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The Rate of Bromate Decomposition in the Simulated Human Stomach and Associated Studies

Final Project Report

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In Part I, it expands the initial Phase I work by evaluating the roles of various co-constituents that could be present in drinking water or in stomach fluids to determine their affect on the rates of reaction in simulated gastric juice at low pH and in the presence of chloride and hydrogen sulfide. The issue being investigated was whether bromate could be decomposed to some degree in the stomach (and in other premetabolism segments such as liver and blood), and therefore reduce the amount that could ultimately reach target organs. If sufficient bromate could be reduced prior to reaching target organs, the actual risk at low environmentally relevant doses would be lower than predicted by the current risk calculation methodologies.

Part II, as a continuation and expansion of the logic of Part I, included scoping studies on analytical methodology and disappearance of bromate in rat whole blood, and rat blood plasma.

Part III included a very preliminary screening of several organic chemicals in the presence of 1) hydrogen chloride to simulate stomach acid conditions, 2) sodium hypochlorite/hypochlorous acid, and 3) chloramines to simulate drinking water conditions. This was done to determine the possibility that some organic contaminants in water could also be chemically modified between the water treatment plant, or in the stomach prior to uptake, and therefore possibly be detoxified in some instances, thus possibly reducing the actual risk from low dose ingestion from drinking water.

Scope and Summary Conclusions

Part I—The Rate of Decomposition of Bromate in the Simulated Human Stomach

Scope

1. Fate of bromate ion in the presence of Cl_2 and ClNH_2 at the low pHs used for prior Phase I bromate ion investigations.
2. Assuming bromate ion reactions with in the presence of Cl_2 and ClNH_2 at the pHs used for prior bromate ion investigations, study the same solutions in the presences of H_2S .

3. Fate of bromate ion in the presence of Fe (II) and Fe (III) at the pH values used for prior bromate ion investigations.
4. Fate of bromate ion in the presence of NO_2^- at the pH values used for prior bromate ion investigations.
5. Fate of bromate ion in the presence of I^- at the pH values used for prior bromate ion investigations.

Part I –Findings

These studies were conducted to determine the chemical effect of ions that could typically be present in stomach contents from ingestion of water and food, and whether they could increase or decrease the basic reaction rates between bromate and hydrogen sulfide under simulated stomach acid conditions. Our prior studies had shown that hydrogen sulfide is present in stomach acid, and half life of decomposition of bromate in acid and $\sim 10^{-4}$ molar hydrogen sulfide and some thiols was on the order of 2 minutes at pH 0.8 and slowed as the pH was raised. This half life is biologically relevant in terms of retention time in the stomach of ingested water or food. As would be expected the rates of decomposition reactions between bromate ion, hydrogen sulfide and reducing agents were generally as fast, or faster than the rates in the absence of the additional reducing agents. However, and more surprisingly, the rates of bromate decomposition with added hypochlorite, chloramines and ferric ion were somewhat slowed, but not significantly relative to the rates when they were absent. This indicates that the decomposition of bromate from consumption of chlorinated or chloraminated drinking water would not be significantly retarded compared to unchlorinated drinking water.

Oxidizing agents: Sodium hypochlorite/hypochlorous acid, chloramines, ferric iron.

Chlorine water solutions indicated a relatively small non significant reduction in the rates of bromate decomposition, which are dominated by the amount of hydrogen sulfide present and whether or not it is in molar excess. Chlorine oxidizes sulfide in competition with bromate, and chlorine also reoxidizes intermediate bromine oxygen species back to bromate. If sufficient hydrogen sulfide is present the effect on bromate reduction is not significant when the chlorine is present at 4 mg/L or less. It should be noted that during ingestion of water, it is likely that much of the chlorine will decompose by reactions in saliva and with TOC organic matter that is present.

Chloramine water solutions showed a somewhat greater effect on bromate reduction rates than chlorine and probably involved the same reoxidation of intermediate bromine oxygen species. However, the rate of oxidation of the bromine oxygen species is probably slower with chloramines rather than free chlorine. Chloramines probably would not react rapidly with organic matter in the saliva or stomach contents.

Ferric iron causes a somewhat slowing of the rate of reaction of hydrogen sulfide with bromate compared to no ferric iron presence. The rates are approximately doubled at all concentrations of hydrogen sulfide, but they do not reduce proportionately as the ferric iron concentrations increase. The rates without hydrogen sulfide were not significantly affected by ferric iron.

Reducing agents studied: iodide, nitrite, ferrous iron.

Iodide ion probably participates in the bromate oxidation reactions, and the rates at each hydrogen sulfide concentration are increased as the iodide concentration increases. Iodide can also react with some of the bromine oxygen intermediates to reduce them to bromide. However, in the low range of 1 or 2 mg/L of iodide and 10^{-4} molar sulfide, the rates of reduction are not significantly different from no iodide. This is probably because the rates without iodide are already very rapid. At 10^{-5} and 10^{-6} molar hydrogen sulfide concentrations, the acceleration of the rates of reduction of bromate are notable as the iodide concentration increases. This is probably because it is present in molar excess as the hydrogen sulfide levels decline and continues to react with residual bromate.

Nitrite reacts rapidly with bromate without hydrogen sulfide, but it is slightly less reactive than hydrogen sulfide. The rate of reduction of bromate with nitrite and hydrogen sulfide is more rapid at all corresponding hydrogen sulfide levels, and it increases with increased nitrite levels. So, nitrite reacts at somewhat similar rates as hydrogen sulfide. The reaction rates with 10^{-4} molar hydrogen sulfide with and without nitrite are virtually identical because the rate is already rapid without nitrite. Rates at lower hydrogen sulfide levels are enhanced by increased nitrite.

Ferrous iron rapidly reacts with bromate without hydrogen sulfide, but it is slightly less reactive than hydrogen sulfide, and also slightly less reactive than nitrite. The rate of reduction of bromate with ferrous iron and hydrogen sulfide is more rapid at all corresponding hydrogen sulfide levels and it increases with increased ferrous iron levels. So, ferrous reacts at somewhat slower rates as hydrogen sulfide. The rates with 10^{-4} molar hydrogen sulfide with and without ferrous iron are virtually identical because they are already rapid without ferrous iron. Rates at lower hydrogen sulfide levels are enhanced by increased ferrous iron.

