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Health Effects from Exposure to High Levels of Sulfate in Drinking Water Study

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Health Studies Branch
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Office of Drinking Water and Ground Water
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Executive Summary

Sulfate is a substance that occurs naturally in drinking water. Health concerns regarding sulfate in drinking water have been raised because of reports that diarrhea may be associated with the ingestion of water containing high levels of sulfate. Of particular concern are groups within the general population that may be at greater risk from the laxative effects of sulfate when they experience an abrupt change from drinking water with low sulfate concentrations to drinking water with high sulfate concentrations.

There are very few scientific reports that address sulfate concentration in drinking water and the effects it may have on the health of those individuals who are exposed. Furthermore, the concerns regarding sensitive populations are based solely on case studies and anecdotal reports. One such potentially sensitive population is infants receiving their first bottles containing tap water, either as water alone or as formula mixed with water. Other groups of people who could potentially be adversely affected by water with high sulfate concentrations include transient populations (i.e., tourists, hunters, students, and other temporary visitors) and people moving from areas with low sulfate concentrations in drinking water into areas with high concentrations.

The objective of the present study was to provide additional information regarding whether sensitive populations (infants and transients) may be adversely affected by sudden exposure to drinking water containing high levels of sulfate. Specifically, we designed a field investigation to recruit 880 infants exposed to naturally occurring high levels of sulfate in the drinking water provided by public water systems and an experimental trial of exposure in adults.

We planned a prospective cohort study of infants born in geographic areas with naturally occurring high levels of sulfate in the drinking water provided by public water systems in New Mexico, South Dakota, and Texas. Infants were to be enrolled at birth and followed for four weeks to determine if there was an association between exposure to drinking water containing varying levels of sulfate and reported cases of diarrhea.

We conducted a pilot study of the planned recruitment methods and study instruments in four counties in South Dakota with high levels of sulfate in the drinking water provided by the public water systems. We approached 72 pregnant women from these counties (served by three community health clinics) about participating in the study, but only eight were eligible based on the study eligibility criteria. Of the eight eligible women, three refused to participate. Only one of the five women who agreed to participate completed all of the study activities.

Because we experienced recruiting problems during the pilot study, we developed a self-administered questionnaire (SAQ) to examine tap water use. The questionnaires were provided to all women who received care during a two-week period from one of 32 Women, Infants and Children (WIC) clinics in New Mexico, South Dakota, and Texas. The clinics were located in geographic areas with a range of sulfate levels (from <100 mg/L to > 1000 mg/L) in the drinking water provided by public water systems. The SAQ asked questions about the source of the women's home tap water,

what mothers of infants ≤3 months old were currently feeding their babies, and how pregnant women planned to feed their infants.

Of the 951 pregnant women who responded to the SAQ, more than half (582 or 61%) reported planning to breast-feed their infants and only 141 (15%) reported that they planned to use infant formula mixed with their home tap water to feed their infants. Of the 542 mothers with infants ≤ 3 months of age, 272 (50%) reported having breast fed their infants.

To determine how many of the 1388 women who completed the SAQ would have been eligible to participate in our study based on the drinking water source and use criteria, we examined the responses of the 1164 women who received their tap water from public water systems and who did not have filters on their home taps. We found that 403 women with infants ≤ 3 months of age and 761 pregnant women met these eligibility criteria. The largest numbers of women who had used or were planning to use tap water to mix infant formula were in the 4 counties with average sulfate levels ≤ 250 mg/L. Of the 365 pregnant women in areas with sulfate levels > 250 mg/L, only 39 (11%) planned to use infant formula mixed with their tap water. Of the 183 women with infants ≤3 months old in areas with sulfate levels > 250 mg/L, only 35 (19%) reported having used infant formula mixed with their tap water. Thus, of the 548 pregnant women and women with infants ≤ 3 months old, only 74 infants were or would be exposed to tap water containing ≥ 250 mg/L sulfate. These results are consistent with our findings during the pilot study and indicate that only a very small number of women who live in areas with high levels of sulfate in the tap water provided by public water systems plan to give this water to their infants.

Another population potentially sensitive to abrupt exposure to high levels of sulfate in drinking water is transient adults (students, visitors, hunters, etc.). To study the effects in adults of suddenly changing drinking water sources from one that has little or no sulfate to one that is high in sulfate, we conducted an experimental study involving volunteers from Atlanta, Georgia, including CDC employees and employees at the U.S. EPA Region IV office. Volunteers were randomly assigned to one of five sulfate exposure groups (i.e., 0, 250, 500, 800, or 1200 mg/L sulfate from sodium sulfate in bottled drinking water) and were provided with bottled drinking water for six days. The bottled water for days 1, 2, and 6 contained plain water, while the bottles for days 3 through 5 contained water with added sulfate. The unfinished or empty bottles were returned and weighed to determine how much water was consumed each day. Volunteers were blinded to the level of sulfate in their drinking water.

One hundred and five study participants were divided among the dose groups as follows: 24 received 0 mg/L sulfate; 10 received 250 mg/L sulfate; 10 received 500 mg/L sulfate; 33 received 800 mg/L sulfate; and 28 received 1200 mg/L sulfate. We analyzed the number of bowel movements recorded each day by study participants. There were no statistically significant differences in the bowel movements among the groups on days 3, 4, 5, or 6. There were also no statistically significant differences in the bowel movements reported when comparing days 1 and 2 (the days when there was no sulfate in the water) with days 3, 4, and 5 within each dose group.

To examine the data for a trend toward increased frequency of reports of diarrhea with increased dose of sulfate, we included the dose as an ordinal variable in a logistic regression model of osmotic diarrhea. There was no statistically significant increase in reports of diarrhea with increasing dose (one-sided $p = 0.099$).

The overall purpose of these studies was to examine the association between consumption of tap water containing high levels of sulfate and reports of osmotic diarrhea in susceptible populations (infants and transients). We were unable to conduct a study of infants because we could not identify enough exposed individuals from which to draw a study population. The results of our SAQ examining tap water use indicated that more than half of the pregnant women who completed the survey planned to breast-feed their infants. Of those who planned to use formula mixed with water, most did not plan to use tap water to mix the formula. In our experimental trials with adult volunteers, we did not find an association between acute exposure to sodium sulfate in tap water (up to 1200 mg/L) and reports of diarrhea.

