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Project Summary

Chromosome Studies on Human Subjects Exposed to Arsenic in Drinking Water

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A 2-year study was carried out on human subjects of various ages and backgrounds who have been drinking water containing 0.05 mg/l (0.05 ppm) or more arsenic for a period of at least 5 years. A control group that had been exposed to arsenic in drinking water at concentrations less than 0.05 mg/l was used for some aberrations and sister chromatid exchanges in the lymphocytes of the two populations with the amount of arsenic in the water they have been drinking. A questionnaire was designed to explore the incidence of skin cancer, fetal wastage, any genetic development abnormalities in the family and related parameters. Several other variables, e.g., coffee, wine and cigarette consumption, sex, residence (rural vs. urban) and exposure to chemicals, smelter and pesticides were taken into consideration. The study involved the city of and areas in the vicinity of Reno, Nevada.

This study on chromosome aberrations (104 exposed and 86 control individuals) and on sister chromatid exchanges (98 exposed and 83 control individuals) did not show that arsenic at a mean level of 0.109 mg/l has any effect on these parameters. Similarly, no effect of arsenic at these modest concentrations was found on other health parameters studied.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction and Summary

Inorganic arsenicals have been implicated in several health problems A partial list includes skin-cancers, liver cirrhosis, gastrointestinal catarrh, hyperkeratosis, polyneuritis, and genetic damage. Many parts of the United States contain rather high quantities of inorganic arsenicals in their drinking water, especially obtained from underground deposits Considering the results from several different studies mostly of non-U S origin, the United States *Environmental Protection Agency* (EPA) set "Interim Drinking Water Regulations" in 1976 which require that drinking water contain less than 0.05 mg/l of total arsenic

In the meanwhile, the EPA has initiated an extensive research program to assess health effects in relation to various levels of arsenic in drinking water. Recent *in vitro* experiments and study of lymphocytes of patients treated for psoriasis with arsenic containing compounds have shown that arsenic induces genetic damage Furthermore, a strong correlation exists between carcinogenic and mutagenic potentials, especially induction of Sister Chromatid Exchanges (SCEs), of chemicals With this in view, a study was initiated in 1978 to find out if any increase in the frequency of chromosome aberrations and SCEs is evident in a population drinking water containing 0 05 mg/l or higher arsenic in comparison to a population drinking water containing less than 0 05 mg/l arsenic

The study centered around the city of Reno and encompassed some neighboring communities, viz. Hidden Valley, Fallon, Virginia Foot Hills, and Fernley Most of the residents of Reno had been drinking

water containing an average of 0 01 ± 0 001 mg/l of arsenic (range <0.005 to 0.045 mg/l) and thus served as control group. Those of other communities, especially Fallon — 50 miles NE of Reno — have an average of 0 109 ± 0.005 mg/l of arsenic (range 0.05 to 0 39 mg/l) in drinking water One individual had been drinking water with 2 275 mg/l arsenic but was not included in further statistical analysis This group served as the "exposed" population or "cases." In order to be included in the study as control the subject was to have been drinking water containing <0 05 mg/l arsenic for at least 5 years, and similarly >0.05 mg/l arsenic for 5 years to be included as "exposed."

A questionnaire was designed to obtain data on the following: sex, ethnic origin, age, occupation, exposure to pesticides and other chemicals, water quality and quantity food and drinking habits, smoking, health status including pains, liver and kidney disease, nerve disorders, arthritis, cancer or ulcers, anemia, bone problems, hair, nail or weight loss, hyperpigmentation of skin, nausea, menstrual irregularities, stillbirths, miscarriages and pregnancy complications A telephone call was used for the first contact and participation was entirely voluntary The questionnaire was completed by personal interview. At that time a water sample from the kitchen tap was obtained to analyze arsenic content A 5-ml blood sample was obtained for chromosome studies.

