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Immunotoxic effects of an industrial waste incineration site on groundwater in rainbow trout (Oncorhynchus mykiss)

Nadjet Benchalgo¹, François Gagné², Michel Fournier¹

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ABSTRACT

The discharge of organic waste from the petrochemical industry into the Mercier lagoons caused major groundwater contamination. The objective of this study was to determine the immunotoxic potential of three groundwater wells at increasing distance from the incinerator dumping site (1.17, 2.74 and 5.40 km). Rainbow Trout were exposed to increasing concentrations of water from three groundwater wells for 14 days. Immunocompetence was characterized by phagocytosis, mitogen-stimulated proliferation of lymphocytes, cell cycle analysis and apoptosis. A significant increase in innate (phagocytosis) and specific immune response (B lymphocyte proliferation) was observed in trout exposed to water collected from the well at 2.74 km. However, phagocytosis activity was suppressed in groups at 1.17 and 5.40 km. The proportion of lymphocytes in S phase was significantly increased in groups at 2.74 and 5.40 km, while lymphocytes in G0/G1 phase were decreased in all three exposure groups. Additionally, dexamethasone (DEX)-induced apoptosis of lymphocytes was significantly reduced in the group at 2.74 km, which suggests decreased lymphocyte turnover. Furthermore, the ratio of DEX-induced apoptosis/apoptosis was lower in the groups at 2.74 and 5.40 km. In summary, our experiments have shown that exposure to the mixture of organic compounds present in Mercier groundwater modulates phagocytosis and cell proliferation, disrupts the cell cycle and reduces the ratio of DEX-induced apoptosis/apoptosis. It is concluded that groundwater collected in the vicinity of an incinerator containment field could impact immunocompetence in fish.

Introduction

An estimated 40,000 m³ of industrial incineration wastes were dumped in an abandoned gravel pit (Mercier City, Quebec, Canada). The industrial waste consisted of used oil and liquid wastes from chemical and petrochemical industries (Poulin et al., 1985). Over the years, the contaminants permeated through the soil and contaminated aquifers, posing a risk to local habitats. In an attempt to alleviate this contamination problem, a groundwater treatment station plant was constructed near the incinerator area to remove and clean up the groundwater in the vicinity of the dumping sites. Poulin et al. (1985) revealed that the extent of the groundwater contamination is delimited by four zones, and the characterization of groundwater concluded the existence of a highly polluted area in the first two areas (1 and 2) (Fig. 1), which occupy an area of 2 km². Two other zones (3 and 4) extend over an area of some 30 km², where the water contains various contaminants at low concentrations.

Preliminary analyses of groundwater samples have detected volatile organic compounds (VOCs) which are the most common groundwater contaminant (Poulin et al., 1985; Golfinopoulos et al., 2001). Polycyclic aromatic hydrocarbons (PAHs), heavy metals and polychlorinated biphenyls (PCBs) were also found (Poulin et al., 1985; BAPE, 1994). Some volatile organohalogens (e.g., vinyl
cytotoxicity are able to trigger apoptosis. Otherwise, disturbances in apoptosis may lead to abnormal development and diseases (Follezou et al., 1999; Igney and Krammu, 2002).

The objectives of this study was to investigate the effects of exposure to groundwater, at 1.17, 2.74 and 5.4 km from the contaminated lagoon area, on the immune system in juvenile Rainbow Trout following 14 days of exposure time. For this purpose, immune function was examined by following changes in phagocytosis and stimulated lymphoblastic proliferation. In addition, cell cycle analysis and the proportion of cells undergoing apoptosis were examined.

1 Materials and methods

1.1 Fish collection and maintenance

Juvenile Rainbow Trout (Oncorhynchus mykiss) (n = 108), weighing (23.1 ± 9.3) g, were obtained from a local fish farm “Les Arpents Verts” located at Sainte-Edwidge-de-Clifton, Quebec, Canada. They were kept for a minimum of 2 weeks in 300 L tanks at 15°C under a photoperiod of 12 hr light/12 hr dark and constant aeration. They were fed daily at a rate of 2% body weight with food pellets.

1.2 Water sampling

The location of the sampling station is shown in Fig. 1. The groundwater samples were collected in the plume of contamination from three wells during May 2010. The wells are located at distances of 1.17, 2.74 and 5.40 km from the lagoons.

