

Biomarkers study in rainbow trout exposed to industrially contaminated groundwater

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Abstract

The spill of liquid industrial waste from chemical and petrochemical industries in Mercier lagoons located 20 km south of Montreal, Quebec, caused a major groundwater contamination by industrial contaminants. The aim of this study was to investigate the toxic effects of Mercier groundwater, following 4 and 14 days of exposure to graded concentrations from three wells at increasing distances 1.2, 2.7 and 5.4 km from the source of contamination. Rainbow trout were examined for several biomarkers of defense [ethoxyresorufin *O*-deethylase (EROD) and glutathione *S*-transferase (GST) activities] and those of tissue damage [lipid peroxidation (LPO) and DNA strand breaks]. The results showed that EROD activity was significantly enhanced in hepatic tissue at 1.2 and 5.4 km, whereas inhibition in activity was observed in group at 2.7 km. Therefore, GST activity was significantly increased at 3.1% concentration for the 2.7 km well. No change in LPO was observed. However, a significant induction of DNA strand breaks in liver was obtained at each distance. In conclusion, the data suggest that the release of these contaminants in groundwater leads to increased biotransformation for coplanar aromatic hydrocarbons and DNA damage in groundwater.

Introduction

The dumping of 40,000 to 170,000 m³ of industrial hydrocarbon waste in an abandoned gravel pit (Mercier, Quebec, Canada) caused severe water supply problems in the region.¹ The main industrial waste consisted of used oil from chemical and petrochemical industries.² In the gravel pit lagoon, the contaminants permeate through soil down to the groundwater. Among the organic compounds found, volatiles organic compounds (VOCs) (Table 1) such as vinyl chloride (chloroethene), trans 1,2-dichloroethylene, 1,1-dichloroethane, 1,2-dichloroethane, cis

1,2-dichloroethene, benzene, chlorobenzene, m+p-xylènes, chloroethane, 1,3-dichloropropane and phenol.¹⁻³ Their physicochemical properties and hydrogeological conditions of the site are responsible of the complexity of contamination problem.⁴ Even if the lagoons are the main source of contamination, the industrial waste incinerator may be considered as well.

Organic industrial wastes containing VOCs, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and metals are usually found at hazardous waste sites.⁵ They are known to produce a variety of adverse effects in organism including, immunotoxicity,^{6,7} genotoxicity⁸⁻¹⁰ and carcinogenesis.¹¹⁻¹³ Those contaminants are able to induce the biotransformation enzyme cytochrome P450 and glutathione *S*-transferase in fish^{12,14-16} and induce lipid peroxidation (LPO) and DNA strand breaks in exposed animals.^{9,17,18}

Although chemical analysis could find some of chemicals in these complex mixtures, it remains difficult to determine the cumulative effects in exposed organisms. The measurement of biomarkers represent a mean to determine the toxicological outcome of exposure to complex industrial contaminants mixtures^{19,20} and give early warning signals about environmental threats of contaminants.²¹ Indeed, exposure to PAHs leads to the induction of cytochrome P4501A1 responsible for ethoxyresorufin-*O*-deethylase (EROD) activity in fish liver and kidney.²²⁻²⁴ Gwinn *et al.*⁹ showed that certain volatile organic compounds (*e.g.* vinyl chloride, dichloroethane) are metabolized via oxidation mediated by CYP 450 system to form electrophilic metabolites which may be detoxified by glutathione *S*-transferase (GSTs).

Glutathione *S*-transferase is an important phase II enzyme that catalyses the conjugation of electrophilic compounds to GSH,²⁵ GST reacts with a wide spectrum of environmental pollutants.^{26,27} It has been reported that the enzyme GST react differently to a variety of compounds.¹⁸ These same authors, reported that GST was inhibited by benzene, whereas its activity was enhanced in animals exposed to 2,4-dichlorophenol.²⁸ Since this reaction consumes reduced glutathione, sustained or increased activity could lead to oxidative stress in cells.

The measurement of LPO levels in animal tissues has been recognized as biomarkers of oxidative damage towards unsaturated phospholipids.^{18,20} LPO is reported to cause loss of cell function under oxidative stress.²⁹ Genotoxicity was determined by evaluating DNA strand breaks and has been proposed as effective biomarkers in assessing the impact of contaminants released into the aquatic environment.³⁰ Sasaki *et al.*³¹ showed hepatic DNA damage in rodents exposed to 1,2-

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Key words: groundwater, ethoxyresorufin *O*-deethylase, glutathione *S*-transferase activity, lipid peroxidation, DNA strand breaks, rainbow trout.

