Journal of Toxicology and Environmental Health, Part A Current Issues

ISSN: 0098-4108 (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/uteh19

Toxicity of monochloramine in rat: An alternative drinking water disinfectant

Mohamed S. Abdel-Rahman , Duck H. Suh & Richard J. Bull

To cite this article: Mohamed S. Abdel-Rahman , Duck H. Suh & Richard J. Bull (1984) Toxicity of monochloramine in rat: An alternative drinking water disinfectant, Journal of Toxicology and Environmental Health, Part A Current Issues, 13:4-6, 825-834, DOI: 10.1080/15287398409530543

To link to this article: https://doi.org/10.1080/15287398409530543

TOXICITY OF MONOCHLORAMINE IN RAT: AN ALTERNATIVE DRINKING WATER DISINFECTANT

Mohamed S. Abdel-Rahman, Duck H. Suh

Toxicology Laboratory, Department of Pharmacology, UMDNJ—New Jersey Medical School, Newark, New Jersey

Richard J. Bull

U.S. Environmental Protection Agency, Health Effects Research Laboratory, Cincinnati, Ohio

Monochloramine (NH_2Cl) is under consideration as an alternative to chlorine as a disinfectant in public water supplies, to avoid trihalomethanes formation. This study was conducted to investigate the toxicity of NH_2Cl (0, 1, 10, 100 mg/l) in drinking water. Glutathione (GSH) content in rat blood was decreased significantly after 4 mo treatment, and the decreases were consistent throughout the treatment period. Treatment groups showed a slight increase in blood osmotic fragility. After acute administration (3 ml) of 20 and 40 mg NH_2Cl/l, blood GSH levels were increased as early as 15 min and the increases were consistent up to 1 h. After 2 h exposure, however, the GSH content returned to the control value. At 3 mo, red-blood-cell count and hematocrit were decreased significantly, while after 10 mo treatment significant decreases in hemoglobin concentration and mean corpuscular hemoglobin were observed. Monochloramine administered in drinking water for 3 mo increased the incorporation of [^{3}H]thymidine into nuclei of rat kidney and spleen in the 1- and 10-mg/l groups, while the incorporation in testes was increased only in the 100-mg/l group. The body weight of rats was decreased significantly in the highest treatment group after 3 mo treatment, and the decrease persisted throughout the period studied. An examination of blood chloroform content in all the groups after 4, 6, 9, and 12 mo showed no significant changes compared to the control.

INTRODUCTION

In recent years, there has been some concern and speculation regarding the use of chlorine as a disinfectant in public water supplies, since chlorine can react with various organic materials contained in natural waste waters to form trihalomethanes (Rook, 1976). Chloroform, one of the trihalomethanes, has been shown to increase the occurrence of cancers in several animal species (Kimura et al., 1971; National Cancer Institute, 1976). Moreover, epidemiological studies reported an increased risk of cancer mortality

Requests for reprints should be sent to Mohamed S. Abdel-Rahman, Toxicology Laboratory, Department of Pharmacology, UMDNJ—New Jersey Medical School, Newark, New Jersey 07103.

Journal of Toxicology and Environmental Health, 13:825–834, 1984

associated with the practice of chlorinating water (Kuzma et al., 1977; Page et al., 1976). This had led to a search for an alternative disinfectant to replace the widespread use of free chlorine.

A number of alternative methods of water disinfection, such as strong oxidizing agents, heat, ultraviolet (UV) irradiation, or change of pH, have been suggested (Calabrese et al., 1978). However, the most commonly discussed alternatives may include chlorine dioxide, chloramine, and ozone. Chlorine dioxide (ClO_2) is a stronger disinfectant than chlorine (White, 1972), and it has the advantage of not causing the formation of trihalomethanes in treated water (Miltner, 1976). However, the potential toxic effects of ClO_2 were observed in rat and chicken. Abdel-Rahman et al. (1979) demonstrated that ClO_2 in drinking water decreased blood glutathione (GSH) after 2 mo treatment. Moreover, alterations in red blood cell morphology from the normal shape to echinocyte and dacrocyte were observed in rat and chicken, respectively (Abdel-Rahman et al., 1979). Furthermore, chlorine dioxide in drinking water altered the incorporation of [^{3}H]thymidine into the nuclei of rat kidney, testes, and small intestine after 3 mo treatment (Abdel-Rahman et al., 1983a).

