NORTH-WESTERN JOURNAL OF ZOOLOGY 8 (2): 358-361
Article No.: 121204

http://biozoojournals.3x.ro/nwjz/index.html

Toxicity tests of chlorinated hydrocarbons on the river mussel, *Unio crassus* (Bivalvia, Unionidae)

Andrei SÁRKÁNY-KISS[1,*], Ildikó HERCZEG[2], Barbara PALOMBI[2], István GRIGORSZKY[2], László ANTAL[2], István BÁCSI[2], Attila MOZSÁR[2], Attila Ferenc KALMÁR[2] and Sándor Alex NAGY[2]

1. Apáthy István Society, Cluj-Napoca, Romania, Str. Gheorghe Dima nr. 33., ap. 18.
2. Department of Hydrobiology, Faculty of Science and Technology, University of Debrecen, H-4010 Debrecen, Egyetem sqr. 1., PO Box 57., Hungary.
* Corresponding author, A. Sárkány-Kiss, E-mail: esarkany@gmail.com

Received: 31. May 2011 / Accepted: 22. April 2012 / Available online: 31. May 2012 / Printed: December 2012

Abstract. The toxicity of three highly volatile organic solvents – tetrachloroethane ($Cl_2HC–CHCl_2$), tetrachloroethylene ($Cl_2C=CCl_2$), trichloroethylene ($ClHC=CCl_2$) – was studied on *Unio crassus* specimens. The tested chlorinated hydrocarbons sink to the bottom of standing water bodies due to their high density; therefore they represent an increased risk for benthic organisms. For a start, all three compounds were applied in 100% saturation. In the case of tetrachloroethylene and trichloroethylene all specimens died within a few days. In the case of tetrachloroethane the intoxication process was much faster, all the specimens died within 4 hours. Tetrachloroethane proved to be the most toxic compound; its diluted solutions were further tested: in the 5% solution all *U. crassus* specimens died after 24 hours. The 1% solution had a strong disturbing effect on the behaviour of the bivalves, which was gradually moderated and eventually stopped on the 33rd day. These results suggest that the examined chlorinated hydrocarbons are strongly toxic to living organisms even in aquatic medium and cause rapid death even in low concentrations.

Keywords: chlorinated hydrocarbons, mussels, toxicity test.

Tetrachloroethane ($Cl_2HC–CHCl_2$), tetrachloroethylene ($Cl_2C=CCl_2$) and trichloroethylene ($ClHC=CCl_2$) were or are used in a wide range of industries as components of paints and pesticides, or as solvents (Lewis 2001). These chemicals are highly effective fat solvents, thus can easily penetrate into the cells of different organisms, therefore they are very strong poisons. As a consequence of their physical and chemical characteristics (small molecular mass, volatility and considerable lipophilicity) all the three chlorinated hydrocarbons are absorbed quickly after their contact with a living organism. In mammals they damage especially the liver cells (Gavino et al. 1983, Sano & Tappel 1990), even their vapours inhaled causing serious intoxication (Toxicological profile for tetrachloroethylene, 1997, Toxicological profile for trichloroethylene, 1997, Toxicological profile for 1,1,2,2-tetrachloroethane, 2008).

Their water solubility differs notably and is generally low. Having higher density than water, they sink to the bottom of standing water, from where they continuously get back, damaging the aquatic organisms (Leighton & Calo 1981, Thomas 1990).

The literature regarding the potential effects of chlorinated hydrocarbons on aquatic ecosystems is relatively scarce. However, it can be presumed, that their harmful effects on aquatic vertebrates are comparable with the symptoms observed in terrestrials vertebrates – as already shown in the case of several fish species (Roberts et al. 1987, Mather-Mihaich & DiGiulio 1991). The specific effects of chlorinated hydrocarbon pollutants on aquatic invertebrates and phytoplankton are not unknown (Pearson & McConnell 1975, Bringmann & Kühn 1980, Ward et al. 1986, Håkanson 1999, Ando et al. 2003, Lukavsky et al. 2011); some organic contaminants causing oxidative stress in molluscs (Cheung et al. 2002), being detectable in the tissues of mussels and oysters (O'Connor et al. 2002, Glynn et al. 2003).

Nowadays, due to the unsuitable storage of industrial waste, chlorinated hydrocarbon contaminations of soil and groundwater are frequent. Certain reports show that there is an increasing risk of appearance of these contaminants (mainly tetrachloroethane, tetrachloroethylene and trichloroethylene) in surface waters due to the movement of polluted groundwater (Santerra Soil Remediation, Project SANT 0901, 2009; Mid-Danubian Environmental and Water Authority, Tender Report, 2010).