Acknowledgments: this study was supported by the Canada Research Chair in Environmental Immunotoxicology (Dr M. Fournier) and Aquatic Contaminant Research Division, Environment Canada (Dr F. Gagné). The authors are thankful to Mr Julien Paquette and Michel Paquin from Ministry of Sustainable Development, Environment and Parks (Quebec) for their precious help in water sampling.

Conflict of interests: the authors declare no conflicts of interests.

Received for publication: 28 October 2013.

Revision received: 13 December 2013.

Accepted for publication: 16 January 2014.

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Journal of Xenobiotics 2014; 4:1991
doi:10.4081/xeno.2014.1991

dichloroethane (EDC). Exposure to benzene leads to single strand breaks in erythrocytes in rodents.¹⁰ However, the toxicity of EDC has yet to be examined in fish. In this context, the present study was carried out to investigate biomarkers enzyme effects of Mercier groundwater exposure on rainbow trout. A set of biomarkers of defense and tissue damage were measured in the liver of trout exposed to groundwater at various distances from the landfill.

Materials and methods

Fish

Juvenile rainbow trout (*O. mykiss*) (total N=216), weighing 23.14±9.3 g, were obtained from a local fish farm *Les Arpents Verts* (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 h light: 12 h dark and constant aeration. They were fed daily at a rate of 2% body weight with food pellets.

Groundwater exposure experiments

The groundwater samples were collected in the summer of may 2010 from three wells at 1.2, 2.7 and 5.4 km from Mercier lagoons (Figure 1). These samples were then stored at 4°C in the dark until exposure. In six plastic vessels (31 cm diameter × 44 cm height, 20 L capacity) filled with 15 L of water sample using polyethylene plastic bags, six groups of 12 juvenile rainbow trout were exposed to increasing concentrations of groundwater (3.1% and 50%) for 4 days and 14 days at 15°C. The control group and the dilution water consisted of UV- and charcoal-treated tap water from the City of Montréal (Quebec, Canada). The exposure experiments were repeated twice and the water was changed twice a week. 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 h before the end of the exposure. Water chemistry (*e.g.*, temperature, pH, oxygen, conductivity, nitrate-nitrite and ammonia) according to standard methods of the *Centre d'Expertise en Analyse Environnementale* of the province of Quebec³ was within acceptable limits. The following

organic VOC parameters were determined: vinyl chloride, trans-1, 2-dichloroethylene, 1,1-dichloroethane, 1, 2-dichloroethane, cis-1, 2-dichloroethene, benzene, and chlorobenzene (Table 2).³²⁻³⁴

Biochemical analyses

Fish were humanely anaesthetized with tricaine methanesulfonate 0.1% (MS222) (Sigma-Aldrich, ON, Canada) after 4 days and 14 days (n=6), in accordance with the recommendations of the animal care committee and length and body weight measured. Condition factor (CF) for each fish was calculated according to the following equation (White and Fletcher, 1985):

$$CF = \text{body weight (g)} / \text{fork length}^3 \text{ (cm)} \times 100 \quad (1)$$

and the hepatosomatic index (HSI) was determined by the following:

$$\text{liver weight} / \text{body weight} * 100 \quad (2)$$

Fish samples were conserved at -80°C until

biochemical analyses. Frozen livers were thawed and homogenized (20 s duration) using a Teflon pestle tissue grinder in an ice-cold homogenization buffer (10 mM Hepes-NaOH, pH 7.4 containing 140 mM NaCl, 1 mM dithiothreitol and 1 mg/mL aprotinin (a protease inhibitor). Aliquots of each homogenate were taken for LPO, DNA strand break determinations and protein concentrations. The remainder of the homogenate was centrifuged at 15,000 g for 20 min at 4°C. The supernatant (S15) was collected to measure 7-ethoxyresorufin-O-deethylase activity, GST activity and proteins. These biomarkers were normalized with both homogenate and S15 protein concentrations as determined by the method of Bradford³⁵ using standards of bovine serum albumin.

