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Effects of Chlorite Exposure on Conception Rate and Litters of A/J Strain Mice

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The use of chlorine has been almost universally accepted as the method of choice for disinfecting potable water supplies. However, recent studies have demonstrated that the interaction of chlorine with ubiquitous and naturally occurring humic acids in water results in the formation of trihalomethanes (U.S. EPA 1975, ROOK 1976) some of which are known or suspected carcinogens (MARX 1974, U.S. EPA 1975, DEROVEN & DIEM 1975a, U.S. EPA 1977a). Among these, chloroform has been shown to produce hepatomas in selected mouse and rat strains in the exposure range of 90 to 447 mg/kg of body weight (ANONYMOUS 1976, ROE 1976).

Extrapolation from animal studies to humans have been criticized because human exposure is of the chronic low level type and equivalent levels of human exposure through drinking water would require the consumption of from 57,000 to 114,000 liters of water per day from the drinking water source with the highest chloroform levels (311 µg/L) identified in the recent EPA assessment of carcinogen levels in 80 municipal water supplies (U.S. EPA 1975).

A number of epidemiological studies have, however, shown a positive association between cancer mortality and the practice of water chlorination (DEROVEN & DIEM 1975b, MILLER 1976, PAGE 1976, OKUN 1976, MASS. DEPT. OF PUBLIC HEALTH 1949, ALAVANJA et al. 1977) with an apparent increase in the risk of gastrointestinal and uterine cancers in urban chlorinated water areas (ALAVANJA et al. 1977). Not all epidemiological studies have shown this association (TUTHILL & MOORE 1978).

In light of the evidence of the carcinogenicity of chloroform and of the fact that chloroform is produced in all water supplies where chlorination is practiced, alternatives to chlorine as a disinfectant method for potable water are being considered by the United States Environmental Protection Agency (U.S. EPA) and chlorine dioxide (ClO_2) is receiving serious consideration (U.S. EPA 1977b, STEVENS et al. 1976).

The use of ClO_2 is one method which would reduce the concentration of trihalomethanes. STEVENS et al. (1976) reported the results of laboratory studies which indicate that when ClO_2

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alone is used for water disinfection, no trihalomethanes are produced. When ClO_2 is generated using excess chlorine as in the usual water treatment practice, the final concentration of trihalomethanes is lower than if chlorine alone is used. However, as STEVENS et al. (1976) indicate, ClO_2 water treatment may result in a new and different array of organic by-products and possible adverse health effects of exposure to these compounds remain to be assessed. Additionally, end products formed as a result of ClO_2 treatment include the oxidants chlorite and chlorate (U.S. EPA 1977b, MILTNER 1977).

Chlorite is formed at the rate of 50 percent of the ClO_2 demand (MILTNER 1977). Blood destruction, nephritis, and methemoglobinemia have developed in humans after acute poisoning with large doses of chlorate (JUNG 1947, RICHARDSON 1937). It was suggested that in chronic poisoning that uremia, anemia, and nephritis might develop (RICHARDSON 1937). Chlorite is thought to be a more potent oxidant stressor to blood than chlorate (HEUBNER & JUNG 1947). HEFFERNAN et al. (1979a,b) reported that chlorite belonged to a class of oxidant compounds that stimulate the generation of hydrogen peroxide (KIESE 1974) resulting in a lowering of reduced glutathione (GSH) levels and the development of compensated hemolytic anemia in animal models. Oral administration of chlorite to mice has been shown to increase the mean corpuscular volume, osmotic fragility, G6PD activity, and the number of acanthocytes at exposure to 100 ppm of chlorite but not at 1.0 or 10.0 ppm (MOORE et al. 1980). Groups at high risk to developing hemolytic anemia from oxidant compounds would include persons with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency (LYNCH 1974, BEUTLER 1972, HANSEN & BENNETT 1964) and newborns (MOORE et al. 1978). The effect of chlorite on human G6PD deficient red cells has been shown *in vitro* to produce decreases in GSH and G6PD activity while markedly increasing methemoglobin (MetHb) levels as compared to human red cells (MOORE & CALABRESE 1980b). The potential effects of chlorite on human newborns may be exaggerated because:

1. Infants consume nearly 3 times as much liquid per unit of body weight than adults (HANSEN & BENNETT 1964).

2. Infants are born with hemoglobin F (fetal hemoglobin) a form that is readily oxidizable to MetHb (LEHMAN et al. 1972).

