Metabolism and Pharmacokinetics of Alternate Drinking Water Disinfectants

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The chlorination of surface waters is known to elevate trihalomethanes; consequently, chlorine dioxide (ClO₂) is being considered as an alternative disinfectant. The primary products resulting from ClO₂ disinfection of waters are chlorites (ClO₂⁻) and chlorates (ClO₃⁻). Studies in rats revealed that ClO₂ is converted to chloride (Cl⁻), ClO₂⁻ and ClO₃⁻. ClO₂⁻ and ClO₃⁻ are excreted as Cl⁻, ClO₂⁻ and Cl⁻, ClO₂⁻, ClO₃⁻, respectively. Radioactivity was rapidly absorbed from the gastrointestinal tract following the administration of ClO₂ orally, and the half-life for the elimination of Cl from the rat was 44 hr, corresponding to a rate constant of 0.016/hr. After 72 hr, radioactivity was highest in plasma, followed by kidney, lung, and stomach. Cl in plasma reached a peak at 2 hr and 1 hr after oral administration of ClO₂ and ClO₃⁻, respectively. Cl excretion was greatest 24 hr after the administration of ClO₂⁻, but in the case of ClO₃⁻, the excretion probably represented saturation of the biotransformation and excretion pathway. A low activity in packed cells compared to plasma was detected in chlorate ingestion, rather than an even distribution in chloride treatment. Chloroform determinations in rat blood after one year indicated that chloroform was significantly higher than control in the 100 and 1000 mg/l ClO₂ groups. However, no significant values were observed in the 1 or 10 mg/l ClO₂ and ClO₃⁻ metabolites group. ClO₂ and its metabolites are eliminated from the body more rapidly than chlorine, and they do not appear to increase trihalomethane concentrations at low dosages.

Introduction

Chlorination is, and always has been, the most widely used method for disinfection of public water supplies. Concern over its possible adverse effects and those of its by-products on humans has increased considerably in recent years. The formation of trihalomethanes such as chloroform (CHCl₃), bromodichloromethane (CHBrCl₂), dibromochloromethane (CHBr₂Cl) and bromoform (CHBr₃) during the chlorination process has become evident (1, 2).

The United States Environmental Protection Agency conducted a comprehensive survey of 80 water supplies in the U.S. for haloegenated organics (3). It was found that the four trihalomethanes—CHCl₃, CHBrCl₂, CHBr₂Cl, CHBr₃—are widespread in chlorinated drinking water in the U.S. and result from chlorination, since these substances were not found in raw waters. The trihalomethanes were not a result of impurities in the chlorine being used but were a result of the reaction of chlorine with precursor. Morris (4) made the point that the number of trihalomethane precursors is extensive and includes ethanol, acetaldehyde and the methyl ketones. Rook (1) and Morris (4) indicated that the bromide ion present in a natural water is rapidly oxidized by aqueous chlorine to hypobromous acid (HOBr), and that this acid reacts with precursor to account for the formation of the brominated species.

Since these trihalomethanes may be a health hazard, chlorine dioxide (ClO₂) is one of the alternative disinfectants being considered because it prevents the formation of trihalomethanes (5, 6). ClO₂ is a more effective oxidizing agent than chlorine and greatly improves the taste of water by avoiding the unpleasant taste associated with chlorination of...
water (7). The unpleasant taste of chlorine in water is caused partially by the build-up of chlorinated phenols.

Miltner (6) has reported that the primary products resulting from ClO₂ disinfection of surface waters include chlorites (ClO₂⁻) and chlorates (ClO₃⁻), which appear in concentrations of 50% and 30% of ClO₂ demand, respectively.

Toxicity studies demonstrated that rats drinking ClO₂, ClO₂⁻ and ClO₃⁻ (Cl compounds) daily for 9 months exhibited depressed red blood cell counts, hemoglobin concentration and packed cell volumes. Also, these Cl compounds in drinking water for three months inhibited the incorporation of ³H-thymidine into nuclei of rat testes (8).

