Residential Water Source and the Risk of Childhood Brain Tumors

Beth A. Mueller,[1,2] Katherine Newton,[1,3] Elizabeth A. Holly,[4] and Susan Preston-Martin[5]

[1]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington USA; [2]Epidemiology Department, School of Public Health and Community Medicine, University of Washington, Seattle, Washington, USA; [3]Center for Health Studies, Group Health Cooperative of Puget Sound, Seattle, Washington, USA; [4]Department of Epidemiology and Biostatistics, University of California San Francisco, California, USA; [5]Department of Preventive Medicine, University of Southern California Los Angeles, California, USA

Gestation may represent a window of susceptibility to transplacental effects of environmental exposures, including chemicals in water. The *N*-nitroso compounds (NNC), a class of chemicals with demonstrated neurocarcinogenic potential, include substances detected in drinking water. We used data from a study of possible risk factors for childhood brain tumors (CBT) to investigate the association of source of residential drinking water during pregnancy and CBT occurrence among offspring. In addition, dipstick measurements were made of nitrates and nitrites in tap water for the subset of women living in the same home they had lived in during their pregnancies. Population-based CBT cases (*n* = 540) and controls (*n* = 801) were identified in three regions including Los Angeles County, and the San Francisco Bay Area of California, and the Seattle-Puget Sound area of western Washington State. Overall, we observed no increased risk of CBT in offspring associated with wells as the source of residential water. However, an increased risk of CBT [odds ratio (OR) = 2.6; 95% confidence interval (CI), = 1.3–5.2] was observed in western Washington among offspring of women who relied exclusively on well water, and a decreased risk of CBT (OR = 0.2; 95% CI, 0.1–0.8) was observed in Los Angeles County. Among the small subset of subjects for whom dipstick measurements of tap water were available, the risk of CBT associated with the presence of either measurable nitrite and/or nitrate was 1.1 (95% CI, 0.7–2.0). Given the crude measurement method employed and because measurements often were obtained years after these pregnancies occurred, the relevance of the dipstick findings is unclear. The lack of consistency in our findings related to residential water source does not support the hypothesis of increased risk related to consumption of well water; however, regional differences in well water content may exist, and the increased risk observed in western Washington deserves further evaluation. *Key words:* childhood brain tumors, drinking water, environmental exposures, nitrates, nitrites. *Environ Health Perspect* 109:551–556 (2001). [Online 18 May 2001]
http://ehpnet1.niehs.nih.gov/docs/2001/109p551-556mueller/abstract.html

The potential association of *N*-nitroso compound (NNC) exposures and the occurrence of childhood brain tumors (CBT) remains unclear, possibly because of the difficulties of measuring these complex environmental exposures in most epidemiologic studies. Accurate assessment of NNCs is difficult because they comprise a large family of chemicals ubiquitous in the environment or produced endogenously from commonly ingested foods. Drinking water may be an important source of nitrate (*1–4*) that can be reduced to nitrite, a potential precursor of endogenously formed NNCs that include recognized carcinogens such as the nitrosamides (*5–7*). Similarly, nitrite ingested in drinking water may react *in vivo* with nitrosable substrates from foods to form NNCs. The maximum allowable levels of nitrate and nitrite (both as nitrogen) set by the U.S. government for public drinking water are 10 mg/L and 1 mg/L, respectively, because of concerns related to the potential risks of methemoglobinemia and possible adverse health conditions including cancer.

High rates of tumor induction have been observed in offspring when sodium nitrite and ethyl urea [precursors of the carcinogenic nitrosamide ethylnitrosourea (ENU)] are present in food and drinking water of pregnant rats, or among pregnant rats fed nitrites plus amines or amides (*8*). There is also some evidence from human studies of a possible association of dietary NNC exposure and CBT occurrence (*9–11*). It is possible that, due to a decreased capacity for DNA repair and a high rate of cell division, the fetal and newborn brain are particularly vulnerable to potential carcinogenic effects of exposures to NNC.

We conducted a case–control study of CBT to evaluate the effects of several exposures, including NNC *in utero* and during early childhood. The purpose of this analysis was to evaluate the association of source of residential drinking water during pregnancy with CBT occurrence among offspring. Because of the possibility that well water, including water from private wells that may be less subject to routine water quality regulation, may contain nitrates or other chemicals possibly associated with CBT occurrence, we examined whether there was an increased risk of CBT associated with reliance on wells as the source of residential water. We also attempted to assess potential exposure to contaminants in residential drinking water by using dipsticks to measure levels of nitrates and nitrites in tap water.

