Health Considerations in the Preparation of Drinking Water from the River Rhine*

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Abstract

Rhine water is used in the Netherlands as a raw water source for the preparation of drinking water. Because of the massive pollution of this river it is questionable whether this source from the qualitative point of view, is suitable for this purpose.

Experimental evidence is presented which indicates that Rhine water shows chronic toxicity to rainbow trout; rainbow trout in Rhine water cannot complete their life cycle in a normal way; Rhine water induces chromosome breaks in the gills of the eastern mudminnow; and that the fraction of aromatic hydrocarbons present in Rhine water induces mutations in bacteria (positive result in the Ames Test).

Based upon these results, the quality criteria set as well as the necessity to use the Rhine as a raw water source, future research should be directed at the determination of the efficiency of water purification processes with regard to the removal of toxic pollutants.

Introduction

The Netherlands, especially the western part, lack sufficient groundwater of good quality to serve as a source for the preparation of drinking water. Rhine water, amongst other sources, is therefore used for the preparation of drinking water.

As is generally known the Rhine has for many years been polluted heavily with numerous toxic substances. These substances have different origins, the main categories being industrial and household wastewater, leaching from land treated with fertilizers and pesticides, and shipping. As a result of this and of the fact that the chemical industry produces an enormous diversity of products, the composition of Rhine water is very complex. In addition, the composition is variable due to the changing composition of the wastewaters, to fluctuating discharges in the river and to seasonal influences. Many of the chemicals present in the Rhine are detrimental to public health and must be removed during the purification process if this water is used for the preparation of drinking water.

It must be emphasized that it cannot be absolutely guaranteed that all harmful substances are always, and under all circumstances, completely removed during the purification processes. Therefore, in the case of incomplete purification, these substances can be ingested via the drinking water. In fact it must be admitted that it is only partly known to what extent toxic compounds are removed during the purification process. With this in mind, the aim must be that raw water, when used as a source for the preparation of drinking water, should not contain substances in toxic concentrations. This statement is in accordance with the philosophy of the International Consortium of Water Supply Undertakings in the Rhine Catchment Area (IAWR, 1973) namely that the Rhine water should be of such a quality that healthy drinking water can be prepared from it simply by means of so-called "natural" (i.e. physical and biological) methods.

The water undertakings in the Netherlands as in most other countries, are legally bound to supply consumers with a good and reliable quality of drinking water. It has, therefore, to be decided which quality of surface water, from a health point of view, is good enough to prepare an absolutely reliable quality of potable water under all circumstances. To answer this question it is necessary to know which requirements have to be met before any surface water can be used as a raw water source for drinking water. Since it is practically impossible to set standards

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for every pollutant present in Rhine water, only a general requirement with respect to the surface water quality is deemed necessary. Based upon statements of the WHO (1966) and the advice of a group of toxicological and environmental experts to the European Committee (Van Genderen, *et al.*, 1973), it is concluded that "if surface water is not toxic for fish and the fish can normally reproduce", such water will be suitable for the preparation of drinking water. This is a practical requirement since it does not demand the complete absence of all toxic substances and it can be experimentally determined. Against this background the following two main questions are applicable:

(1) Does Rhine water contain substances or mixtures of substances in such quantities (concentrations) that chronic exposure to these is or can be harmful to water organisms?

(2) If this question is answered in the affirmative, then the second question remains, namely, whether Rhine water can be sufficiently purified so that the quality of the drinking water prepared from it, will not be harmful to the consumer during any period of his life.

To date research in the KIWA laboratory has been directed at the first question. Therefore, a series of experiments, mainly with fish, has been performed to determine the toxicological quality of Rhine water. Rainbow trout were used as the experimental animal, not only for practical reasons but also because this fish species is very sensitive to toxicity.

