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Effect of Some Monocyclic Aromatic Hydrocarbons on Freshwater Invertebrates

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Petrochemical compounds frequently get into organic industry wastewaters, and, through them, to surface and underground waters. If the concentration of compounds is high, the biodegradation is disabled and the compounds persist in the soil and the water for a long time (Evans et al. 1991; Mc Lelland 1991).

Petrochemical compounds have a strong toxic effect on aquatic organisms. It is known that hydrocarbons are rapidly taken up by gill tissues and through them to all other organs (Cairns et al. 1976). By autoradiography Lee et al. (1977) demonstrated that radioactive aromatic compounds were not metabolised by the molluscs. Crustaceans also lack the ability to metabolise aromatic hydrocarbons (Lee et al. 1977). As a result of that the aromatic compounds were stored in the digestive gland of this invertebrate. In contrast, vertebrate systems have enzymes in the microsomes of the liver that hydroxylate aromatic compounds (Cairns et al. 1976).

There is data on the influence of petrochemical compounds on aquatic organisms (Cairns et al. 1976; Fogels and Sprague 1977; Das and Konar 1988; Holcombe et al. 1987; Moring and Rose 1997; Metcalfe et al. 1997; Pelletier et al. 1997; Sheedy et al. 1998), but not many deal with monocyclic aromatic hydrocarbons (Erben 1978; Capuzzo et al. 1988; Vittozzi and De Angelis 1991; Erben and Pišl 1993). That is why we chose, for our research, petrochemical compounds from the group of monocyclic aromatic hydrocarbons. All of them consist of the basic hexagon ring with three aromatic bonds that cause these compounds to be unreactive.

Acute toxicity of 10 aromatic hydrocarbons was tested during 96 hr (Parrish 1985). We wanted to find out how toxicity depends upon the position of different radicals on the benzene ring. We studied toxic influence of the mentioned compounds on four freshwater invertebrates, crustaceans *Asellus aquaticus* L. and *Gammarus fossarum* Koch. and snails *Amphimelania holandri* Fer. and *Viviparus viviparus* L., inhabiting freshwaters throughout Europe.

Nowadays lots of attention is paid on the estimation of danger caused by production, distribution, usage and storage of chemical compounds. It is necessary

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to know about their characteristics and possible toxic effects that they could have on the organisms in the nature. That is the reason to find which concentrations of which chemical compounds are a real or potential danger for aquatic and terrestrial ecosystems.

We have frequently witnessed the dying of various animal species after the accidental spills of petrochemical compounds from the petrochemical industry in Zagreb into the river Sava and other waterways. In this paper we, therefore, attempted to extend our earlier examinations (Erben 1978; Erben and Pišl 1993) of toxic influence of aromatic hydrocarbons on freshwater invertebrates.

MATERIALS AND METHODS

In the experiment we used as test organisms freshwater snails (*A. holandri* FÉR. and *V. viviparus* L.) and freshwater crustaceans (*A. aquaticus* L. and *G. fossarum* KOCH.). The animals were collected from the streams and rivers on the outskirts of Zagreb. The chemical analysis of waters has shown that water was free from petrochemical compounds and other toxicants. The animals were acclimated to laboratory conditions in dechlorinated tap water for 24 hr. The specimens, chosen for the experiment, were healthy and in good condition.

The experiments were carried out in aerated glass dishes, each containing 2 L of dechlorinated tap water. All dishes were covered with glass plates. There were 10 animals per dish, and the number of dishes corresponded to the number of concentrations examined. One dish was used as a control and received dechlorinated tap water only. The test solutions and control water were renewed daily (semi–static test). The experiment lasted for 96 hr and it was repeated 3 times. The animals' condition and behaviour were also checked daily and the animals were not fed during the experiment.

The following aromatic hydrocarbons were used in the experiment: 1,2,4-trichlorobenzene ($C_6H_3Cl_3$), dichlorobenzene ($C_6H_4Cl_2$), chlorobenzene (C_6H_5Cl), styrene (C_8H_8), α-methylstyrene (C_9H_{10}), benzene (C_6H_6), ethylbenzene (C_8H_{10}), trimethyl benzene (C_9H_{12}), xylene ($C_{24}H_{30}$) and toluene (C_7H_8). All were obtained form INA-OKI Zagreb, Croatia (organo-chemical industry) and were 99% pure. Each compound was added to the test dishes by stirring it evenly into the water with a glass rod.