Part II – Preliminary Analyses of Bromate and Bromide Reduction in Rat Blood and Plasma

Scope

This was a preliminary study to determine whether sufficient chemical reactivity between bromate and rat whole blood, and between bromate and rat blood plasma, occurred and could be detected. It also explored the preparation of dosed blood and plasma samples and whether their stability was sufficient to allow frozen storage and several days transport from the toxicology laboratory to the analytical laboratory. It was also intended to determine whether IC-ICP/MS analyses were sufficiently sensitive and robust to be used in indicated future pharmacokinetic model development research.

Findings

These preliminary studies verified the hypothesis that bromate would be reactive in blood and demonstrated that bromate was rapidly diminished in fresh rat blood and plasma, and also that the IC-ICP/MS analysis technique provides the opportunity to carry out very sensitive analyses of bromate and bromide in biological fluids. This screening work is continuing in preparation for

a proposal that is being developed to produce a quantitative pharmacokinetic model of the reductive metabolism of bromate at very low drinking water doses in the principal body components of the GI tract to liver, blood, kidney, thyroid and testis; the latter three are the target organs for the high dose carcinogenicity of bromate in rats, and possibly humans. Stability studies are being extended to verify results obtained in the initial experiments, and to determine whether chemical quenching could be a useful technique to be used in controlling the chemical reactions of bromate in body fluids reactions prior to analyses of residual bromate and bromide.

Part III Reactivity of Toxic Organic Chemicals in Water and Simulated Gastric Juice

Scope

The 6 organic chemicals were scheduled for rapid screening to determine whether they would react sufficiently in drinking water and simulated stomach acid so that their toxicology at low intake doses from drinking water would be reduced from high dose predictions. The goal was to make a rough determination of the rate of conversion, if any, of acrylamide, epichlorohydrin, styrene, diazinon, carbaryl/sevin, and cyanazine in the presences of undisinfected drinking water, hydrochloric acid (simulated gastric juice), chloramines, and hypochlorite. The chemicals were studied in those media and analysed by ES/MS electrospray mass spectrometry to determine reaction products.

Findings

Aldicarb was not reactive in water, but it reacted rapidly with chloramines to produce 2 major products. It also reacted with HCL and NaOCl/HOCl to produce several products at a slower rate. Chlorination was not observed.

Cyanazine was stable in water, but hydrolyzed in HCL producing a carboxylic acid and also an amine by another path. Products were slowly formed with NaOCl/HOCl, but no reactions were detected with chloramines.

Diazinon was stable in water, but hydrolyzed in HCl. Reactions, including chlorination, were noted with HCl and NaOCl/HOCl.

Acrylamide was stable in water, HCl and NaOCl/HOCl. A product was formed in chloramines.

Epichlorohydrin reacted in HCl to form several products, but no reactions were detected with water, chloramines or NaOCl/HOCl.

The ES/MS analytical technique that was utilized had the potential to generate artifact products so several of the detected products could well not have occurred from the reactions in water. It is likely that much of the chemistry observed from cyanazine, diazinon and epichlorohydrin was reflective of the conditions of exposure to the water solutions of the various chemicals.

Synopsis of Experimental Studies and Results

Part I-- The Rate of Decomposition of Bromate in the Simulated Human Stomach

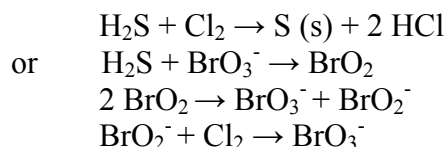
Results: The report includes some information from the previous progress report for context.

Free Chlorine

In the absence of H₂S free available chlorine slows the rate of bromate ion reduction because the initial reaction of bromate ion and H⁺ and Cl⁻ forms bromine-containing intermediates, which can react with Cl₂ to re-form bromate ion.

There is only a small effect of even high concentrations of chlorine on the bromate reaction with 10⁻⁴ M H₂S added to the 0.170 M HCl and free available chlorine solution as shown by the observed 1 – 5 minute half-lives. There is about a 100 fold molar excess of H₂S (not considering the electron balance), so that any intermediates formed from the reaction of H₂S and bromate ion are quickly reduced, which minimizes the possibility of re-forming bromate ion.

However, bromate ion is re-formed when 10⁻⁵ or 10⁻⁶ M H₂S is added to the HCl and Cl₂ solution. The half-life increases from 14 minutes in the absence of Cl₂ to 50 minutes in the presence of 10 mg/L Cl₂. The re-formation of bromate ion occurs because there is only a small excess of H₂S in the presence of 10⁻⁵ M H₂S and no excess in the presence of 10⁻⁶ M H₂S. The excess of Cl₂ as compared to H₂S results in Cl₂ reoxidizing BrO₂ and BrO₂⁻ to reform bromate ion before H₂S completely reduces bromate ion. Therefore, the apparent rate of bromate ion reduction is slower. The rate of bromate ion reduction with hydrogen sulfide in the presence of an oxidizing agent, such as Cl₂, may appear to decrease because of these oscillating reactions:



These equations show how hydrogen sulfide reduces bromate ion to form BrO₂. This intermediate disproportionates to form bromate ion and BrO₂⁻. The BrO₂⁻ reacts with an oxidizing agent, such as HOCl, to reform bromate ion. This results in an increase of the half-life (5 to 21 to 273 minutes or 1 to 50 to 341 minutes).

These equations show that an oxidizing agent such as Cl₂, can regenerate bromate ion in the presence of H₂S, because H₂S is also reacting to form oxidized species such as sulfur. The decrease of available H₂S to react with bromate ion decreases the actual rate of bromate ion reduction. These effects on the rate of bromate ion reduction are dependent on the following rates of hydrogen sulfide loss as shown in the differential equations:

$$\begin{array}{l} \text{Rate of H}_2\text{S loss: } k_{\text{H}_2\text{S}} [\text{H}_2\text{S}] [\text{Cl}_2] \\ \text{Rate of H}_2\text{S loss: } k_{\text{BrO}_3^-} [\text{BrO}_3^-] [\text{H}_2\text{S}] \end{array}$$

In conclusion, the results from the experiments show that free available chlorine, a strong oxidizing agent, has little or no effect on the apparent rate of bromate ion reduction in the presence of 10^{-4} M H_2S . However, the oxidizing agent has a significant effect on bromate ion reduction in the absence of H_2S and in the presence of 10^{-5} or 10^{-6} M H_2S . The effects on bromate ion reduction are dependent on the rates of competing chemical reactions. In two earlier real gastric juice samples, the measured H_2S concentration was approximately 10^{-4} molar. The results of the experiments in the absence of H_2S (column labeled 0 M H_2S) show that free available chlorine appears to slow the rate of bromate ion reduction. The rate of bromate ion reduction is slowed because the initial reaction of bromate ion and H^+ and Cl^- form bromine-containing intermediates that can react with Cl_2 to re-form bromate ion. It should be noted that free chlorine in drinking water would likely react rapidly with saliva and gastric juice/stomach organic chemicals TOC and reducing agents to be further removed and the effective concentration in the stomach would be less than in the original water.