7-Ethoxyresorufin-O-deethylase

7-ethoxyresorufin-O-deethylase activity was measured according to Gagné and Blaise³⁶ method. The reaction mixture contained 50 µL of S15 and 160 µL of 50 µM 7-ethoxyresorufin in 10 mM Tris-HCL, pH 7.4 and Tween 20. The reaction was started by the addition of 10 µL

Table 1. Samples of water collected in campaign 2010 from 15 wells in order to study the quality of Mercier groundwater. Analysis showed that approximately half of wells (7 wells) have concentrations of volatiles organic compounds higher than detection limit [(the concentration values (µg/L)]. Vinyl chloride concentrations within a well ranged up to 0.5 µg/L and a maximum concentration of 85 µg/L was recorded in one well. Analyses were provided by the *Centre d'Expertise en Analyse Environnementale* of the province of Quebec, and determined according to their standard methods (CAEQ,2010)

Compounds	N° of Wells							DLM
	94-7R	7121	94-1S	94-5S	5M-81-B	94-6R	PW-09	
Chlorure de vinyl	3.5	-	85	0.7	0.5	4.0	0.5	0.1
Trans-1,2-Dichloroethylene	0.42	-	0.4	-	-	2.0	-	0.08
1,1-Dichloroethane	0.17	-	2.2	-	-	0.2	-	0.08
Cis-1,2- Dichloroethene	0.25	-	7.0	-	-	0.37	-	0.05
Benzene	0.15	-	-	-	0.08	0.29	-	0.05
1,2-Dichloroethane	0.1	-	-	-	0.5	0.41	-	0.1
m +p-Xylenes	-	0.21	-	-	-	-	-	0.14
Chloroethane	-	-	1.7	-	-	-	-	0.1
1,1-Dichloroethene	-	-	5.7	-	-	-	-	0.1
1,3-Dichloropropane	-	-	0.5	-	-	-	-	0.1
Chlorobenzene	-	-	0.56	-	-	-	-	0.05

Table 2. Summary of analytical results of groundwater sites at 1.2, 2.7 and 5.4 km sampled for 61 organic contaminants (data present only compounds with concentration above limit detection).

Compounds (µg/L)	Distance wells (km)			Detection limit	Criteria groundwater (µg/L)
	1.2	2.7	5.4		
Vinyl chloride	-	4.00	-	0.10	2.40 ³³
Trans-1,2-Dichloroethylene	-	2.00	-	0.08	50 ³⁴
1,1-Dichloroethane	-	0.40	-	0.10	1500 ³⁴
Cis-1,2-Dichloroethene	-	0.37	-	0.05	50 ³³
1,2-Dichloroethane	-	2.00	-	0.10	55 ³⁴
Benzene	-	0.29	-	0.05	2.2 ³³

Values of measurements during sampling in may 2010. (-) No detected.

NADPH 1 mM. The mixture was incubated at room temperature for 30 and 60 min where fluorescence of resorufin was measured using 535 nm (excitation) and 635 nm (emission) filters. Calibration was achieved by comparing the rate of fluorescence change in the samples with fluorescence of resorufin standards. Enzyme activity (EROD) was expressed in nmol of resorufin/min/mg of proteins.

Lipid peroxidation

Lipid peroxidation was measured according to the thiobarbituric acid method (Wills, 1987). A volume of 150 μ L of the homogenate were mixed with 300 μ L of 10% trichloroacetic acid solution containing 1 mM FeSO₄ and 150 μ L of 0.67% thiobarbituric acid. The mixture was allowed to stand in a water bath (70°C) for 10 min. Standards solutions were prepared with 0.001% of tetramethoxypropane for calibration. The thiobarbituric acid reactants (TBARS) were measured by fluorescence at 540 nm (excitation) and 590 nm (emission). Results were expressed in nmol of malonaldehyde equivalents per mg of proteins.

DNA strand breaks

DNA strand breaks were determined using the alkaline precipitation.³⁷ Briefly, 25 μ L of the homogenate was added to 200 μ L of SDS solution 2% containing 40 mM NaOH, 10 mM Trisma base and 10 mM EDTA. An equal volume of 0.12 M KCl was added to the mixture which was allowed to stand in water bath 60°C for 10 min, and then cooled at 4°C for 30 min; to precipitate SDS associated nucleoproteins and genomic DNA. The mixture was then centrifuged at 8000 g for 5 min. DNA present in the supernatant were measured by mixing 50 μ L of the supernatant with 150 μ L of Hoechst dye at a concentration of 1 μ g/mL in 0.4M NaCl, 4 mM sodium cholate and 0.1 M tris-acetate pH 8.5. Fluorescence was then assessed using 360 nm (excitation) and 460 nm (emission) filters. DNA quantification was measured with standard solutions of Salmon sperm DNA. Results were expressed as μ g DNA/mg of proteins.

