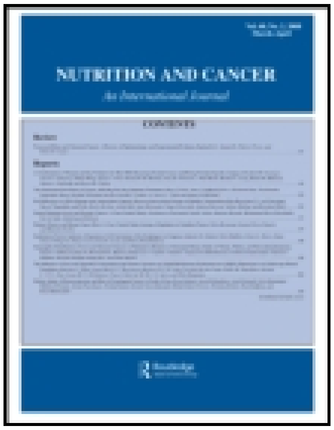

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Geographic Distribution of Liver and Stomach Cancers in Thailand in Relation to Estimated Dietary Intake of Nitrate, Nitrite, and Nitrosodimethylamine

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It is our working hypothesis that the high rate of the liver and gastric cancers in North and Northeast Thailand is associated with increased daily dietary intake of nitrate, nitrite, and nitrosodimethylamine (NDMA). Samples of fresh and preserved Thai foods were systematically collected and analyzed from 1988 to 1996 and from 1998 to 2005. Consumption frequencies of various food items were determined on the basis of a dietary questionnaire given to 467 adults (212 males and 255 females) from 1998 to 2005. Food consumption data for the preceding and current year were collected and intakes (day, week, and month) of nitrate, nitrite, and NDMA were calculated. The trends in liver and stomach cancer age-standardized incidence rates (ASR) in four regions of

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Thailand were compared with the dietary intake of nitrate, nitrite, and NDMA in those same geographic regions. Mean daily intakes of nitrate of 155.7 mg/kg, of nitrite of 7.1 mg/kg, and of NDMA of 1.08 μg/kg per day were found. Significant differences in dietary nitrate, nitrite, and NDMA intakes were seen between various Thai regions ($P < 0.0001$), and these corresponded to the variations in liver and stomach cancer ASR values between the regions. Dietary factors are likely to play key roles in different stages of liver and stomach carcinogenesis in Thailand.

INTRODUCTION

Liver and gastric cancers are the most common malignancies in the world. In Thailand, primary liver cancer, which comprises both hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA), accounts for 16.3% of all new cancers in males, making it the leading cancer in males, and 5.5% in females, making it the third leading cancer in females (1,2,3). Geographically, liver cancer incidence is highest in the Northeast for both genders (ASR male = 88.0, female = 35.4) and lowest in the South for both genders (ASR male = 6.6, female = 1.5). (Table 1) Stomach cancer is the sixth most common malignancy in Thai males and the ninth most common in Thai females, with estimated ASR incidence rates of 4.1 for men and 2.8 for women. There is a 2- to 3-fold regional variation, with highest rates in the North (Chiang Mai and Lampang combined) for both sexes, with ASR of 5.4 (6.45 males and 4.35 females) and lowest rates in the South (Table 2). The overall male:female incidence ratio varies from 1.68 in the Northeast to 1.48 in the North to 1.05 in the South. The comparison of time trends of ASR for the hepatic and gastric cancers indicate geographic variation in the country and also variation in males and females.

The etiological factors that have been associated with these cancers pertain to lifestyle and dietary habits. Risk factors for liver cancer include hepatitis B virus infection, liver fluke Opisthorchis viverrini (OV), aflatoxins, alcohol, smoking, and dietary exogenous and endogenous nitroso compounds (1). Risk factors for stomach cancer are dietary components including lower consumption of fresh vegetables and fruits; high intake of salt in preserved food; and high intake of nitrate, nitrite, and nitrosamines and Helicobacter pylori (HP) infection (2).

Because N-nitroso compounds, either exogenous or endogenous, have been mentioned as possible environmental carcinogens and are known to induce tumors in the liver and stomach of experimental animals (3–5), we have analyzed nitrosodimethylamine (NDMA) and its precursors, nitrate and nitrite, in representative and frequently consumed preserved and cooked foods collected from all 4 regions in Thailand. Of particular interest were high-risk liver and stomach cancer regions. We also obtained dietary information from populations of 4 regions of Thailand to investigate the potential association between liver and stomach cancers and nitrate, nitrite, and NDMA consumption.

