Does calcium in drinking water modify the association between nitrate in drinking water and risk of death from colon cancer?

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ABSTRACT

The objective of this study was to explore whether calcium (Ca) levels in drinking water modified the effects of nitrate on colon cancer risk. A matched case–control study was used to investigate the relationship between the risk of death from colon cancer and exposure to nitrate in drinking water in Taiwan. All colon cancer deaths of Taiwan residents from 2003 through 2007 were obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health. Controls were deaths from other causes and were pair-matched to the cases by gender, year of birth and year of death. Information on the levels of nitrate-nitrogen (NO_3-N) and Ca in drinking water have been collected from Taiwan Water Supply Corporation (TWSC). The municipality of residence for cases and controls was assumed to be the source of the subject's NO_3-N and Ca exposure via drinking water. We observed evidence of an interaction between drinking water NO_3-N and Ca intake via drinking water. This is the first study to report effect modification by Ca intake from drinking water on the association between NO_3-N exposure and risk of colon cancer mortality.

Key words | calcium, colon cancer, drinking water, effect modification, nitrate

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INTRODUCTION

Nitrate in drinking water originates from numerous natural and man-made sources, including waste waters and agricultural and urban runoff. Nitrogen fertilizer is the largest contributor to anthropogenic nitrogen globally and has been implicated as an even more important source of drinking water nitrate in rural areas (Fields 2004). The US Environmental Protection Agency (EPA) has established a maximum contaminant level (MCL) in drinking water of 10 mg/L as nitrate-N to protect infants from developing methemoglobinemia (Ward *et al.* 2005). However, the effectiveness of this regulatory limit for preventing other health risks such as cancer has not been adequately studied (De Roos *et al.* 2003).

Nitrate may act as a procarcinogen, interacting with amines and amides in the stomach to form a variety of N-nitroso compounds (NOC) (nitrosation), most of which are potent animal carcinogens (Tricker & Preussmann 1991), after reduction of nitrate to nitrite in saliva (Walker 1990). Several studies support a direct relationship between nitrate intake and endogenous formation of NOC. High nitrate levels in drinking water have been associated with increased excreted N-nitrosoproline levels in urine (Moller *et al.* 1989; Mirvish *et al.* 1992). Nitrate administered via drinking water was shown to be directly correlated with concentration of total NOC in feces (Rowland *et al.* 1991). In addition, populations with high rates of esophageal,

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gastric and nasopharyngeal cancer excrete high levels of N-nitrosoproline (Lu *et al.* 1986; Kamiyama *et al.* 1987; Yi *et al.* 1993). These results demonstrate a contribution of drinking water nitrates in nitrosation and suggest that nitrate intake may be used as a surrogate for exposure to target tissues to NOC (De Roos *et al.* 2003).

NOC are potent animal carcinogens, inducing tumors at multiple organ sites including the colon (Bogovski & Bogovski 1981; Ward *et al.* 2005). NOC were shown to produce tumors in several animals species tested, and it is likely that humans are also affected (Ward *et al.* 2005). However, few epidemiologic studies have been conducted to address the association of nitrates in drinking water with cancer risk and most of these studies focused on gastric cancer with mixed results (Forman 1989; Cantor 1997; Yang *et al.* 1998; Gulis *et al.* 2002).

Our hypothesis is derived from animal experiments in which rats (Mirvish *et al.* 1987) and hamsters (Germann *et al.* 1991) had higher rates of intestinal tumors after administration of NOC either in drinking water or by injection. Given the biological plausibility for a role of NOC in risk of development of colon cancer and widespread exposure to nitrate in the population, there is a surprising deficit of epidemiologic data concerning the possible association of nitrates in drinking water with colon cancer. One ecologic study conducted in Slovakia found a positive association between drinking water nitrates and colon cancer rates (Gulis *et al.* 2002). Other ecologic studies reported no association with colon cancer (Geleperin *et al.* 1976; Jensen 1982; Morales Suarez-Varela *et al.* 1995). A prospective cohort study of Iowa women found that municipal drinking water nitrate levels were associated with an elevated risk of colon cancer that did not consistently increase with exposure (Weyer *et al.* 2001). De Roos *et al.* (2003) conducted a case–control study in Iowa. No association of colon cancer with measures of nitrate in public water supplies, including average nitrate and the number of years with elevated average nitrate levels, was observed. However, a positive association was observed in a subpopulation of high meat or low vitamin C consumers (De Roos *et al.* 2003). From a case–control study conducted in Wisconsin women, McElroy *et al.* (2008) reported that nitrate exposure from drinking water was not significantly associated with colorectal cancer risk overall. However, a 2.9-fold increased risk of proximal colon cancer was observed (McElroy *et al.* 2008).

