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Aqueous exposure to Aroclor 1254 modulates the mitogenic response of Atlantic salmon anterior kidney T-cells: Indications of short- and long-term immunomodulation

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Abstract

Polychlorinated biphenyls (PCBs) exist as persistent organic pollutants in numerous river systems in the United States. Unfortunately, some of these rivers are sites of active Atlantic salmon restoration programs, and polychlorinated biphenyls have been implicated as ancillary factors contributing to failed salmon restoration. Here, we investigate the immediate and chronic effects of intermediate duration aqueous PCB exposure (1 or 10 $\mu g\,L^{-1}$ Aroclor 1254) on the mitogen-stimulated lymphoproliferative response of Atlantic salmon anterior kidney leukocytes (AKLs). A short-term study was designed to examine immunomodulation in Atlantic salmon smolts immediately following 21 days of aqueous exposure, while a long-term study evaluated chronic impacts in the mitogen response in parr 15 months post-exposure as larvae. The proliferative response of AKLs to the mitogens concanavalin A (CON A), phytohemaglutinnin-P (PHA-P), pokeweed mitogen (PWM), and lipopolysaccharide were used as an indice of immunomodulation. The proliferative response to the T-cell mitogens CON A and PHA-P was significantly increased in the 10 $\mu g\,L^{-1}$ group ($n = 10$; $P = 0.043$ and 0.002, respectively) immediately following exposure of smolts. Additionally, The PHA-P response was significantly increased in the 1 $\mu g\,L^{-1}$ exposure group ($n = 10$, $P = 0.036$). In fish treated as larvae and tested 15 months later, the PHA-P sensitive populations exhibited elevated proliferation in the 1 and 10 $\mu g\,L^{-1}$ groups ($n = 12$, $P < 0.04$) relative to the vehicle control while the PWM response was significantly increased ($n = 12$, $P = 0.036$) only in the 10 $\mu g\,L^{-1}$ treated groups. These results demonstrate an immunomodulatory effect of PCBs on T-cell mitogen sensitive populations of lymphocytes in Atlantic salmon as well as long-term immunomodulation in PHA-P and PWM sensitive populations.

Keywords: Atlantic salmon; *Salmo salar*; Fish; Aroclor; PCB; AhR; Mitogenesis; T-cell; Immunomodulation

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1. Introduction

Precipitous declines in Atlantic salmon (*Salmo salar*) and other anadromous fishes were observed in New England rivers of the North Eastern United States following the Industrial Revolution (ca. 1850–1910). Population decreases were so profound that these fish were extirpated from southern New England. Substantial management efforts during the last century focused on the restoration of these highly valued populations. Current restoration programs involve stocking hatchery-reared larvae or smolts and the operation of fish elevators and ladders at hydroelectric dams that occlude up stream passage in migratory waters (Parrish et al., 1998). Despite these active restoration programs, Atlantic salmon populations have failed to recover to historical levels. Genetically distinct populations of Atlantic salmon in eastern Maine rivers that began to decline in the 1970s have recently been listed as endangered (King et al., 2001). Effluent from paper mills, agricultural and industrial run-off, and other point and non-point sources of pollution may affect the overall health of resident and migrating salmon. Aquatic contaminants including polychlorinated biphenyls (PCBs) have been implicated as likely contributing factors to failed salmon restoration (Waring and Moore, 2003; Arsenault et al., 2004; Lerner et al., unpublished data).

Polychlorinated biphenyls are highly stable anthropogenic chemicals historically manufactured in mixtures called Aroclors. Due to their widespread use and improper disposal in recent decades, PCBs now exist as persistent organic pollutants (POPs) in numerous river systems in the United States. These include New England rivers with active salmon restoration programs such as the Dennys River, Maine and the Millers River, Massachusetts that are annually stocked with Atlantic salmon larvae and smolts. Exposure to PCBs has been reported to modulate both cell-mediated and humoral responses in laboratory animals and humans (Tryphonas et al., 1991; Harper et al., 1993; Luster and Rosenthal, 1993; Mayura et al., 1993; Tryphonas, 1994; Yoo et al., 1997; Regala et al., 2001; Smits et al., 2002; Sures and Knopf, 2004). Additionally, immunomodulation associated with PCB exposure has been observed in numerous wild aquatic animals including harbor seals (Ross et al., 1996), piscivorous birds (Grasman et al., 1996), and Chinook salmon (Arkoosh et al., 1994).

