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Detection Limits of a Biological Monitoring System for Chemical Water Pollution Based on Mussel Activity

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Based on their ability to accumulate a variety of chemicals, mussels have been widely adopted as useful indicators of pollutant contamination in chemical surveillance programs (PHILIPS 1976; GOLDBERG et al. 1978; DAVIES + PIRIE 1980; NAS 1980; JENSEN et al. 1981). However, sentinel organisms living close to input sources of pollutants may respond rapidly to changes in pollutant flux. Several studies have shown that some bivalves are capable of detecting high pollutant levels and are able to avoid pollutant stress by valve closure (SALANKI + VARANKA 1978; MANLEY + DAVENPORT 1979). Although such effective avoidance behaviour may interfere with the results of accumulation monitoring programs, the rapidity and reversibility of the valve closure suggest that this response may be of use in detecting arising toxic conditions of the water.

In the present paper an automatic early warning system based on the valve response of the fresh water mussel, Dreissena polymorpha, is described. Results on the sensitivity of the system to various chemicals are presented and compared to those of a monitoring system based on fish respiration.

MATERIALS AND METHODS

Indicator Organisms. The fresh water mussel Dreissena polymorpha was chosen, as this species has a widespread occurrence in Dutch surface waters and is used for accumulation studies (MARQUENIE 1981). Moreover, in contrast to most bivalves, it shows no periodic activity (SAMANT + AGARWAL 1978). Specimens, 3.0 -3.5 cm in length, were collected from the storage basin of the water works of the city of Dordrecht (The Netherlands). The mussels were cleaned and subsequently maintained under laboratory conditions prior to experimentation. During this acclimatization period (3 days minimally) the organisms were fed with green algae (Chlorella spec.).

Monitoring Apparatus. The monitoring system is based on the detection of the behaviour of mussels in terms of shell valve movements, using an electronic device developed by TNO-Central Laboratory (SCHURING + GEENSE 1972). In this device one valve is fixed, whereas the other valve transfers its action to a coil

moving in an oscillating electromagnetic field generated by a fixed coil. The current in the receiving coil is subsequently converted to a voltage proportional to the valve displacement.

In order to automate the system, the voltages indicating the valve displacements of 6 mussels were compared to a threshold value corresponding with a 0.25 mm gap between the shell halves. The information on the open or closed position of the valves were transformed to logical 1 or 0 levels compatible with a 6 bit parallel input port of a simple digital computer (Explorer-85, Netronics R & D Ltd.). The interface card also provides a 30 sec pulse to a single bit port to serve a software interrupt in timing the sample interval. The computer program was written in Microsoft BASIC, allowing a choice of response evaluation. In the experiments a mussel was considered to respond to pollutant stress when the valves were closed for 10 times the sample interval, which corresponds with approx. 5 min. Any moment at which at least 4 out of 6 animals were responding, an alarm condition was recognized. Additionally, at regular intervals the percentage of time the individual mussels were closed, as well as the rhythmic activity, was printed on a Teletype model 38 ASR.

Experimental Design. For each toxicity test a group of 6 mussels was placed in a glass basin containing Dutch Standard Water (CANTON + SLOOFF 1982) of 18 $\pm$ 1°C that was circulating at a flow of 240 L/h. After recovery from the stress as a result of the handling, the toxicants were added at a constant rate by an injector system (perfusor V, Braun). The same chemicals as have been used in an earlier study on fish respiration were tested (SLOOFF 1979). Some of these chemicals were chosen because they are being discharged in the rivers or shipped in large quantities, others were selected to represent different chemical groups. To avoid solubility problems different stock solutions, test volumes (5-20 L) and dosing speeds were used, depending on the chemical tested. If necessary, the stock solutions were prepared with dimethylsulfoxide (DMSO), adding maximmaly 0.13 mL DMSO/L (<10% of the detection limit). To arrive at an appropriate test duration, the dosing rate was calculated to reach a level of the LC_{50} value for fish (Brachydanio rerio; SLOOFF 1979) within 8 h.

Each chemical was tested in tripliclate to determine the detection limit, which was defined as the lowest test concentration than causes an alarm. To study the possibility of the occurrence of adaptation to chemicals, mussels were held in solutions of cadmium chloride and pentachlorophenol at a concentration of 10% of the detection limits for 4, 8 and 16 days under continuous flow conditions, the test concentration was increased continuously to evoke an alarm situation.

During the experiments the actual concentrations were not measured and all data presented are based on nominal concentrations of the test chemicals.

RESULTS

In Table 1 the detection limits of the biological monitoring system are given for the test chemicals as determined in three test runs. The maximum difference between the results of the test runs varies from a factor 1.4 for trichloroethylene to 3.7 for pentachlorophenol with a mean of 2.6. This indicates a variability comparable to that of common acute toxicity tests. At the start of the experiments the valves were gaping continuously.

After a certain period of toxicant addition, the animals initially showed a testing behaviour, characterized by a gradual increase of valve closure periods. Finally, they responded by a total closure of the shell valves. For most compounds, the concentration range at which the testing behaviour occurred was generally narrow. A broad range of testing behaviour is reflected by a relatively high variability in the test results (e.g. chloroform, pentachlorophenol).

From Table 2 it is evident that the threshold concentration may increase significantly for some compounds by adaptation or intoxication as a result of pre-exposure to low toxicant levels. For instance, the sensitivity of the mussel system to Cd^{2+} decreased up to a factor 4 after pre-exposition.