A composite or grab volume of 100 L of groundwater samples were collected at the sites and the samples were stored at 4°C until exposure. The physico-chemical parameters of pH, conductivity and temperature were determined according to standard methods of Quebec’s Centre d’expertise en analyse environnementale (ISO/CEI 17025). VOC parameters were vinyl chloride, trans-1,2-dichloroethylene, 1,1-dichloroethane, 1,2-dichloroethane, cis-1,2-dichloroethene, benzene and chlorobenzene (gas chromatography/mass spectrometry (GC/MS)) was used as a detection method.

1.3 Exposure experiments

Six plastic vessels (31 cm diameter × 44 cm height, 20 L capacity) were filled with 15 L of water sample using polyethylene plastic bags. Six groups of 12 juvenile rainbow trout were distributed to each tank and were exposed to provide a better mechanistic insight. Examining cell cycle alteration provides an opportunity to assess the early signals of cytotoxicity, while apoptosis provides early warning signals of environmental disturbance (Osman et al., 2012). The signals arising within the cells following cytotoxicity are able to trigger apoptosis. Otherwise, disturbances in apoptosis may lead to abnormal development and diseases (Follezou et al., 1999; Igney and Krammu, 2002).

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to increasing concentrations of groundwater (3.1%, 12.5%, 25% and 50%) for 14 days at 15°C. The water was changed twice a week. The control group and the dilution water consisted of UV- and charcoal treated tap water from Montreal City (Quebec, Canada). The exposure experiments were repeated twice. The fish were fed 3 times weekly with commercial fish pellets and the extra food was removed after 10 min. The feeding was stopped 24 hr before the end of the exposure. During the experiment, parameters such as temperature, dissolved oxygen content, pH and conductivity were measured daily. Data on nitrite, nitrate and ammonia concentrations were analyzed according to standard methods (CAEQ, ISO/CEI 17025).

All chemicals were obtained from the Sigma Chemical Company (Ontario, Canada) unless specifically identified. Fish (n = 6) were humanely anaesthetized with tricaine methane sulfonate (MS222) at 0.1% concentration after 14 days in accordance with the recommendations of the animal care committee. The head-fork length and body weight were measured. The anterior kidney was removed under sterile conditions with a 2-mL glass grinder (Wheaton Scientific, New Jersey, USA) containing 1 mL of sterile RPMI 1640 (Sigma-Aldrich, Ontario, Canada) supplemented with heparin (10 U/mL; Organon Tecknika, Ontario, Canada), HEPES (10 mmol/L), penicillin/streptomycin (100 U/mL and 100 mg/mL respectively), and 10% fetal bovine serum. The cell suspension of head-kidney was centrifuged at 485 × g for 30 min and washed twice in RPMI media without heparin. The ratio of live/dead cells was assessed microscopically with a hemacytometer (Bright-line, Pennsylvania, USA) using trypan blue exclusion (0.4%) and the suspension was adjusted to 1 × 10⁶ live cells/mL.

1.4 Phagocytosis

Phagocytic activity was assessed using fluorescently labelled beads in accordance with the protocol of Brousseau et al. (1998). A volume of 500 μL of each leukocyte suspension was incubated for 18 hr at 15°C with fluorescent latex beads (diameter of 1.71 μm; Polysciences, Pennsylvania, USA) at a 100:1 bead-to-cell ratio. After the incubation period, the cell suspensions were layered over 4 mL of RPMI supplemented with 3% bovine serum albumin and centrifuged at 150 × g at 4°C for 8 min to remove the free beads adsorbed at the surface of cell membranes. The cell pellets were resuspended and fixed in 0.5% formaldehyde and 0.2% sodium azide in phosphate-buffered saline (PBS). Cells were then analyzed using a flow cytometer (Becton Dickinson, California, USA) and 5000 events were recorded. Two endpoints determined the percentage of macrophages that engulfed one bead or more (phagocytic activity) or three beads or more (phagocytic efficiency). The data were expressed as a percentage of cells that engulfed one bead or more and three beads or more.