The studies described in this report were conducted to provide information on metabolism and pharmacokinetics of chlorine, chlorine dioxide, chlorite and chloride in the rat. Also, the effect of ClO₂ in drinking water on the formation of CHCl₃ in rat blood and various organs was studied.

Methods

Synthesis of ³⁶Cl Compounds

H³⁶Cl was obtained from New England Nuclear. This material, with a radionuclidic purity of > 99% was used as the source of ³⁶Cl. The potassium chlorate (K³⁶ClO₃) was synthesized according to the method of Abdel-Rahman et al. (9). The generation of ⁴⁰ClO₂ from K³⁶ClO₃ was accomplished by the reaction (1). ⁴⁰Cl₂ was formed by the reaction (2).

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2K³⁶ClO₃ + (COOH)₂ + 2H₂SO₄ \rightarrow 2⁴⁰ClO₂ + 2KHSO₄ + 2CO₂ + 2H₂O \quad (1)
\]

KMnO₄ + 16H³⁶Cl \rightarrow 2K³⁶Cl + Mn³⁶Cl₂ + 5⁴⁰Cl₂ + 8H₂O (2)

The purity of the synthesized K³⁶ClO₃, which is an essential intermediate step to the production of ClO₂, was 95%, as determined by fast neutron activation analysis.

Kinetics of Chlorine Dioxide, Chlorite, Chlorate and Chlorine in the Rat

Four groups of four male Sprague-Dawley rats (230 ± 20 g) each were used in these experiments. ³⁶Cl compounds were administered by gavage as follows: 3 ml of 100 mg/l ³⁶ClO₂ (0.7 μCi) was administered to each rat, producing a dose of 1.5 mg/kg body weight; 3 ml of 10 mg/l ³⁶ClO₂⁻ (0.17 μCi) was administered to each rat, producing a dose of 0.15 mg/kg body weight; 3 ml of 5 mg/l ³⁶ClO₂⁻ (0.85 μCi) was administered to each rat, producing a dose of 0.065 mg/kg body weight; 3 ml of 250 mg/l H²⁰Cl (0.45 μCi) was administered to each rat, producing a dose of 3.26 mg/kg body weight.

Heparinized blood samples were collected at 5, 10, 20, 30 and 60 min, and 2, 4, 8, 24 and 48 hr by orbital sinus puncture. The blood was centrifuged at 1000g for 15 min to separate the red blood cells from the plasma. Then the radioactivity was counted in a liquid scintillation counter (Beckman LS 7500), and the rate constant and T½ of absorption and elimination from plasma were calculated for each compound.

Distribution of Cl Compounds in the Rat

After 72 hr following the administration of ³⁶Cl compounds as described previously, rats were killed by decapitation, and blood was collected in heparinized tubes. Tissue specimens of stomach, testes, lung, kidney, duodenum, ileum, spleen, liver, bone marrow, carcass and skin were prepared for the determination of ³⁶Cl content by liquid scintillation spectrometry as follows: Whole blood and packed cells were prepared according to the method of Mahin et al. (10). Samples of wet tissue weighing up to 200 mg and samples of bone marrow up to 50 mg were placed in individual glass LSC vials with Teflon or polyethylene-lined caps. Then 2 ml Protosol was added to each vial, which was capped tightly to prevent loss of solvent. Samples were solubilized by heating overnight at 50°C, then cooled at room temperature; afterwards 0.2 ml of 30% H₂O₂ was added for decolorization. The samples were heated at 50°C for 30 min, then cooled, and 15 ml of Aquasol-2 was added.

Sample quench effects were corrected by using the method of channel ratios, and efficiency was greater than 80%.

Metabolism and Excretion Studies

Four groups of four male Sprague-Dawley rats/group were used in these studies. Each group was administered orally the same concentrations of ³⁶Cl compounds described above. Animals were housed in modified Roth all-glass metabolism chambers for the collection of expired air and fecal and urine samples. The radioactivity of the total ³⁶Cl compounds was measured. The analysis of radioactive metabolites (Cl, ClO₂⁻, ClO₃⁻) was performed as described in a previous report, (9), and measured as a percentage of the initial dose.
Effect of Chlorine Dioxide, Chlorite and Chlorate on the Formation of Chloroform in Rat Blood and Organs

Male Sprague-Dawley rats (50–170 g) were used in this experiment. The animals were housed in a controlled environment (temperature, 22°C; humidity 50%) with a 12 hr light/dark cycle beginning at 6:00 A.M. Food was available ad libitum. Groups of rats were allowed to drink doubly distilled water containing 10 or 100 mg/l. ClO₂, ClO₂⁻ or ClO₃⁻ for 20 hr/day, 7 days/week for 12 months. ClO₂ was generated daily and water concentrations determined by the DPD (diethyl-p-phenylenediamine) method of Palin (11).