The arsenic content of water was analyzed by the Nevada State Health Laboratory using atomic absorption as well as by colometric technique Chromosome preparations were carried out by culturing whole blood in medium 1A (GIBCO) for 48 to 72 hours For SCEs the cells were treated with BrdU for two rounds of replication. Routine colcemid, hypotonic treatments were used for harvesting Giemsa stain was used on slides used for chromosome aberrations and fluoresent plus giesma technique for SCE analysis. Attempts were made to analyze up to 200 cells for various types of aberrations (gaps, breaks, exchanges or chromatid and chromosome type) and at least 25 cells for SCE analysis). Quality control was always exercised to assure reproducibility of results of all studies.

A total of 211 cases were included in the study. The population samples in the control and exposed groups expressed considerable, though not perfect, similarities in life style Relevant information for the control (86 volunteers) and "exposed" (105 volunteers) populations, respectively, are as follows age in years = 29 56 ± 1 5 vs. 42 20 ± 1 6; water consumed (glasses per day) = 4 35 ± 0 2 vs. 5.12 ± 1.6; coffee consumed (cups per day) = 3.30 ± 0.4 vs. 4.09 ± 0.3; % males = 58 1 vs. 58.3, % exposed to pesticides = 7 0 vs 9 5; % exposed to smelter = 1.2 vs 2 9; % exposed to chemicals = 1 2 vs 2 9; % cigarette smokers = 16.3 vs 15 2, % wine drinkers = 25.6 vs 12.4; % residents of rural Nevada = 31 4 vs. 92.4. Statistical analysis of the control vs. exposed population showed no significant differences with respect to sex, exposure to pesticides, smelter, chemicals or smoking However, there were more wine drinkers among control than exposed individuals (X^2 = 4.66 on ldf, p = 0.03) and more exposed individuals came from rural than urban sample (x^2 = 74 56 on ldf, p<0.001) The mean age of the control group (29.6 years) was also significantly (p<0.001) less than that of the exposed group (42 2 years). The mean water consumption or coffee consumption was not significantly different.

For various reasons, only 191 cases were found suitable for the two types of cytogenetic analysis. Of these, 190 cultures used for analysis of chromosome aberrations were derived from 86 control and 104 exposed volunteers Two hundred cells per individual were analyzed in every case except for 16 cases. The study of SCEs was based on a sample size of 181 individuals, 83 in control group and 98 in the exposed. A sample of 50 cells per case was analysed for SCEs for the first 62 cases and 25 cells per case thereafter.

Results

A total of 18,240 cells were studied for chromosome aberrations among the 86 control individuals and 19,264 cells from the lymphocytes of 104 exposed. The frequency of chromosome aberrations in the entire population studied ranged between 0.00 to 0.13 aberrations/cell, with a mean of 0.033 ± 0.002 aberrations/cell in the control population and 0.026 ± 0.002 aberrations/cell in the exposed group. The frequency of aberrations, though lower in the exposed population than the control, is none-the-less within normal range for both The frequency of SCEs, however, was similar in the two groups. The range for the entire group was 5.84 to 15.28 SCEs/cell with an average of 8.301 ± 0 113/cell for the control group and 8.306 ± 0.141/cell in the exposed group.

Detailed information on multiple regression analysis of chromosome aberrations and SCEs as function of arsenic exposure and other independent variables is provided in Table 1.

Statistical interpretation of the data showed that the six multiple regressions using chromosome aberrations as a dependent variable were all significant, with 10 9%-13.0% of the variance being explained by the 10 independent variables as a group. The urban population sample showed significantly higher aberration frequency than the rural group There was also some indication that coffee consumption may increase the frequency of chromosome aberrations. However, arsenic exposure showed no relationship with chromosome aberrations in our sample.