We were not able to conduct the dose-response studies that were requested as part of the Safe Drinking Water Act Amendments of 1996. Instead, we convened an expert workshop whose members reviewed the available literature and addressed a series of questions about the health effects from exposure to sulfate in drinking water: 1) Do reported studies suggest that a certain sulfate level would not be likely to cause adverse effects?, 2) Does the literature support acclimatization or adaptation?, 3) Can an infant dose-response study be done anywhere in the U.S. or Canada?, 4) Is there enough scientific evidence that there adverse health effects from sulfate in drinking water to support regulation? The workshop was held in Atlanta, Georgia on September 28, 1998. The workshop report accompanies this document.

Introduction

Sulfate is a substance that is often found in drinking water. Health concerns regarding sulfate in drinking water have been raised because of reports of diarrhea associated with the ingestion of water containing high levels of sulfate. Available data suggest that people acclimate rapidly to the presence of sulfates in their drinking water. However, there are groups within the general population that may be at greater risk from the laxative effects of sulfate when they experience an abrupt change from drinking water with low sulfate concentrations to drinking water with high sulfate concentrations.

One such potentially sensitive population is infants receiving their first bottles containing tap water, either as water alone or as formula mixed with water. A series of three case histories from Saskatchewan reported by Chien *et al.* (1968) suggested that infants may experience gastroenteritis, including diarrhea and dehydration, upon their first exposure to water that contains high levels of sulfate. The three infants discussed in the report were symptom-free until their families moved to areas with water supplies that contained high levels of sulfate (650 to 1150 mg/L). Interestingly, the infants developed diarrhea when they were given water from these new sources. Stools from two of the infants tested negative for bacterial pathogens, ova, and parasites; and the diarrhea subsided when alternative water sources were used. In light of these reports, the authors suggested that sulfate levels are important with respect to their laxative effect on babies. They recommended that water be screened for sulfate content if a sample is submitted for assessment of suitability for infant feeding.

Other groups of people who could potentially be adversely affected by water with high sulfate concentrations are transient populations (i.e., tourists, hunters, students, and other temporary visitors) and people moving to areas with high sulfate concentrations in drinking water from areas with low sulfate concentrations. This concern is based primarily on anecdotal reports rather than on published studies. For example, an analysis of 300 responses to an informal survey conducted by the North Dakota Department of Health suggested that water with sulfate levels $\geq$ 750 mg/L was considered laxative by most consumers (Peterson, 1951). (Peterson noted that a high concentration of magnesium sulfate was even more likely to have a laxative effect on consumers than was a high concentration of sodium sulfate.) He also noted that reports of a laxative effect of water with a low concentration of sulfate could have been from people new to the area, whereas reports of no laxative effect in areas of high sulfate concentration could have come from people acclimated to sulfate exposure.

In another informal report, Moore (1952) evaluated data collected in North and South Dakota on well water quality. The data from South Dakota included 67 wells with 1000 to 2000 mg/L sulfate and indicated that the water was at least tolerable as drinking water with no apparent extensive physiologic effect. The data from North Dakota included information from 248 private drinking water wells. As the concentration of sulfate in these wells increased, more adults reported a laxative effect. For example, for well water containing <200 mg/L sulfate, only 22% of consumers reported that their water had a laxative effect. Water containing high ($\geq$ 1000 mg/L) concentrations of magnesium sulfate affected 62% of consumers. Neither of these reports suggested that the

population affected considered the laxative effect of their drinking water to be an adverse health issue.

Experimental studies of the association between exposure to sulfate and subsequent diarrhea have been conducted in pigs and piglets and in human adults. For example, groups of 10 artificially-reared (using a mechanical "auto-sow") neonatal piglets were provided diets containing 0, 1200, 1600, or 2000 mg of added inorganic sulfate (as anhydrous sodium sulfate)/L of diet for 28 days (Gomez *et al.,* 1995). Sulfate concentrations of $\geq$1800 mg/L of diet caused persistent, but nonpathogenic diarrhea in the piglets. Growth was not affected in any of the exposure groups. A study by Veenhuizen *et al.* (1992) found no adverse effect on nursery pig performance (e.g., mean weight gain, feed consumption, water consumption, prevalence of diarrhea) when the pigs were fed concentrations of up to 1,800 mg of sodium sulfate, magnesium sulfate, or a combination of sodium and magnesium sulfate/L of diet for 16 or 18 days.

In another study, Veenhuizen (1993) conducted a water quality survey of 54 swine farms in Ohio in which water samples were analyzed for concentrations of sulfates and total dissolved solids, and found that sulfate concentrations ranged from 6 to 1600 mg/L. There was no association between sulfate concentration and the prevalence of diarrhea on the farms.

Heizer *et al.* (1997) provided four healthy adult subjects with drinking water containing increasing levels of sulfate (0, 400, 600, 800, 1000, and 1200 mg/L from sodium sulfate) for six consecutive 2-day periods. In a single-dose study, six other volunteers received water with 0 or 1200 mg/L sulfate for two consecutive 6-day periods. In the dose-range study, there was a decrease in mouth-to-anus appearance time (using colored markers) with increasing sulfate concentration. In the single dose study, there was a significant increase in stool mass for the six days of exposure to sulfate compared to the six days without exposure. None of the study subjects reported diarrhea.

While the studies mentioned above address the acute effects of sulfate on adult human intestinal function and provide animal data that can be extrapolated to people, it has not been feasible to conduct an experimental study to verify the reported effects of exposure to high levels of sulfate on human infants. Esteban *et al.* (1997) conducted a field study in 19 South Dakota counties to determine the risk for diarrhea in infants exposed to high levels of sulfate in tap water compared to the risk for diarrhea in those unexposed. In this study, there was no significant association between sulfate ingestion and the incidence of diarrhea for the range of sulfate concentrations studied (mean sulfate level 264 mg/L; range 0 - 2,787 mg/L). In addition, there was no dose-response or threshold effect, and the results suggested that breast milk has a more significant laxative effect than does sulfate in drinking water. However, because the sample size was small (274 infants) and the age of the infants ranged from 6.5 weeks to 30 weeks, it is probable that some of the infants could have been exposed (and become acclimated) to drinking water containing high levels of sulfate prior to being enrolled in the study.

