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Exposure of *Daphnia magna* to trichloroethylene (TCE) and vinyl chloride (VC): Evaluation of gene transcription, cellular activity, and life-history parameters

Magali Houde [a,*], Mélanie Douville [a], Pierre Gagnon [a], Jim Sproull [b], François Cloutier [c]

[a] Environment Canada, Science and Technology Branch, Centre Saint-Laurent, 105 McGill Street, Montreal, QC, Canada H2Y 2E7
[b] Environment Canada, Science and Technology Branch, Prairie and Northern Laboratory for Environmental Testing, 5320 122 St. NW, Edmonton, AB, Canada T6H 3S5
[c] Environment Canada, Environmental Protection Operations Directorate, 105 McGill Street, Montreal, QC, Canada H2Y 2E7

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ABSTRACT

Trichloroethylene (TCE) is a ubiquitous contaminant classified as a human carcinogen. Vinyl chloride (VC) is primarily used to manufacture polyvinyl chloride and can also be a degradation product of TCE. Very few data exist on the toxicity of TCE and VC in aquatic organisms particularly at environmentally relevant concentrations. The aim of this study was to evaluate the sub-lethal effects (10 day exposure; 0.1; 1; 10 μg/L) of TCE and VC in *Daphnia magna* at the gene, cellular, and life-history levels. Results indicated impacts of VC on the regulation of genes related to glutathione-*S*-transferase (*GST*), juvenile hormone esterase (*JHE*), and the vitelline outer layer membrane protein (*VMO1*). On the cellular level, exposure to 0.1, 1, and 10 μg/L of VC significantly increased the activity of JHE in *D. magna* and TCE increased the activity of chitinase (at 1 and 10 μg/L). Results for life-history parameters indicated a possible tendency of TCE to affect the number of molts at the individual level in *D. magna* ($p=0.051$). Measurement of VG-like proteins using the alkali-labile phosphates (ALP) assay did not show differences between TCE treated organisms and controls. However, semi-quantitative measurement using gradient gel electrophoresis (213–218 kDa) indicated significant decrease in VG-like protein levels following exposure to TCE at all three concentrations. Overall, results indicate effects of TCE and VC on genes and proteins related to metabolism, reproduction, and growth in *D. magna*.

1. Introduction

Trichloroethylene (TCE) is a highly volatile organic compound (VOC) with a global utilization as degreasing agent for automotive and metal industries as well as minor usage in dry cleaning, textile manufacturing, and production of adhesives and co-polymers (CCME, 2007). Several degradation products are known for TCE including both isomers of dichloroethylene (DCE) and vinyl chloride (VC) (Olaniran et al., 2004). Vinyl chloride is also an industrial solvent primarily used to manufacture polyvinyl chloride. TCE and VC are on the List of Toxic Substances of the Canadian Environmental Protection Act (CEPA, 2014) and a priority pollutants listed by the US Environmental Protection Agency (USEPA, 2014a, 2014b). Industrial discharges, landfill leaching, improper storage/disposal as well as atmospheric transport and deposition are important routes of entry in the aquatic environment for these two VOCs (CCME, 1999).

TCE tends to evaporate to the atmosphere following its release onto soil surfaces. Depending on the nature of the substrate and the frequency of the releases, TCE can also gradually penetrate into subsurfaces and reach low conductivity layers (Schwille, 1988). The vertical migration of TCE into soil, its absorption by soil particles and the creation of a TCE pool in the saturated zone can act as TCE contamination sources that will gradually dissolve into the groundwater. TCE has been reported in concentrations up to 90 μg/L in surface water across Canada (CCME, 2007) and was one of the most frequently detected chlorinated solvents in groundwater in the United States (Moran et al., 2007). High levels (i.e., up to 21 000 μg/L) of TCE have also been widely found in groundwater from specific landfill, disposal, industrial, and superfund sites across North America (CCME, 2007). These results led to important environmental concern for long-term exposure to humans and aquatic organisms. Estimated half-lives for TCE were up to 12 days in freshwater ecosystems (Lay et al., 1984) and 28 days in the

* Correspondence to: Centre Saint-Laurent, Environment Canada, 105 McGill Street, Montreal, QC, Canada H2Y 2E7. Fax: +1 514 496 7398.
E-mail addresses: magali.houde@ec.gc.ca (M. Houde), melanie.douville@ec.gc.ca (M. Douville), pierre.gagnon@ec.gc.ca (P. Gagnon), jim.sproull@ec.gc.ca (J. Sproull), francois.cloutier@ec.gc.ca (F. Cloutier).

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marine environment (Wakeham et al., 1983).

Most toxicity studies for TCE and VC have been conducted on mammalian models which have shown carcinogenic effects in rodents and epidemiologic data indicated that TCE may also cause cancer in humans (Chiu et al., 2013; US EPA, 2000). Additionally, exposure of avian cells and embryos have shown toxicity of TCE on cardiac output (Drake et al., 2006; Makwana et al., 2010). In aquatic organisms, impacts of TCE and VC on algal growth (Brack and Rottler, 1994; Lukavský et al., 2011; Nam and An, 2010) and of TCE on the density and chlorophyll content of phytoplankton (Bácsi et al., 2012) have been found. Moreover, studies have reported oxidative stress in freshwater clams (Vidal et al., 2001), metabolic perturbations during fish embryogenesis (Viant et al., 2005) and cellular changes in rainbow trout chronically exposed to TCE (Heining and Hoffman, 1993). These tests were conducted using concentrations above (i.e., mg/L) the 5 μg/L maximum allowable concentrations for TCE in drinking water and the Canadian Water Quality Guidelines for the Protection of Aquatic Life established at 21 μg/L (Health Canada, 2005).