Using SCE as a dependent variable, all the six multiple regressions were statistically significant However, only 11.1-13.6 percent of the total variance in SCE was explained by the 10 independent variables as a group Specifically, smokers exhibited more SCEs than non-smokers However, arsenic concentration in drinking water showed no effect on the frequency of SCEs

The average arsenic content in the exposed group in this study is more than twice (exactly 218%) of the highest limit mandated by the EPA. The quantity of water consumed is also higher for the exposed populations since most of it was derived from farming communities in rural Nevada The mean age of the exposed group is also higher than that of the control (p<0.001), which may dispose the exposed group to higher frequency of chromosome aberration In spite of these factors, our data do not show an increased incidence of chromosome aberrations or SCEs in the population exposed to modest quantities of arsenic in drinking water

Conclusions

These studies show that arsenic when consumed in modest quantities (at least up to 0.1 mg/l) in drinking water for up to 5 years does not appear to cause any detectable increase in the frequency of chromosome aberrations or SCEs in the lymphocyte cultures Besides, in the limited population surveyed no evidence of increased incidence of other serious health effects were observed.

Table 1. *Summary of Multiple Linear Regression Analyses of Chromosome Aberrations and Sister Chromatid Exchanges as Functions of Arsenic Exposure and Other Independent Variables*

Dependent Variable	Standardized Regression Coefficients for Independent Variables[1]													F^1	r^1
	ARSENIC	ARSGRP	ARSWAT	SEX	AGE	PEST	SMELT	CHEM	COFFEE	LOGCOFF	WINEGRP	CIGSGRP	CITYGRP	(For overall regression)	
SCE	061			-097	047	-119	002	-043	151		030	168+	134	2 23+	11 0
		052		-098	049	-114	007	-046	149		031	167+	134	2 21+	11 1
			163+	-102	052	-136	010	-046	157		011	170+	171	2 65*	13 1
	057			-093	044	-117	006	-050		129	025	183+	129	2 12+	11 0
		054		-094	044	-112	011	-052		129	026	181+	133	2 11+	11 0
			164+	-097	046	-134	014	-052		139	006	185+	169	2 55*	13 0
CRABNOR	-013			-003	026	035	057	-066	162+		-058	065	316*	2 62*	12 8
		-007		-003	025	033	056	-066	163+		-058	065	319*	2 62*	12 8
			040	-004	023	027	057	-065	164+		-062	067	339*	2 65*	13 0
	-018			009	067	030	070	-068		028	-042	110	311*	2 18+	10 9
		-006		009	065	028	069	-067		028	-042	111	317*	2 17+	10 9
			033	008	063	023	070	-066		029	-045	113	334*	2 19+	11 0
CRABLOG	-014			-020	-010	059	096	-071	092		-062	041	281*	1 94+	9 8
		-043		-021	-007	057	094	-072	092		-063	040	263*	1 96+	9 9
			020	-020	-013	054	095	-070	093		-063	043	296*	1 95+	9 8
	-017			-013	014	056	103	-072		014	-052	068	278	1 81	9 2
		-043		-014	017	054	102	-072		013	-054	067	262	1 83	9 3
			016	-013	010	052	103	-071		015	-053	069	293	1 81	9 2

[1] *Significance levels indicated as follows + -0 01 <p<0 05, * - p<0 01*

Codes — ARSENIC = Arsenic in mg/l, ARSGRP = Arsenic exposed or control groups, ARSWAT = ARSENIC X glasses of water/day, SEX = sex of subject (female = 0, male = 1), AGE = age in years, PEST = unexposed (=0) or exposed (=1) to pesticides, SMELT = unexposed (=0) or exposed (=1) to smelter, CHEM = unexposed (=0) or exposed (=1) to chemicals, COFFEE = cups of coffee consumed/day, LOGCOFF = Log_{10} (COFFEE + 1), WINEGRP = nonconsumers (=0) or consumers (=1) of wine, CIGSGRP = cigarette consumption, non-smokers (=0) or smokers (=1), CITYGRP = residents of rural Nevada (=0) or urban Nevada (=1), SCE = sister chromatid exchanges/cell, CRABNOR = chromosome aberrations/cell, CRABLOG = Log_{10} (100 X CRABNOR + 1)

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The complete report, entitled "Chromosome Studies on Human Subjects Exposed to Arsenic in Drinking Water," (Order No. PB 85-125 821; Cost: $10.00, subject to change) will be available only from:
National Technical Information Service
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