The aim of this work was to study the effects of three short-chain chlorinated hydrocarbons on the typical benthic organism *Unio crassus*. We also wanted to find out whether *Unio* species are sensitive test organisms for these highly volatile, low water soluble compounds, which – despite of their physicochemical properties – are referred to as possible contaminants of aquatic environments.

In the course of the laboratory tests, six–eight years old *Unio crassus* specimens were used. The test animals were collected from the Târnava Mică River (Kis-Küküllő, Transylvania, Romania), in early winter (with the permit of the Romanian National Agency for Environmental Protection – Mures, 53/05.10.2011). After collection, the 70 individuals were acclimatised to 16 °C water. This temperature was maintained during the experiments.

To test the toxicity of the three substances in different concentrations their 100 litres aquatic solutions were used. Sediment was not placed into the aquariums, in order not to modify the effects of the chemicals.

For the first rough toxicity tests, 100 % saturated solvents were prepared. To reach the 100 % saturation 9.4 ml tetrachloroethylene, 93.24 ml trichloroethylene, respectively 177.99 ml tetrachloroethane were mixed with tap water. Considering the initial results, tetrachloroethane was used in the further tests in 5 % (8.81 ml / 100 l water), and 1 % solution (1.76 ml / 100 l water). Aquariums with untreated tap water served as controls. 10 – 10 specimens were placed into each aquarium except the case of 5 % and 1 % tetrachloroethane solutions, where only five – five individuals were used, because more specimens could reduce the accuracy of the observations and note takings, especially in very short intervals.

During the experiments the activity of the incurrent and excurrent siphons was observed, and their state was marked on a scale of 0-4. The dead individuals were noted as "0"; "1" was used for closed siphon; "2" was used when siphons were open a crack, "3" meant siphons opened to mid-position; "4" meant fully open siphons; furthermore the relaxed muscle that moved when touched was also noted as "0.5". Besides observing the activity of siphons, the motion of the shells and their opened or closed state, moreover the motion of the foot were also noted.

The untreated specimens ordinarily kept their incurrent and excurrent siphons fully open (4) and actively coursed the water through the mantle cavity. Every 5 – 10 minutes they powerfully closed the siphons (1), then they immediately opened them tight. From time to time they opened the shells and pushed out their foot and moved actively. Certain individuals occasionally opened the siphons only a crack (2) or to mid-position (3) (Fig. 1, 2; open circles).

In the 100 % saturated tetrachloroethylene solution more specimens stretched the foot, trying to escape_in the first minutes. Shortly they convulsively closed the shells, the foot got stuck and they were not able to pull it back anymore. Although in the first hour two specimens opened the siphons a crack, they closed it soon again. Five specimens died on the fifth day, other two - two individuals died on the fifth and sixth day and the tenth individual perished on the seventh day (Fig. 1; filled squares).

In the case of 100 % saturated trichloroethylene solution all the specimens closed the shells and siphons as a reaction. Four days later the muscles of seven individuals relaxed, they opened the shells and died. Till the end of the fifth day the remaining three individuals died also (Fig. 1; filled triangles).

The bivalves in the 100 % saturated tetrachloroethane solution immediately closed the shells and siphons and remained motionless. Two and a half hours later five individuals relaxed the muscles, opened the shells and died. Three and a half hours later another specimen died, after four hours another three and after four and a half hours the last individual perished as well (Fig. 1, 2; filled diamonds). The fast progress of the process shows that the intoxication was very strong.

The individuals placed in the 5% saturated tetrachloroethane solution were very active in the first 50 minutes, opened and closed the siphons; moreover some individuals opened the shells and stretched the foot. On the second day all the individuals opened the shells, stretched their foot and died (Fig. 2; filled squares). Contrary to the previous cases, the set of death occurred simultaneously in all individuals.

In the 1% saturated tetrachloroethane solution the activity of the bivalves approached much more the behaviour of the control individuals. However, under the effect of the toxic material the specimens closed the siphons, but later they opened them repeatedly and coursed actively the solution through the mantle cavity. During the 35 days of observation, the activity of the individuals became more and more similar to the untreated ones (Fig. 2; filled triangles). From the 33rd day the individuals behaved the same way as the control ones.

