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Immunotoxicological Effects of Environmental Contaminants in Teleost Fish Reared for Aquaculture

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1. Introduction

Contamination is one of the major problems associated with the environmental sciences. Many of the environmental pollutants affect to the different aquatic animals to certain degree depending on the toxic substance, concentration, self-life and animal behaviour and biology. Direct ingestion of environmental contaminants and bioaccumulation of toxic substances in bivalves, crustaceans, molluscs or fish for human supply is a serious task to consider in human nutrition. Furthermore, it is known that to provide the necessary proteins that need and will need the world's population must intensify efforts in production of both proteins of plant origin and animal origin. Among the latter is predicted that aquaculture will be one of the fields over the coming years will increase. In this regard, aquaculture is trying for some decades to compensate this negative balance for human consumption. Among the important issues to consider in the aquaculture business the impact of the environmental contaminants in the species produced for humans need to be controlled by the farmer. In this specific field, most of studies have evaluated the toxic effects in terms of fish viability or induction of tumors using different fish models. However, relevant fish species for aquaculture are less used in these experiments. Moreover, the impact of the environmental contaminants in the immune response of these fish, and consequently in the disease resistance, have received much less attention.

2. Overview of the teleost fish immune response

Fish are the first group of vertebrate animals with both innate and adaptive immune responses and are essential for proper understanding of the immune system and its evolution. The fish adaptive immune responses are less effective than in mammals because they are poikilotherms and completely dependent on the environmental temperature. Therefore, the importance of the innate immune response is more relevant, but not exclusive, in the fish disease resistance to pathogens. Overall, the mechanisms and molecules involved in the immune response are quite well conserved during the immune system evolution. However, there are major differences in terms of haematopoietic organs structure and function as well as in leucocyte distribution and function (Figure 1).

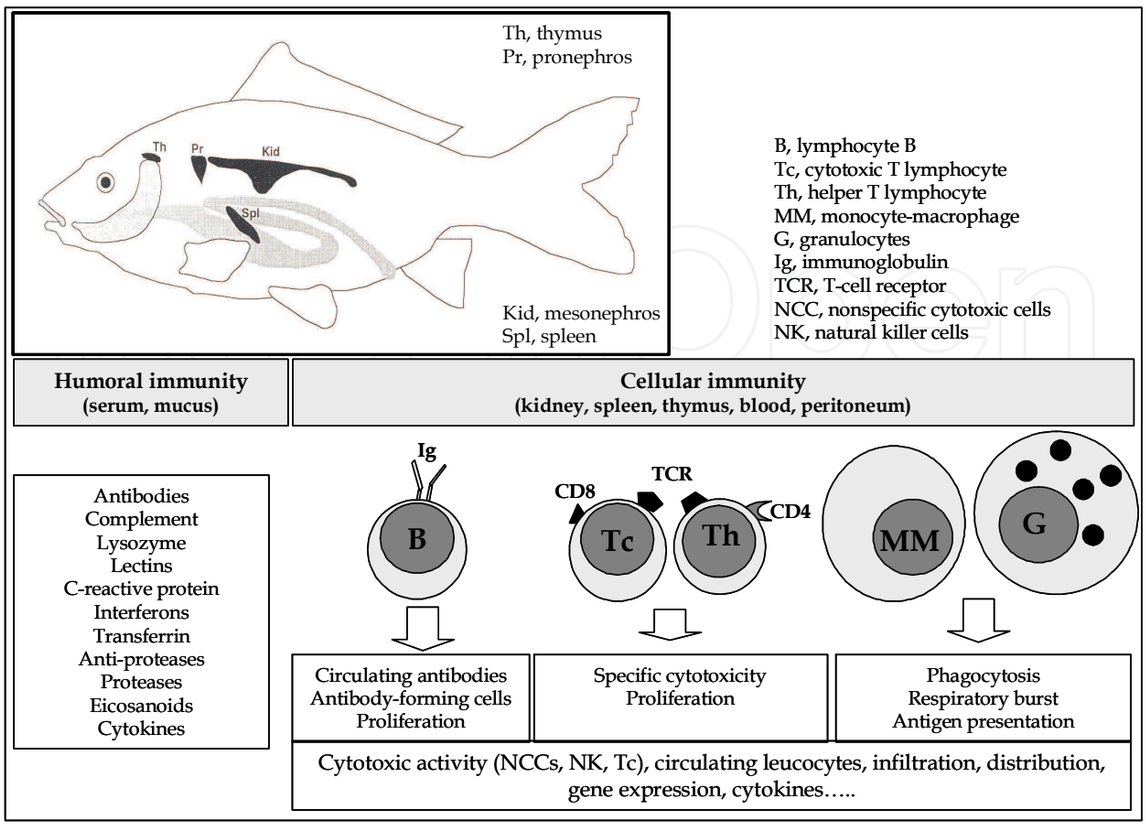

Fig. 1. Fish immune system organization (from Manning, 1998) and representative humoral and cellular immune responses used in immunotoxicological studies.