Glutathione S-transferase

Glutathione S-transferase activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as the co-substrate. A volume of 50 μ L of S15 was mixed with 1 mM GSH and 1 mM CDNB substrate in 50 mM Hepes-NaOH, pH 7.4, containing 100 mM NaCl. The mixture was incubated at 15°C for 0, 5, 15, 25 and 45 min. Activity was expressed by the increase in absorbance at 340 nm per min per milligram of protein.

Statistical analyses

The data were expressed as the mean with the standard error. In each experience, differences between control and groups of fish exposed to Mercier groundwater were evaluated

by one-way analysis of variance (ANOVA) followed by *post-hoc* test ($P \leq 0.05$). The calculations were performed using Statistica for Windows (Version 7.0, StatSoft Inc., 1995). Correlation was performed using Pearson test. Discriminant function analysis was performed to determine the well distance characteristics. Significance was set at $P \leq 0.05$.

Results

Chemical analysis

Among the 61 volatile organic compounds measured, vinyl chloride (4 μ g/L), trans-1,2-dichloroethylene (2 μ g/L), 1,1-dichloroethane (0.4 μ g/L), 1,2-dichloroethane (2 μ g/L), cis-1,2-dichloroethene (0.37 μ g/L) and benzene (0.29 μ g/L) were found at concentrations above the detection limit at 2.7 km (Table 2).

Morphological parameters

Neither trout weights, lengths nor CF and HSI showed any significant differences in sampling sites after 4 and 14 days of exposure time (Table 3). However, significant correlations were found between weights and CF ($r = 0.91$, $P \leq 0.05$), between lengths and CF ($r = 0.96$, $P \leq 0.05$) in groups at 5.4 km.

Biomarker responses

Phase I and II biotransformation activities

EROD activity was significantly increased in fish exposed to groundwater at 1.2 km (50%)

and 5.4 km (3.1%) following 4 and 14 days exposure respectively reaching 1.8-fold inductions. However, a significant inhibition in EROD activity was observed in fish exposed to groundwater at 2.7 km for all times of exposure at (3.1%) and (50%) concentrations (Figure 2). GST activity in the liver of fish exposed at 3.1% groundwater concentration from the site located at 2.7 km raised significantly after 4 days, reaching 2.1-fold relative to control (Figure 3). However, at (50%) groundwater concentration, GST activity returned to 1.3-fold with respect to control suggesting saturation. Indeed, measurement of GST activities following 14 days exposure to groundwater at (3.1%) and (50%) revealed no significant effects although the mean activity reached 1.2 and 1.6-fold respectively compared to control. The samples collected from groundwater at 1.2 km and 5.4 km did not trigger the increase in GST activity compared to control.

Biomarkers of tissue damage

Lipid peroxidation expressed by TBARS levels in liver of trout were no significantly different compared to control trout (Figure 4). This result was observed for all concentrations tested. Positive correlation was obtained between LPO and GST ($r = 0.88$, $P \leq 0.05$) in group at 5.4 km (Table 4). Exposure of trout to groundwater at 1.2 km caused a significant elevation of DNA strand breaks reaching 1.2-fold after 4 days exposure at (50%) concentration and (2-fold) after 14 days exposure at (3.1%) water concentration (Figure 5). At 5.4 km distance from the lagoons, a much

