

Maternal cured meat consumption during pregnancy and risk of paediatric brain tumour in offspring: potentially harmful levels of intake

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Abstract

Objective: To describe the relationship between specific levels of nitrite intake from cured meat consumption during pregnancy and the relative risk of paediatric brain tumours in the offspring.

Design: Exposure data were previously collected for a population-based case-control study of paediatric brain tumours; data on nitrite content were obtained by a comprehensive literature review of surveys of residual nitrite content in cured meats published in the USA and Canada. The level of nitrite intake for each mother was predicted by year of pregnancy based on survey results. Dose-response was evaluated both categorically and continuously using polynomial and quadratic spline regression.

Setting: The US west coast: Los Angeles County, the San Francisco–Oakland Bay Area and the Seattle–Puget Sound area.

Subjects: There were 540 cases diagnosed between 1984 and 1990 at ages varying from 0 to 19 years, and 801 controls frequency-matched by geographic area, age and birth year.

Results: In general, survey results suggest a trend of decreasing nitrite levels in cured meats over time. We observed a moderate increase in brain tumour risk in the offspring of mothers with relatively low levels of nitrite consumption from cured meats during pregnancy, and a two- to three-fold risk increase in offspring of mothers who consumed 3 mg day⁻¹ nitrite from cured meats (about 125 g day⁻¹ of cured meat consumption throughout the pregnancy).

Conclusions: A substantial risk of paediatric brain tumour appears to be associated with relatively high levels of maternal cured meat consumption during pregnancy. A more scientifically valid approach than a literature review to estimate nitrite intake from cured meats and data from a large group of highly exposed subjects would be useful in determining potentially harmful levels.

Keywords
Sodium nitrite
Cured meat products
Diet surveys
Brain tumours
N-nitroso compounds
Childhood neoplasms

It has been suggested that N-nitroso compounds (NOC) formed by *in vivo* reaction, mainly in the stomach, between nitrites and secondary or tertiary amines or amides may be a major contributor to human cancer risk¹ and that half of all human NOC exposure is from endogenous formation². Cured meats may be the most important source of human NOC exposure because of the high level of reactivity produced by high concentrations of nitrite that form around bits of cured meat in the stomach³. One group of NOC, the nitrosoureas, causes neurogenic tumours of various histological types in several species^{4,5} and is most effective when exposure is transplacental⁶. In monkeys, the histological distribution of tumours produced by

transplacental exposure to ethylnitrosourea (ENU) is similar to the histological distribution of human paediatric brain tumours, which are mostly glial (i.e. various types of gliomas, such as astrocytomas), and no tumours are produced by postnatal exposure⁷.

We previously reported a significant dose-response association between paediatric brain tumour risk and maternal consumption of cured meats during pregnancy in a large, population-based case-control study³. The odds ratio (OR), an estimate of relative risk in retrospective studies, was significantly elevated for the highest quartile of exposure (based on the distribution of exposure among all subjects) which corresponded to an

average nitrite consumption from cured meats greater than 1.28 mg day⁻¹. Nitrite levels for individual types of cured meats were based on dietary conversion software provided by Dr Geoffrey Howe (Columbia University School of Public Health, New York) and used published nitrite contents for the cured meats in our study.

Since publishing our initial findings, we have attempted to determine a more valid level of exposure at which relative risk becomes significantly elevated. Because of the widely publicized health concerns associated with NOC, it is reasonable to expect that, over time, meat manufacturers have independently reduced nitrite levels in their products through thermal processing, adding reducing agents such as ascorbate, lowering pH levels, and using higher storage temperatures and longer storage times^{8–10}. Thus, a single estimate of nitrite content may not be appropriate in a study such as ours with an exposure period that spanned three decades. We therefore conducted our own literature search and developed a database of published nitrite levels by year of publication. We then determined predicted nitrite levels for each type of cured meat based on year of publication and used these predicted levels to assign nitrite exposure to our study subjects based on year of pregnancy. We reanalysed our data using these new exposure levels and chose fixed cut-off points for categorical analysis rather than basing the cut-off points on the distribution of exposure. Finally, we related nitrite exposure levels to units of cured meat consumption to express potentially harmful consumption levels in a more intuitive manner.

