Environ. Sci. Technol. **1998**, *32,* 1799−1805

Genotoxic and Ecotoxic Effects of Groundwaters and Their Relation to Routinely Measured Chemical Parameters

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The primary aim of the present investigation was to study possible adverse effects of groundwater from an aquifer south of Austria's capital, Vienna, and to relate these toxicological effects to routinely measured physical/chemical parameters. Fourty-three water samples were tested for genotoxic and ecotoxic effects. For genotoxicity testing the *Salmonella*/microsome assay, the micronucleus test with primary rat hepatocytes and micronucleus tests with plants (*Tradescantia*, *Vicia faba*) were used. In ecotoxicity tests, algae (*Selenastrum capricornutum*), water cress (*Lepidium sativum*), and water flea (*Dapnia magna*) were studied as target organisms. In genotoxicity assays, 10 samples (23%) gave a weak positive response with a single end point, but only one sample (2%) was genotoxic in three different test systems. Thirty-six samples (86%) caused adverse effects in ecotoxicity assays. Plants (algae and water cress) were more sensitive than daphnie. No correlations between toxic effects and physical/chemical parameters were detected. The genotoxicity experiments indicate presently a low risk from genotoxic compounds. The ecotoxic (especially phytotoxic) properties of many water samples raise concern about their suitability for irrigation purposes. The lacking correlation between results from toxicity tests and physical/chemical data indicates that it is presently impossible to predict toxic properties from routine physical/chemical measurements with a sufficient level of safety. It is therefore important to include biological toxicity assays in groundwater monitoring programs.

Introduction

Groundwater is an important source for drinking and irrigation water supplies. The contamination of aquifers with substances originating from agricultural and industrial activities or waste disposal has raised much public concern during the last years (*1*). Scientific and regulatory efforts are presently focused on the identification and quantification of single chemicals and summary parameters that are considered to be harmful for man or the environment. This approach is based on the assumptions (a) that all harmful compounds are known and accessible to chemical analysis and (b) that the compounds present in the environment interact according to a certain model (e.g., additivity). A review of the present literature (*2*) reveals that only a minor fraction of the organic substances present in water has been identified. Antagonistic and synergistic effects of water contaminants are not predictable a priori, and the toxicological properties of many water contaminants are unknown.

Therefore, it was proposed to use biological toxicity tests, which were initially developed for the safety evaluation of chemicals, directly for environmental samples. Although many reports exist on toxic properties of aqueous media (for reviews, see refs *3*−*6*), only a very few studies have been published about adverse toxicological effects of groundwater (*7*−*12*).

The primary objective of this study was to investigate if DNA-damaging (genotoxic) compounds are present in one of the largest groundwater reservoirs of Austria located south of the capital Vienna and to clarify if contaminated drinking water might be responsible for the slightly elevated cancer rate observed in this region. It is well-known that the majority of carcinogens act by genotoxic mechanisms (*13*). Therefore, short-term genotoxicity tests are used to identify carcinogens prior to long-term animal studies. We applied a battery of genotoxicity assays with different target organisms, namely, the *Salmonella*/microsome assay (*14*), the micronucleus test with primary rat hepatocytes (*15*), and micronucleus assays with plants (*Tradescantia* (*16*) and *Vicia faba* (*17*)) to detect genotoxic and probably carcinogenic properties of groundwater from the investigated area.

These tests were supplemented with ecotoxicity assays to identify adverse effects toward plants and invertebrates that may result from agricultural use of the groundwater (e.g., irrigation). Standardized ecotoxicity tests were conducted with algae (*Selenastrum capricornutum*; (*18*), water cress (*Lepidium sativum*; *19*), and water flea (*Daphnia magna*; *20*).

Finally, we compared the results from toxicity tests with physical/chemical parameters routinely measured according to Austrian Federal regulations (*21*). The primary aim was to identify compounds responsible for toxic effects. The second aim was to clarify if it is possible to substitute expensive toxicity assays with the measurement of selected chemical parameters.