This study is the first to determine and to report on the mean daily dietary intake of these substances in Thailand. The semiquantitative information gathered was used to calculate crude estimates of the total dietary nitrate, nitrite, and exogenous NDMA intake of the Thai population as a whole and regionally. The regional variation in the daily intake of nitrate, nitrite, and NDMA is discussed including the effect of tobacco use and possible effect of vitamins as modifying factors.

MATERIAL AND METHODS

Food Samples

Samples of fresh food and foodstuffs, including preserved, dried, salted and fermented samples, were collected from the homes of local residents and at local markets in various villages in the North, Northeast, Central, and South regions of Thailand. The diet samples were collected in covered stainless steel containers and kept on ice during transit. The systematic collection of samples took place from February 1988 to January 1996 and from April 1998 to December 2005. Some samples were analyzed in Thailand at the Mahidol, Chiang Mai, and Mahasarakham Universities. Most of the samples were analyzed at the former American Health Foundation in New York. Some NDMA data on food samples were provided by the

TABLE 1
Age-standardized incidence rate of liver cancer in Thailand, time trends from 1988–1997[a]

	Male			Female		
Region	1988–1991 ASR	1992–1994 ASR	1995–1997 ASR	1988–1991 ASR	1992–1994 ASR	1995–1997ASR
North[b]	49.8	21.3	23.55	37.4	11.2	11.55
Northeast	94.8	87.5	88.0	39.4	37.2	35.4
Central	9.7	11.0	14.4	2.7	3.4	3.9
South	10.7	6.4	6.6	1.9	1.4	1.5

[a] Abbreviation is as follows: ASR, age-standardized incidence rates.
[b] Average of ASR incidence in Chiang Mai and Lampang, rate per 100,000. Data from Refs. 1, 2, and 3.

TABLE 2
Age-standardized incidence rate (ASR) of stomach cancer in Thailand, Time trends from 1988–1997

	Male			Female		
Region	1988–1991 ASR	1992–1994 ASR	1995–1997 ASR	1988–1991 ASR	1992–1994 ASR	1995—1997ASR
North[a]	10.7	7.7	6.45	6.1	4.9	4.35
Northeast	5.2	3.2	3.2	2.3	1.9	1.9
Central	4.2	4.8	4.9	2.2	2.9	3.7
South	3.2	2.2	1.9	1.0	1.3	1.4

[a] Average of ASR incidence in Chiang Mai and Lampang, rate per 100,000. Data from Refs. 1, 2, and 3.

International Agency for Research on Cancer (IARC) toxicology laboratory in Lyon, France. All analyses were done with a view of consumption frequency and with a consideration of a method used.

Analysis of Nitrate and Nitrite

Nitrate concentrations were determined in food samples (3–7 g) by a standardized colorimetric method using diazotization with sulfanilamide and coupling with N-(1-naphthyl)ethylenediamine to form a colored azo-dye, which was measured by ultraviolet spectroscopy at 540 nm. Nitrate was measured after its reduction to nitrite in a column containing granulated cadmium copper. The set-up was either manual or semiautomated (5,6).

Analysis of NDMA

The methods used here were described in detail previously (5). In brief, about 10 g of food sample (if solid) was minced into small pieces and accurately weighed and homogenized in a mixing blender. 50 ml phosphate-citric acid buffer at pH 4.5 was then added, followed by 175 mg ascorbic acid and 1 ml of a 5 ppm solution of N-nitrosodipropylamine as an internal standard. The blended mixture was transferred to a 250-ml flask to which 150 ml buffer and 700 mg ascorbic acid were then added. The mixture was stirred for 2 h and then filtered through Celite 545. The aqueous filtrate was extracted 4 times with 300 ml dichloromethane (DCM). The pooled DCM extracts were dried over anhydrous sodium sulfate, concentrated to 3 to 5 ml, chromatographed on basic alumina with 200 ml DCM, and carefully concentrated to 0.5 to 1 ml prior to gas chromatographic (GC) analysis. Under these conditions, recovery of volatile nitrosamines was between 60–90% depending on the compound and the type of food samples analyzed. A Hewlett-Packard Model 5890 GC coupled to a model 610 Thermal Energy Analyzer (TEA, Thermo Electron, Waltham, MA) was used. Analyses were carried out on a 2 mm I.D. × 12 ft by 1/4 inch (o.d.) glass column packed with 10% carbowax 20 M on Chromosorb W-HP (80/100 mesh). The flow rate of the carrier gas (helium) was 35 ml/min. The temperatures of the injection port and column oven were 225°C and 175°C, respectively.