Methods

The US West Coast Childhood Brain Tumour Study has been described in detail elsewhere³. Briefly, the study included 540 cases and 801 controls, aged 0–19 years, from 19 counties in three West Coast regions: Los Angeles County, 13 counties in western Washington state, including the Seattle–Puget Sound area, and five counties in the San Francisco–Oakland metropolitan area. Cases were diagnosed from January 1984 to December 1990 (Seattle, San Francisco) or through June 1991 (Los Angeles). Controls were recruited from the same three geographic areas using random digit dialing¹¹ and frequency-matched to cases by area, gender and birth year.

Reference ages were assigned based on diagnosis ages of cases within each stratum defined by the matching variables. As part of a larger interview that primarily emphasized prenatal and childhood exposure to NOC, mothers were asked about their intake of 47 food items relevant to the NOC hypothesis during the past year and during their pregnancies. Data on the frequency of consumption and portion sizes (measured using abstract food models) were converted to average daily grams of consumption using a dietary conversion software

program provided by Dr Geoffrey Howe of Columbia University¹².

Several sources were used to gather all surveys of NOC or NOC precursor content in commercially available cured meats published in the USA or Canada since 1965. Initially, Medline was searched using keywords ‘nitrite’, ‘nitrate’, ‘N-nitroso compounds’, and ‘cured meat’ to find relevant literature, most of which was published in food science journals. From the articles retrieved, relevant articles in older journals and journals not covered by Medline were found. We also searched Food and Drug Administration publications available on microfiche and contacted various institutions (US Department of Agriculture Food Safety and Inspection Division, American Meat Institute, National Livestock and Meat Board, Dr Walter Fiddler at the Agricultural Research Service) to determine if any other survey data had been published and to obtain available documentation. Results from studies that altered the product, e.g. NOC added to test measurement methods, were excluded.

A total of 85 surveys were reviewed; 26 of these reported sodium nitrite (hereafter referred to as nitrite) levels using several different testing methods: spectrophotometric⁸, colorimetric^{13–16}, diazotization¹⁷, the Griess–Saltzman method^{18–23} and methods recommended by the Association of Official Analytical Chemists^{24–27}. Methods were not specifically stated for 10 surveys contained in six references^{9,28–32}. In tabulating survey results, we classified meats into five categories that corresponded to those used in our case–control study interview: bacon (fried and raw); sausage; hot dogs; ham; and other cured meats, which included lunch meats, meat loaves and pork. Hot dog varieties used in the surveys included beef, chicken, pork, fish and cheese. Approximately 30 varieties of sausage were surveyed, the most common being Vienna, salami, mettwurst, summer, Lebanon bologna, liver, pepperoni, Polish and knockwurst. Ham preparation methods included raw, canned, smoked, cooked and fried. Curing methods used were dry, immersion and pumped.

Original data published in the surveys, if available, were used for analysis. Nineteen references provided original data, three provided only summary data (averages and/or ranges) and four provided a combination of original and summary data. When only summary data were available, average nitrite levels weighted by sample sizes were used. Polynomial regressions (SAS/STAT Version 6.04, SAS Institute, Cary, NC) of residual nitrite level on survey publication year, weighted by the number of meat samples surveyed and excluding obvious outliers, were performed to determine the best-fitting model and to compute residual nitrite (in mg kg⁻¹) predicted by publication year for each category of cured meat. ‘Publication year’ was defined as the year the survey was published, even if the survey was cited in a subsequently published article; e.g. if a survey was

Table 1 Median and midsread (25th–75th percentiles) residual nitrite levels (ppm) in cured meats according to published surveys by year of publication and category of cured meat

Cured meat category	1970–1975			1976–1980			1981–1985			1986–1991		
	<i>n</i> *	Median	Midsread	<i>n</i>	Median	Midsread	<i>n</i>	Median	Midsread	<i>n</i>	Median	Midsread
All combined	408	14	(7–39)	518	34	(15–58)	110	14	(8–38)	66	30	(18–47)
Bacon†	85	25	(12–51)	310	40	(22–63)	47	28	(14–45)	49	27	(14–40)
Sausage‡	98		–	36	13	(3–25)	24	8	(6–12)	0		–
Hot dogs§	33	14	(7–21)	30	35	(18–51)	13	22	(13–42)	17	47	(28–65)
Ham	44	18	(7–54)	83	16	(6–62)	11	8	(3–52)	0		–
Other cured meats¶	148	10	(4–40)	59	28	(14–43)	15	9	(5–14)	0	12	(10–20)

* Number of meat samples surveyed.

