United States
Environmental Protection
Agency

Health Effects
Research Laboratory
Research Triangle Park NC 27711

Research and Development

EPA/600/S1-87/008 May 1988

EPA

Project Summary

Formation and Distribution of Organic N-Chloramines from the Ingestion of Chlorinated Drinking Water

Frank E. Scully, Jr., and Daniel E. Sonenshine

The chemical reactions that hypochlorite undergoes in the body when chlorinated water is ingested have received very little attention. Because amino nitrogen compounds are important components of the average diet, the reactions of hypochlorite with amino compounds in the stomach were investigated.

Stomach fluid was recovered from Sprague-Dawley rats that had been fasted for 48 hr and administered 4 mL deionized water. The chlorine demand of the stomach fluid was determined. An average volume-independent demand of 2.7 mg chlorine was measured. At doses below 40 mg/L chlorine reducing reactions appeared to account for reduction of all oxidizing species within 15 min as measured by the FAS-DPD titrimetric method.

At least part of the chlorine demand is associated with amino acids present in the stomach fluid. Amino acids were identified and quantified in the stomach fluid by pre-column derivatization with ortho-phthalaldehyde and high-pressure liquid chromatography (HPLC).

When stomach fluid is chlorinated to concentrations of chlorine between 200 and 1000 mg/L, organic N-chloramines are formed. After derivatization of chlorinated stomach fluid with dansyl sulfinic acid, fluorescent derivatives of chloramines were separated by HPLC. Three chloramino acid derivatives, N-chloroalanine, N-chloroglycine, and N-chlorophenylalanine, were identified by co-chromatography with known standards using two chromatographic methods.

The yield of a chloramine that would form in stomach fluid on administration of hypochlorite to animals was determined using tritiated piperidine and doses of 200 and 1000 mg/L chlorine. Yields of tritiated N-chloropiperidine (NCP) in recovered stomach fluid were 70% and 42%, respectively, of the theoretical amount expected.

The stability of ^{36}Cl-N-chloropiperidine was examined at typical pHs found in stomach fluid (pH 2-7). N-Chloropiperidine was found to transfer its chlorine atom slowly to unchlorinated amines at pHs below 3 with a half-life at 37°C (pH 2.35) of 292 min. ^{36}Cl-N-chloropiperidine slowly undergoes isotope exchange in a phosphate buffer (0.01 *M* at pH 2.5) with 0.1 *M* chloride without equally fast decomposition of the chloramine. However, both chlorine transfer and isotope exchange are too slow to be of significance in the toxicological studies reported here.

When ^{36}Cl-N-chloropiperidine is incubated with rat stomach fluid at 37°C for 30 min, 34% is reduced to ^{36}Cl-chloride, and 66% reacts with organic components in the fluid to form a mixture of ^{36}Cl-chlorinated organic compounds of unknown identity.

A series of pharmacokinetic studies was conducted in male and female

Sprague-Dawley rats employing ^{3}H-N-chloropiperidine and ^{36}Cl-chloropiperidine as test compounds and ^{3}H-piperidine and ^{36}Cl-chloride as control compounds. Studies showed that tritiated compounds were absorbed into blood, excreted, and distributed in tissues in a similar manner. However, ^{36}Cl-activity was retained in the tissues of animals administered ^{36}Cl-N-chloropiperidine to a much greater extent and eliminated at a much slower rate than from animals given ^{36}Cl-chloride. A relationship between the retention of the ^{36}Cl-activity in the pharmacokinetic studies and formation of ^{36}Cl-chloroorganic compounds *in vitro* is discussed.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Over the past 10 years it has been recognized that chlorine, used to disinfect drinking water, reacts with trace organic compounds dissolved in natural waters to produce by-products which may have adverse health effects in humans. Consequently, evaluation of the potential health effects of water disinfection of these by-products and determination of the quantities of these compounds typically ingested by the population at large. Water treatment policies have been primarily concerned with minimizing the concentrations of these trace contaminants, particularly the trihalomethanes.

In the United States the average person drinks between 2 and 3 liters of water each day which may typically contain between 1 and 2 mg/L of the residual chlorine oxidant such as aqueous chlorine (Cl_2). By comparison with the U.S. Environmental Protection Agency's (EPAs) Maximum Contaminant Level for chloroform, the molar concentration of chlorine in a drinking water containing 2 mg/L is 30 times higher. Furthermore, when drinking water is ingested, it enters a medium with a total organic carbon (TOC) content that is several orders of magnitude higher than that found in natural waters. However, little attention has been given to the possible reactions of hypochlorous acid which may take place in the organic-rich medium of the stomach on ingestion of chlorinated drinking water.