Objective

The objective of the present study was to provide additional information regarding whether sensitive populations (infants and transients) may be adversely affected by sudden exposure to drinking water containing high levels of sulfate. Specifically, we designed a field investigation of infants exposed to naturally occurring high levels of sulfate in the drinking water provided by public water systems, and an experimental trial of exposure in adults.

Materials and Methods

The protocols described in this report were reviewed and approved by an expert panel organized specifically to review this work and by the Institutional Review Boards (IRBs) of CDC, Battelle Centers for Public Health Research and Evaluation, and the Texas Department of Health.

A. Infants

We attempted to conduct a prospective cohort study of newborn infants whose mothers did not plan to breast-feed, but who planned to feed their infants formula mixed with tap water and who planned to stay home with their infants for at least four weeks after giving birth. Unfortunately, because we were not able to identify many new mothers who planned to feed their infants formula mixed with tap water, we were unable to conduct the study. Below is a brief description of how we planned to conduct this study.

Study Design

We planned a prospective cohort study of infants born in geographic areas with naturally occurring high levels of sulfate in the drinking water provided by public water systems in New Mexico, South Dakota, and Texas. Infants were to be enrolled at birth and followed for four weeks to determine if there was an association between exposure to drinking water containing varying levels of sulfate and reported cases of diarrhea.

It may be difficult for new mothers to identify diarrhea in their infants. Also, a new baby's stools may be irregular in consistency for their first few days of life, making it difficult to identify a "normal" elimination pattern. To allow the babies' digestive systems time to stabilize and to prevent the infants from being immediately exposed to tap water, we planned to provide the mothers with ready-to-feed formula. The new mothers would be instructed to use this formula until the baby was 14 days old, and then to switch to powdered formula (that we would provide) that would be mixed with their home tap water for the second two weeks of the study.

Sample size

The baseline for diarrhea in infants from all causes is 13% (personal communication, Center for Infectious Disease, Centers for Disease Control and Prevention). With $\propto = 0.05$ and 80% power, a ratio of unexposed:exposed of 1:1, and assuming that the risk ratio for exposed compared with

unexposed is approximately 2, we will needed a sample size of approximately 100 mothers and infants per sulfate group. We planned to recruit approximately 110 mothers and infants per group to allow for an anticipated 10% loss to follow-up.

Participant Recruitment

For a woman to be included in our study, she had to meet the following criteria: (1) Be accessible through a clinic that offers prenatal care, (2) Be 35 to 36 weeks pregnant (to exclude severely premature infants and to be able to interact with the woman at her next prenatal visit), (3) Be planning not to breast-feed her baby, (4) Be planning to feed her infant concentrated formula mixed with her home tap water (which could be boiled), (5) Be planning to be at home with her infant for at least one month, (6) Be living in a home (private house, apartment, mobile home, etc.) that was served by a public water supply and that did not have a water filtration system, and (7) Be able to read the study instruments in either English or Spanish.

Using historic water quality data for secondary drinking water constituents (including concentrations of sulfate, chloride, copper, fluoride, iron, manganese, and total dissolved solids) provided by the states and Geographic Information System (GIS) techniques, we generated maps of the geographic distribution of each water system that simultaneously identified the level of sulfate in the water and the size of the population each system served. Using the GIS maps, the number of expected births in the participating states, the inclusion criteria we have described, and the assumption that 25% of infants are not breast-fed, we planned a 6-month recruiting period. We planned to enroll a maximum of 880 infants, 110 each from water systems within the following ranges of sulfate: < 250 mg/L (baseline or comparison group), 251-500 mg/L, 501-700 mg/L, with a maximum of 550 infants from water systems with sulfate levels greater than 701 mg/L.

Data collection

Each mother would be instructed to complete a 4-week diary describing her baby's food intake and elimination patterns beginning as soon as the baby was born and continuing until the end of the baby's 4th week. In order to limit misclassification of diarrheal illness in infants, we included specific questions about the baby's stools rather than asking whether the baby had diarrhea.

To verify the sulfate levels in a particular water system, we planned to request water samples for each water system from which we recruited study participants. We planned to collect, store, ship, and analyze the samples according to the methods described in the National Primary Drinking Water Regulations--Sulfate; Proposed Rule (59 FR 65586) using state laboratories certified under EPA's drinking water laboratory certification program.

Statistical analysis

We planned to examine the average number of episodes of diarrhea, as reported by the mothers, in infants living in homes using tap water containing various levels of sulfate. The magnitude of the association between the consumption of sulfates in drinking water and the incidence of diarrhea was to have been estimated by calculating the relative risk for diarrhea in each of the high-sulfate groups (251-500 mg/L, 501-700 mg/L, 701-1100 mg/L, and >1101 mg/L sulfate) as compared with the baseline group (0 - 250 mg/L sulfate).

Infant pilot study

We conducted a pilot study of the planned recruitment methods and study instruments in four counties in South Dakota. Local Public Health Nurses, who already had rapport with clinic patients, were hired and trained to recruit study participants and conduct the activities associated with the study.

In the 4 counties (served by 3 Community Health Clinics which also provided the Women, Infants, and Children [WIC] program services), 72 women were approached about participating in the study during their prenatal visit to the public health clinics. Of these 72 women, 30 were ineligible because they planned to breast-feed their infant, 23 were ineligible because they planned to use water other than tap water to mix infant formula, and 11 were ineligible because they did not meet other eligibility criteria.

Of the eight women who were eligible to participate in the study, three refused, five agreed to participate, and one completed all the study activities. Of the four women who did not complete the study activities, two switched to bottled water as the source of water to mix with infant formula, one chose to use ready-to-feed formula after two weeks of using the powdered formula, and the fourth moved out of the area.