A combination of chlorine and ammonia treatment can be used as an alternative disinfectant to the use of free chlorine (Dowty et al., 1975; Eaton et al., 1973). This combination produces mainly monochloramine (NH_2Cl) and to a lesser extent dichloramine ($NHCl_2$), which do not form trihalomethanes or produce much lower concentrations of trihalomethanes (Symons et al., 1977; Brodtman and Russo, 1979). Although NH_2Cl is reported to be a less effective biocide compared to free available chlorine (Environmental Protection Agency, 1978), it shows prolonged duration of disinfective action, since residual chlorine is supplied slowly from NH_2Cl treatment. This practice also reduces unpleasant tastes and odors caused by chlorinated aromatic compounds normally present in chlorine treated water (Symons et al., 1977).

Chloramines have been found to cause the formation of methemoglobin, depression of the hexose monophosphate pathway, shortened erythrocyte survival, and hemolysis in hemodialyzed uremic patients (Eaton et al., 1973). Shih and Lederberg (1976) reported monochloramine to be a weak mutagen when reversion of Trp C to Trp$^+$ in strain 168 of *Bacillus subtilus* was used as an indicator. Recently, Abdel-Rahman et al. (1982a) demonstrated that there was no significant teratogenic effect in rats treated with NH_2Cl in drinking water daily for 3 mo prior to and throughout gestation.

The studies described in this report were conducted to provide information on blood glutathione, osmotic fragility, hematologic parameters, and chloroform formation in rats treated chronically with NH_2Cl in drinking water. In addition, the effect of NH_2Cl on the incorporation of [^{3}H]thymidine into the nuclei of rat liver, kidney, testes, small-intestinal mucosa, and spleen was studied.

MATERIALS AND METHODS

Preparation of NH_2Cl Solution

Chlorine gas (Matheson, E. Rutherford, N.J.) was bubbled into double-distilled water and the concentration was determined according to the diethyl-*p*-phenylene diamine (DPD) method of Palin (1967). Monochloramine solution was synthesized by the addition of stock chlorine and ammonium hydroxide to a bicarbonate buffer of pH 9.0, as described in a previous report (Abdel-Rahman et al., 1982a). The concentration of NH_2Cl was then determined by the DPD method of Palin (1967). This method also detects any small amounts of chlorine or di- or trichloramines present in the solution. The levels of chlorine and dichloramine were less than 1% of the NH_2Cl concentration, and no trichloramine was found in the solution.

Animals

Sprague-Dawley male rats (150–170 g; Taconic Farms, Germantown, N.Y.) were used in this study. The animals were maintained on a 12-h light/dark cycle with constant temperature (25°C) and relative humidity (50–55%). Animals drank 0, 1, 10, or 100 mg NH_2Cl/l in deionized water daily, and food was available *ad libitum*. Rat body weight was measured during the treatment.

Determination of Blood Glutathione and Osmotic Fragility

Heparinized blood was collected by cardiac puncture at 2, 4, 6, 8, 10, and 12 mo after the NH_2Cl treatment. For the acute experiments, 4 rats were given a single dose (3 ml) of 0, 10, 20, or 40 mg NH_2Cl/l by gavage, and blood was taken at 15, 30, 60, and 120 min after administration. Blood glutathione was determined by the method of Beutler et al. (1963). The osmotic fragility curve for rat blood was prepared with different concentrations of phosphate-buffered saline (PBS) (Dacie, 1977). The concentration of 0.45% PBS was used for the calculation of the osmotic fragility.

Hematologic Analysis of Blood Cell Compartment

Heparinized blood was obtained every month for analysis of blood cell compartment from rats treated with NH_2Cl. A Coulter counter (Model S) was used to determine white-blood-cell (WBC) and red-blood-cell counts (RBC), hemoglobin percent (HGB), hematocrit percent (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

[^{3}H]Thymidine Incorporation in Rat Organs

Methyl-[1′,2′-^{3}H]thymidine (110 Ci/mmol, New England Nuclear) was administered intraperitoneally (0.5 μCi/g body weight) to rats treated with

NH_2Cl for 3 mo. The rats were sacrificed by decapitation after 8 h following the administration. [^{3}H]Thymidine incorporation into the nuclei of rat liver, kidney, testes, small-intestinal mucosa, and spleen was determined by the method described by Abdel-Rahman et al. (1983a).