Firstly, the immune tissues are quite different since fish lack the bone marrow and lymphatic nodules (Manning, 1998). Thus, pronephros (anterior/head-kidney) is the main lympho-haematopoietic tissue in fish, whilst the posterior part or mesonephros is mainly excretory and the first site for development and B cells production. Thymus is the main tissue for T cells development and maturation whilst spleen is the main secondary lymphoid tissue in fish. Other important site for the immune response is the mucosal associated-lymphoid tissue (MALT), disperse in the skin, gill and gut. The leucocyte-types present in fish are quite similar between vertebrates but with some specific differences (Meseguer et al., 1994; Secombes et al., 2005; Miller et al., 1998; Rombout et al., 2005). Thus, fish lymphocytes are responsible for the production of antibodies (B cells) and the specific cellular immune response (T cells). B lymphocytes express and secrete immunoglobulin M (IgM), respond to the mitogen lipopolysaccharide (LPS) and constitute about 30% of the circulating lymphocytes. T lymphocytes are mainly detected in the thymus, express the T-cell receptor (TCR) and proliferate with the mitogens concanavalin A and phytohemagglutinin (PHA). They are responsible for the humoral and cellular immune response against T-dependent antigens by the different populations of CD4+ (Th or helper) and CD8+ (Tc or cytotoxic). Moreover, there are also subpopulations of fish lymphocytes lacking proper cell markers, Ig or TCR, and constitute the natural killer (NK) cells (Shen et al., 2002). By other side, monocyte-macrophages are the leucocytes displaying similar characteristics to both mammalian circulating monocytes and tissular macrophages.

Moreover, they are mainly localized in kidney and spleen where they concentrate the ingested particles and aggregate in melano-macrophage (MM) centres. Granulocytes can be divided in neutrophils, eosinophils and basophils according to their staining properties but in the case of fish the distribution and functions do not fit well with their mammalian counterparts. Monocyte-macrophages and some granulocytes form the phagocytic cells involved in phagocytosis of particulated antigens and in production of a machinery of lytic enzymes and the respiratory burst reaction, in which very toxic reactive oxygen species (ROS) and nitrogen intermediates (RNI) are produced. Finally, nonspecific cytotoxic cells (NCCs) are involved in the lysis of tumor cells, virus-infected cells and parasites in a similar fashion than the mammalian NK cells (Evans et al., 1984). However, they are a heterogeneous population (lymphocytes, granulocytes and/or monocyte-macrophages) and therefore some authors talk of nonspecific cytotoxic activity more than a cellular type or population (Cuesta et al., 1999).

The humoral immune response is a compilation of proteins and glycoproteins with defense functions found in the fish plasma and other body fluids such as mucus or sexual products (Kaattari & Piganelli, 1997). The complement system, in plasma and mucus, shows classical, alternative and lectin activation pathways with levels 5-10 times higher than in mammalian species with most of its components detected and characterized (Holland & Lambris, 2002). Direct lytic activity against bacteria, virus and parasites is the most relevant and studied function but it also acts as opsonin, chemotactic and neutralize endotoxins (Boshra & Sunyer, 2006). An important bacteriolytic enzyme is the lysozyme, mainly found in eggs, mucus, plasma and leucocytes (Magnadottir, 2006). There are also other innate immune factors such as acute phase proteins (C-reactive protein CRP), antimicrobial peptides, interferon (IFN), lectins, proteases, protease inhibitors or eicosanoids (Secombes, 1996; Aranishi, 1999; Bayne & Gerwick, 2001; Robertsen, 2006; Cammarata et al., 2007; Cuesta et al., 2008a). Finally, and the most interesting in fish, Ig are the major component of the adaptive humoral immune response. Fish were thought to have only one immunoglobulin isoform, the IgM. The fish IgM is tetrameric instead of pentameric as it occurs in mammals. Both membrane and soluble forms are observed by alternative processing of the mRNA (Wilson et al., 1990). Igs are found in the membrane of the B lymphocytes and this can be used to separate Ig+ and Ig- cells. The Ig functions are antigen neutralization, precipitation, opsonization and activation of the classical pathway of the complement system. In the last years, the presence of other Ig isoforms (IgD, IgZ or IgT) is throwing some light into the repertoire of fish immunoglobulins and their evolution in vertebrates (Hsu et al., 2006; Hikima et al., 2011).

3. Immunotoxicological effects of environmental contaminants

Environmental contaminants are widely distributed in aquatic environments. Although many of them are prohibited or restricted most of them are very persistent in the nature. Field and semi-field experiments are good to have suspicions about the contaminant presence but the setup of laboratory experiments with controlled parameters and precise and pure compounds are strictly necessary to understand the impact on fish immune response and their potential mechanisms. In line with the immunotoxicological studies in mammals, most of fish studies have evaluated the immune response (Figure 1) by measuring the macrophage functions (i.e. phagocytosis and ROS production), lymphoproliferative responses, host disease resistance, antibodies (circulating antibody

levels or antibody-forming cell numbers), number of circulating leucocytes, lymphoid organ cellularity and weights (Luebke et al., 1997; Bols et al., 2001).

3.1 Heavy metals

Heavy metals in aquatic environments are receiving more and more attention. Among the adverse effects, they can produce mortality, alteration of sexual maturation or immunodeficiency. Some heavy metals may transform into the persistent metallic compounds with higher toxicity, which can be bioaccumulated in the organisms and magnified in the food chain, thus threatening human health (Zhou et al., 2008).

