Bull. Environm. Contam. Toxicol. 23,517-523 (1979)

Detection Limits of a Biological Monitoring System Based on Fish Respiration

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Increased pollution of the surface water has led to the development of more advanced methods for pollution detection aimed at the protection of human health and aquatic life. Most pollution monitoring programmes have still been orientated towards chemical and physical methods. However, these methods do not meet the requirements for an operational surveillance system related to toxic compounds (POELS 1975). Since living organisms will respond to every possible substance or mixture of substances at some level, the biological techniques are playing an increasingly important role in predicting and controlling water pollution (CAIRNS et al 1977). Recently several biological monitoring systems have been reviewed (SLOOFF 1977). Some of these systems show a promising future, such as the systems based on the respiratory activity in fish. Although several automatic recording devices to detect the breathing movements of fish have been proposed (HEATH 1972), the method using dual external electrodes is considered to have the best application possibilities since the fish is allowed a maximum amount of freedom under test conditions (SPOOR et al 1971, WALLER + CAIRNS 1972, MORGAN 1976). This paper discusses the value of such a monitoring system based on several laboratory tests.

MATERIALS AND METHODS

As biological indicator rainbow trout (Salmo gairdneri) was used, obtained from a commercial fish hatchery. After being transported in oxygenated plastic containers to a fish holding facility, the fish were maintained under laboratory conditions for at least two weeks prior to use. During the experiments each fish was kept in a test chamber of approximately 2.5 L, supplied with continuously flowing dechlorinated tap water (20 $\pm$ 1°C, pH: 8.0 $\pm$ 0.2.; dissolved oxygen content: 9.0 $\pm$ 0.5 mg/L; hardness: 3.6 meq/L; flow-rate: 30 l.h-1).

All experiments were carried out under conditions of a fixed natural photoperiod to allow diurnal variation in respiration patterns. Dawn occurred gradually between 6.00 and 7.00 h., dusk between 20.00 and 21.00 h. To minimize disturbances a separate experimental room was constructed, which was closed during the

0007-4861/79/0023-0517 $01.40

tests. The waterflow and the toxicant addition were regulated outside this room. To avoid reponses due to floor vibrations the test chambers were placed on a heavy iron table which rested on a separate concrete foundation. Futhermore all electrical and water connections were flexible to minimize the transmission of wall vibrations.

The test chambers were designed according to the electrode chamber of SPOOR et al. (1971) consisting of a glass tube in which two stainless steel mesh electrodes are placed at the opposite ends, covering the total cross-section of the chamber. From each of these mesh electrodes, one wire is connected to a shielding cable leading to a high quality frequency selective preamplifier, which was operated at a gain of approximately 10.000, amplifying signals between 0.3 and 10Hz. Subsequentely, the amplified signals of each fish alternately pass via a multiplex time switch system to a frequency filter to suppress possible disturbances or large variation in the level of the recorded baseline caused by other movements of the fish. The filter was operated with low and high cut-off frequency limits from 0.5 Hz to 5.0 Hz. At the time of the experiments, the data were collected by a multichannel paperrecorder. At the present stage the system is automatized using a timer counter and a calculator.

Since the respiration patterns at different times of the day for the same fish are heterogeneous, each fish was used as its own standard to allow for individual variations. To compensate for the diurnal variations the critical values were determined as the minimum and maximum respiration frequency during a light and a dark period recorded during one min. each hour after the acclimatization of the fish to the experimental conditions. It was presumed that the fish recovered from the initial stress by being netted and transferred into the test chambers within 3-4 days. After recording the standard breathing signals, the toxicants were added continuously into the waterflow by an injector system during 48 h. In case the respiration frequency of at least three fourth of the test fish exceeded the predetermined individual critical values at the same hourly interval, it was considered as an indication of a toxic condition of the water.

At least three different concentrations of each toxicant were tested to establish the detection limit of the biological monitoring system, which is defined as the lowest concentration at which a toxic condition is developed within 24 h after toxicant administration. To compare the obtained detection limits with common LC_{50} values, additional experiments with zebrafish (Brachydanio rerio) were carried out. For this purpose 10 fishes were exposed for 48 h in 10 L aquaria to each toxicant concentration; the flow rate of these closed dynamic systems amounted to 6 L.h-1. During the experiments the fish were not fed.

and do not reflect much higher exposures which can be tolerated over short time periods such as those associated with spill events. Therefore the scenario according to DAWSON et al. (1975) is used, which is based on a man weighing 70 kg consuming 2.5 L of water a day: LD_{50} in $mg.kg.^{-1}$ (rat) x 70 kg : 2.5 L. Even when the threshold concentrations are assigned by applying a 1000 fold safety factor, most of the selected chemical will be detected before reaching harmful levels for man.