Determination of Chloroform Content in Blood

Heparinized blood was collected by cardiac puncture after 4, 6, 9, and 12 mo treatment. Chloroform concentration in blood was determined as described by Abdel-Rahman et al. (1982b).

Statistical Analysis

Treatment effects were analyzed by analysis of variance (ANOVA). Duncan's multiple-range test was applied for paired comparisons, and the significant values are reported at the $p < 0.05$ level.

RESULTS

Blood was collected in heparinized tubes by cardiac puncture at 2, 4, 6, 8, 10, and 12 mo from groups of rats drinking NH_2Cl. Blood glutathione and osmotic fragility were determined as described in the methods section.

The results of blood glutathione determination are shown in Table 1. At 4 mo, glutathione content decreased significantly in the 1- and 100-mg NH_2Cl/l groups. After 6 and 12 mo treatment, a significant decrease in the glutathione contents was observed in all treatment groups.

Blood osmotic fragility results are summarized in Table 2. Increased osmotic fragility was observed in the 10- and 100-mg/l groups after 2 mo treatment. Although the osmotic fragility was increased in all treatment groups throughout the treatment period, the significant values compared to control group were observed only in the 100- and 10-mg/l groups after 6 and 10 mo treatment, respectively.

TABLE 1. Effect of NH_2Cl in Drinking Water on Rat Blood Glutathione

Treatment time	Control	NH_2Cl level		
		1 mg/l	10 mg/l	100 mg/l
2 mo	41.5 ± 0.9[a]	45.3 ± 0.8	46.4 ± 2.3	44.0 ± 1.9
4 mo	43.6 ± 2.8	31.8 ± 1.3[b]	37.6 ± 2.7	34.9 ± 1.7[b]
6 mo	55.8 ± 2.6	42.5 ± 1.9[b]	44.7 ± 2.0[b]	43.0 ± 2.4[b]
8 mo	43.6 ± 3.2	35.4 ± 2.2	39.1 ± 6.2	32.5 ± 5.5
10 mo	47.1 ± 3.6	36.5 ± 3.4	58.7 ± 2.8[b]	49.8 ± 5.0
12 mo	46.9 ± 2.7	40.0 ± 1.7[b]	35.7 ± 0.3[b]	36.5 ± 1.5[b]

[a] Values represent the mean ± SE (mg%) from 4 rats/group.
[b] Significantly different from control ($p < 0.05$), analysis of variance.

TABLE 2. Effect of NH_2Cl in Drinking Water on Osmotic Fragility in the Rat

Treatment time	Control	NH_2Cl level		
		1 mg/l	10 mg/l	100 mg/l
2 mo	43.8 ± 4.0[a]	53.9 ± 8.5	62.9 ± 4.1[b]	55.6 ± 3.6[b]
4 mo	49.4 ± 4.5	47.7 ± 8.9	65.9 ± 5.9	63.1 ± 5.5
6 mo	33.5 ± 2.7	36.1 ± 6.4	41.8 ± 8.4	56.0 ± 4.0[b]
8 mo	58.7 ± 3.2	69.5 ± 11.7	59.6 ± 5.8	64.8 ± 5.0
10 mo	50.3 ± 6.1	43.4 ± 3.9	70.1 ± 6.4[b]	61.1 ± 4.0
12 mo	48.0 ± 8.5	54.2 ± 8.0	61.1 ± 9.0	66.9 ± 3.3

[a]Values represent the mean ± SE (% hemolysis) from 4 rats/group.
[b]Significantly different from control ($p < 0.05$), analysis of variance.

The control values at 30 and 60 min were not determined in Tables 3 and 4, because it has been observed that GSH concentration and hemolysis in control blood were not changed during the 2-h incubation period (Abdel-Rahman et al., 1979). The control values for 15 and 120 min were used in calculations for statistical analysis at 30 and 60 min, respectively. The early effect of NH_2Cl on rat blood glutathione after acute exposure is shown in Table 3. In the first 15 min, blood glutathione was increased significantly in the 20- and 40-mg/l groups, while the 10-mg/l group was without any change compared to the control. At 30 and 60 min, the glutathione content increased significantly in all treatment groups. However, after 2 h exposure, the GSH concentration returned to normal values in all treatment groups. In the same acute experiment, osmotic fragility was without any change in the treated groups (Table 4).