Self-administered questionnaire

Because we experienced recruiting problems during the pilot study conducted in South Dakota, we developed a self-administered questionnaire (SAQ) made up of previously approved questions from the infant study instruments we had planned to use to determine tap water use. We provided English and Spanish versions of the SAQ to 32 WIC clinics that offered prenatal care in New Mexico, South Dakota, and Texas. The clinics were located in areas with a range of sulfate levels in the drinking water provided by the local public water systems

The questionnaires were given to all women who came to the clinic during a two-week period, and included questions about the source of their home tap water, what mothers of infants $\leq$3 months old were currently feeding their babies, and how currently pregnant women planned to feed their new infants.

B. Transient Populations

Study design

To study the effects on adults of suddenly changing drinking water sources from one that has little or no sulfate to one that is high in sulfate, we conducted an experimental study involving volunteers from Atlanta, Georgia, including CDC employees and employees at the U.S. EPA Region IV office. The sulfate concentration in the drinking water in Atlanta is very low, thus study volunteers were not already acclimated to the presence of sulfate in their drinking water. Potential volunteers were ineligible to participate in the study if they had traveled outside the U.S. within the last two weeks before the interview, if they had any acute illnesses (e.g., flu or food-borne illness) or if they had chronic medical problems that could affect bowel movements (e.g., irritable bowel syndrome).

Sample Size

The baseline for diarrhea in adults is 14% (i.e., at any point in time, 14% of adults will have had diarrhea in the last month). With 95% confidence and 80% power, a ratio of unexposed:exposed of 1:1 and assuming that the risk ratio for exposed compared with unexposed is approximately 4, we will needed a sample size of 25 per sulfate group. To account for participant drop out during the study and still have sufficient power to detect differences in reports of diarrhea among the groups, we attempted to recruit 150 volunteers, 30 each for the following sulfate exposure groups: 0 mg/L, 250 mg/L, 500 mg/L, 800 mg/L, 1200 mg/L.

Participant recruitment

We attempted to recruit 150 volunteers from the National Center for Environmental Health Campus and the Environmental Protection Agency Region 4 office in Atlanta, GA using signs posted in building lobbies, a center-wide e-mail message, and personal requests. Participation was very limited for a number of reasons, including: 1) out of town travel, 2) concern about how having diarrhea would affect work or evening activities, 3) personal distaste regarding the request to report bowel movements, 4) concern about drinking "contaminated" water.

Data collection

For this experiment, we added different levels of sulfate (from anhydrous sodium sulfate, Sigma Chemical Company, St. Louis, MO) to bottled drinking water. The sodium salt was chosen to be consistent with the studies conducted by Heizer *et al.* (1997) and to avoid the confounding that could occur from exposure to magnesium sulfate (magnesium is also a laxative that might be present in tap water).

Volunteers were randomly assigned to one of 5 sulfate exposure groups (i.e., 0, 250, 500, 800, or 1200 mg/L sulfate from sodium sulfate) and were provided with bottled drinking water for

6 days. The bottled water for days 1, 2, and 6 contained plain water, and the bottles for days 3 through 5 contained the added sulfate. The unfinished or empty bottles were returned and weighed to determine how much water was consumed each day. Volunteers were blinded to the level of sulfate in their drinking water.

Study participants were also requested to complete a short daily diary describing the following: any changes in smell, taste, or appearance of their drinking water compared to what they normally drank, any visits to health care professionals, any medication they took for diarrhea, anyone in their household who was currently ill with vomiting or diarrhea, any work or recreational activity missed because of illness, and the number, size, and consistency of their bowel movements.

We conducted two trials of exposure to sulfate in drinking water. In the first trial, the 63 participants who completed the study were divided among the dose groups as follows: 10 received 0 mg/L sulfate; 10 received 250 mg/L sulfate; 10 received 500 mg/L sulfate; 18 received 800 mg/L sulfate; and 15 received 1200 mg/L sulfate. In the second trial, the 42 participants who completed the study were divided among the dose groups as follows: 14 received 0 mg/L sulfate; 15 received 800 mg/L sulfate; and 13 received 1200 mg/L sulfate.

Statistical Analysis

Using SAS statistical software (univariate analyses and logistic regression), we examined water consumption, the relative frequency of diarrhea, and the number and description of bowel movements reported in the different dose groups.

Results

A. Infant Study

The 37 WIC clinics participating in the survey study draw their clients from 15 counties with varying average levels of sulfate in the drinking water provided by local public water systems. Of the 1,388 SAQs completed by women attending the 32 public health clinics included in our study, 1184 were completed in English and 204 in Spanish; one thousand one hundred and two (79%) women reported that their home tap water came from a public water system, and 143 (10%) had some type of filter on their home tap. One thousand fifty three (76%) women reported that at least one person in her household drinks water from the tap.

Of the 951 pregnant women who completed the SAQ, 582 (61%) reported that they planned to breast-feed their new infant and 141 (15%) reported that they planned to use infant formula mixed with their home tap water. Of the 542 women with infants $\leq$3 months old who completed the SAQ, 272 (50%) reported that they had breast-fed their infant and 272 (50%) reported that they had used infant formula mixed with their home tap water. In addition, 357 (66%) reported that they would not use tap water to mix infant formula in the future. A chi-square analysis showed that a pregnant woman's plans to use tap water to mix infant formula was associated with the level of sulfate in her

home tap water ($\chi^2 = 19.03$, $p < 0.001$). A chi-square analysis of the three lower doses combined into one group and compared with the highest exposure group was also statistically significant (($\chi^2 = 15.53$, $p < 0.001$).

To determine how many of the women who completed the SAQ would have been eligible to participate in our study (based on the tap water source and use information), we examined the responses of women who received their tap water from public water systems and who did not have filters on their home tap. The result was that 403 women with infants ≤ 3 months of age and 761 pregnant women met these eligibility criteria (Table 1).

Among the 761 pregnant women who responded to the SAQ (and who received tap water from a public water system and did not have a filter on their home tap), only 123 (16%) reported planning to use tap water to mix formula for their infants. The largest numbers of women who had used or were planning to use tap water to mix infant formula (and who received tap water from a public water system and did not have a filter on their home tap) were in the 4 counties with average sulfate levels ≤ 250 mg/L. Of the 365 pregnant women in areas with sulfate levels > 250 mg/L, 226 (62%) planned to use infant formula. However, only 39 (11%) planned to use infant formula mixed with their tap water. Of the 183 women with infants ≤3 months old in areas with sulfate levels > 250 mg/L, 151 (83%) used infant formula, but only 35 (19%) reported having used infant formula mixed with their tap water. Thus, of the 548 pregnant women and women with infants ≤ 3 months old, only 74 infants had been or would be exposed to tap water containing ≥ 250 mg/L sulfate.