We have previously reported a protective effect of Ca intake via drinking water against colon cancer (Yang *et al.* 1997a). However, none of the previous studies has explored whether Ca levels in drinking water might modify the association between NO_3-N exposure and health outcomes. If substantial effect modification by Ca levels in drinking water exists, the true magnitude of the association between NO_3-N exposure and colon cancer may be obscured. Furthermore, better knowledge of the modifying factors will help in public policy-making, risk assessment and standard setting.

The objective of this study was to explore whether Ca levels in drinking water modify the effects of NO_3-N on colon cancer risk.

MATERIALS AND METHODS

Study area

Taiwan is divided into 361 administrative districts, which will be referred to herein as municipalities. These are the units that will be subjected to statistical analysis. Excluded from the analysis were 30 aboriginal townships and 9 islets which had different life-styles and living environments (the diets of people in these municipalities are generally rich in fiber, antioxidants and nitrosation inhibitors, which may yield beneficial properties and act in a way against colon carcinogenesis). This elimination of unsuitable municipalities yielded 322 municipalities.

Socioeconomic factor

Each municipality in Taiwan was assigned to a degree-of-urbanization category from 1 to 8 based on the urban–rural classification of Tzeng & Wu (1986), which takes into account variables such as population density, age composition, economic activity and family income, educational level, environment and health service-related facilities. A municipality with the highest urbanization score, such as the Taipei metropolitan area, was classified in category 1, whereas mountainous areas with the lowest score were

assigned to category 8. The urbanization index used in this study serves as a proxy for a large number of explanatory variables such as socioeconomic status and differential exposures to environmental conditions, which are related to the etiology of mortality. For the analyses, the urban–rural classification was further divided into 4 levels: I, metropolitan (categories 1 and 2); II, city (categories 3 and 4); III, town (categories 5 and 6); and IV, rural (categories 7 and 8).

Subject selection

Data on all deaths of Taiwan residents from 2003 through 2007 were obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health which is in charge of the death registration system in Taiwan. For each death, detailed demographic information including gender, year of birth, year of death, cause of death, place of death (municipality) and residential district (municipality) were recorded on computer tapes. The case group consisted of all colon cancer deaths occurring in subjects between 50 and 69 years of age (International Classification of Disease, ninth revisions [ICD-9], code 153). In all, 3,707 colon cancer deaths with complete records satisfied this criterion.

The control group consisted of all other deaths excluding those deaths which were associated with gastrointestinal diseases. The deaths excluded were those caused by malignant neoplasms of stomach (code 151), malignant neoplasm of small intestine, including duodenum (code 152), malignant neoplasm of colon (code 153), malignant neoplasm of rectum, rectosigmoid junction and anus (code 154), gastric ulcer (code 531), duodenal ulcer (code 532), peptic ulcer, site unspecified (code 533), gastrojejunal ulcer (code 534), and gastrointestinal hemorrhage (code 578). Subjects who died from bladder (Morales Suarez-Varela *et al.* 1993; Weyer *et al.* 2001), lung (Hoffmann *et al.* 1994), esophagus (Yang 1980; Wu *et al.* 1993; Cantor 1997), liver (Mitacek *et al.* 2008), head and neck (Herity *et al.* 1981; Andre *et al.* 1995) cancers, and non-Hodgkin lymphoma (NHL) (Ward *et al.* 1996; Cantor 1997; Gulis *et al.* 2002) were also excluded from the control group because of previously reported associations with nitrate or NOC exposure. Control subjects were pair matched to the cases by gender, year of birth and year of death. Each matched control was selected randomly from the set of possible controls for each case. The most frequent causes of death amongst the controls were diabetes mellitus (12.3%), chronic liver disease and cirrhosis (7.2%), breast cancer (3.6%), acute myocardial infarction (3.6%) and motor vehicle traffic accidents of unspecified nature (3.5%).

