Science of the Total Environment 408 (2010) 3148–3152

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Assessing the ecotoxicity of vinyl chloride using green alga *P. subcapitata*, nematode *C. elegans*, and the SOS chromotest in a closed system without headspace

Sun-Hwa Nam, Youn-Joo An *

*Department of Environmental Science, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, Republic of Korea*

ARTICLE INFO

*Article history:*
Received 8 July 2009
Received in revised form 8 March 2010
Accepted 13 March 2010

*Keywords:*
Vinyl chloride
Ecotoxicity
*Pseudokirchneriella subcapitata*
*Caenorhabditis elegans*
SOS chromotest

ABSTRACT

The ecotoxicity of vinyl chloride (VC) was evaluated using green alga, nematode, and the SOS chromotest. The green alga and nematode tested were *Pseudokirchneriella subcapitata*, and *Caenorhabditis elegans*, respectively. Because of the tendency of VC to escape from an aqueous exposure medium to the air phase, all tests in the present study were performed in a closed system without headspace to minimize the losses of VC. Previous studies on VC toxicity were performed in an open system or closed system with headspace. VC inhibits the growth of *P. subcapitata*. The 48-h IC50 value for *P. subcapitata* exposed to VC was calculated to be 5.15 mg/L. The survival of *C. elegans* was not influenced at concentrations of up to 60 mg/L; however, VC has an adverse effect on the reproduction of *C. elegans*. In a stress-related gene expression test using *C. elegans*, a significant and concentration-dependent expression of heat shock protein 16.2 was observed, indicating that VC induces the stress of *C. elegans* at the genetic level. The results of the SOS chromotest using *Escherichia coli* PQ37 showed an $IF_{max}$ value of 1.11, indicating that VC is not genotoxic. The present study demonstrated that VC has an adverse effect on the algal growth and reproductive and genetic levels of *C. elegans*. A closed system without headspace is an effective method of testing the aquatic toxicity of volatile organic compounds such as VC.



## 1. Introduction

Vinyl chloride (VC) is a known human carcinogen (WHO IARC, 2007; US EPA IRIS, 2000; ECB ESIS). VC does not occur naturally, and is produced commercially by chemical reactions of hydrogen chloride and acetylene (ATSDR, 2006). VC is primarily used to manufacture the polymer polyvinyl chloride (PVC) (ATSDR, 2006; Gu, 2003; PIMs, 1997); PVC materials are broadly used in pipes, furniture, construction materials, and packaging products (ATSDR, 2006).

VC is a daughter compound from the breakdown of chlorinated organic solvents, such as tetrachloroethylene (PCE), trichloroethylene (TCE), and *cis*-1,2-dichloroethylene (*cis*-1,2-DCE) (ATSDR, 2006; Gu, 2003). PCE and TCE are widely used as degreasing agents and dry cleaning fluids; therefore, some sites contaminated by PCE and TCE may be also polluted by VC metabolized by the bacterial degradation of these solvents (ATSDR, 2006; Gu, 2003). In particular, VC is generally more toxic than PCE, TCE, or *cis*-1,2-DCE (WHO IARC, 2007). Based on the assessment of the Integrated Risk Information System (IRIS), the International Agency of Research on Cancer (IARC), and the European Chemical Substances Information System (ESIS) (WHO IARC, 2007; US EPA IRIS, 2000; ECB ESIS), it is carcinogenic via several routes of exposure (oral, inhalation, and dermal).

Some countries have established standards for VC levels in ambient water quality or drinking water (US EPA, 2006a, b; Ministry of the Environment, Japan, 2006; WHO, 2006). For instance, the U.S Environmental Protection Agency (EPA) recommends that the national water quality criteria of VC for human health are 0.025 μg VC/L for consumption of water and organism, and 2.4 μg VC/L for consumption of aquatic organism only (US EPA, 2006b). The guideline value of VC for human health is 2 μg/L in Japan (Ministry of the Environment, Japan, 2006). The World Health Organization (WHO) suggests a VC guideline of 0.3 μg/L for drinking-water quality (WHO, 2006). A drinking-water standard of 2 μg/L for VC was presented by the US EPA (US EPA, 2006a). The main potential source of VC in drinking water is PVC water pipes with high VC content (WHO, 2006). VC has been detected in a concentration range of 0.8–4.7 μg/L in four major rivers in Korea (MEV, NIER, 2006).

