Toxicological Effects of Chlorine Dioxide, Chlorite and Chlorate

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Review of the available literature obtained from both acute and chronic experiments utilizing rats, mice and chickens treated with ClO₂, ClO₂⁻ and ClO₃⁻ in drinking water has demonstrated alterations in hematologic parameters in all species tested. The effects were usually dose related and marked changes occurred only at the higher dosages (up to 1000 mg/l.). In chronic studies, rats have been given ClO₂ at doses of up to 1000 mg/l., and NaClO₂ or NaClO₃ at up to 100 mg/l., in their drinking water for one year. Treatment groups receiving ClO₂, ClO₂⁻ or ClO₃⁻ showed alterations in erythrocyte morphology and osmotic fragility; at higher dosages mild hemolytic anemia occurred. An examination of blood glutathione content and RBC enzymes involving glutathione formation showed a dose-related diminution of glutathione in chlorine compound treated groups. The higher oxidative capacity of the chlorine compounds resulting in the decreased erythrocytic glutathione might well be the principal biochemical event leading to the other hematological alterations.

More recent data show that ClO₂, ClO₂⁻ and ClO₃⁻ alter the incorporation of ³H-thymidine into the nuclei of various organs of the rat. These data suggest the possibility of increased turnover cells of the gastrointestinal mucosa and inhibited DNA synthesis in several organs. In the latter category, most concern revolves around whether or not the apparent depression of DNA synthesis in the testes is associated with depressed spermatogenesis and reproductive toxicity in the male rat.

Introduction

Institution of disinfection to drinking water treatment has been one of the key successes of public health policy. In the United States almost complete dependence has been placed on chlorine for primary disinfection. In recent years, however, it has become clear that treatment of drinking water with chlorine results in the formation of trihalomethanes (1, 2). One of the trihalomethanes, chloroform, has been shown to be carcinogenic in mice and rats (3).

These observations stimulated a search for a means to minimize the formation of these products. One of the most attractive alternatives is to substitute disinfection methods that do not promote the formation of chlorinated by-products.

Chlorine dioxide is a very attractive alternative to chlorine as a disinfectant. For all intents and purposes its disinfectant properties are equivalent to or perhaps exceed those of chlorine (4). Chlorine dioxide does not react with phenol to produce the same taste and odor problems that result from chlorine treatment (5). It has the added advantage that formation of trihalomethanes does not occur (6, 7).

Prior to the recent consideration of chlorine dioxide as a primary disinfectant for potable water, very little information existed concerning its tox-
Hematologic Effects

Rats, mice and chickens have been treated with ClO₂, ClO₂⁻ and/or ClO₃⁻ in drinking water at concentrations ranging from 1 to as high as 1000 mg/l. A relatively consistent picture has been observed across species. In the rat, Heffernan et al. (9) demonstrated that chloride concentrations of 100 mg/l. and above resulted in decreased red blood cell counts, hemoglobin concentrations and packed cell volume at 30 and 60 days of exposure. These effects tended to be reversed by 90 days of exposure despite continued treatment with sodium chloride. These data were extended to the mouse by Moore and Calabrese (10) with very similar results. Abdel-Rahman et al. (11) studied the effects of ClO₂ and ClO₃⁻ as well as ClO₂⁻ in both the rat and chicken for periods of up to 9 months. Signs of anemia observed in shorter term exposures of 30 to 60 days (12) had substantially disappeared in this interval with ClO₂ and ClO₃⁻. However, the results obtained with ClO₃⁻ at 9 months indicated 13 and 28% decreases in RBC at 10 and 100 mg/l. This represented a considerably greater effect than had been observed at shorter time intervals.

Accompanying the relatively small decreases in RBC count is subclinical evidence of increased red cell destruction. This was first reported by Heffernan et al. (9) in the cat, where significant decreases in the half-life of erythrocytes were observed at concentrations of ClO₂⁻ of 100 mg/l. of drinking water and above. No effect was observed at 10 mg ClO₂⁻/l. Other investigators have provided evidence of distortions in erythrocyte morphology in mice (10) as well as the rat and chicken (12). These changes were quite marked at concentrations of 100 mg ClO₂⁻/l. ranging from mildly crenated erythrocytes which maintained their discoid shape to crenated spheres (echinocytes). With ClO₃⁻ treatment, codocytes (Mexican hat-shaped) were also observed in the rat. In the chicken, the characteristic abnormality was the dacrocyte, which was evident primarily with ClO₂ treatments of 100 and 1000 mg/l. Early studies had somewhat paradoxically indicated that ClO₂, ClO₂⁻ and ClO₃⁻ decreased osmotic fragility of erythrocytes isolated from treated animals (12). Subsequent experiments have shown this effect is most likely due to the oxidation of thiol groups resulting in the precipitation of hemoglobin to give falsely low indications of hemolysis (13). This is also consistent with the in vitro observations of Heffernan et al. (14) that a complete loss of measurable pigment (both hemoglobin and methemoglobin) occurred at high concentrations of ClO₂⁻.