Considering the continuous release of TCE and VC in aquatic ecosystems and the lack of information on the impacts of these solvents at environmentally relevant concentrations, a study was designed to evaluate the toxicity of these VOCs on the gene transcription, the cellular activity, and the life-cycle (i.e., growth, molting, reproduction, and survival) of *Daphnia magna. D. magna* is a widely distributed freshwater crustacean and a model organism in ecotoxicology. This zooplankton is well representative of lower trophic level organisms. Chemical analyses of TCE and VC and related metabolites were conducted in parallel to monitor degradation and ensure adequate exposure of *D. magna*.

2. Material and methods

2.1. Chemical analyses

The stability of testing conditions at concentrations ranging from 1.0 to 0.0001 μg/L of vinyl chloride (CAS 75-01-4) and trichloroethylene (CAS 79-01-06) were studied at various times (0–48 h) using a standardized method for volatile organic compounds (PNLET, 2011). The presence of possible breakdown products such as 1,1-dichloroethylene, trans-1,2-dichloroethylene, and cis-1,2-dichloroethylene were also monitored in solutions.

Solutions of TCE at each concentration level was prepared in water. These aqueous solutions were prepared by adding a small volume of a methanol aliquot of the analytes to the water such that the methanol concentration never exceeded 0.1%. Eleven time periods were investigated (in h): 0, 1, 2, 3, 4, 5, 6, 8, 24, 30, 48. Each beaker was covered with a watch glass and left at 20 °C (Daphnia Testing temperature). At the sampling time point 10 mL was subsampled and dispensed into a headspace vial which was sealed with a septum cap and crimp cap for analysis by gas chromatography with mass spectrometric detection using a Headspace Sampler. Stock standard solutions of TCE, VC, 1,1-dichloroethylene, trans-1,2-dichloroethylene, and cis-1,2-dichloroethylene were obtained from Supelco (Bellefonte, PA, USA) and were diluted in methanol and water. The methanol used was Omnisolv HR-GC grade from EMD Millipore (Billerica, MA, USA) and the water used was City of Edmonton tap water treated to remove chlorine. This water is used for culturing and toxicity testing of *D. magna*.

The analytical method used is based on US EPA method 8260 for VOCs by Gas Chromatography/Mass Spectrometry. The concentration of TCE and VC in water were determined by gas chromatography with mass spectrometric detection using an Agilent 6890 gas chromatograph (GC), Agilent 5973 Mass Spectrometric Detector (MSD) and EST Markelov HS 9000 Headspace Sampler (Table S1). The GC column was a 60 m DB-624, 0.32 mm I.D., 1.8 μm film thickness with helium carrier gas and the following temperature program and settings: Inlet temperature 150 °C, Initial temperature 40 °C, Initial time 3.00 min, ramp 8 °C/min to 90 °C, hold at 90 °C for 4.00 min, ramp 6.00 °C/min to 120 °C. The MSD temperature settings were Transfer line 250 °C, Quad 150 °C, and MSD Source was held at 230 °C. The MS detector was operated in the Positive Ion Electron Impact. Selected Ion Monitoring (mass-to-charge ratio, m/z) and detection limits for the analytical method used are listed in Table S2.

As required at each time period and for each concentration level studied, 10 mL of water sample was placed into a vial and heated in the platen of the headspace sampler at 90 °C for 10 min after which an aliquot was injected into the gas chromatograph. VOCs in samples were identified by comparing the retention times and detector responses of peaks in the sample ion chromatograms to the retention times and responses of analyte peaks in standard ion chromatograms. The standard ion chromatograms were obtained by analyzing a series of calibration standards under the same conditions as the samples and these were used to calibrate the instrument and quantitate the analyte levels in the samples. Method blanks were analyzed with the samples and results indicated that there was no carry-over or cross-contamination.

2.2. Culture maintenance

Genetically homogenous *D. magna* were cultured in growth chamber following Environment Canada's method (Environment Canada, 1990). Cultures were kept at 20 ± 1 °C with a photoperiod of 16 h light: 8 h dark. Organisms were fed green algae *Pseudokirchneriella subcapitata* (concentration: 3.85×10^5 cells/mL) and YCT preparation (yeast–cerophyll–trout chow, concentration: 0.0125 g/L) every day. All experiments were performed under the same constant temperature and diurnal lighting conditions.

2.3. Toxicity assays

For sub-lethal exposure, neonates (< 24 h) from 21 day-old females were exposed for 10 days to both solvents following OECD guidelines (OECD, 2008). This period includes the different instar stages and the production of the first brood. Chronic exposures to TCE and VC (concentrations of 0.1, 1, and 10 μg/L) were performed in a biological hood using four groups of 10 daphnids in sealed containers. Concentrations were based on environmental levels detected in river water (Quebec, Canada) (Laliberté, 2005, 2010). Culture medium was used as a control group. Two additional control groups of daphnids (one for TCE and one for VC) were exposed to methanol as the commercial standards used were diluted in this solvent at different percentages (TCE: 0.0002%; VC: 0.0005%). Considering the volatile properties of the studied compounds, renewal of the media was conducted every 24 h in order to ensure the continuous exposure of *D. magna*. New stock solutions were prepared at every media renewal and water temperature, conductivity, dissolved oxygen, pH, and hardness were monitored.

Time of first molt, number of molts, time of first brood, and number of offspring were monitored each day and used as reproductive parameters. Growth was evaluated with the body length ($n = 10$/treatment) which was defined as the distance from the upper edge of the compound eye to the base of the tail spine and evaluated using a digital image analyzing system (Leica M165c Stereomicroscope, Wetzlar, Germany). Six replicates of 2–3 *D. magna* per treatment were preserved in RNALater for genomics and the same quantity stored at −80 °C for biomarker analyses.