Experimental Protocol

Sample Sources. From 1994 to 1996, 43 samples were taken from 26 locations in the investigation area, some of them (locations 3, 12, 17, 22, 29, 35, and 40) repeatedly in different years. The sample codes used in this paper are composed by a number indicating the location followed by the sampling year (e.g., 3-94 location 3 in 1994). The majority of samples were taken from wells whose water is currently not used as human drinking water (locations 3−5, 12, 13, 16−19, 22, 24, 29, 39, and 109). In addition, five local water works (locations 35, 101, 102, 104, and 105) and one reclamation plant for groundwater contaminated by a landfill (location 40) were included in this study. At locations 35, 105, and 40, different

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S0013-936X(97)01049-3 CCC: $15.00
Published on Web 05/09/1998

TABLE 1. Water Treatment Processes at the Water Works (35, 105) and at the Groundwater Reclamation Plant (40)

location	sample code	remarks
waterworks		
35	35a	raw water (well 2)
	35b	raw water (well 3)
	35c	drinking water (after activated charcoral filtration)
105	105a	raw water
	105b	after stripping
	105c	after activated charcoral filtration
groundwater reclamation plant		
40	40a	raw water
	40b	after activated charcoral filtration
	40c	after UV irradiation

methods for water purification were applied (details in Table 1).

Existing taps or a mobile motor pump were used to sample water into 25-L polyethylene containers after a 15-min preflow period to avoid stagnating water. Previous investigations have shown that the container material does not affect the outcome of toxicity tests (*22*). The samples were transported to the laboratories within 4 h and processed immediately after arrival.

Chemicals. Positive controls for genotoxicity assays were benzo[*a*]pyrene (*Salmonella*/microsome assay TA98 and TA100 with metabolic activation), sodium azide (*Salmonella*/microsome assay TA98 without metabolic activation), 2-nitrofluorene (*Salmonella*/microsome assay TA100 without metabolic activation), aflatoxinB1 (micronucleus assay with primary rat hepatocytes), maleic acid (micronucleus assay with *Tradescantia*), and 1,2-dibromoethane (micronucleus assay with *V. faba*). All compounds were obtained from from Sigma (St. Louis, MO). Positive control for all ecotoxicity assays was potassium dichromate p.a. from Merck (Darmstadt, Germany).

Bacto tryptone, bacto agar, and minimal essential media (MEM) were from Difco (Detroit, MI). Nutrient broth no. 2 used for the overnight cultures was from Oxoid (Basingstoke, U.K.). Epidermal growth factor (EGF) and collagenase came from Sigma (St. Louis, MO). Arochlor 1254-induced (500 mg/kg body weight) rat liver S9 was obtained from Organon Teknika (protein concentration 32 mg/mL, Durham, NC). Solvents such as acetone, hexane, ethanol, and DMSO were p.a. quality and came from Merck (Darmstadt, Germany).

The nutrients for algal growth (described in ref *18*) and the chemicals for the dilution water for crustaceans (described in ref *20*) were purchased from Merck (Darmstadt, Germany).

Test Organisms. *Salmonella typhimurium* strains TA98 and TA100 were obtained from B. N. Ames (Berkeley, CA). Female Fisher 344 rats came from Harlan (Netherlands). *Tradescantia* 4430 clones were a gift from T. S. Ma (Macomb, IL). They were propagated in a growth chamber with a 16/8 h day/night cycle and 50% humidity. *V. faba* beans were a gift from A. Murin (Bratislava, Slovakia).

D. magna S. (crustacea) were obtained from IRCHA (Centre de Recherche 91 Vert le Petit, France). *S. capricornutum* P. (algae) came from the Institute for Plant Physiology (University of Göttingen), and *L. sativum* L. (cress) were purchased from Austrosaat (Vienna).

Salmonella/Microsome Assay. *Concentration of Water Samples.* Concentration of water samples was performed according to the procedure recommended by the U.S. EPA (*23*): XAD resins [prefabricated XAD-2 and XAD-7 columns (5 g/30 mL) from Applied Separations, Lehigh Valley, PA] were activated with 100 mL of hexane−acetone (85:15) followed by 100 mL of bidistilled water (Haraeus Destamat). Approximately 50 L of freshwater samples were aspirated (flow rate ≈ 100 mL/min) without pH adjustment through the XAD columns. The columns were dried with nitrogen and eluted with 100 mL of hexane−acetone (85:15). The solvent was evaporated in a Rotavapor (Büchi), and the final volume was adjusted with DMSO (p.a.) to obtain a concentration factor of 1:10000. Concentrated samples were stored in liquid nitrogen. As a control for the concentration procedure, similar concentrates were made with bidistilled water for every new batch of XAD resins and solvents. These controls were always devoid of mutagenicity.