We examined historical drinking water analysis data for the counties represented by the clinics participating in the SAQ. In many areas where sulfate concentrations were high, the concentrations of other constituents that affect the organoleptic quality of drinking water tended to be close to or above the levels suggested by the National Secondary Drinking Water Standards (NSDWS) (40 CFR 143.3) (see Appendix). For example, in one county in South Dakota, the average levels of drinking water constituents that affect the taste of the water were: sulfate, 841 mg/L; total dissolved solids, 1462 mg/L (suggested level 500 mg/L); manganese 0.31 mg/L (suggested level 0.05 mg/L); iron, 0.59 mg/L (suggested level 0.3 mg/L); fluoride, 1.53 mg/L (suggested level 2.0 mg/L), and chloride, 125 mg/L (suggested level 250 mg/L).

B. Adult Study

The results of the two sulfate trials were similar; therefore, we have reported the combined results. One hundred five study participants were divided among the dose groups as follows: 24 received 0 mg/L sulfate; 10 received 250 mg/L sulfate; 10 received 500 mg/L sulfate; 33 received 800 mg/L sulfate; and 28 received 1200 mg/L sulfate. The demographic information for the study population was as follows: the mean age of participants was 42 years; the majority (62%) was female; the races included in the study population were white (80%),

Table 1: Planned and previous use of tap water to mix infant formula by women who are currently pregnant and by women who have an infant ≤ 3 months old (and who received their tap water from public water systems and who did not have filters on their home taps).

Mean Sulfate Level (mg/L)	Number of Counties	Number of SAQs	Women with Infants ≤ 3 mos.		Pregnant Women	
			n	Formula Mixed With Tap Water[1]	n	Formula Mixed With Tap Water[2]
0 - 250 mg/L	4	682	220	83 (38%)	396	84 (21%)
251 - 500 mg/L	5	455	123	20 (16%)	219	17 (8%)
501 - 750 mg/L	3	135	27	7 (26%)	76	11 (14%)
> 751 mg/L	3	116	33	8 (35%)	70	11 (16%)
TOTAL	15	1388	403	118 (29%)	761	123 (16%)

[1] Number of women with infants ≤ 3 mos. of age who had used powdered formula mixed with water and who used tap water.
[2] Number of pregnant women planning to used powdered formula mixed with water and who are planning to use tap water.

black (13%), and Asian/Pacific Islander (7%). Ninety-five percent of the participants were non-Hispanic.

During the study period, no one reported taking medication for diarrheal illness and no one visited a health care professional for anything other than routine medical care. In addition, no one reported missing work or recreational activities because they had diarrhea.

Because the presence of sulfate affects the organoleptic quality of drinking water, we examined reported differences in smell or taste of the water provided to study participants compared to the water they normally drank. Among study participants who reported no difference in taste or smell (compared to the water they normally drank) on days 1 and 2, the numbers of people who reported a difference on days 3, 4, or 5 by dose group were: 4 (25%) at 0 mg/L; 4 (57%) at 250 mg/L; 2 (50%) at 500 mg/L; 15 (79%) at 800 mg/L; and 14 (82%) at 1200 mg/L.

We analyzed the number of bowel movements recorded each day by study participants, and found that there were no statistically significant differences in the mean number of bowel movements among the groups on days 3, 4, 5, or 6. Neither were there statistically significant differences in the mean number of bowel movements reported when comparing days 1 and 2 (the days when there was no sulfate in the water) with days 3, 4, and 5 within each dose group.

Because the sulfate dose for each individual was dependent upon his or her water consumption, we examined the amount of water consumed by the different dose groups (see Figure 1). Water consumption varied across the days of the study for all dose groups. The average water consumption in the 500, 800, and 1200 mg/L dose groups tended to decrease on days 3, 4, and 5 (when there was sulfate in the drinking water). All exposed groups consumed more water on day 6 (no sulfate in the drinking water) than on day 5 (the last exposure day).

The frequencies of diarrhea reported by individuals exposed to varying levels of sulfate in their drinking water are presented in Table 2. Three different definitions of diarrhea were used: increase in stool volume compared to normal, change in stool consistency to liquid or paste, and increase in volume/change in consistency. Because the effect of sulfate is dependent on both the amount of water consumed and the weight of the participant (dose/kg of body weight), we used logistic regression to examine the reported frequency of diarrhea (using the three different definitions described above) by sulfate dose ([concentration of sulfate in water x volume of water consumed on days 3, 4, and 5]/body weight). The results of the logistic regression are presented in Table 3. Sulfate dose was not a statistically significant predictor of diarrhea in any of the models.

To examine the data for a trend toward increased frequency of reports of diarrhea with increased sulfate intake, we included the dose as an ordinal variable in a logistic regression model of osmotic diarrhea. There was no statistically significant increase in reports of diarrhea with increasing dose (one-sided $p = 0.099$).

Table 2. Number and percent of study participants reporting diarrhea on days 3, 4, or 5 of the study (limited to those individuals who reported normal stool volume on days 1 and 2 and who did not have family members who experienced vomiting or diarrhea on days 3, 4, or 5) following exposure to varying levels of sulfate in their drinking water.

Sulfate Dose (mg/L)	n	Osmotic Diarrhea[1] No.[4] (%)	Diarrhea[2] No.[4] (%)	Diarrhea[3] No.[4] (%)
0	24	2/18 (11)	6/16 (38)	5/14 (36)
250	10	0/9	1/7 (14)	1/6 (17)
500	10	1/8 (12)	4/9 (44)	3/8 (38)
800	33	4/26 (15)	7/20 (35)	8/17 (47)
1200	28	5/27 (18)	6/17 (35)	6/17 (35)

[1] Reported as an increase in stool volume on days 3, 4, or 5 and limited to those who reported a normal stool volume on days 1 and 2.