In real gastric juice, the measured H_2S concentration in two earlier tests was approximately 8×10^{-5} M (~ 3 mg/L), and the half-life of bromate ion reduction was between 120 and 175 minutes. The estimated half-life for bromate ion reduction in 0.170 M HCl, 2 mg/L free available chlorine, and $\sim 8 \times 10^{-5}$ M H_2S is estimated to be ~ 5 minutes (estimated by comparing half-lives for other free available chlorine concentrations and 10^{-4} M H_2S). The estimated half-life of bromate ion reduction in 0.170 M HCl and the absence of free available chlorine is ~ 5 minutes (2 minutes for 10^{-4} M H_2S and 14 minutes for 10^{-5} M H_2S).

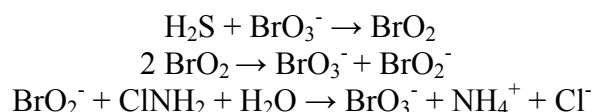
Thus, the concentration of free available chlorine typically found in drinking water should have little effect on bromate ion reduction in the stomach. The significant differences in half-lives between real gastric juice and the 0.170 M HCl, 2 mg/L free available chlorine, and $\sim 8 \times 10^{-5}$ M H_2S (difference in half-lives is between 115 and 170 minutes) is most probably due to unidentified real gastric juice species and/or negative synergisms among gastric juice components, or possibly higher pH.

Chloramine

The disinfectant and mild oxidizing agent, ClNH_2 , can be found in drinking water distribution systems at residual concentrations of approximately 0.1 – 2 mg/L. Monochloramine is not as strong an oxidizing agent as HOCl/Cl_2 . However, it would also survive longer than chlorine in saliva and in the presence of organic TOC.

ClNH_2 slows the rate of bromate ion reduction in the absence of H_2S somewhat more than the thermodynamically stronger oxidizing agent, Cl_2 , probably due to the initial reaction of bromate ion and H^+ and Cl^- or Br^- to form bromine-containing intermediates, such as BrO_2^- . Bromite ion is oxidized by ClNH_2 to re-form bromate ion.

Bromate ion is probably being reformed in the presence of 10^{-4} M H_2S and ClNH_2 . The effect is small because there is an excess of H_2S ; the decrease in the rate is greater in the presence of ClNH_2 compared to HOCl . However, there is still enough intermediate present to reform some bromate ion. Hydrogen sulfide in the presence of an oxidizing agent, such as ClNH_2 , may appear to decrease the rate because of the following partial oscillating reactions:



This set of reactions proposes that hydrogen sulfide can reduce bromate ion to form BrO_2 . The BrO_2 reacts to reform bromate ion and BrO_2^- . The BrO_2^- can react with an oxidizing agent, such as ClNH_2 , to also reform bromate ion.

With 10^{-5} M H_2S and ClNH_2 , there is a small increase in the rate of bromate ion reduction as compared to the presence of only 10^{-5} M H_2S ; therefore, the intermediates that are formed cannot be completely removed by H_2S , which instead results in the reformation of bromate ion.

In conclusion, these experiments show that ClNH_2 , a mild oxidizing agent, causes a small decrease in the rate of bromate ion reduction in the presence of 10^{-4} M H_2S . There is a greater decrease in the rate in the presence of ClNH_2 as compared to HOCl . At 10^{-5} M H_2S , ClNH_2 causes a small increase in the rate of bromate ion reduction, while HOCl causes a significant decrease in the rate. With 10^{-6} M H_2S and ClNH_2 the rate of bromate ion reduction is decreased, but less than chlorine. ClNH_2 without hydrogen sulfide results in a significant decrease in the rate of bromate ion reduction. The half-life for bromate ion reduction in 0.170 M HCl , 4 mg/L monochloramine, and $\sim 8 \times 10^{-5}$ M H_2S is estimated to be ~ 9 minutes (9 minutes for 10^{-4} M H_2S and 9 minutes for 10^{-5} M H_2S). The estimated half-life of bromate ion reduction in 0.170 M HCl , $\sim 8 \times 10^{-5}$ M H_2S , and the absence of monochloramine is ~ 5 minutes (2 minutes for 10^{-4} M H_2S and 14 minutes for 10^{-5} M H_2S). Therefore, the consumption of drinking water containing as much as 4 mg/L monochloramine should have little effect on the rate of bromate ion reduction in the stomach, and chloramines at 4 mg/L would be a relatively worst case scenario for actual drinking water.

Iodide Ion Experiments

The reducing agent, I^- , is present in seafood, milk, and table salt. The average dietary consumption of I^- is approximately 200 μg per day.

Iodide ion, increases the rate of bromate ion reduction in a dose dependent manner, but hydrogen sulfide is a better reducing agent than iodide for bromate ion. Figure 1 is a plot of \log bromate ion half-life (in minutes) vs. \log iodide ion concentration (mg/L); it is linear and the equation of the line is $y = -0.85x + 2.06$. This indicates that the order of the reaction with respect to iodide ion is 0.85.

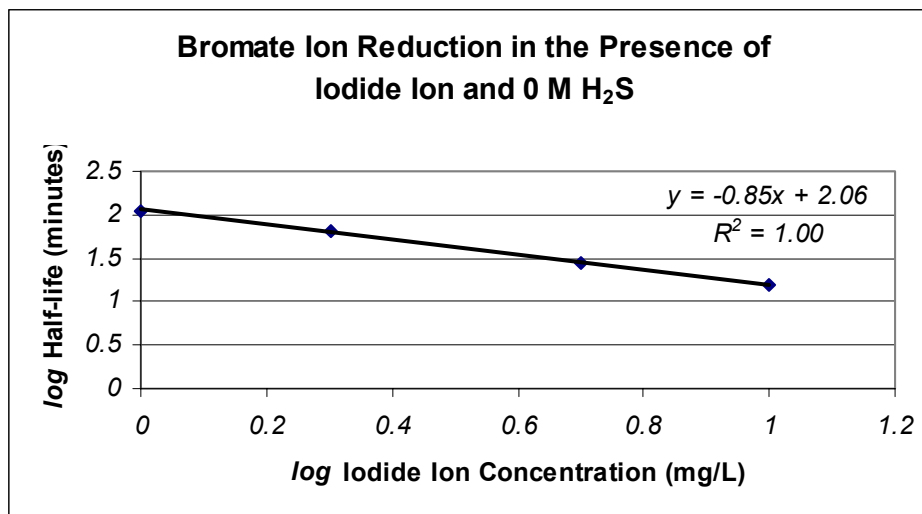
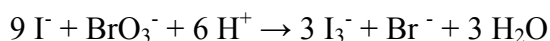