Chromium (Cr) is a naturally occurring element found in rocks, animals, plants, and soil, predominantly in its insoluble trivalent form [Cr(III)]. Unfortunately, excessive industrialization and other anthropogenic activities have led to the global occurrence of soluble Cr (VI) in concentrations above permissible levels (Velma et al., 2009). The very scarce data *in vitro* have demonstrated that incubation of common carp (*Cyprinus carpio*) leucocytes with 2-200 μM hexavalent chromium showed depressed lymphocyte proliferation upon mitogen induction, as well as phagocytic functions, at much lower concentrations that produced cytotoxicity or cell death (Steinhagen et al., 2004). Moreover, neutrophils changed their morphology and reduced the amount of ROS and RNI. *In vivo* studies are more abundant and diverse and have also demonstrated the direct negative effects on fish leucocyte function and viability. Thus, tilapia (*Oreochromis mossambicus*) specimens exposed to sublethal doses of Cr-containing tannery effluents suffered a decreased antibody production, serum lysozyme activity and production of ROS and RNI by peripheral blood leucocytes (Sudhan & Michael, 1995; Prabakaran et al., 2007). Tilapia specimens exposed for 28 days with 0.5 and 5 mg Cr (VI)/L also decreased the disease resistance to bacterial infection and non-specific and specific immune response whilst the exposure with 0.05 mg Cr (VI)/L produced the opposite effects (Prabakaran et al., 2006). In another study, the spleen weight and the lymphocyte and leucocyte counts were significantly reduced by chronic exposure to Cr (III) and Cr (VI), producing the hexavalent form the greatest inhibitions (Arunkumar et al., 2000). In *Tilapia sparrmanii,* acute or chronic water exposures to potassium dichromate (0.098 mg/L) produced general haematological disorders including thrombocytopenia (Gey van Pittius et al., 1992). Moreover, and depending on the pH, fish showed leucocytosis and leucopenia at acidic and basic pH values, respectively (Wepener et al., 1992). In another more extensive study, the freshwater fish *Saccobranchus fossilis* were exposed for 28 days to 0.1-3.2 mg Cr (IV)/L and showed important changes in humoral and cellular immune responses and disease resistance (Khangarot et al., 1999). Concretely, they found a significant increase in the spleen size accompanied by an increment of spleenic lymphocytes. However, the number of plaque-forming cells and the phagocytic activity was reduced in spleen and head-kidney leucocytes. On the other hand, at blood level, the number of lymphocytes was decreased, but neutrophils and thrombocytes were increased, as well as the level of circulating antibodies and resistance to *Aeromonas hydrophila* infections. Otherwise, in plaice (*Pleuronectes platessa*), Cr-treatment increased the number of melano-macrophage centres but reduced their size (Kranz & Gercken, 1987). In the case of common carp and brown trout (*Salmo trutta* L.), 38 weeks of exposure with potassium dichromate diminished the primary and secondary humoral responses being the carp more susceptible to the heavy metal (O'Neill, 1981). In other kind of studies, the chromium exposure was carried out by dietary

intake and resembling the food chain bioaccumulation. In this case, rainbow trout (*Oncorhynchus mykiss*) fed diets containing 1540 to 4110 ppb Cr showed increased serum lysozyme activity as well as respiratory burst and phagocytic activity of macrophages in a dose- and time-dependent manner (Gatta et al., 2001).

Mercury (Hg), and derivatives such as methylmercury, are also important contaminants in aquatic environments inducing organ lesions, neurological, haematological and immunological disorders (Sweet & Zelikoff, 2001). First evidences, in rainbow trout, described a decrease in the number of mucous-producing cells and mucus production after exposure to mercury and methylmercury, which can be associated to impaired immunity (Lock & Overbeek, 1981). Afterwards, serum C-reactive protein was increased in freshwater murrel (*Chana punctatus*) (Ghosh & Bhaattacharya, 1992) and major carp (*Catla catla*) (Paul et al., 1998) by exposure to mercury. However, plasmatic lysozyme of plaice was decreased after exposure to sublethal doses of mercury (Fletcher, 1986). In sharp contrast, blue gourami (*Trichogaster trichopterus*) showed increased kidney and plasma lysozyme activity, but at the same time reduced the production of agglutinating specific antibodies after chronic exposure to 0.045 or 0.09 mg $Hg2^+$/L (Low & Sin, 1998). Further evidences have been obtained *in vitro*. Blue gourami lymphocytes incubated with mercury showed increased proliferation at low dosages, which was reversed by higher levels (>0.045 mg/L) (Low & Sin, 1998). In the marine fish *Sciaenops ocellatus*, mercury treatment (≤10 μM) produced a high-dose inhibition and a low-dose activation of leukocytes as determined by Ca-mobilization and tyrosyne phosphorilation of proteins (MacDougal et al., 1996). More recently, in the European sea bass (*Dicentracrchus labrax*), *in vitro* treatment with $HgCl_2$ induced apoptosis in head-kidney macrophages as well as reduced the ROS production and the benefits of macrophage-activating factors (MAF) (Sarmento et al., 2004).

Cadmium (Cd) is a nonessential heavy metal causing great toxicity. Among the first observations, Robohm (1986) found that Cd treatment inhibited the antibody levels in cunners (*Tautogolabrus adspersus*) and enhanced the antibody levels and chemotactic activity of peritoneal exudate cells in striped bass (*Morone saxatilis*). In rainbow trout exposed to 2 ppb of Cd, the same level found in some contaminated waters, the lysozyme activity was unaffected while the macrophage functions, phagocytosis and production of ROS, were significantly impaired (Zelikoff et al., 1995). These authors also demonstrated that Japanese medaka (*Oryzias latipes*) leucocytes increased their production of ROS and phagocytic functions without any change in many haematological parameters or antibody levels (Zelikoff et al., 1996). In the European sea bass, while *in vivo* exposure had a similar inhibitory effect on phagocytic functions the *in vitro* treatment produced an increment (Bennani et al., 1996). In the case of juvenile common carp experimentally infected with the blood parasite, *Sanguinicola inermis* (Trematoda: *Sanguinicolidae*) there were tissue changes and while the counts of neutrophils, eosinophils and thrombocytes increased in the thymus the number of neutrophils in the pronephros was reduced due to $Cd2^+$ treatment (0.1 mg/L) (Schuwerack et al., 2003). More recently, the Cd exposure has been related to the increase of melano-macrophage centres on several fish tissues (Suresh, 2009). In the hybrid tilapia (*Oreochromis niloticus* × *O. aureus*), the Cd exposure increased the lysozyme activity but greatly reduced the alternative complement activity (Wu et al., 2007).