Experimental Results and Discussion

Experiment A: Determination of the chronic toxic quality of Rhine water and its effects on rainbow trout

General description of the experimental design

Rainbow trout (*Salmo gairdneri,* R.) were purchased from a trout hatchery and kept in quarantine for 2 months prior to the experiment. During the experiment the trout were kept in 500 ℓ capacity stainless steel basins through which Rhine water flowed and compared with a control group which was exposed under identical experimental conditions to a very good quality of groundwater. The Rhine water was untreated except for a sedimentation period of 1 h. Water flow was 2 to 8 ℓ/min and the water temperature in the test basins of both groups corresponded to the river water temperature. The water was well aerated with compressed air. The fish were fed with artificial food (Trouvit). For both groups the amount of food per kg fish was identical as was the number of fish per litre of water. The experiment started in October 1975 and was completed in April 1977.

Results and discussion

The results after 18 months' exposure of the rainbow trout to either Rhine or groundwater are shown in Table 1. As can be seen from this table a number of significant differences was observed between rainbow trout exposed to either Rhine water

TABLE 1
EFFECTS ON A NUMBER OF PARAMETERS IN RAINBOW TROUT AFTER 18 MONTHS EXPOSURE TO RHINE WATER AS COMPARED WITH CONTROL (mean of 20 to 30 fish, with standard error of mean)

Parameter	Control	Rhine water	%-age of control
bodyweight (g)	631,5 ± 181,8	370,3 ± 82,4**	−41,4
somatic liver index (1)	1,13 ± 0,23	1,50 ± 0,21***	+32,7
somatic kidney index (1)	0,75 ± 0,19	0,85 ± 0,17	+13,3
somatic spleen index (1)	0,109 ± 0,039	0,148 ± 0,055**	+35,8
hemoglobin content blood (g/100 mℓ)	4,32 ± 0,65	2,95 ± 0,54***	−31,7
hematocrit value (vol %)	47,2 ± 7,3	35,9 ± 7,3***	−23,9
glucose content blood (mg/100 mℓ)	41,9 ± 9,7	62,5 ± 11,8***	+49,2
ureum content serum (mg/100 mℓ)	0,7 ± 2,1	7,3 ± 1,8*	−16,2
APDM activity per mg liver protein (2)	4,81 ± 2,13	4,18 ± 1,64	−13,0
APDM capacity per liver (2)	3 832 ± 1 658	2 688 ± 1 240**	−29,9
APDM capacity per liver relative to body weight (2)	6,34 ± 2,52	7,16 ± 2,83	+12,9
GPT of liver (mU/mℓ) (3)	627 ± 218	862 ± 259**	+37,5
LDH of serum (mU/mℓ) (4)	627 ± 385	940 ± 501*	+50,1
AF of serum (mU/mℓ) (5)	201 ± 123	250 ± 74	+24,4
Acetylcholine esterase activity in brain tissue (mU/mg protein)	88,9 ± 14,8	65,3 ± 10,9***	−26,6

(1) somatic tissue index: $\frac{\text{tissue mass (g) x 100}}{\text{body mass (g)}}$

(2) Aminopyrinedemethylase activity expressed as nmol formaldehyde produced per hour

(3) Glutamate pyruvate transaminase

(4) Lactate dehydrogenase

(5) Alkaline fosfatase

* P < 0,1
** P < 0,05
*** P < 0,005

or groundwater. These differences were of such a nature and extent that it was concluded that the Rhine water contained substances which caused a series of chronic toxic effects on rainbow trout. As an earlier identical experiment showed similar results (Poels and Strik, 1975) it may be concluded that the chronic toxic effects on rainbow trout are characteristic for Rhine water.

Experiment B: Determination of the effects of Rhine water on the egg and juvenile development of rainbow trout

General description of the experimental design

Green fertilized eggs were purchased from a trout hatchery and put in a trout egg incubator. Half of the eggs were exposed to Rhine water and the other half to groundwater under identical experimental conditions. The water temperature was kept constant at 12°C. Mud particles in Rhine water up to 50 μm were removed by a cotton candle filter. After hatching the juveniles were transferred to all-glass flow-through aquaria. Intermediate groups were formed by transferring juveniles hatched in Rhine water to groundwater and from groundwater to Rhine water. The experiment was performed between January and May 1976. A duplicate experiment was also carried out.