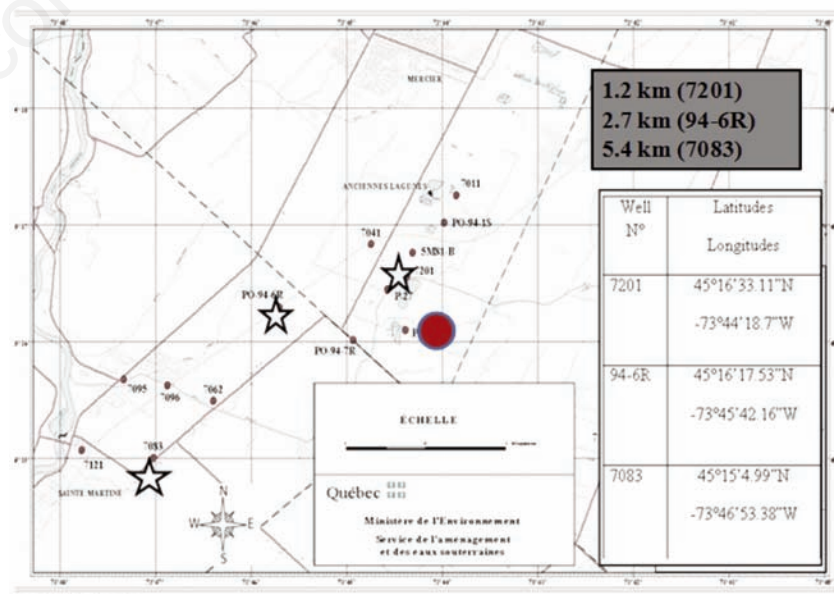


Figure 1. Map with sampling points around Mercier city in campaign may 2010. The wells exposure 7201, 94-6R and 7083 are located at distances (1.2, 2.7 and 5.4 km) from the lagoons. (Modified Map provided by the Ministry of Sustainable Development, Environment and parks of Quebec, 2009).

stronger induction (reaching 3.5 fold induction at 50%) compared to control was observed after 14 days. However, DNA Strand breaks were somewhat lower (1.0 and 1.3 fold) in liver of fish exposed to 3.1% and 50% groundwater respectively at 2.7 km for 4 days. A slight raise was observed after 14 days exposure (1.57 and 2.02 fold) compared to control. In addition, significant correlation was found in groups at 2.7 km between DNA strand breaks and LPO levels ($r=0.99$, $P 0.05$) (Table 3). However, negative correlation was between DNA strand breaks and EROD activity ($r=0.91$, $P 0.05$) (Table 4).

Discriminant function analysis

In the aim to describe the biochemical effects of chronic exposure (14 days) to contaminated groundwater on the liver of rainbow trout, discriminant function analyses were performed (Figure 6). The main biomarkers were identified on X and Y-axis. Discrimination function analysis of the biochemical responses revealed that all three wells at 2.7 km, 5.4 km and 1.2 km and the controls were correctly classified: 81%, 83%, 62% and 100% correctness respectively. EROD was the main biomarker that discriminated the sites.

Discussion

The aim of this study was to investigate the biochemical effects of exposure to groundwa-

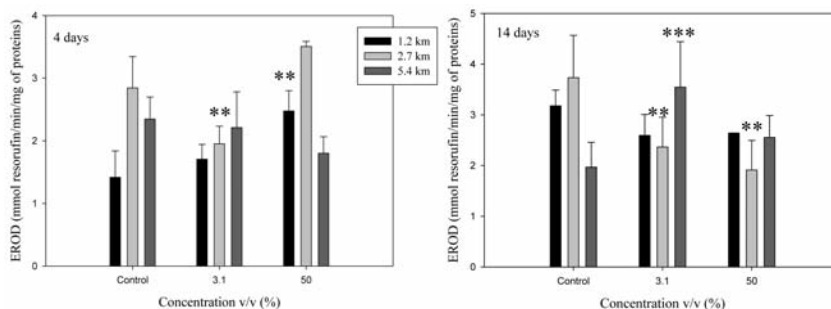


Figure 2. Ethoxyresorufin O-deethylase (EROD) activity in trout liver after 4 and 14 days exposure to groundwater at 1.2 km, 2.7 km and 5.4 km. Data are presented as mean±standard error. Asterisks indicate significant difference from controls (** $P<0.01$; *** $P<0.001$).

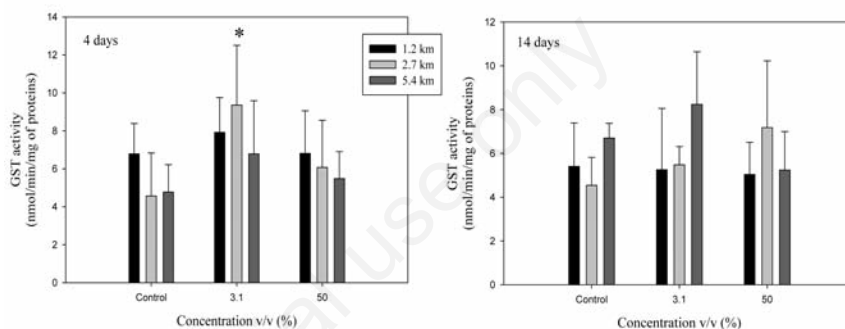


Figure 3. Glutathione S-transferase activity in trout liver after 4 and 14 days exposure to groundwater at 1.2 km, 2.7 km and 5.4 km. Data are presented as mean±standard error. Asterisks indicate significant difference from controls (* $P<0.01$).