However, the continuous operation of the monitoring system seems to be questionable for most natural waters. Based on the maximum concentrations of 25 organic and 12 inorganic compounds of known toxicity incidentically measured in surface waters, only in 12 cases the toxicity of the compounds would have been potentially detected, whereas in at least 8 of these 12 cases the calamity would have been revealed by the occurrence of fish kills at the same time SLOOFF + ZOETEMAN 1976).

Taking into account these considerations, the monitor will be suitable only to prevent aquatic ecosystems from serious damage by monitoring industrial and domestic effluents, and may be also a helpful tool in protecting to some extent the human health by the capacity to detect many toxicants which may occur in water to be used for drinking water supply at levels which are harmful to mammals. Therefore the most successful application seems to be monitoring waters at strategic points as is shown in figure 1, in conjunction with other biological test systems (e.g. Ames-test for mutagenicity screening) combined with adequate multidedectional chemical monitoring methods.

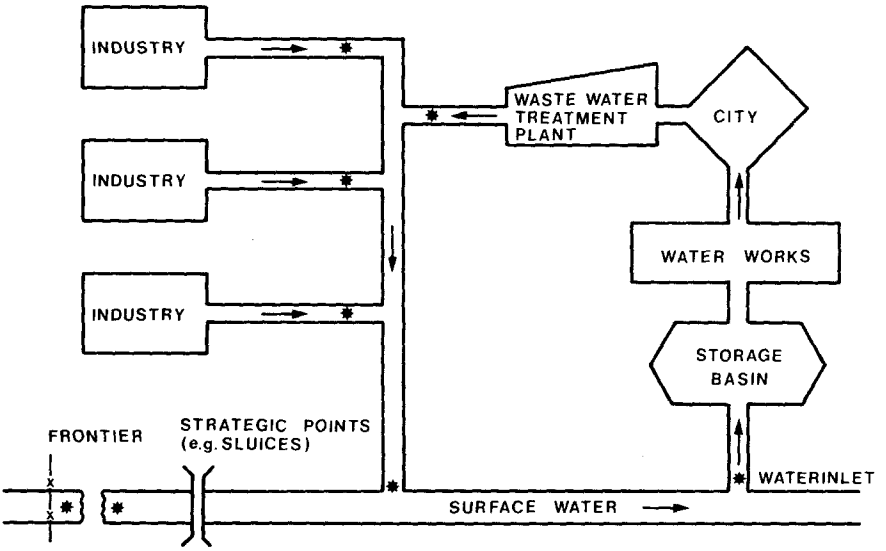

Fig. 1. Schematic presentation of application possibilities for in-stream biological monitoring systems.

RESULTS AND DISCUSSION

In most cases the toxicants caused an increase of the breathing rate of the fish in sublethal concentrations, even up to 2-4 fold the normal rate. Such responses were obtained more often during the dark interval than during the light interval, which is in accordance with the findings of CAIRNS et al (1973). This may be due to the fact that the breathing rates were generally lower and often less variable during the dark interval, resulting in closer upper and lower critical values at night. Besides it is also possible that the sensitivity of the fish to the toxicants is subjected to a circadian periodicity (CAIRNS et al 1973). In table 1 the detection limits of the biological monitoring system of 13 chemicals are compared with the corresponding LC_{50}-values for fish. Considering the fact that the LC_{50}-values are based on exposure times twice as long as used the biological monitoring studies, it is obvious that the described system is a much more sensitive tool in predicting developing toxic conditions of the water, than the common LC_{50} measurements. MORGAN (1976) suggested that the response limit of fish sensors lies between 5 and 10 percent of the 48 h LC_{50}, using a similar biological monitor system. The results of this study indicate a more broad range of these ratio's, varying from 1% (cadmium) to 30% (acrylonitrile, cyanide, lindane) of the 48 h LC_{50}.

The detection limits of the monitor could be related to standards for chronic exposure (CAIRNS et al 1973). For that reason the ratio's (LC_{50}-acute exposure/detection limit) may be compared with the ratio's (LC_{50}-acute exposure/no-toxic effect level), which are shown in table 2. Since a change of the respiratory activity of fish due to toxins has to be regarded as a toxic effect, it will be not surprizing that a major part of chemicals will pass the monitor undetected in quantities which are not completely safe biologically. However, as both ratio's are merely in the order of 1 to 100 it seems likely that the monitor is capable to detect most toxicants at levels which are at the utmost a tenfold of the no-toxic effect level. Nevertheless, when the detection limits are compared with the no-toxic effect levels for the same compounds (table 3), it should be recognized that the system is probably only reliable in the prevention of accidental spills or environmental changes that produce acutely toxic conditions.