Mutagenicity Assay. Salmonella/microsome assays were carried out as standard plate incorporation tests (*14*) with *S. typhimurium* strains TA98 and TA100. A 100 μL sample of overnight cultures was plated with concentrated water samples (four doses on 3 plates: 100, 50, 25, and 12.5 μL), 2 mL of top agar, and 0.5 mL of S9 mix (4%) or 0.5 mL of phosphate buffered saline (PBS) on Vogel−Bonner selective agar plates and incubated for 2 days at 37 °C. Mutant colonies were counted manually.

Micronucleus Assays with Primary Rat Hepatocytes. *Sample Preparation.* Native water samples were used to prepare minimal essential media (MEM, pH 7.4) that was sterilized with a 0.2 μm Sartorius filter.

Micronucleus Assay. The micronucleus assay with primary rat hepatocytes was performed as described in ref *15*. Hepatocytes were isolated from female Fisher 344 rats by the in situ two-step collagenase perfusion technique. The isolated hepatocytes were plated on collagen coated plastic dishes and cultured in MEM supplemented with nonessential amino acids for 3 h (37 °C, 5%CO_2, 95% humidity). The medium was exchanged for freshly prepared medium supplemented with insulin (10^{-7} M). After a 20-h incubation period, the prepared water samples were added to the hepatocyte cultures to yield concentrations of 100, 10, 1, and 0.1%. About 3 h later the medium was removed, the dishes were washed twice with fresh medium, the medium supplemented with epidermal growth factor (EGF) was added to stimulate cell division, and the cultures were incubated for 48 h.

For the determination of micronuclei and of the mitotic index, the cells were fixed with cold methanol−acetic acid (3:1, 5 min), rinsed with distilled water (2 min) and, air-dried. The cells were stained with DAPI (0.2 g/mL, 30 min), washed with McIlvaine buffer (2 min), briefly rinsed with distilled water, and mounted in glycerol. For each concentration, two dishes with 2000 cells were evaluated.

Micronucleus Assays with Plants. *Tradescantia Assays.* Experiments with *Tradescantia* were performed according to ref *24*. Fifteen cuttings were exposed for 30 h to native water samples and to two dilutions with tap water (1:1 and 1:3). The inflorescences were fixed for 24 h in ethanol−acetic acid (3:1) and stored in 70% ethanol. Per experimental point, five slides with early tetrad cells were stained with acetocarmine, and 300 cells/slide were evaluated under 400× magnification.

Vicia faba Assays. Experiments with *V. faba* were performed according to ref *17*. Three seedlings with secondary

roots of ≈2 cm were exposed for 48 h to native water samples and to two dilutions with tap water (1:1 and 1:3). The root tips were fixed for 48 h in ethanol−acetic acid (3:1) and stored in 70% ethanol.

For micronucleus analysis, the fixative was removed with distilled water (15 min), and the roots were hydrolyzed in 5 N HCl (1 h) and washed with distilled water (15 min). Per experimental point, three slides were stained with acetoorceine, and 1000 cells/slide were evaluated under 400× magnification.

Ecotoxicity Assays. Ecotoxicity tests with *D. magna* S. (crustacea) were performed according to ref *20*. Forty organisms were exposed for 24 h to native water samples and dilutions of 1:2, 1:3, and 1:4 in four parallel experiments. After the test period, all daphnids with swim inhibition were counted.

The algal tests (*S. capricornutum* P.) were performed according to ref *18*. Algal cultures were exposed for 72 h to native water samples and dilutions of 1:2, 1:4, 1:6, 1:8, 1:12, 1:16, 1:24, and 1:32 in three parallel experiments. The growth of the cultures was measured with a culture counter in 24-h periods. The growth curves were integrated to obtain the algal growth and to measure the inhibition in relation to control cultures.

L. sativum L. (cress) tests were performed according to the method of Neururer (*19*). A total of 100 seedlings of *Lepidium* was exposed for 48 h to native water samples and dilutions of 1:2, 1:3, 1:4, 1:5, 1:6, 1:8, and 1:10 in four parallel experiments. The root length was measured manually.

Statistical Methods. Data from the *Salmonella*/microsome assay were evaluated with the nonlinear regression model described in refs *22* and *25*. The micronucleus assays were analyzed according to the suggestions of ref *26* with Kruskal−Wallis tests (non-parametric ANOVA) for differences between treatment groups and Dunn's multiple comparisons for differences to the negative control.