After one year, rats were killed by decapitation and blood collected in heparinized tubes. Tissue samples of liver, kidney, spleen, brain, and testes were homogenized in isotonic sucrose medium. Tissue homogenate or blood sample (3 ml) was extracted by 5 ml pentane. The pentane layer was then separated by centrifugation at 1000g for 5 min. A gas chromatograph equipped with an electron capture detector was used for the quantitation of chloroform in each sample, with bromodichloromethane being used as the internal standard.

Results

Kinetics of Cl Compounds in the Rat

The rate constant and T₁/₂ for absorption and elimination from plasma of Cl compounds are shown in Table 1. The rate constant for absorption was highest for ⁰³ClO₂ (3.77 ± 0.24/hr), followed by ⁰³ClO₃⁻, ⁰³ClO₂⁻, and lowest for HO⁻³Cl (0.157 ± 0.001/hr). The T₁/₂ for absorption, which is inversely proportional to the rate constant, was longest for HO⁻³Cl (4.21 ± 1.31 hr), followed by ⁰³ClO₂⁻, ⁰³ClO₂⁻, and was shortest for ⁰³ClO₂ (0.18 ± 0.01 hr). For elimination from plasma, the rate constant was highest for ⁰³ClO₂⁻ (0.02 ± 0.002/hr), followed by ⁰³ClO₃⁻, ⁰³ClO₂ and HO⁻³Cl (0.009 ± 0.001/hr). The T₁/₂ elimination from plasma was longest for HO⁻³Cl (77.0 ± 8.8 hr), followed by ⁰³ClO₂, ⁰³ClO₃⁻, and ⁰³ClO₂⁻ (35.2 ± 3.0 hr).

Distribution of Cl Compounds in the Rat

The distribution of ³⁰Cl compounds after 72 hr from the time of oral administration is shown in Table 2. Values are expressed as a percentage of

### Table 1. Kinetic constants of chlorine dioxide, chlorite, chlorate and chloride in the rat.*

| Treatment       | Absorption, hr⁻¹ | Elimination, hr⁻¹ |
|-----------------|------------------|-------------------|
|                 | Rate constant    | T₁/₂              | Rate constant    | T₁/₂              |
| ClO₂ (100 mg/l.)| 3.77 ± 0.24      | 0.18 ± 0.01       | 0.0158 ± 0.0008  | 43.9 ± 2.3        |
| ClO₂⁻ (10 mg/l.)| 0.198 ± 0.06     | 3.50 ± 1.06       | 0.0197 ± 0.0017  | 35.2 ± 3.0        |
| ClO₃⁻ (5 mg/l.) | 0.399 ± 0.151    | 1.74 ± 0.66       | 0.019 ± 0.003    | 36.7 ± 5.8        |
| HO⁻³Cl (250 mg/l.)| 0.157 ± 0.001   | 4.42 ± 1.31       | 0.009 ± 0.001    | 77.0 ± 8.8        |

*Values were calculated from the time course of the elimination from rat plasma; n = 4 rats per treatment.