Methods

The methods of the U.S. West Coast CBT study, a population-based, case–control study, have been described previously in detail (*9,12*). Briefly, data for 19 counties from three cancer registries were used to identify all children younger than 20 years of age who were diagnosed with primary tumors of the brain, cranial nerve, or meninges (ICDO 1976 codes 191.0–192.1). Subjects resided at the time of their diagnoses in the geographic regions of San Francisco, California, and western Washington State, including the Seattle-Puget Sound area during 1 January 1984–31 December 1990, and in Los Angeles County, California, during 1

January 1984–30 June 1991. A control group was obtained using a random digit dial (RDD) procedure (13,14) to identify children without tumors in a ratio of 1:1 in Los Angeles, and 2:1 in San Francisco and Seattle. In Seattle and San Francisco, controls were frequency-matched to cases by birth year and sex. In Los Angeles, cases and controls were individually matched on these criteria. Inclusion required that the child's physician be contacted before contacting the child's mother, that the biological mother consent to be interviewed in either English or Spanish, and that the family have a telephone present in their home. Approvals from the Institutional Review Boards of all participating institutions were received before the study was conducted.

Of the 813 cases identified, 762 (94%) were determined to be eligible. Of these, 540 (71%) were interviewed. Reasons for nonparticipation included physician refusal (3%), parental refusal (10%), parents moved from geographic area or were not traceable (16%), or parents unable to complete the questionnaire for other reasons (1%). Using RDD, 6,170 of the 6,990 residences contacted (88%) were screened for eligibility. Interviews were conducted with 801 (67%) mothers of the 1,079 children determined to be eligible as controls. Of the eligible controls, reasons for nonparticipation included parental refusal (20%), parents not traceable (3%), or parents unable to complete the questionnaire for other reasons (2%). Response levels for cases and controls were similar in all three geographic regions. A comprehensive in-person interview was conducted with mothers of all subjects using a detailed questionnaire to obtain information on demographic factors and potential exposures from the time of pregnancy with the index child to the date of the child's tumor diagnosis, or a similar date (the "reference date") for the control children. Exposure information included potential sources of environmental NNCs via tobacco smoke, hobby and occupational activities, and diet during the mother's prenatal period and during the subject's early childhood. Maternal consumption during the index pregnancy of 47 food items relevant to the NNC hypothesis (major dietary sources of nitrate, nitrite, vitamins C and E) was assessed with a dietary recall questionnaire and abstract food models to gauge portion size (9). Use of prenatal vitamins or other vitamin supplements during the index pregnancy also was ascertained.

Mothers were asked about the sources of their household water (public water, well, spring, rainwater/cistern, street tank, river, pond/lake, other) during the prenatal and early childhood period. They were then asked to estimate what proportion of water consumed during their pregnancies was bottled. In addition, if the mother was living in the same residence where she resided during her pregnancy with the index child, dipstick measurement of nitrate and nitrite in the tap water was conducted by the interviewer. Dipsticks were kept enclosed in airtight containers and refrigerated at the regional study centers until needed. They then were transported to interviews in a dry thermos to avoid contamination by ambient air moisture until water testing. The presence of any nitrates and/or nitrites and the semiquantitive determination of these ion levels (none detected, 10, 25, 50 100, 250, 500 mg/L NO_3^-; none detected, 1, 5, 10, 20, 40, 80 mg/L NO_2^-, as provided by the Merckoquant dipsticks; Merck, Darmstadt, Germany) were measured in the tap water in homes of mothers of 119 of the 540 children with brain tumors (22%) and 191 of 801 control children (24%).

Initially, we conducted stratified analysis with adjustment for potential confounders using Mantel-Haenszel methods to evaluate residential water source (public water only, well water only, mixture of public and well water, mixture of public and other water source) and the risk of CBT occurrence. Women who reportedly consumed any bottled water during their pregnancies were then excluded and the analyses repeated in an attempt to refine our measurement of water consumption. Subsequently, unconditional logistic regression was used to obtain odds ratio (OR) estimates of the relative risk of CBT and their associated 95% confidence intervals (CI) (15) for questionnaire information about residential water source and dipstick measurements of nitrates and nitrites. Factors evaluated for their potential effects on the relationships of interest included the child's age and sex and mother's education, prenatal smoker status, reference/diagnosis date (before 1989, 1989 or later), ethnicity, and geographic area. Only those factors that appreciably changed risk estimates were retained in final analyses. Unless otherwise indicated, all final risk estimates presented are adjusted for child's age (< 5; 5–9; 10–14; ≥ 15 years) and sex. In addition, stratum-specific analyses within histologic categories [astrocytomas and gliomas, morphology codes 9380–9384, 9400–9421, 9424–9442; primitive neuroectodermal tumors (PNET), codes 9362, 9470–9473, 9500; and all other histologies combined] were conducted.