Copper (Cu) is an essential nutrient but intensive use against fungal infections has shown to become a contaminant in some aquatic environments with immunosuppressive effects in general. *S. fossilis* fish exposed to sublethal Cu concentrations (0.056 to 0.32 mg/L) adversely

affected the humoral and cell-mediated immune system parameters (Khangarot et al. 1988; Khangarot & Tripathi, 1991) and reduced the fish resistance to *A. hydrophila* infections (Khangarot et al., 1999). European sea bass exposed to copper also showed an inhibited phagocytosis and ROS production both *in vivo* and *in vitro* (Bennani et al., 1996). Similar findings were also recorded in other experimental fish such as rainbow trout, goldfish (*Carassius auratus*), *Puntius gonionotus* or *Colossoma macropomum* (Hetrick et al. 1979; Knittel, 1981; Muhvich et al., 1995; Shariff et al., 2001; Lugo et al., 2006). Both *in vitro* and *in vivo* data have also demonstrated a decrease in the NCC activity and phagocytic responses in zebrafish (*Danio rerio*) (Rougier et al., 1994). Strikingly, further studies in common carp have shown increased humoral and cellular immune responses after Cu treatment (0.1-2.5 mg/L) (Dautremepuits et al., 2004a, 2004b). Very recently, Cu-incubation of trout macrophages up-regulated the expression of immune-relevant genes (interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNFα), serum amyloid A (SAA) and trout C-polysaccharide binding protein (TCPBP)) trying to understand the mechanisms and regulation of the immune response by heavy metals (Teles et al., 2011).

The immunotoxic impact of other heavy metals in fish has received less attention. Thus, zinc (Zn) was able to induce lymphoproliferation and NK-cell activity against tumor cells in common carp pronephros (Ghanmi et al., 1989, 1990). In zebrafish kidney leucocytes, Zn treatment increased the NCC activity and reduced the phagocytic responses both *in vitro* and *in vivo* (Rougier et al., 1994). $MnCl_2$ treatment also increased lymphoproliferation and NK cell activity in carp (Ghanmi et al., 1989, 1990). By contrast, Ni exposure reduced the lymphoproliferative response in medaka and deeper analysis led to the authors to suggest that the targets were the T-cells since neither the LPS-induced B-cell proliferation and antibody-forming cells were unaffected (Luebke et al., 1997). Arsenic (As) reduced the leucocyte respiratory burst, expression of some immune-relevant genes and disease resistance in zebrafish (Hermann & Kim, 2005; Nayak et al., 2007) in a similar fashion than in the catfish *Clarias batrachus* (Ghosh et al., 2007; Datta et al., 2009).

3.2 Polycyclic aromatic hydrocarbons (PAHs)

Aquatic environments are usually contaminated by PAHs derived form industry or petroleum, which produce external abnormalities, somatic mutations, cancer and immunodepression (Skupinska et al., 2004). The most toxic and the best studied are 7,12-dimethylbenz[a]anthracene (DMBA), benzo[a]pyrene (BaP) and 3-methylcholanthrene (3-MC) (Davila et al., 1995). In fish, as in mammals, the immunotoxicological effects are somehow contradictory and depend on the dose and time of exposition.

Liquid creosote (3-10 µl/L), containing PAHs, exposure of rainbow trout produced decreased respiratory burst of head-kidney leucocytes but increased phagocytic activity and percentage of Ig+ cells at short exposition times (Karrow et al., 2001). However, after 28 days, respiratory burst and phagocytic activity returned to control levels while the count of B cells remained decreased. The use of the heavily polluted Elizabeth River (Virginia, USA) has been extensively used for immunotoxicological evaluations. In the case of mummichogs (*Fundulus heteroclitus*), contamination produced a decrease in the levels of circulating IgM, both total and specific, and NCC activity while the plasmatic lysozyme was increased (Faisal et al., 1991a; Frederick et al., 2007). Moreover, lymphoid cells expressed higher levels of lysozyme and COX-2 (cyclooxygenase-2), the last as indicator of macrophage activation. Native fish (*Leiostomus xanthurus* and *Trinectes maculates*) from this river also showed lower