Nitrate-nitrogen (NO_3-N) and Ca levels

Information on the levels of NO_3-N and Ca in each municipality's treated drinking water supply was obtained from the Taiwan Water Supply Corporation (TWSC) (TWSC/ROC 1991), to which each waterworks is required to submit drinking water quality data including the levels of nitrates and Ca. Four finished water samples, one for each season, were collected from each waterworks. The samples were analyzed by the waterworks laboratory office using standard methods (cadmium reduction method and spectrophotometric method, respectively). Since the laboratory office examines nitrate and Ca levels on a routine basis using standard methods, it was thought that analytical variability was minimal. Among the 322 municipalities, 70 were excluded as they had more than one supply of drinking water and the exact population served by each could not be determined. Their details are provided in earlier publications (Yang *et al.* 1998, 2000; Yang 1998). The final complete data comprised NO_3-N and Ca data from 252 municipalities. Ca remains reasonably constant for long periods of time and is quite a stable characteristic of a municipality's water supply (Bell & Doege 1984). Data collected were the annual mean levels of NO_3-N and Ca for the year 1990. The municipalities of residence for all cases and controls were identified from the death certificate and it was assumed that drinking water was the source of the subjects' nitrate and Ca exposure. The levels of NO_3-N and Ca of each municipality were used as an indicator of exposure to NO_3-N and Ca for an individual residing in that municipality.

Statistics

In the analysis, the subjects were categorized into one of the three NO_3-N exposure categories: low (the lowest 50th percentile among controls; <0.38 ppm); medium (50th–75th

percentile among controls; 0.39–0.57 ppm); and high (above the 75th percentile among controls; 0.60–2.86 ppm). Conditional logistic regression was used to estimate the association between NO_3-N levels present in drinking water and colon cancer risk. Odds ratio (OR) and their 95% confidence intervals (95% CI) were calculated using the low exposure group as the reference group (Breslow & Day 1980). The association between drinking water NO_3-N levels and risk of colon cancer was stratified by Ca levels in drinking water. The analyses were performed using the SAS software (version 8.2; SAS Institute, Inc., Cary, North Carolina). All statistical tests were two-sided and values of $p < 0.05$ were considered statistically significant.

RESULTS

A total of 3,707 colon cancer cases with complete records were collected for the period of 2003–2007. Of the 3,707 cases, 2,087 were males and 1,620 females. The majority of both cases (78.0%) and controls (74.1%) were married. Cases had a higher rate (45.0%) of living in metropolitan municipalities than controls (38.2%). Both cases and controls lived in municipalities in which more than 90% of the population were served by a waterworks. The mean NO_3-N concentration in the drinking water of the colon cancer cases was 0.45 mg/L (SD = 0.46). Controls had a mean NO_3-N exposure of 0.43 mg/L (SD = 0.47). Cases (45.8%) were less likely to have lived at a residence served by drinking water with high levels (≥34.7 mg/L) of Ca than the controls (49.5%) (Table 1).

Of the 252 studied municipalities, 64 (25.4%) had NO_3-N levels <0.12 mg/L (the lowest 25th percentile); 73 (29.0%) had NO_3-N levels of 0.12–0.38 mg/L (25th–50th percentile); 52 (20.6%) had NO_3-N levels of 0.39–0.60 mg/L (50th–75th percentile); and 63 (25.0%) had NO_3-N levels >0.60 mg/L (above 75th percentile). Table 2 shows the distribution of cases and controls and OR with respect to the levels of NO_3-N in drinking water. The crude OR was significantly higher than 1.0 for the group with the highest levels of nitrate in their drinking water (OR = 1.22, 95% CI = 1.01–1.36). Adjustments for possible confounders only slightly altered the ORs. The adjusted ORs (95% CI) were 1.02 (0.90–1.15) for the group with water nitrate levels between 0.39 and 0.57 mg/L and 1.16 (1.04–1.30) for the group with nitrate levels of 0.60 mg/L or more. There was a significant trend towards an elevated risk of death from colon cancer with increasing nitrate levels in drinking water (X^2 for trend = 13.26, $p = 0.001$).

