

## A review: dietary and endogenously formed *N*-nitroso compounds and risk of childhood brain tumors

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### Abstract

Maternal dietary exposure to *N*-nitroso compounds (NOC) or to their precursors during pregnancy has been associated with risk of childhood brain tumors. Cured meat is one source of exposure to dietary NOC and their precursors. Most epidemiological studies that have examined the role of maternal consumption of cured meats during pregnancy have found a significant positive association between maternal intake of cured meat and the risk of childhood brain tumor (CBT). NOC consist of two main groups, *N*-nitrosamines and *N*-nitrosamides. The carcinogenicity profiles of NOC suggest that *N*-nitrosamides rather than *N*-nitrosamines are the compounds that may be associated with CBT and that they should be investigated more closely in epidemiological studies. We present a review of the chemical and carcinogenic properties of NOC in connection with the findings of case–control studies. This approach may be helpful in determining the essential information that must be collected in future epidemiological studies on CBT.

**Abbreviations:** NOC – *N*-nitroso compounds; NO<sub>x</sub> – nitrogen oxides; VNA – volatile *N*-nitrosamines; NVNA – non-volatile *N*-nitrosamines; NDMA – *N*-nitrosodimethylamine; NDEA – *N*-nitrosodiethylamine; NPYR – *N*-nitrosopyrrolidine; NPIP – *N*-nitrosopiperidine; MNU – Methylnitrosourea (*N*-nitroso-*N*-methylurea); ENU – Ethylnitrosourea (*N*-nitroso-*N*-ethylurea); NTHZ – *N*-nitrosothiazolidine; NHMTCA – *N*-nitroso-2-(hydroxymethyl)-thiazolidine-4-carboxylic acid; NTCA – *N*-nitrosothiazolidine-4-carboxylic acid; NTHZCA – *N*-nitrosothiazolidine-4-carboxylic acid and related homologues.

### Introduction

Brain tumors are the most common solid tumors in children. The incidence rate of childhood tumors of the brain, brain stem, and spinal cord, hereafter referred to as childhood brain tumor (CBT), in the USA, Europe and Japan lies between 24 and 27 cases per million children per year [1, 2]. The occurrence of CBT is highest in children under 5 years of age, and the relative 5-year survival rate from the time of diagnosis is 65% [3–5]. Similar to adult brain tumors, the majority of brain tumors in children are

also gliomas, but histologically, childhood brain tumors differ markedly from cancers in adulthood [6]. The most common gliomas in children are astrocytomas and primitive neuroectodermal tumors (PNET) [7].

The etiology of CBT is largely unknown. The few established risk factors include ionizing radiation (e.g., *in utero* X-rays [8–10] or radiotherapy for leukemia [11, 12]) and predisposing genetic syndromes such as neurofibromatosis or Von-Recklinghausen's disease [13, 14]. However, these risk factors explain only a small fraction of diagnosed CBT.

In this review we examine a major hypothesis that has been investigated extensively in epidemiologic studies over the last two decades: that *N*-nitroso compounds (NOC) are etiologically related to CBT. This CBT-NOC

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hypothesis stems from the observation in animal studies that *N*-alkylnitrosoureas, (which are *N*-nitrosamides, one of two major subgroups of NOC) induced brain tumors in the offspring of pregnant rodents and monkeys [15–18]. Several epidemiological studies have found maternal intake of cured meats during pregnancy to be associated with elevated risk of CBT. Cured meats are a major dietary source of preformed NOC and their precursors. Although the amount of nitrite used to cure meat has been reduced in the past two to three decades in most of the meats, this decrease does not correlate with the incidence of CBT. In fact, CBT occurrence has been reported to be increased by 35% in the US between 1973 and 1994, resulting likely from changes in diagnosis methods available [5, 19].

While non-dietary sources of exogenous exposure to NOC exist (tobacco products, cosmetics, drugs, agricultural chemicals, and certain occupational settings such as rubber, leather, and metal machining industries [20–22]), we focus here on dietary exposures. Diet can provide preformed NOC and can also provide precursors (typically sodium nitrite, amines and amides) for the endogenous formation of both *N*-nitrosamines and *N*-nitrosamides. Preformed *N*-nitrosamides are unlikely to occur in food because of their instability. Clarity in the epidemiologic literature has been hampered by lack of information about NOC in general, their precursors in food, and their endogenous formation, as well as confusion in relation to the two classes of NOC, the nitrosamines and nitrosamides.

In this article, we review case-control studies published between 1982 and 2004 on CBT and the role of maternal and childhood dietary exposure to NOC. We also describe the chemistry and properties of NOC in some detail, as this is important for the evaluation/review of the CBT-NOC hypothesis.

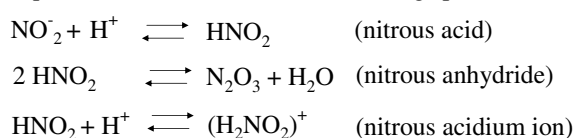
## ***N*-nitroso compounds**

### *Background*

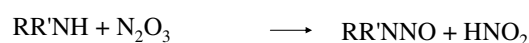
Based on their chemical structure, NOC are divided into two major groups: *N*-nitrosamines and *N*-nitrosamides. Properties and differences of *N*-nitrosamines and *N*-nitrosamides are listed in Table 1. Both groups of NOC are characterized by a nitroso group (–N=O) attached to a nitrogen atom (–N–N=O). Both are formed by the reaction of a nitrite compound with amines or amides. *N*-nitrosamines are derived from nitrosation (reaction with nitrite) of secondary amines containing dialkyl, alkylaryl and diaryl substituents (see Equation (2) in schematic diagram). *N*-nitrosamides are

derived from nitrosation of amides such as *N*-alkylamides, *N*-alkylureas, and *N*-alkylcarbamates [23] (see Equation (3) in schematic diagram). Under acidic conditions [24], nitrite forms intermediate nitrosating species such as nitrous anhydride and the nitrous acidium ion (N<sub>2</sub>O<sub>3</sub>, (H<sub>2</sub>NO<sub>2</sub>)<sup>+</sup>, respectively) [22, 23, 25] (see Equation (1) in schematic diagram). *In vitro*, the nitrosation of amines is accelerated by thiocyanates [26]; the nitrosation of amides is catalyzed by citrate and other organic acids. Organic acids and thiocyanates are compounds which occur naturally in foods. Organic acids are also used as food additives [26–29], and thus could be present to enhance nitrosation. Under neutral conditions, nitrosation can take place via bacteria catalyzed processes. Bacterial nitrosation can for example occur at neutral pH in the hypochlorhydric stomach in individuals with chronic gastritis [22]. Hypochlorhydria facilitates the bacterial overgrowth in the stomach with nitrate-reducing bacteria. The formed nitrite can then promote the formation of NOC, catalyzed by bacterial strains with nitrosating activity [30]. Nitrosation inhibitors are vitamins C and E, which are natural compounds in fruits, vegetables, and grains. They inhibit nitrosation by reducing nitrite to nitric oxide (NO), which is not directly a nitrosating agent [25, 31, 32]. Certain types of vegetables are high in nitrate which can be reduced to nitrite by bacteria in the saliva. However, only about 5% of ingested nitrate is reduced to nitrite via this pathway [33], and as mentioned above, vegetables also provide nitrosation-inhibiting antioxidants such as vitamins C and E.