The hematologic analysis of blood cell compartment was performed every month, but only after 3 and 10 mo treatment were significant changes in the blood compartment observed in the treated groups. After 3 mo treatment, significant decreases in red-blood-cell count (RBC) and hematocrit

TABLE 3. The Early Effect of NH_2Cl on Rat Blood Glutathione after Acute Exposure

Treatment	Time (min)			
	15	30	60	120
Control	43.0 ± 1.5[a]	–[c]	–	40.3 ± 1.5
10 mg/l	44.0 ± 2.4	46.4 ± 2.5[b]	50.7 ± 1.1[b]	41.1 ± 3.5
20 mg/l	50.3 ± 1.4[b]	49.5 ± 1.1[b]	50.4 ± 2.3[b]	40.6 ± 3.0
40 mg/l	48.7 ± 2.8[b]	50.5 ± 1.5[b]	53.8 ± 2.2[b]	46.8 ± 6.8

[a]Values represent the mean ± SE (mg%) from 4 animals/group.
[b]Significantly different from control ($p < 0.05$), analysis of variance.
[c]Not determined.

TABLE 4. The Early Effect of NH_2Cl on Rat Blood Osmotic Fragility after Acute Exposure

Treatment	Time (min)			
	15	30	60	120
Control	48.0 ± 10.0[a]	–[b]	–	48.1 ± 11.2
10 mg/l	45.4 ± 6.9	35.3 ± 5.2	23.7 ± 3.9	31.4 ± 4.7
20 mg/l	54.4 ± 6.2	37.0 ± 5.5	44.9 ± 5.5	37.8 ± 4.7
40 mg/l	44.9 ± 12.5	30.1 ± 10.8	33.1 ± 12.0	28.8 ± 10.2

[a]Values represent the mean ± SE (% hemolysis) from 4 rats/group.
[b]Not determined.

(HCT) were observed in the 10- and 100-mg NH_2Cl/l groups (Table 5). However, after 10 mo treatment in the hemoglobin concentration (HGB) and mean corpuscular hemoglobin (MCH) were decreased significantly only in the 100-mg/l group (Table 6).

Table 7 summarizes the incorporation of [^{3}H] thymidine into the nuclei of rat organs (liver, kidney, testes, intestinal mucosa, and spleen) following 3 mo treatment. The results in kidney and spleen reveal that NH_2Cl increased the incorporation of [^{3}H] thymidine in the 1- and 10-mg NH_2Cl/l groups. In testes, about a threefold increase of [^{3}H] thymidine incorporation compared to the control was observed in the 100-mg/l group.

The weights of rats drinking NH_2Cl daily for 1 yr are given in Table 8. As little as 3 mo treatment with 100 mg NH_2Cl/l resulted in a significant decrease in body weight compared to the control. This decrease of body weight persisted throughout the treatment period, while no change was observed in the 1- and 10-mg/l groups.

Blood chloroform was measured in the control and treated animals after 4, 6, 9, and 12 mo. No significant values of chloroform level compared to the control were observed in rat blood during the treatment (Table 9).

DISCUSSION

The present investigation revealed that glutathione content in rat blood was decreased after treatment with NH_2Cl. The decrease of blood GSH

TABLE 5. Effect of NH_2Cl on Rat Blood Cell Compartment after 3 Months Treatment

Treatment	RBC (10^6/mm^2)	HGB (g%)	HCT (%)	MCV (FL)	MCH (PG)	MCHC (%)
Control	9.1 ± 0.2[a]	15.0 ± 0.3	45.9 ± 0.6	40.0 ± 0.9	14.8 ± 0.3	32.8 ± 0.3
1 mg/l	8.7 ± 0.2	15.5 ± 0.2	44.4 ± 1.0	42.8 ± 2.2	17.1 ± 0.7	34.9 ± 0.4
10 mg/l	8.3 ± 0.2[b]	15.1 ± 0.2	41.0 ± 0.6[b]	42.0 ± 2.3	17.8 ± 0.6	36.9 ± 0.6
100 mg/l	8.3 ± 0.1[b]	14.7 ± 0.3	40.3 ± 0.7[b]	39.8 ± 1.8	17.1 ± 0.7	36.4 ± 0.2

[a]Values represent the mean ± SE from 4 animals/group.
[b]Significantly different from control ($p < 0.05$), analysis of variance.

