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Ecotoxicological characterization of wastewater from a Magnesium Industry

Norsk Hydro A/S, Porsgrunn, Norway

Norsk institutt for vannforskning NIVA

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Abstract:

An ecotoxicological evaluation of wastewater from Norsk Hydro A/S magnesium industry was carried out in connection with the establishment of a similar plant in Canada. The toxic effect of the wastewater was low after pH adjustment on separation of the precipitate. However, the remaining chlorinated benzenes and octachlorostyrene should be of concern because of the tendency for bioacumulation.

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Investigation of ecotoxicological effects of wastewater from a Magnesium industry (Norsk Hydro A/S, Porsgrunn, Norway)

3 July 1986

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SUMMARY AND CONCLUSIONS

An ecotoxicological characterization of wastewater from Norsk Hydro A/S magnesium industry in Porsgrunn, Norway was carried out to provide data for assessment of environmental effects of a similar, projected industry in Canada.

Short term toxictity tests were carried out with bacteria, algae, a zooplankter (Daphnia magna) and fish. A test for chronic toxicity was perfomed with Daphnia magna, (reproduction test). Genotoxicity was investigated with the Ames test.

Bioaccumulation of chlorinated hydrocarbons from the wastewater was studied in a test with rainbow trout.

The wastewater samples were adjusted to pH 6.5 and the formed precipitate removed before testing. Chemical analysis showed that the pre-treatment removed much of the chlorinated hydrocarbons present in the wastewater. The remaining concentrations were; hexachlorobenzene 7 µg/L and pentachlorobenzene 3 µg/L. Tri- and tetra chlorobenzenes and octachlorostyrene were also present.

The toxicity of the wastewater was wery low. No effect was found on rainbow trout at concentrations up to 50%. The wastewater induced a short lag-phase in the growth of an alga, (Selenastrum capricornutum) but no effect was found on the growth rate at concentrations up to 10%. Higher concentrations could not be tested because of interference by the salinity of the wastewater on the freshwater algae. No toxicity was recorded on bacteria with the Microtox test.

The Ames test did not reveal any genotoxic effects.

The reproduction of Daphnia magna was not affected by wastewater up to 5.6% concentration. The salinity of the wastewater prevented testing at higher concentrations.

Tests with algae and Microtox showed that the wastewater was more toxic if the precipitate formed after pH-adjustment was not removed. This indicates that the most toxic compounds were adsorbed by the precipitate.

The wastewater contains several persistent chlorinated hydrocarbons that are bioaccumulative. The bioconcentration factor for hexachlorobenzene in rainbow trout was estimated to 6,500. Also penta, tetra and trichlorobenzene and octachlorobenzene accumulated in fish.

The results of the characterization suggest that the major concern regarding environmental effects of discharge of wastewater from this type of magnesium plant should be the discharge of bioaccumulative, persistent compounds such as chlorinated benzenes and styrenes.

The tested wastewater is based on seawater, and effects of very high concentrations of this wastewater on fresh-water organisms could not be tested. However, we conclude that the toxicity of the wastewater to aquatic organisms is low after pH-adjustment and removal of precipitate.

1. INTRODUCTION

In connection with plans for establishment of a magnesium plant on the shores of St. Lawrence River in Quebec, Environment Canada, EPS, Quebec Region requested NIVA to conduct a basic ecotoxicological evaluation of the discharge from a similar magnesium plant in Norway (Norsk Hydro A/S, Porsgrunn). The testing was in accordance with regulatory requirements and include tests for LC_{50}-96h with rainbow trout, EC_{50}-15 min with Photobacterium (Microtox), EC_{50} 24h with Daphnia magna (motility) and EC_{50} 15-20 days with Daphnia magna (reproduction).

In addition to the toxicity tests, bioaccumulation of organochlorides and genotoxicity (AMES test) were examined. The samples for testing were supplied by Norsk Hydro A/S.

The chemical analysis, Microtox test and Ames test were carried out at Center for Industrial Research (SI). Other tests were performed at the Norwegian Institute for Water Research (NIVA).

2. SAMPLING AND PREPARATION OF SAMPLES

Samples for the tests were collected from the effluent from the primary scrubbers in the chlorination plant. According to Norsk Hydro this gives the most representative sample of effluent. The chlorination plant is a part of the larger magnesium production facilities. In the chlorination plant melted magnesium chloride is produced by the reaction between magnesium oxide, coke and chlorine in shaft ovens. The off gases contain carbon monoxide, carbon dioxide, chlorine, hydrogen chloride, various salts and traces of chlorinated hydrocarbons. The off gases are treated in large gas scrubbers where most of the contaminants are transfered to the scrubber water. According to Norsk Hydro, the effluent from the primary scrubbers contains all the contaminants of environmental importance. Other effluents which contribute to the total amount of wastewater will mostly lead to a dilution, slight alkalization or some addition of suspended solids.

The first sample (wastewater sample 1) was collected by an automatic sampler over a four day period from 7-10 January 1986. The effluent sampler was adjusted to give approximately 150 L over the four days.

Thus, the sample represents a mean over the sampling period. The composite sample was treated to remove free chlorine and adust pH to 5.6, in accordance with instructions from Environment Canada.

When pH was adjusted by addition of NaOH, a precipitate began to develop at pH 2.1. No further adjustment of the concentrated sample was therefore made. Instead pH was adjusted after dilution to the different concentrations used in the tests.

The chlorine content was analysed with a Lovibond Nessleriser comparator. No immediate colour reaction was observed, but after 15 minutes the comparator indicated 0.9 ppm of chlorine. After addition of 100 mL 0.25N $Na_2S_2O_3 \cdot 5H_2O$ to 130 L, no more chlorine was detected. However, after storage at 5°C over night, H_2S developed as a result of the thiosulphate addition.

The wastewater sample 1, was used for preliminary tests with algae, Daphnia and fish. pH was adjusted to pH 6.5-7.0 with NaOH after dilution in the different test media. This caused the development of a precipitation at all concentrations down to 1% wastewater.

After discussion with representatives from Norsk Hydro and Environment Canada, it was decided that a new sample should be taken, and prepared in a different way before testing.

Wastewater sample 2 was collected by an automatic sampler over the seven day period 28 January - 4 February 1986. The sample was mixed in a 200 L stainless steel container. The pH was adjusted to 5.6 with addition of 4 N NaOH and the sample was allowed to settle for 2.5 hours. The supernatant was then decanted, and the sludge collected in separate bottles. Approximately 20 L of the decanted water was collected in a stainless steel container for shippment to Canada. 120 L was transported in glass bottles and transported to NIVA for toxicity testing.