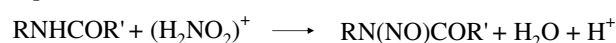
### Equation 1: Formation of the nitrosating species



### Equation 2: Formation of *N*-nitrosamines



### Equation 3: Formation of *N*-nitrosamides



*Scheme 1.* Schematic diagram of NOC formation (adapted from [22,25]).

### *Carcinogenicity of *N*-nitrosamines and *N*-nitrosamides*

*N*-nitrosamines can be divided into two categories: volatile (VNA) and non-volatile (NVNA). NVNA are mostly hydroxylated compounds, including nitrosated derivatives of amino acids. The total estimated daily

Table 1. Properties and differences between *N*-nitrosamines and *N*-nitrosamides

Property	<i>N</i> -nitrosamines	<i>N</i> -nitrosamides
Chemical structure	NO-group attached to nitrogen atom	NO-group attached to nitrogen atom
Formation milieu <sup>a</sup>	Generally under acidic conditions [24]	Under acidic conditions
Formation catalyst	Thiocyanates [29]	Citrate and other organic acids
Formation inhibitor	Redox compounds (ascorbate, tocopherols)	Redox compounds (ascorbate, tocopherols)
Stability/decomposition	<ul style="list-style-type: none"> <li>• Relatively stable in water</li> <li>• Most are stable under neutral conditions [30]</li> </ul>	<ul style="list-style-type: none"> <li>• Unstable in aqueous solvents [49, 93]</li> <li>• Unstable under neutral and alkaline conditions [35, 43, 49]</li> <li>• Thermally unstable (decompose readily at &gt; 100 °C) [49]</li> </ul>
Carcinogenicity	<ul style="list-style-type: none"> <li>• Decompose under UV light</li> </ul>	<ul style="list-style-type: none"> <li>• Decompose under normal light and UV light</li> </ul>
	Require enzymatic activation by cytochrome P-450 enzymes to act as carcinogen	Do not require enzymatic activation to act as carcinogen; often act at site of occurrence
Tumor induction in rodents	Lung, liver, bladder, esophagus, pancreas, nasal cavity, kidney, trachea [36]	Lymphatic system, central nervous system [36], stomach, gastrointestinal tract, bone [40, 94]
Organ specificity	Yes	Suggested [22]
Neurocarcinogens in animal studies	No	Yes (ENU)
Chemical determination	Gas chromatography/thermal energy analyzer (GC/TEA)	High-performance liquid chromatography/photolytic interface/thermal energy analyzer (HPLC/TEA) [95] combined with gas chromatography/mass spectrometry (GC/MS) for structure confirmation [58, 59]

<sup>a</sup> NOC in general are also formed via bacterial nitrosation at neutral pH, and via nitric oxide (NO) formation during inflammation [22].

dietary exposure to NVNA is several hundred times higher than to VNA (10–100 *versus* 0.3–2.0  $\mu\text{g}$ , respectively) [23]. However, most dietary NVNA identified to date are biologically inactive [23, 34], and consequently research on the occurrence of *N*-nitrosamines in food has focused more on VNA than on NVNA [23]. VNA are low molecular weight and cyclic compounds such as *N*-nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR) and *N*-nitrosopiperidine (NPIP).

The carcinogenic properties of *N*-nitrosamines have been extensively investigated [35–38]. They have been shown to induce tumors of the liver, lung, esophagus, bladder, pancreas, and other sites [35–39] in various animal species [35, 39]. Over 30 different animal species are responsive to *N*-nitrosamine carcinogenesis, with most bioassays conducted in mice, hamsters and rats [38, 39]. *N*-nitrosamines are highly organotropic, animal species specific, and induce tumors at specific sites independent of the route of administration [39]. The *N*-nitrosamines that have been tested induced cancer in various organs in animals but never in the brain or spinal cord. *N*-nitrosamines require metabolic activation via cytochrome P450s to act as carcinogens.

*N*-nitrosamides are direct alkylating compounds and can lead to DNA adducts at the site of their occurrence. Druckrey and colleagues tested *N*-nitrosoureas (a subgroup of *N*-nitrosamides) for oncogenic characteristics in animals and found that several types are carcinogenic and that they mostly induced tumors in the nervous system

[35]. Lijinsky reported that *N*-nitrosamides (alkylnitrosoureas or alkylnitrosocarbamates) also induce tumors of the stomach, gastrointestinal tract and bone [40]. With regard to carcinogenic effects of *N*-nitrosamides, experiments on endogenous formation of alkylnitrosoureas have been conducted by Ivankovic and Preussmann in prenatal rats. These experiments showed that administration of ethylurea (an alkyurea) and nitrite together during pregnancy resulted in malignant tumors of the nervous system in the offspring, whereas when ethylurea or nitrite were given separately, no tumor development occurred [41, 42]. These results clearly demonstrate that ethylnitrosourea forms *in vivo* in animals and induces brain cancer.

#### *Kinetics of N-nitrosamide formation and stability of N-nitrosamides*

Studies on the kinetics of *N*-nitrosamide formation by Mirvish showed that the initial reaction rate of *e.g.*, methylurea nitrosation to yield *N*-nitroso-*N*-methylurea is proportional to methylurea and nitrite concentration at pH 2 and increased about 10-fold for each 1-unit drop in pH. However, these kinetics are modified by catalysts, such as *e.g.*, organic acids, which may be present *in vivo* and can therefore influence the endogenous formation rate of *N*-nitrosamides [22, 25, 43].

The stability of *N*-nitrosamides *in vivo* has been investigated by Swann and Magee using animal

experiments. Their experiments in rats after intra-venous injection showed that the half-life of *N*-ethyl-*N*-nitrosourea (ENU) in blood is about 5–6 min and that of *N*-methyl-*N*-nitrosourea (MNU) is approximately 1 min [44, 45]. This time period is likely to be long enough for *N*-nitrosamides to reach the brain, supported by the fact that brain tumors are induced endogenously by administering ethylurea plus nitrite, as described above.

### NOC in foods

Food may contain preformed NOC as a result of food processing. Preformed dietary NOC have been found mainly in food that contains nitrite and/or has been

exposed to nitrogen oxides (NO<sub>x</sub>), such as nitrite-cured and smoked meat and fish, cheese and beer [46, 47] (Table 2).