Study Population and Methods

From 1998 to 2005, a dietary survey was conducted in Thailand. Random samples of the population, aged 19 to 60+ yr, in selected rural and semiurban and urban communities, were recruited and were offered a small incentive to participate. In total, 572 males and females agreed to participate (out of 920 people who were approached), all of whom were interviewed about their lifestyle and dietary habits. Incomplete responses to several questions (specifically food amount assessment per day, per week, and per month) reduced the total study population to 467, 212 males and 255 females. The extensive questionnaire also included personal data, living conditions (type of home, presence of indoor plumbing, presence of indoor cooking facilities, food storage, refrigeration, if any, etc.), social status, education, present occupation or profession, medical history with a focus on digestive disorders, smoking history, and family history of illnesses.

The questionnaire was pilot tested for content and clarity by 10 individuals as representatives of recruited participants and not included in this study. They completed the questionnaire for reliability for food consumption frequency and participated in a focus group. Their comments were used to make slight changes. The validity and reliability of this questionnaire was assessed by comparing results of administrations of this instrument, 3 wk apart, each time to 10 different residents.

Dietary Questionnaire

Ninety-seven different types and varieties of foods, all of which were chosen for their high concentration of nitrate, nitrite, and NDMA and because they were usually consumed quite frequently, were included in the dietary portion of the interview. Food amounts were assessed per day, per week, or per month. Real food samples, frequently a duplicate portion method, were used in the estimation of food amounts. For the cooked dishes, the components were classified using a recipe as explained by a family member or by cooks from the local eateries. The calculations of the daily dietary intake of nitrate, nitrite and NDMA utilized the results from previous analytical studies of food composition in specific Thai communities (5,7,8–10); the calculations of NDMA also utilized information published by IARC (9). Nitrate and nitrite in drinking water were not included in our calculations of dietary intake because drinking water analyses were not done systematically throughout all 4 regions of Thailand. The questions regarding the consumption of beer and alcoholic beverages were included in the questionnaire. A vast majority refused to answer these questions with exception to those pertaining to beer. Therefore, the responses on ethanol consumption were not available for analysis. The consumption of the beer, due to its content of nitrate, nitrite, and NDMA, was included in this study. Although we tested the presence of several different N-nitroso compounds (5,7), we decided to estimate only the dietary intake of NDMA.

Statistical Methods

The analysis of variance (ANOVA) model was used in the comparison of nitrate, nitrite, and NDMA levels between the various major food groups and in the comparison of intake of these compounds in males and females, among people of different age groups, working in different occupations, living in different geographical areas, and among current smokers, ex-smokers, and nonsmokers. This analysis was followed by the Bonferroni multiple comparison procedure to compute the confidence interval estimates of the differences in the pairs of means (11).