Results and discussion

Mortality figures for eggs exposed to Rhine water and groundwater are shown in Fig. 1. It appears that before the "eyed egg" stage the mortality of the eggs in Rhine water is significantly higher compared with the groundwater group, while after this stage there is no difference. Table 2 shows that the body length of the embryos present in Rhine water just before hatching is significantly smaller compared with the groundwater group. Fig. 2 shows the mortality of the juvenile rainbow trout exposed to Rhine water, groundwater and the intermediate groups. It is clear that during the experimental period Rhine water was acutely toxic to juvenile rainbow trout and that several developmental stages were delayed. It may be concluded that eggs exposed to Rhine water show a higher mortality rate in the juvenile stage. The results indicate that rainbow trout eggs and juveniles exposed to Rhine water do not show normal development. This will lead to an inability to complete their reproductive cycle in a normal way.

TABLE 2
LENGTH OF THE TROUT EMBRYOS JUST BEFORE HATCHING AFTER EXPOSURE TO GROUNDWATER AND RHINE WATER (n = 25)

	Ground water	Rhine water	P
Mean length (cm)	1,412 ± 0,062	1,238 ± 0,061	< 0,005

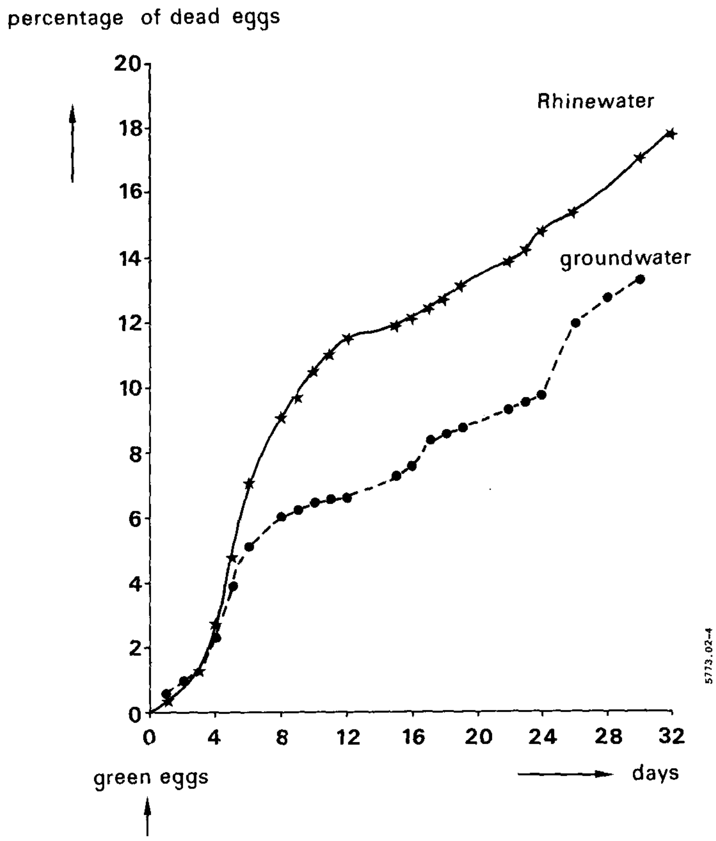

Figure 1
Mortality of rainbow trout eggs during the egg-stage of eggs exposed to either groundwater or Rhine water