chemotactic and phagocytic activities that those kept in clean waters, and this suppression was reversed by maintenance in clean waters for several weeks (Weeks & Warinner, 1984; Weeks et al., 1986). Treatment of rainbow trout with 10-70% sewage plant effluents (containing PAHs among other contaminants) also reduced the number of circulating lymphocytes but increased their *in vitro* proliferation capacity (Hoeger et al., 2004). Strikingly, this effluent failed to alter any other immune functions such as respiratory burst, phagocytosis, lysozyme activity, leucocyte populations other than lymphocytes and *A. salmonicida*-specific IgM production. Intraperitoneal (ip) injection of diesel oil-based drilling mud extracts produced no effect on IgM levels and complement activity, suppression of serum lysozyme and elevated head-kidney lymphocyte proliferation in response to phytohemagglutinin (Tahir & Secombes, 1995). Petroleum-containing sediments also affected the immune response of flounder (*Pseudopleuronectes americanus*) since the number of melano-macrophage centres were diminished (Payne & Fancey, 1989). Deeper studies have evaluated the effects of heavy oil contamination (3.8 g/L for 3 days) in the Japanese flounder (*Paralichthys olivaceus*) using cDNA microarrays (Nakayama et al., 2008). They have found an alteration of expression in immune-related genes including down-regulation of immunoglobulin light chain, CD45, major histocompatibility complex class II antigens and macrophage colony-stimulating factor precursor, and up-regulation of interleukin-8 and lysozyme. Moreover, *in vitro* incubation with oils, pure and single PAHs, of European sea bass plasma produced significant changes in lysozyme and alternative complement activities indicating that these contaminants caused changes in the production of them by the leucocytes but also directly affects the enzymatic activity (Bado-Nilles et al., 2009). Similarly, PAHs mixture spiked-sediments (10 mg/kg dry wt) failed to change the serum lysozyme but reduced the ROS activity of kidney leucocytes of dab (*Limanda limanda*) (Hutchinson et al., 2003) while decreased the number of circulating lymphocytes (Khan, 2003). In the marine fish spot, *L. xanthurus*, exposed to PAH-contaminated sediments the T-lymphocyte proliferation was suppressed but the B-cell proliferation was greatly increased (Faisal et al., 1991b). Rainbow trout fed diets containing 0.66 or 7.82 μg PAH mixtures/g bw/day resulted in suppressed disease resistance against bacteria (Bravo et al., 2011).

Regarding the effects of single and pure PAHs, injections of DMBA (0.6 or 12.7 mg/kg body weight-bw) depressed the number of plaque-forming cells in head-kidney and spleen to T-independent antigens in Chinook salmon (*Oncorhynchus tshawytscha*) (Arkoosh et al., 1994). Injection of tilapia (*Oreochromis niloticus*) with DMBA (25 or 75 mg/kg bw) produced hipocellularity in spleen and head-kidney whilst phagocytosis and respiratory burst activity were not altered unless mortality occurred (Hart et al., 1998) similarly to the unaffected trout phagocytosis (Spitsbergen et al., 1986). By contrast, i.p. injection of 1-100 mg DMBA/kg bw to oyster toadfish (*Opsanus tau*) resulted in a peritoneal macrophage activity suppression in essentially a linear fashion, whereas NCC activity was virtually obliterated at all dosages (Seeley & Weeks-Perkins, 1997). BaP suppressed B cell immunity in tilapia at 15 mg/kg while increased at 25 mg/kg (Smith et al., 1999). Injection of 5-50 mg/kg also produced important histological changes in pronephros (reduction of lymphoid elements and augmentation of immune cells in apoptosis) and while the phagocytic activity was unaltered the respiratory burst was reduced (Holladay et al., 1998). In Japanese medaka, BaP injection (2–200 mg/kg bw) greatly reduced lymphocyte proliferation and number of antibody-forming cells (Carlson et al., 2002, 2004). In European sea bass, ip injection of BaP (20 mg/kg bw) significantly depressed the leucocyte phagocytosis and completely abrogated the ROS

production (Lemaire-Gony et al., 1995). In rainbow trout, BaP and BaA (benzo(a)anthracene) injection failed to significantly change the phagocytic activity (Walczak et al., 1987). Finally, 3-MC injection (40 mg/kg bw) into common carp increased the proliferative ability of resting circulating lymphocytes, rainbow but reduced their proliferative activity with the B- and T- mitogens, as well as the macrophage respiratory burst (Reynaud et al., 2002, 2003; Reynaud & Deschaux, 2005). Similarly, trout exposed to 3-MC increased the serum C-reactive protein 10-20-fold but not affected the IFN activity of leucocytes, measured as the resistance to bluetongue virus (Winkelhake et al., 1983).

3.3 Organochlorinated (OCs) contaminants

This group of contaminants comprises many of the most toxic and persistent compounds for aquatic environments such as DDT and relatives, lindane, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs or dioxins) or polychlorinated dibenzofurans (PCDFs or furans). These are common contaminants in water ecosystems and their residues still have toxic consequences including immunotoxicity, reproductive deficits, teratogenicity, endocrine toxicity and carcinogenicity (Ahlborg et al., 1994). Unfortunately, although OC levels detected in fish worldwide seems to be declining they still should be lowered to decrease risk for human consumers (Gómara et al., 2005).

DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane), and its metabolites DDE (p,p'-DDE and o,p-DDE), are among the most important OCs in agricultural and aquatic environments. However, though no information exists regarding the direct effect of DTT on fish immunology some data are available about its derivatives. Thus, o,p-DDE treatment (10 ppm) of Chinook salmon, at fertilisation and hatch stages, failed to affect viability and growth but these fish still suffered immunosuppression one year later as consequence of the contamination (Milston et al., 2003). *In vitro*, p,p'-DDE (0-15 mg/L) produced a reduction in lymphocyte-granulocyte viability, by increasing the percentage of apoptotic cells, and in lymphocyte proliferation, in both spleen and head-kidney that was also observed *in vivo* (59 ppm exposure) (Misumi et al., 2005). By contrast, marine gilthead seabream leucocytes incubated with p,p'-DDE (5 ng to 50 mg/ml) failed to change their viability and main innate cellular immune parameters but up-regulated the expression of some immune genes (IL-1beta, TNFalpha, MHCIalpha, MHCIIalpha, Mx, TLR9, IgM and TCRalpha) indicating only effects at genetic level but not in function (Cuesta et al., 2008b).