Table 1. The sensitivity of a biological monitoring system based on the valve closure response of mussels to toxicants. A, B and C represent the detection limit as measured in three test runs, arranged in increasing order.

Test compounds	Detection limit of the monitoring system in mg/L		
Cadmium (chloride)	0.20	0.35	0.56
Copper (sulphate)	0.018	0.025	0.046
Cyanide (potassium)	0.40	0.59	0.60
1,3-Dichlorobenzene	1.4	1.4	4.3
Chloroform	43.2	125.7	150.2
Phenol	14.6	17.8	46.9
Pentachlorophenol	0.14	0.36	0.52
Toluene	6.1	10.0	12.1
Xylene	11.9	17.2	19.4
Trichloroethylene	8.0	9.7	11.4
Hexachlorobutadiene	0.15	0.23	0.41
γ-Hexachlorocyclohexan	0.06	0.11	0.16

Table 2. Effect of pre-exposure to Cd^{2+} (0.033 mg/L) and PCP (0.033 mg/L) on the detection limit of these compounds.

	Detection limit of the monitoring system in mg/L			
Pre-exposure time (days)	0	4	8	16
Cadmium (chloride)	0.37	0.73	1.63	1.44
Pentachlorophenol	0.32	0.62	0.45	0.60

DISCUSSION

All chemicals tested resulted in a valve closure response of Dreissena polymorpha. The observations on the reaction to isolate itself from da effects of toxic concentrations confirms that this organism is capable acting as an efficient indicator of chemical pollution. Comparing the results with those previously obtained with an early warning system ba respiration of trout (Salmo gairdneri) (SLOOFF 1979), which is conside the most sensitive system presently available (VAN HOOF 1980), the mussel appears to be slightly less susceptible to most compounds (Table 3). Comparing the data on cadmium, copper and cyanide with those obtained from experiments with other warning systems with fish, crustaceans, algae, and bacteria (VAN HOOF 1980), the mussel system mostly yields lower detection levels. In all cases a lower threshold was found for copper, an important trace element in the heme pigment of molluscs. A high response rate to copper was also found by MANLEY + DAVENPORT (1979), who observed

Table 3. A comparison of the average detection limits of early warning systems based on respiratory frequency of fish (SLOOFF 1979) and valve response of mussels.

Compounds	Detection limit in mg/L		
	Fish	Mussels	Ratio
Copper (sulphate)	0.06	0.03	0.5
Trichloroethylene	5	9.7	1.9
γ-Hexachlorocyclohexane	0.04	0.11	2.8
Toluene	2.5	9.4	3.8
Cyanide (potassium)	0.13	0.53	4.1
1,3-Dichlorobenzene	0.5	2.4	4.8
Pentachlorophenol	0.07	0.34	4.9
Hexachlorobutadiene	0.05	0.26	5.2
Chloroform	20	106	5.3
Phenol	4	26.4	6.6
Xylene	2	16.2	8.1
Cadmium (chloride)	0.025	0.37	14.8

an initial interruption of the normal valve movements of several marine bivalves at 0.021 - 0.14 mg Cu^{2+}/L. According to SALANKI + VARANKA (1976) even an extraordinary low level as 10 ng $CuSO_4$/L (!) resulted in a decrease of the duration of the active periods of the fresh water mussel Anodonta cygnea. The detection limit (Table 2) may be increased by prior exposure to low toxicant levels. A similar observation was made by DAVENPORT + MANLEY (1978), who found an 8-fold decrease of the susceptibility to copper based on the behavioural response of Mytilus edulis, after acclimatization to 0.02 mg Cu^{2+}/L for 10 days. In practice, the biomonitor will be applied in streams that will always contain a variety of toxic compounds. Therefore, to avoid a significant decrease in the sensitivity of the system, a regular and frequent replacement of the mussels is necessary.

Although the presented biomonitor is probbly somewhat less sensitive to toxicants than the system based on fish respiration, there are several reasons in favour of the mussel system. (1) As a consequence of a more direct way of measuring a response, the mussel system is less susceptible to noise problems that may result in false alarms at an unacceptable rate (GRUBER et al. 1980). (2) In contrast with the fish respiration, additional experiments showed that changes in temperature and photoperiod do not interfere with the behaviour of the mussels. This makes it applicable without the necessity to control important environmental conditions. (3) The mussel monitor is more easy to handle as the replacement of the organisms takes less time, no individual control recordings are required and feeding does not interrupt the measurements. (4) Moreover, used in natural waters the mussels are self maintaining. Hence, the mussel system is more feasible for wide-spread industrial applications than the fish respiration system, as it is more reliable and can be installed and operated at much lower costs (ca. 20%).

A point of interest is whether or not the range of environmental toxicant concentrations may initiate a valve closure response and so may bias the results of bioaccumulation studies in possible biological chemical monitoring programmes. In this study the mussel was very sensitive to copper, resulting in a detection limit varying from 0.018 to 0.046 mg Cu^{2+}/L. Taking the levels of copper in the river Rhine for example, the mean concentration of total copper is about 0.01 mg Cu^{2+}/L during the last few years (RIJKSWATERSTAAT, 1980). As the environmental copper levels encountered by the mussels are only slightly less than the concentration range at which valve closure is occurring, it may be expected that in the vicinity of point sources the feeding behaviour of the mussels is interrupted and pollutants may not accumulate to full extent.

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