The results from ecotoxicity tests (*D. magna* S., *S. capricornutum* P., *L. sativum* L.) were compared with the negative control groups using multiple *t*-tests to obtain the lowest dilution without inhibitive effects.

Results and Discussion

Genotoxicity. The results of genotoxicity tests are summarized in Table 2. Only one sample (17-94) gave positive results in all three genotoxicity assays. Ten samples (3-94, 12-94, 18-94, 19-94, 22-94, 35a-96, 40a-94, 40a-96, 40b-94, 40c-94) were genotoxic in a single assay. Eight genotoxic samples were detected with the *Salmonella*/microsome assay, mainly with strain TA100 after metabolic activation (six samples). Further genotoxic effects were found with primary rat hepatocytes (five samples) and the *Tradescantia* micronucleus test (one sample). An inhibition of cell divisions (mitotic delay) in rat hepatocytes was caused by four samples (29-95, 35a-94, 35a-95, 35b-96).

The highest response was obtained with sample 17-94 (29.9 revertants/L, 1.8/3.8 times increased micronucleus frequencies in rat hepatocytes/*Tradescantia* inflorescences). The other positive results were between 23.8 and 7.3 revertants/L and 1.6−1.3 times increased micronucleus frequencies.

At present only a very few reports exist on the genotoxicity of natural groundwater (*7, 8, 10, 12*). In The Netherlands (*7, 8*), measurements were conducted with the *Salmonella*/microsome assay, and an investigation in the United States was performed with the SOS-Chromotest (*10*). Various cytogenetic end points in *Allium cepa* were measured in a Slovenian study (*12*), but groundwater was used only as a negative control for wastewater and surface water samples.

In a survey of 10 cities in The Netherlands with drinking water prepared from groundwater without chlorine treatment, mutagenicity was measured in 18 out of 29 (62%) samples (*8*). All raw groundwaters assayed in another Dutch investigation (*7*) were mutagenic in the *Salmonella*/microsome assay. It is interesting to note that in this study from The Netherlands positive results were obtained mainly with strain TA98 and metabolic activation whereas in the present investigation genotoxicity was detected almost exclusively with TA100 and metabolic activation. In an investigation of U.S. groundwaters with the SOS-Chromotest (*10*), no statistical analysis was performed, but 51 out of 122 (42%) samples had higher relative activities than the background level (0.04 unit/L). In the present study, we measured genotoxic activities in 26% of the samples, which is considerably lower than the proportion of positive results (42−100%; *7, 8, 10*) in the studies cited above. The low portion of positive results was unexpected because the measurements were performed with a battery of three assays, which detects more genotoxins than a single assay. These results indicate a low level of contamination with genotoxic compounds of the investigated aquifer.

This assumption is further supported by a comparison of the genotoxic potencies in Table 2 with the results from previous investigations with the *Salmonella*/microsome assay. The highest genotoxic potencies measured there (*7, 8*) exceed 100 revertant/L, which is approximately 5 times higher than the values measured in the present study. For the micronucleus assay with primary rat hepatocytes, no comparative groundwater data are available, but measurements with river water caused an induction of micronucleus frequencies 1.6−2.6 times higher than the background (*15*), which is approximately 1.5 times higher than the values (1.3−1.8) obtained in the present study.

At several sampling locations (3, 12, 17, 22, 29, 35, and 40), measurements were repeated 1−3 times after approximately 1 year. The results show that the groundwater quality is highly variable. Sampling location 17 is an illustrative example: At this location, significant effects were measured in 1994 in all genotoxicity assays, but no genotoxic effects were found in the following years (1995 and 1996). In almost all cases the highest activities were detected in 1994.

At three locations (35, 40, and 105), various water treatment processes were applied to purify the groundwater. At 105 no genotoxicity was detected in the untreated water. At location 35, contaminants causing mitotic delay or micronucleus induction in rat hepatocytes were efficiently removed by activated carbon filtration. The results from the groundwater purification plant 40 are less consistent: In 1994 and 1995, genotoxicity was detected after activated carbon filtration, although in 1995 no genotoxicity was found in the raw water. The purified water after UV irradiation was, with one exception (1994), devoid of genotoxic activity.

The results from water works (locations 35, 101, 102, 104, and 105) indicate that it is at present unlikely that genotoxic compounds cause a health risk for people consuming drinking water from these sources.