Lindane (gamma-hexachlorocyclohexane) is another OC that have focused much of the attention. Dietary intake of lindane (10-1000 ppm) failed to affect the spleen weight, serum and mucus antibody levels and phagocytosis in the common carp though most of the tissues reflected great contamination (Cossarini-Dunier, 1987; Cossarini-Dunier et al., 1987). In rainbow trout, intraperitoneal injection of lindane (10-100 mg/kg bw) greatly depressed the number of antibody-secreting cells, serum lysozyme levels, respiratory burst activity and myeloperoxidase (contributes together with ROS and RNI to pathogen killing), proliferating capacity of B cells, but not of T cells, and its percentage in the head-kidney but at the same time increased the plasmatic ceruloplasmin, an acute phase protein (Dunier & Siwicki, 1994; Dunier et al., 1994). The same group also demonstrated that oral administration of lindane (1 mg/kg) for 30 days significantly decreased the respiratory burst activity of head-kidney leucocytes but unaffected the lymphocyte proliferation and number of circulating B lymphocytes in a similar way to the previous data in carp (Cossarini-Dunier et al., 1987; Dunier et al., 1994). Moreover, they have also demonstrated that these negative effects can be reversed by the *in vitro* addition of nitrogranulogen (Siwicki & Dunier, 1994) or dietary

intake of vitamin C (Dunier et al., 1995). Lindane bath of Nile tilapia also reduced the counts of circulating leucocytes, phagocytic activity and antibody levels (Khalaf-Allah, 1999). *In vitro*, lindane (2.5-100 μM) treatment was able to increase ROS production in rainbow trout head-kidney phagocytes and MAF (macrophage activating factors) production by peripheral blood leucocytes, in both cases depending on the dose and with contradictory results (Betoulle et al., 2000; Duchiron et al., 2002a, 2002b). These studies also demonstrated that low lindane concentrations increase the cytoplasmatic cAMP but high doses increase the intracellular Ca2+, and these two factors contribute to the dual effects of induction/reduction of the leucocyte immune functions produced by lindane treatment in leucocytes (Betoulle et al., 2000; Duchiron et al., 2002a, 2002b). In gilthead seabream, head-kidney leucocyte incubation (5 ng to 50 μg/ml) with lindane failed to significantly change the leucocyte viability (by necrosis and apoptosis) and innate cellular immune functions (phagocytosis, respiratory burst and cell-mediated cytotoxicity) but strikingly increased the expression of many immune-related genes (IL-1beta, TNFalpha, MHCIalpha, MHCIIalpha, Mx, TLR9, IgML and TCRalpha) (Cuesta et al., 2008b).

PCBs, with theoretically 209 distinct congeners, may be divided into those with coplanar geometry, the most toxic as they bind and activate AhR (hydrocarbon receptors) and CYP1A (cytochrome P4501A) expression, while noncoplanar congeners can interfere with AhR signalling but also affect cells via AhR-independent pathways (Duffy & Zelikoff, 2006). Immunotoxicological effects of PCB mixtures, such as Arochlor, have been evaluated in fish. Thus, Aroclor 1254 depressed plaque-forming cells in head-kidney and spleen to a T-independent antigen in Chinook salmon after ip injection (Arkoosh et al., 1994). However, it failed to modulate the innate disease resistance and antibody production by oral administration of environmental doses in the same fish (Powell et al., 2003). In Artic charr (*Salvelinus alpinus*), diets containing 100 mg Aroclor 1254/kg diet resulted in increased disease susceptibility to furunculosis (Maule et al., 2005). In Atlantic salmon (*Salmo salar*), by contrast, water exposure with 1-10 μg/L produced increased T lymphocyte proliferation at short and long-term (Iwanowicz et al., 2005). In rainbow trout, while the C-reactive protein levels in serum were increased the leucocyte IFN and NCC activities were unchanged (Winkelhake et al., 1983; Cleland & Sonstegard, 1987). Another study using Aroclor 1248, in the brown bullhead (*Ameiurus nebulosus*), have provoked a decrease in the bactericidal activity and antibody titers (Iwanowicz et al., 2009). PCBs mixture (Aroclor 1242, 1254 and 1260) failed to modify lysozyme and ROS activity in *L. limanda* (Hutchinson et al., 2003). Regarding the effects of pure PCBs, the congener 126 has been the most studied. PCB 126 injection (0.01-1 μg/g bw) to Japanese medaka reduced the antibody forming cell numbers (Duffy et al., 2002) but either reduced or increased the phagocyte-mediated ROS production at 3 or 14 days post-treatment, respectively (Duffy et al., 2003). Dietary administration (100 ng/g bw) to European eel (*Anguilla anguilla*) completely abrogated the production of specific antibodies against a parasite (Sures & Knopf, 2004). PCB 126 also produced a reduction of phagocyte respiratory burst and NCC activities in channel catfish (*Ictalurus punctatus*) at (0.01–1 mg/kg bw) (Rice & Schlenk, 1995). In the bluegill sunfish (*Lepomis macrochirus*), the coplanar PCB 126 (0.01 or 1.0 μg/g bw) also slightly affected the B-lymphocyte proliferation while the noncoplanar PCB 153 (5.0 or 50.0 μg/g bw) significantly reduced the phagocyte-mediated respiratory burst activity and the B- and T- lymphocyte proliferation (Duffy & Zelikoff, 2006). Strikingly, short incubation of rainbow trout head-kidney leucocytes with PCB 126 (1 μM) increased the expression of IL-1β gene and failed to abrogate the LPS effects on gene regulation (Quabius et al., 2005). The PCB Clophen A50 (0.4-2 μg/egg) injected into the eggs of rainbow trout with

pathogenic bacteria resulted in a higher disease resistance than those injected with the bacteria suggesting a direct effect on the immune response (Ekman et al., 2004).