Nitrosation of amines or amides in food is most likely to occur during the curing process of meat. Meat is cured to prevent *clostridium botulinum*, an anaerobic bacterium that, under the proper conditions (such as vacuum-packed meat), can produce the potentially fatal toxin that causes botulism. Curing also modifies the taste of meat and preserves the bright red color of hemoglobin which otherwise would oxidize and turn brown [48]. The salt used to cure meat contains sodium nitrite that can nitrosate amines or amides in meat, resulting in the formation of NOC. Smoking or drying of food is performed technologically by exposing food

Table 2. Examples of concentrations of *N*-nitroso compounds and nitrosatable alkylureas in food

Food products (place of analysis)	<i>N</i> -nitroso compound	[µg/kg] = ppb	Reference
	<i>VNA</i>	–	
Cured meats (US)	NDMA, NPYR	ND–320	Nitrite Safety Council (1980) [96]
Cooked bacon (US)	NTHZ	ND–32	Kimoto <i>et al.</i> (1982) [97]
Fried bacon (US)	NTHZ	ND–2.0	Pensabene and Fiddler (1983) [98]
Fried bacon (US)	NDMA, NPYR, NTHZ	ND–4.2	Vecchio <i>et al.</i> (1986) [52]
Fried bacon (US)	NDMA, NPYR	ND–65	Canas <i>et al.</i> (1986) [99]
Smoked meats, fish (Canada)	NTHZ	ND–241	Sen <i>et al.</i> (1986) [100]
Cured meats (Canada)	NPYR, NPIP, NDMA, NDEA	ND–55	Sen <i>et al.</i> (1980) [101]
Cured meats (Germany)	NDMA, NPYR	0.5–>5	Spiegelhalter <i>et al.</i> (1980) [102]
Sausage products (Germany)	NDMA, NPIP	ND–1.8	Tricker <i>et al.</i> (1991) [103]
	<i>NVNA</i>		
Smoked and cured sausage (Germany)	NHMTCA	110–400	Tricker <i>et al.</i> (1984) [104]
Smoked and cured sausage (Germany)	NTCA	180–210	Tricker <i>et al.</i> (1984) [104]
Bacon fried (Germany)	NHMTCA	ND–2100	Tricker <i>et al.</i> (1984) [104]
Bacon fried (Germany)	NTCA	ND–520	Tricker <i>et al.</i> (1984) [104]
Smoked meats, fish (Canada)	NTCA	ND–13700	Sen <i>et al.</i> (1986) [100]
	<i>N-nitrosoalkylureas (MNU equivalents or MNU confirmed)</i>		
Bacon smoked and fried (Germany)	MNU (equivalent) <sup>a</sup>		Mende <i>et al.</i> (1991) [57]
	Under chemical conditions <sup>b</sup>	1790–3150	
	Under simulated gastric conditions	3.5	
Boiled cauliflower	MNU (equivalent) <sup>a</sup>		Mende <i>et al.</i> (1991) [57]
	Under chemical conditions	4	
	Under simulated gastric conditions <sup>c</sup>	1.4	
Fish (herring) (Canada)	MNU (GC/MS confirmed)	<0.03 to 68.8 <sup>d</sup>	Sen <i>et al.</i> (2001) [59]
	Under simulated gastric conditions <sup>c</sup>	(<0.3 to 688 ng/10 g)	
Fish sauce (Canada)	MNU (GC/MS confirmed)	0.4–142.4 <sup>d</sup>	Sen <i>et al.</i> (2001) [59]
	Under simulated gastric conditions <sup>c</sup>	(2–712 ng/5 g)	
Japanese pickled radish (Canada)	MNU (GS/MS confirmed)	<0.03 to 7.2 <sup>d</sup>	Sen <i>et al.</i> (2001) [59]
	Under simulated gastric conditions <sup>c</sup>	(<0.3 to 72 ng/10g)	
Cured meats (Canada)	MNU (GS/MS confirmed) Under simulated gastric conditions <sup>c</sup>	0.2–2.6 <sup>d</sup>	Sen <i>et al.</i> (2000) [58]
		(2–26 ng/10 g)	

VNA = volatile *N*-nitrosamines; NVNA = non-volatile *N*-nitrosamines; MNU = methylnitrosourea.

<sup>a</sup> The concentrations listed in these references are reflecting DNA alkylating activity corresponding to MNU and are therefore presented as MNU equivalents.

<sup>b</sup> Under chemical conditions (on average): pH = 1.0, nitrite concentration 100 mM. The difference to simulated gastric conditions is the lower pH value and the excessive concentration of nitrite.

<sup>c</sup> Under simulated gastric conditions; by Sen *et al.* (2001): pH 0.8–1.5, nitrite concentration 1.59 mM; by Sen *et al.* (2000): pH 0.8–2.5, with and without additional nitrite added.

<sup>d</sup> ppb calculated from original data in publication; original data in parenthesis.

to warm air or smoke containing a higher percentage of nitrogen than normal air to prevent oxidation of fatty acids in food, thus preventing rancidity, but also acting as potential nitrosating agents to amines or amides. Unlike *N*-nitrosamines, *N*-nitrosamides are very unstable, especially under neutral and alkaline conditions, and they decompose under heat and light exposure (see Table 1) [22, 35, 43, 49]. Consequently, it is unlikely that preformed *N*-nitrosamides are abundant in large quantities in food.

Since the discovery of the formation of NOC in food and their carcinogenic properties, extensive efforts have been undertaken to decrease the amount of nitrite used for curing meat and the content of nitrogen oxides used in the heating process during beer production. Starting in the 1970s, the amount of sodium nitrite added to meat has been reduced from 200 to 120 ppm, and vitamin C has been added as a nitrosation inhibitor [50]. In 1986, regulations in the US further restricted sodium nitrite levels to 100 ppm [51]. These steps resulted in an 80% decrease in two major volatile *N*-nitrosamines in fried bacon (NPYR and NDMA, combined, decreased from 100 to 21 ppb) [22, 52]. A literature review of published surveys of sodium nitrite levels in cured meat products from 1970 to 1990 suggested that sodium nitrite levels had decreased in all products investigated except hot dogs [53], although valid up-to-date survey data in this area are lacking. *N*-nitrosamine concentrations in beer were greatly reduced by sequestering the malt from fuel gases used to heat the malt during beer production. This reduced the levels of *N*-nitrosamines in beer from 50 to 5 ppb [40].

The nitrites in food may form *N*-nitrosamides in the presence of appropriate substrates. A variety of constituents in foods are nitrosatable precursors for *N*-nitrosamide formation, such as amides and amide-like compounds [23], including ureas and guanidines [24, 43, 54, 55]. For example, meat contains high concentrations of the guanidines creatine and creatinine [54]. As mentioned above, preformed *N*-nitrosamides are unstable and unlikely to exist in large quantities in food. Therefore, accurate measurement and identification is difficult to achieve. However, potential *N*-nitrosamide precursors in food can be estimated by *in vitro* experiments under extreme chemical conditions (low/acidic pH and excessive nitrite concentrations). For this, food is treated with nitrite and the resulting *N*-nitrosamides are then measured. Laboratory experiments on food conducted by Mirvish *et al.* revealed that treatment of fried bacon and salted dried fish with a large excess of nitrite under extreme acidic conditions resulted in the formation of methylurea from creatinine. Methylurea is an alkylurea and alkylureas are readily converted to

*N*-nitroso-alkylureas (e.g., methylnitrosourea, MNU) [25, 56] when nitrosated in laboratory experiments (see Table 2).