Temperature, hardness and pH of the test solutions and control water were determined every 24 hr by routine procedure (APHA 1985). Physico-chemical characteristics of the solutions were as follows: temperature 18±1°C; pH 7,0-7,8; hardness 324,8-359,0 mg $CaCO_3$/L.

Statistical processing of data was carried out by means of the Probit method. The relationship between the Probit values derived from the percent of deaths and logarithms of concentrations and time was established through multivariate linear regression analysis (Wardlaw 1987).

RESULTS AND DISCUSSION

All the species used in this research are beta-mesosaprobic indicators (Sladeček 1973) that live in relatively clean waters, free of the petrochemical compounds examined. The animals were well adapted to laboratory conditions and there was no mortality in the control group during the experiment. Animals were not fed during the experiment (Carr and Neff 1981; Stuart and Robertson 1985).

We used compounds that are partly soluble in and lighter than water so they were floating on the surface, making a thin layer. Therefore the aeration was introduced in all the tests to prevent a double action – toxicity and disabling gas exchange (Das and Konar 1988). Without aeration the water would quickly become turbid, particularly when animals die; a large number of bacteria appear, and a skin-like layer appears on the surface of the water which also decreases the exchange of gases (Erben 1982; Evans et al. 1991). In the case of snails, this surface layer was even thicker due to the mucus produced by the animals.

The measured values of pH and total hardness, in our tests, do not allow concluding about their effects on the change in toxicity of the examined compounds.

All the animals involved in our experiments showed behavioural changes that were consistent with other reports (Bhale et al. 1989; Vittozzi and De Angelis 1991; Rice et al. 1997). During the acute toxicity test at lower concentrations, the snails were more active than normal and reacted to a needle sting in the foot by a fast retraction into the shell. At higher concentrations, the snails were inert and reacted more slowly to irritations. The day before death, the snails turned feet up and reacted to mechanical provocations only with slight movements. The crustaceans also reacted with behavioural changes. During intoxication all the crustaceans showed various degrees of excitement and convulsive reactions being typical reactions to the unfavourable conditions.

One of the aims of our research was to find how toxicity depends upon the type and the position of different radicals on the benzene ring. Our results (Table 1) show that the most toxic agents are the aromatic hydrocarbons with chlorine as a radical. From the ones without chlorine the most toxic was α-methylstyrene. Toluene and xylene had the lowest toxicity. In general, toxicity of different compounds differs between the species; the freshwater crustaceans were more sensitive than the freshwater snails. We suppose that the greater mass of snails and the strength of their shells afford better protection than the thin chitin cuticle of the freshwater crustaceans.

Increases in concentration caused increased mortality, except for toluene, benzene, α-methylstyrene, chlorobenzene, 1,2,4-trichlorbenzene with *A. holandri* and toluene and xylene with *V. viviparus*. Increases in the time of exposure caused increased mortality, as expected (Parrish 1985; Vittozzi and De Angelis 1991).

Table 1. The mean of the LC_{50} values for the examined species estimated by Probit method.