The average person's daily diet includes a minimum of 30 to 45 grams of protein. Through the action of digestive enzymes in the stomach, proteins are broken down into peptones, large polypeptides, and about 15% amino acids. Since stomach fluid contains high concentrations of organic amino nitrogen compounds, and since hypochlorite reacts rapidly with these types of compounds to form chloramines, it was hypothesized that, upon ingestion, chlorinated water would react rapidly with amino nitrogen compounds in the stomach to produce N-chloramino by-products which may be distributed throughout the body. Therefore, two main objectives were identified at the beginning of the project:

1. To determine if organic N-chloramines can form in the stomach upon ingestion of hypochlorous acid and inorganic chloramines.
2. To determine what chemical reactions chloramines, both organic and inorganic, may undergo in the stomach and whether they can be absorbed into the bloodstream for circulation to other parts of the body.

N-Chloropiperidine (NCP) was used extensively in this project as a model to study the reactions of chloramines in the stomach. Initially, this compound was chosen for four reasons. First, the parent amino, piperidine, had been identified in drinking water and had been shown to be excreted in urine at the rate of 5 mg/L/day by normal human males. It was, therefore, recognized as an endogenous amine in humans. Secondly, the oxidizing power of NCP was recognized to be similar to that of other organic monochloramines, but it is a relatively stable chloramine. From an experimental standpoint this property makes it possible to track the fate of this chloramine in the body. The mono-N-chlorinated derivatives of primary amines and ammonia are not as stable below pH 8 and are converted to their dichloramino analogues by chlorine exchange. NCP does not undergo this reaction because it has no remaining exchangeable hydrogens that can be replaced with chlorines. The third reason NCP was chosen was that conditions for chromatographing NCP directly without extensive work-up or pre-column derivatization have been developed. The analysis of the compound in aqueous solution can be carried out simply and rapidly.

The fourth reason NCP was selected was because both a tritium-labeled N-chloropiperidine (^{3}H-NCP) and a ^{36}Cl-labeled N-chloropiperidine (^{36}Cl-NCP) could be synthesized. Chloramines may act as either chlorinating agents or as aminating agents. Therefore, if the pharmacokinetics of absorption and excretion of ^{3}H-NCP differed from those of this control compound, tritiated piperidine, it might be inferred that chloramines acted as aminating species in biological systems. On the other hand, if the pharmacokinetics of ^{36}Cl-NCP differed from its control compound, $^{36}Cl^{-}$, it might be inferred that chloramines acted as chlorinating agents in the body.

Recently, a series of studies on the toxicity and pharmacokinetics of ^{36}Cl-labeled hypochlorous acid and ^{36}Cl-labeled monochloramine were reported. These studies showed that the ^{36}Cl used in these studies is retained in non-fasted animals much longer than ^{36}Cl-enriched chloride. Therefore, it is important to determine what chemistry that can take place in the stomach can account for this greater degree of retention.

Part of the impetus for this research is the previous observation that at least one organic N-chloramine, NCP, is mutagenic by Ames' assay, is cytotoxic, and induces chromosomal aberrations in mammalian cells the frequency of which is proportional to the concentration of NCP.

In addition to NCP, N-chloroglycine was used to probe the reactions of hypochlorite and the stabilities of chloramines in the stomach. N-Chloroglycine is a relatively stable chloramino acid formed from glycine. The chloroglycine is not present in the stomach because of ingestion or as a product of proteolytic activity, but glycine would be. It can then serve as a precursor for formation of chloroglycine by reacting with ingested hypochlorus acid or another chloramine.

Recently, a method for the derivatization and analysis of organic N-chloramines in dilute aqueous solution was described. In the method, solutions containing N-chloramines were reacted with 5-dimethylaminonaphthalene-1-sulfinic acid ($DANSO_2H$) to produce highly fluorescent sulfonamide derivatives (dansyl derivatives) which could be analyzed by HPLC. In the present study, this derivatization method is used to detect the formation of N-chloroglycine

in stomach fluid, but results are corroborated by chromatography of an underivatized chloramine (NCP) and its radiolabeled counterpart.