Ecotoxicity. The results of ecotoxicity tests are summarized in Table 3. Ecotoxic effects were detected in 86% (36 out of 42) of the samples; 26% (11 out of 42) caused severe effects (see Table 4) that are comparable to industrial effluents. Plants (algae and cress) were generally more sensitive than daphnie. Only sample 17-96a was very toxic for crustaceans.

Repeated measurements at the same sample locations demonstrated a highly variable outcome, similar to the results of genotoxicity tests. The strongest effects were detected in 1995 and 1996. During the investigation period (1994−1996), ecotoxic effects decreased at sampling sites 3 and 29; at sites 12, 17, and 22 increasing toxicity was detected.

It is possible to use the inhibition of root growth of cress as an indication if water is usable for irrigational purposes

TABLE 2. Results from Genotoxicity Assays[a]

			micronucleus assays (increase over background)		
	*Salmonella*microsome assay[b]			plants	
sample	(revertant/L)	strain	primary rat hepatoctes[c]	*Tradescantia*[d]	*Vicia faba*[e]
3-94	–		1.3*	·	–
3-95	–		·	–	–
4-94	–		–	·	–
5-94	–		–	–	–
12.94	–		1.6**	·	·
12-95	–		–	–	–
13-94	–		–	·	–
16-94	–		–	–	–
17-94	29.9**	TA100+S9	1.8**	3.8*	·
17-95	–		–	–	–
17-96a	–		–	–	·
17-96b	–		–	–	·
18-94	7.3** 10.2**	TA98+S9, TA100+S9	–	–	·
19-94	10.3*	TA100-S9	–	·	–
22-94	15.9*	TA100+S9	–	–	·
22-95	–		–	–	–
24-94	–		·	·	–
29-94	–		·	–	–
29-95	–		m	–	·
35a-94	–		m	·	–
35a-95	–		m	·	–
35a-96	–		1.5**	·	·
35b-95	–		–	·	–
35b-96	–		m	·	·
35c-95	–		–	·	–
35c-96	–		–	·	·
39-94	–		–	·	·
40a-94	18.3*	TA100+S9	–	–	·
40a-95	–		–	–	·
40a-96	13.7*	TA100+S9	–	·	–
40b-94	23.8*	TA100+S9	–	–	·
40b-95	–		1.4**	–	·
40b-96	–		–	·	·
40c-94	21.3**		–	–	·
40c-95	–		–	–	·
40c-96	–		–	·	·
101-96	–		–	–	·
102-96	–		–	–	·
104-96	–		–	–	·
105a-96	–		–	–	·
105b-96	–		–	–	·
105c-96	–		–	–	·
109	–		–	–	·

[a] Negative results ($p > 0.05$) are indicated by –; (*) results statistically significant at $0.01 \leq p \leq 0.05$; (**) results statistically significant at $p \leq 0.01$; · means not tested. [b] Positive controls: 5 μg/plate benzo[*a*]pyrene (TA98 with S9, 346 ± 130 revertant/plate; TA100 with S9, 337 ± 28 revertant/plate), 1 μg/plate sodium azide (TA98 without S9, 823 ± 93 revertant/plate), and 7.5 μg/plate 2-nitrofluorene (TA100 without S9, 222 ± 36 revertant/plate). [c] Positive control: 10^{-11} mol/L aflatoxineB1 (19.5 ± 0.4 micronuclei/1000 cells, 2.4 times over background). Mitotic inhibition (statistically significant ($p \leq 0.05$) reduction of dividing cells) is indicated by m. [d] Positive control: 2 mmol/L maleic acid (6.2 ± 2.8 micronuclei/100 tetrads, 2.6 times over background). [e] Positive control: 10 mmol/L 1,2-dibromoethane (8.0 ± 2.0 micronuclei/1000 cells, 114 times over background).

(*27*). According to this criteria, 63% of the investigated samples was estimated to have adverse effects on agriculture; 14% of the samples should not be used for agricultural purposes.

An inhibition of algal growth was measured with samples from three water works (104-96, 105a-96, 109-96). It was possible to remove the toxic compounds at location 105 by air stripping; at the other two water works, the drinking water was not purified. The water of 109-96 is not used as drinking water. It is not possible to predict human health hazards from the algal toxicity observed at water works 104, but the well should remain under observation.

In 1994, an increase of algal toxicity due to activated carbon filtration was observed at the groundwater reclamation plant (location 40). In the following years (1995 and 1996), it was possible to reduce crustacean toxicity with activated carbon filtration and UV irradiation. According to our knowledge, this is the first systematic investigation of ecotoxic effects of groundwater. It is therefore not possible to compare the results of the present study with data from other countries.