While the effects of PCB exposure on immune function have been investigated in a few species of salmonids, much of this research has specifically focused on functional aspects of B-cell populations (Arkoosh et al., 1994; Stehr et al., 2000). Other work has considered the aspects of disease susceptibility (Powell et al., 2003; Jacobson et al., 2003) or the sensitivity of T-cells to PCB exposure during in vitro culture (Miller et al., 2002; Sweet et al., 1998). Assays that quantify the proliferative responses of leukocytes to lectins including concanavalin A (CON A), phytohemagglutinin-P (PHA-P), pokeweed mitogen (PWM), and lipopolysaccharide (LPS) are commonly used to gage functional immune status (Gehrs et al., 1997; Ottinger and Kaattari, 1998). They are useful measures of lymphocyte responsiveness as the mitogens employed in these assays are polyclonal stimulators of specific lymphocyte populations. This approach permits the evaluation of treatment effects on specific lymphocyte populations. The mitogens CON A and PHA-P stimulate T-cell populations, LPS stimulates B-cells, while PWM stimulates both B and T-cell populations (Kehrer et al., 1998). These mitogenesis assays have not been employed to measure immune status in salmonids exposed to PCBs. Additionally, few studies have investigated both the short- and long-term effects of PCB exposure in salmonids. In the present study, we investigated the short- and long-term effects of intermediate duration aqueous Aroclor 1254 exposure on the mitogen-stimulated leukoproliferative response in Atlantic salmon exposed to environmentally relevant concentrations of this PCB mixture.

2. Methods

2.1. Fish maintenance

Atlantic salmon were obtained from the White River National Fish Hatchery (Bethel, VT, USA) and transferred to the Conte Anadromous Fish Research Center (Turners Falls, MA, USA) as parr in October 2002 or eggs in February 2003. Parr were reared in 1.6 m diameter tanks with Connecticut River water at a flow rate of 6–8 L min^{-1} under natural photoperiod at $10 \pm 1\,^{\circ}C$. For 6 weeks through December to January, water temperatures were reduced to $4 \pm 1\,^{\circ}C$ to provide parr with a “winter” event. Animals were

fed to satiation twice daily until PCB exposure as smolts.

Eggs and fry were maintained in covered egg trays under 24 h darkness until yolk absorption, then under natural photoperiod with a flow rate of 1–2 L min^{-1} using dechlorinated city water at $7 \pm 1\,^{\circ}C$. After PCB treatment and upon absorption of the yolk sac, larvae were placed in 1 m diameter tanks, provided fresh water at a flow rate of 2–4 L min^{-1}, and fed to satiation. Temperatures were allowed to fluctuate with the ambient conditions of the rearing water, which is provided directly from the Connecticut River. These animals were maintained in this manner through the Spring of 2004 at which time the fish that did not smolt were separated and held until June 2004 for tissue sampling as described below.