A third sample, wastewater sample 3, was collected by automatic sampling in the period 24 - 28 February. The sample was prepared the same way as sample 2 and used for the bioaccumulation test with rainbow trout.

The wastewater samples were stored in a stainless steel tank equipped with a stirrer. Subsamples for the different tests were taken out after mixing. All test were started within 2 days after the samples were received.

3. CHEMICAL CHARACTERIZATION

3.1 Analytic procedures

Wastewater sample 2, which was used for toxicity tests, was analysed for chlorinated hydrocarbons, which were extracted with 15-20 mL cyclohexane in the sampling bottles using magnetic stirring for two hours. The cyclohexane phase was separated, washed several times with rinsed water to remove inorganic halogen, and dried over sodium sulphate. The extract was then concentrated about ten times at 50^{0}C under a gentle stream of nitrogen and treated with concentrated sulphuric acid in order to remove the non-persistent organic compounds The gas chromatograph was equipped with an electron capture detector (ECD), and the quantification was based on standard curves using three different standard concentrations of each compound. The total amount of extractable persistent chlorinated compounds (EPOCl) were measured using neutron activation analysis (NAA). These analysis were carried out at the Institute For Energy (IFE) at Kjeller, Norway.

3.2 Results

The results of the chemical characterization of wastewater sample 2 are shown in table 1. Among the identified components are hexachlorobenzene (HCB), pentachlorobenzene (QCB), tetrachlorobenzenes (TeCB) trichlorobenzenes (TCB) and octachlorostyrene (OCS). The same components have been identified in the receiving water of the factory (Baumann Ofstad et al. 1978). A chromatograph of a wastewater sample 3 is shown in figure 3.

The results show the composition of the wastewater after precipitation. Analysis carrried out at the factory laboratory showed that the precipitation that followed the pH-adjustment removed more than 91% of the HCB and more than 50% of the QCB and OCS from solution. The relative reduction of different chlorinated hydrocarbons by adsorption to the precipitate will depend on the solubility and total concentration.

Table 1. Concentrations of chlorinated hydrocarbons identified in wastewater sample 2 after neutralization and precipitation.

Compound	Concentration (µg/L)
Octachlorostyrene	0.5
Hexachlorobenzene	7
Pentachlorobenzene	3
1,2,3,4 tetrachlorobenzene	1.4
1,2,4,5 tetrachlorobenzene	0.2
Trichlorobenzene	0.3

4. TOXICITY TESTS

4.1 Bacteria

4.1.1 Test method

The toxicity of wastewater samples 1 and 2 was tested on bioluminiscent bacteria with the Microtox system. The method is based on the emmittance of light from luminiscent bacteria, and the toxicity expressed as the concentration of sample (%) causing 50% reduction in light output.

The test of sample 1 was performed on the whole sample (with precipitate) after pH adjustment, while sample 2 was tested after removal of the precipitate.

4.1.2 Results

Toxic effects on the bacteria were found only in wastewater sample 1, which gave an EC_{50} (5 min)=10%. The results are listed in table 2.

Table 2. Results of Microtox test. The EC-50 values are based on 4 concentrations and 4 parallels. The values are given as the effective concentration of toxicant in % causing a 50% decrease in the light output.

Sample	pH in sample	pH after adjustment	EC_{50} (5 min) %	EC_{50} (15 min) %
Sample 1	1.7	7	10	11
Sample 2	6.1	-	n.o.	n.o.
Sample 2	6.1	7	n.o.	n.o.

n.o. = No observed toxicity

4.2. Algae

4.2.1. Test method

The toxicity test with algae was carried out according to the OECD Guidelines (OECD 1981), with the green alga Selenastrum capricornutum Prinz as test organism. Since the wastewater is based on sea water, it was necessary to account for the effect of salinity. Tests with natural seawater showed that the growth rate of Selenastrum was reduced 18% when 5% seawater was added to the growth medium and 44% with 10% sea water.

When the wastewater was tested, seawater was added to all cultures to give cultures with the same concentration of seawater (10%), corresponding to a salinity of 3.5 g/L. 10% seawater was also added to the inoculum culture to adapt the test algae to a higher salinity.

The growth medium was 10% Z8 in 10% natural sea water. The composition of 10% Z8 is given in Appendix 1.

The incubation conditions were as follows:

- Light intensity: 50 $\mu E\ m^{-2} s^{-1}$ (10.5 $W\ m^{-2}$) measured with a 180 π sensor, continuous light.
- Light quality: Fluorescent tubes (daylight type)
- Temperature: $20^{0}C$

- Aeration: Continuous shaking

- Culture volume: 50 mL

The inoculum culture was started 3 days before the test was started, to obtain exponentially-growing cells.

The inoculum culture was diluted with seawater enriched growth medium to a cell density of 10^7 cells L^{-1}. To 100 mL of this algal suspension, sea water, 10% Z8 and wastewater were added to obtain 200 mL samples with different wastewater concentrations (table 3).

Table 3. Scheme for preparation of wastewater concentration series.

Wastewater %	Algae susp. mL	Seawater mL	10%Z8 mL	Wastewater mL
0 (control)	100	20	80	0
1.0*	100	18.0	80	2.0
1.8	100	16.4	80	3.6
3.2	100	13.6	80	6.4
5.6	100	8.8	80	11.2
10.0	100	0	80	20

* This concentration was only tested on sample 1.

At the first test, with wastewater sample 1, pH was adjusted to pH 7.0 after dilution in all samples before addition of the algal suspension. At the test with sample 2, no further pH adjustment was made.

The growth of algae was measured by daily counting with a Coulter Counter. In sample 1, precipitation at all concentrations made counting with Coulter Counter impossible, and as an alternative measure of biomass, chlorophyll content was measured after 4 days.

The average cell number (or chlorophyll concentration) in the three parallell cultures was calculated for each day.

4.2.2. Results

The test with wastewater sample 1, showed significant reduction of algal growth at all concentrations of wastewater in the concentration range 1% to 10%. The chlorophyll values after 3 days are shown in table 4.

Table 4. Chlorophyll concentrations in algal cultures after 3 days at various concentrations of wastewater sample 1.

Wastewater conc. %	Chlorophyll conc. µg/L	% of control
0 (Control)	381	100
1.0	169	44
1.8	171	45
3.2	214	56
5.6	128	34
10.0	30	8

The concentration of chlorophyll at the beginning of incubation was approximately 2 µg/L. The data do not show a normal concentration/effect relationship., which might be a result of the physical effect of the precipitate that was formed after pH-adjustment.