Figure 1. Plot of *log* bromate ion half-life (in minutes) vs. *log* iodide ion concentration (mg/L)

The order of less than one shows that iodide ion is competing with other reducing agents to remove bromate ion. The reduction of bromate ion in the presence of acid and iodide ion is represented by the balanced stoichiometric equation:



With 10^{-4} M H₂S, 1 or 2 mg/L of I⁻ slightly decreases the rate of bromate ion reduction as compared to the absence of I⁻. The effect is minimal because the reaction is already fast in the presence of 10^{-4} M H₂S and HCl alone.

I⁻ affects the rate of bromate ion reduction in the presence of 10^{-5} or 10^{-6} M H₂S. The order of the reaction with respect to iodide ion is 0.6. The order of less than one indicates that iodide ion is competing with hydrogen sulfide to reduce bromate ion. Comparison of the 0.6 order in the presence of 10^{-6} M H₂S and the 0.85 order in the absence of H₂S shows that iodide ion has even less effect on the rate of bromate ion reduction in the presence of 10^{-6} M H₂S. Under these conditions, hydrogen sulfide is a better reducing agent than iodide for bromate ion.

The bromate ion reduction rate in the presence of 10^{-5} or 10^{-6} M H₂S and iodide ion increases as the concentration of iodide ion increases probably because there is excess iodide ion as compared to hydrogen sulfide and bromate ion. The iodide ion and hydrogen sulfide both react with bromate ion and its intermediates quickly. This results in an overall increase in the rate of bromate ion reduction. Therefore, a reducing agent such as iodide ion in the presence of 10^{-6} M H₂S has only a small effect on the rate of bromate ion reduction.

The decrease in the rate of bromate ion reduction does not occur with the addition of 5 or 10 mg/L I⁻ because there is a large excess of iodide ion. The excess iodide ion can reduce bromate ion and its intermediates before bromate ion can be regenerated.

In conclusion, iodide ion, a reducing agent, has a small effect on bromate ion reduction at 10^{-4} M H_2S . The effect is small because the rate of bromate ion reduction in the absence of iodide is already fast. The addition of iodide ion, in the presence of only HCl, **increases** the rate of bromate ion reduction because iodide ion reduces bromate ion and its intermediates **faster** than does chloride ion alone. However, hydrogen sulfide is a better reducing agent than iodide ion. Addition of iodide ion to 10^{-5} and 10^{-6} M H_2S has mixed effects on the rate of bromate ion reduction. The addition of 1 or 2 mg/L iodide ion decreases the rate as compared to the absence of iodide ion. However, the rate of bromate ion reduction is faster in the presence of 5 or 10 mg/L iodide ion and hydrogen sulfide as compared to the absence of iodide ion, probably because there is enough iodide ion present to reduce bromate ion and its intermediates before bromate ion can be regenerated.

A comparison of synthetic gastric juice with and without the addition of iodide ion shows that iodide ion has little effect on the rate of bromate ion reduction. The estimated half-life for bromate ion reduction in 0.170 M HCl, 1 mg/L I^- , and $\sim 8 \times 10^{-5}$ M H_2S is estimated to be ~ 10 minutes (5 minutes for 10^{-4} M H_2S and 23 minutes for 10^{-5} M H_2S). The estimated half-life of bromate ion reduction in 0.170 M HCl, $\sim 8 \times 10^{-5}$ M H_2S , and the absence of I^- is ~ 5 minutes (2 minutes for 10^{-4} M H_2S and 14 minutes for 10^{-5} M H_2S). Therefore, the consumption of foods or water containing as much as 1 mg/L iodide ion should have little affect on the rate of bromate ion reduction.

Nitrite

Nitrite ion (NO_2^-) is consumed daily in processed meats such as hot dogs and bacon. The average intake of nitrite ion is 20 – 40 mg per day. Nitrite ion is also recycled to the stomach through the saliva, partly from catabolism of proteins. Nitrite can also participate in nitrosation of organic nitrogen species that could be present in the stomach under acidic conditions.

In the absence of H_2S (column labeled 0 M H_2S) nitrite ion greatly **increases** the rate of bromate ion reduction. A plot of \log bromate ion half-life (in minutes) vs. \log nitrite ion concentration (mg/L) is shown in Figure 3. The plot is linear and the equation of the line is $y = -1.07x + 1.33$. This indicates that the effect of nitrite ion is first order, in contrast to an order of less than one for iodide ion. The order of one indicates that the reaction of nitrite ion with bromate ion is the main reaction occurring and that bromate ion loss is roughly proportional to the nitrite ion concentration.

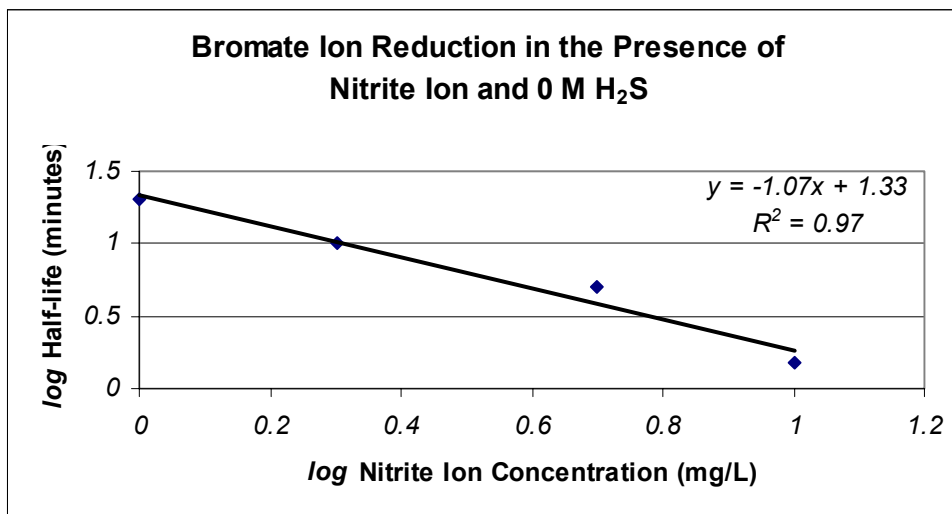
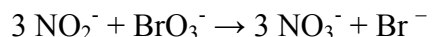


Figure 2. Plot of *log* bromate ion half-life (in minutes) vs. *log* nitrite ion concentration (mg/L)

The increased rate of bromate ion reduction occurs because nitrite ion is a good reducing agent comparable to sulfide under these conditions, and importantly, the nitrite ion reacts with bromate ion and also its intermediates to minimize the regeneration of bromate ion. The stoichiometric reaction of nitrite ion and bromate ion is:



Bromate ion reacts with nitrite ion to form BrO_2^- . In an acidic solution, BrO_2^- reacts with nitrite ion to form HOBr , which reacts with nitrite ion to form bromide ion. Each step in the bromate ion reduction mechanism occurs by the loss of two electrons.