Table 3. Morphometric data of trout exposed to groundwater at 1.2 km, 2.7 km and 5.4 km.

Well	Measures	4 days of exposure			14 days of exposure		
		Control	3.1%	50%	Control	3.1%	50%
1.2 km	Weight (g)	27±8	32±9	30±4	31±8	33±4	31±6
	Length (cm)	13±2	14±2	14±1	13±1	14±1	13±1
	Factor condition (FC)	1.3±0.2	1.0±0.1	1.1±0.1	1.4±0.2	1.3±0.1	1.5±0.2
	Hepatosomatic index (HIS)	1.1±0.3	1.2±0.8	0.8±0.3	1.2±0.6	1.2±0.4	1.1±0.3
2.7 km	Weight (g)	31±6	33±9	35±7	32±9	31±6	33±8
	Length (cm)	13±1	13±1	13±1	14±1	13±1	13±1
	Factor condition (FC)	1.3±0.1	1.3±0.2	1.4±0.2	1.4±0.1	1.4±0.0	1.4±0.1
	Hepatosomatic index (HIS)	1.0±0.3	1.1±0.6	1.2±0.4	1.1±0.6	1.0±0.3	1.3±0.6
5.4 km	Weight (g)	33±9	36±4	34±9	38±7	33±3	34±6
	Length (cm)	15±1	16±1	15±1	14±1	14±0	14±1
	condition (FC)	1.1±0.1	1.0±0.1	1.0±0.1	1.1±0.1	1.3±0.1	1.4±0.0
	Hepatosomatic index (HIS)	1.1±0.7	1.1±0.2	1.2±0.3	1.1±0.3	1.0±0.2	1.1±0.3

Values reported as mean±standard error.

Table 4. Biomarkers correlation in rainbow trout exposed for 4 and 14 days to Mercier groundwater at 1.2, 2.7 and 5.4 km.

	DNA damage	GST activity	Lipid peroxidation	EROD activity
DNA damage	-	-	0.99* (2.7/4d)	-
Lipid peroxidation	-	0.88* (5.4/14d)	-	-
EROD activity	-0.91* (2.7/14d)	-	-	-

GST, glutathione S-transferase; EROD, ethoxyresorufin O-deethylase. *Only significant correlations are shown ($P 0.05$).

ter at increasing distance from an industrial dumping site (Mercier lagoons) in rainbow trout and determine the behavior of the contamination plume in groundwater.

The concentrations of volatile organic compounds (*e.g.*, benzene, dichloroethane and vinyl chloride) measured in samples at 2.74 km were 0.29 $\mu\text{g L}^{-1}$, 2 $\mu\text{g L}^{-1}$ and 4 $\mu\text{g L}^{-1}$ respectively, which indicates that the plume is more concentrated at this distance (Table 2). The presence of VOCs in the groundwater could lead to serious health threats. The chronic exposure of organisms to low levels of VOCs can cause carcinogenesis,³⁸ *i.e.* vinyl chloride and benzene are widely recognized human carcinogens.^{39,8} However, DNA damage was more important at the closest and farthest sampling distance (*i.e.* 1.2 and 5.4 km). Hence, the complexity of the fate and transport of chemical mixtures in groundwater makes it difficult to suggest causative chemicals responsible for the observed effects.

The CF and HSI of the trout were homogeneous in all exposure groups which removes size-related influences in the biomarker responses. It also suggests that the observed response were not immediately threatening at the fish morphological level. Fish from polluted environments generally show an increase in the HSI, exposition to PAHs or others substances which involved biotransformation leads normally to an elevation of this index.⁴⁰ However, no significant increase in HSI was observed after 14 days of exposure time. May be the exposure period was not long enough to see HIS variations.