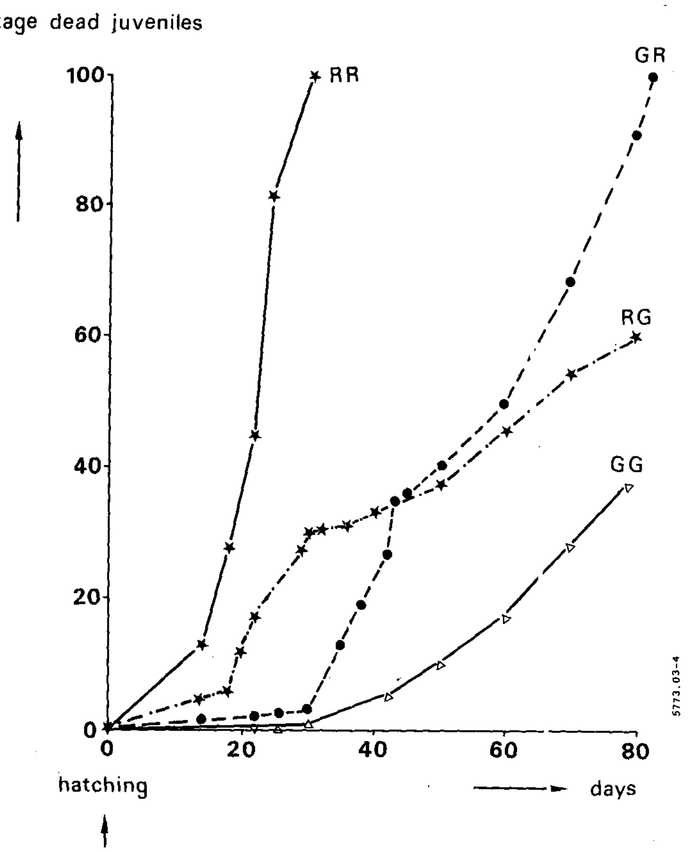

Figure 4
Mortality of rainbow trout larvae in the several experimental groups after hatching

Experiment C: Induction of cytogenetic effects of Rhine water in fish

General description of the experimental design

Two groups of 15 eastern mudminnows *(Umbra pygmaea)* each were exposed to either Rhine water or groundwater in 100 ℓ glass flow-through aquaria under identical experimental conditions. Mud particles up to 1 μm in Rhine water were removed by cotton filters, the water temperature was 12°C and sodium chloride was added to the groundwater until the same conductivity as Rhine water was attained. The conductivity of Rhine water varied between 850 and 1 020 μS during the experimental period. The water flow was 2 ℓ/min. After 3, 7 and 11 days 5 mudminnows were removed and treated with colchicine for 6 h. Squash preparations were made from the gills according to the solid tissue technique (Kligerman and Bloom, 1977). From each fish, 50 metaphase-chromosome preparations were analysed for the presence of chromosome breaks.

Results and discussion

Table 3 shows the results of the exposure of eastern mudminnows to Rhine water and groundwater respectively.

These results indicate that after 3 days' exposure to Rhine water a significant increase occurred in the number of chromosome breaks in the gills compared with the control group. In addition, exposure to Rhine water for 11 days induced significantly more chromosome breaks than after 3 and 7 days.

The results strongly suggest that during the experimental period Rhine water contained substances which were able to induce chromosome breaks in the gills of the eastern mudminnow (Prein *et al.*, 1978).

Experiment D: Induction of mutations in bacteria by Rhine water extracts

General description of the experimental design

The bacterial mutagenicity test was performed according to Ames *et al.*, (1975), using the *Salmonella typhimurium* tester strain TA 100. In order to detect substances which are mutagenic after metabolic activation, a liver-homogenate in the form of the S9-mix was added. 60 ℓ Rhine water and 60 ℓ groundwater were extracted and fractionated according to Meijers *et al.* (1978). The fractions of aromatic bases, aromatic hydrocarbons, aliphatic hydrocarbons and oxygenated compounds were evaporated to complete dryness and subsequently dissolved in 1 mℓ dimethylsulfoxide; 0,1 mℓ was tested by the Ames Test for mutagenic potency. Dimethylnitrosamine was used as a positive control.