RESULTS

The 5 major categories of foodstuffs with their values of nitrate, nitrite, and NDMA were summarized (Table 3). ANOVA method of testing equality of means between the categories of food groups, namely, preserved food, preserved vegetables, fresh vegetables, fruit, cooked food, and beer, were performed for nitrates and nitrites. For equality of nitrate means the ANOVA test yielded the test statistics and P value as $F(5, 893) = 1180.66$, $P < 0.0001$, indicating that the mean for the nitrate concentrations are different for the 6 categories of food groups. For nitrate, the mean concentration of fresh vegetables was significantly greater than the mean in preserved vegetables; in cooked food, it was significantly greater than in preserved food; and in fresh vegetables, it was significantly greater than in fruit. For nitrite means, the ANOVA test yielded the test statistics and P value as $F(5, 893) = 77.94$, $P < 0.0001$, indicating again that the mean for the nitrite concentrations were also different for the food groups. For nitrite, the mean concentration in preserved vegetables was significantly greater than in fresh vegetables; in preserved food, it was significantly greater than in cooked food; and in fresh vegetables, it was significantly greater than in fruit. ANOVA method was used for equality of mean testing for NDMA among the different subcategories of preserved food, namely, pork and beef, fermented fish, salted and dried fish, and preserved vegetables. The ANOVA test yielded the test statistics and P value as $F(3, 457) = 488.82$, $P < 0.0001$, indicating again that the mean for NDMA concentrations were also different for the food groups. The mean concentration of NDMA in fermented fish food was significantly greater than in pork and beef; in salted and dried fish food, it was significantly greater than in pork and beef; and in fermented fish food, it was significantly greater than in salted and dried fish. The nitrate content in preserved meat was high (Table 3). The various fermented fish from the 4 regions of Thailand contained nitrate concentrations ranging from 16 to 118 mg/kg. The nitrate content of salt-preserved foodstuffs were found to be high and varied. A high of 6,322 mg/kg nitrate was found in some samples of salted dried beef, whereas most of the preserved meats had mean concentrations of 169 ± 50 mg/kg of nitrate (Table 3).

The contribution of different foods to daily intake of nitrate, nitrite, and NDMA was also determined (Table 4). Preserved and fresh vegetables contributed 82% of the total nitrate (Table 4). The most important sources were fermented beans ("Tau-chiau"), beetroot, potato, cabbage, spinach, tomato, cucumber, celery, cauliflower, lettuce, and other vegetables. Meat cured products (including sausage and bacon) contributed 15% to the dietary nitrate intake (Table 4). Meat and fish products

TABLE 3
Nitrate, nitrite and nitrosodimethylamine (NDMA) in Thai food from the 4 regions of the country[a]

Food	Type	No. of Samples	Nitrate mg/kg	Nitrite mg/kg	NDMA μg/kg
Preserved food	15(13×)	195	169 ± 50	10.7 ± 3.9	2.1 ± 0.52
Pork and beef [b]					2.7 ± 0.65
Fermented fish					7.9 ± 3.20
Salted and dried fish					6.5 ± 3.10
Preserved vegetables	8(5×)	40	$1{,}184 \pm 104$	6.8 ± 2.6	8.4 ± 4.2
Fresh vegetables	72(4×)	288	$1{,}245 \pm 345$	6.0 ± 3.2	ND
Fruit	10(3×)	30	36 ± 16	0.4 ± 0.3	ND
Cooked food (on plate)	57(5×)	285	220 ± 52	6.1 ± 4.8	ND
Beer	2(30×)	60	55 ± 12.1	2.6 ± 0.9	0.20 ± 0.1

[a]Abbreviation is as follow: NDMA, nitrosodimethylamine. Mean concentrations ± SD in mg/kg for nitrate and nitrite, and in μg/kg for NDMA.

[b]Includes sausages, bacon, and cured meat only. ND = not detected (below detection limit).

TABLE 4
Contribution of different foods to the average daily intake, for both sexes, of nitrate, nitrite, and nitrosodimethylamine (NDMA) in Thailand 1998–2005

	Nitrate		Nitrite		NDMA	
Source	mg	%	mg	%	μg	%
Vegetables: Fresh, preserved and fermented	127.7	82	0.41	5.8	0.242	25
Meat and fish products: Cured meat, fermented fish and meat, salted dried meat and fish	23.3	15	6.69	94.2	0.638	69.6
Fruits	3.7	2.3	ND		ND	
Others	1.0	0.7	ND		0.200	5.4
Total	155.7	100.0	7.1	100.0	1.08	100.0

contributed about 94% of the total intake of dietary nitrite (Table 4), and these included cured meat, salted and dried fish, and squid. The intake from fermented vegetables was about 5.8%.