The test with wastewater sample 2 showed much less effect on the algae. The growth curves for the cultures at the highest tested concentrations are shown in fig. 1. The curves show that there was a lag-phase during the first day in all cultures including the control. With addition of wastewater there was even a small decrease in cell number during the first day. However, the remaining cells were able to grow at the same rate as in the control.

The area under the growth curve from 0 to 72 hours, and the maximum growth rate (between 48 and 96 hours) are shown in table 5.

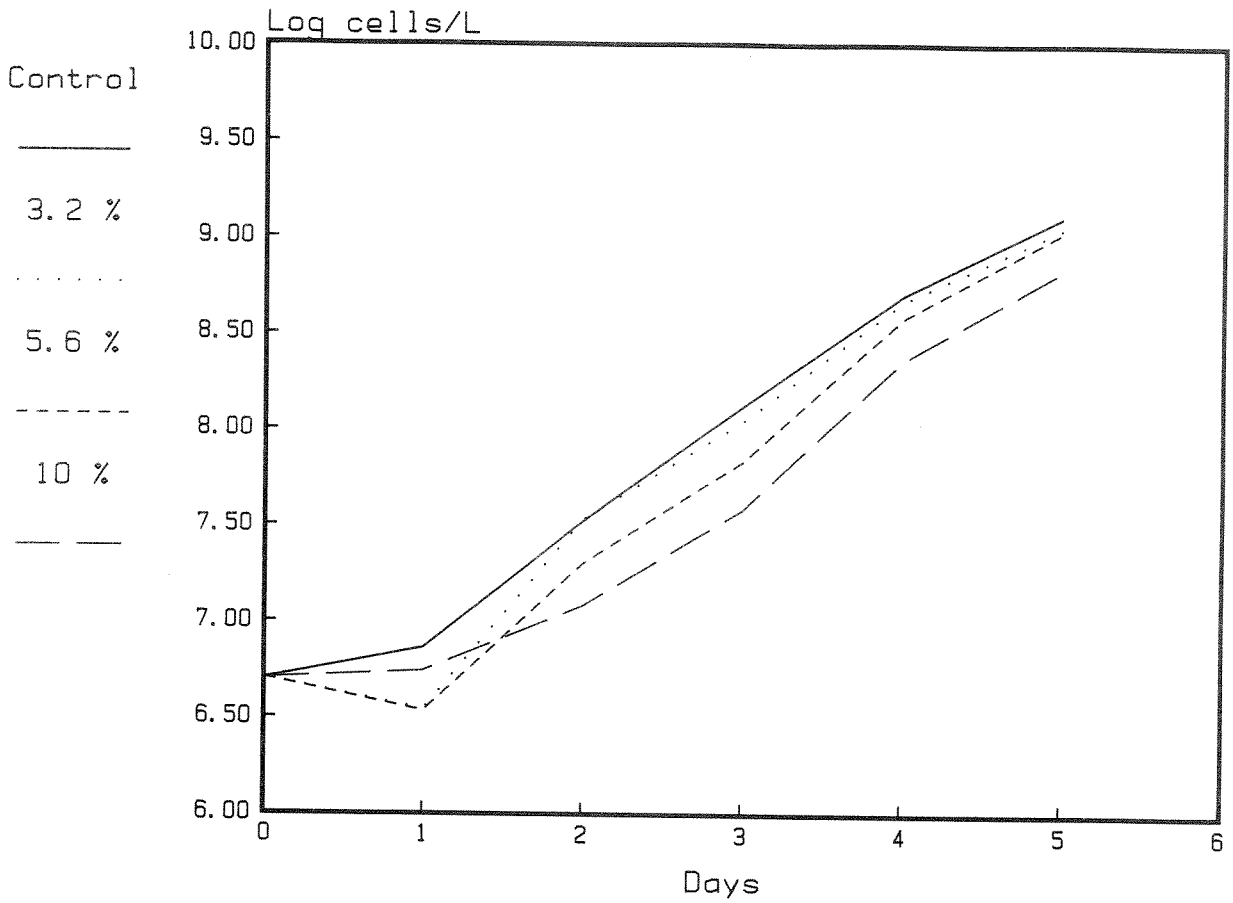

Figure 1. Growth curves of Selenastrum capricornutum at various concentrations of wastewater sample 2.

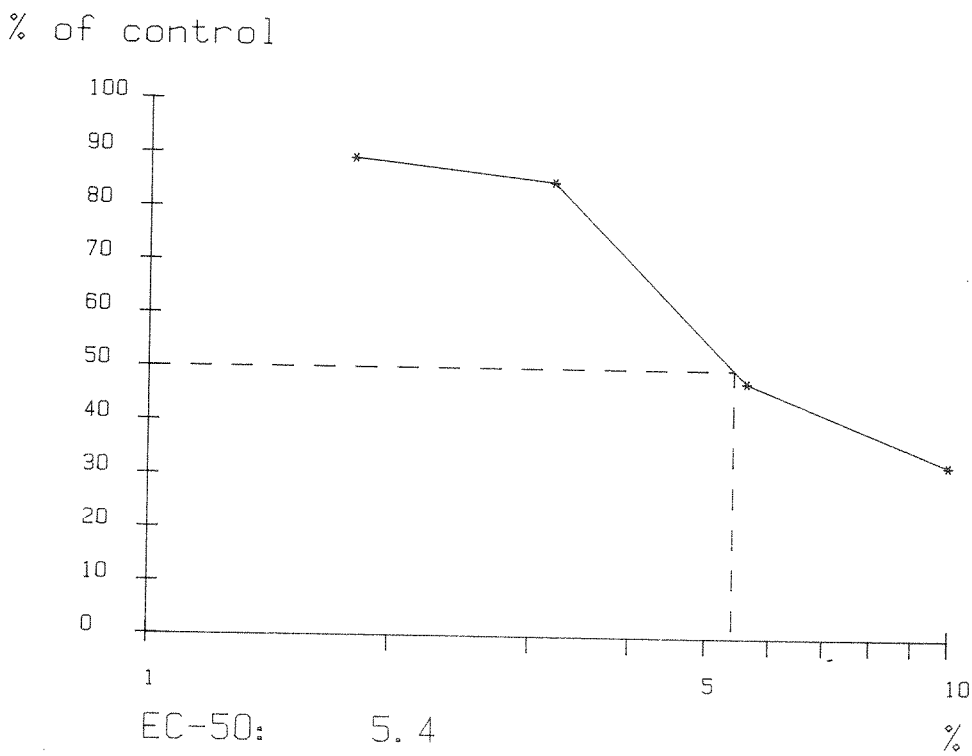

Figure 2. Effect of wastewater sample 2 on the area under growth curve in cultures of Selenastrum capricornutum.