Of the 252 studied municipalities, 67 (26.6%) had Ca levels ≤24.4 mg/L (the lowest 25th percentile); 60 (23.8%) had Ca levels of 25.1–34.9 mg/L (25th–50th percentile); 62 (24.6%) had Ca levels of 35.0–49.8 mg/L (50th–75th percentile); and 63 (25%) had Ca levels >50.0 mg/L (above 75th percentile). The association between NO_3-N levels in drinking water and colon cancer risk among those with

Table 1 | Characteristics of the study population

Characteristics	Cancer cases ($n = 3,707$)	Controls ($n = 3,707$)
Enrollment municipality	252	252
Gender		
Male	2,087 (56.3%)	2,087 (56.3%)
Female	1,620 (43.7%)	1,620 (43.7%)
Age (years)		
50–54	736 (19.9%)	736 (19.9%)
55–59	732 (19.7%)	732 (19.7%)
60–64	913 (24.6%)	913 (24.6%)
65–69	1,326 (35.8%)	1,326 (35.8%)
Marital status		
Single	177 (4.7%)	214 (5.8%)
Married	2,927 (78.0%)	2,747 (74.1%)
Ever married	603 (16.3%)	746 (20.1%)
Urbanization level of residence (%)[a]		
Metropolitan	1,669 (45.0%)	1,417 (38.2%)
City	808 (21.8%)	802 (21.7%)
Town	834 (22.5%)	931 (25.1%)
Rural	396 (10.7%)	557 (15.0%)
NO_3-N level (mg/L)		
Mean ± SD[b]	0.45 ± 0.46	0.43 ± 0.47
<Median (0.38)	1,921 (51.8%)	2,027 (54.7%)
≥Median	1,786 (48.2%)	1,680 (45.3%)
Ca level (mg/L)		
Mean ± SD[b]	33.31 ± 19.38	34.48 ± 19.53
<Median (34.6)	2,009 (54.2%)	1,872 (50.5%)
≥Median	1,698 (45.8%)	1,835 (49.5%)

[a]The urbanization level of each municipality was based on the urban–rural classification scheme of Tzeng & Wu (1986).
[b]Standard deviation.

Table 2 | Odds ratios (OR) and 95% confidence intervals (CI) for colon cancer death in relation to nitrate levels in drinking water, 2003–2007

	Nitrate, mg/L (median)		
	<0.38 (0.05)	0.39–0.57 (0.44)	≥0.60 (1.00)
No. of cases	1,921	730	1,056
No. of controls	2,052	732	923
Crude OR[a]	1.0	1.07 (0.94–1.20)	1.22 (1.01–1.36)
Adjusted OR[b]	1.0	1.02 (0.90–1.15)	1.16 (1.04–1.30)
		X^2 for trend = 13.26, $p = 0.001$	

[a]Odds ratio adjusted for age and gender.
[b]Adjusted for age, gender, marital status, urbanization level of residence.

high (≥median) and low (<median) Ca intake via drinking water is shown in Table 3. There was a suggestion of interaction between drinking water nitrate and Ca intake, in that individuals with the highest NO_3-N exposure and low Ca intake from drinking water had a 1.37-fold increased risk of colon cancer (OR = 1.37; 95% CI = 1.11–1.69), whereas those with similar nitrate exposure whose drinking water Ca intake was above the median had no statistically significant increased risk (OR = 1.11, 95% CI = 0.96–1.29).

DISCUSSION

This study used a death certificate-based case–control study to examine whether Ca levels in drinking water modified the effects of NO_3-N in drinking water on risk of colon cancer mortality. We found that the risk of colon cancer mortality associated with high NO_3-N levels in drinking water was elevated among those with low Ca intake from drinking water.

Our findings suggest that it might be important to consider the levels of Ca in drinking water in the evaluation of the relationship between NO_3-N exposure and risk of colon cancer mortality. To our knowledge, this is the first study to report an effect modification by Ca intake from drinking water in the association between NO_3-N exposure and risk of colon cancer mortality. Antioxidants that inhibit endogenous nitrosation include vitamin C and alpha-tocopherol, which can reduce nitrite to NO (Bartsch *et al.* 1988). No experimental study has examined the modulating effect of Ca on colon carcinogenesis specifically induced by nitrate. Nonetheless, our results suggest that Ca may act in a similar way to vitamin C and alpha-tocopherol, which inhibited endogenous nitrosation caused by intake of nitrate from drinking water, and therefore individuals who had low levels of Ca intake via drinking water may be at increased risk of exposure to NOC and colon cancer mortality.