Chlorinated dioxins, as typified by the most potent isomer TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), are also very toxic for fish. Injection of 0.1-10 µg TCDD/kg bw to rainbow trout resulted in very little changes in humoral and cellular immune responses (Spitsbergen et al., 1986). However, while the C-reactive protein levels in serum were increased the leucocyte production of IFN was unchanged (Winkelhake et al., 1983). In common carp, TCDD injection produced histological alterations including increase of melano-macrophage centres and reduction of lymphocyte numbers (van der Weiden et al., 1994). Further studies have also evaluated fish tissue alterations and CYP1A staining patterns have been described in European flounder (*Platichthys flesus*) and gilthead seabream (Grinwis et al., 2000; Ortiz-Delgado & Sarasquete, 2004).

Some studies have also evaluated the immunotoxicological effect of other OCs. In the case of furans (PCDF), most authors have focused on other fish toxicity tests rather than in immunotoxicology. Endosulfan exposure produces developmental and neurological disorders and acts as endocrine disruptor. Rainbow trout leucocyte treatment with endosulfan inhibited the lymphoproliferative activity where the B-cells were more sensitive than the T lymphocytes (O'Halloran et al., 1996). In another study, crimson-spotted rainbowfish (*Melanotaenia fluviatilis*), golden perch (*Macquaria ambigua*) and Murray cod (*Maccullochella peelii*), but not silver perch (*Bidyanus bidyanus*), leucocytes showed decreased phagocytosis after endosulfan treatment (10 mg/L) (Harford et al., 2005). *In vivo* treatment of Nile tilapia for 96 h at 7 ppb produced an increased phagocytosis and ROS production by spleen leucocytes, IgM levels and production of IL-2-like, but at the same time reduced the spleen viability and relative weight (Tellez-Bañuelos et al., 2009, 2010).

3.4 Organophosphorous pesticides (OPs)

OPs are insecticides used world-wide as an alternative to the persistent and more bioaccumulative OCs. They are potent neurotoxic and immunotoxic since are irreversible acetylcholinesterase inhibitors (Galloway and Handy, 2003). Malathion exposure (0.2-0.8 mg/L) of medaka resulted in reduced number of antibody-forming cells but unchanged circulating leucocyte numbers and T-cell proliferation (Beaman et al., 1999). Vaccinated Nile tilapia exposed to malathion or diazinon presented lower blood cell counts, phagocytosis and antibody levels than those unexposed (Khalaf-Allah, 1999). Diazinon exposure of bluegill had biphasic effects with immune response increases at low concentrations and depressions at high dosages (Dutta et al., 1997). In Nile tilapia, Girón-Pérez et al., (2007, 2008, 2009) have showed that diazinon altered the spleen counts and lymphocyte proliferation, serum IgM and lysozyme levels, phagocytic activity and respiratory burst depending on the exposure dose and time. Chlorpyrifos displayed little immunotoxicity, although there was a dose-dependent reduction in Murray cod lymphocytes (Harford et al., 2005). Nile tilapia exposed to the LC50 failed to change blood parameters but the phagocytic activity was significantly reduced (Girón-Pérez et al., 2006). Chlorpyrifos exposure produced an up-regulation of hsp60, hsp70 and hsp90 genes, related to the cellular stress response in Chinook salmon. Moreover, the cytokine (IL-1b, TGF-beta, Mx and insulin growth factor (IGF)-I) gene expression was unaltered or down-regulated but not affected the virus susceptibility of the fish (Eder et al., 2008, 2009). Dichlorvos and trichlorfon insecticides have been used in aquaculture against ectoparasites in the past. Trichlorfon exposure decreased the serum lysozyme, lymphocyte proliferation, respiratory burst and

phagocytosis of common carp leucocytes (Siwicki et al., 1990; Dunier et al., 1991) but unchanged the production of specific antibodies (Cossarini-Dunier et al., 1990). Water exposure to dichlorvos failed to change the specific IgM production but altered other serum innate immune parameters (Dunier et al., 1991). Edifenphos and glyphosate exposure reduced the lymphocyte proliferation, antibody-producing cells and circulating IgM levels in Nile tilapia (el-Gendy et al., 1998). Glyphosate exposure of silver catfish (*Rhamdia quelen*) resulted in decreased phagocytosis and resistance to disease (Kreutz et al., 2010).

3.5 Pyrethorids

Pyrethroids are extensively used insecticides since they are very stable and produce low mammalian toxicity but this is very high for aquatic animals (Bradbury & Coats, 1989). Among them, deltamethrin injection to *Ancistrus multispinis* increased peritoneal leucocyte numbers and production of RNI by macrophages (Pimpão et al., 2008). Short exposure to deltamethrin (30 min., 1-4 μg/L) of rainbow trout resulted in decreased serum lysozyme and IgM levels (Siwicki et al., 2010). Water exposure of rohu (*Labeo rohita*) to alpha-permethrin produced a reduction in lysozyme activity and resistance to bacteria (Nayak et al., 2004). Rainbow trout exposure to cypermethrin failed to alter any of the immune parameters (Shelley et al., 2009). Esfenvalerate exposure produced an up-regulation of hsp60, hsp70 and hsp90 stress genes, down- or non-regulated cytokines and unaffected the virus susceptibility of the Chinook salmon (Eder et al., 2008, 2009). Using microarrays, delta smelt (*Hypomesus transpacificus*) exposure to esfenvalerate produced alterations in the expression of genes associated with immune responses, along with apoptosis, redox, osmotic stress, detoxification, growth and development (Connon et al., 2009).