Table 5. Effect of wastewater sample 2 on the area under growth curves and growth rate of Selenastrum capricornutum

Wastewater conc. (%)	Area under growth curve	Inhibition %	Growth rate doubl./day	Inhibition %
0 (Control)	94.3	0	2.0	0
1.8	84.0	11	2.0	0
3.2	80.0	15	1.9	5
5.6	44.9	52	2.1	-5
10.0	30.5	67	2.0	0

The results show, that the area under the growth curve during 72 hours incubation was clearly affected by the wastewater at concentrations above 5%. This is entirely a result of the lag-phase that was induced by the wastewater. The maximum growth rate was not affected.

The data for area under the growth curves are plotted against concentration in figure 2. The EC_{50}-value obtained from the plot is 5.4% (54 mL/L).

4.3. Daphnia - acute toxicity

4.3.1 Test method

A 48-hour acute toxicity test with Daphnia magna was carried out on wastewater sample 1, according to OECD Guidelines for Testing of Chemicals (Test 202, 4 April 1984). Test medium was natural water, supplemented with salts according to ISO 6341, 1982, to give a final hardness of approx. 250 mg $CaCO_3$/L, and the proportions Ca/Mg=4 and Na/K=10 on a weight basis. The composition of the natural water is described in table 6.

The test medium was mixed with various amounts of wastewater in 50 mL glass vials, four vials for each concentration. A parallel series with seawater instead of wastewater was also prepared as controls to account for effects of varying salinity. The pH of all mixtures was adjusted to 7.5 with addition of NaOH.

The neutralization caused a precipitation at 3.2% and higher concentrations of wastewater. The precipitated material formed a loose sediment on the bottom of the vials.

5 young (less than 24 hours) Daphnia magna were added to each vial, and the vials were incubated at 20±1°C. The light intensity was 900 lux with 16/8 h light/dark periods.

The motility of the Daphnia was checked after 24 and 48 hours.

4.3.2 Results

There was no mortality in the controls and in 10% seawater but the animals seemed less active than in the control. At 18% seawater, no effect was observed after 24 hours, but only 1 out of 20 animals showed motility after 48 hours. No effect of the wastewater was found at concentrations below 10%. At 10% wastewater 4 animals (20%) were immobilized after 48 hours. At 18% concentration, the wastewater caused immobilization of 15 of the daphnia (75%). Thus, the test showed that it is not possible to clearly distinguish the effect of the wastewater from the effects of pure seawater. The slightly increased immobilization in 10% wastewater indicates an incipient toxic effect at this concentration, the effect at 20% concentration is less than the effect of 20% seawater.

Because of the insignificant acute effect of wastewater on Daphnia magna, the test was not repeated with wastewater sample 2.

4.4 Daphnia magna - reproduction

4.4.1 Test method

The reproduction test with Daphnia magna was carried out on wastewater sample 2. The test method followed the OECD Guidelines for Testing of Chemicals (202, 4. April 1984).

The test medium and incubation conditions were as described under paragraph 4.3.1. The test was carried out in 600 mL vessels containing test medium with various concentrations of wastewater.

A test with sea water had shown that 10% seawater caused a significant reduction of the reproduction and 12.5 % mortality of the females during 21 days. (2.5% in the control). Therefore the test with wastewater 2 was restricted to concentrations from 0.56-5.6%.

The daphnia were fed with a mixture of green alga (Chlorella vulgaris) and Tetramin fish food. The amount of food was as follows:

Tetramin suspension (20 g/L COD): 0.02 mL pr. individual
Algae: 1. week: 15 x10^6 cells pr. individual
2. week: 30 x10^6 cells pr. individual
3. week: 60 x10^6 cells pr. individual

Test vessels: 400 ml volume of test vessels in 600 ml glass beakers.
10 numbers of test organisms per vessel
4 vessels per test concentrations.

Further details of the test are shown in the scheme below:

Day	0	3		6		8		11		13		15		17		19	
Time schedule		old	new	old	new	old	new	old	new	old	new	old	new	old	new	old	new
pH	8	7.8	7.8	7.8	8.0	7.8	8.0	7.8	8.8	7.8	8.0	7.8	8.0	7.8	7.8	7.8	8.0
O_2 mg/L	9.3	8.1	9.2	8.5	9.3	8.5	9.4	8.8	9.0	8.6	9.2	8.6	9.3	8.6	9.4	8.5	9.3
medium renewal	x		x		x		x		x		x		x		x		x
food	x		x		x		x		x		x		x		x		x

4.4.2 Results

The results of the reproduction test are summarized in table 6. The number of offspring was lower in the control than in all tested concentrations of wastewater (0.56%-5.6%) and 5.6% seawater. Reproduction appeared to be stimulated by the increased ionic strength of the medium caused by the addition of sea water. The average number of offspring at all tested concentrations of wastewater was 82-94 per animal compared to 75 in the control. Thus the test did not show any inhibitive effect of the wastewater on the reproduction of Daphnia in the concentration range tested.

Table 6. Result of reproduction test of Daphnia magna with wastewater sample 2.

Concentration in dilution water	Number of newborn mean x	St. dev. $Sx = \Sigma(x-x)^2 / n-1$	C.V.(%) $\frac{Sx}{x} \times 100$
0.56 % wastewater	82.5	10.1	12.2
1.0 % -- " --	85.3	9.8	11.5
1.8 % -- " --	94.4	4.8	5.1
3.2 % -- " --	92.8	6.1	6.6
5.6 % -- " --	84.3	11.1	13.2
5.6 % seawater control	89.3	3.8	4.3
Control (dilution water)	75.3	4.0	5.4

4.5 Rainbow trout

4.5.1 Test method

Acute toxicity was tested by using underyearlings (0+) of trout (Salmo gairdneri Richardson) of 7.5 g mean weight. Chemical data for the natural lakewater used in the test are shown in table 7. The experiments were made in a semistatic system in glass aquaria with 20 L of solution. Fresh solutions were made every day, and the fish were transferred by a dip net to the new solution. The experiments were run for 4 days in order to obtain a 4d LC_{50}-value. The temperature was kept at 9± 1°C, and there was a slight areation of the test chambers. 4 fish were held at each concentration, and mortality and symptoms of stress were noted. A control with a mixture of seawater and freshwater

of approximately the same salinity as the highest test concentration (50% wastewater) was run parallel with the other test concentrations.

In a preliminary test with wastewater sample 1, pH was adjusted to 7.3 by addition of NaOH after mixing with the lakewater. Wastewater sample 2 was adjusted to pH 5.6 before dilution, and no further adjustment was made.