The results of the experiments in the presence of 10^{-4} M H_2S show that the addition of nitrite ion has little or no apparent affect on the rate of bromate ion reduction because the rate is already very rapid in the presence of 10^{-4} M H_2S and HCl . The absence of an oxidizing agent or one-electron reduction process results in no regeneration of bromate ion in the presence of 10^{-4} M H_2S , HCl , and nitrite ion. Therefore, the rate of bromate ion reduction appears to remain constant for all nitrite ion concentrations.

With 10^{-5} M H_2S the addition of nitrite ion **increases** the rate of bromate ion reduction. At 10^{-5} M H_2S there is an excess of hydrogen sulfide and nitrite ion as compared to bromate ion. The combined excess of both reducing agents react faster with bromate ion as compared to the reaction of bromate ion with the individual reducing agents; the half-life decreases from 20 minutes in the presence of 1 mg/L NO_2^- and the absence of H_2S or 14 minutes in the presence of 10^{-5} M H_2S and the absence of NO_2^- to 5 minutes with both 10^{-5} M H_2S and 1 mg/L NO_2^- .

With 10^{-6} M H_2S and nitrite ion, there are mixed effects on the rate of bromate ion reduction; increasing concentrations of nitrite ion will have a greater than first order effect on bromate ion reduction. The effect of nitrite ion concentration on the rate of bromate ion reduction can be described by the following rate law:

$$\text{Rate} = k_1 [\text{BrO}_3^-][\text{NO}_2^-] + k_2 [\text{BrO}_3^-][\text{NO}_2^-]^2$$

With 10^{-6} M H_2S , nitrite ion increases the rate of bromate ion reduction compared to only 10^{-6} M H_2S ; the half-life for bromate ion reduction in the presence of 5 mg/L NO_2^- and 10^{-6} M H_2S is 7 minutes, while it is 32 minutes with 10^{-6} M H_2S and absence of nitrite ion. The presence of 10^{-6} M H_2S and nitrite ion decreases the rate of bromate ion reduction compared to only nitrite ion. The half-life for bromate ion reduction in 1 mg/L NO_2^- without H_2S is 20 minutes, while it is 30 minutes in 1 mg/L NO_2^- and 10^{-6} M H_2S . This probably occurs because there is less available hydrogen sulfide to react with bromate ion and its intermediates, and nitrite ion reacts with bromate ion and its intermediates at a slower rate as compared to hydrogen sulfide.

In conclusion, the addition of nitrite ion has little effect on bromate ion reduction in the presence of 10^{-4} M H_2S because the rate in the presence of only 10^{-4} M H_2S is already rapid. There is an increase in the rate of bromate ion reduction without H_2S because nitrite ion rapidly reduces bromate ion and its intermediates. With 10^{-5} M H_2S , nitrite ion and hydrogen sulfide both remove bromate ion and its intermediates, which results in an increased rate of reduction as compared to the reaction of bromate ion with the individual reducing agents. With 10^{-6} M H_2S and nitrite ion, the bromate ion reduction rate increases as compared to the absence of nitrite ion, but decreases as compared to the absence of H_2S . This is most a result of excess bromate ion as compared to hydrogen sulfide and a slower rate of reaction between nitrite ion and bromate ion.

Comparison of synthetic gastric juice with and without the addition of nitrite ion shows that nitrite ion increases the rate of bromate ion reduction. The estimated half-life for bromate ion reduction in 0.170 M HCl, 10 mg/L NO_2^- , and $\sim 8 \times 10^{-5}$ M H_2S is estimated to be $\sim 1 - 1.5$ minutes (1 – 1.5 minutes for 10^{-4} M H_2S and 10^{-5} M H_2S). The estimated half-life of bromate ion reduction in 0.170 M HCl, $\sim 8 \times 10^{-5}$ M H_2S , and the absence of NO_2^- is ~ 5 minutes (2 minutes for 10^{-4} M H_2S and 14 minutes for 10^{-6} M H_2S). Therefore, consumption of foods or water containing as much as 10 mg/L nitrite ion should have little affect on the rate of bromate ion reduction in the stomach. This is considerably above the drinking water standard of 3 mg/L as nitrite.

Fe (II) Experiments

Ferrous ion (Fe^{2+}) is commonly consumed in foods such as meats, poultry, and fish (PDR Health, 16 May 2005). The average intake of total iron (ferric and ferrous ion) is between 6 and 12 mg per day.

Iron(II), a reducing agent, increases the rate of bromate ion reduction in the absence of H_2S . Figure 5 shows that the plot of \log bromate ion half-life (in minutes) versus \log iron(II) concentration (mg/L) is linear and the equation of the line is $y = -0.58x + 1.97$, which corresponds to the order of the reaction with respect to iron(II) of 0.6.