[2] Reported as paste-like or liquid stools on days 3, 4, or 5 and limited to those who reported normal stool consistency on days 1 and 2.

[3] Reported as change in stool bulk or consistency on days 3, 4, or 5 and limited to those who reported normal stools on days 1 and 2.

[4] (Number of people reporting diarrhea on days 3, 4, or 5)/(Number of people who reported normal stools on days 1 and 2 and who did not report a family member ill with vomiting or diarrhea).

Table 3. Parameter estimates, standard error, and p values for logistic regression models of diarrhea reported by study participants (limited to those individuals who reported normal stool volume on days 1 and 2 and who did not have family members who experienced vomiting or diarrhea on days 3, 4, or 5) following exposure to sulfate in drinking water.

Variables in Model	n	Parameter Estimate	Standard Error	p
Model 1[1]				
Intercept	85	-1.825	0.430	< 0.01
Dose		4.629 E-7	7.189 E-6	0.94
Model 2[2]	67			
Intercept		-0.523	0.349	0.13
Dose		-1.64 E-6	6.587 E-6	0.80
Model 3[3]				
Intercept	60	-0.529	0.364	0.15
Dose		1.415 E-6	6.585 E-6	0.83

[1] Osmotic diarrhea reported as an increase in stool volume on days 3, 4, or 5 and limited to those who reported a normal stool volume on days 1 and 2.

[2] Reported as paste-like or liquid stools on days 3, 4, or 5 and limited to those who reported normal stool consistency on days 1 and 2.

[3] Reported as change in stool bulk or consistency on days 3, 4, or 5 and limited to those who reported normal stools on days 1 and 2.

We further examined the lowest three doses to determine if the response was statistically the same across all three groups, and estimated a generalized linear model for the number of "successes" or episodes of diarrhea in each of the lower sulfate dose groups (0, 250, and 500 mg/L). Since the number of episodes of osmotic diarrhea was lowest in the 250 mg/L dose group, we performed a test to determine whether that group truly differed from the 0 and 500 mg/L groups. We used a one-tailed test to coincide with our alternative hypothesis that the number of episodes of osmotic diarrhea was lower in the 250 mg/L dose group than in the other two dose groups. The resulting p-value was .09, suggesting a flat dose response at those sulfate levels.

When the lowest doses (0, 250, and 500 mg/L) were collapsed into one group, 3 (9%, n = 35) individuals reported an increase in stool volume, 34 (33%, n = 103) individuals reported a change in stool consistency, and 11 (34%, n = 32) reported a change in stool volume or consistency (on days 3, 4, or 5, with no report of household members having diarrhea or vomiting). Compared with the data for the 800 mg/L and 1200 mg/L doses presented in Table 2, there was an increase in reports of osmotic diarrhea with increasing dose.

Discussion

The purpose of these studies was to provide information about the health effects (primarily osmotic diarrhea) in sensitive populations (infants and transients) of exposure to high levels of sulfate in drinking water. The most efficient method available to examine this question would be to conduct an experimental trial in which a subject consumes a known amount of sulfate in water and reports any subsequent cases of osmotic diarrhea. However, there are a number of difficulties associated with conducting this type of study. First, it is difficult for study participants to identify osmotic diarrhea. Second, while there is some agreement about a baseline incidence of diarrhea in adults, it is likely to be under-reported and there is no gold standard with which to compare our control group. Third, it is difficult for study participants to consume a large volume of water containing very high levels of sulfate because of the adverse effect sulfate has on the organoleptic quality of the water. Finally, it would not be ethical to conduct an experimental trial involving exposure of infants to a substance that may cause diarrhea and subsequent dehydration.

For the infant study, we planned to conduct a prospective cohort study of newborn infants. We planned to identify highly exposed populations and recruit pregnant women living in the relevant geographic areas. We anticipated that approximately 75% of women would be ineligible to participate because they were planning to breast-feed their infants. We also anticipated that, of those 25% of women who did not plan to breast-feed, 50 % would not be eligible (either because their tap water came from a source other than a public water system or because they had a filter on their home tap) or would refuse to participate.

During the pilot study conducted in South Dakota, only eight of 72 (11%) women were eligible to participate. Of the five women (63% percent of those eligible) who agreed to participate, two decided to use bottled water to mix infant formula and one decided to use ready-to-feed formula after starting the study. Thus, plans to breast-feed, to use ready-to-feed formula, or to use water other than tap water prevented 67 (93%) of the women from being eligible to enter or complete the study.

Most (74%) of the women approached during the pilot study were not eligible to participate because either they planned to breast-feed their new infant or they did not plan to use their tap water to mix infant formula. This finding suggested that very few infants were actually exposed to tap water containing high levels of sulfate. In order to gain more specific information concerning the number of infants actually exposed, we developed a self-administered questionnaire (SAQ) to determine how many women planned to use tap water to mix infant formula for their new babies.

The results from the SAQ were that more than half (61%) pregnant women reported planning to breast-feed their infants. In addition, of those who planned to use infant formula mixed with water, most (84%) planned to use water other than tap water. These results were consistent with our findings from the pilot study and indicated that only a very small number of women who live in areas with high levels of sulfate in the tap water provided by public water systems planned to give this water to their infants.

The geographic areas where there were high levels of sulfate in the public water systems tended to be rural areas that were sparsely populated. In addition, the historical water quality data indicated that many of the geographic areas with high levels of sulfate in the water provided by public water systems also had high levels of other constituents that adversely affect water quality (see Appendix). Our SAQ results indicated that a very small number of women living in these areas planned to give their babies formula mixed with tap water. Thus, it would not be practical to conduct a study of the effects of sulfate on diarrhea in infants by recruiting a study population exposed to naturally occurring high levels of sulfate in the drinking water provided by public water systems. However, because there is a concern about exposing young infants to tap water containing high levels of ions, including sulfates, a conservative approach would be to recommend that individuals using their tap water to feed infants have their water tested for sulfate content or choose an alternative water source known to be low in sulfate.