Mende *et al.* investigated the amount of methylnitrosourea (MNU) equivalents formed in food under chemical conditions. (Reporting a result in the form of equivalents is commonly done when the exact chemical structure of the compound of interest cannot be identified with the analytical method available but it is certain that it belongs to, e.g., the *N*-nitrosamide group). Under chemical conditions, processed fish and meat products had the highest levels of MNU equivalents and plant products had the lowest [57] (see Table 2). Recently, Sen *et al.* developed a method for the determination of MNU from cured meats combined with spectrometric confirmation [58]. From this method, new data on confirmed MNU formation from food have finally been published [59] and indicate that precursors of *N*-nitrosamides actually do exist in food.

#### *Endogenous formation of N-nitrosoureas*

To estimate the endogenous formation of *N*-nitrosamides in the human stomach, food has been nitrosated in the laboratory under simulated gastric conditions which include slightly less acidic pH than used in experiments under chemical conditions, combined with physiologic concentrations of nitrite. This method has also been applied in the studies by Sen *et al.* [58, 59] and Mende *et al.* [57] mentioned above, and MNU activity was found in cured meats, fish and plant products as indicated by the formation of low but detectable levels of MNU. This suggests that gastric conditions can lead to the formation of at least moderate amounts of MNU endogenously.

As mentioned earlier, creatine and creatinine can be metabolized *in vivo* to ureas by enzymatic de-imidation reactions. Several nitrosated ureas have been detected in human urine [54] which suggests the possibility that compounds susceptible to nitrosation are present *in the body*.

Results from an *animal study* conducted by Maragos *et al.* suggested that micromolar amounts of *N*-nitroso-trimethylurea (a *N*-nitrosoalkylurea) can be endogenously formed in the pig stomach after administration of the amide precursor trimethylurea and nitrite. Nitrite was administered in a concentration similar to human intra-gastric nitrite concentrations [60]. Mirvish and Chu [61, 62] conducted experiments on endogenous *N*-nitrosoalkylurea formation in rats by adding nitrite and the *N*-nitroso-precursors methyl- and dimethylurea to food and drinking water. The majority of the rats developed tumors, mostly of the CNS. This finding

indicates that *N*-nitrosamides formed in the stomach seem to cross the blood brain barrier and reach the brain.

Shephard *et al.* assessed the risk of endogenous NOC formation from dietary precursors in the human stomach using a mathematical model [54]. Results from these studies indicate that nitrosation of food precursors in the stomach could represent a potentially important source of endogenously formed *N*-nitrosamides including *N*-nitrosoureas.

In summary, experimental data to date suggest that *N*-nitrosamides can cause tumors of the central nervous system, and that human exposure to *N*-nitrosamides can result from direct ingestion of foods that contain *N*-nitrosamide precursors and from endogenous formation in the stomach during the digestive process, as shown in studies in rats [61].

### Epidemiological studies

Studies have addressed three principal hypotheses in relation to dietary intake by the mother (during pregnancy) or child and risk of CBT: cured meat intake; vegetable intake as *precursors* of nitrite; and intake of fruits, vegetables and vitamin supplements as *inhibitors* of nitrosation. All the epidemiological studies carried out to date are case-control studies.

#### *Limitations of existing epidemiological studies*

There are several limitations to the studies we have detailed in this report, primarily the reliance on recall in case-control studies. Differential recall can result in biased risk estimates. For example, case mothers may recall and/or report exposure differently than control mothers because of the experience of having a sick child. This is a particular concern when the exposure is perceived to be unhealthy, and cured meats seem likely to fall into that category. Some studies have attempted to evaluate this type of bias, but doing so is logistically difficult. A small study by Wilkins and Bunn compared diet recall between case and control mothers for two time periods, 4.5 years apart, and found no significant case-control differences for recall of dietary nitrite or nitrate for either mother's diet during pregnancy or for child's diet [63]. This study indicates that there is little evidence that such bias exists for the association of maternal diet and childhood brain tumor. However, using controls with serious health conditions thought to be not causally associated with diet may minimize the chance for recall bias in studies of dietary intake of NOC and brain tumor risk but, to date, no such studies

have been reported, and use of such controls may introduce other biases.

The potential for biased control selection is another limitation to the case-control study approach. Study participants from a control population tend to be more educated therefore have higher income and thus may have higher socioeconomic status (SES) than non-participants [64]. This is especially true when random digit dialing is used for control selection [65]. Of the studies we reviewed, five [66–70] used random digit dialing but did adjust for SES analytically. Nonetheless, if cured meat consumption is associated with lower SES, risk estimates from these studies could be artificially inflated by residual, uncontrolled SES confounding. Studies currently under analysis should be able to evaluate whether or not cured meat intake relates to SES.

All but one of the studies reviewed, on both maternal and child's diet did not use full-length dietary questionnaires covering a wide range of foods. Shortened food lists were used in the interest of brevity, but important foods may have been omitted, such as fruits and vegetables and sources of antioxidants (*e.g.*, fortified foods). Further, three studies [67, 71, 72] did not report on use of vitamin supplementation. These omissions result in an inability to control for the seemingly protective effects of fruits, vegetables, and vitamins. As pointed out by Blot *et al.* if these dietary components are inversely related to dietary NOC intake, the elevated risk estimates that have been observed for NOC could be the result of uncontrolled confounding [73].

#### *Mother's diet*

##### *Maternal intake of cured meat during pregnancy*

We reviewed 10 major epidemiological studies [9, 66–72, 74, 75] that have examined the role of maternal consumption of cured meats during pregnancy. Specific cured meats investigated included ham, bacon, hot dogs, sausage, and lunch meat. Analyses were reported for individual cured meat types, all cured meats combined, and/or both.

Eight [9, 66–71, 74] of the ten studies found a significant positive association between the frequency of maternal intake of cured meat (individual or combined cured meats) and the risk of CBT (see Table 3). The odds ratios (OR) for moderate to high cured meat intakes ranged from 1.1 to 2.3, with one study reporting an OR of 6.04 [71]. Seven of the eight were from the US [9, 66–71] and the eighth was from Australia [74]. In the study by Bunin *et al.* [69] a significant interaction between cured meat consumption and household income was found, with an increased risk of CBT observed only in the low income group. The authors discussed in detail

Table 3. Overview of epidemiological case-control studies on childhood brain cancer and MATERNAL diet during pregnancy