1.5 Lymphocyte proliferation

Cells were plated at 5 × 10⁵ cells/well in round-bottom microplates and incubated with 20μg/mL of phytohemagglutinin (PHA), a mitogen activator of lymphocyte T, and with 250 μg/mL of lipopolysaccharide (LPS), a mitogen activator of lymphocyte B. After 72 hr of incubation, 0.5 μCi/well of [³H]-methyl thymidine (MP Biomedical, Ohio, USA) was added, and plates were incubated for a further 18 hr at 15°C. Cells were harvested with a semi-automatic cell harvester (Skatron Instruments As, Lier, Norway) on a fibreglass filter (Skatron Instruments As, Norway). Radioactivity was measured with a microbeta Trilux scintillation counter (Perkin Elmer, Toronto, Canada). The raw data were expressed as counts per min (CPM), and the results were presented as stimulation index (SI) calculated as follows:

\[ SI = \frac{\text{CPM stimulated cells (PHA or LPS)}}{\text{CPM unstimulated cells}} \times 100 \]

1.6 Cell cycle

Cell cycle analysis was determined by flow cytometry. Briefly, a volume of (5 × 10⁵) cells was centrifuged for 10 min at 450 × g with the brake on low. The supernatant was removed and 500 μL of ice-cold 70% ethanol was added and cells were centrifuged for 5 min at 450 × g. Cells were then washed twice in PBS and resuspended in 0.5 mL of PBS containing PI (50 μg/mL) and RNase (100 μg/mL). Cells were incubated at room temperature for 30 min. The DNA histogram results showed the percentage of cells in G0/G1, S and G2/M phases under total percentage, using Cell Quest Pro software (Becton Dickinson). At least 5000 events were acquired for each sample. Human tumor cells (K562) were used as a positive control for the cell cycle assay.

1.7 Apoptosis

To determine in vitro apoptosis, a commercial kit was used (Vybrant Apoptosis Assay Kit #4 from Molecular Probes, USA). It contains YO-PRO-1 and propidium iodide nucleic acid stains. The principle of this kit is to detect apoptosis as compared to cell membrane permeability; the green fluorescent YO-PRO-1 dye can enter apoptotic cells, but not propidium iodide. We added 10 μmol/L of YO-PRO-1 and 0.1 mg/mL of propidium iodide to 0.5 × 10⁶ cells/mL. After the incubation period of 30 min at 4°C in the dark, stained cells were analyzed by flow cytometry using green fluorescence emission for YOPRO-1 (488 ± 30 nm emission) and red fluorescence for propidium iodide (610 ± 20 nm band pass). For each sample the fluorescence of 10,000 events was recorded. Dexamethasone was used as an inducer of apoptosis at 10 μmol/L.

1.8 Statistical analyses

The data were expressed as a mean with standard error. In each experiment, differences between the control and
groups of fish exposed to Mercier groundwater were evaluated by one-way analysis of variance (ANOVA) followed by a post-hoc test ($p \leq 0.05$). Analysis was performed using Statistica for Windows (Version 7.0, StatSoft Inc., 1995). Correlation was performed using Pearson-moment procedure, and factorial analyses were determined using principal component and discriminant function methods. Significance was set at $p \leq 0.05$.

2 Results

The results reveal that Mercier groundwater at 2.74 km from the lagoons has a higher degree of contamination than water at 1.17 and 5.40 km (Table 1). This suggests that groundwater treatment was effective at 1.17 km but the contamination persists at 2.74 km distance. Discriminant analysis confirmed that the site at 2.74 km distance from the lagoons was more contaminated and was different from the other sites. The biomarkers that discriminated the 2.74 km site were phagocytosis activity, LPS- and PHA-stimulation lymphocyte proliferation, and the cell cycle (more explanation in Section 2.4).

### Table 1: Chemical composition of Mercier groundwater

<table>
<thead>
<tr>
<th>Compound</th>
<th>Distance† wells</th>
<th>Detection limit</th>
<th>Criteria groundwater (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.17 km</td>
<td>2.74 km</td>
<td>5.4 km</td>
</tr>
<tr>
<td>Vinyl chloride (µg/L)</td>
<td>&lt; 0.10</td>
<td>4.00</td>
<td>&lt; 0.10</td>
</tr>
<tr>
<td>trans-1,2-Dichloroethylene (µg/L)</td>
<td>&lt; 0.08</td>
<td>2.00</td>
<td>&lt; 0.08</td>
</tr>
<tr>
<td>1,1-Dichloroethane (µg/L)</td>
<td>&lt; 0.10</td>
<td>0.40</td>
<td>&lt; 0.10</td>
</tr>
<tr>
<td>cis-1,2-Dichloroethene (µg/L)</td>
<td>&lt; 0.05</td>
<td>0.37</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>1,2-Dichloroethane (µg/L)</td>
<td>&lt; 0.10</td>
<td>2.00</td>
<td>&lt; 0.10</td>
</tr>
<tr>
<td>Benzene (µg/L)</td>
<td>&lt; 0.05</td>
<td>0.29</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Values of measurements during sampling in may 2010. † Distance between the wells and contaminated site.