### Table 2. Chlorine dioxide, chlorite, chlorate and chloride distribution in the rat.

| Tissue          | ⁰³ClO₂ (100 mg/l.) | ⁰³ClO₂⁻ (10 mg/l.) | ⁰³ClO₃⁻ (5 mg/l.) | HO⁻³Cl (250 mg/l.) |
|-----------------|-------------------|--------------------|-------------------|--------------------|
| Plasma          | 0.72 ± 0.02       | 0.55 ± 0.038       | 0.68 ± 0.09       | 0.77 ± 0.04        |
| Packed cells    | —b                | 0.63 ± 0.11        | 0.23 ± 0.02       | —                  |
| Whole blood     | —                 | 0.64 ± 0.01        | 0.57 ± 0.05       | —                  |
| Kidney          | 0.81 ± 0.15       | 0.30 ± 0.06        | 0.42 ± 0.07       | 0.39 ± 0.03        |
| Lung            | 0.74 ± 0.15       | 0.37 ± 0.04        | 0.45 ± 0.07       | 0.34 ± 0.02        |
| Stomach         | 0.70 ± 0.15       | 0.40 ± 0.07        | 0.46 ± 0.05       | 0.29 ± 0.03        |
| Jejunum         | 0.29 ± 0.07       | 0.51 ± 0.01        | 0.34 ± 0.04       | 0.29 ± 0.03        |
| Jejunum         | 0.48 ± 0.09       | 0.17 ± 0.03        | 0.21 ± 0.02       | 0.14 ± 0.02        |
| Liver           | 0.38 ± 0.09       | 0.06 ± 0.03        | 0.20 ± 0.03       | 0.20 ± 0.01        |
| Spleen          | 0.25 ± 0.04       | 0.22 ± 0.02        | 0.29 ± 0.04       | 0.23 ± 0.02        |
| Bone marrow     | 0.16 ± 0.03       | 0.09 ± 0.03        | 0.15 ± 0.03       | 0.40 ± 0.01        |
| Testes          | —                 | 0.39 ± 0.04        | 0.45 ± 0.07       | 0.37 ± 0.02        |
| Skin            | —                 | 0.38 ± 0.06        | 0.42 ± 0.10       | 0.32 ± 0.03        |
| Carcass         | —                 | 0.25 ± 0.04        | 0.21 ± 0.02       | 0.18 ± 0.003       |

*Values represent mean ± SE as percentage of the initial dose from four rats per treatment after 72 hr.

bNot determined.
the highest testes, marrow, bone highest liver, kidney, skin, carcass estin stomach, ileum, packed 22 Table 36Cl dose from four a Values Treatment 36ClO2-(100 mg/l.) 36ClO2-(100 mg/l.) 36ClO2-(5 mg/l.) b None Treatment Urine 36ClO2-(10) 0Cl02 36CIO3-(5) a Values H036C1 In Treatment amg/l. was not initial dose. The distribution of 36ClO2 was highest in the kidney, followed by lung, plasma, stomach, ileum, liver, duodenum, spleen and bone marrow, while the distribution of HO36Cl was highest in plasma, followed by bone marrow, kidney, testes, lung, skin, duodenum, spleen, stomach, liver, carcass and ileum. The distribution of 36ClO2 was highest in plasma, followed by stomach, testes, skin, lung, duodenum, kidney, carcass, spleen, ileum, bone marrow and liver. In the case of 36ClO2-, plasma was highest, followed by stomach, lung, testes, kidney, skin, duodenum, spleen, ileum, carcass, liver and bone marrow.

In whole blood, when plasma was separated from packed cells, radioactivity was divided more or less evenly between the plasma and packed cells (0.55 ± 0.038 and 0.63 ± 0.11% of initial dose, respectively) for 36ClO2-, whereas for 36ClO3-, radioactivity preferentially accumulated in the plasma (0.68 ± 0.09% of initial dose vs. 0.23 ± 0.02% in the packed cells).

Metabolism of Cl Compounds

The administered 36Cl compounds are found as various metabolites in rat urine, as shown in Table 3. After 72 hr from the time of administration, 36ClO2 and 36ClO3 are found in urine as Cl-, ClO2- and ClO3- whereas 36ClO2- is found as Cl- and ClO2-. In all three cases, Cl- is the major metabolite (approximately 87, 84 and 64% of the total radioactivity found in urine for 36ClO2, 36ClO2- and 36ClO3-, respectively).