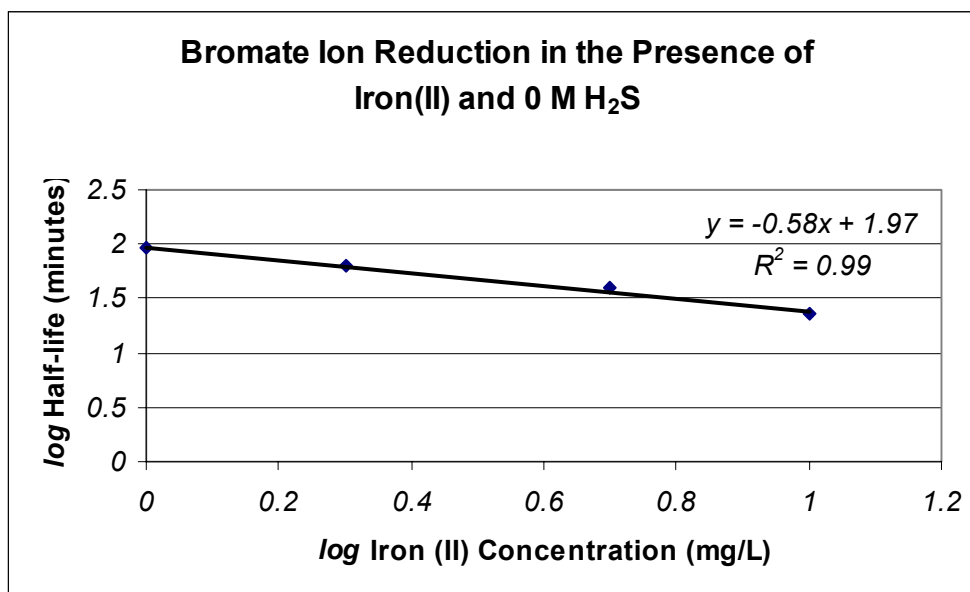
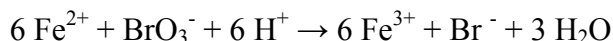
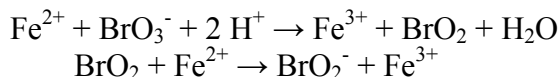


Figure 3. Plot of *log* bromate ion half-life (in minutes) vs. *log* iron(II) concentration (mg/L)

This indicates that iron(II) is competing with other reducing agents to remove bromate ion. The stoichiometric reaction of iron(II) and bromate ion in the presence of acid is:

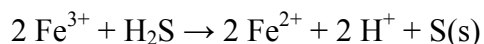


The initial steps in the reaction of bromate ion with iron(II) in the presence of acid most probably are:



These mechanistic steps suggest that excess iron(II) and acid react with bromate ion to form BrO_2 and iron(III). The BrO_2 reacts with excess iron(II) in the presence of acid to form BrO_2^- . The BrO_2^- will react with excess iron(II) and acid to eventually form bromide ion. The increased rate of bromate ion reduction occurs because iron(II) is a good reducing agent and it reacts with bromate ion, BrO_2 , and BrO_2^- and minimizes regeneration of bromate ion.

The bromate ion reduction rates with 10^{-4} and 10^{-5} M H_2S are constant even in the presence of increasing iron(II) concentrations. The bromate ion half-life in the presence of 10^{-4} M H_2S and iron(II) is 1 – 1.5 minutes and the half-life in the presence of 10^{-5} M H_2S and iron(II) is 9 ± 1 minutes. The rate of reduction with changing iron(II) concentrations is nearly constant because of a series of competing reactions. The reaction of iron(II) and bromate ion or BrO_2 forms iron(III); the iron(III) that is formed can react with and remove hydrogen sulfide:



Reaction of iron(III) and hydrogen sulfide results in the removal of hydrogen sulfide and formation of iron(II) and sulfur. The iron(II) that is formed can reduce additional bromate ion

and BrO_2^- . This cycle results in a steady-state bromate ion reduction rate with iron(II) that is constant in the presence of 10^{-4} and 10^{-5} M H_2S .

The increase in the bromate ion reduction rate in the presence of 10^{-4} M H_2S or 10^{-5} M H_2S and iron(II) is minimal as compared to the presence of only 10^{-4} M H_2S or 10^{-5} M H_2S ; the respective half lives are 1 – 1.5 minutes and 2 minutes. The bromate ion half-life in the presence of 10^{-5} M H_2S and iron(II) is 9 ± 1 minutes compared to 14 minutes with 10^{-5} M H_2S alone. The effect is minimal probably because some hydrogen sulfide is removed by the reaction of iron(III) and hydrogen sulfide. However, there is still sufficient hydrogen sulfide available to rapidly reduce bromate ion, BrO_2^- , and BrO_2^- , resulting in a rapid bromate ion reduction rate.

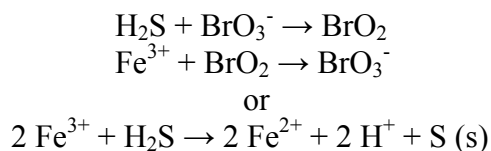
In conclusion, bromate ion reduction in the presence of 0.170 M HCl and iron(II) is more rapid compared to only 0.170 M HCl because iron(II) quickly reduces bromate ion, BrO_2^- , and BrO_2^- to minimize the reformation of bromate ion. In the presence of 10^{-4} , 10^{-5} , and 10^{-6} M H_2S , both hydrogen sulfide and iron(II) quickly reduce bromate ion, which increases the overall bromate ion reduction rate. The bromate ion reduction rates with 10^{-4} and 10^{-5} M H_2S are constant even with increasing iron(II) concentrations, probably because iron(II) reacts with bromate ion to form iron(III) and the iron(III) reacts with hydrogen sulfide to re-form iron(II). This cycle results in constant availability of reducing agents and bromate ion half-lives.

A comparison of synthetic gastric juice with and without the addition of iron(II) shows that iron(II) increases the rate of bromate ion reduction. The estimated half-life for bromate ion reduction in 0.170 M HCl, 10 mg/L Fe^{2+} , and $\sim 8 \times 10^{-5}$ M H_2S is estimated to be ~ 3 minutes (1 – 1.5 minutes for 10^{-4} M H_2S and 8 minutes for 10^{-5} M H_2S). The estimated half-life of bromate ion reduction in 0.170 M HCl, $\sim 8 \times 10^{-5}$ M H_2S , and the absence of Fe^{2+} is ~ 5 minutes (2 minutes for 10^{-4} M H_2S and 14 minutes for 10^{-5} M H_2S). Therefore, consumption of foods or water containing as much as 10 mg/L iron(II) should have little effect on the rate of bromate ion reduction in the stomach with sulfide present.

Fe (III)

Ferric ion (Fe^{3+}), an oxidizing agent is commonly consumed in foods such as dairy products and vegetables. The average uptake of total iron (ferric and ferrous ion) is between 6 and 12 mg per day. It is seldom present in water above 0.3 mg/L as total iron due to adverse taste.

The rate of bromate ion reduction in the presence of iron(III) and 10^{-4} , 10^{-5} , or 10^{-6} M H_2S , decreases as compared to the presence of only H_2S . The possible sets of reactions that may decrease the rate of bromate ion reduction are:



The above equations suggest that bromate ion is reduced by hydrogen sulfide to form BrO_2 , and the BrO_2 can react with iron(III) to re-form bromate ion. This would result in less available hydrogen sulfide to react with bromate ion. If, the reaction of hydrogen sulfide with bromate ion is more rapid, then the bromate ion reduction rate is controlled by the first 2 equations. However, if the reaction of hydrogen sulfide with iron(III) is more rapid, the bromate ion reduction rate is controlled by the 3rd equation.