Results

A slight majority of cases were male (55%), and the largest age group consisted of those < 5 years of age at diagnosis (38%; Table 1). A majority of cases were diagnosed with astroglial tumors (57%) and nearly equal

Table 1. Characteristics of all subjects with childhood brain tumors and their controls and characteristics of those with residential tap water tested for nitrate and nitrite.

| | All interviewed subjects | | | | Subjects with water tested[a] | | | |
| | Cases (n = 540) | | Controls (n = 791) | | Cases (n = 119) | | Controls (n = 191) | |
Characteristic	No.	%	No.	%	No.	%	No.	%
Age at diagnosis								
< 5 years	205	38.0	302	37.7	68	57.1	97	50.8
5–9 years	149	27.6	227	28.3	25	21.0	40	20.9
10–14 years	99	18.3	152	19.0	18	15.1	24	12.6
15–19 years	87	16.1	120	15.0	8	6.7	30	15.7
Ethnicity								
White	313	58.0	532	66.5	79	66.4	139	72.8
Black	42	7.8	41	5.1	6	5.0	10	5.2
Hispanic	147	27.2	183	22.9	29	24.4	35	18.3
Asian	29	5.4	29	3.6	3	2.5	6	3.1
Native American	5	0.9	6	0.8	0	0.0	1	0.5
Other	4	0.7	9	1.1	2	1.7	0	0.0
Male	298	55.2	448	55.9	65	54.6	105	55.0
Reference year								
1984–1988	361	66.9			79	66.4		
1989–1991	179	33.1			40	33.6		
Mother's education								
< 12 years	114	21.1	109	13.6	21	17.6	18	9.4
High school	75	13.9	111	13.9	17	14.3	30	15.7
Some college	252	46.7	365	45.6	50	42.0	80	41.9
College graduate	99	18.3	215	26.9	31	26.1	63	33.0
Study region								
Seattle	134	25.0	281	35.0	32	26.9	59	30.9
San Francisco	102	19.0	205	26.0	19	16.0	54	28.3
Los Angeles	304	45.0	315	39.0	68	57.1	78	40.8
Histologic category								
Astroglial	308	57.0	—	—	64	53.8	—	—
PNET	107	19.8			24	20.2		
Other	125	23.0			31	26.1		

[a]Tap water tested only if subject resided in same home she had lived in during index pregnancy.

proportions had PNET (20%) or other histologic tumor types (23%).

Similar proportions of mothers of cases (25%) and controls (27%) resided in the home they had lived in during their pregnancy with the case or control child. Subjects for whom dipstick measurements were obtained had generally similar characteristics as their respective larger case or control group with the exception that a greater proportion of subjects with water tests were from the youngest age group.

The majority of case and control mothers reported public water as the major source of residential water during their pregnancy with the index child and up through the child's first year of life. No increased risk of CBT was associated with use of any well water (reported by 9% of both cases and controls) relative to use of at least some public water (OR = 1.1; 95%CI, 0.8–1.7, adjusted for age, sex, and region; data not shown). The proportions of subjects reporting well water as the sole source of residential water varied by study region (Table 2). Overall, having well water as the exclusive source of residential water during pregnancy was not associated with an increased risk of CBT in offspring relative to use of public water (OR = 1.2; 95% CI, 0.8–2.2). Because well water content may have differed by region, we examined the association of reliance on well water during pregnancy and CBT occurrence separately for each study region. Exclusive use of well water was reported by 1% of cases and 4% of controls in Los Angeles (OR = 0.2; 95% CI, 0.1–0.8), by 1% of cases and 2% of controls in the San Francisco area (OR = 0.7; 95% CI, 0.1–6.6), and by 14% of cases and 6% of controls in the Seattle region (OR = 2.6; 95% CI, 1.3–5.2).

Because women with known residential water contamination may be likely to drink bottled water instead of tap water, these analyses were repeated after excluding subjects who reported they had used any bottled water during their pregnancies. Although there was no difference in the proportion of use of bottled water reported by cases and controls within a region, bottled water usage varied markedly by region, ranging from 37% of women in Los Angeles to 15% and 7% of women in San Francisco and Seattle, respectively. The risks of CBT associated with exclusive reliance on well water among women who did not use bottled water were 0.1 (95% CI, 0.01–0.9) in Los Angeles, 0.7 (95% CI, 0.1–6.5) in San Francisco, and 2.8 (95% CI, 1.3–5.9) in Seattle (data not shown).