Results and discussion

Results are shown in Table 4. It appears that the fractions of aromatic hydrocarbons and aromatic bases from Rhine water

TABLE 3
CHROMOSOME BREAKS IN GILL CELL PREPARATIONS OF *UMBRA PYGMAEA* EXPOSED FOR 3, 7 AND 11 DAYS TO EITHER RHINE WATER OR GROUNDWATER

Exposure time (days)	Groundwater		Rhine water	
	percentage of metaphases with at least 1 break*	number of breaks per metaphase	percentage of metaphases with at least 1 break*	number of breaks per metaphase
3	7,6 ± 2,6	0,08 ± 0,04	14,8 ± 2,7**	0,16 ± 0,03**
7	2,0 ± 1,4	0,02 ± 0,01	13,2 ± 2,3**	0,14 ± 0,02**
11	8,0 ± 3,2	0,08 ± 0,03	28,0 ± 4,7**	0,31 ± 0,06**

* mean percentage with standard deviation
**P < 0,005

TABLE 4
MUTAGENICITY OF RHINE WATER AND GROUNDWATER FRACTIONS IN THE SALMONELLA-MICROSOME MUTAGENICITY TEST

Waterfraction	Groundwater		Rhine water	
	number of mutants with S9-mix	number of mutants without S9-mix	number of mutants with S9-mix	number of mutants without S9-mix
Aromatic bases	113	110	220	133
Aliphatic hydrocarbons	121	111	111	101
Aromatic hydrocarbons	136	110	388	328
Oxygenated compounds	111	103	105	97
Dimethylnitrosamine	308	158		
Blank	140	130		

show an increase in the number of mutations compared with the groundwater extracts. However, only the fraction of aromatic hydrocarbons shows a statistically significant increase in the number of mutations. This means that in 6 ℓ Rhine water sufficient mutagenic aromatic hydrocarbons are present to induce a significant increase in mutations according to the Ames Test.

General discussion and conclusions

The experimental evidence collected so far clearly indicates the following:

(1) Rhine water is chronically toxic to rainbow trout.

(2) Rainbow trout cannot complete their life cycle in a normal way in Rhine water.

(3) Rhine water induces chromosome breaks in the gills of the eastern mudminnow.

(4) The fraction of aromatic hydrocarbons present in Rhine water is able to induce mutations in bacteria.

Although these results are only valid for the experimental periods it may be concluded that Rhine water has potentially negative biological effects on water organisms.

The answer to the first question "Does Rhine water contain substances or mixtures of substances in such quantities that chronic exposure to these is or can be harmful to water organisms" is therefore in the affirmative.

The question now is how to judge this statement with regard to the use of Rhine water as a raw material for the preparation of drinking water. It is believed that the quality of Rhine water is such that the possibility that toxic substances may reach the consumer is not excluded. As already mentioned the normal purification processes of surface water does not remove all toxic substances completely.

The experiments, however, were performed with fish and, up till now, little is known about the relation of the susceptibility of fish to toxicity *vis-à-vis* human susceptibility. Fish generally respond to lower concentrations of toxic substances than human beings, probably because of their larger dependence on the water quality (Dawson *et al.*, 1975). However, most of the chronic toxic effects found with rainbow trout also occur in man because of the resemblance in the cellular metabolism and structural organisation of most tissues and organs of fish and man. This holds also for the cytogenetic effects on the eastern mudminnow and the induction of mutations in the bacteria since the structures, in which the genetic information is laid down, are principally identical for all living organisms.

This means that a watchful eye should be kept and further research done on the toxicologic quality of Rhine water as a source for the preparation of drinking water.

The second question has now become of great importance: "Can Rhine water be sufficiently purified so that the quality of the drinking water prepared from it, will not be harmful to the consumer during any period of his life?"

It is hoped that an answer to this question will be found in the next few years. To this end two lines of research will be pursued:

(1) To determine the toxicological quality of drinking water prepared from Rhine and other surface waters; and

(2) To screen every conventional purification process for efficiency of removal of the toxicological active substances.

Acknowledgements

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