Table 7. Chemical composition of lakewater used for toxicity and bioaccumulation tests with rainbow trout. Mean values.

Parameter		Parameter	
pH	6.3	Ca mg/L	3.7
Conductivity, mS/m at 25^{0}C	3.2	Mg, mg/L	0.41
Colour, mg Pt/L	21	Na, mg/L	1.1
COD (perm. no.), mg O/L	4.0	K, mg/L	0.35
Total nitrogen, µg N/L	300	Cd, µg/L	0.30
NO_3, µg/L	180	Cu, µg/L	2.0
Total phosphorous, µg P/L	4.5	Zn, µg/L	<10
Cl, mg/L	1.3	Pb, µg/L	1.0
Hardness, mg $CaCO_3$/L	11	Al, µg/L	110

4.5.2 Results

In the test with wastewater 1, a brownish precipitate was formed after pH adjustment. The precipitate was held partly in suspension by the fish movements and the areation. The 4-day exposure resulted in 25% mortality at the highest concentration tested (25%). No mortality was observed in 25% seawater. The 4 days LC_{50} was thus above 25% or 250 mL/L of wastewater sample 1. The surviving fish in 25% wastewater showed symptoms of stress with increased respiration frequency and abnormal swimming movements.

Because of the low toxicity that was observed at the preliminary test only two concentrations (25 an 50%) were used in the final test with wastewater sample 2.

During the 4-day exposure, no mortality or specific signs of stress occurred. Thus the acute toxicity is low, and the 4d LC_{50} is above 50%

dilution (i.e.>500 mL wastewater/L). Higher concentrations might cause osmotic stress due to the content of sea water in the waste and were therefore not tested.

5. BIOACCUMULATION TEST

5.1 Test method

The bioaccumulation test was carried out as a semistatic test in glass aquaria containing 160 L of solution. 18 underyearlings (0+) of rainbow trout (7.5 g mean weight) were exposed to a test solution containing 10% wastewater sample 3, and 90% freshwater (see table 7.) at 10±1°C. The test duration was 21 days, and each third day the fishes were transferred to another aquarium containing a solution prepared 24 hours beforehand.

Shortly before the transfer, the fishes were fed with a pelleted dry food in order to keep the fish in good condition.No mortalities occurred during the exposure and no signs of stress were observed.

Each third day, three fishes were removed, and 200-300 mL samples of the new test solution and the test water after three days in the aquarium were taken for analysis. The fish samples were wrapped in aluminium foil and kept at -20°C. The water samples were kept in glass bottles with glass stoppers at +4°C after addition of 20 mL cyclohexane.

5.2 Analytic procedure

5.2.1 Water samples

The procedure for analysis of water samples is decribed in section 3.1.

5.2.2 Fish samples

All the whole fishes sampled after three and six days exposure and three control fishes were homogenized and extracted twice using a mixture of cyclohexane/isopropanol (1:1). The rest of the fish samples were analyzed individually with the same technique. The cyclohexane phase was isolated by adding rinsed water, and the cyclohexane extract was washed with water and treated with concentrated sulphuric acid. The fat content of the samples was determined by evaporating the solvent from an aliquote of the extract, and weighing the residue.

5.3 Calculations

The bioconcentration factor (BCF) was calculated as:

$$BCF = \frac{C_{F_{st}}}{C_{W_{st}}}$$

where $C_{F_{st}}$ = concentration of test component in fish (µg/g wet weight) at "steady state"

and $C_{W_{st}}$ = concentration of the test component in water (µg/g =µg/ml) at "steady state".

"Steady state" for a component is reached when the amount of the component in the fish is at a constant level during several days. The water concentration($C_{W_{st}}$) is the average concentration of the test component during the time of "steady state".

5.4 Results

The results of analysis of fish and water samples are presented in table 8. Figure 3 shows typical gas chromatographs of the non-polar extracts isolated from the concentrated test-water and a fish sample (F-7-1, 18 days exposure). The identified components are marked on the figures. The rest of the peaks represent unknown chlorinated (brominated) persistent compounds.

Table 8. Concentrations of chlorinated hydrocarbons in fish and water samples during the bioaccumulation test with rainbow trout

Sample	Day no.	Fat%	1,2,4-TCB	1,3,5-TCB	1,2,3,4-TeCB	1,2,4,5-TeCB	QCB	HCB	OCS	PCB	EPOCl	identified % of EPOCl	
3 fishes	0	4.1	nd	nd	nd	nd	nd	0.002	nd	0.05	0.4	10	20
3 fishes	3	7.1	0.02	0.01	0.09	0.04	0.24	0.39	0.03	0.05	1.3	48	7
3 fishes	6	6.1	0.02	0.01	0.11	0.05	0.36	0.64	0.04	0.05	1.0	90	13
1 fish	12	9.6	0.03	0.03	0.13	0.08	0.49	1.0	0.07	0.20	1.3	109	23
1 fish	12	7.6	0.02	0.02	0.11	0.07	0.51	1.1	0.07	0.22	2.1	65	8
1 fish	12	9.5	0.02	0.03	0.12	0.07	0.54	1.2	0.08	0.20	2.3	69	8
1 fish	18	6.7	0.01	0.02	0.07	0.04	0.49	1.3	0.09	0.20	1.8	88	13
1 fish	18	7.5	0.01	0.02	0.08	0.05	0.48	1.4	0.08	0.15	1.5	110	20
1 fish	18	7.9	0.02	0.02	0.07	0.04	0.51	1.3	0.09	0.21	2.0	81	12
1 fish	21	7.8	0.02	0.02	0.10	0.05	0.51	1.2	0.08	0.24	1.6	108	20
1 fish	21	9.1	0.02	0.03	0.09	0.05	0.54	1.4	0.10	0.27	1.8	100	20
1 fish	21	5.8	nd	nd	0.05	0.04	0.41	1.3	0.10	0.28	1.8	82	12
1 fish	21	4.3	nd	nd	0.04	0.04	0.32	1.3	0.11	0.28	2.4	63	8
1 fish	21	8.3	0.02	0.02	0.07	0.05	0.42	1.1	0.08	0.24	1.9	75	10
1 fish	21	7.9	0.01	0.03	0.07	0.04	0.46	1.2	0.08	0.22	2.3	66	7