Despite their inherent limitations (Morgenstern 1982), studies of the ecological correlation between mortality and environmental exposures have been used widely to generate or discredit epidemiological hypotheses. Before any conclusion based on such a mortality analysis is made, the completeness and accuracy of the death registration system need to be evaluated. Since it is mandatory to register death certificates at local household registration offices, the death registration in Taiwan is complete. Although causes of death may be misdiagnosed and/or misclassified, the problem has been minimized through the improvement in the verification and classification of causes of death in Taiwan since 1972. Furthermore, malignant neoplasms, including colon cancer, were reported to be one of the most unequivocally classified causes of death in Taiwan (Chen & Wang 1990). Because of a fatal outcome, it is believed that all colon cancer cases from high or low levels of NO_3-N and Ca exposure in drinking water had

Table 3 | Odds ratios for colon cancer by levels of nitrate and Ca in drinking water

	NO_3-N level (median) (mg/L)								
	≤0.38 (0.05)			0.39–0.57 (0.44)			≥0.60 (1.00)		
Calcium (mg/L)	Cases	Controls	OR[a] (95% CI)	Cases	Controls	OR[a] (95% CI)	Cases	Controls	OR[a] (95% CI)
≥34.6	744	844	1.00	192	234	0.89 (0.72–1.11)	791	723	1.11 (0.96–1.29)
<34.6	1,177	1,208	1.02 (0.89–1.16)	538	498	1.09 (0.93–1.28)	265	200	1.37[b] (1.11–1.69)

[a]Adjusted for age, gender, marital status, urbanization level of residence.
[b]p value for interaction on the multiplicative scale <0.05.

access to medical care regardless of geographical location in recent years.

Of greater concern is whether the relative levels of nitrate in the period around 1990 correspond to the relative levels occurring in periods 20–30 years earlier. This is important since it is likely that exposure to causal factors would precede cancer mortality (temporality). Nitrate contamination in public water supplies in Taiwan was due principally to the use of nitrogen fertilizers in areas of arable farming (Yang *et al.* 1997b). The historical levels of nitrates are not available for the study areas. However, it is assumed that the correlation between the levels of 1990 and levels in the past 20–30 years would be high since a municipality's urban development is gradual (the agricultural areas decreased gradually). Therefore we feel that the nitrate levels in 1990 were a reasonable indicator of historical levels occurring over the past 20–30 years.

Migration from a municipality of high nitrate and Ca exposure to one of low nitrate and Ca exposure or vice versa may have introduced misclassification bias and bias in OR estimates (Gladen & Rogan 1979; Polissar 1980). The individuals included in the present study were subjects whose residence and place of death were in the same municipality. In the event of a death in Taiwan, there is a social custom that the decedent's family always considers the death to have occurred in the municipality where the person was born. Therefore, the decedent's residence, place of birth and place of death are likely to be listed as the same municipality, although the place of birth information was not available for this data set. We believe that this ameliorates the migration problem (Yang 1998). In addition, mobility is age dependent, and diseases usually occur with a higher incidence amongst older groups and proximate to the location of the environmental 'cause' (Polissar 1980). However, neighboring water sources tend to possess similar chemical composition (Flaten 1991), and hence even if an individual moved, the change in exposure to nitrate and Ca in drinking water would probably not be significant provided that the new residential municipality is near their old residential municipality (or moving within the same municipality). Also Taiwan's population is rather stable in terms of mobility compared with populations in most Western industrialized countries (Yu *et al.* 2006). It was reported that more than 90% of rural residents lived in the municipality in which they were born for their entire life (Wu *et al.* 1989). Further, urbanization levels were included as a control variable in the analysis. Since it is conceivable that municipalities with similar urbanization levels may have similar migration rates, this probably minimized the migration problem in our study. Furthermore, any misclassification of exposure is most likely to be nondifferential, which would reduce the estimated magnitude of association rather than introduce a positive bias in the estimation.

Since the measure of effect in this study is mortality rather than incidence, migration during the interval between colon cancer diagnosis and death must also be considered. During this period, cancer diagnosis may influence a decision to migrate and may have possibly introduced bias. Data are not available for the differences in survival rates of colon cancer patients between high and low nitrate (or Ca) exposure areas. If there is a trend toward migration to more urban areas or lower nitrate (Ca) exposure areas because of proximity to medical care (and thus a better survival rate for colon cancer), for example, a spurious association between nitrate (Ca) exposure and colon cancer death would have been noted. Two aspects of this study presumably minimized this possibility. First, migration due to colon cancer diagnosis would be unlikely, since for this cohort of decedents the subject's occupational status would weigh against a move requiring a job change late in life. Second, the study subjects in the present study were between the ages of 50 and 69, and it is generally assumed that the elderly are likely to remain in the same residence during the last 20 years of their life (Rademacher *et al.* 1992).