2.2. PCB exposure

To investigate the short- and long-term effects of intermediate duration, aqueous Aroclor 1254 exposure on the mitogen-stimulated leukoproliferative response in Atlantic salmon, animals were exposed to a low or high dose of the PCB mixture as either yolk-sac larvae or smolts in two separate experiments. During March 2003, 21 days post-hatch (dph) Atlantic salmon yolk-sac larvae were exposed to 1 or 10 $\mu g\,L^{-1}$ of Aroclor 1254 (Lot # 124–191-B; Accustandard, New Haven, CT) or vehicle control (0.0001% methanol) in replicate tanks for 3 weeks. Concentrated solutions were delivered via a peristaltic pump (Cole-Parmer Instrument Co., Vernon Hills, IL, USA) and mixed in head tanks with dechlorinated city water to deliver the desired concentrations of chemicals on a continuous basis and under flow through conditions. All tank water was passed through an activated carbon canister (approximately, 10 kg of carbon) prior to discharge. The ratio of carbon to water flow and PCB levels was calculated to give a five-fold higher removal rate than necessary to remove all PCB's. During the exposure water temperature was maintained at $7 \pm 1\,^{\circ}C$. After exposure animals were held for 15 months as described above until sampling. Ambient water temperatures were $17 \pm 1\,^{\circ}C$ on the sampling date.

During April 2003, replicate tanks of Atlantic salmon smolts were aqueously exposed to 1 or 10 $\mu g\,L^{-1}$ of Aroclor 1254 or vehicle control (0.0001% methanol) in their rearing tanks for 3 weeks. Chemical delivery was the same as described above for larvae. During the exposure water temperature was maintained at $10 \pm 1\,^{\circ}C$ and animals were fed a maintenance diet once every other day. Food was withheld 24 h prior to sampling. Animals were sampled immediately following exposure as described below.

Anterior kidneys were harvested from five smolts from each replicate tank for each treatment during May 2003 for the short-term study and six parr per replicate tank in June 2004 in the long-term study. All fish were anesthetized in buffered 50 ppm tricaine methanesulfonate (pH 7.0) and bled via the caudal vessels with heparinized syringes within 3 min of capture. Fish carcasses were pooled by treatment group, frozen at $-80\,^{\circ}C$ and shipped to Spectrum Analytical Inc. (Agawam, MA, USA) for PCB body burden analysis. Whole fish body burden concentrations of Aroclor 1254 were determined using gas chromatography (EPA method # SW846 8082) after ultrasonic extraction (EPA method # SW846 3550B).

2.3. Leukocyte isolation

Unless otherwise noted, all media components were obtained from Sigma Chemical Company (St. Louis, MO).

Anterior leukocytes were isolated and processed as described by Harms et al. (2000). Briefly, anterior kidney tissue was aseptically removed from Atlantic salmon and placed into L-15 medium supplemented with 2% fetal bovine serum (FBS; Atlanta Biologicals, Norcross, GA) 100 U mL^{-1} penicillin, 100 $\mu g\,mL^{-1}$ streptomycin, and 10 U mL^{-1} sodium heparin (Processing medium, PM). Following the fragmentation of the kidney tissue into a single-cell suspension and subsequent separation from settled tissue fragments, cells were pelleted by centrifugation at $500 \times g$ for 10 min at $4\,^{\circ}C$. Cells were washed by suspension in PM followed by centrifugation as above, suspended in PM, and then layered on 51% Percoll in Hanks Balanced Salt Solution (HBSS) without phenol red, pH 7.2. The cells on Percoll were centrifuged at $500 \times g$ for 40 min at $4\,^{\circ}C$ and the leukocyte fraction was removed from the medium/Percoll interface. Leukocytes were pelleted and washed as described above and then suspended in PM for counting. The number of viable leukocytes isolated from each fish was determined by

trypan blue exclusion (0.1% trypan blue in PM), and the cells were pelleted as described above. Leukocytes were suspended at 2×10^7 viable cells mL^{-1} in L-15 supplemented with 5% FBS, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (Culture medium, CM) and loaded into 96-well tissue-culture plates at 1×10^6 cells $well^{-1}$. All tissues and cell suspensions were kept cold in an ice bath during processing.