† References 9, 13–20, 23, 26–29, 31.

‡ References 8, 13, 14, 20, 25, 26, 28.

§ References 8, 13, 14, 20–22, 24–26, 28.

|| References 13, 14, 20, 25, 26, 28, 31.

¶ References 8, 13, 14, 20, 25, 26, 28, 30, 31.

originally published in 1970 but was cited in an article published in 1975, 'publication year' for data from that survey was 1970. Log-transformed data better satisfied the homoscedasticity and normality regression assumptions than non-transformed data.

For each category of cured meat, average daily nitrite intake during pregnancy was calculated using average daily consumption of each category and nitrite intake per kilogram of consumption (i) predicted by pregnancy year plus one (to account for publication lag time) using the derived regression equations described above, and (ii) based on the Howe dietary conversion software. Total nitrite intake from cured meats was derived by summing nitrite intake over all cured meat categories. Unconditional logistic regression (Epilog Plus Version 3.99,

Epicer Software, Pasadena, CA) stratified by geographic area, gender, diagnosis (or reference) age and birth year was used to compute maximum likelihood estimates of ORs and 95% confidence intervals for relating maternal nitrite intake from cured meats to risk of paediatric brain tumour. Race and socioeconomic status (SES) were evaluated as potential confounders and effect modifiers. Both variables, however, had little effect on OR estimates when included as possible confounders, and OR estimates did not differ substantially by race or level of SES. Therefore, we present results that are unadjusted for and unstratified by race and/or SES. Cut-off points for categorical analysis were set a priori at 0, 0.5, 1, 2 and 3 mg. Using these same values as knots, quadratic spline regression with linear restriction of the upper tail was

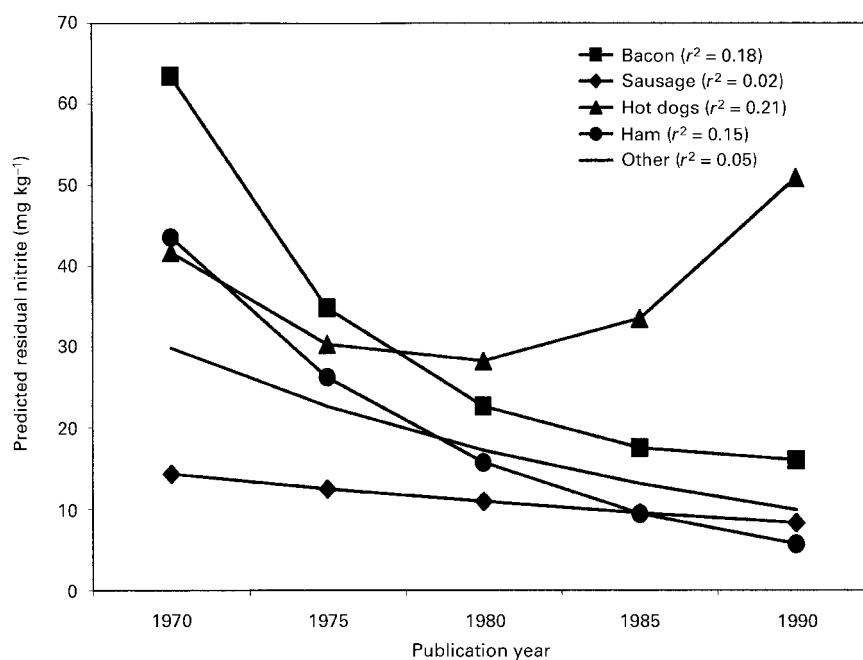


Fig. 1 Residual nitrite levels in cured meats predicted by publication year of survey literature, 1970–1991. Each point represents predicted mg nitrite/kg cured meat product for a given year. Trends were significant for bacon ($P = 0.001$ for linear term, 0.003 for quadratic), ham ($P = 0.0001$), other cured meats ($P = 0.001$) and hot dogs ($P = 0.007$ for linear term, 0.005 for quadratic)