Performance of Individual Assays. The *Salmonella*/microsome assay and the micronucleus test with primary rat hepatocytes were of almost equal sensitivity (6 and 5 positive samples, respectively) in this investigation, but each assay detected genotoxicity in different samples. This indicates that both test systems complement each other. Micronucleus assays with plants (*Tradescantia*, *V. faba*) were less sensitive (respectively 1 and no positive samples). This result was unexpected because previous investigations demonstrated that plant micronucleus assays are very sensitive toward environmental genotoxins (*28, 16*). In quantitative terms, the *Tradescantia* micronucleus assay is approximately 10 times more sensitive toward ionizing radiation than the micronucleus assay with primary rat

TABLE 3. Results from Ectoxicity Assays[a]

sample	*Daphnia magna*	*Selenastrum capricornutum*	*Lepidium sativum*
3-94	–	***	***
3-95	–	–	*
4-94	*	***	**
5-94	–	–	**
12-94	–	*	*
12-95	*	**	***
13-94	–	*	**
16-94	–	–	*
17-94	*	*	*
17-95	–	**	*
17-96a	***	**	–
17-96b	–	**	*
18-94	–	*	–
19-94	*	***	***
22-94	–	–	–
22-95	*	**	*
24-94	–	–	*
29-94	–	*	*
29-95	–	–	*
35a-94	–	*	**
35a-95	–	–	*
35a-96	–	–	*
35b-95	–	–	*
35b-96	–	–	*
35c-95	·	·	·
35c-96	–	–	–
39-94	*	*	*
40a-94	–	**	–
40a-95	–	–	**
40a-96	–	–	**
40b-94	–	***	*
40b-95	–	–	**
40b-96	–	*	*
40c-94	–	***	–
40c-95	–	–	–
40c-96	–	–	*
101-96	–	–	–
102-96	–	–	–
104-96	–	**	*
105a-96	–	**	–
105b-96	–	–	–
105c-96	–	–	–
109	–	**	*

[a] Negative results ($p > 0.05$) are indicated by –. For the classification of ecotoxicity test results, see Table 4. · means not tested. Positive controls were potassium dichromate.

TABLE 4. Classification of the Results from Ecotoxicity Assays

classification of the effect	symbol	lowest dilution without inhibition of test organisms: *Daphnia magna*	*Selenastrum capricornutum*	*Lepidium sativum*
not significant	–	1	1	1
low	*	1:2	1:2–1:6	1:2–1:4
high	**	1:3	1:8–1:16	1:5–1:6
severe	***	1:4	1:20	1:8

hepatocytes (*29, 30*). This may not be true for water samples because the hepatocytes are immersed in the solution whereas the plants have to absorb it through their xylem. Further comparative studies are necessary to clarify this point. It is also possible that former validation studies were biased due to the restricted number of agents tested or that the results of this study are singular due to the special composition of the groundwater samples.

The *L. sativum* test was the most sensitive ecotoxicity assay (30 positive samples) in our investigation. *S. capricornutum* was almost of equal sensitivity (23 positive samples); only 8 samples were toxic to *D. magna*. This may lead to the conclusion that the investigated area is contaminated with herbicidal rather than insecticidal compounds. But it is also possible that the difference in sensitivity is due to different exposure conditions (*L. sativum*, 48 h; *S. capricornutum*, 72 h, *D. magna*, 24 h).

TABLE 5. Correlation between Results of Toxicity Tests

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- - -  S P E A R M A N   C O R R E L A T I O N   C O E F F I C I E N T S  - - -

@_DAPH          .3444*

@_CRESS        -.1575     -.0189

@SAL            .1091     -.0286     -.2903

@HEP           -.1229      .2723      .2484     -.0265

@TRAD           .0470      .5410**   -.2123      .3284      .5554**

                @_ALGEA    @_DAPH    @_CRESS     @SAL       @HEP

* - Signif. LE .05     ** - Signif. LE .01     (2-tailed)
" . " is printed if a coefficient cannot be computed


@_ALGEA   growth inhibition of Selenastrum capricornutum (green algae)
@_DAPH    immobilization of Daphnia magna (waterflea)
@_CRESS   inhibitiion of root growth of Lepidium sativum (watercress)
@SAL      Salmonella/microsome assay
@HEP      micronucleus assay with primary rat hepatocytes
@TRAD     Tradescantia micronucleus assay
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The genotoxicity and ecotoxicity assays applied in this study use different target organisms and toxic end points to detect a wide range of toxic effects. Nevertheless, it is possible that two test systems give similar results because of similar biological processes. DNA damage, for example, can lead to mutations but also to the death of the target organism. Therefore, we compared the results from the toxicity assays with correlation analysis to detect commonalities between the responses of the different test systems (Table 5).