2.4. Mitogen stimulation

Anterior kidney leukocytes (AKLs) were plated such that each 96-well plate contained cells from all treatment groups to account for possible plate effect variability. A volume of 50 μL mL^{-1} of CM with or without mitogen (control) was added to the wells immediately after cells were plated. Mitogen concentrations used to stimulate the isolated leukocytes were as follows: 10 μg mL^{-1} CON A, 10 μg mL^{-1} PHA-P, 50 μg mL^{-1} PWM, or 100 μg mL^{-1} LPS from *Escherichia coli* 0111:B4. Mitogen-treated and control wells were replicated in triplicate and plating was performed with the plates on ice. Following plating, leukocytes were incubated in humidified chambers at 10 °C under atmospheric conditions. The mitogenic response was measured on the fourth day of incubation post-mitogen stimulation based on pre-determined optimum mitogen response kinetics of Atlantic salmon AKLs for CON A, PHA-P, and PWM in our laboratory (Fig. 1). The mitogenic response was measured as described below.

2.5. Mitogenesis assay

The mitogen-induced proliferative response was evaluated using the BrdU-based ELISA at room temperature as described by Gauthier et al. (2003) with minor modifications. Briefly, 24 h prior to the ELISA, leukocytes were treated with 25 μL $well^{-1}$ of sterile-filtered 65 μM BrdU in L-15 and incubated for an additional 24 h. Following incubation with BrdU, cells were washed with 100 μL $well^{-1}$ DPBS and fixed for 15 min with 1% paraformaldehyde in DPBS (pH 7.2). Cells were washed three times as above and the cell membranes were porated by a 30 s exposure to 50 μL $well^{-1}$ of 0.01% polyoxyethylene-sorbitan monolaurate (Tween-20) in DPBS. Cells were

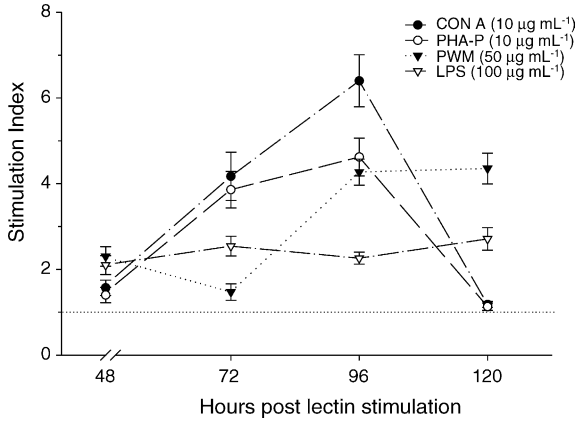

Fig. 1. Temporal mitogen response of Atlantic salmon AKLs. Leukocytes were isolated from >2-year-old salmon ($n = 6$) and stimulated with Con A, PHA-P, PWM, or LPS to determine the day of peak mitogenesis. Cells were cultured under atmospheric conditions at 10 °C for 120 h post-lectin stimulation. Error bars denote standard error from the mean. Maximum stimulation indices for Con A and PHA-P occurred 96 h post-stimulation.

washed three times and the wells were then blocked with 250 μL $well^{-1}$ of blocking buffer (DPBS containing 1% bovine serum albumin). Blocking buffer was removed and replaced with 50 μL $well^{-1}$ of 0.2 U mL^{-1} anti-bromodeoxyuridine Fab fragments labeled with horseradish peroxidase (Roche Chemicals) in blocking buffer and incubated for 1 h. Cells were washed five times with 100 μL $well^{-1}$ DPBS and 50 μL $well^{-1}$ of a substrate solution (4.8 mL, 10 mM citrate buffer (pH 4) with 200 μL, 18.2 mM 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 5 μL, 30% hydrogen peroxide) was added according to Ottinger and Kaattari (1998). The optical density (405 nm) of the solution in each well was determined at 5 min increments over a period of 20 min using a UV-Thermo Kinetic Microplate Reader (Molecular Devices Corporation, Sunnyvale, CA).

Stimulation index (SI) values were calculated as the replicate mean optical density for a given set of mitogen-treated leukocytes divided by the replicate mean optical density of the associated mitogen free (control) leukocytes. Stimulation index values were calculated for all time points and the maximum SI (mSI) value for a given pair of mitogen-treated and control leukocytes was defined as the highest SI value determined for the four time points.