For the adult study, we conducted a modified experimental trial. Study participants were randomly assigned to a sulfate dose level and were blinded to the dose they were receiving. However, at the high levels of sulfate, study participants could taste the change in their drinking water, and so could tell that they were in an exposed group. It is not clear whether, or in which direction, this knowledge might bias the responses provided by the study participants. It is interesting to note that some of the participants who were in the control group complained that the water tasted bad and they weren't sure they would be able to complete the study.

Given the limitations of the study design, we examined the incidence of diarrhea in study participants exposed to different levels of sulfate in their drinking water in two ways. First, we examined the number of reports of diarrhea across exposure groups using three different definitions of diarrhea (see Table 2). There was an increase in the number of people who reported osmotic diarrhea in the most highly exposed groups (800 mg/L and 1200 mg/L) compared to the controls (0 mg/L), but the differences were not statistically significant. There were no associations between sulfate dose and the number of reports of diarrhea when the other two definitions of diarrhea were used. These results are consistent with the results on human subjects reported by Heizer *et. al.* (1997) (i.e., water containing 1200 mg/L sulfate from sodium sulfate produced mild increases in stool weight, decreases in self-reported stool consistency, but no complaints of diarrhea).

Because water consumption varied for each individual in the study, we also examined the sulfate dose as the amount of sulfate consumed per kg of body weight. We used logistic regression analysis to examine the association between sulfate dose (mg/kg body weight) and reports of diarrhea using the three definitions (see Table 3). There were no statistically significant dose-response associations between sulfate dose and reports of diarrhea.

The study participants exposed to 250 mg/L sulfate actually reported fewer incidents of diarrhea than the control group (0 mg/L sulfate) did. Similar to the conclusions drawn by Heizer, *et. al.*, (1997) and Chien *et al.* (1968), our conclusion is that it is unlikely that exposure to sulfate in drinking water at concentrations below 600 mg/L would cause diarrhea in people. Thus, the 0 mg/L, 250 mg/L, and 500 mg/L exposures may all be below a threshold dose required to produce a biological effect, and, if so, a dose-response effect within this low range would not be expected.

Study participants were not requested to drink a specified amount of water. Because we were concerned that the changes in the organoleptic quality of the water would result in lower consumption among people in the higher dose groups, we examined water consumption (see Figure 1). The average water consumption decreased during the exposure period (days 3-5) for all exposure groups. There was no pattern in the average water consumption by the control group over the 6-day study period.

We were not able to conduct the dose-response studies that were requested as part of the Safe Drinking Water Act Amendments of 1996. Instead, we convened an expert workshop whose members reviewed the available literature and addressed a series of questions about the health effects from exposure to sulfate in drinking water: 1) Do reported studies suggest that a certain sulfate level would not be likely to cause adverse effects?, 2) Does the literature support acclimatization or adaptation?, 3) Can an infant dose-response study be done anywhere in the U.S. or Canada?, 4) Is there enough scientific evidence of adverse health effects from sulfate in drinking water to support regulation? The workshop was held in Atlanta, Georgia on September 28, 1998. The workshop report accompanies this document.

Conclusion

The purpose of this project was to examine the association between consumption of tap water containing high levels of sulfate and reports of osmotic diarrhea in susceptible populations (infants and transients, i.e., those acutely exposed). We were unable to conduct a study of infants because we could not identify enough exposed individuals from which to draw a study population. The results of our SAQ examining tap water use indicated that most pregnant women who completed the survey planned to breast-feed their infants. Of those who planned to use formula mixed with water, most did not plan to use tap water to mix the formula. In our experimental trials with adult volunteers, we did not find a significant dose-response association between acute exposure to sodium sulfate in water (up to 1200 mg/L) and reports of diarrhea. However, we did find a weak (not statistically significant) increase in reports of diarrhea at the highest dose level when it was compared to the combined lower doses.

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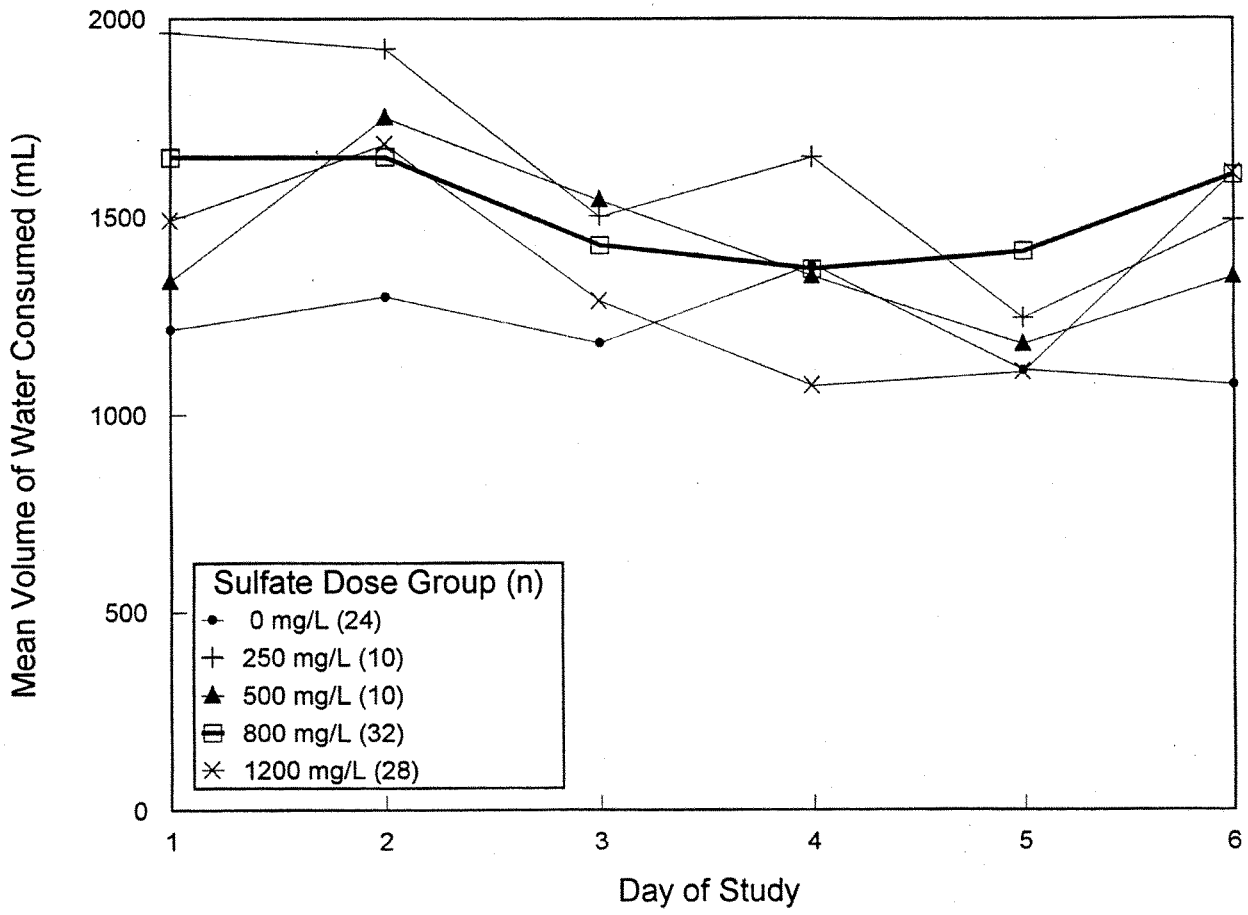
Figure 1: Mean volume of water in mL consumed by volunteers in the two adult trials on each day of the study.