The data obtained in this study would suggest the presence of chemicals able to activate the Ah receptor. Indeed, EROD activity of groundwater exposure, were significantly enhanced over fish from the control at 1.2 km (50%) and 5.4 km (3.1%), but was reduced at 2.7 km for all times of exposure (at 3.1%) and (50%) concentrations. The reduction of EROD activity in fish at site 2.7 km could be due to hepatotoxic damage which may inhibit the liver cells production of this enzyme or we hypothesized the presence of the blocker (antagonist) for AhR which reduce the enzyme activity mediated normally by AhR in trout. Whyte *et al.*⁴¹ suggested that toxicity caused by xenobiotics accumulation and their metabolites might inhibit enzyme production leading to an inhibition of EROD activity. Biotransformation of vinyl chloride, dichloroethane and benzene, the chemicals identified in groundwater samples at 2.7 km, are under cytochrome P450 2E1,⁴² as well as through glutathione conjugation.^{43,44} Trans and cis 1,2-dichloroethylene inhibit their own metabolism *in vivo* by inactivation of the metabolizing enzyme presumably the CYP 450 isoform CYP 2E1.¹⁶ The induction of GST activity as for CYP450 is regulated, in part by the Ah receptor.²⁵ Indeed, an increase

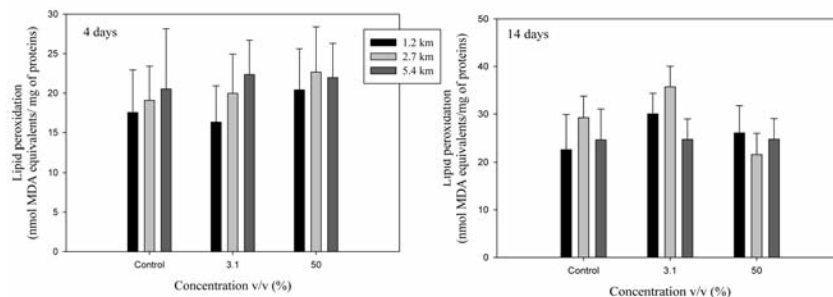


Figure 4. Lipid peroxidation in trout liver after 4 and 14 days exposure to groundwater at 1.2 km, 2.7 km and 5.4 km. Data are presented as mean \pm standard error. No significant difference was observed (ANOVA $P > 0.05$).

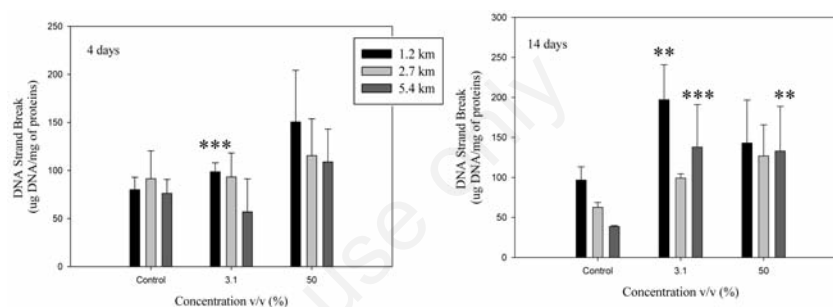


Figure 5. DNA strand break in trout liver after 4 and 14 days exposure to groundwater at 1.2 km, 2.7 km and 5.4 km. Data are presented as mean \pm standard error. Asterisks indicate significant difference from controls (** $P < 0.01$; *** $P < 0.001$).