2.6. Statistical analysis

Data were tested for normality using the Shapiro–Wilks *W*-test and homogeneity of variance via the Brown–Forsythe test of homogeneity of variances. Mitogen data were analyzed using a one-way ANOVA and significant differences between group means were determined using the Duncan multiple range test. All statistical analyses were performed using Statistica 6.1 (StatSoft Inc.). Differences were considered statistically significant when $P \leq 0.05$.

3. Results

3.1. Short-term effects of PCB exposure

Whole body burden analysis of experimental Atlantic salmon from the short-term experiment demonstrated that aqueous exposure led to an accumulation of PCBs in a dose dependant fashion. No detectable levels of PCBs were measured in the vehicle control group. Mean PCB concentrations in the treated groups were 173 $\mu g\,kg^{-1}$ (S.E. = 34) and 529 $\mu g\,kg^{-1}$ (S.E. = 13) for the 1 and 10 $\mu g\,L^{-1}$ groups, respectively. The sensitivity of detection was 11.49 $\mu g\,kg^{-1}$ (S.E. = 0.25). Measured concentrations in the experimental fish were in the range of PCB levels reported in wild salmon from contaminated sites (500–8000 $\mu g\,kg^{-1}$; Coleman, 2001). Plasma cortisol levels were not significantly affected by PCB exposure (Lerner et al., unpublished data).

Atlantic salmon smolts sampled immediately following the 3-week PCB exposure exhibited statistically significant differences in mitogen stimulation respective to treatment. The T-cell mitogens CON A and PHA-P elicited increased cell proliferation in the 10 $\mu g\,L^{-1}$ group ($n = 10$; $P = 0.043$ and 0.002, respectively) compared to control fish. Additionally, the PHA-P response was significantly increased in the 1 $\mu g\,L^{-1}$ group ($n = 10$, $P = 0.036$). Stimulation indices suggest a dose-dependent functional response by these cell populations in respect to PCB concentration (Fig. 2). Qualitatively, mean cell yields of viable AKLs were lower than vehicle control fish in both PCB treatment groups from the short-term experiment. Percent viable AKLs decreased by 42.7 and 47.1% in fish exposed to 1 or 10 $\mu g\,L^{-1}$ Aroclor 1254, respectively.

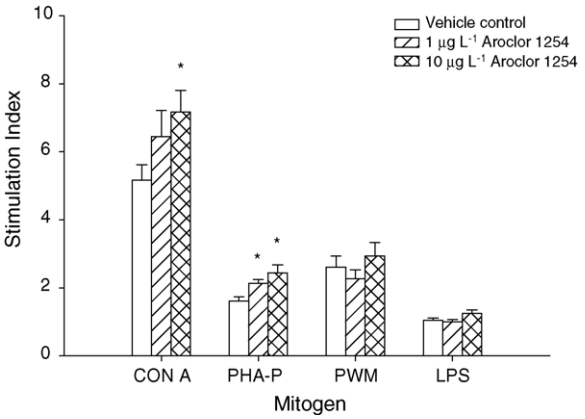

Fig. 2. Mitogen profiles of AKLs isolated from Atlantic salmon smolts immediately following a 3-week Aroclor 1254 exposure. Statistical differences from the vehicle control are indicated by an asterisk (*); one-way ANOVA and Duncan's multiple range test, $P < 0.05$; $n = 10$ fish per treatment. Error bars represent the standard error from the mean.