Appendix

Organoleptic Secondary Drinking Water Constituents and Tap Water Use for Infant Formula: Summary

Organoleptic Secondary Drinking Water Constituents and Tap Water Use for Infant Formula: Summary

County (No. PWS)	Drinking Water Constituents: Mean and Range							No. Clinics	No. SAQs[3] Eng/Sp	Women with Infants (≤ 3 mos.)			Pregnant Women			
	Chloride MCL[1]: 250 mg/L	Copper MCL: 1 mg/L	Fluoride MCL: 2.0 mg/L	Iron MCL: 0.3 mg/L	Manganese MCL: 0.05 mg/L	Sulfate MCL: 250 mg/L	TDS[2] MCL: 500 mg/L			n	Powdered Formula		n	Breast-Feed n	Powdered Formula	
											n	Tap Water[4]			n	Tap Water[5]
New Mexico																
Bernalillo (28)	44 0-225	.	0.54 0.1-2.97	0.09 0-0.1	0.05 0.05-0.05	116 19-360	481 102-1000	8	112/71	48	26	14 (29%)	132	94	47	14 (11%)
Chaves (6)	42 12-35	.	1.08 0.73-1.90	0.10 (1value)	0.08 (1 value)	318 46-462	.	1	81/9	19	17	1 (6%)	56	28	35	2 (4%)
Dona Ana	.	.	.	.	.	165 16-283	.	1	18/20	13	8	0	26	14	12	0
Edd (8)	102 5-467	.	0.63 0.22-0.83	.	.	328 41-687	.	2	98/9	41	36	7 (19%)	55	30	31	1 (2%)
McKinley (4)	46.5 28-65.1	.	1.49 1.25-2	0.11 0.02-0.2	0.04 0.02-0.05	789 224-2187	760 (1 value)	1	33/3	10	6	4 (40%)	19	13	9	3 (16%)
Otero (13)	199 37-356	.	0.36 0.15-0.78	.	.	506 146-820	.	1	65/2	14	10	3 (30%)	33	20	17	2 (6%)
Socorro (2)	51.2	.	0.5	0	0	899 96-1702	.	1	31/6	10	8	0 (0%)	26	18	15	5 (19%)
South Dakota (Data from treated [T] water samples if that data was provided, otherwise average of other types of samples [R=raw, RT=raw treated, RC=raw composite, RTC=raw treated composite with limited treatment])																
Beadle (10)	125 21-353	.	1.53 0.72-2.58	0.59 0.1-1.6	0.31 0.02-2.26	841 193-1255	1463 509-2093	1	43/0	13	10	4 (40%)	25	12	15	3 (12%)
Brown (8)	87 32-235	.	0.85 0.16-1.64	0.13 0.0-0.3	0.18 0.02-0.63	409 171-1101	1011 486-1849	1	33/0	3	3	3 (100%)	26	12	21	11 (42%)
Spink (5)	161 6-476	.	2.07 0.06-5.17	0.6 0.1-2.1	0.23 0.02-0.45	737 10-1213	1608 36-2794	1	24/0	6	6	4 (67%)	14	11	8	3 (21%)
City of Pierre (5)	96 11-213	.	0.65 0.25-2.03	0.69 0.2-2.0	1.33 0.02-3.48	528 235-1105	1304 462-2032	1	44/0	7	5	0 (0%)	29	15	19	6 (21%)
City of Rapid City (13)	8 2-26	.	0.47 0.17-1.42	0.1 0.1-0.3	0.025 0.02-0.04	96 14-433	324 142-697	1	325/0	99	80	54 (68%)	173	112	86	49 (27%)

Texas																
Midland/ Ector (14)	473 216-1386	0.011 0.006-0.04	1.67 0.8-3.75	0.06 0.01-0.33	0.006 0.002-0.008	450 277-916	1606 950-3381	4	147/40	45	38	4 (11%)	72	24	54	3 (4%)
Hays (8)	32 16-73	0.014 0.006-0.039	2.2 0.3-3.1	0.11 0.02-0.48	0.050 0.002-0.35	534 38-1648	1030 322-2697	2	33/2	15	12	5 (42%)	11	10	2	0 (0%)
Travis (1)	61	0.006	0.6	0.02	0.008	46	243	11	97/39	60	35	15 (43%)	64	44	38	18 (%)
													761	**457**	**409**	**123**

[1]Maximum Contaminant Level; U.S. Environmental Protection Agency Secondary Drinking Water Standards.
[2]Total Dissolved Solids
[3]Number of completed Self-Administered Questionnaires (SAQs)
[4]Number of women using tap water (PWS or community well) to mix infant formula for their infants ≤3 months of age
[5]Number of pregnant women planning to use tap water (PWS or community well) to mix infant formula
. Data not available