Risk estimates for the Seattle area were recalculated after stratifying subjects by prenatal use of vitamins or consumption of cured meat products, two modifiable dietary factors previously demonstrated to be associated with decreased and increased risks of CBT (9), and by histologic type. The risk of CBT associated with well water as the sole source of household water, relative to public water, among subjects whose mothers had used prenatal vitamins was 2.2 (95% CI, 0.9–5.0); among those without prenatal vitamin use the risk was 9.8 (95% CI, 1.0–120; Table 3). Among subjects whose mothers reported consuming fewer than 5 servings of cured meat per week, the risk of CBT in their offspring associated with exclusive use of well water was 1.8 (95% CI, 0.7–4.6); among those with 5 or more servings per week, this risk was 6.9 (95% CI, 1.2–69). The elevated odds ratio in the Seattle area for CBT associated with exclusive well water use was not restricted to any single histologic category, although the number of subjects available within each category was limited. Because of the small number of subjects available for these stratum-specific analyses, crude risk estimates are presented. The small number of well users in Los Angeles and San Francisco precluded similar analyses in these regions.

Among the 310 subjects for whom dipstick measurements were obtained, nitrates were detected in a greater proportion of tap water samples from control (22%) than case (13%) residences (OR = 0.6; 95% CI, 0.3–1.1; Table 4). Although the semiquantitative measurement levels provided by the dipstick provided only an estimate of the true nitrate content, few households had tap water measurements of 50 mg/L or greater (with 45 mg/L nitrate ion equivalent to 10 mg/L nitrate-nitrogen, the maximum allowable in public drinking water in the United States), and the proportions of case (2%) and control (1%) residences within this category were similar. Nitrites were detected in samples from 11% of cases and 2% of controls (OR = 8.8; 95% CI, 2.1–46). Similar proportions of

Table 2. Risk of childhood brain tumor in offspring associated with source of residential water during pregnancy.

Residential water source[a]	Cases (n = 540)		Controls (n = 801)		OR	95% CI
	No.	%	No.	%		
All regions[b]						
Public water only	479	90.7	709	89.9	1.0	—
Well water only	21	4.0	30	3.8	1.2	0.8–2.2
Public + well water	23	4.4	38	4.8	1.1	0.6–1.8
Public + other	5	0.9	12	1.5	0.7	0.3–2.1
Los Angeles area[c]						
Public water only	286	96.3	289	92.9	1.0	—
Well water only	2	0.7	11	3.5	0.2	0.1–0.8
Public + well water	7	2.4	9	2.9	0.8	0.3–2.2
Public + other	2	0.7	2	0.6	1.0	0.1–7.6
San Francisco area[c]						
Public water only	93	93.9	191	94.6	1.0	—
Well water only	1	1.0	3	1.5	0.7	0.1–6.6
Public + well water	3	3.0	5	2.5	1.2	0.3–5.4
Public + other	2	2.0	3	1.5	1.4	0.2–8.4
Seattle area[c]						
Public water only	100	75.8	229	83.0	1.0	—
Well water only	18	13.6	16	5.8	2.6	1.3–5.2
Public + well water	13	9.8	24	8.7	1.3	0.6–2.6
Public + other	1	0.8	7	2.5	0.3	0.1–2.7

[a]Excludes three cases and six controls with water source unknown, and nine cases and six controls reporting other sources or mixtures of sources. [b]Adjusted for child's age, sex, and region. [c]Adjusted for child's age and sex.

Table 3. Risk of childhood brain tumor in offspring associated with exclusive use of well water during pregnancy among Seattle area subjects with selected characteristics.[a]

	Cases (n = 118)	Controls (n = 247)	OR[b]	95% CI
Dietary characteristic				
Prenatal vitamins				
None used	4/19	2/31	9.7	1.0–120
Used prenatal vitamins	14/108	14/216	2.2	0.9–5.0
Cured meats consumed				
< 5 servings/week	10/82	14/197	1.8	0.7–4.4
≥ 5 servings/week	8/36	2/50	6.9	1.2–69
Histologic category				
Astroglial tumors	9/66	11/122	1.6	0.6–4.4
PNET	3/23	1/59	8.7	0.6–464
Other	6/29	4/66	4.0	0.9–21

[a]Restricted to cases and controls reporting either well or public water as the sole source of residential water. [b]Risk associated with well water, relative to public water as the sole source of household water.

cases (24%) and controls (22%) had any measurable nitrates and/or nitrites detected in their tap water (OR = 1.1; 95% CI, 0.7–2.0). When women who reported use of bottled water were excluded from this analysis, risk of CBT associated with presence of nitrates/and or nitrites in residential water was 2.1 (95% CI, 0.98–4.4).