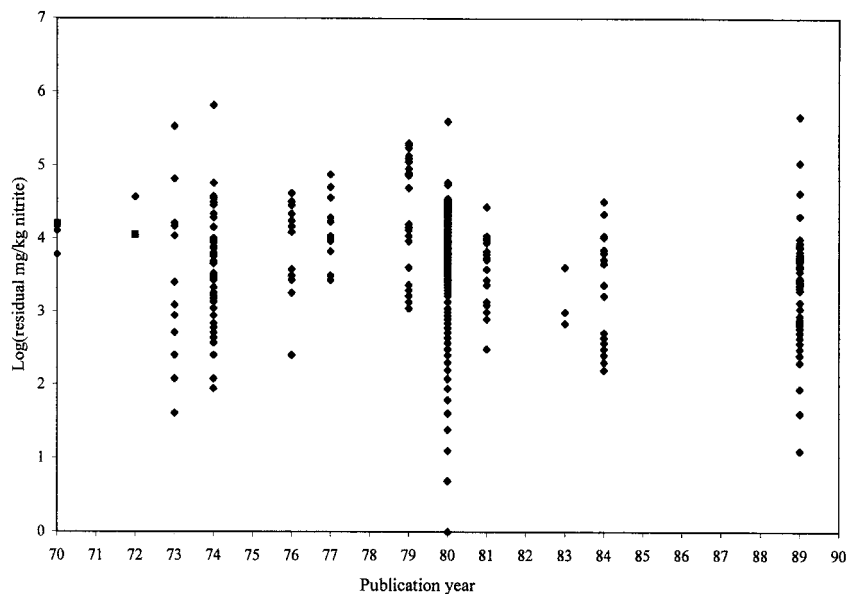


Fig. 2 Log(residual nitrite) in bacon by publication year of survey. Each point represents log(reported mg nitrite/kg bacon) from a single survey. Linear and quadratic terms in test for trend were significant ($P = 0.001$ for linear, 0.003 for quadratic)

used to fit a smooth curve over the range of literature-based nitrite exposure³³. The spline model was compared to a model containing only the linear component of exposure by the likelihood ratio test. All tests were two-sided with a 0.05 significance level.

Results

Within our target time period of 1965 to 1991 (the birth year range of our study subjects), no published nitrite surveys were available prior to 1970, nor were all years in the time period represented. Within samples defined by meat and year, sample sizes ranged from one to 260. In this context, 'sample size' refers to the number of meat samples summed over all surveys performed on a given type of meat in a given year. Residual ranges (in ppm) were very wide, particularly for bacon (Table 1). There were two outlying values of residual ppm for bacon and three for hot dogs that were omitted from regression analyses to predict nitrite exposure by year. As shown in

Fig. 1, there were decreasing trends in predicted nitrite levels by survey publication year for bacon (regression coefficient for linear term [β] for log(ppm) = -0.61 , standard error [SE] = 0.19, $P = 0.001$; for quadratic term [β^2] = 0.003, SE = 0.001, $P = 0.004$), ham ($\beta = -0.10$, SE = 0.021, $P = 0.0001$), sausage ($\beta = -0.03$, SE = 0.016, $P = 0.09$) and 'other cured meats' ($\beta = -0.05$, SE = 0.017, $P = 0.001$) and, after 1980, an increasing trend for hot dogs ($\beta = -0.77$, SE = 0.28, $P = 0.007$; $\beta^2 = 0.005$, SE = 0.002, $P = 0.005$). Percentages of variation accounted for by the regressions (r^2) are reported in the figure. Despite the significant regression coefficients for all cured meats but sausage, the low r^2 values indicate that very little of the variability in nitrite levels reported in the surveys is explained by publication year. This is illustrated for bacon in Fig. 2.