Table 5 shows the regional variation of the estimated daily intake of nitrate from selected foods. The ANOVA method of testing equality of means between the 4 different geographical regions—North, Northeast, Central, and South in Thailand—were performed for nitrates and nitrites. For equality of nitrate means, the ANOVA test yielded the test statistics and P value as $F(3, 464) = 5798.9007$, $P < 0.0001$, indicating that the mean for the nitrate concentrations were different in the 4 geographical regions. For nitrite means, the ANOVA test yielded the test statistics and P value as $F(3, 464) = 6406.6968$, $P < 0.0001$ and for NDMA P value as $F(3,464) = 8333.5085$, $P < 0.0001$, indicating again that the mean for the nitrite and NDMA concentrations were also different. For equality between the different age groups in Thailand, the nitrate, nitrite, and NDMA means the ANOVA test yielded the test statistics as $F(2, 465) = 1030.4161$, $F(2, 465) = 28135863.44$, and $F(2, 465) = 1245838814$, respectively, all giving $P < 0.0001$, indicating that the mean for nitrate, nitrite, and NDMA concentrations were different in the 3 age groups. In general, nitrate intake from food was highest in the North and Northeast regions and lowest in the South region. The differences in nitrate intake from food are mainly due to an increased consumption of preserved vegetables and preserved meats in the North and Northeast regions relative to the South region. The consumption of high nitrate, low ascorbic acid vegetables was also significantly greater in the North and Northeast regions than in the South region. The mean daily nitrate intake in male diets was 155.7 mg/kg and in female diets was 154.2 mg/kg (Table 5). However, the group of younger adults had a higher intake than older individuals. Among the 4 different subcategories of occupation, namely, agriculture, service, small business, and housewives, the ANOVA test yielded the test statistics and P value as $F(3, 464) = 11754355.28$, $P < 0.0001$; $F(3,464) = 44695.39755$, $P < 0.0001$; and $F(3, 464) = 854.5657093$, $P < 0.0001$, indicating again that the mean for nitrates, nitrites, and NDMA were significantly different among the occupations, being highest in housewives and agriculture workers and lowest in small business and service professions.

For the ANOVA method between the 3 different groups namely smokers, ex-smokers, and those that never smoked, the ANOVA test yielded the test statistics as $F(2, 465) = 14.84165$, $F(2,465) = 348.6546$, and $F(2,465) = 3.438392$, respectively, giving $P < 0.0001$ for nitrates and nitrites and $P = 0.03$ for NDMA, indicating that the mean for the nitrate, nitrite, and NDMA concentrations were different in the 3 groups. The smokers had higher nitrate intake than ex-smokers or nonsmokers.

The mean daily dietary intake of nitrite was higher in males than females (7.8 mg vs 6.4 mg) (Table 5), with the largest differences being in the younger group, in agricultural workers, and in current smokers. Geographically, the nitrite dietary intake was highest in the North and Northeast, largely as a result of a higher consumption of sausages and bacon. Dietary intake of NDMA was mainly provided by fermented pork (Nam moosom), fermented fish (Pla-salid, Pla-ra, etc.), salted and dried fish (Larb-pla, Pla-siu, etc.), and mixed fermented vegetables and fermented beans (Tau-chiau). Another source of NDMA was cooked sausages. Beer contributed to the daily dietary intake of NDMA. Daily dietary intake of NDMA was higher for males (1.200 μg) than females (0.960 μg) (Table 5). Demographics indicated that NDMA intake was higher in middle age, in agriculture workers, and in current smokers. The NDMA intake was significantly higher in the Northeast and North than in the other regions, particularly among the smokers.

DISCUSSION

This is the first study conducted in Thailand to determine the mean total dietary intake of nitrate, nitrite, and NDMA by geographical region and to find a positive association between the intake of these compounds and liver and stomach cancer rates between the highest and lowest regions of Thailand. The mean intake of each of the 3 compounds in Thailand obtained from this study were substantially higher than those from the United States, Japan, and other developed countries where dietary intake of nitrate ranges from 48 to 121 mg/day, of nitrite ranges from 1.4 to 5.3 mg/day, and of NDMA ranges from 0.12 to 1.8 μg/day (12–15). The specific hypothesis underlying the present study was that the higher rates of liver and stomach cancer in Northeast and North regions of Thailand would be associated with increased exposure to nitrate, nitrite, and NDMA in