Excretion of Cl Compounds

The total of the 36Cl compounds recovered in the urine and feces 72 hr after administration was determined for each compound and calculated as the percentage of the initial dose. In the case of 36ClO2, 75% of the recovered dose was found in the urine, while 25% was found in the feces. For 36ClO2-, 87 and 13% was found in urine and feces, respectively; and for HO36Cl, 76 and 24% was found in urine and feces, respectively (Table 4).

Table 4. Excretion of chlorine dioxide, chlorite, chlorate and chlorine in the rat.

| Treatment | Percentage of initial dose* |
|-----------|-----------------------------|
|           | Urine | Feces |
| 36ClO2 (100 mg/l.) | 30.81 ± 0.8 | 10.10 ± 1.7 |
| 36ClO2 (10 mg/l.) | 34.51 ± 1.4 | 4.75 ± 1.0 |
| 36ClO2 (5 mg/l.) | 40.14 ± 2.14 | 3.14 ± 1.0 |
| HO36Cl (250 mg/l.) | 21.52 ± 2.51 | 7.09 ± 0.24 |

*Values represent the mean ± SE as percentage of the initial dose from four rats per treatment, throughout the 72 hr studied. 36Cl was not detected in expired air.

Table 5. Effect of chlorine dioxide, chlorite and chlorate on the formation of chloroform in rat blood and organs.

| Treatment* | Concentration, mg/l. | n | Organ | Effects |
|------------|----------------------|---|-------|---------|
| ClO2       | 10                   | 4 | Blood | No effect |
|            | 100                  | 7 | Blood | Increased |
|            | 10                   | 4 | Liver, kidney, spleen, brain | No effect |
|            | 10                   | 4 | Testes | Increased |
| ClO2-      | 100                  | 7 | Liver, testes, brain | Increased |
| ClO3-      | 100                  | 7 | Blood | No effect |
|            | 100                  | 9 | Liver | Increased |
|            | 100                  | 9 | Liver | Increased |

*Rats were treated with chlorine dioxide and its metabolites daily for one year.
PHARMACOKINETICS OF CHLORINE COMPOUNDS

$^{36}$Cl compounds were not found in the expired air of any treatment group throughout the 72 hr time period.

Effect of Cl Compounds on the Formation of Chloroform in Rat Blood and Organs

Table 5 shows the effects of chronic treatment with chlorine dioxide and its metabolites for one year on the formation of chloroform in rat blood, liver, kidney, spleen, brain and testes. A concentration of 100 mg/l. ClO$_2$ daily in the drinking water caused an increase in chloroform levels in blood, liver, testes and brain. A concentration of 10 mg/l. ClO$_2$ increased chloroform levels in rat testes, while levels in blood, liver, kidney, spleen and brain remained unchanged. Treatment of rats with 100 mg/l. ClO$_2^-$ for one year elevated chloroform levels in the liver and brain, while the levels in blood were not affected. Treatment with 100 mg/l. ClO$_3^-$ increased liver but not blood chloroform concentrations after one year.

Discussion

From the kinetics study, it is apparent that ClO$_2$ and its metabolites (ClO$_2^-$ and ClO$_3^-$) are more rapidly absorbed into the bloodstream than HOCl. The effect is very pronounced when comparing ClO$_2$ itself with HOCl. Likewise, HOCl is more slowly eliminated from rat plasma than ClO$_2$ or its metabolites.

The distribution study revealed that although plasma and whole blood contain the highest activity, the bone marrow contained far less in $^{36}$ClO$_2$, $^{36}$ClO$_2^-$ and $^{36}$ClO$_3^-$ treatment, while HOCl had a higher activity in bone marrow. The high activity in testes, even after 72 hr, indicates a possible pharmacologic action at this site. Effects of these compounds on the reproductive system are currently in progress in our laboratory.

It is important to indicate that after 72 hr, high amounts of radioactivity were still found in the stomach, duodenum and ileum.

The Cl compounds studied are eliminated mainly by kidney and intestinal routes, since no radioactivity was found in expired air. For HO$^{36}$Cl and $^{36}$ClO$_2$ treatment, about 75% of the recovered dose was found in the urine; in the case of $^{36}$ClO$_2^-$ and $^{36}$ClO$_3^-$, about 90% of the recovered dose was in the urine.

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