Categorical dose-response relating average daily nitrite (in mg) consumption from cured meats to paediatric brain tumour risk is shown in Table 2. With estimated nitrite intake based on our literature review, there is an

Table 2 Comparison of odds ratios (OR) and 95% confidence intervals (CI) at fixed categories of maternal nitrite exposure from consumption of cured meats during pregnancy by source of nitrite estimation, US West Coast Childhood Brain Tumour case-control study, 1984–1991

Average daily nitrite from cured meats (mg)	Time-specific nitrite estimates from literature review			Nitrite estimates from dietary conversion software		
	No. of cases (%)	No. of controls (%)	OR (95%CI)	No. of cases (%)	No. of controls (%)	OR (95%CI)
0	102 (20)	161 (20)	1.0	102 (20)	161 (20)	1.0
0.01–0.49	293 (57)	499 (63)	1.1 (0.8, 1.5)	377 (73)	601 (75)	1.2 (0.9, 1.6)
0.50–0.99	68 (13)	72 (9)	1.9 (1.2, 2.9)	27 (5)	24 (3)	2.3 (1.3, 4.4)
1.00–1.99	28 (5)	43 (5)	1.3 (0.8, 2.3)	6 (1)	10 (1)	1.3 (0.5, 3.3)*
2.00–2.99	12 (2)	13 (2)	1.8 (0.8, 4.1)	2 (0.4)	0 (0)	–
≥ 3.0	11 (2)	9 (1)	3.0 (1.2, 7.9)	0 (0)	1 (0.1)	–

* Includes two cases and one control with exposure ≥ 2 mg day⁻¹.

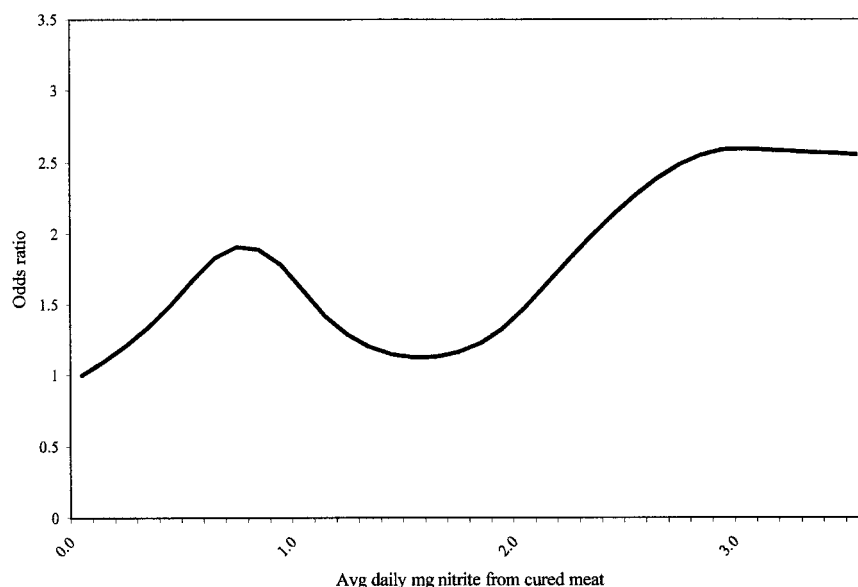


Fig. 3 Fitted odds ratio of paediatric brain tumour associated with maternal intake of nitrite (average mg day^{-1}) from cured meats during pregnancy with time-specific predicted nitrite levels based on the literature review. Restricted quadratic spline with knots at 0.5, 1, 2 and 3

indication of increasing OR with increasing exposure and significantly increased OR at exposure levels of at least 3 mg day^{-1} . Among subjects with nitrite intake levels of at least 3 mg day^{-1} , median cured meat consumption was 124 g day^{-1} . This roughly corresponds to three hot dogs (1 hot dog = 45 g), 20 slices of bacon (1 slice cooked bacon = 6.3 g), five pork sausage links (1 link = 24 g), over four slices of lunch meat (1 slice = 28.35 g) or one cup of diced ham (1 cup = 135 g)³⁴. With estimated nitrite based on the dietary conversion software, categorical dose–response using the same exposure cut-off points could not be adequately evaluated because very few mothers were in the highest categories of exposure. At intermediate exposure levels ($0.5\text{--}0.99 \text{ mg day}^{-1}$), the OR was somewhat higher using the dietary conversion software than using the time-specific literature-based estimates of nitrite intake (OR = 2.3 vs 1.9).