With iron(III) and 10^{-4} , 10^{-5} , or 10^{-6} M H_2S the bromate ion reduction rate at each hydrogen sulfide concentration is relatively constant even in the presence of increasing iron(III) concentrations. The half-lives in the presence of 10^{-4} , 10^{-5} , and 10^{-6} M H_2S and iron(III) are 5 ± 1 minutes, 40 ± 3 minutes, and 87 ± 3 minutes respectively

In conclusion, the addition of iron(III) to 0.170 M HCl decreases the rate of bromate ion reduction, and it also decreases the rate especially at the lower hydrogen sulfide concentrations. The constant bromate ion reduction rate results because iron(III) reacts with hydrogen sulfide to form iron(II) and iron(II) reacts with bromate ion to reform iron(III). This cycle of reactions results in a constant (steady-state) bromate ion reduction rate.

The half-life for bromate ion reduction in 0.170 M HCl, 10 mg/L Fe^{3+} , and $\sim 8 \times 10^{-5}$ M H_2S is estimated to be ~ 13 minutes (6 minutes for 10^{-4} M H_2S and 42 minutes for 10^{-5} M H_2S). The estimated half-life of bromate ion reduction in 0.170 M HCl, $\sim 8 \times 10^{-5}$ M H_2S , and the absence of Fe^{3+} is ~ 5 minutes (2 minutes for 10^{-4} M H_2S and 14 minutes for 10^{-5} M H_2S). Therefore, consumption of foods containing as much as 10 mg/L iron(III) may result in a small decrease on the rate of bromate ion decomposition.

ICP-MS Calibration Experiments

These preliminary studies by ICP-MS and ICP-MS/MS were used to determine if bromate ion could be measured accurately and reproducibly with an ICP-MS and the comparability of IC and ICP MS results. They were carried out by the Southern Nevada Water Authority laboratories in Henderson, Nevada. Calibration standards and bromate ion samples were measured in 0.017 and 0.0017 M HCl. A correlation coefficient of 0.996 was achieved for four separate calibration curves at 0.017 M HCl; an error of 11 % and a relative standard deviation of 5 % was achieved. At 0.017 M HCl, a correlation coefficient of 0.9998 was achieved for four separate calibration curves at 0.0017 M HCl; an error of 13 % and a relative standard deviation of 6 % was achieved.

ICP-MS/MS

This preliminary study was designed to compare the bromate ion half-lives measured with an ion chromatograph and LC-MS/MS. The LC-MS/MS data show that the measured bromate ion half-life in 0.170 M HCl ranged from 90 – 245 minutes. The average bromate ion half-life for the data points for the two samples was 160 minutes. This is in good agreement with the 153 minute bromate ion half-life measured with an ion chromatograph. The standard deviation of the half-lives measured by LC-MS/MS was 60 minutes. The standard deviation is larger than anticipated, because a detailed protocol was not developed.

Part II Preliminary Analyses of Bromate and Bromide Reduction in Rat Blood and Plasma

This work was carried out by in the laboratories of Jeff Fisher at the University of Georgia and at the Southern Nevada Water Authority (SNWA) with reprogrammed funds provided from AwwaRF 3025, and supplemented with reprogrammed funds from an existing project on bromate reactivity in simulated gastric juice from the National Water Research Institute, and in-kind support from SNWA.

This work was undertaken as a preliminary investigation of the chemical reduction of bromate in rat blood and rat blood plasma. It also involved initial development of an analytical method that would be sensitive enough for application in the future to detailed studies of *in vivo* and *in vitro* kinetics and metabolism of bromate in animals under normal conditions of intake. Its goal was to determine the feasibility of conducting those detailed studies that would determine the consequences of consumption of bromate in drinking water at low doses. The ideal experiments will require the ability to measure the rates of disappearance of bromate and simultaneous appearance of bromide.

These preliminary studies demonstrated that bromate was rapidly diminished in fresh rat blood and plasma, and also that the IC-ICP/MS analysis technique provides the opportunity to carry out very sensitive analyses of bromate and bromide in biological fluids. This screening work is continuing in preparation for a proposal that is being developed to produce a quantitative pharmacokinetic model of the reductive metabolism of bromate at very low drinking water doses in the principal body components of the GI tract to liver, blood, kidney, thyroid and testis. The latter three are the target organs for the high dose carcinogenicity of bromate in rats, and possibly humans.

Experimental Procedures

Three sets of measurement experiments and multiple iterations were conducted using blood and plasma samples prepared by the University of Georgia. They are briefly described below.

- 1) Blood was collected from 4 rats using heparinized syringes and transferred to collection tubes. In the first set, 3 mL of whole blood was spiked with 100 uL of 10 ug/L bromate. In the second set 3 mL of whole blood was spiked with 100 uL of 1 ug/L of bromate. The spiked whole blood was separated into 4 microcentrifuge tubes, held for 0, 5, 10 and 20 minutes, respectively, and then frozen in liquid nitrogen to stop the reactions.
- 2) In this set of experiments, plasma collected from whole rat blood was spiked and processed as in #1 above, then frozen as above in liquid nitrogen.
- 3) In this set of experiments, whole rat blood was spiked with bromate, then centrifuged to separate plasma and then the plasma was held 0, 5, 10 and 20 minutes and frozen in liquid nitrogen.

All samples were shipped to SNWA for analysis.

The analyses were run using ion chromatography coupled with inductively coupled plasma mass spectrometry (IC-ICP/MS) after suitable sample preparations. The procedure involves using IC to selectively separate the bromate and bromide ions from the mass of the blood or plasma sample matrix. The plasma samples were treated with acetonitrile to precipitate proteins and lipids. After concentration of a sample with nitrogen and dilution with water, it was injected into the ICP/MS for analysis of bromate and bromide.

The analytical procedure was developed and then verified using spiked standard samples. The method reporting limit was 5 ng/mL from a 1 mL plasma sample. The instrument detection limit was about 1 ng/mL.

Results

Bromate concentrations spiked into fresh whole rat blood decayed by about 80% to 100% within 10 minutes of mixing and processing. Spikes ranged from the equivalent of 0.03 mg/L to 0.320 mg/L (30 ng/mL to 320 ng/mL).