TABLE 6 Effect of NH_2Cl on Rat Blood Cell Compartment after 10 Months Treatment

Treatment	RBC ($10^6/mm^3$)	HGB (g%)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (%)
Control	7.2 ± 0.2[a]	14.7 ± 0.5	43.4 ± 1.6	52.5 ± 1.9	20.0 ± 0.1	34.2 ± 0.2
1 mg/l	7.4 ± 0.4	14.8 ± 0.8	41.7 ± 0.7	52.0 ± 1.7	19.6 ± 1.0	35.9 ± 2.2
10 mg/l	7.2 ± 0.4	13.0 ± 1.2	39.5 ± 1.0	49.7 ± 3.0	18.2 ± 2.6	33.1 ± 3.1
100 mg/l	7.4 ± 0.1	11.5 ± 0.4[b]	38.4 ± 0.6	45.0 ± 1.5	15.4 ± 0.4[b]	30.3 ± 1.5

[a] Values are the mean ± SE from 4 rats/group.
[b] Significantly different from control ($p < 0.05$), analysis of variance.

TABLE 7. Effect of NH_2Cl on [^{3}H]Thymidine Incorporation into Nuclei of Rat Organs

Treatment	Liver	Kidney	Testes	Intestinal mucosa	Spleen
Control	1.07 ± 0.04[a]	0.21 ± 0.03	0.98 ± 0.18	11.06 ± 1.75	0.57 ± 0.14
1 mg/l	1.70 ± 0.19	0.60 ± 0.08[b]	2.37 ± 0.30	18.00 ± 4.95	1.86 ± 0.38[b]
10 mg/l	1.57 ± 0.36	0.81 ± 0.21[b]	2.13 ± 0.72	8.09 ± 3.19	1.48 ± 0.41[b]
100 mg/l	1.89 ± 0.35	0.50 ± 0.08	2.95 ± 0.93[b]	10.63 ± 2.28	1.25 ± 0.33

[a] Values represent the mean ± SE as DPM/μg DNA from 4 rats/group given [^{3}H]thymidine (0.05 μCi/g body weight) after drinking NH_2Cl for 3 mo.
[b] Significantly different from control ($p < 0.05$), analysis of variance.

TABLE 8. Effect of NH_2Cl in Drinking Water on Rat Body Weight

Treatment time	NH_2Cl level			
	Control	1 mg/l	10 mg/l	100 mg/l
1 mo	354.6 ± 5.9[a]	358.8 ± 6.4	348.9 ± 6.6	343.4 ± 6.6
2 mo	405.4 ± 7.3	417.0 ± 6.9	407.3 ± 8.3	389.0 ± 8.8
3 mo	438.0 ± 11.0	452.0 ± 6.3	444.0 ± 8.4	401.0 ± 9.7[b]
4 mo	480.4 ± 7.9	493.4 ± 11.1	478.8 ± 8.3	437.8 ± 15.5[b]
5 mo	495.4 ± 10.0	515.6 ± 9.7	482.7 ± 14.0	457.2 ± 16.1[b]
6 mo	532.5 ± 12.0	531.0 ± 10.6	516.1 ± 14.7	477.9 ± 17.2[b]
9 mo	567.8 ± 14.1	549.2 ± 14.4	532.8 ± 15.6	477.4 ± 18.7[b]
11 mo	574.3 ± 14.6	564.6 ± 14.4	543.3 ± 19.5	499.2 ± 18.7[b]
12 mo	569.7 ± 13.2	577.3 ± 18.9	535.1 ± 14.0	473.2 ± 28.3[b]

[a] Values represent the mean ± SE (g) from 4 rats/group.
[b] Significantly different from control ($p < 0.05$), analysis of variance.