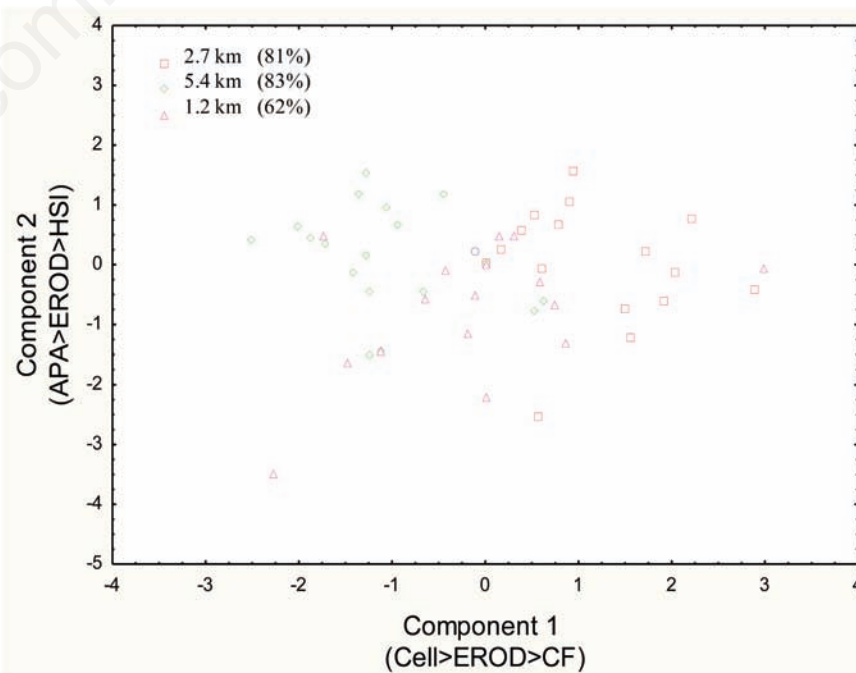


Figure 6. Results for discriminant function analyses for 14 days exposure to Mercier groundwater at 1.2 km, 2.7 km and 5.4 km. CF, condition factor; HSI, hepatosomatic index; Cell, cellularity; ethoxyresorufin-*O*-deethylase, APA and EROD activity.

in hepatic GST activity has been reported in several studies after fish exposure to PAHs, PCBs and certain VOCs.^{17,45,46} Various substrates involved in GST activity are recognized by the binding site of hydrophobic substrates.²⁷ Nevertheless, the enzyme GST has been reported to respond differently to different substances. For example, Otitoloju *et al.*¹⁸ reported that enzyme was inhibited by benzene, while Qian *et al.*⁴⁷ reported increased GST activity in liver of Crucian carp (*Carassius auratus*) injected with chlorobenzene. In our study, a significant increase displayed in the phase II biotransformation in trout exposed to 3.1% groundwater at 2.7 km after 4 days exposure. The GST response to groundwater after 4 days exposure in all groups shows a bell-shaped trend with an initial increase in activity, but significantly only at 2.7 km. Accordingly, the low enzyme activities after 14 days in fish from 1.2 km, 2.7 km and 5.4 km could be associated with deficiency to compensate for oxidative stress, possibly due to high levels of pollutant exposure.⁴⁸ Since LPO was not significantly affected and was not related to GST activity, we cannot support the argument that decreased GST activity was associated to oxidative stress.

Oxidative stress can be mediated by numerous organic contaminants, including, halogenated hydrocarbons, PAHs, and dioxins.¹⁸ In the current study, LPO levels in trout exposed to groundwater at all distance increased slightly even if non-significantly. Although LPO was positively correlated to GST, the change in GST activity seemingly did not influence LPO under analysis of covariance.

DNA damage was measured by the levels of soluble strand breaks and can be used as tools to monitor genotoxicity in organisms.⁴⁹ At 4 days exposure, an initial increase in DNA strand breaks was observed in trout exposed to 50% groundwater at 1.2 km. Although, after 14 days of exposure to groundwater at 5.4 km, the amount of DNA were more elevated (reaching 3.4-fold) with respect to control. In fact, Devaux *et al.*⁵⁰ observed that the chub (*Lenciscus cephalus*) caught in Rhone River close to an industrial area presented DNA damage in erythrocytes. Genotoxic effect seems to be reflected by metabolites effects of chemicals on DNA, including their capabilities to lead to DNA strand. According to Gwinn *et al.*⁹ which reported that EDC, with its strong electrophilic affinity to DNA appears to induce DNA damage. Vinyl chloride oxidation produces an epoxide intermediate which reacts with a variety of cellular nucleophiles including DNA and glutathione (GSH).¹⁶ Mattes *et al.*⁸ reported likewise that DNA damage of vinyl chloride is due to the formation of the extremely reactive epoxide chlorooxirane during the oxidative metabolism of VC by CYP 450 in the liver. Unfortunately, we did not measure CYP2E1 activity in this study. However, DNA damage is

not correlated with EROD ($r = 0.91$, $P < 0.05$), this suggests that VOCs are associated with genotoxicity.

Conclusions

The data revealed that exposure to contaminated groundwater stimulated both phases I and II biotransformation activities as evidenced by EROD and GST activities. The water samples were also genotoxic which was seemingly not related to oxidative stress given the lack of LPO changes. This study provides some insights on the toxicity of groundwater contaminated by industrial waste to rainbow trout.

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