Intake of nitrate from drinking water and dietary sources may result in increased exposure to NOC through endogenous nitrosation (Moller *et al.* 1989; Mirvish *et al.* 1992). The principal dietary nitrate sources are vegetables. Vegetables also contain vitamin C and other nitrosation inhibitors (Bartsch *et al.* 1988), and therefore, high intakes may not result in high rates of formation of NOC (Coss *et al.* 2004). Dietary intakes of red and processed meat are of particular importance in the formation of fecal NOC (Bingham 1999; Bingham *et al.* 2002). There is unfortunately no information available for assessing the dietary nitrate sources from vegetables and meat for individual subjects in this study. However, there is no reason to believe that there would be any correlation between the sources of

dietary nitrate and the levels of nitrate in drinking water. Furthermore, Chilvers *et al*. (1984) indicated that when the concentration of waterborne nitrate is high, drinking water contributes substantially to total nitrate intake and the potential for nitrite and NOC formation may be increased. It is thus proposed that individuals with higher daily nitrate intake from drinking water and lower intakes of nitrosation inhibitors may be at an elevated risk of colon cancer.

Dietary Ca is the main source of Ca intake. The principal dietary Ca sources are dairy products, dried small fish, chicken eggs, soybean curd and soybean sauce (Pan *et al*. 1992). There is unfortunately no information available for assessing the dietary Ca sources for individual subjects in this study. However, there is no reason to believe that there would be any correlation between the sources of dietary Ca and the levels of Ca in drinking water. In Taiwan, the mean daily intake of dietary Ca is only 81.9% of the recommended daily intake (Lee *et al*. 1991). One may hypothesize that waterborne Ca can make an important contribution to the total daily intake for subjects with insufficient Ca intake (Yang *et al*. 1997a). It is thus proposed that individuals with higher daily nitrate intake from drinking water and lower intakes of Ca from drinking water may be at increased risk of colon cancer mortality.

Screening has been shown to be effective in reducing the incidence of, and mortality from, colorectal cancer (Pignone *et al*. 2002). Unfortunately, there is no information available on the prevalence of screening utilization for individual study municipalities and therefore it could not be adjusted for directly in the analysis. However, there is no reason to believe that there would be any correlations between the habits of undergoing colon cancer screening and nitrate levels in drinking water. We therefore think that the degree to which not controlling for the screening prevalence may have affected our results is not significant.

Some potential limitations of this study need to be noted. The information on nitrate or Ca concentration of drinking water was not obtained individually from the subjects, but estimated from their concentrations in the public drinking water supply of their residential municipality. The lifetime residential history and the identification of the primary drinking water source (municipal water supply or private well water) of each subject are also not available even though both cases and controls lived in municipalities in which more than 90% of the population were served by a waterworks (92.94 and 91.32% for cases and controls, respectively). Our study may thus have been limited by potential exposure misclassification. While these sources of misclassification are important, such misclassification of exposure is most likely to be nondifferential (i.e. unlikely to be associated with colon cancer), which would reduce the magnitude of association rather than introduce a positive bias in the association. Furthermore, our results might have been confounded by the fact that no information on other potential risk factors such as physical activity and meat consumption was available (Slattery 2004; Chao *et al*. 2005). However, there is no reason to believe that there would be any correlation between these risk factors and the levels of nitrates and Ca in drinking water.

The nitrate concentration in drinking water in Taiwan is below the guideline value recommended by the World Health Organization (1984) of 10 mg/L. This guideline was not based on estimates of cancer risk. In addition, there is no scientific evidence to justify firm conclusions about the safety of any concentration of nitrate in water with regard to cancer risk. Forman (1989) noted that although environmental nitrate exposure probably plays a role in the development of cancer, it does not show a rate-limiting effect.

In summary, our data suggest that Ca in drinking water modified the effects of nitrate exposure on risk of colon cancer mortality. Future studies should increase the precision of the estimation of the individual's intake of nitrates and Ca, through both food and water, and control for confounding factors, especially personal risk factors such as physical activity and meat consumption.

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