Among the 51 subjects who reported exclusive use of well water, dipstick measurements of tap water were conducted for only 14 households. Of these, 2/8 cases (25%) and 1/6 controls (17%) had measurable nitrates, a difference that was not statistically significant. None of these samples contained measurable nitrites.

We examined whether tap water with measurable nitrates or nitrites was observed more commonly among households using well water than those reporting public water as the sole source of residential water (Table 5). In the Seattle region, 3/13 specimens (23%) from homes reporting well water as the sole source of residential water and 2/70 specimens (3%) from homes reporting use of public water contained measurable nitrates. In San Francisco no nitrates were measured in the single specimen available from a residence with well water. None of the samples from Los Angeles were from homes with reported reliance on well water. However, 45/146 (31%) and 6/71 (8%) of samples from homes with public water in Los Angeles and San Francisco, respectively, contained measurable nitrates. Los Angeles and San Francisco were the only regions where nitrite was detected in tap water specimens.

Discussion

An increased risk of CBT was observed among children in western Washington whose mothers reported well water as the sole source of household water supply during their pregnancies. No increased risk was measured in the San Francisco area, and a decreased risk was observed in Los Angeles, although well water use was relatively uncommon in these regions. In a previous case–control study of CBT in children living in the United States and Canada, no increased risk of CBT associated with self-reported use of well water was found (10).

The possible association of drinking water nitrates with adult brain tumor occurrence has been examined more frequently than for childhood brain tumors. A German case–control study of adult brain tumors reported no association of nitrate exposure and tumor occurrence (4) based on an analysis of individual mean potential nitrate exposures estimated from residential drinking water sources, as provided by water treatment plants and health authorities in the study region. The mean nitrate levels for both cases and controls in that study were approximately 16 mg/L. Dipstick measurements of tap water nitrates were also obtained at the time of interview and were reported to be comparable between cases and controls. In apparent contrast, an ecologic study comparing cancer incidence in different areas of northern England reported an increased incidence of adult brain and central nervous system tumors, but not gastric or esophageal cancer, in areas with higher drinking water nitrate levels (mean of 29.8 mg/L) relative to areas with lower levels (mean of 2.4 mg/L) (20).

The lack of consistency in our findings concerning residential water source across the three study regions deserves comment. If our results represent associations with a specific substance present in well water, then it is unlikely that it would exert a different effect in Los Angeles than in Seattle. Well water content probably varies across the three regions of our study, suggesting the possibility that our observed association in the Seattle area reflects some substance present only in that region. Of the substances that might be present in well water for which a relationship with brain tumors has been hypothesized, nitrates and nitrites are reasonable candidates, as levels of these substances in well water reflect the land use practices of the surrounding area and thus are likely to vary by region. Results of our analyses based on dipstick measurements of these substances, however, do not strongly support this. It is also possible that household use of well water, as measured in this study, is a proxy for some other factor that may be related to brain tumor occurrence. For example, rural or farm residents more often rely on well water and may also have other exposures for which we have no

Table 4. Presence of measurable nitrates or nitrites by dipstick measurement of tap water in residences of children with brain tumor and their controls.

Nitrates/nitrites	Cases (n = 119) No.	%	Controls (n = 191) No.	%	OR	95% CI
Level of nitrates						
None detected	104	87.4	150	78.5	1.0	—
10 mg/L	9	7.6	30	15.7	0.4	0.1–1.0
25 mg/L	4	3.4	9	4.7	0.6	0.2–2.4
50[a]–100 mg/L	2	1.6	2	1.0	1.4	0.1–15
Any nitrates measured[b]	15	12.6	41	21.5	0.6	0.3–1.1
Level of nitrites						
None detected	106	89.1	188	98.4	1.0	—
1 mg/L	8	6.7	3	1.6	4.7	1.1–23
5 mg/L[a]	3	2.5	0	0	Undefined	
10 mg/L	2	1.7	0	0	Undefined	
Any nitrites measured[b]	13	10.9	3	1.6	8.8	2.1–46
Presence of measurable nitrates and/or nitrites						
All households measured[c]	28	23.5	42	22.0	1.1	0.7–2.0
Excluding bottled water users[c,d]	18	22.2	19	13.8	2.1	0.98–4.4

[a]Levels of these ions equivalent to public drinking water standards in the United States are 45 mg/L nitrate (10 mg/L nitrate-nitrogen) and 1.5 mg/L nitrite (1mg/L nitrite-nitrogen). [b]Adjusted for child's age. [c]Adjusted for child's age and sex. [d]Excluding 36 cases and 51 controls whose mothers reported use of any bottled water during pregnancy, and 2 cases and 2 controls with missing data.