2.1 Phagocytosis

Trout weights, lengths and leukocyte concentrations were not significantly changed by the incinerator groundwater effluent after 14 days of exposure time (Table 2). Exposure to Mercier groundwater showed two different immune responses: immune suppression for wells at 1.17 and 5.40 km and immune stimulation for well at 2.74 distance km. In fish exposed to groundwater at 1.17 km, a significant reduction in both immunoactivity (> 1 bead) and immuno-efficiency (> 3 beads) was found at concentrations of 3.1% and 25%, respectively. In fish exposed to groundwater at 2.74 km, a significant increase in immunoactivity and efficiency was observed at 12.5% concentration. In fish exposed to the groundwater at 5.40 km (Fig. 2), phagocytosis was suppressed at concentrations of 12.5% and 25%.

2.2 Lymphocyte proliferation

There was no apparent response in lymphocyte proliferation of total lymphocytes in fish exposed to well water at 1.17 km. However, exposure to the water at 2.74 km led to a significant reduction in proliferation of PHA-stimulated lymphocytes at 12.5% concentration. Both lymphocyte B and T proliferation were significantly enhanced at 50% water concentration after 14 days of exposure at 5.40 km (Fig. 3).
Stimulation index

PHA LPS

Control 3.1 12.5 25 50

Effluent concentration (%)

Fig. 3 Lymphocyte proliferation stimulated with PHA (T lymphocytes) and with LPS (B lymphocytes) in Rainbow Trout exposed for 14 days to Mercier groundwater (at 1.17, 2.74 and 5.40 km). Asterisks indicate significant difference from controls (*p<0.05; ***p < 0.001).

2.3 Cell cycle

Fish exposed to Mercier groundwater at 1.17, 2.74 and 5.40 km displayed significant disturbance in cell cycle compared to controls (Fig. 4). Indeed, a significant decrease in the number of cells in the G0/G1 phase was observed at 1.17, 2.74 and 5.40 km. This was accompanied by a significant increase in the proportion of cells undergoing DNA synthesis (S phase) at 50% concentration of effluent at 2.74 km and at 3.1%, 25% and 50% concentration of effluent at 5.40 km.

2.4 Apoptosis

The proportion of apoptotic leucocytes was also determined in fish exposed to the groundwater samples (Table 3). After 14 days, there was no significant effect in groups at 1.17 and 5.40 km. However, the percentage of apoptotic lymphocytes significantly dropped at 3.1% concentration for groundwater at 2.74 km. In the attempt to determine the susceptibility of lymphocytes to undergo apoptosis, we treated lymphocytes to a known inducer of apoptosis (dexamethasone or DEX). The level of the response to DEX induction appears higher in lymphocytes than in macrophages (Fig. 5). The correlation analysis revealed that apoptosis was correlated with cell cycle, phagocytosis and lymphocyte proliferation (Table 4). Negative correlation between apoptosis and phagocytosis (r = -0.83, p ≤ 0.05) was observed. On other hand, the correlation between proportion of apoptotic macrophages and the proportion of cell cycle in phase G2/M in groups at 1.17 km was significantly positive (r = 0.90, p ≤ 0.05) indicating that the correlation between the proportion of apoptotic lymphocytes and the proportion of cell cycle in phase G2/M in groups at 2.74 km was significantly negative (r = -0.92, p ≤ 0.05). Moreover, a negative correlation was found between apoptosis of lymphocytes and both T and B lymphocyte-induced proliferation in groups at 5.40 km (r = -0.92, p ≤ 0.05 and r= -0.94, p ≤ 0.05) respectively.