Three statistically significant associations were found, but the Spearman rank correlation coefficients were generally low (<0.56):

(A) *Tradescantia* micronucleus assay–*Daphnia magna* immobilization.

(B) *Tradescantia* micronucleus assay–*Selenastrum capricornutum* growth inhibition.

(C) *Daphnia magna* immobilization–*Selenastrum capricornutum* growth inhibition.

The first two correlations are due to the fact that only one sample (17-94) was positive in the *Tradescantia* micronucleus assay; the same sample was also highly toxic in the other test systems. The correlation coefficient between the results of the *Daphnia* and the algae ecotoxicity assays is very low ($r = 0.34$).

These calculations indicate that the results from the individual tests are statistically independent. Each test system gives a unique information, and it appears that the applied test battery is well suited for the toxicity testing of groundwater.

Relation between Toxicity Test Results and Routinely Measured Chemical Parameters. Toxicity assays with environmental samples have the advantage that the chemical identity of the constituents do not have to be known a priori. Nevertheless, it was often argued that these assays are too costly and that a similar amount of information can be derived from chemical analysis. In practice, almost exclusively chemical/physical parameters are measured in routine monitoring programs. This was the reason we examined the relationship between the results of toxicity assays and physical/chemical parameters routinely measured by Austrian governmental agencies (*21*). Table 6 lists the parameters that were measured in the investigated area. Additional

TABLE 6. Physical/Chemical Parameters Routinely Measured in the Study Area According to Austrian Legislation

parameter	unit	abbreviation[a]	statistical evaluation (y/n)	insufficient no. of measurements	values lower than detection limit
conductivity (25 °C)	μS/cm	CONDUCT	y		
O_2	mg/L	O2	y		
BOR	mg/L	BOR	y		
NH_4^+	mg/L	NH4	y		
NO_2^+	mg/L	NO2	y		
NO_3^+	mg/L	NO3	y		
Cl^+	mg/L	Cl	y		
SO_4^-	mg/L	SO4	y		
HCO_3^-	mg/L	HCO3	y		
o-PO_4	mg/L	O_PO4	y		
$KMnO_4^-$ usage	mg/L		n	+	
dissolved organic carbon	mg/L	DOC	y		
total organic carbon	mg/L	TOC	y		
benzene	μg/L		n	+	
toluene	μg/L		n	+	
xylene	μg/L		n	+	
adsorbable organic halide	μg/L	AOX	y		
purgeable organic halide	μg/L	POX	y		
tetrachloroethene	μg/L	TETRA	y		
trichloroethene	μg/L	TRI	y		
1,1,1-trichloroethane	μg/L	111-TRI	y		
tetrachloromethane	μg/L		n		+
chloroforme	μg/L		n		+
tribromomethane	μg/L		n		+
bromodichloromethane	μg/L		n		+
dibromochloromethane	μg/L		n		+
dichloromethane	μg/L		n		+
1,1-dichloroethene	μg/L		n		+
1,2-dichloroethane	μg/L		n	+	+
trichlorofluoromethane	μg/L		n	+	+
atrazine	μg/L	ATRAZINE	y		
desethylatrazine	μg/L	DES_ATR	y		
desisopropylatrazine	μg/L		n		+
simazine	μg/L		n	+	+
alachlor	μg/L		n	+	+
lindane	μg/L		n		+
metolachlor	μg/L		n	+	+
cyanazine	μg/L		n	+	+
prometryn	μg/L		n	+	+
propazine	μg/L		n	+	+
terbutylazine	μg/L		n	+	+
sebutylazine	μg/L		n	+	+
pendimethaline	μg/L		n	+	+
terbutryne	μg/L		n	+	+
aldrine and dieldrine	μg/L		n		+
chlordane (all isomers)	μg/L		n		+
heptachlor	μg/L		n		+
hexachlorbenzene	μg/L		n		+
endrine	μg/L		n	+	+

[a] Abbreviations used in Table 7.

information was obtained from measurements at the groundwater treatment plant (location 40) and from the Hygiene Institute (University of Vienna), which analyzed the samples from water works 101, 102, 104, 105, and 109. Many parameters listed in Table 6 were not included into statistical analysis, either because not enough measurements were available or because the concentrations were lower than the detection limit.