Table 5. Number of household water samples within nitrate or nitrite category by reported residential water source and study region.

Nitrates/nitrites	Los Angeles[a] Public (n = 146) No. (%)	San Francisco Public (n = 71) No. (%)	Well (n = 1) No. (%)	Seattle Public (n = 70) No. (%)	Well (n = 13) No. (%)
Level of nitrates					
None detected	101 (69.2)	65 (91.5)	1 (100)	68 (97.1)	10 (76.9)
10 mg/L	31 (21.2)	5 (6.8)	0	1 (1.4)	2 (15.4)
25 mg/L	12 (8.2)	1 (1.5)	0	0	0
50 mg/L[b]	1 (0.7)	0	0	1 (1.4)	1 (7.7)
100 mg/L	1 (0.7)	0	0	0	0
Level of nitrites					
None detected	139 (95.2)	62 (87.3)	1 (100)	70 (100)	13 (100)
1 mg/L	7 (4.8)	4 (5.6)	0	0	0
5 mg/L[b]	0	3 (4.2)	0	0	0
10 mg/L	0	2 (2.8)	0	0	0
Any nitrates/nitrites measured	51 (34.9)	14 (19.7)	0	2 (2.9)	3 (23.1)

[a]All samples in Los Angeles were from homes reporting public water as the sole source of residential water. [b]Levels of these ions equivalent to public drinking water standards in the United States are 45 mg/L nitrate (10 mg/L nitrate-nitrogen) and 1.5 mg/L nitrite (1mg/L nitrite-nitrogen).

information. Our ability to examine how the relationship of well water and CBT occurrence may have been influenced by other factors for which we did have information was limited by small numbers. We did, however, attempt to evaluate whether the well water relationship varied by farm residence in Seattle: in this area, the risk of CBT associated with exclusive use of well water, relative to public water only, was elevated regardless of whether the subject lived on a farm (OR = 2.9; 95% CI, 0.3–29) or had no farm exposure during the first 6 months of life (OR = 2.2; 95% CI, 0.8–5.7). (Both estimates are unadjusted, with exact confidence intervals.)

Residential water supply is a potentially important source of dietary nitrates in areas where water nitrate levels are high. However, water represents only a single source of ingested dietary nitrate, and the proportion of total nitrate ingested from drinking water varies considerably by nitrate concentration. Water nitrate levels < 50 mg/L are responsible for < 30% of total ingested nitrate (*3*). Furthermore, many dietary factors known to inhibit nitrosation, including vitamins C and E, and substances contained in some fruits and vegetables (*16*) may reduce or negate the effects of drinking water nitrate among individuals with a diet rich in these items. It has been hypothesized that if increased risk of tumor exists, it may be evident only among population subgroups already compromised by low dietary intake of vitamin C and other antioxidant-containing fruits or vegetables (*17*). The fetal brain may have an enhanced susceptibility to tumorigenesis, perhaps due to the rapid division of neural cells and/or a decreased capacity to repair alkylation-induced DNA damage (*18,19*) that results from exposure to NNCs. Thus, prenatal dietary and drinking water effects might be of particular importance for childhood cancer. It is possible that infants born to women with diets deficient in these substances and who ingested high levels of drinking water nitrate while pregnant may have an even greater susceptibility. It is also possible that prenatal consumption of greater quantities of foods thought to contain NNCs (such as cured meats), in combination with high levels of drinking water nitrate may increase risk. Our ability to further investigate this potential interaction with modifiable dietary factors was limited; however, the directions of the risk estimates we observed for the well water–CBT associations are consistent with this scenario when the data are stratified by selected prenatal dietary factors.

We observed no increased risk of CBT associated with the presence of measurable nitrates in tap water; in fact, a greater proportion of controls than cases had nitrates detected in their water. Nitrites were detected infrequently; however, they were detected more often in water from case households than control households. Considerable variation existed in the level of nitrate detection by study region. In western Washington nitrates were measured more often in water from households using wells than those with public water, a comparison we were unable to conduct in other regions. The proportion of samples with detectable nitrates from homes relying on wells in western Washington, however, was less than that observed for samples from homes relying on public water in Los Angeles.