Publications and place of study	Tumor group studied	Dates of diagnosis and age of children included in study	Number of cases and controls	Results
Preston-Martin <i>et al.</i> (1982) [9] US (Los Angeles county)	Childhood brain tumors	Diagnosed during 1972–1977 0–14 years old	209 cases 209 controls	<p><b>Cured meats</b></p> <ul style="list-style-type: none"> <li>– OR = 2.3 for <i>high</i> consumption of all <i>cured meats</i> combined (<math>\geq 2</math> times per week);</li> <li>– OR = 1.2 for <i>moderate</i> consumption of all <b>cured meats</b> combined (once per month to once per week) (trend <math>p = 0.008</math>; reference category: “low cured meat intake”).</li> </ul> <p><b>Vegetables, fruits and fruit juices</b></p> <ul style="list-style-type: none"> <li>– No significant association between maternal consumption of any of six <b>vegetables</b> high in nitrates and CBT.</li> </ul> <p><b>Vitamin supplements</b></p> <ul style="list-style-type: none"> <li>– OR = 0.6 (<math>p = 0.12</math>) for consumption of <b>vitamin supplements</b> during pregnancy (reference category: “no vitamin use”).</li> </ul>
Preston-Martin <i>et al.</i> (1996) [66] US (West Coast)	Childhood brain tumors	Diagnosed during 1984–1991 0–19 years old	540 cases 801 controls	<p><b>Cured meats</b></p> <ul style="list-style-type: none"> <li>– OR = 2.1 (95% CI: 1.3–3.2) for <i>cured meat</i> consumption <math>&gt; 7</math> times per week;</li> <li>– OR = 1.4 (95% CI: 1.0–2.0) for <i>cured meat</i> consumption 4–7 times per week (trend <math>p = 0.003</math>; reference category: “never consumed cured meat”).</li> </ul> <p><b>Vegetables, fruits and fruit juices</b></p> <ul style="list-style-type: none"> <li>– OR = 1.0 (95% CI: 0.7–1.3) for highest quartile of consumption of <i>nitrate</i> from <i>vegetables</i> (<math>p = 0.97</math>; reference category: “lowest quartile”).</li> </ul> <p><b>Vitamin supplements</b></p> <ul style="list-style-type: none"> <li>– Daily use of <i>prenatal vitamins</i> throughout pregnancy decreased risk: OR = 0.5 (95% CI: 0.4–0.8) (trend <math>p = 0.004</math>; reference category: “never took daily”).</li> </ul>
Schymura <i>et al.</i> (1996) (abstract only) [71] US (New York)	Childhood brain tumors	Timeframe of diagnosis not reported 0–14 years old	338 cases 676 controls	<p><b>Cured meats</b></p> <ul style="list-style-type: none"> <li>– OR = 1.3 (95% CI: 1.0–1.8) for <i>hot dogs</i> consumption once per week; – OR = 2.0 (95% CI: 1.1–3.7) for <i>hot dogs</i> consumption 2–3 times per week; – OR = 6.0 (95% CI: 1.9–19.3) for <i>other cured meats</i> consumption once per week; (reference: <math>&lt; \text{once/week}</math>).</li> </ul> <p><b>Vegetables, fruits and fruit juices</b></p> <ul style="list-style-type: none"> <li>– OR = 0.4 (95% CI: 0.1–1.0; trend <math>p = 0.06</math>) for highest quartile of consumption of <i>vegetables</i>;</li> <li>– OR = 1.5 (95% CI: 0.6–3.7; trend <math>p = 0.4</math>) for highest quartile of consumption of <b>fruit</b>; (reference categories: “lowest quartiles”).</li> </ul>
McCredie <i>et al.</i> (1994) [74] Australia	Childhood brain tumors and cranial nerve tumors	Diagnosed during 1985–1989 0–14 years old	82 cases 164 controls	<p><b>Cured meats</b></p> <ul style="list-style-type: none"> <li>– OR = 2.5 (95% CI: 1.1–5.7) for highest quartile of consumption of <i>cured meats</i> (trend <math>p = 0.01</math>; reference category: “lowest quartile”).</li> </ul> <p><b>Vegetables, fruits and fruit juices</b></p> <ul style="list-style-type: none"> <li>– OR = 0.4 (95% CI: 0.1–1.0; trend <math>p = 0.06</math>) for highest quartile of consumption of <i>vegetables</i>;</li> <li>– OR = 1.5 (95% CI: 0.6–3.7; trend <math>p = 0.4</math>) for highest quartile of consumption of <b>fruit</b>; (reference categories: “lowest quartiles”).</li> </ul>

Table 3. (Continued)

Publications and place of study	Tumor group studied	Dates of diagnosis and age of children included in study	Number of cases and controls	Results	Cured meats	Vegetables, fruits and fruit juices	Vitamin supplements
Cordier <i>et al.</i> (1994) [75] France	Childhood brain tumors	Diagnosed during 1985–1987 0–15 years old	75 cases 113 controls		<p>– OR = 1.0 (95% CI: 0.4–2.7) for <i>ham</i>; – OR = 0.6 (95% CI: 0.2–1.8) for <i>sausage</i>; – OR = 0.6 (95% CI: 0.3–1.4) for <i>salami</i> and <i>pate</i>; when consumed at least once a week (reference categories: “less than once a week”).</p> <p>– OR = 1.1 (95% CI: 0.6–2.0; trend <math>p = 0.8</math>) for highest quartile of <i>total cured meats</i> intake (reference category: “lowest quartile of intake”). – OR = 1.7 (95% CI: 1.0–2.9) for consumption of <i>bacon</i> at least once per week (<math>p &lt; 0.05</math>; reference category: “less than once per week”).</p>	<p>– OR = 0.3 (95% CI: 0.1–1.0) for <i>peppers</i> (<math>p</math> trend <math>&lt; 0.02</math>); – OR = 0.8 (95% CI: 0.3–1.9) for <i>apples</i>, – OR = 0.5 (95% CI: 0.2–1.1) for <i>orange juice</i>, when consumed at least once a week (reference categories: “less than once a week”).</p> <p>– OR = 0.4 (95% CI: 0.2–0.7; trend <math>p = 0.005</math>) for highest quartile of frequency of <i>vegetable</i> intake (reference category: “lowest quartile of intake”). – OR = 0.3 (95% CI: 0.1–0.6; trend <math>p = 0.003</math>) for highest quartile of frequency of <i>fruit and fruit juice</i> intake (reference category: “lowest quartile of intake”).</p>	<p>– OR = 0.6 for any consumption of <i>multivitamin supplements</i> during first 6 weeks of pregnancy (<math>p = 0.02</math>; reference category: “no consumption”).</p>
Bunin <i>et al.</i> (1993) [68] US and Canada	Medulloblastoma / Primitive neuro-ectodermal brain tumors	Diagnosed during 1986–1989 0–6 years old	166 cases 166 controls		<p>– OR = 1.7 (95% CI: 0.8–3.4; trend <math>p = 0.10</math>) for highest quartile of frequency of <i>cured meats</i> intake (reference category: “lowest quartile of intake”). – OR = 2.3 (95% CI: 0.8–6.3; trend <math>p &lt; 0.05</math>) for highest quartile of frequency of <i>cured meats</i> intake in low income group (reference category: “lowest quartile of intake”).</p>	<p>– OR = 0.7 (95% CI: 0.3–1.4; trend <math>p = 0.3</math>) for highest quartile of frequency of <i>fruit and fruit juice</i> intake (reference category: “lowest quartile of intake”). – OR = 0.6 (95% CI: 0.3–1.3; trend <math>p = 0.3</math>) for highest quartile of frequency of <i>vegetable</i> intake (reference category: “lowest quartile of intake”).</p>	<p>– OR = 0.3 (95% CI: 0.1–1.3) in low income group and 1.3 (95% CI: 0.4–4.7) in high income groups for any <i>multivitamin supplement</i> use. (<math>p &lt; 0.10</math> for interaction with income; reference category: “no supplement use”).</p>
Bunin <i>et al.</i> (1994) [69] US and Canada	Astrocytic glioma	Diagnosed during 1986–1989 0–6 years old	155 cases 155 controls				