3.2. Long-term effects of PCB exposure

Mitogen profiles of AKLs isolated from Atlantic salmon parr in the long-term experiment also exhibited significant differences in cell proliferation as a result of PCB exposure. Leukocytes isolated from fish exposed to 10 $\mu g\,L^{-1}$ Aroclor 1254 had significantly elevated

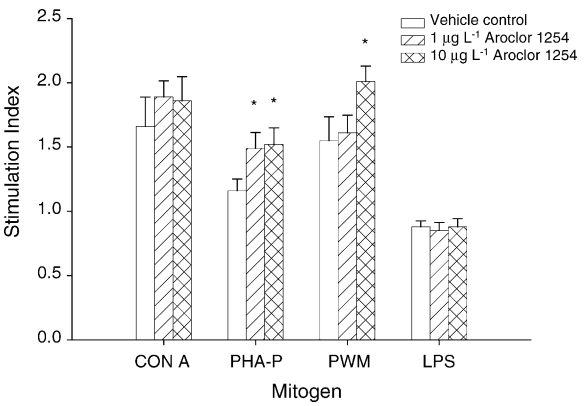

Fig. 3. Mitogen profiles of AKLs isolated from Atlantic salmon parr 15 months following a 3-week Aroclor 1254 exposure as 21 dph larvae. Statistical differences from the vehicle control are indicated by an asterisk (*); one-way ANOVA and Duncan's multiple range test, $P < 0.05$; $n = 12$ fish per treatment. Error bars represent the standard error from the mean.

mitogen responses to PHA-P ($n = 12$, $P = 0.032$) and PWM ($n = 12$, $P = 0.036$) relative to control fish. A significant increase in cell proliferation was also observed following PHA-P stimulation ($n = 12$, $P = 0.038$) exposed to 1 $\mu g\,L^{-1}$ Aroclor 1254 (Fig. 3). Differences between mean viable cell yields were less than 2.5% in fish sampled 15 months post-exposure.

4. Discussion

Numerous studies have demonstrated the immunotoxicity of various PCB congeners. In mammals PCB exposure is associated with thymic atrophy (Grasman and Whitace, 2001), leukopenia, shifts in T-cell populations (Lai et al., 1995), decreased antibody production (Tryphonas et al., 1991), and differential sensitivity of lymphocytes to lectin stimulation (Segre et al., 2002). In the case of salmonids, laboratory studies have primarily focused on B-cell effects in Chinook salmon. In particular juvenile Chinook salmon exposed to sublethal doses of Aroclor 1254 exhibit a suppressed primary and secondary plaque forming-cell (B-cell) response to the T-cell-independent antigen TNP-LPS (Arkoosh et al., 1994). The present study investigates the short-term effects of aqueous Aroclor 1254 exposure on the lymphoproliferative response of Atlantic salmon immunocytes during smolting. Moreover, it also evaluates putative, long-term immune dysfunction (altered lymphocyte mitogenesis) in parr sampled 15 months post-exposure as 21 dph larvae. Results from this work clearly demonstrate an immunomodulatory effect on T-cell mitogen (CON A and PHA-P) sensitive populations of lymphocytes in Atlantic salmon as well as long-term modulation of PHA-P and PWM sensitive populations.

Aroclor 1254 exposure leads to apoptosis and decreased viability of murine splenocytes (Yoo et al., 1997; Jeon et al., 2002). Similarly the induction of apoptosis in lake trout T lymphocytes has been reported following in vitro exposure to Aroclor 1254 (Sweet et al., 1998; Miller et al., 2002). In the present study, we observed a qualitative reduction in the number of total anterior kidney leukocytes isolated from Atlantic salmon immediately following a 3-week aqueous exposure. While the tissue source of leukocytes in the present study differed from the studies cited above, the lower cell yields from Aroclor-treated salmon may be the result of similar lymphotoxic effects. Direct comparisons between these studies are not possible, however, due to the differences in exposure time and cellular environment (in vitro versus in vivo). While direct lymphotoxic effects of Aroclor 1254 would explain AKL depopulation, effects on the supportive stromal cells and microenvironment of this hemopoietic organ are possible as well. The lack of differences in total viable AKLs in the long-term study suggests that cell depopulation is not permanent, and that 15 months is adequate time for AKLs to repopulate. It is unknown, however, whether relative proportions of leukocyte (sub)populations reached that of pre-treatment values.