Fresh plasma spiked with bromate showed rapid decay of bromate to non detection whereas commercial plasma yielded no chemistry and virtual 100% recovery.

Conclusions

These results indicate rapid loss of bromate from fresh whole rat blood and fresh rat blood plasma, and no loss from commercial (aged?) plasma. These are important findings that will be essential in design of future detailed studies that hopefully will be conducted. These results and the analytical methods that were partly developed demonstrate the feasibility of conducting pharmacokinetic studies of bromate in rat blood and also organ cells, and by extension in other animals and human blood.

Part III Reactivity of Toxic Organic Chemicals in Water and Simulated Gastric Juice

Many toxic synthetic organic chemicals are present in the environment due to spills, usage, and manufacturing. The goal of this preliminary screening study was to observe the decomposition of 3 pesticides (aldicarb, cyanazine, diazinon) and three industrial chemicals (acrylamide, epichlorohydrin, and styrene) in extreme solutions that mimic drinking water and gastric juice. The latter three were chosen because they are components of coagulant aid polymers used in drinking water treatment. This would give indications of whether some of these substances may be converted to other products during water treatment and in transit, and prior to their uptake after ingestion. The data would allow preliminary indications of reactivity, and preliminary identification of the decomposition by-products.

A protocol was developed to measure the chemicals using an electrospray ionization mass spectrometer (ESI-MS). The baseline aqueous reactions were observed by analyzing the mass spectrum of the chemicals in triply distilled water (TDW). The chemicals were mixed with NaOCl/HOCl (pH 7.50), ClNH₂ (pH 7.50), or 0.170 M HCl (pH 0.80). Mass spectra were analyzed for each chemical 5 minutes, 24 hours, 48 hours, and 72 hours after mixing. The

measurement times were chosen in order to study the immediate reactions of the chemicals in water and a longer period of time that exceeds normal residence time in the stomach for the acidic media. The by-products of the chemical decomposition reactions with NaOCl/HOCl and ClNH_2 in water simulate drinking water conditions, and HCl chemistry may represent reactions that could occur in stomach conditions. The detection limits using ESI/MS were fairly high so some products that formed might not have been detected, and some artifact formation probably occurred in the ionization stage.

Results of Organic Chemicals Degradation Screening

Aldicarb

The reaction of aldicarb with HCl, ClNH_2 , and NaOCl/HOCl resulted in the formation of new products. The parent peak for aldicarb was not observed indicating that aldicarb decomposes before vaporization in the ESI-MS. Two products formed in water include a nitrile and a product of cyclization of a fragment. The reactions of aldicarb with HCl and HOCl show decomposition products, but there is no indication of chlorination of aldicarb in the presence of HCl and HOCl. However, the reaction of aldicarb with ClNH_2 after 5 minutes forms two chlorinated products one of which by addition of HCl to the C=N bond. In NaOCl/HOCl 5 products forming over time were indicated.

Cyanazine

Cyanazine was stable in water. Cyanazine hydrolyzed in HCl solution to the carboxylic acid, and a product indicating cleavage of the secondary amine was also detected. New products were not found in reactions with ClNH_2 and no changes in the peak intensity of the parent compound occurred. The reaction of cyanazine with NaOCl/HOCl does form several new products in a time dependent manner. The reaction of cyanazine with 99 mg/L NaOCl/HOCl after 48 hours forms a decomposition product, which is a multi-chlorinated compound.

Diazinon

Diazinon was stable in water, but readily hydrolyzed in HCl to cleave the thiophosphate moiety. Reaction with ClNH_2 and HOCl result in the formation of several new products that were tentatively identified in some cases. The reaction of diazinon with HOCl after 5 minutes forms a chlorinated compound of mass 311 and after 24 hours forms a chlorinated compound of mass 187. The reaction of diazinon with ClNH_2 after 5 minutes forms chlorinated or multi-chlorinated monomers and dimers of masses 315, 373, 433, 489, and after 48 hours forms chlorinated or multi-chlorinated monomers and dimers of masses 549, 606, 664, 724, and 782.

Acrylamide

Acrylamide was stable in water, HCl and NaOCl/HOCl. Reaction of acrylamide with ClNH_2 results in partial formation of an unidentified product of mass, 94. Chlorination of acrylamide was not observed in the presence of HCl, NaOCl/HOCl, or ClNH_2 .

Epichlorohydrin

Reaction of epichlorohydrin with HCl results in the formation of new products that were not identified. There was no observed reaction of epichlorohydrin with water, ClNH_2 or HOCl . Chlorination of epichlorohydrin is not observed in the presence of HCl, HOCl , or ClNH_2 .

Styrene

Styrene could not be studied in the ESI/MS system due to solubility and analytical difficulties.

Publications and Presentations

- Jason Keith, Gilbert Pacey, Joseph Cotruvo, Gilbert Gordon. Experimental Results from the Reaction of Bromate Ion with Synthetic and Real Gastric Juices, in Bromate Toxicity including Mechanisms of Cancer Induction: State of the Science Report and Health Research Strategy, Joseph Cotruvo and Richard Bull, eds, AwwaRF 3025, and *Toxicology*, 2005, in press.
- Presentations to the NWRI research advisory board meetings held in Costa Mesa, California on 29 – 30 April 2005, and 14-16 October 2005.
- A manuscript entitled “Measurement of Bromate Ion in High Chloride Solutions Using Ion Chromatography” has been prepared for submission to the scientific journal, *Analytica Chimica Acta*
- Oscar Quinones, Shane Snyder, Joseph Cotruvo, Jeff Fisher and Richard Bull. Analysis of Bromate and Bromide in Blood, in Bromate Toxicity including Mechanisms of Cancer Induction: State of the Science Report and Health Research Strategy, Joseph Cotruvo and Richard Bull, eds, AwwaRF 3025, and *Toxicology*, 2005, in press.
- Masters Thesis, Jason Keith, Miami University, August 2005.

We wish to express our sincere gratitude to the National Water Research Institute, the Southern Nevada Water Authority, the International Bottled Water Association, and the American Water Works Association Research Foundation for their support of these studies aimed at examining the premise that the actual risk from ingestion of small quantities of toxic chemicals may be mitigated by pre systemic metabolism chemical processes that can occur in the stomach and GI tract and in the blood. These results have demonstrated that the concept is valid in numerous instances when chemically reactive substances are involved. We appreciate their confidence and willingness to allow us to explore these basic and applied issues that are fundamental to arriving at drinking water regulations that are realistic and also protective of health.