Table 7 shows the Spearman rank correlation coefficients between toxicity tests and physical/chemical measurements. Considering qualitative (statistical significance) and quantitative (correlation coefficients) criteria, we conclude that no causal relationship exists between the results from toxicity assays and the measured physical/chemical parameters.

It was unexpected that the phytotoxic activities of the water samples could not be related to the presence of herbicides. Only the concentrations of atrazine and its degradation product desethylatrazine were high enough to include them in statistical analysis (Table 6). It is possible that toxic effects in plants were caused by herbicides or degradation products that are not included into routine measurements.

The results of the present study demonstrate that it is was not possible to predict toxic effects of groundwater from the investigation area from physical/chemical measurements. This is in accordance with findings from other studies: Noordsij (*7*) and Kool (*8*) tried to relate the results of *Salmonella*/microsome assays to organic summary parameters (dissolved organic carbon (DOC), volatile halogenated organics (VOX), extractable halogenated organics (EOX), ...) and could not find any association between chemical and toxicological parameters. Also with water samples of other origins, in most cases it was not possible to find a relationship between physical/chemical parameters and genotoxic prop-

TABLE 7. Correlation between Physical/Chemical Parameters and Toxic Properties (Spearman Rank Correlation Coefficients)

- - - SPEARMAN CORRELATION COEFFICIENTS - - -						
AOX	-.1237	-.0563	-.1690	.0383	.0000	.7071
ATRAZINE	.1910	.1713	.3289	-.1374	-.0335	-.1670
BOR	.0880	-.1850	.3656	-.1611	-.1565	-.2108
CL	-.3522	-.1989	.3230	.2636	.0684	-.2266
DES_ATR	.3268	.1706	.2038	-.0011	-.1573	-.2250
DOC	-.2847	-.3265	.3902	-.3050	-.3598	-.3750
HCO3	-.2824	-.1825	.2423	-.1578	.1315	-.1983
CONDUCT	-.1799	-.1392	.4558*	-.0799	.0705	-.2772
NH4	-.1757	-.1450	.4293*	-.0102	-.2127	-.1631
NO2	-.0222	-.0459	.2548	-.0226	.1602	-.1195
NO3	.1034	.2197	.0324	.1629	.1183	-.0802
O2	.0438	-.0120	.3756*	-.1430	.2199	.1659
O_PO4	.0059	-.2049	.2423	-.3552	-.1293	-.2451
POX	-.4023	-.3253	.0993	.0879	-.0554	.2739
SO4	-.3711	-.2699	.0303	.2759	-.2618	-.1700
111-TRI	-.2228	-.2092	-.1037	.2833	-.0024	.1170
TETRA	.1413	.0418	-.0269	.0889	.1059	.2704
TOC	-.1523	.	.0546	.1808	-.2798	.
TRI	-.1885	-.3012	-.0719	.1407	-.1571	-.1444
	@_ALGEA	@_DAPH	@_CRESS	@SAL	@HEP	@TRAD

* - Signif. LE .05 ** - Signif. LE .01 (2-tailed)

" . " is printed if a coefficient cannot be computed

@_ALGEA	growth inhibition of Selenastrum capricornutum (green algae)
@_DAPH	immobilization of Daphnia magna (waterflea)
@_CRESS	inhibitiion of root growth of Lepidium sativum (watercress)
@SAL	Salmonella/microsome assay
@HEP	micronucleus assay with primary rat hepatocytes
@TRAD	Tradescantia micronucleus assay

erties, probably due to the fact that only a very low portion of organic matter in water samples is accessible to chemical analysis (*5*, *6*).

In light of the results of the present study, we suggest that biological toxicity tests should be included in routinely performed monitoring studies of groundwater, especially if the water is used for human consumption. Further research is needed to identify the compounds responsible for genotoxic and ecotoxic properties of groundwater, for example, by bioassay directed chemical analysis.

Acknowledgments

This study was supported by the Austrian Federal Ministry for Environment, Youth and Family; the Federal Ministry for Health and Consumer Protection; and the Federal Ministry for Agriculture and Forestry.

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Received for review December 4, 1997. Revised manuscript received March 26, 1998. Accepted April 2, 1998.

ES971049G