While this type of work has not been previously conducted in Atlantic salmon, the observation of an increased proliferative response to T-cell specific mitogens (CON A and PHA) is not unprecedented. Increased mitogenesis in rats (Smialowicz et al., 1989), mice (Wu et al., 1999; Segre et al., 2002), birds (Smits et al., 2002), and fishes (Arkoosh et al., 1996) has been documented following experimental or environmental exposure to PCBs. The toxicity of Aroclor 1254 to lymphocytes has been well established in a number of animal models including salmonids. T lymphocytes are one of the most sensitive cell populations to this mixture of PCBs (Sweet et al., 1998; Miller et al., 2002). For this reason, a treatment-associated increase in T-cell mitogenesis may seem contrary to expectations. These observations, however, are not unfounded and may be explained by the following: (1) the Aroclor treatment may lead to the selection of T-cell subpopulations more responsive to mitogen stimulation, (2) regulatory T-cell-like populations that down-regulate the proliferative response may be the target of PCB toxicity (Arkoosh and Kaattari, 1987), (3) cytokine and cytokine receptor expression may be altered thus leading to a modulated proliferative response (Kwon et al., 2002; Segre et al., 2002), or (4) tyrosine kinase-mediated cell signaling may be affected (Canesi et al., 2003). Certainly a combination of these explanations is possible as well. These mechanisms could explain our observations in both the short- and long-term experiments in the present study.

In recent years, the long-term effects of some immunotoxicants in fish have been investigated. Age of exposure is a critical factor in regards to long-term

immunomodulatory effects in mammals. For instance, rats exposed to immunotoxicants during early life stages display immune responses that are more severely and persistently altered than those exposed during adult life (Holladay and Smialowicz, 2000). Chinook salmon exposed to *o,p*-dichlorodiphenyldichloroethylene (*o,p*-DDE) during early life history exhibit splenic B-cell immunomodulation for at least 1 year post-treatment (Milston et al., 2003). Similarly, long-term immune dysfunction has been reported in rainbow trout exposed to aflatoxin during early and late developmental stages. Immunomodulation is evident for a minimum of 2 years post-exposure and manifests as an increased proliferative response of AKLs to PWM (Ottinger and Kaattari, 2000). Interestingly, this is the same physiological response observed in the current study induced by larval exposure to Aroclor 1254. As suggested by the authors above, early life stage exposure may lead to a reduced capacity to immunoregulate.

Toxic equivalency (TEQ) values are often assigned to explain the toxic potential of PCB congeners relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Coplanar PCBs are regarded as the most toxic congeners as they bind the aryl hydrocarbon receptor (AhR) and induce subsequent changes in gene expression similar to TCDD. The non-coplanar congeners affect cellular mechanisms via AhR-independent pathways. Similarities exist between immunocyte dysfunction associated with PCB exposure and exposure to other chemicals that interact with the aryl hydrocarbon receptor. Direct comparisons of TCDD and the coplanar PCB126 have demonstrated similar suppressive effects on IgM and IL-5 production in mice. Likewise, in vivo exposure of these chemicals reduces the total number of thymocytes and leads to a shift in the dominant subpopulations of T-cells (Pan et al., 2004). The mixture Aroclor 1254 contains both coplanar and non-coplanar congeners. It is known to interact with the AhR but leads to less pronounced AhR-mediated cellular effects than the more potent AhR ligands including TCDD, benz[*A*]pyrene, and aflatoxin (Bannister et al., 1987). Given the fact that Aroclor 1254 and TCDD toxicities are in part mediated through a common receptor, it is likely that some of the mechanisms leading to immunomodulation are similar (Kerkvliet, 1995).