To describe the overall effects of exposure to contaminated groundwater on the immune system, cell cycle and apoptosis in Rainbow Trout, factorial and discriminant function analyses were performed (Fig. 6). The factorial analysis revealed that 78% of the total variance was ex-
Table 3  Apoptosis in lymphocytes and macrophages of Rainbow Trout exposed for 14 days to Mercier groundwater

<table>
<thead>
<tr>
<th>Distance</th>
<th>Parameters</th>
<th>Control</th>
<th>3.10%</th>
<th>12.50%</th>
<th>25%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.17 km</td>
<td>Lymphocytes apoptosis</td>
<td>2.6 ± 0.3</td>
<td>3.0 ± 0.5</td>
<td>2.2 ± 0.2</td>
<td>2.1 ± 0.6</td>
<td>2.3 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Macrophages apoptosis</td>
<td>1.5 ± 0.1</td>
<td>2.4 ± 1.1</td>
<td>1.8 ± 1.2</td>
<td>1.4 ± 0.5</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Induced apoptosis of lymphocytes</td>
<td>8.0 ± 2.6</td>
<td>17.7 ± 4.0***</td>
<td>13.3 ± 3.6</td>
<td>8.9 ± 3.3</td>
<td>9.9 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Induced apoptosis of macrophages</td>
<td>3.0 ± 1.9</td>
<td>5.4 ± 2.2**</td>
<td>3.5 ± 1.3</td>
<td>3.3 ± 1.5</td>
<td>2.1 ± 1.3</td>
</tr>
<tr>
<td>2.74 km</td>
<td>Lymphocytes apoptosis</td>
<td>3.0 ±0.7</td>
<td>1.6 ± 0.7**</td>
<td>1.5 ±0.4**</td>
<td>2.7 ±1.0</td>
<td>1.9 ±0.8**</td>
</tr>
<tr>
<td></td>
<td>Macrophages apoptosis</td>
<td>2.5 ± 0.9</td>
<td>3.4 ± 3.2</td>
<td>11.6 ± 3.4*</td>
<td>15.5 ±7.3</td>
<td>7.8 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>Induced apoptosis of lymphocytes</td>
<td>11.3 ± 3.2</td>
<td>13.9 ± 2.3</td>
<td>11.6 ± 3.7</td>
<td>20.1±7.4**</td>
<td>10.8 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>Induced apoptosis of macrophages</td>
<td>1.9 ± 1.0</td>
<td>3.4 ± 2.1</td>
<td>2.7 ± 1.1</td>
<td>2.6 ± 1.1</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td>5.40 km</td>
<td>Lymphocytes apoptosis</td>
<td>6.4 ± 2.7</td>
<td>6.4 ± 2.2</td>
<td>6.2 ± 0.7</td>
<td>6.9 ± 4.3</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Macrophages apoptosis</td>
<td>8.0 ± 5.8</td>
<td>5.9 ± 1.4</td>
<td>5.0 ± 1.5</td>
<td>6.0 ± 2.5</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Induced apoptosis of lymphocytes</td>
<td>6.4 ± 1.6</td>
<td>9.1 ± 4.0***</td>
<td>7.8 ± 1.4</td>
<td>7.4 ± 2.0</td>
<td>4.8 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Induced apoptosis of macrophages</td>
<td>2.8 ± 1.4</td>
<td>2.4 ± 0.5</td>
<td>2.0 ± 0.4</td>
<td>2.5 ± 0.7</td>
<td>1.9 ± 0.6</td>
</tr>
</tbody>
</table>

Data are expressed as the percentage of apoptotic cells. Asterisks indicate significant difference from controls (*p < 0.05; **p < 0.01; ***p < 0.001).

Fig. 4  Effects of the exposure to Mercier groundwater (at 1.17, 2.74 and 5.40 km) on the cell cycle of leucocytes in Rainbow Trout (14 days). Asterisks indicate significant difference from controls (*p < 0.05; **p < 0.01; ***p < 0.001).