TABLE 9. Effect of NH_2Cl in Drinking Water on the Formation of Chloroform in Rat Blood Following Chronic Exposure

Treatment time	Control	NH_2Cl level		
		1 mg/l	10 mg/l	100 mg/l
4 mo	0.0 ± 0.0[a]	–[b]	0.0 ± 0.0	0.2 ± 0.2
6 mo	2.7 ± 2.5	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2
9 mo	0.2 ± 0.2	0.0 ± 0.0	2.2 ± 2.2	0.0 ± 0.0
12 mo	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.0 ± 0.0

[a]Values represent the mean ± SE as ng/ml from 4 rats/group.
[b]Not determined.

could be related to the oxidative stress of NH_2Cl and the protective role of GSH against damage caused by oxidants (Hill et al., 1964). However, blood GSH concentrations were increased after acute exposure to NH_2Cl. The increase in GSH level is most likely related to an increase in the activity of RBC glutathione reductase to compensate for the oxidation stress of NH_2Cl during the first hour after acute exposure. In a similar experimental condition, Abdel-Rahman et al. (1979, 1983b) demonstrated that chlorine dioxide and chlorine decreased rat blood glutathione after acute exposure. The discrepancy observed in the present study may be explained by the oxidative property of NH_2Cl, which releases residual chlorine slowly and enhances the activity of glutathione reductase. Further study is in progress in our laboratory to confirm this phenomenon.

The present data show that erythrocytic fragility was increased after 2 mo treatment with NH_2Cl. The increased osmotic fragility may be explained by the oxidation of thiol groups and the formation of disulfide bonds on the red-cell membrane, causing hemolysis of erythrocytes. A depressed hemolysis in controls at 6 mo might be correlated with the elevated glutathione levels exhibited by this group at this time period. A lack of dose-response or time-dependent effect in GSH and hemolysis with NH_2Cl administration is in agreement with other chlorine compounds such as chlorine dioxide and chlorine (Abdel-Rahman et al., 1979, 1983b).

An analysis of red-cell compartment demonstrated that rats drinking NH_2Cl for 3 mo exhibited depressed red-blood-cell counts and packed cell volume. Also, the decreases in hemoglobin concentration and mean corpuscular hemoglobin (MCH) were observed after 10 mo treatment. Since MCH is one of the erythrocyte indices that define the size and hemoglobin content of the red blood cell, a lower MCH value indicates some degree of damage to the erythrocytes.

[^{3}H]Thymidine incorporation studies have shown that NH_2Cl increased DNA synthesis in kidney, testes, and spleen (Table 7). The data in this report are in agreement with studies of chlorine, while ClO_2 decreases DNA synthesis in kidney and testes after 3 mo treatment (Abdel-Rahman et al., 1983a, 1983b).

It has been demonstrated that chlorite (ClO_2^-), a metabolite of ClO_2, elicited the production of hydrogen peroxide (H_2O_2) (Heffernan et al., 1979). Harrison and Schultz (1976) presented the involvement of myeloperoxidase (MPO) and its cofactor (H_2O_2) in the peroxidation of chloride ions to hypochlorous acid (HOCl). This HOCl may then react with organic compounds to form chloroform. Although NH_2Cl is metabolized to chloride (Cl^-) in the rat (Abdel-Rahman et al., 1983c), NH_2Cl releases residual chlorine slowly and may produce H_2O_2 at the minimal level, which may not influence the MPO system to form chloroform *in vivo* Table 9).

REFERENCES

Abdel-Rahman, M. S., Couri, D., and Bull, R. J. 1979. Kinetics of ClO_2 and effects of ClO_2, ClO_2^-, and ClO_3^- in drinking water on blood glutathione and hemolysis in rat and chicken. *J. Environ. Pathol. Toxicol.* 3:431-449.

Abdel-Rahman, M. S., Berardi, M. R., and Bull, R. J. 1982a. Effect of chlorine and monochloramine in drinking water on the developing rat fetus. *J. Appl. Toxicol.* 2:156-159.

Abdel-Rahman, M. S., Couri, D., and Bull, R. J. 1982b. Metabolism and pharmacokinetics of alternate drinking water disinfectants. *Environ. Health Perspect.* 46:19-23.

Abdel-Rahman, M. S., Couri, D., and Bull, R. J. 1983a. Toxicity of chlorine dioxide in drinking water. *J. Environ. Pathol. Toxicol.* in press.

Abdel-Rahman, M. S., Suh, D. H., and Bull, R. J. 1983b. Pharmacodynamics and toxicity of chlorine in drinking water in rat. *J. Appl. Toxicol.* in press.