3. Infants have a lower capacity than adults to enzymatically reduce MetHb to Hb (EMERSON 1972) .

4. Newborn infants are characteristically low in vitamin E (EMERSON 1972), an important antioxidant compound because there is little placental transfer of vitamin E.

There is also evidence that the fetus may be at increased risk to oxidant stressors. SHUVAL & GRUENER (1977) demonstrated that nitrites can be transferred to the fetus *in utero* causing

an elevation of MetHb in fetal blood. When administered in drinking water to pregnant dams, 2000-3000 mg/L of $NaNO_2$ resulted in a decreased litter size, increased mortality of pups in the first three weeks, and a lagging of growth rate despite having equal birthweights to controls. Since chlorite is a potent oxidant, this study was designed to determine the effect on pups of pregnant (A/J) mice exposed to sodium chlorite in their drinking water.

MATERIALS AND METHODS

Ten to 12 week old female A/J mice were grouped by ten in an 11 1/2" L x 7 1/2" W x 5" D polycarbonate breeding cage for one week in order to synchronize ovulation (WHITTEN 1959). Females were than randomly mated one to one with A/J males of a similar age and checked for vaginal plugs on the mornings of the next four days. Males were removed from females at the appearance of plugs. Date of plugging, as well as weight of sire and dam were recorded. Females that were not plugged were grouped for a week before breeding again.

Plugging was considered to be day one of pregnancy and females were placed in fresh breeding cages with wood chips for the duration of their pregnancy and lactation. At plugging, females were randomly assigned to either a control group which was given distilled water or a treatment group which was given 100 ppm sodium chlorite [Matheson, Coleman and Bell, Norwood, OH 45212] in distilled water. A dose of 100 ppm ClO_2^- was selected since other studies (MOORE et al. 1980a, HEFFERNAN et al. 1979a,b) have shown that effects on hematological parameters are first observed at concentrations of chlorite reaching 50 to 100 ppm. Females remained on the specified water during gestation and laction periods and water intake was monitored during this time.

At parturition each litter was examined and data collected. Individual birth weights were taken and pups were weighed once a week until weaning at 28 days. Statistical analysis was performed on the data collected for each litter as well as water consumption and rate of positive pregnancy for each group.

RESULTS

The means, standard deviations, and t-test results are presented for control and treatment groups in Table 1. The average number of pups born alive in each litter was 5.4 for controls and 5.0 for the treatment group ($p=0.339$). The average number of pups alive at weaning (4 weeks after birth) was greater at 4.2 pups for controls than for the 3.4 pups in the treatment group ($p=0.24$). In contrast to the number of living pups, the average number of pups per litter dead at birth was 1.5 for controls and 2.0 for treatment animals ($p=0.25$). The average number of pups per litter that died after birth but before

TABLE 1

Differences Between Control (0.00 ppm chlorite) and Treatment (100 ppm chlorite) Groups for Ten Variables

VARIABLE	Control Group Mean	S	N	Treatment Group Mean	S	N	t-test* t	df	P
Live Litter Size	5.4	2.5	21	5.0	3.3	12	0 42	31	0.339
No. Alive at Weaning	4.2	2.4	19	3.4	3.7	12	0.73	29	0.237
Av. Pup Weaning-Weight	12.5	1.6	16	10.7	2.4	7	2.06	21	0.026
Birth-Weaning Growth Rate	0.408	0.055	16	0.336	0.082	7	2.11	21	0.024
Gestation Time	20.2	0.68	9	19.71	1.30	12	1.16*	17.1*	0.110
Breeding Weight of Mother	21.84	2.16	13	21.03	1.57	12	1.07	23	0.149
Age Mother at Parturition (weeks)	18.0	3.9	21	18.5	4.21	12		----------	
Average Live Birth Litter Weight	1.27	0.380	20	1.17	0.113	10	1.15*	24.7*	0.130
No. Dead at Birth	1.50	2.01	20	2.00	2.04	12	-0.68	30	0.252
No. Survivors Dying Before Weaning	1.32	1.77	19	1.90	2.02	10	-0.81	27	0.214

*: t-separate statistic, with adjusted degrees of freedom, are presented since the variances of the control and treatment groups are shown to be unequal by the F-test (α =0.10). Results are based on a one-sided test (H_A: mean for controls litters > mean for treatment litters).

t: S=standard deviation; N=number of litters.

weaning was 1.3 for controls and 1.9 for the treatment group ($p=0.214$).