Table 3  Apoptosis in lymphocytes and macrophages of Rainbow Trout exposed for 14 days to Mercier groundwater

plained by the following parameters, which exhibited high factorial weights (> 0.7): apoptosis, lymphocyte proliferation and cell cycle (Fig. 6a). LPS-induced proliferation, G2/M and apoptosis were clustered together, suggesting that these biomarkers were closely associated (Fig. 6a). A discriminant function analysis of the immune responses, cell cycle and apoptosis revealed that all three wells and the controls were correctly classified, at 78%, 92%, 73% and 100% correctness, respectively (Fig. 6b). The well at 2.74 km differed most from the control and the other wells. The sites at 1.17 km and 5.40 km were closer in behavior to the control, and hence less toxic. Phagocytic activity (P1), phagocytic efficiency (P2), and PHA and LPS-induced proliferation were the main biomarkers that discriminated the sites. Table 5 shows the fold change values of various biomarkers measured in the study and summarizes the characteristics of different aspects of the contaminants present in Mercier groundwater with respect to their immunotoxic and neoplasia risks.

3 Discussion

In this study, we investigated the immunotoxic effects of groundwater at increasing distance from an industrial dumping site (Mercier incinerator) in Rainbow Trout. A discriminant function analysis used to classify and determine the similarities or differences between study sites revealed that the sites farthest away from (5.40 km) and closest to (1.17 km) the lagoons were closer in behavior to the controls. This suggests that these water samples were less immunotoxic towards fish, and this is corroborated by the contaminant levels in groundwater (Table 1). Measured concentrations of volatile organic compounds (e.g., benzene, dichloroethane and vinyl chloride) were above the detection limit in groundwater at 2.74 km (Table 1). However, their effects on aquatic organisms are less reported. Based on the chemical contamination data, it appears then that the culprit contaminants are other than those measured in this study or that they perhaps act in a cumulative manner as a mixture.

Various chemicals from the environment can disrupt immune system function through multiple mechanisms (Ren et al., 2013). Groundwater samples at 2.74 km caused...
Table 4  Correlation between biomarkers in Rainbow Trout exposed for 14 days to Mercier groundwater

| Weight Length Phagocytic activity Phagocytic efficiency PHA LPS Apoptosis G0/G1 S G2/M |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Weight         | 0.94 A         | 0.93 A         |                |                | 0.95 A         | 0.94 A         |                |                |
| Length         | 0.94 A         | 0.95 A         |                |                |                | 0.95 A         |                |                |
| Phagocytic activity | 0.83 A         |                |                |                |                |                |                |                |
| Phagocytic efficiency |            |                |                |                |                |                |                |                |
| PHA            | 0.92 C         |                |                |                |                |                |                |                |
| LPS            | 0.94 C         |                |                |                |                |                |                |                |
| Apoptosis      | 0.89 B         | 0.90 A         |                |                |                |                |                |                |

A: 1.17 km; B: 2.74 km; C: 5.40 km.

Only significant correlations are shown (* p < 0.05).

Table 5  Summary table to characterize the different aspects of the immunotoxic risk of contaminants

<table>
<thead>
<tr>
<th>Distance</th>
<th>Phagocytosis</th>
<th>Lymphocyte T</th>
<th>Lymphocyte B</th>
<th>G0/G1, S phase</th>
<th>Apoptosis</th>
<th>Immunotoxic risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.17 km</td>
<td>(-) 0.71</td>
<td>/</td>
<td>/</td>
<td>G0/G1: (-) 0.87</td>
<td>Normal: /</td>
<td>Non specific immunosuppression</td>
</tr>
<tr>
<td></td>
<td>(-) 0.68</td>
<td>/</td>
<td>/</td>
<td>S: /</td>
<td>DEX-induced: 2.2</td>
<td></td>
</tr>
<tr>
<td>2.74 km</td>
<td>1.13</td>
<td>(-) 0.54</td>
<td>/</td>
<td>G0/G1: (-) 0.91</td>
<td>Normal: (-) 0.63</td>
<td>Non specific immunosuppression</td>
</tr>
<tr>
<td></td>
<td>1.33</td>
<td>/</td>
<td>/</td>
<td>S: 2.42</td>
<td>DEX-induced: 1.76</td>
<td>Specific immunosuppression</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Arrest: Yes</td>
<td>Neoplasia risk</td>
<td></td>
</tr>
<tr>
<td>5.40 km</td>
<td>(-) 0.94</td>
<td>2.45</td>
<td>2.35</td>
<td>G0/G1: (-) 0.85</td>
<td>Normal: /</td>
<td>Non specific immunosuppression</td>
</tr>
<tr>
<td></td>
<td>(-) 0.85</td>
<td>/</td>
<td>/</td>
<td>S: 1.57</td>
<td>DEX-induced: 1.42</td>
<td>Specific immunosuppression</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Arrest: Yes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values indicated fold change calculated by dividing the data from the experimental group by the data from the control group. “/” indicates no significant response.