Abdel-Rahman, M. S., Waldron, D. M., and Bull, R. J. 1983c. A comparative kinetics study of monochloramine and chlorine in rat. *J. Appl. Toxicol.* 3:175-179.

Beutler, E., Duran, O., and Kelly, B. 1963. Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.* 61:882-888.

Brodtman, N. W. Jr. and Russo, P. J. 1979. The use of chloramine for reduction of trihalomethanes and disinfection of drinking water. *J. Am. Water Works Assoc.* 71:40-42.

Calabrese, E. J., Moore, T. S., and Tuthill, R. W. 1978. The health effects of chlorine dioxide as a disinfectant in potable water: A literature survey. *J. Environ. Health* 41:26-31.

Dacie, J. V. 1977. Tests for hemolytic disease: Osmotic fragility of erythrocytes. In *Laboratory Medicine Hematology*, eds. J. B. Miale, pp. 1020-1021. St. Louis: C.V. Mosby.

Dowty, B. J., Charlisle, D. R., and Laseter, J. L. 1975. New Orleans drinking water sources tested by gas chromatography-mass spectrometry. *Environ. Sci. Technol.* 9:762-765.

Eaton, J. W., Dolpin, C. F., Kjellstrand, C. M., and Jacob, H. S. 1973. Chlorinated urban water: A cause of dialysis-induced hemolytic anemia. *Science* 181:463-464.

Environmental Protection Agency. 1978. Interim Primary Drinking Water Regulations. "Control of organic chemical contaminants on drinking water." *Fed. Reg.* Part II:5756-5780.

Harrison, J. E. and Schultz, J. 1976. Studies on the chlorinating activity of myeloperoxidase. *J. Biol. Chem.* 251:1371-1374.

Heffernan, W. P., Guion, C., and Bull, R. J. 1979. Oxidative damage to the erythrocyte induced by sodium chlorite, *in vivo*. *J. Environ. Pathol. Toxicol.* 2:1487-1499.

Hill, A. S. Jr., Haut, A., and Wintrose, M. M. 1964. The role of non-hemoglobin proteins and reduced glutathione in the protection of hemoglobin from oxidation *in vitro*. *J. Clin. Invest.* 43:17-26.

Kimura, E. T., Ebert, D. N., and Dodge, P. W. 1971. Acute toxicity and limits of solvent residues for sixteen organic solvents. *Toxicol. Appl. Pharmacol.* 19:699-704.

Kuzma, R. J., Kuzma, D. M., and Buncher, C. R. 1977. Ohio drinking water source and cancer rates. *Am. J. Public Health* 67:725-729.

Miltner, R. J. 1976. The effect of chlorine dioxide on trihalomethanes in drinking water. Master thesis, University of Cincinnati, Cincinnati, Ohio.

National Cancer Institute. 1976. *Report on the Carcinogenesis Bioassay of Chloroform, NCI.* Springfield, Va.: National Technical Information Source No. PB264018/AS.

Page, T., Harris, R. H., and Epstein, S. S. 1976. Drinking water and cancer mortality in Louisiana. *Science* 193:55-57.

Palin, A. T. 1967. Methods for the determination in water, of free and combined available chlorine, chlorine dioxide and chlorite, bromine, iodine and ozone using diethyl-*p*-phenylene diamine (DPD). *J. Inst. Water Eng.* 21:537-547.

Rook, J. J. 1976. Haloforms in drinking water. *J. Am. Water Works Assoc.* 68:168-172.

Shih, K. and Lederberg, J. 1976. Chloramine mutagenesis in *bacillus subtilis. Science* 192:1141-1143.

Symons, J. M., Carswell, J. K., Clark, R. M., Dorsey, P., Geldreich, E. E., Heffernan, W. P., Hoff, J. C., Lore, O. T., McCabe, L. J., and Stevens, A. A. 1977. *Ozone, Chlorine Dioxide and Chloramines as Alternatives to Chlorine for Disinfection of Drinking Water. State-of-the-Art.* Water Supply Research, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

White, G. C. 1972. *Handbook of Chlorination*, pp. 744-750. New York:Van Nostrand Reinhold.

Received June 3, 1983
Accepted October 24, 1983