Sample	Day no.	Fat%	1,2,4-TCB	1,3,5-TCB	1,2,3,4-TeCB	1,2,4,5-TeCB	QCB	HCB	OCS	PCB	EPOCl	identified % of EPOCl
Water	0	-	0.02	0.01	0.01	0.01	0.14	0.21	0.03	nd	-	-
" old	3	-	0.01	0.01	0.01	0.01	0.03	0.16	0.02	nd	-	-
" new	3	-	0.03	0.01	0.08	0.04	0.26	0.48	0.03	nd	-	-
" old	6	-	nd	nd	0.01	0.01	0.03	0.15	0.01	nd	-	-
" new	6	-	0.01	0.01	0.01	0.01	0.11	0.42	0.02	nd	-	-
" old	9	-	nd	nd	nd	nd	0.04	0.21	nd	nd	-	-
" new	9	-	nd	nd	nd	0.02	0.03	0.12	0.01	nd	-	-
" old	12	-	nd	nd	nd	nd	0.04	0.24	0.01	nd	-	-
" new	12	-	nd	nd	nd	nd	0.03	0.39	nd	nd	-	-
" old	15	-	nd	nd	nd	nd	0.03	0.18	0.01	nd	-	-
" new	15	-	nd	nd	nd	nd	nd	0.05	nd	nd	-	-
" old	18	-	nd	nd	nd	nd	0.02	0.15	0.01	nd	-	-
" new	18	-	nd	nd	nd	nd	0.01	0.07	0.01	nd	-	-
" old	21	-	nd	nd	nd	nd	0.02	0.12	0.01	nd	-	-

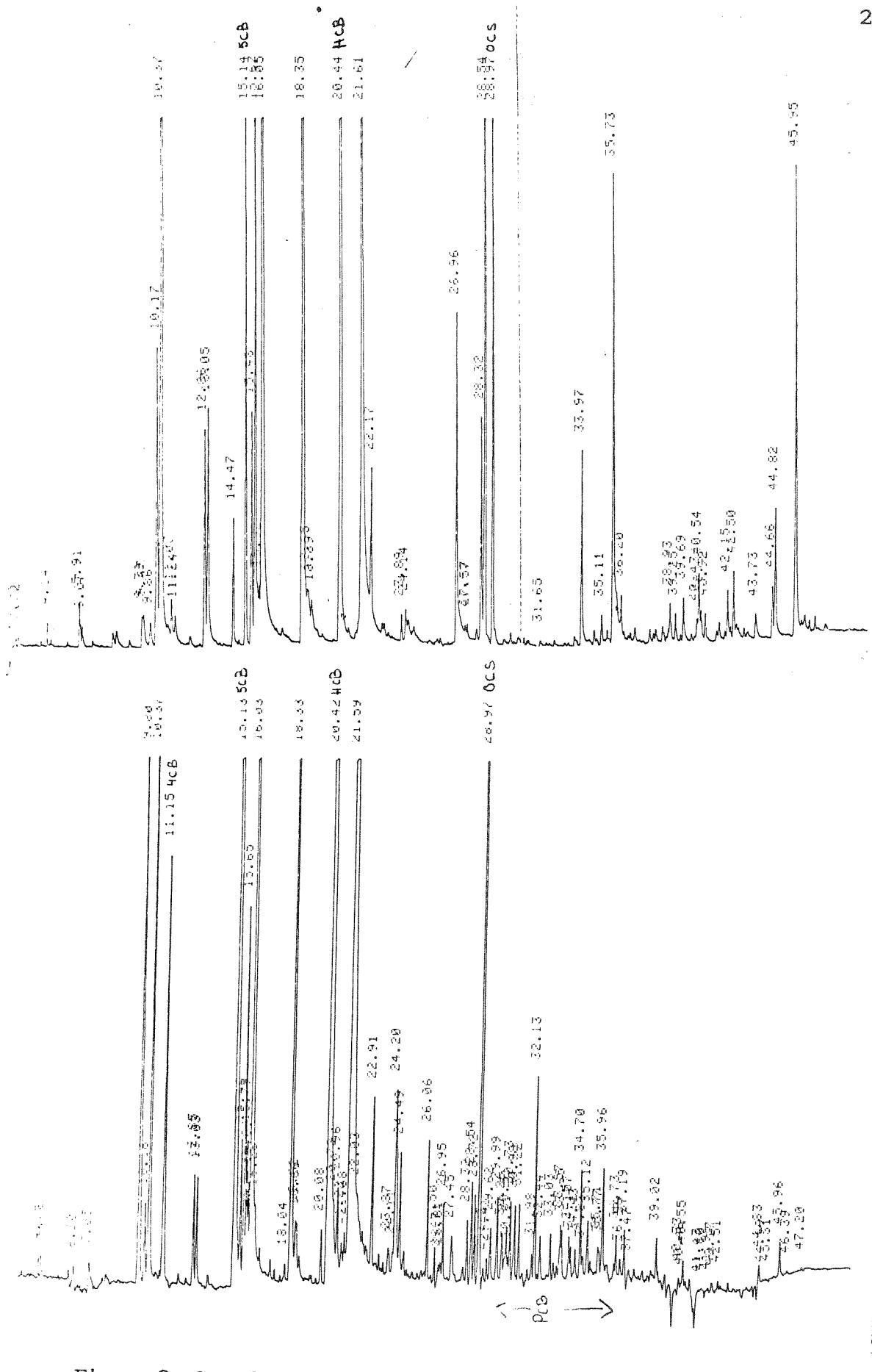

Figure 3. Gas chromatographs of non-polar extracts of wastewater (above) and fish (below) after 18 days exposure to 10% wastewater.

Tri-, tetra-, penta- and hexa-chlorobenzenes (TCB, TeCB, QCB, HCB) and octachlorostyrene (OCS) were found in the test water. When compared with the fish extracts, there seems to be a relatively high degree of bioaccumulation of these compounds in fish. In addition to the chlorinated benzenes and styrenes, polychlorinated biphenyls (PCB) were found in the fish, also before exposure to the wastewater. The concentration of PCB in the test water was under the detection level.

The identified components explain 50-100% of the extractable persistent organically bound chlorine (EPOCl) as indicated in table 8. This is in agreement with results from investigations of biota from the receiving water of the factory (Baumann Ofstad et al. 1978), while the identified compounds in the control fish explain a smaller part of the EPOCl, which is typical for fish from uncontaminated areas.

The analysis of water samples show that the concentrations of the chlorinated hydrocarbon components were not constant during the experiment. The relatively large variations indicate an uneven distribution of the compounds in the test system which probably is a result of chemical precipitation that occurred in the aquarium. Even after vigorous stirring some of the particulate material which will adsorb much of the chlorinated hydrocarbons, may not have been evenly distributed.

There is also a tendency of decreasing concentrations of the identified chlorinated compounds during the experiment, which indicates that some of these were lost from the wastewater sample during storage. The concentrations in a wastewater sample that was analysed by the end of the experiment showed concentrations that are approximately 10x the concentrations found in the aquarium with 10% wastewater, which indicates that these are correct.