T lymphocytes express the AhR and are the most sensitive immunocyte population to TCDD and other polyaromatic hydrocarbons (PAHs) (Kerkvliet et al., 2002). Low doses of TCDD have been shown to affect thymocyte development and hence T-cell diversity (Nohara et al., 2000; Laiosa et al., 2003). TCDD has also been reported to modulate the proliferative response of T-cells. For instance, an increased proliferative response to the T-cell mitogen CON A with a corresponding increase in IL-2 was reported by Lundberg et al., 1990. This is similar to the observations reported by Exon et al. (1985) in rats due to PCB exposure. Likewise, there are well-documented associations between increased proliferative responses of T-cells in mixed leukocyte reactions following TCDD exposure. In some cases, increased synthesis of cytokines including IL-2 and IL-12 accompany the corresponding increased T-cell proliferation (Vorderstrasse and Kerkvliet, 2001). Recent evidence from mice indicated that activation of the AhR directly induces IL-2 mRNA synthesis in naïve T-cells (Jeon and Esser, 2000).

Dendritic cells responsible for producing accessory molecules necessary for coordinating immune responses have also been shown to be targets of TCDD toxicity (Vorderstrasse et al., 2003). Differentiation of monocytes into dendritic cells is also deleteriously affected by PAH exposure (Laupeze et al., 2002). Evidence of dendritic-like cells has been reported in cartilaginous fishes and Atlantic salmon based on morphological characteristics and immunohistochemical techniques, but the cell markers required for conclusive identification are lacking (Rumfelt et al., 2002; Koppang et al., 2003). Given the complexity of the teleost immune system, the presence of dendritic cells or a functionally similar population of regulatory cells, as seen in mammals, would not be unexpected. Likewise, while not currently described, regulatory T-cells or analogous suppressor cells may be present and could be targets of PCB toxicity.

While some PCB congeners are AhR agonists, recent evidence suggests that some of the PCB congeners present in Aroclor mixtures modulate immune function in an AhR independent manner (Smithwick et al., 2003; Pan et al., 2004). Activation of the cytokine expression regulator, nuclear factor-κβ (NF-κβ), is observed as a result of PCB exposure both in vitro and in vivo, and this mechanism has been associated with the pro-inflammatory actions of PCBs (Hennig et al.,

2002). Depression of LPS-induced proliferation following PCB exposure has recently been attributed to decreased expression of cyclin D2 (Smithwick et al., 2004). The effects of PCBs vary according to age, developmental stage, sex, species, route, dose, and length of exposure and PCB mixture (Monosson, 1999; Kodavanti et al., 2001; Duffy et al., 2002). In any case, it is clear that the interaction of TCDD and PCBs with immunocytes and immunoregulatory cells is complex and still poorly understood. Despite exhaustive research in mammalian models only a few mechanisms of immunotoxicity have been elucidated for these compounds. Mechanisms of modulation are even less clear in teleosts. Recent findings indicate that cytokine modulation may be an underlying cause of the resulting immunomodulation in mammals. While cytokine expression was not measured in the current study, disruption of these messenger molecules may occur in Atlantic salmon as a result of PCB exposure that would explain the observed immunomodulation. It is apparent that future work with teleosts should follow the mammalian example and redirect focus to examine the potential effects of PCBs on cytokine expression.

Regardless of the mechanisms or cell populations affected by intermediate duration exposure to Aroclor 1254, the net result is significant alteration of the physiological response of Atlantic salmon immunocytes. The fact that PCB exposure as smolt and larvae induces a chronic immunomodulation may be of consequence for salmon restoration. Concentrations of PCBs in the range or higher than those measured in the current study have been reported in wild Atlantic salmon from New England rivers, purporting that wild salmon accumulate PCBs in a natural setting (Coleman, 2001). Additionally, Arkoosh et al. (2001) demonstrated that Aroclor 1254 exposure leads to a higher pre-disposition to infection and subsequent disease in Chinook salmon at environmentally relevant concentrations. Consequently, PCB exposed fish may be less capable of mounting an appropriate immune response upon encounters with pathogens. To date, field studies have not been conducted to determine if similar immunological effects occur in wild Atlantic salmon. Further work is necessary to verify that the observed immunomodulation leads to immune compromised individuals, and that this laboratory observation occurs in nature.

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