When VC is released in water, it is expected to have an adverse effect on aquatic organisms. To the best of our knowledge, the available data for ecotoxicity of VC are extremely limited, including studies on 6 fish, 2 arthropoda, 2 protozoa, 1 nematoda, and 1 plant species, from late the 1970s to the mid 1990s (OECD SIDS, 2001; Euro chlor, 1999; Nam, 2008; EC, 2000; Brown et al, 1977; Sauvant et al., 1995a, b, c). Toxicity to fish was evaluated for *Brachydanio rerio*, *Lueciscus idus*, *Lepomis macrochirus*, *Micropterus salomoides*, *Oryzias latipes* (OECD SIDS, 2001) and *Esox lucius* (Brown et al., 1977), The 96-h median lethal concentration (LC50) values in fish assays were in a range of 210 (for *B. rerio*) to 1,220 (for *L. macrochirus*) mg/L (OECD

* Corresponding author. Tel.: +82 2 2049 6090; fax: +82 2 2201 6295.
*E-mail address:* anyjoo@konkuk.ac.kr (Y.-J. An).

0048-9697/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2010.03.022

SIDS, 2001). The 48-h LC50 using the arthropoda *Moina macrocopa* and *Gyraulus convexiusculus* was reported to be greater than 20 mg/L and 10 mg/L, respectively (Nam, 2008). Effect on protozoa were tested with *Uronema parduczi* (EC, 2000) and *Tetrahymena pyriformis*, and 36-h median inhibitory concentration (IC50) value for *T. pyriformis* was calculated to be 520 mg/L (Sauvant et al., 1995a, b, c). Toxicity tests using nematode *Panagrellus redivivus* (Euro chlor, 1999) and green algae *Scenedesmus quadricauda* (OECD SIDS, 2001) has been performed, but since the toxicity data were obtained in an open system or a closed test unit with a headspace, they did not seem to consider the partitioning of VC from the aquatic phase to the gas phase. There is a high possibility that the previous toxicity data of VC for aquatic organisms could be underestimated due to the decreased exposure concentration in the water phase. For this reason, a suitable test method is needed for assessment of volatile VC and to produce reliable ecotoxicity data for it.

In the present study, the effects of VC were assessed using *Pseudokirchneriella subcapitata* and *Caenorhabditis elegans*. The genotoxicity of VC was assessed by a stress-related gene expression test using *C. elegans* and the SOS Chromotest using *Escherichia coli* PQ37. All tests were conducted in a closed system without headspace to prevent the loss of VC out of the aqueous phase. The selection of bioassays was based on the following criteria. First of all, the species used in this study (e.g. *P. subcapitata* and *C. elegans*) were not used in previous tests of VC's aquatic toxicity. Second, the genome of multicellular organism *C. elegans* has been completely sequenced. We focused on the genetic effect of the maximum exposure concentrations of VC with a stress-related gene expression test. Accordingly, we conducted the SOS chromotest, keeping the genetic toxicity of VC in mind.

## 2. Experimental

### 2.1. Test chemicals

Vinyl chloride ($C_2H_3Cl$, 99.9% purity; Sigma-Aldrich, St. Louis, MO, USA) was obtained as a methanolic solution of 2000 μg/mL, which is commercially available. It was diluted in a suitable exposure media of each test species to obtain a range of exposure concentrations of VC. The selected physicochemical properties of VC are listed in Table 1. The maximum exposure concentrations of VC correspond to the maximum acceptable concentration of methanol for each test species.

The reagents used for the stress-related gene expression test were TRIzol reagent (Invitrogen, USA), isopropanol (Amresco, USA), 10× DNase I buffer (Takara Bio Inc., Japan), DNase I (RNase-free) (Takara Bio Inc., Japan), phenol–chloroform–isoamyl alcohol mixture (Fluka, Switzerland), Oligo dT primers (Promega, USA), PCR PreMix solution (Bioneer, Korea), primers (Bioneer, Korea), and ethidium bromide (EtBr) (Amresco, USA). The reagents for SOS Chromotest are *O*-nitrophenyl-β-galactopyranoside (ONPG), *p*-nitrophenyl-phosphate disodium (PNPD), tris(hydroxymethyl) aminomethane, and 4-nitriquinoline 1-oxide (4NQO), all purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