The concentrations of chlorinated compounds in fish levelled off after 12 days, and the period between 12 and 21 days has been used for calculation of "steady-state" levels. The concentrations in fish of the three most important compounds are presented in table 9, together with the average water concentrations for the same period and the resulting bioconcentration factors. Also included are data from Oliver et al. (1983), who investigated the accumulation of chlorinated benzenes in rainbow trout.

The bioaccumulation factors for the various chlorinated benzenes in the wastewater are lower, but of the same order of magnitude as found by Oliver et al. (1983). The value for HCB is similar that reported by

Neely (1974), who estimated the bioconcentration factor in rainbow trout to 8000. Thus the results of the bioaccumulation test seem to give a realistic impression of the bioaccumulation potential of the persistent chlorinated hydrocarbons present in the wastewater.

Table 9. Bioconcentration factors in rainbow trout for the chlorinated persistent compounds in 10% wastewater (sample 3). The concentrations in water are mean values for days 11-21.

Compound	Conc. at "steady state"		BCF	
	Water (µg/L)	Fish (µg/g)	this study	Oliver et al 1983
OCS	0.01	0.09	9000	-
HCB	0.2	1.3	6500	20000
QCB	0.03	0.47	16000	20000
1,2,3,4 TeCB	0.01	0.08	8000	12000
1,2,4,5 TeCB	0.01	0.05	5000	13000

6 GENOTOXICITY

6.1 Test method

Reverse mutation assays (Ames test) were carried out on wastewater sample 1 (with precipitate after pH adjustment) and sample 2 (precipitate removed). The samples were tested directly and after concentration 500%. One liter sample was extracted (stirred 1 hour at room temperature) with 150+100 mL cyclohexan. The extracts were combined, evaporated to near dryness, and 2 mL dimetylsulfoxide (DMSO) added. The concentrated samples obtained were termed extract I (sample 1) and extract II (sample 2). The strains of Salmonella typhimurium were TA 98 and TA 100. Further details are given in appendix 2.

6.2 Results

The results of the tests are shown in table 10. and 11. No mutagenic activity was observed in any of the strains under the test conditions employed.

TABLE 10
Mutagenicity testing of water samples in the Salmonella strain TA 98. The results are expressed as number of revertants per plate (mean of two plates). The spontaneous mutations are mean of five plates.

Sample	Volum	REVERTANTS PER PLATE					
		TA 98					
		+ S9			- S9		
		Test 1	Test 2	X	Test 1	Test 2	X
Sample I	1 ml	1	4	3	3	2	3
	2 ml	3	0	2	7	2	5
Sample II	1 ml	2	1	2	4	7	6
	2 ml	6	5	6	4	1	3
Extract I 27/1-86	20 µl	9	3	6	2	5	4
	50	7	9	8	12	9	11
	100	19	10	15	11	9	10
Extract II 10/2-86	20 µl	5	13	9	1	4	3
	50	4	9	7	3	11	7
	100	9	11	10	7	12	10
Spontaneous		29	33	31	25	29	27
1-NP 100 ng BaP 5 µg		498	377		602	588	

TABLE 11
Mutagenicity testing of water samples in the Salmonella strain TA 100. The results are expressed as number of revertants per plate (mean of two plates). The spontaneous mutations are the mean of five plates.

Sample	Volum	REVERTANTS PER PLATE					
		TA 100					
		+ S9			- S9		
		Test 1	Test 2	X	Test 1	Test 2	X
Sample I	1 ml	23	39	31	9	6	8
	2 ml	19	26	23	7	29	18
Sample II	1 ml	10	24	17	19	16	18
	2 ml	15	19	17	4	21	13
Extract I 27/1-86	20 µl	26	11	19	12	29	21
	50	13	15	14	11	16	14
	100	21	16	19	15	22	19
Extract II 10/2-86	20 µl	20	26	23	33	17	25
	50	31	11	21	31	22	27
	100	29	19	24	16	24	20
Spontaneous		154	131	143	150	109	130
1-NP 100 ng BaP 5 µg		1294	1411		877	1012	

7. DISCUSSION

The investigated wastewater from this magnesium factory is very acid, and pH adjustment before discharge into a fresh water recipient will probably be required to avoid ecological damage because of acidification. The toxicity tests with bacteria and algae have showed that toxic compounds in the wastewater are removed efficiently with the precipitate that forms after neutralization. The reduced toxicity may be a result of the removal of chlorinated benzenes that is obtained during sedimentation.

Some of the test organisms (algae and daphnia) were affected by the salinity of the seawater based wastewater and the interference of salts made reliable tracing of other toxic effects difficult at high concentrations of wastewater. However, no effects were found on bacteria, and there was no lethality of rainbow trout at 50% concentration of the wastewater. We conclude, therefore, that the acute toxicity of the wastewater after pH-adjustment and removal of precipitate is very low.

Experiments with sea water showed effects on Daphnia at concentrations 10% or higher, and the effect of the wastewater on reproduction could therefore not be conducted in more concentrated wastewater. However, no effects were found in the concentration range tested (up to 5.6 %). The effects on reproduction of Daphnia are caused primarily by the salinity of the wastewater. Chronic effects on Daphnia of other components of the wastewater have not been demonstrated.

The concentrations of identified persistent chlorinated hydrocarbons found in the wastewater are not high enough to cause acute toxic effects. The concentration of HCB after pH adjustment and precipitation was 7 µg/L. Laseter(1976) observed no lethality of crayfish (Procamberus salmoides) and fish (Poecilia latipinna and Micrpoterus salmoides) exposed to concentrations of HCB up to 20 µg/L for 10 days. Histological effects on the kidney and gallbladder was, however found in Micropterus salmoides exposed to 25 µg/L. Geike (1978 a) found effects of HCB on the enzyme activity in the protozooa Tetrahymena pyriformis down to 1 µg/L. The photosynthetic activity of the green alga Chlorella pyrenoidosa was reduced 33% by 10 µg HCB/L (Geike 1978 b).

The toxicity of chlorinated benzenes has been shown to be inversely proportional with the water solubility (Wong et al. 1984). Tests performed by U.S. EPA (1980), referred by Zaroogian et al. (1985),

showed that LC_{50} for the mysid Mysidopsis bahia was 160 µg/L for pentachlorobenzene and 330-1460 µg/L for tetrachlorobenzenes. The EC_{50} values for the green alga (Ankistrodesmus falcatus) reported by Wong et al. (1984) are higher (1,25 mg/L for 5CB, 3-5 mg/L for 4CB, 6-9 mg for TCB and 20-65 mg/L for DCB).