The control litters had an average live birth litter weight of 1.27 g compared with 1.17 g for the treatment group ($p=0.13$). Two variables where statistical significance was reached includes the averaged weight of pups at weaning and the average birth to weaning growth rate. The birth to weaning growth rate was 0.336 g/day for treatment animals while being significantly greater at 0.408 g/day for the control group ($p=0.024$). Further, the average weight at weaning was 10.7 g for the treatment group and 12.5 g for the control group ($p=0.026$).

The conception rate was also determined for control and treatment groups. All females positive for vaginal plugs were randomly placed into control and treatment groups. Not all plugged females conceived or produced litters. The percent of dams that were plugged that also produced litters were defined as the conception rate. The conception rate for the treatment group was 39 percent and for controls was 56 percent.

Since differences between groups in the weight of dams, age, gestation time, or the amount of water consumed might have influenced the development of pups, these variables were measured. The gestation time of dams was not statistically different between control and treatment groups ($p=0.110$). The average breeding weight of the treatment and control dams was 21.03 and 21.84 g, respectively ($p=0.149$), while the age of mothers at parturition was 18 weeks for controls and 18.5 weeks for the treatment group. Finally, the level of water intake between control and treatment groups (Table 2) was measured. The water consumed during lactation is usually greater than that consumed during gestation, so these figures are presented separately. The control dams consumed an average of 5.9 mL/day during gestation and 15.6 mL/day during lactation. The treatment group consumed 6.2 mL/day of chlorite-treated water during gestation and 15.3 mL/day during gestation. The difference between treatment and control groups in the amount of water consumed was not significant for gestation period ($p=0.08$) or lactation period ($p=0.87$).

DISCUSSION

Chlorine dioxide is presently being considered by the U.S. EPA (1977) to replace chlorine as a disinfectant in many municipal water supplies. However, a byproduct of ClO_2 disinfection is chlorite ion, and this powerful oxidant was shown in recent reports to produce a compensated hemolytic anemia in animal models (HEFFERNAN 1979a,b, MOORE & CALABRESE 1980a) and to adversely effect human G6PD deficient red cells more than normal human red cells *in vitro* (MOORE & CALABRESE 1980b). Other high risk groups to oxidants would include fetuses and newborns

TABLE 2

Average Water Consumption (mL/day) During Gestation and Lactation for Treatment vs. Control (A/J Mice)

Period of Water Consumption		Treatment	Control
Gestation	x	6.2	5.9
	s	0.97	1.15
	n	20	22
Lactation	x	15.3	15.6
	s	3.06	2.86
	n	9	21

x = average; s = standard deviation; n = number of dams conceiving on treatment or control regimen.

(SHUVAL & GRUENER 1977, HANSEN & BENNETT 1964). This study has demonstrated that there are no effects of ingested chlorite ion (100 ppm) on pregnant dams when measuring gestation time, breeding weight, or age at parturition. Consequently, these variables do not appear to confound the results. Additionally, there is no significant difference in litter size which also rules this out as a confounding factor in influencing weaning weight and birth-weaning growth rate. Although the number of pups alive at weaning was somewhat greater for the controls, this difference agains was not significant. It would be expected that fewer mice in a litter either at birth or prior to the weaning process would result in greater weight gains (EPSTEIN, 1978). However, there were fewer in the treatment groups alive at weaning, yet they weighed significantly less on the average than controls, and had less weight gain. These results appear similar to the work of SHUVAL & GRUENER (1977) who reported that pups of dams which drink water with nitrite (1000-3000 mg/L) experienced increased mortality in the first three weeks, and lagged behind in growth despite having equal birth weights to controls. The conception rate of the dams drinking chlorite-treated water was reduced 17% compared to controls. These findings indicate that chlorite ion at 100 ppm concentration is capable of reducing the conception rate of A/J mice and of retarding the growth rate of A/J pups through weaning. Levels of 100 ppm chlorite represent a 10 to 100 fold increase over expected concentrations in drinking water and additional studies are being undertaken to determine minimal health effect levels. Further, these studies should be performed on other species of animals to provide a more accurate assessment of potential health effects on humans. Until such studies are completed, it seems reasonable to exercise caution in introducing ClO_2 in the nation's water supplies where chlorite formation in the 0.5 to 1.0 ppm range is likely (MILTNER 1977) and where 10.0 ppm has been achieved (HEFFERNAN, Personal Communication).

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