Discussion

When rats were treated with either the tritium-labeled organic chloramine, NCP, or its tritium-labeled parent amine, piperidine, the radioactivity was rapidly eliminated from the body. Except in the case of the male animals administered ^{3}H-NCP (where plasma decay and excretion rates were faster than in the other studies), plasma decay rates were similar for both compounds and in all cases less than 5% of the label remained in the animal at the end of the 120-hr study period. The study of the pharmacokinetics of ^{3}H-NCP in male rats is being repeated to determine if the apparent deviation from the kinetics observed in the other studies is real. These data suggest that the ^{3}H-labeled compound is absorbed into blood and excreted in both studies is being treated in the same manner. Tentative identification of ^{3}H-piperidine in urine and tissues from animals administered ^{3}H-NCP suggests that the chloramine, ^{3}H-NCP, is rapidly being dechlorinated to its parent amine, ^{3}H-piperidine (the control compound), which is then absorbed into blood and excreted by the body with the same kinetics as the control compound.

By contrast, the kinetics of ^{36}Cl-NCP plasma decay and excretion differed dramatically from those of the control compound, ^{36}Cl-chloride. The half-life of elimination of $^{36}Cl^{-}$ (53 hr in males and 50 hr in females), was comparable to that found in other studies (51.9 hr). However, the rate of elimination of ^{36}Cl-chloride from plasma was approximately twice as fast as elimination of ^{36}Cl-activity after administration of ^{36}Cl-NCP (half-lives of 173 hr and 116 hr, respectively, in male and female rats). By the end of the test period less than 50% of the radioactivity had been excreted in ^{36}Cl-NCP-treated animals. These results suggest that the ^{36}Cl-activity on administration of the ^{36}Cl-labeled chloramine is being treated in the body differently than the $^{36}Cl^{-}$ chloride.

At first glance the results of the pharmacokinetic studies involving ^{3}H-NCP might seem to contradict the results using ^{36}Cl-NCP. However, taken together, these data suggest that the ^{36}Cl-label originally associated with the administered chloramine is rapidly becoming associated with another molecule in the stomach and this new chlorinated compound is exhibiting different pharmacokinetics than chloride.

To support this hypothesis, we have examined the *in vitro* reactions of ^{36}Cl-NCP. As described in the full report it was observed that at concentrations of 576 ppm (Cl_2) ^{36}Cl-NCP reacts with organic constituents in stomach fluid to form a new ^{36}Cl-chloroorganic fraction of compounds that is chemically distinct from either ^{36}Cl-NCP or ^{36}Cl-chloride. It would appear that this ^{36}Cl-chloroorganic fraction is likely to account for the distribution and retention of the ^{36}Cl-activity in the pharmacokinetic study of ^{36}Cl-NCP.

In a comparative study of the pharmacokinetics of $HO^{36}Cl$ and $NH_2{}^{36}Cl$, investigators found that ^{36}Cl-activity was eliminated from plasma, distributed, and excreted from fasted Sprague-Dawley rats at rates similar to that of ^{36}Cl-chloride.

However, a pharmacokinetic study of $HO^{36}Cl$ is complicated by the fact that the labeled hypochlorous acid undergoes extremely rapid isotope exchange with unlabeled chloride. Other studies found that the rate of isotope exchange is dependent on the concentrations of HOCl, chloride, and hydrogen ion and measured a third order rate constant at 27°C of $3.16 \times 10^{13}\ M^{-1}min^{-1}$. In the stomach, which can contain as much as 0.1 *M* chloride, this reaction would be greater than 99% complete in 8.7×10^{-4} sec, assuming an initial pH of 7.0, a temperature of 27°C and no competing reactions. Consequently, the similarity between the pharmacokinetics of $HO^{36}Cl$ and ^{36}Cl-chloride is likely to be due to the rapid formation of ^{36}Cl-chloride from $HO^{36}Cl$ by isotope exchange with unlabeled chloride in the stomach.

On the other hand, it was observed that the half-life for elimination of ^{36}Cl-activity from non-fasted male rats administered $HO^{36}Cl$ was twice (88.5 hr) that of the half-life for elimination from fasted rats (44.1 hr). Unfortunately, the half-life of ^{36}Cl-chloride in non-fasted rats is unknown. However, in light of the observed formation of a ^{36}Cl-chloroorganic fraction when ^{36}Cl-NCP is mixed with stomach fluid, it is possible that $HO^{36}Cl$ reacts rapidly with the higher concentration of food-based organic compounds in the stomachs of non-fasted rats before it has the opportunity to undergo isotope exchange.