Biochemical Effects

Early reports had documented the ability of ClO₂⁻ to oxidize hemoglobin to methemoglobin by high intraperitoneal doses (320 mg/kg) (15).

Heffernan et al. (9) confirmed these observations using intraperitoneal doses in the rat and bolus oral doses of ClO₂⁻ in the cat. However, in studies where ClO₂, ClO₂⁻ or ClO₃⁻ was included in the drinking water for up to several months, no significant increases in methemoglobin concentrations resulted with doses as high as 1000 mg/l. in the rat, mouse or chicken (9, 10, 12).

Therefore, although a real hazard with large bolus doses, methemoglobinemia appears to be an unlikely hazard at concentrations of ClO₂, ClO₂⁻ and ClO₃⁻ which might be anticipated to be associated with drinking water treatment.

Heffernan et al. (9) reported that ClO₂⁻ incubated with RBC in vitro depleted these cells of glutathione and increased the level of hydrogen peroxide produced as measured by catalase complex 1 formation. These in vitro studies clearly demonstrated that virtually complete loss of glutathione occurred before there was significant accumulation of methemoglobin. Additionally, these studies demonstrated a biphasic consumption of added ClO₂⁻, one phase of which was associated with hydrogen peroxide generation and glutathione depletion and the other with hemoglobin oxidation. The changes in erythrocyte morphology by scanning electron microscopy in vitro paralleled exactly the progression observed with ClO₂ treatment in subsequent in vivo experiments (10, 12).

From these data it was clear that ClO₂⁻ possesses...
the types of activity associated with chemicals that produce oxidative hemolysis in vivo (16–18).

Red blood cell glutathione concentrations have proven to be the most sensitive measure of ClO₂⁻, ClO₂⁻ and ClO₃⁻ induced hematological effects in vivo as well. Heffernan et al. (9) observed significant depletions of glutathione and increases of 2,3-diphosphoglycerate in the red cells of rats exposed to as little as 50 mg of ClO₂⁻/l. of drinking water. Unlike the effects on RBC counts and hemoglobin concentrations, tolerance does not develop to ClO₂⁻-induced depletion of glutathione. Abdel-Rahman et al. (11, 12), confirmed this result with ClO₂⁻ and extended the observations to ClO₂ and ClO₃⁻. Like ClO₂⁻, ClO₃⁻ produced stable depression of red cell glutathione concentrations with continued treatment at 10 and 100 mg/l. On the other hand, depression of red cell glutathione levels by ClO₂⁻ tends to disappear with continued exposure (11, 13).

The recovery or nonrecovery of glutathione levels with continued treatment in vivo appeared at least partially dependent upon a complex set of interactions with redox systems operating in the rat erythrocyte (19). The reversibility of the ClO₂⁻ effect appears to result from increased levels of glutathione reductase activity in the red cell following 6 months of treatment. In the case of ClO₃⁻ there appears to be no such adaptive increase in this enzyme level. With ClO₂⁻ the glutathione reductase levels do increase to levels comparable to those observed with ClO₂. However, this adaptive change appears to be offset by significantly depressed levels of catalase in the red cell with ClO₂⁻ treatment.

The importance of the changes in enzyme levels in the adaptation to chronic ClO₂⁻ exposure has been indirectly supported by the data of Heffernan et al. (9, 14). First, the addition of glucose to red cells treated with ClO₂⁻ in vitro partially protects against depletion of glutathione. Glutathione peroxidase is the principal means of disposing of hydrogen peroxide in the red cell (17). Glucose serves as the carbon source which donates the reducing equivalents necessary for the reduction of oxidized glutathione (17, 20).

Secondly, in vivo treatment with ClO₂⁻ greatly enhances the formation of ClO₂⁻-induced formation of hydrogen peroxide in vitro. Clearly in vivo treatment with ClO₂⁻ reduces the ability of the red cell to dispose of hydrogen peroxide. Although the animal is not seriously ill at levels of ClO₂⁻, ClO₂⁻ or ClO₃⁻ considerably exceeding those contemplated in drinking water treatment, the body's ability to adapt to oxidant stress is compromised at relatively low concentrations of these chemicals.