TABLE 5
Mean daily intake of nitrate, nitrite and nitrosodimethylamine (NDMA) in categories of different variables in Thailand 1998–2005

Variable	Category	No. of subjects	Nitrate (mg)	Nitrite (mg)	NDMA (μg)
Sex	Male	212	155.7 ± 6.20	7.8 ± 0.312	1.200 ± 0.05
	Female	255	154.2 ± 6.10	6.4 ± 0.256	0.960 ± 0.03
P value			0.0133	<0.0001	<0.0001
Age	20–39	140	168.0 ± 6.72	8.9 ± 0.445	0.910 ± 0.04
	40–59	233	158.6 ± 7.93	6.8 ± 0.340	1.420 ± 0.07
	>60	94	126.5 ± 5.06	5.9 ± 0.236	0.620 ± 0.03
P value			<0.0001	<0.0001	<0.0001
Occupation	Agriculture	195	156.1 ± 7.80	8.8 ± 0.440	1.210 ± 0.06
	Service/other	40	151.0 ± 7.55	8.0 ± 0.40	0.901 ± 0.04
	Small business	54	144.0 ± 7.20	7.6 ± 0.38	1.100 ± 0.05
	Housewives	178	158.3 ± 7.91	6.7 ± 0.33	1.101 ± 0.05
P value			<0.0001	<0.0001	<0.0001
Geographical area	North	131	194.4 ± 7.78	9.5 ± 0.38	1.410 ± 0.06
	Northeast	112	187.6 ± 7.50	8.8 ± 0.35	1.590 ± 0.06
	Central	127	129.6 ± 5.18	6.2 ± 0.25	0.950 ± 0.04
	South	97	98.7 ± 5.94	4.5 ± 0.18	0.650 ± 0.02
P value			<0.0001	<0.0001	<0.0001
Smoking	Never-smoked	238	155.2 ± 7.76	7.5 ± 0.37	1.050 ± 0.05
	Ex-smokers	26	151.7 ± 7.58	8.2 ± 0.41	0.945 ± 0.05
	Current smokers	203	158.5 ± 7.92	8.5 ± 0.43	1.350 ± 0.07
P value			<0.0001	<0.0001	0.03

these same regions. The Northeast region, which had the highest liver cancer incidence and mortality rates from liver cancer, also had the highest intake of NDMA, whereas the South region had the lowest NDMA intake and lowest liver cancer and stomach cancer incidence rates. The North region, which had the second highest incidence of liver cancer and the highest incidence of stomach cancer, had significantly higher intakes of nitrate and nitrite but slightly lower intake of NDMA than the Northeast region. These results seem to suggest that regional variation in nitrate, nitrite, and NDMA might be associated with the regional variation in liver and stomach cancer incidence. Studies in Italy and England have found a weak association between exposure to dietary nitrate and gastric cancer (12,16) but did not address liver cancer.

In the mechanism of carcinogenesis, dietary nitrate is reduced to nitrite by the flora of the mouth and stomach at high pH and then reacts with amines and is converted to carcinogens. Approximately 20% of salivary nitrate is reduced to nitrite by the oral flora, and this accounts for 80% of the gastric nitrite (17,18). Salivary nitrate and nitrite levels are positively correlated with the high prevalence of stomach cancer in endemic regions in other countries (19). Correlation has been observed with nitrate levels in food, regional drinking water (20), and salty diet (21). In addition, salty foods predispose to H. pylori colonization and premalignant lesions (21). H. pylori antibodies have been shown to be present at early ages in people from a rural Thai community, with a prevalence as high as 75% being seen in middle age category groups (22). More recently, several studies have reported an association between H. pylori infection and gastric cancer (2,21,22).