Other investigators also studied the pharmacokinetics of 370 mg/L (as Cl_2) $NH_2{}^{36}Cl$ in male Sprague-Dawley rats. They note that the ^{36}Cl-activity in the plasma reached a peak 8 hr following adminstration. The ^{36}Cl-activity remained at a plateau from 8 to 48 hr after administration before it was eliminated with a rate constant similar to that of chloride. Consequently, over 70% of the amount of radioactivity administered was retained in the animals at the end of the 5-day test-period. The rate of isotope exchange between $NH_2{}^{36}Cl$ and unlabeled chloride has not been measured. Therefore, it is possible to determine how much of the label was lost by this reaction. However, because of the differences between the kinetics of $NH_2{}^{36}Cl$ and $HO^{36}Cl$ and ^{36}Cl-chloride, the greater degree of retention of the label in the study involving $NH_2{}^{36}Cl$ is likely to be due to retention of the chloramine with organics in the stomach to form a ^{36}Cl-chloroorganic fraction similar to the one found in the reaction of ^{36}Cl-NCP with rat stomach fluid.

Studies showed that hypochlorite reacts with fetal calf serum to produce products that inhibit division of porcine aortic vascular endothelial cells, but do not kill them. A reduction in cell growth of at least 20% compared with controls was noted when any combination of fetal bovine serum and NaOCl was preincubated in the growth medium.

In the present study radiolabeled NCP in fetal calf serum was incubated with BHK-2 cells or 3T3 NIH cells. At approximately the same concentrations of active chlorine used (≈20 mg/L as Cl_2), the tritium activity from ^{3}H-NCP was taken up by cells in 15 min, although much of the activity was lost over the remaining 4 hr. ^{36}Cl-activity was accumulated within 30 min in 3T3 NIH cells to a much greater extent after incubation of the cells with ^{36}Cl-NCP than after incubation with ^{36}Cl-chloride. Evidence was obtained that there was a time-dependent accumulation of a very small amount of cell nuclei.

The ^{36}Cl-chloroorganic fraction formed when ^{36}Cl-NCP is incubated with rat stomach fluid is believed to be due to a reaction of the chloramine with proteinaceous components of the stomach fluid. Since both hypochlorite and chloramines are chlorinating agents, and since the chloramine is incubated with MEM containing 2% fetal calf serum in the cell culture studies, it is possible that products similar to the ^{36}Cl-chloroorganic

fraction are being formed which are being taken up into the cell in the present study and which account for the inhibition of growth in the studies with porcine aortic endothelium cells.

Conclusions

Based on results of this study, it may be concluded that the rates of reaction of hypochlorite with amines are sufficiently fast that organic chloramines can be formed on ingestion of aqueous hypochlorite. In low concentration they are short-lived and appear to be reduced to non-oxidizing species. However, organic chloramines appear to undergo subsequent reaction with other organics in stomach fluid to form new covalently bonded chlorine compounds, probably chlorocarbon compounds. It is possible that these compounds are intermediates in the detoxification and/or elimination of active chlorine compounds in the body. On the other hand, the pharmacokinetic data suggests that much of ^{36}Cl-labeled organic fraction is retained in the body after five days. Since these compounds have not yet been characterized, their health effects are unknown. However, they appear to be the end-products for active chlorine compounds in the body and, as such, related to the health effects of both hypochlorite and inorganic chloramine disinfectants. On the other hand, the fact that a chloramine can be absorbed into blood is remarkable in itself and suggests that direct toxicological effects of chloramines cannot be ignored.

Frank E. Scully, Jr. and Daniel E. Sonenshine are with Old Dominion University, Norfolk, VA 23508.

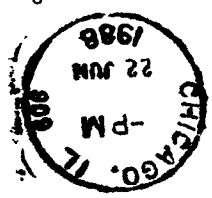
***Frederick C. Kopfler** is the EPA Project Officer (see below).*

The complete report, entitled "Formation and Distribution of Organic N-Chloramines from the Ingestion of Chlorinated Drinking Water," (Order No. PB 88-103 742/AS; Cost: $14.95, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650

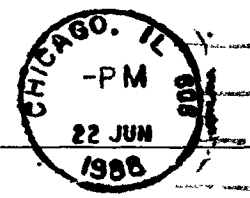
The EPA Project Officer can be contacted at:

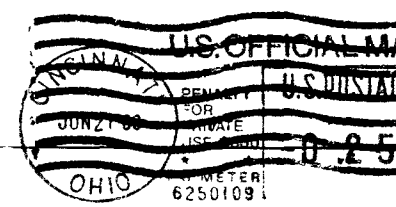
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711



United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268





Official Business
Penalty for Private Use $300

EPA/600/S1-87/008

0000329 PS
U S ENVIR PROTECTION AGENCY
REGION 5 LIBRARY
230 S DEARBORN STREET
CHICAGO IL 60604

☆U.S GOVERNMENT PRINTING OFFICE 1988—548-013/8