Our results based on dipstick measurements of nitrate or nitrite in tap water must be interpreted with caution for several reasons. First, we sampled household tap water only for a small portion of subjects. Second, our measurements were obtained after the *in utero* exposure window hypothesized to be relevant for the children under study and thus may provide only a crude measure of possible exposure. Levels of these substances are likely to vary temporally even within the same water supply source. Finally, we have only a crude measurement of the extent to which household tap water was ingested by subjects. We attempted to refine the analyses to address this last issue by excluding mothers who reported they had consumed any bottled water during their pregnancies.

The information we obtained on residential water source was available for nearly all subjects and was relevant to the *in utero* time period in which waterborne contaminants might exert a significant influence. Well water contamination by nitrates from waste and fertilizers is an increasing problem in many areas (*1,21*), and levels of nitrate-nitrogen exceeding 10 mg/L have been documented in many farming areas including parts of Washington State (*22,23*). Thus, our finding of an increased risk of brain tumor associated with well water exposure in this particular geographic area of our study is of interest. If drinking water nitrates are associated with an increased risk of cancer, some potential pathways have been suggested. Increased levels of HPRT variant frequencies (*24*) and sister chromatid/chromosome breaks (*25*) have been measured in the peripheral blood lymphocytes of subjects exposed to high levels of drinking water nitrate, suggesting some routes for genotoxic and cytogenetic effects. High levels of nitrate also are considered by some to indicate the presence of other contaminants such as pesticides or metals in the water (*22*), but we have no data on these factors.

Reliance on the mother's recollection of residential water source, often from many years previously, is a limitation. Even if the source of residential water is accurately reported, women may have consumed water from other sources outside the home for which we have no data. However, it is likely that those who used private wells recalled their source of household water fairly accurately given the routine maintenance activities that may be required. Although public water supplies may include water from wells, we allowed respondents to define "well water" for themselves. It is possible that only those who relied on private domestic wells were aware of wells as a source of their drinking water and thus well water use was reported, for the most part, by women using water from private wells. In general, nitrate levels in private wells are not monitored by public health agencies as frequently as in public water sources, and it is conceivable that nitrate levels in private wells are relatively high. A recent report by the U.S. Geological Survey (USGS) indicates that although only 1% of public water supplies contained excess nitrates, 9% of domestic water wells, and 21% of shallow wells beneath farm lands exceeded the nationally accepted standard of 10 mg/L (*23*). An earlier report from Iowa indicated that 18% of that state's private rural wells exceeded this nitrate level, while up to 35% of shallow wells of less than 15 m exceeded 10 mg/L (*26*). A recent report of data for areas of eastern Washington State indicated 8–26% of wells that were monitored (for those > 300 feet and < 300 feet deep, respectively) exceeded this standard for nitrate (*22*). Both the USGS report (*23*) and data from Washington (*22*) also demonstrated a trend of increasing levels of nitrate in wells from 1970 to 1992, particularly in agricultural areas. This increase is likely to have occurred as a result of a concomitant increase in the use of nitrate-containing fertilizer (*22,23*). Thus, an increasing proportion of domestic wells supplying households in the United States may exceed nationally accepted levels for these substances.

Although these results do not provide strong support of an association of drinking water nitrates with CBT occurrence, they do suggest the possibility that in at least one of the regions studied, some factor related to well water use may have an association. In view of the substantial proportion of the population exposed to well water in some areas, evidence of increasing levels of contamination by nitrates (and possibly other contaminants), and a plausible biological mechanism for NNC-induced damage to fetal brain resulting in CBT, it will be important to further clarify this relationship.

REFERENCES AND NOTES

1. Johnson CJ, Kross BC. Continuing importance of nitrate contamination of ground water and wells in rural areas. Am J Ind Med 18:449–456 (1990).
2. Moller H, Landt J, Jensen P, Pedersen E, Autrup H, Jensen OM. Nitrate exposure from drinking water and

diet in a Danish rural population. Int J Epidemiol 18(1):206–212 (1989).

3. Chlivers C, Inskip H, Caygill C, Bartholomew B, Fraser P, Hill M. A survey of dietary nitrate in well-water users. Int J Epideimol 13(3):324–331 (1984).

4. Steindorf K, Schlehofer B, Becher H, Hornig G, Wahrendorf J. Nitrate in drinking water. A case-control study of primary brain tumours with an embedded drinking water survey in Germany. Int J Epidemiol 23(3):451–457 (1994).

5. Harris CC. Biochemical and molecular effects of N-nitroso compounds in human cultured cells: an overview. IARC Sci Publ 84:20–25 (1987).