Table 3. (Continued)

Publications and place of study	Tumor group studied	Dates of diagnosis and age of children included in study	Number of cases and controls	Results	Cured meats	Vegetables, fruits and fruit juices	Vitamin supplements
Kubin <i>et al.</i> (2000) [72] Israel	Childhood brain tumors	Diagnosed during 1984–1993 0–18 years old	300 cases 574 controls		– OR = 1.1 (95% CI: 0.8–1.6; trend $p = 0.5$ ) for high total <i>cured meats</i> intake (reference category: “low intake”).	– OR = 1.3 (95% CI: 0.8–1.9; trend $p = 0.4$ for high <i>vegetable</i> intake. – OR = 1.2 (95% CI: 0.7–1.8; trend $p = 0.5$ ) for high <i>fruit</i> intake (reference category: “low intake”).	
Kuijten <i>et al.</i> (1990) [67] US (Delaware Valley)	Astrocytic gliomas	Diagnosis during 1980–1986 0–14 years old	163 cases 163 controls		– OR = 2.0 (trend $p = 0.04$ ) for frequent ( $\geq 9$ times per week) total <i>cured meat</i> intake (reference category <9 times/week)		
Sarasua and Savitz (1994) [70] US (Denver)	Brain tumor	Diagnosed during 1976–1983 0–14 years old	45 cases 206 controls		– OR = 2.3 (95% CI: 1.0–5.4; no trend $p$ -value provided) for <i>hot-dog</i> consumption $\geq$ once/week (reference category: < once/week). – OR = 1.0 (95% CI: 0.5–2.1; no trend $p$ -value provided) for <i>ham, bacon, sausage</i> $\geq$ once/week (reference category: < once/week). – OR = 0.4 (95% CI: 0.2–0.8; no trend $p$ -value provided) for <i>lunch meat</i> consumption $\geq$ once/week (reference category: < once/week). – OR = 2.2 (95% CI: 0.6–8.1; no $p$ -value provided) for hot dog consumption $\geq$ once/week combined with <i>no vitamin supplement</i> use (reference category: <1 hot dog/week + no vitamin use)		

RR = relative risk; OR = odds ratio; statistical significance is defined as  $p < 0.05$ .

several possible explanations for this finding, such as selection bias and random variation [69].

The two studies that failed to find an association were studies from France and Israel [72, 75]. The small study from France observed non-significant reduced risk for several types of cured meats [75]. The study from Israel [72] did not find an association with cured meats/sausages or with nitrate or nitrite intake.

#### *Maternal intake of vegetables*

Vegetables contribute to the dietary intake of nitrate which is a precursor in endogenous NOC formation. Nitrate, which can be reduced to nitrite by bacteria in the saliva, can act as a nitrosating agent *in vivo* in the stomach. Only about 5% of ingested nitrate is reduced to nitrite via this pathway [33]. Vegetables high in nitrate include spinach, collards, turnip greens, eggplant, beets, celery, and radishes. Vegetables also provide nitrosation-inhibiting antioxidants such as vitamins C and E. Mirvish points out that the excess amount of vitamin C in vegetables generally causes them to be endogenous nitrosation inhibitors rather than promoters [22]. Only a few vegetables, such as beets and celery, have especially high nitrate but low ascorbate contents [76]. Because of the well-established role of vitamin C in inhibiting the nitrite to NOC reaction, several studies have examined the association between maternal intake of vegetables and risk of CBT. None of the seven studies investigating vegetables found positive associations [9, 66, 68, 69, 72, 74, 75]. Four [68, 69, 74, 75] of these studies found reduced risks related to vegetable intake; in two studies the relationship was significant [68, 75] and in one study the relationship approached significance [74]. Preston-Martin *et al.* also investigated nitrate intake from vegetables and did not find an association with CBT risk in either direction [66]. The remaining three studies found no association between CBT risk and vegetables [9, 66, 72].

#### *Maternal intake of fruit*

Three of five studies [68, 69, 72, 74, 75] that asked questions on *maternal* fruit and/or fruit juice intake observed decreased risk [68, 69, 75], which was significant in only one of the studies [68].

#### *Maternal intake of antioxidants from foods and from vitamin supplements*

Only four studies estimated maternal intake of antioxidant nutrients from the diet (*e.g.*, vitamins C and E) [68, 69, 72, 75], and two found a protective relationship [68, 69]. One found no association with dietary vitamin C and E [75] and one [72] found a significant positive

association with dietary vitamin E. None summed the antioxidants in foods with those provided by supplements, and none reported fruits and vegetables separately in non-users of vitamin supplements. In addition, none of the studies investigated other sources of antioxidants, such as fortified cereals or fortified drinks.

Four studies examined the association between vitamin supplements and CBT risk [9, 66, 68, 69]. All four found a reduced risk associated with supplement use, and in two of the four the association was significant [66, 68]. In addition, a multi center international study [77, 78] found a strong and highly significant effect, with risk declining linearly with increasing duration of supplement use during pregnancy; these findings were largely driven by the large US component of this study [66].

In summary, most studies observed reduced CBT risk associated with antioxidant intake from vegetables, fruits, and vitamin supplements. Further, vegetable intake or nitrate intake from vegetables does not appear to be a risk factor for CBT. This is consistent with findings from several studies on stomach cancer (the stomach is where endogenous formation of NOC from ingested nitrate would take place), which have consistently shown that the consumption of fresh vegetables is associated with decreased risk [76, 79, 80].

#### *Child's diet*

##### *Child's intake of cured meat*

We reviewed seven studies that examined children's consumption of cured meats in relation to CBT risk (see Table 4) [9, 69, 70, 72, 75, 81, 82]. One study found a statistically significant positive association [9] and results from two studies [70, 81] suggested a positive association with the odds ratios ranging from 2.0 to 2.3 for moderate to high consumption. One study found a suggestive but non-significant reduced risk related to intake of cured meats [75]. In the study by Preston-Martin *et al.*, the child's consumption of cured meats had little effect on CBT risk once the correlation between mother's and child's diet was taken into account [9]. McCredie *et al.* [81] found a suggestive increased risk with child's "high" cured meat intake (OR, 2.0, 95% CI, 0.5–7.1) but very few subjects were exposed at this level (9 cases, 9 controls). Sarasua and Savitz found a significant interaction between cured meats and vitamin supplement use, with increased risk only among nonusers of supplements. However, the sample sizes in these subgroups were small (6–9 subjects) resulting in wide confidence intervals [70]. Bunin *et al.* [69] did not observe any noteworthy associations between child's intake of cured

Table 4. Overview of epidemiological case-control studies on childhood brain cancer and CHILD's diet