The literature about the effects of chlorinated hydrocarbons on molluscs, especially mussels is scarce. Studies focus on polychlorinated biphenyls (PCBs), chlorinated pesticides (CPs) or dichlorodiphenyltrichloroethane (DDT) mainly in marine environment (Cheung et al. 2002, O'Connor et al. 2002, Glynn et al. 2003). On the other hand, there are studies focusing on the effect of nutrients – especially nitrate and ammonia enrichment – on freshwater mussels (Patzner & Muller 2001, Aug-

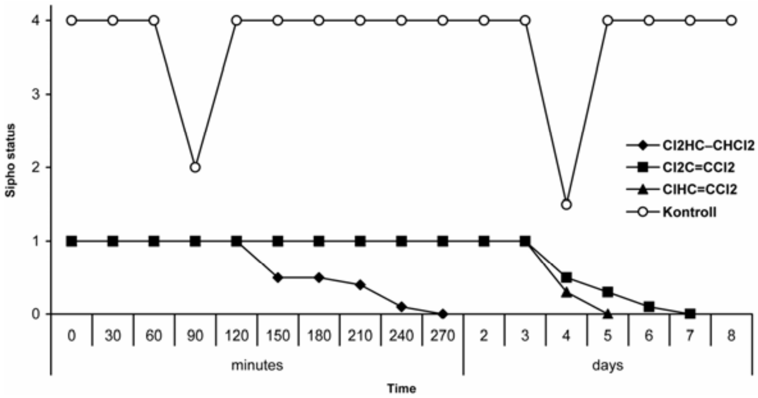

Figure 1. The behaviour of *U. crassus* bivalves in control and 100 % saturated tetrachlorethylen, trichloroethylene and tetrachloroethane solvents.

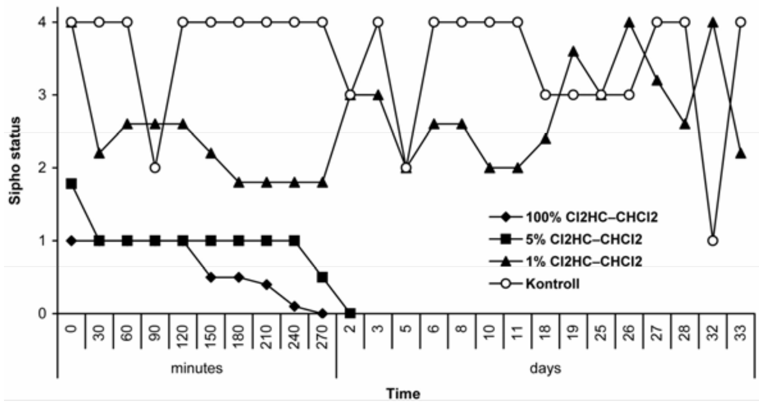

Figure 2. The behaviour of *U. crassus* bivalves in 100 %; 5 % and 1% saturated solution of tetrachloroethane.

spurger et al. 2003, Wang et al. 2007, Douda 2010). There is much less information about the effects of the short-chain chlorinated hydrocarbons on molluscs in continental aquatic habitats (Kruse & Kruger 1989, Thiebaud et al. 1994).

Our results clearly demonstrate that all three studied chlorinated hydrocarbons are strongly toxic to the tested organism. In addition, the behaviour of the bivalves was markedly altered by the chlorinated hydrocarbons. Siphon activity is commonly used as a toxicity warning system because it provides preliminary information about the magnitude of the toxicity. Englund & Heino (1996), after examining the effects of trichlorophenol on *Anodonta anatine*, and failing to find a connection between treatments and siphon activity, concluded that the activity of siphons depends on many factors. In our experiments, the saturated chlorinated hydrocarbon solutions had strong effects within a short time on the test animals.

Tetrachloroethane showed the strongest toxicity; therefore the experiments were continued with its diluted solutions, even the 5 % saturated tetrachloroethane solution causing irreversible damage. The 1 % solution had a strong disturbing effect on the *U. crassus* specimens in the first days, but from the 19th day the activity of the individuals markedly approached the control ones; from the 33rd day they behaved the same way as the control individuals. It can be concluded, that the toxic effects were reversible in the case of this highly diluted solution. As tetrachloroethane is a highly volatile chemical, we assume that the concentration could have decreased to a great extent during the observation period, which permitted the reestablishment of the state of the test animals close to normal.

These preliminary experiments prove that *U.*

crassus is a very sensitive indicator of the tested organic solvents. Further experiments are needed to establish the effective concentration (EC) and inhibitory concentration (IC) values of the compounds as well as to determine their concentration and distribution in the mussel tissues. On the basis of these early data it can be concluded that *Unio* species are suitable for indicating chlorinated hydrocarbon contamination even in very low concentrations.

Acknowledgements. This work was supported by TÁMOP-4.2.2-08/1-2008-0012.

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