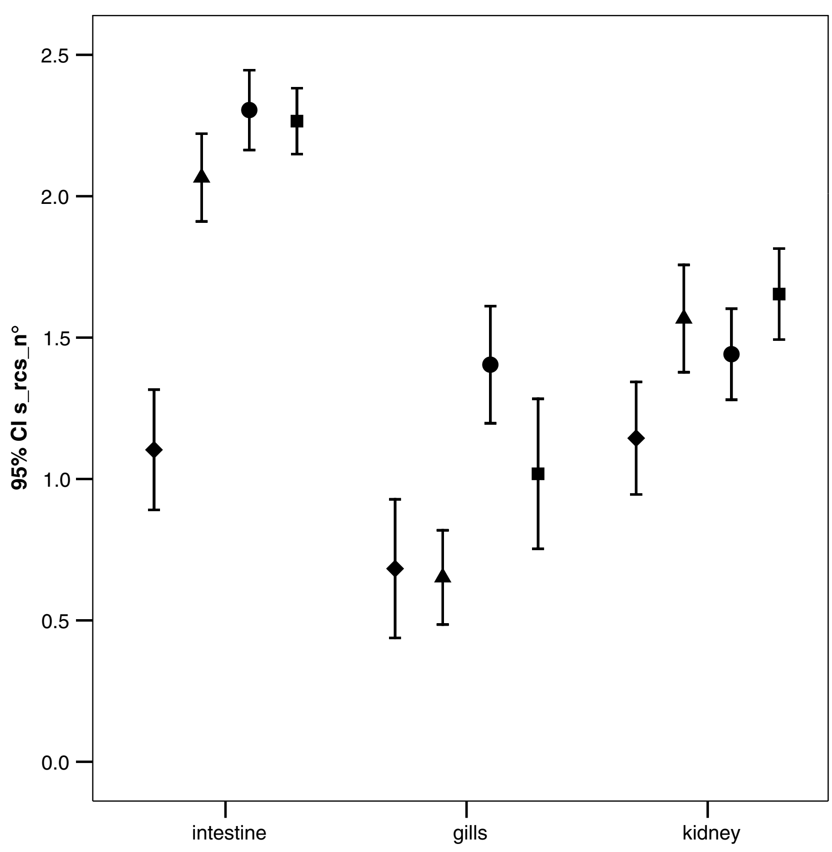

Fig. 1. 95% Confidence interval of the RCs number (projected to 25000 μm^2 and square root transformed) in relation to the organ and to the herbicide concentration (tank). ♦, ctr; ▲, tank 1; ●, tank 2; ■, tank 3.