**Table 1**
Physicochemical properties of vinyl chloride (PIMs, 1997; US EPA, 2000).

| Property | Vinyl chloride |
|---|---|
| CAS registry no. | 75-01-4 |
| Molecular formula | $C_2H_3Cl$ |
| Molecular weight | 62.5 |
| Color/form | Colorless gas |
| Odor | Mild ethereal odor |
| Boiling point (°C) | −14 |
| Melting point (°C) | −154 |
| Vapor pressure (mm Hg at 20 °C) | 2530 |
| Water solubility (mg/L at 25 °C) | 2400 |
| Henry's law constant (atm $m^3$/mol at 25 °C) | 0.0278 |
| Log Koc | 1.376 |
| Log Kow | 1.62 |
| BCF | 3.55 |
| Half-life (hour) | 360 |

### 2.2. Algal assay

*P. subcapitata* (UTEX 1648) was obtained from the Korea Institute of Toxicology (KIT) and the Korea Research Institute of Chemical technology (KRICT) in Daejeon, Korea. It was grown in an algal medium as in OECD guideline 201 (OECD, 2006) for several months. The growth inhibition of *P. subcapitata* was tested for 48 h in OECD algal medium, according to the method of Blaise and Ferard (2005) and Mayer et al. (2000), with slight modifications. The test duration of 48 h was set to guarantee maximum growth and to stabilize the pH (Mayer et al., 2000). All cultures were shaken in a rotary shaker kept at 24 ± 2 °C, pH 8.0 ± 1.0 and 100 rpm under continuous fluorescent light (approximately 4000 lx). Precultures were shaken in the 250-mL borosilicate glass flasks with air-permeable stoppers for the exponential growth phase. Test cultures were kept in a U-bottom plate (ID 7 mm, height 10 mm, volume 0.3-mL for each well) with a silicon septum and cover, and the wells were completely filled with exposure solution. The initial algal density of $3 \times 10^4$ cells/mL was inoculated in each well in five replicates. The cells were counted with a hemacytometer (Paul Marienfeld GmbH&Co.KG, Lauda-Königshofen, Germany) under a stereomicroscope (Motic SMZ-168) at 400-power magnification. The exposure concentrations were 0.625, 1.25, 2.5, 5, and 10 mg VC/L. The 0.5% v/v methanolic solution was used as a solvent control. The preliminary experiment proved that 0.5% v/v methanolic solution has a negligible effect on the growth of *P. subcapitata* (data not shown).

### 2.3. Nematode assay

*C. elegans* (wild type, Bristol strain N2) were taken from Animal Genomics laboratory, Konkuk University in Seoul, Korea, having been harvested in nematode growth medium for several months. *E. coli* strain OP50 was supplied as a food source. Previous to the test, eggs in old K-agar plates were placed on new ones and cultured in a dark incubator at 20 ± 1 °C for 3 days. Using these synchronized adult worms, a 72-h toxicity test was performed in K-medium (with *E. coli* strain OP50) in accordance with the method of Williams and Dusenvery (1990), with the following modifications. The ten individuals were transferred to a flat-bottom plate (ID 17 mm, height 17 mm, volume 3.8-mL for each well) in triplicate. The exposure solution completely filled up the wells, and the plate was covered with a silicon septum and lid. The measurement was performed with a needle to identify the dead species under a dissecting microscope. Before the test, solvent control of VC was established with 3% v/v methanolic solution, considering the negligible effect of methanol on the survival of *C. elegans*. The exposure concentrations of VC were 10, 20, 30, 40, 50, and 60 mg/L.

### 2.4. Stress-related gene expression test

*C. elegans* exposed to VC for 72 h was used in a stress-related gene expression test. The exposure concentrations of VC were 10, 20, 30, 40, 50, and 60 mg/L. The solvent control was 3% v/v methanolic solution. The Ribonucleic acid (RNA) isolation of *C. elegans* was prepared by using the TRIzol reagent, chloroform, isoprophanol, 75% ethanol, and RNase-free water (diethylpyrocarbonate, DEPC) according to a standard protocol from Animal Genomics laboratory, Konkuk University in Seoul, Korea. The deoxyribonucleic acid (DNA) elimination from total RNA was conducted with 10× DNase I buffer, DNase I (RNase-free), DEPC, phenol–chloroform–isoamyl alcohol mixture, sodium acetate, chilled ethanol, and 70% ethanol following the manufacturer's standard instructions (Takara Bio Inc., Japan). The