These considerations focus attention on populations known to be sensitive to oxidants, for example, those deficient in glucose-6-phosphate dehydrogenase. Such individuals are known to be considerably more sensitive to oxidant stress than normal humans. As will be discussed in other papers in this symposium, erythrocytes isolated from such individuals are three to four times as susceptible to ClO₂⁻-induced damage than normal human red cells (21). In this regard, Michael et al. (22) conducted a human study in a small town which utilized ClO₂⁻ in combination with chlorine as a primary disinfectant during the summer months. Concentrations of ClO₂⁻, ClO₂⁻ and ClO₃⁻ were not closely controlled but the total of their concentrations averaged 5–7 mg/l. In this situation normal humans were not apparently affected. However, the only individual in the community deficient in glucose-6-phosphate dehydrogenase experienced a substantial decrease in hemoglobin levels and hematocrit. With only one case it is difficult to conclusively ascribe this change to ClO₂⁻ or its by-products, but it does suggest reason for concern about sensitive populations.

Evidence of Damage to Other Tissues

Research examining nonhematologic effects of ClO₂⁻, ClO₂⁻ and ClO₃⁻ is sparse. Distribution and clearance of ³⁶Cl derived from ClO₂⁻, ClO₂⁻ or ClO₃⁻ is widespread throughout all organs including bone marrow (12, 23, 24).

There is some evidence of macromolecular binding in the liver. These findings raised the question of whether or not cellular types in addition to the red cell are similarly targeted by these chemicals. The gastrointestinal mucosa is the obvious first potential target of residual disinfectant species. Abdel-Rahman et al. (11) examined the incorporation of ³H-thymidine into nuclei of cells of the intestinal mucosa of the rat as a measure of the turnover of these cells. At levels of 10 mg/l. of ClO₂⁻ and ClO₂⁻ in drinking water for 3 months, there were significant increases in ³H-thymidine incorporation, implying an increased turnover of intestinal epithelium. No effect was observed with equivalent concentrations of ClO₃⁻. Unfortunately, lower levels of exposure to ClO₂⁻ and ClO₃⁻ were not examined.

Examination of other organs revealed that ClO₂⁻ treatment inhibited ³H-thymidine incorporation into nuclei of cells in the kidney and testes of the rat. ClO₂⁻ produced similar effects in the testes and markedly inhibited ³H-thymidine incorporation in the liver at 10 and 100 mg/l. ClO₂⁻ produced significant effects only in the testes. At 100 mg/l, ClO₂⁻ also

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inhibited the incorporation of $^{3}$H-thymidine into the gastrointestinal mucosa and ClO$_2$ produced a smaller increase than had been observed with 10 mg/l. These data suggest that at high doses in the intestinal mucosa and at lower doses in testes and liver, ClO$_2$ and/or its by-products is interfering with DNA synthesis. The significance of these results is presently difficult to determine. But since a large portion of the DNA synthesis ongoing in testes is associated with spermatogenesis, these results cannot be lightly dismissed. Consequently, it is essential that this work be followed up with studies of the reproductive toxicity of these chemicals and be extended to lower doses.

**Conclusions**

It is clear from this brief review that substantial questions remain to be answered concerning the safety of ClO$_2$ as a primary disinfectant of drinking water. Although ClO$_2$, ClO$_2^-$ and ClO$_3^-$ have been used extensively in drinking water treatment, actual human exposure has been limited in the past because the concentrations proposed for taste and odor control are much lower than required for primary disinfection. Europeans have used ClO$_2$ as a primary disinfectant, but again levels which have been used are quite low because disinfectants are employed after much of the organic material has been removed from the water by granular activated carbon, a practice not common in the U.S. Consequently, only in certain very limited circumstances have epidemiological evaluations been possible and appropriate to the use of ClO$_2$ as a primary disinfectant. Consequently, it appears that further experimental work will be necessary to more clearly identify and quantify the hazards involved with its use.

Three major areas of concern over the use of ClO$_2$ as a disinfectant have been identified by research performed to date. First, there is a need to study human populations which display higher levels of sensitivity to oxidant chemicals. Second, efforts must be made to determine the significance of increased turnover of the epithelium of the gastrointestinal tracts at low doses of ClO$_2$ and ClO$_2^-$. This tissue normally turns over quite rapidly. On the other hand, cellular damage followed by regeneration has been associated with increased susceptibility to chemical carcinogens in other organs such as the liver (25). Third, and perhaps most important, is the question of potential reproductive effects of ClO$_2$ and its by-products implied, but not established, by the reduced incorporation of $^{3}$H-thymidine into testicular DNA. The fact that this effect occurred at quite low doses of ClO$_2$, ClO$_2^-$ and ClO$_3^-$ raises the level of concern considerably. It should be noted that similar effects were not observed with HOC1 (11).

In summary, there is little conclusive evidence to prevent the use of ClO$_2$ as a disinfectant at present. On the other hand, there are scattered pieces of indirect evidence that would advise caution about instituting the use of ClO$_2$ as a primary disinfectant in place of chlorine. This is complicated by the fact that the total concentration of ClO$_2$, ClO$_2^-$ and ClO$_3^-$ might reach several parts per million in the absence of removing background organic material to be an effective disinfectant. It is obvious that this question can only be finally resolved through further research.

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