6. Leaf CD, Wishmnok JS, Tannenbaum SR. Mechanisms of endogenous nitrosation. Cancer Surv 8:323–334 (1989).

7. Correa P. A human model of gastric carcinogenesis. Cancer Res 48:3558–3560 (1988).

8. Tomatis L, Ponomarkov V, Turosov V. Effects of ethylnitrosourea administration during pregnancy on three subsequent generations of BDVI rate. Int J Cancer 19(2):240–248 (1977).

9. Preston-Martin S, Pogoda JM, Mueller BA, Holly EA, Lijinsky W, Davis RL. Maternal consumption of cured meats and vitamins in relation to pediatric brain tumors. Cancer Epidemiol Biom Prev 5:599–605 (1996).

10. Bunin GR, Kuijten RR, Boesel CP, Buckley JD, Meadows AT. Maternal diet and risk of astrocytic glioma in children: a report from the Childrens Cancer Group (United States and Canada). Cancer Causes Control 5:177–187 (1994).

11. Sarasua S, Savitz DA. Cured and broiled meat consumption in relation to childhood cancer. Denver, Colorado (United Sates). Cancer Causes Control 5:141–148 (1994).

12. Norman MA, Holly EA, Ahn DK, Preston-Martin S, Mueller BA, Bracci PM. Prenatal exposure to tobacco smoke and childhood brain tumors: results from the United States West Coast Childhood Brain Tumor Study. Cancer Epidemiol Biomark Prev 5:127–133 (1996).

13. Hartge P, Brinton LA, Rosenthal JF, Cahill JI, Hoover RN, Waksberg J. Random digit dialing in selecting a population-based control group. Am J Epidemiol 120:825–833 (1984).

14. Lele C, Holly EA, Roseman DS, Thomas DB. Comparison of control subjects recruited by random digit dialing and area survey. Am J Epidemiol 140(7):643–648 (1994).

15. Breslow NE, Day NE. Statistical Methods in Cancer Research. Volume I: The Analysis of Case-control Studies. IARC Sci Publ 32 (1980).

16. Steinmetz KA, Potter JD. A Review of vegetables, fruit and cancer. I. Epidemiology. Cancer Causes Control 2:325–357 (1991).

17. National Research Council. Nitrate and Nitrate in Drinking Water. Washington DC:National Academy Press, 1995.

18. Rice JM, Ward JM. Age dependence of susceptibility to carcinogenesis in the nervous system. Ann NY Acad Sci 381:274–289 (1982).

19. Krokan H, Haugen A, Myrnes B, Guddal PH. Repair of premutagenic DNA lesions in human fetal tissues: evidence for low levels of O6-methylguanine-DNA methyltransferase and uracil-DNA glycosylase activity in some tissues. Carcinogenesis 4(12):1559–1564 (1983).

20. Barrett JH, Parslow RC, McKinney PA, Law GR, Forman D. Nitrate in drinking water and the incidence of gastric, esophageal, and brain cancer in Yorkshire, England. Cancer Causes Control 9:153–159 (1998).

21. Macilwain C. U.S. report raises fears over nitrate levels in water [News]. Nature 377(6544):4 (1995).

22. Ryker SJ, Jones JL. Nitrate Concentrations in Ground Water of the Central Columbia Plateau. USGS Open File Report 95–445. Tacoma, WA:U.S. Geological Survey, 1998. Available: http://wa.water.usgs.gov/ccpt/pubs/ofr-95-445.html (cited 5 April 2001).

23. Mueller DK, Hamilton PA, Helsel DR, Hitt KJ, Ruddy BC. Nutrients in Ground Water and Surface Water of the United States – An Analysis of Data Through 1992. Water-Resources Investigations Report 95-4031. Denver, CO:U.S. Geological Survey, 1995.

24. van Maanen JMS, Welle IJ, Hageman G, Dallinga JW, Mertens PLJM, Kleinjans JC. Nitrate contamination of drinking water: relationship with HPRT variant frequency in lymphocyte DNA and urinary excretion of N-nitrosamines. Environ Health Perspect 104:522–528 (1996).

25. Txezou A, Kitsiou-Tzeli S, Galla A, Gourgiotis D, Papageorgiou J, Mitrou S, Molybdas PA, Sinaniotis C. High nitrate content in drinking water: cytogenetic effects in exposed children. Arch Environ Health 51(6):458–461 (1996).

26. Kross BC, Hallberg GR, Bruner DR, Cherryholmes K, Johnson JK. The nitrate contamination of private well water in Iowa. Am J Public Health 83:270–272 (1983).