Publications and place of study conduction	Tumor group studied	Dates of diagnosis and age of children included in study	Number of cases and controls	Results	
				Cured meats	Vegetables, fruits and fruit juices Vitamin supplements
Preston-Martin <i>et al.</i> (1982) [9] US (Los Angeles county)	Childhood brain tumors	Diagnosed during 1972-1977 0-14 years old	209 cases 209 controls	- Child's consumption of <i>cured meats</i> associated with tumor development: OR = 2.3 (high consumption) (trend $p = 0.01$ ; reference category: "low consumption"). But highly correlated to mother's diet; once this was taken into account, childhood consumption had little effect. - OR = 0.7 (95% CI: 0.2-3.0) for ever consumed any <i>cured meats</i> (reference category: never consumed cured meats).	
Cordier <i>et al.</i> (1994) [75] France	Childhood brain tumors	Diagnosed during 1985-1987 0-15 yrs old	75 cases 113 controls		- OR = 0.6 (95% CI: 0.1-3.0) for ever consumed <i>fruit</i> and OR = 0.5 (95% CI: 0.1-2.0) for never consumed <i>orange juice</i> (reference categories: ever consumed). Investigated child's intake of four <i>fruits and fruit juices</i> . No significant associations observed. - OR = 1.3 (95% CI: 0.8-2.3; trend $p = 0.2$ ) for high <i>vegetable</i> intake, - OR = 1.2 (95% CI: 0.8-2.1; trend $p = 0.4$ ) for high <i>fruit</i> intake (reference categories: low intake).
Bunin <i>et al.</i> (1994) [69] US and Canada	Astrocytic glioma	Diagnosed during 1986-1989 0-6 years old	155 cases 155 controls	Investigated child's intake of six <i>cured meats</i> . No significant associations observed. - OR = 1.0 (95% CI: 0.7-1.5; trend $p = 0.9$ ) for high total <i>cured meat</i> intake (reference category: "low intake")	Investigated child's intake of <i>vitamin supplements</i> . No significant associations observed.
Lubin <i>et al.</i> (2000) [72] Israel	Childhood brain tumors	Diagnosed during 1984-1993 0-18 years old	300 cases, 574 controls		
Sarasua and Sawitz (1994) [70] US (Denver)	Brain tumor	Diagnosed during 1976-1983 0-14 years old	45 cases 206 controls	- OR = 2.1 (95% CI: 0.7-6.1; no $p$ -value provided) for <i>hot dog</i> consumption $\geq$ once/week (reference category: <once/week). - OR = 6.8 (95% CI: 2.5-18.5; no $p$ -value provided) for <i>hot dog</i> consumption $\geq$ once/week combined with <i>no vitamin supplement use</i> (reference category: hot dog consumption < once/week + no supplementation).	

Table 4. (Continued)

Publications and place of study and conduct	Tumor group studied	Dates of diagnosis and age of children included in study	Number of cases and controls	Results	Cured meats	Vegetables, fruits and fruit juices	Vitamin supplements
McCredie et al. (1994) <sup>a</sup> [81] Australia	Childhood brain tumor	Diagnosed during 1985–1989 0–14 years old	82 cases 164 controls	– OR = 2.0 (95% CI: 0.5–7.1; no trend <i>p</i> -value provided) for high cured meat consumption (reference category: “no consumption”). – RR = 1.1 (95% CI: 0.6–2.3; <i>p</i> = 0.7) for cured meat consumption > 1 serving per week (reference category: ≤ 1 serving per week) – RR = 1.1 (95% CI: 0.7–1.8; trend <i>p</i> = 0.6) per serving/day.	– OR = 0.4 (95% CI: 0.1–1.1; no trend <i>p</i> -value provided) for high blended or solid fruit consumption (reference category: “no consumption”). – RR = 0.2 (95% CI: 0.1–0.6; <i>p</i> = 0.009) for fruit juice consumption of > 1 glass per week (reference category: ≤ 1 glass per week) – RR = 0.9 (95% CI: 0.4–1.9; trend <i>p</i> = 0.8) for vitamin C supplement consumption of > 1 per week (reference category: ≤ 1 per week) – RR = 0.8 (95% CI: 0.4–1.9; trend <i>p</i> = 0.6) per 100 mg/day.	– OR = 0.5 (95% CI: 0.2–1.4; no trend <i>p</i> -value provided) for high vitamin syrup consumption (reference category: “no consumption”). – RR = 0.9 (95% CI: 0.4–1.9; trend <i>p</i> = 0.8) for vitamin C supplement consumption of > 1 per week (reference category: ≤ 1 per week) – RR = 0.8 (95% CI: 0.4–1.9; trend <i>p</i> = 0.6) per 100 mg/day.	
Howe et al. (1989) <sup>a</sup> [82] Canada (Toronto)	Childhood brain tumor	Diagnosed during 1977–1983 0–19 years old	74 cases 138 controls	– RR = 1.1 (95% CI: 0.6–2.3; <i>p</i> = 0.7) for cured meat consumption > 1 serving per week (reference category: ≤ 1 serving per week) – RR = 1.1 (95% CI: 0.7–1.8; trend <i>p</i> = 0.6) per serving/day.	– OR = 0.4 (95% CI: 0.1–1.1; no trend <i>p</i> -value provided) for high blended or solid fruit consumption (reference category: “no consumption”). – RR = 0.2 (95% CI: 0.1–0.6; <i>p</i> = 0.009) for fruit juice consumption of > 1 glass per week (reference category: ≤ 1 glass per week) – RR = 0.9 (95% CI: 0.4–1.9; trend <i>p</i> = 0.8) for vitamin C supplement consumption of > 1 per week (reference category: ≤ 1 per week) – RR = 0.8 (95% CI: 0.4–1.9; trend <i>p</i> = 0.6) per 100 mg/day.	– OR = 0.5 (95% CI: 0.2–1.4; no trend <i>p</i> -value provided) for high vitamin syrup consumption (reference category: “no consumption”). – RR = 0.9 (95% CI: 0.4–1.9; trend <i>p</i> = 0.8) for vitamin C supplement consumption of > 1 per week (reference category: ≤ 1 per week) – RR = 0.8 (95% CI: 0.4–1.9; trend <i>p</i> = 0.6) per 100 mg/day.	

<sup>a</sup> Investigated child's diet only; RR = relative risk; OR = odds ratio; statistical significance is defined as *p* < 0.05.

meats and CBT risk. In some of the studies respondents were queried on only a small number of food items and thus the child's diet may not have been accurately assessed.

#### Child's intake of antioxidants from foods and vitamin supplements

Two [81, 82] out of five studies [69, 72, 75, 81, 82] that investigated child's intake of fruit or fruit juice found an association with reduced risk, with one study being statistically significant [82]. None of the studies investigated other sources of antioxidants, such as fortified cereals or fortified soft drinks. With regard to child's vitamin supplement intake, one [75] out of four studies [69, 75, 81, 82] observed significantly reduced risk and one observed non-significant decreased risk for high vitamin syrup intake [81]. The remaining two studies [69, 82] observed no association between child's intake of vitamin supplements and risk of CBT.