Since the biological monitoring system is aimed at the protection of the human health in the scope of drinking water resources as well, the results are also compared with literature data on LC_{50}- values for rats (table 1). In comparison with these data the monitor was capable to detect the selected compounds far below the critical levels for rats. To extrapolate these results to man, existing drinking water limits are not appropriate, since they are designed to protect against chronic ingestion of toxicants

TABLE 1

THE RELATIVE SENSITIVITY OF A BIOLOGICAL MONITORING SYSTEM WITH DUAL EXTERNAL ELECTRODES BASED ON CHANGES IN RESPIRATION FREQUENCY OF FISH TO SEVERAL ORGANIC AND INORGANIC COMPOUNDS

Nr.	Substances tested	Toxicity to fish (model experiments)		Toxicity to mammals (based on literature data)	
		Detection limit of the biological monitoring system using rainbow trout, defined as the conc. (mg/L) at which an alarm is caused within 24 h	LC_{50}-48 h for zebrafish in mg/L	LD_{50} for rat, oral in mg/kg b.w.	Threshold conc. in mg/L for man, defined as LD_{50} (rat) in mg/kg b.w. x 70 kg: 2.5 L x $\frac{1}{1000}$ (see text)
1	Acrylonitrile	5	15	81	2.3
2	Cadmium(chloride)	0.025	2.5	150	4.2
3	Chloroform	20	100	2000	56
4	Copper(sulphate)	0.06	0.6	120	3.4
5	Cyanide(potassium)	0.13	0.44	10	0.3
6	o/m-Dichloorbenzene	0.5	10	500	14
7	γ-Hexachlorocyclohexane	0.04	0.12	76	2.1
8	Hexachlorobutadiene	0.05	1	200	5.6
9	Pentachlorophenol	0.07	0.4	180	5.1
10	Phenol	4	60	530	14.8
11	Toluene	2.5	25	5850	163.8
12	Trichloroethylene	5	60	4920	137.8
13	Xylene	2	20	4000	112

Furthermore the river Rhine has recently been monitored continuously by means of a rheotaxis model (POELS 1975) over a period of more than 2 yr, in which period the monitor did not once yield an alarm level, whereas still many toxic chemicals were found to be present and occasionally in potentially hazardous amounts. Hereto it has to be emphasized that such compounds may be mutagenic and/or carcinologenic, or require bioactivation into reactive metabolites before exerting a toxic action or are not available for uptake by fish at the time of exposure (KOEMAN et al. 1977).

TABLE 2

PERCENTUAL DISTRIBUTION OF THE RATIO'S (LC_{50}-ACUTE EXPOSURE/ NO-TOXIC EFFECT LEVEL) AND (LC_{50}-ACUTE EXPOSURE/DETECTION LIMIT) FOR A NUMBER OF ORGANIC AND INORGANIC CHEMICALS.

		Distribution of the ratio's in %			
		1-10	10-100	100-1000	1000
$\frac{LC_{50}\text{-acute exposure}}{\text{no toxic effect level}}$	(n=77[1])	37.7	41.5	15.6	5.2
$\frac{LC_{50}\text{-acute exposure}}{\text{detection limit}}$	(n=13)	50	46	4	0

1) after CANTON + SLOOFF (1978)

TABLE 3

COMPARISON OF THE DETECTION LIMIT OF THE BIOLOGICAL MONITORING SYSTEM AND THE NO-TOXIC EFFECT LEVELS FOR SOME ORGANIC AND INORGANIC CHEMICALS.

Compounds	Detection limit in µg/l	No-toxic effect level in µg/l	Detection limit / no-toxic effect level
Cadmium(chloride)	25	2[1)]	12.5
Copper(sulphate)	60	14[1)]	4.3
Cyanide(potassium)	130	26[1)]	5
γ-Hexachlorocyclohexane	40	2[1)]	20
Hexachlorobutadiene	50	3[2)]	16.7
Pentachlorophenol	70	5[1)]	14
Trichloroethylene	5000	18[1)]	278

1) after CANTON + SLOOFF (1978)
2) after LEEUWANGH et al. (1975)

ACKNOWLEDGEMENTS

This publication is partly based on the work which was supported by the Commission of the European Communities under contract nr. 111-75-1-ENV. The author is greatly indebted to Mr. E. Rab and Mr. T.P. Spierenburg for their technical advice and to Mr. R. Baerselman for his assistance.

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