In the Northeast and North, two high-risk regions for liver cancer, the daily intake of dietary nitrate, nitrite, and NDMA is highest (Table 5). The Northeast is known to be endemic for liver fluke OV, and people consume fermented fish and meat more frequently than in the other regions. Because some regions, particularly the Northeast, are economically impoverished, the diet contains inadequate consumption of fresh fruits and vegetables containing the vitamins necessary to inhibit endogenous nitrosation among individuals infected with OV (5,10). This is in addition to the limited intake of ascorbic acid resulting in deficiencies of vitamins A and E, which we reported earlier (5). The effects of smoking in lung and digestive cancers, and even in liver cancer, have been documented in several epidemiological surveys and in our earlier report (23). Because NDMA is a gastric and liver carcinogen, it would be prudent to conduct bioassays for endogenous formation of nitrosamines among the people infested with OV (23). The major carcinogenic nitrosamines in cigarette smoke are the tobacco-specific nitrosamines (TSNA), which induce tumors in the lung, liver, and digestive tracts (23). A British study found lower levels of nitrate in smokers than in nonsmokers and for smoking to have an inhibitory effect on salivary uptake at higher levels of dietary nitrate intake (12).

In Thailand, the consumption of cigarettes and per capita sales has increased (23). Over 50% of all cigarettes and cigars consumed in Thailand are hand-rolled. These products rank especially high in smoke yield of tar and nicotine (24). A recent case-control study reported a close relationship between infection with the liver fluke OV, smoking, and CCA. The majority of the individuals in the study reported smoking cigarettes using local tobacco. Thus, the habit of smoking local tobacco or even chewing betel nut, which is mixed with the local tobacco, has emerged as a risk factor for CCA and possibly for HCC and gastric cancer (25,26). Research has to be undertaken to determine TSNA levels in these local tobacco products. In the analysis of duplicate portions, dietary nitrate intake was slightly higher in males than in females, but dietary nitrite was larger in males than females (Table 5). The dietary intakes of nitrate and nitrite in older people were lower than in the younger people. We found distinct differences in intake of nitrate, nitrite, and NDMA between geographical regions but not a significant difference between the occupational categories, with agricultural and housewives slightly elevated. Current smokers had higher dietary intake of nitrate, nitrite, and NDMA than ex-smokers and nonsmokers (Table 5).

In the present study, nitrate intake from the drinking water was not included in nitrate intake calculations but could be potentially very high. This is a potential limitation of our study. We did not include the values found in the water in our estimates because drinking water analyses have not been done in all regions of Thailand. The evaluation of nitrate levels in a group of randomly selected drinking water wells in the Northeast showed high concentrations, ranging from 80 to 366 mg/l in most of them. Water samples from the major waterways, the Chao-Praya and Mekong Rivers, contained from 40 to 1,210 mg/l and 22 to 1,150 mg/l, respectively, of nitrate. Some rural communities living along these rivers use these waters casually for cooking, and as a result, nitrate was also found in cooked food. Boiling reduces nitrate content in vegetables, as nearly 50% of this compound is passed into stock during boiling (17). However, the stock is used for soups or other meal preparation, and therefore, the nitrate is still ingested. A recent study in China (20) found cancer risk to be associated with the consumption of drinking water from the nitrate polluted wells. The intake of nitrate in drinking water should be further investigated in all regions in Thailand. Another potential limitation of our study was that we were unable to assess alcohol consumption with the exception of beer. Although the consumption of the beer was freely admitted, we did not see the same attitude toward admission for intake of other alcoholic beverages. Due to insufficient responses on the questionnaire (only 11 out of 476 individuals) and because of an inhibition to these questions among the population, there were inadequate data to permit statistical analysis of alcohol consumption. Reports from other countries have associated the consumption of alcohol with liver and stomach cancers (26–29).

In summary, we estimated exposures to dietary nitrate, nitrite, and NDMA in the various regions of Thailand as a potential explanation for the strong regional differences in liver and stomach cancer, and we did find that the regions with the highest intakes of these compounds were those with the highest incidence of liver and stomach cancers. We also suggest that nitrate exposure from pickled vegetables and regional drinking waters might contribute further to the regional variability in liver and stomach cancers. A case-control study in North Thailand is underway initiated by our preliminary results, to correlate the incidence rate of hepatic and gastric cancers with the daily dietary intake of nitrate, nitrite, and NDMA. In addition, biomarkers are needed as to the uptake and the extent of endogenous formation of nitrosamines in the population with the high rates of hepatic and gastric cancers.

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