The toxicity of octachlorostyrene has been investigated by Tarkpea et al (in press). The 96 h LC_{50} for the crustacea Nitocra spinipes was 68 µg/L.

Based on the toxicity tests that have been performed on the wastewater from the magnesium plant, and the data on toxicity of the identified persistent organic compounds in the wastewater, we conclude that the acute toxicity of the wastewater after pH-adjustment and precipitation is very low. The concentration of the major persistent chlorinated organic compound, HCB, in the concentrated wastewater is, however, in the range that has been shown to cause sublethal effects.

The bioaccumulation potential of several of the chlorinated hydrocarbons, particularly hexachlorobenzene, pentachlorobenzene and octachlorostyrene that has been demonstrated in the accumulation test with rainbow trout and several other studies, is obviously the most important factor to consider when the concequences of discharge of the wastewater is assessed. These compounds have also been found in biota from the receiving water of the factory from which the samples were obtained. (Baumann Ofstad et al (1978). The biological half lives of hexachlorobenzene and octachlorstyrene are estimated to 60-70 days and 90-120 days, respectively, in a recent study by Norheim and Roald (1985).

8 REFERENCES

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APPENDIX 1. COMPOSITION OF GROWTH MEDIUM 10% Z8 FOR ALGAE

Salt	Concentration	Element	Concentration
$NaNO_3$	46.7 mg/L	N	8.40 mg/L
NA_2CO_3	2.1 "	P	0.55 "
K_2HPO_4	3.1 "	K	1.40 "
$Ca(NO_3)_2$ 4 H_2O	5.9 "	Ca	1.00 "
$MgSO_4$ 7 H_2O	2.5 "	Mg	0.25 "
EDTA	0.37 "	Fe(III)	58 µg/L
$FeCl_3$ $6H_2O$	0.28 "	Mn	8.1 "
$MnSO_4$ 4 H_2O	22.3 µg/L	B	5.4 "
H_3BO_3	31 "	J	0.64 "
KBr	1.2 "	Zn	0.66 "
KJ	0.83 "	Cd	0.56 "
$ZnSO_4$ 7 H_2O	2.87 "	Mo (VI)	0.49 "
$Cd(NO_3)_2$ 6 H_2O	1.54 "	Cu	0.32 "
$(NH_4)_6Mo_7O_{24}$ 4 H_2O	0.88 "	Co	0.30 "
$CuSO_4$ $5H_2O$	1.25 "	Ni	0.30 "
$Co(NO_3)_2$ 6 H_2O	1.46 "	Al	0.27 "
$NiSO_4(NH_4)_2SO_4$ 6 H_2O	1.98 "	W (VI)	0.19 "
$Al_2(SO_4)_3K_2SO_4$ 24 H O	4.74 "	Cr (III)	0.05 "
Na_2WO_4 2 H_2O	0.33 "	V	0.05 "
$Cr(NO_3)_3$ 9 H_2O	0.41 "		
V_2O_5	0.09 "		

APPENDIX 2. AMES TEST PROCEDURE

(1) The top agar is melted in a water bath and the histidin/biotin solution added.

(2) Top agar is dispensed (2 mL) into sterile disposable 13x100 mm glass tubes with a sterile disposable pipette. Tubes are kept in a 45 ^{0}C water bath.

(3) Test strain (0.1 mL), test compound (10µL - 200 µL), and 0.5 mL sterile S-9 mix (if desired) are added to top agar in that order (two to three parallell plates).
Top agar is mixed by vigorous rolling of tubes between hands and the mixture is poured onto agar plates. The tube is tapped gently on the side of the plate to get the last drop of liquid. The plate is left to dry on a horizontal (watered) bench to dry for 10 minutes to get evenly distribution of bacteria on the plates.

(4) All the plates are inverted and incubated for two days at 37 ^{0}C.

(5) Revertant colonies are counted with a New Brunswick Biotran II Automatic Colony Counter. Plates with less than approximately 150 colonies are counted manually.

(6) Known mutagens are included in each experiment. Benzo(a)pyrene, 3-nitropyrene (NP), or 2-aminoanthracene (2-AA) are used as controls.

(7) Genetic/biochemical markers are cheked every 4 week, or if the known mutagens are giving an unexpected result.

(8) In case of a non-linear dose-response curve, the background lawn of bacteria on the plate is studied in a dissecting microscope. Toxic effects result in reduced growth of the background lawn.

Media preparation, S-9 mix and strain maintenance

(1) Agar and NaCl for the top agar are weighted out in 100 mL bottles. Water is added, the solution is then autoclaved and kept at +4 ^{0}C. Just prior to use, the agar is melted in a water bath and 10 mL of 0.5 mM histidine/0.5 nM biotin is added.

(2) Glucose minimal agar plates (Vogel Bonner) containing 20 mL are prepared as described by Ames et al. (1). Plates are stored in

plastic bags at +4 ^{0}C.

(3) Test strains are stored at -70 ^{0}C in 1 mL aliquotes (1). Nutrient broth cultures are prepared by scraping off bacteria from the frozen permanent. 10 mL of nutrient broth (antibiotic medium, Difco) is inoculated and grown overnight (16 h) in a shaker (200 r.p.m.) at 37 ^{0}C.

(4) S-9 is prepared according to Ames (1). Male Wistar rats (200 g) are injected with Aroclor 1254 (500 mg/kg) i.p. 5 days prior to preparation. The rats are given drinking water ad libitum and food (1) until 12 hours before sacrifice when the food is removed. On the fifth day the rats are decapitated and homogenate (S-9) prepared under sterile conditions. Rat livers are placed in beakers containing 10 mL 0.15 M KCl. The livers are weighed and transfered to beakers containing 3 volumes 0.15 M KCl (3 mL /g wet liver) minced with scissors, homogenized (on ice) with an Ultra Turrax for 4 sec. and further homogenized in a Potter Elvehjem apparatus with a teflon pestle. The homogenate is centrifuged for 10 minutes at 9000 xg. The supernatant (S-9) is distributed in portions in plastic tubes, quickly frozen in dry ice and stored at -80 ^{0}C.

(5) S-9 mix (according to Ames et al. (1)). S-9 mix contains per mL: S-9 (0.02 - 0.12 mL) standard amount 0.08 mL, $mgCl_2$ (8 µmoles), KCl 8 µmoles) and sodium phosphate, pH 7.4 (100 µmoles). S-9 mix is prepared each day and kept on ice.

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(1) B.N. Ames, J. McCann and E. Yamasaki, Mutation Res., 31 (1975) 347.