The linear trend relating literature-based average daily mg nitrite consumption from cured meats (as a continuous variable) to relative risk of paediatric brain tumour was highly significant (β (SE) = 0.22 (0.08); $P = 0.008$). Although quadratic spline regression did not significantly improve the fit to the data ($P = 0.09$), the dose–response curve suggests a moderate (maximum OR = 1.9) increase in paediatric brain tumour OR at low levels of maternal nitrite exposure (up to 1 mg day^{-1}) followed by another OR increase beginning at about 1.5 mg day^{-1} with an OR of 2.5 at 3 mg day^{-1} (Fig. 3).

Evaluation of time as an effect modifier would have been useful to confirm an association between higher nitrite exposure and increased risk. However, this was not possible due to the relationships among age, birth era and effects of prenatal risk factors. One of the case inclusion requirements in our study population was diagnosis no

earlier than 1984. Thus, subjects born in the earliest years were the oldest subjects and all of the youngest subjects were born no earlier than 1980. Since prenatal risk factors would be expected to act early in life, increased risk from nitrite among the earliest birth year subjects is likely to be obscured by the fact that these were also the oldest subjects.

Discussion

Other studies besides ours have reported elevated risk related to maternal cured meat consumption; however, all of them had limitations that prevented valid quantification of potentially harmful levels of nitrite and/or cured meat intake: significant increasing dose–response was demonstrated but amounts of consumption were not reported³⁵, dose–response analysis was not reported³⁶, clear evidence of dose–response was not present³⁷, or dose–response was restricted to low-income subjects only³⁸. Because of the high prevalence of exposure to cured meats – 80% of all mothers in our study ate some cured meat while pregnant – we felt it was important to try to determine a level of nitrite and corresponding cured meat consumption at which risk of paediatric brain tumour became significantly increased. In doing so, we found it necessary to consider chronological trends by type of cured meat. Our study subjects were born between 1965 and 1991, a particularly active period in governmental and consumer interest in nitrite in cured meats². The surveys we reviewed suggest that meat manufacturers responded by significantly reducing nitrite levels in all products except hot dogs, in which nitrite levels seem to have increased after 1980 (Fig. 1).

Literature review may be the only method available to

obtain data such as human nitrite intake from cured meats; however, this approach does have a serious scientific limitation in that the surveys are not controlled so that they are consistent and directly comparable to each other. For example, residual nitrite levels in cured meats vary considerably depending on processing and storage methods, yet most surveys are conducted shortly after processing and therefore do not account for the reduction in residual nitrite that occurs during storage². Longer storage times increase depletion but refrigeration inhibits the depletion process³⁹. Also, residual nitrite analysis can be quite variable, even when restricted to a single method. Adding to the variability of estimates based on several surveys are differences in curing, preparation and testing methods; laboratories in which the surveys are conducted; and sources of meat samples. This high degree of variability is reflected in the low r^2 values shown in Fig. 1 and is illustrated for bacon in Fig. 2, indicating that relatively little of the variation in residual nitrite (mg kg^{-1}) is accounted for by publication year. Nevertheless, the significant coefficients from regressions of year on nitrite levels suggest that predicting nitrite exposure by year probably improves upon exposure estimates that ignore the timing of the exposure.

While we acknowledge that our estimates of nitrite levels in cured meats are not ideal, this is the first attempt, to our knowledge, to quantify potentially harmful levels of nitrite exposure and estimate corresponding cured meat consumption during pregnancy as it relates to paediatric brain tumour risk in offspring. Our conclusion is that a two- to three-fold increase in risk occurred at a relatively high level of nitrite exposure (e.g. corresponding to eating three hot dogs a day throughout pregnancy), but that much lower levels of exposure were also associated with moderately increased risk. Our observations could be improved upon by other studies that confirm the dose–response relationship we noted and by more scientifically valid estimates of residual nitrite levels in cured meats. Studies that include more subjects with relatively high exposure are necessary to increase the confidence in risk estimates for these exposure levels; however, that seems unlikely given the decline in nitrite levels that has occurred in cured meats over past decades.

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