an induction in macrophage phagocytosis activity. Conversely, a decrease in phagocytosis activity and efficiency was observed at 1.17 and 5.40 km. According to the literature, phagocytosis activity can be enhanced or depressed under chemical exposure. The stimulation in phagocytosis observed may result in the loss of regulation in the immune system and can lead to adverse outcomes including cancer, while chemical-induced immune suppression can lead to increased susceptibility to infectious diseases (Descotes et al., 2004). A previous analysis of groundwater has also shown the presence of not only VOCs but also PAHs and PCBs as well (BAPE, 1994). Those compounds can generate various types of immunotoxic effects (i.e., immunosuppression and stimulation) depending on the dose (Lacroix et al., 2001; Veraldi et al., 2006; Bado-Nilles et al., 2011; Danion et al., 2011).
Our results demonstrate a different proliferative response in trout after exposure to Mercier groundwater. Data showed that the proliferative responses of T lymphocytes, but not of B lymphocytes, were significantly reduced at 2.74 km. This suggests that T cells (PHA-induced) were more sensitive to humoral response than B cells (LPS-induced), and hence antibody production would be more affected. Proliferation responses of both T and B lymphocytes at 5.40 km were increased by 2.45 times relative to the control. This may be related to compensatory proliferation induced in response to cell loss observed in apoptosis which was induced in that event.

It is known that alterations in the cell cycle provide an early warning signal of cytotoxicity (Peng et al., 2012). Contaminants can suppress the proliferation of cells as a consequence of arresting cell cycle division. In this study, the results indicated disruption of the normal cell cycle of lymphocytes upon exposure to groundwater near the Mercier incinerator at all three distances 1.17, 2.74 and 5.40 km. In fact, the distribution of exposed cells between G0/G1, S, and G2/M, apoptosis induced (50%) in the group at 2.74 km. The cells could be arrested in S phase to repair DNA damage before mitosis occurs (Binkova et al., 2000). The data on the cell cycle supports the hypothesis that it is probably a disturbance in the various signals that regulate cell cycle transition, such as transcription of many genes involved in DNA replication that is responsible for the effects observed.

Programmed cell death, or apoptosis, is a controlled process used to eliminate damaged cells in vertebrates and invertebrates (Kiss, 2010; Franco et al., 2009). Nevertheless, immunosuppressive pollutants can disrupt the natural balance of leucocytes (Rajaram et al., 1995; McConkey et al., 1988); conversely, the inhibition of apoptosis can lead to several diseases (Banerjee et al., 2012). In this study, apoptosis was decreased at the 2.74 km site with a concomitant decrease in lymphocyte proliferation. This suggests that damaged cells are removed from the system by apoptosis less often and that acquired and humoral aspects of the immune system in fish are compromised. In contrast, apoptosis was not inhibited in macrophages at 2.74 km, indicating that the turnover of cells responsible for phagocytosis was unchanged. Reduced apoptosis has been largely linked to the occurrence of cancer, where an excessive proliferation or failure of cell death was a hallmark of cancer (Manna et al., 2011) and apoptosis follows when DNA damage is too important for cell survival (Osman et al., 2012; Armstrong et al., 2011; Marabini et al., 2011; Nigro et al., 2002). Moreover, the low ratio of DEX-induced apoptosis/apoptosis observed in (Fig. 5) suggests that some chemicals could increase the resistance of lymphocytes or macrophages to apoptosis. Constant proliferation with less apoptosis could contribute to cancer development. These observations indicate that apoptosis may be used as a sensitive and promising biomarker for the detection of sublethal effects of chemicals in the aquatic environment.

4 Conclusions

This study provides some insight into the immunotoxic effect on Rainbow Trout of groundwater contaminated by incineration waste from industrial activities. The data suggest that chemicals were able to account for the observed effects. A number of effects were observed, including a modulation of phagocytic activity and an alteration of lymphocyte proliferation. Additionally, this study suggests that these contaminants affect the cellular cycle of leucocytes and apoptosis of macrophages and lymphocytes.
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References


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