Species	Compound	LC_{50} % v/v			
		24 hr	**48 hr**	**72 hr**	**96 hr**
Gammarus fossarum	1,2,4-Trichlorobenzene	0,015	0,003	0,001	0,0006
	Dichlorobenzene	0,017	0,006	0,003	0,002
	Chlorobenzene	0,030	0,015	0,010	0,007
	Styrene	0,275	0,049	0,018	0,009
	α-methylstyrene	0,055	0,045	0,040	0,037
	Benzene	0,237	0,067	0,032	0,019
	Ethylbenzene	0,102	0,060	0,044	0,035
	Trimethyl Benzene	0,171	0,073	0,044	0,031
	Toluene	0,170	0,103	0,070	0,057
	Xylene	0,156	0,100	0,078	0,065
Asellus aquaticus	1,2,4-Trichlorobenzene	0,031	0,014	0,009	0,007
	Dichlorobenzene	0,037	0,017	0,011	0,008
	Chlorobenzene	0,040	0,027	0,020	0,016
	Styrene	0,123	0,078	0,069	0,057
	α-methylstyrene	0,294	0,168	0,121	0,096
	Benzene	0,357	0,186	0,126	0,096
	Ethylbenzene	0,337	0,227	0,180	0,152
	Trimethyl Benzene	0,459	0,280	0,210	0,171
	Toluene	0,606	0,321	0,221	0,170
	Xylene	0,469	0,278	0,204	0,164
Amphimelania holandri	1,2,4-Trichlorobenzene	0,208	0, 143	0,115	0,099
	Dichlorobenzene	0,159	0,052	0,027	0,017
	Chlorobenzene	0,590	0,452	0,101	0,030
	Styrene	0,702	0,249	0,136	0,089
	α-methylstyrene	0,621	0,270	0,165	0,117
	Benzene	1,543	1,097	0,205	0,164
	Ethylbenzene	2,963	1,185	0,693	0,474
	Trimethyl Benzene	0,806	0,575	0,472	0,410
	Toluene	3,250	1,755	1,223	0,947
	Xylene	1,269	0,910	0,749	0,653
Viviparus viviparus	1,2,4-Trichlorobenzene	1,900	0,567	0,279	0,169
	Dichlorobenzene	0,621	0,223	0,123	0,080
	Chlorobenzene	0,738	0,305	0,182	0,126
	Styrene	5,663	3,208	2,300	1,831
	α-methylstyrene	5,646	3,679	2,864	2,398
	Benzene	5,908	4,608	3,984	3,493
	Ethylbenzene	6,484	4,972	4,256	3,812
	Trimethyl Benzene	6,896	5,290	4,530	4,058
	Toluene	31,680	12,690	7,439	5,090
	Xylene	21,878	10,427	6,760	4,970

There was a strong and positive correlation between the concentrations of all the compounds examined and the mortality of freshwater crustaceans. Also, there was a positive and strong correlation between the time of exposure and mortality for all the concentrations chosen. The weakest correlations were found for the concentration of 0,03% v/v of toluene for *G. fossarum* (r = 0,7746) and 0,1% v/v α-methylstyrene for *A. aquaticus* (r = 0,8733).

For freshwater snails there is a strong and positive correlation between the mortality of animals and the concentrations of all the compounds examined except toluene and xylene for *V. viviparus,* and toluene, benzene, α-methylstyrene, chlorobenzene and 1,2,4- trichlorobenzene for *A. holandri*. Also there is positive and strong correlation between the time of exposure and mortality for all the concentrations chosen. The weakest correlations for *A. holandri* (r = 0,8944, r = 0,8866, r = 0,8540) were found for the 0,07% v/v concentration of 1,2,4-trichlorbenzene, 0,2% v/v ethylbenzene, 1% v/v toluene, respectively. For *V. viviparus* the weakest correlations (r = 0,8944, r = 0,7746) were found for the 1% v/v concentration of styrene and 1,5% v/v concentration of α-methylstyrene, respectively.

In the natural water ecosystem, the evaporation of aromatic hydrocarbons will depend upon the temperature of the water, air pressure, wind, water currents etc. (Hites and Eisenreich 1987) and that is not a case in the experimental conditions. In the laboratory, the system is closed and the animals have constant contact with the toxicant. The first contact with the toxicants caused considerable poisoning of the animals, creating a thin film on the surface of their bodies (Erben and Pišl 1993). The same occurs in natural conditions in the case of sudden discharge of petrochemical compounds into the recipient (i.e. discharge from factories, ships, cisterns etc.).

Simultaneous multiple species testing allows direct comparison of species sensitivity, since each species is exposed to nearly identical conditions within each exposure tank and it allows side-by-side comparisons of behavioural effects between species (Holcombe et al. 1987). Future studies should focus on the behavioural toxicology bioassay because it may be valuable in comparing and predicting the mode of action of new or unknown toxicants in freshwater invertebrates.

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