3.6 Organotins

Organotin compounds or stannanes are chemical compounds based on tin (Sn) with hydrocarbon substituents showing different toxic effects. TBT (triorganotins) is specially important since it has been widely used as marine anti-biofouling agent. Injection of 0.01-1 mg TBT (tributyltin)/kg bw of channel catfish altered leucocyte counts, NCC, phagocytic and respiratory burst activities, production of specifc antibodies and number of antibody-produceing cells (Rice et al., 1995). TBT treatment signinficantly reduced the lymphocyte numbers in spleen, the thymus volume and the leucocyte NCC activity in European flounder (*P. flesus*) (Grinwis et al., 2000). In rainbow trout, *in vitro* incubation with 2.5-500 ppb TBT and DBT (dibutyltin) reduced the lymphoproliferation activity in pronephros and spleen but failed to affect the NCC activity showing DBT higher toxicity than TBT (O'Halloran et al., 1998). *In vitro* incubation of several Australian fish head-kidney leucocytes with TBT or DBT depressed the phagocytic activity and reduced the numbers of lymphocytes and granulocytes (Harford et al., 2005).

3.7 Other chemicals

Herbicides are still widely used and end in aquatic environments producing many physiological alterations but little studies have focused on their immunotoxicological effects in fish. Herbicides mixture, containing atrazine, simazine, diuron and isoproturon, exposition of goldfish increased spleen and head-kidney ROS production and serum lysozyme but reduced the specific antibodies and resistance to bacterial infections (Fatima et al., 2007). Atrazine exposure of silver catfish resulted in decreased phagocytosis and

resistance to disease (Kreutz et al., 2010) whilst failed to do so in common carp (Cossarini-Dunier et al., 1987; Cossarini-Dunier & Hattenberger, 1988). Phenols are another group of toxics. Phenol, pyrocatechol and hydroquinone decreased the cell-mediated cytotoxic activity of spleen lymphocytes in common carp (Taysse et al., 1995), pentachlorophenol reduced macrophage production of cytokines in goldfish (Chen et al., 2005) but activated phagocytosis and unaltered other immune functions and disease resistance in rainbow trout (Shelley et al., 2009). Endocrine disrupting chemicals produce population decline, an increasing incidence of cancer, inhibition of reproductive function, and developing disruption of the immune and nervous systems. However, there are very limited data concerning the role of endocrine disrupting chemicals on aquatic organism, including the fish immune response. Zebrafish embryos exposed for 3 days to 17α-ethynyestradiol, permethrin, atrazine and nonylphenol (0.1-12.5 μg/L) altered the expression of immune-relevant genes (TNFα, IFN, IL-1β, IL-8, CXCL-Clc, CC-chemokines, iNOS, etc.) indicating their single and combined effects upon fish immune response (Jin et al., 2010).

4. Conclusion

As described above, most of the aquatic contaminants have shown either activations or suppressions in the immune response that greatly varied with the exposure route, time, dosage and fish specie with many similarities to immunotoxicological data in mammals. Therefore, although researchers do not have precise contamination biomarkers in aquatic animals some conclusions may rise: i) heavy metals contamination is usually followed by metallothionein overexpression (Misra et al., 1989; Hansen et al., 2007; Costa et al., 2009); ii) OCs exposure is concomitant to decreased number and size of melano-macrophage centres (Schmitt et al., 2005; Hinck et al., 2007); iii) immunotoxicological effects due to PHAs and PCBs are generally parallel to an increase in the activity of the detoxification proteins cytochrome P4501A (CYP1A), through the involvement of aryl hydrocarbon receptors (AhR), and/or EROD (ethoxyresorufin-O-deethylase) (Lee & Anderson, 2005; Duffy & Zelikoff, 2006; Reynaud & Deschaux, 2006; Bravo et al., 2011); and iv) further and deeper studies are needed to understand the real effect of environmental contaminants in fish and the mechanisms for toxicity. Moreover, looking at the fish species studied and those subjected to aquaculture, most of the data come from wild fish, salmonids and cyprinids but other major species are almost ignored. Even further, most of the studies focus on freshwater fish and very little is known for marine species. These aspects should be covered by future works to progress in the understanding of the immunotoxicological effects and mechanisms and the consequences and risks they may have on human consumers as consequence of the bioaccumulation.

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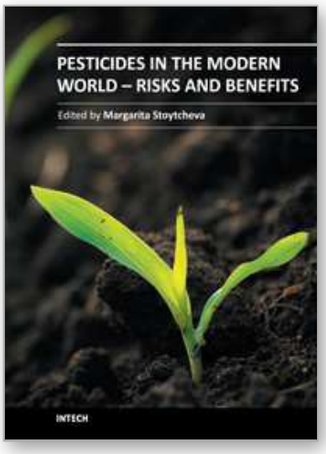

Pesticides in the Modern World - Risks and Benefits

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This book is a compilation of 29 chapters focused on: pesticides and food production, environmental effects of pesticides, and pesticides mobility, transport and fate. The first book section addresses the benefits of the pest control for crop protection and food supply increasing, and the associated risks of food contamination. The second book section is dedicated to the effects of pesticides on the non-target organisms and the environment such as: effects involving pollinators, effects on nutrient cycling in ecosystems, effects on soil erosion, structure and fertility, effects on water quality, and pesticides resistance development. The third book section furnishes numerous data contributing to the better understanding of the pesticides mobility, transport and fate. The addressed in this book issues should attract the public concern to support rational decisions to pesticides use.

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