#### Discussion

Hypotheses regarding dietary factors in CBT risk have focused on cured meats and associated NOC, and on antioxidant inhibitors of nitrosation and their fruit and vegetable sources. Of these, the association with cured meats has received the most investigation and the most support from case-control studies. Eight of 10 case-control studies found significant positive associations with maternal cured meat intake. Less evidence exists for an association with child's cured meat intake, and there is no evidence of a positive association with nitrate from vegetables in maternal diet. However, considerable evidence suggests that antioxidants are associated with decreased risk of CBT. One study assessed the levels of nitrate in household drinking water, another source of nitrite, but found that they were uniformly low and were unrelated to risk of CBT [66]. Drinking water has been reported to only be a major source of nitrite if its levels of nitrate are extraordinarily high [83].

It should be noted that brain tumors include a variety of histological types which differ in sex ratio, age at onset, etc. and therefore might also differ in their etiologies. All but three [67–69] of the studies reviewed here have investigated the association of cured meat intake with CBT combining all histological types of brain tumors and most have found elevated risk. Three studies have investigated the association of certain histologic types of CBT (e.g., astrocytoma or primitive neuroectodermal brain tumor) with maternal dietary intake of cured meats. For astrocytic gliomas, cured meat intake was associated with elevated and significant or nearly signif-

icant risk [67, 69]. For PNET one study found an elevated but non-significant risks for total cured meat, although risk was significantly elevated for some types of cured meat [68]. Therefore, the epidemiologic data available to date suggests that risk does not differ by tumor histology. However, future epidemiological studies should investigate the association of cured meat intake and CBT by tumor type, if sample size allows this.

Most of the epidemiological studies tried to investigate the association between CBT risk and dietary intake of cured meats considering only *N*-nitrosamines as the causative compounds. Only one of those studies mentioned the possibility that *N*-nitrosamides actually could be responsible for the association seen [66]. The observed associations of maternal cured meat intake and increased risk of CBT may not be explainable with the *N*-nitrosamine hypothesis, but rather with a *N*-nitrosamide hypothesis. As noted above in the review of the chemistry of NOC, *N*-nitrosamines have not produced tumors of the brain or spinal cord in animal studies. Given the chemical characteristics and the record of carcinogenicity from laboratory and animal studies of those two groups of NOC, it seems clear that the *N*-nitrosamides should be investigated more closely with regard to CBT.

Perhaps even more important than *N*-nitrosamides versus *N*-nitrosamines is the issue of endogenous formation. Laboratory results strongly suggest that *N*-nitrosoureas can be formed endogenously in the acidic medium of the stomach. Therefore, endogenous formation of *N*-nitrosamides is a topic that needs more research attention. Very few studies have attempted to collect information about the conditions that permit nitrosation in the body. The only study that considered endogenously formed *N*-nitrosamides, was conducted by Preston-Martin *et al.* [66]. However, the authors note that they were unable to evaluate sources of exposure to alkylamides since no data on alkylamide contents in food was available. To investigate if endogenous formation of *N*-nitrosamides could be associated with CBT in epidemiologic studies, knowledge about the alkylamide content of food would be required. Only with that information can it become feasible to investigate this question. If more extensive laboratory data would exist on *N*-nitrosamide precursors in various food items, estimations on potential endogenous formations could be made. Another approach would be to use hemoglobin adducts as biomarkers for alkylnitrosourea formation resulting from the consumption of cured meats which has been investigated by Gurney *et al.* [84]. Although the results from that pilot study were negative, continued research in that field might lead to the development of useful

biomarkers in the future. With regard to endogenous formation of NOC in general, information about the consumption of mixed meals needs to be collected in epidemiological studies. For example, if nitrites from cured meats were consumed, were inhibitors present in the stomach at the same time? Was orange juice consumed with bacon? Were antioxidant vitamin supplements or fruits/vegetables consumed with hot dogs? Such information would need to be collected in future studies.

It is known that *N*-nitrosamides act at the site of their occurrence. Thus if they are formed endogenously in the stomach, they might cause stomach cancer. This has been shown by Ohgaki *et al.* [85] who reported methylation of DNA in the digestive tract of rats chronically exposed to *N*-nitroso-*N*-methylurea. However, the *N*-nitrosamides formed endogenously in the mother's stomach could also be transported transplacentally to the brain of the embryo or fetus, passing the blood brain barrier, and resulting in brain tumor development in the child. This has been demonstrated in animal studies [41, 84] using rats, hamsters and minipigs. Rodent and human placentas are very similar in their anatomy, and *N*-nitrosamides pass the blood-brain barrier in humans as well.

It also could be hypothesized that precursors from ingested food are transported transplacentally to the brain of the embryo where they then form cancerous *N*-nitrosamides on site, in the embryonic brain. However, the prevailing pH of 7.32 [86] in cerebrospinal fluid and the presence of antioxidants such as vitamin C [87] would not necessarily favor this formation.

In summary, a plausible hypothesis on the pathway of formation of brain tumors in the offspring after *in utero* exposure to NOC is that *N*-nitrosamides – either preformed or their precursors – are transported to the fetus transplacentally [88]. There they may cause tumors only in the brain because of suggested organ specificity of *N*-nitrosamides [22]. Other potential reasons for the brain tumor specificity are the fact that fetal brain cells are rapidly dividing during early stages of pregnancy and are believed to be highly sensitive to neoplastic changes [89], and/or because fetal brain tissue lacks alkyltransferase which repairs DNA adducts [90–92].

## Conclusion

*N*-nitrosamides, rather than *N*-nitrosamines, are likely to be the group of NOC that can cause brain tumors in human fetuses. Only *N*-nitrosoureas (which are *N*-nitrosamides) have caused brain tumors in rodents and

monkeys whereas *N*-nitrosamines have caused cancer in various animal species in a variety of organs but never in the brain. The epidemiologic literature is quite consistent in finding increased risk of CBT with maternal intake of cured meats. However, further clarification will be enhanced by the collection of dietary data on the full range of foods, fortified foods and supplements; by increased data on *N*-nitrosamide precursors in foods; by attempts to quantify the potential for endogenous formation by identifying the simultaneous ingestion of nitrites, the protein components they interact with (such as amides, ureas and guanidines), and antioxidants; and by consideration of potential confounders and effect modifiers, particularly socio-demographic factors and antioxidant or fruit/vegetable intake.

### Dedication

We dedicate this manuscript to the memory of Dr Willie Lijinsky who died in February 2004. Willie had a long and distinguished career focusing on chemical carcinogenesis and cancer prevention. His work provided many insights that were critical in epidemiologic studies of cancer. For example, he was the first to show that polycyclic aromatic hydrocarbons were present in char-broiled meat. Most of his career focused on *N*-nitroso compounds. He was one of the first to demonstrate the formation of nucleic acid adducts from a carcinogen, dimethylnitrosamine. His comprehensive studies on the carcinogenicity of *N*-nitroso compounds still form the basis of mechanistic investigations which continue to elucidate tissue specific carcinogenic effects of these compounds. His studies on nitrosation of common drugs were also ahead of their time and demonstrated the potential carcinogenic hazards of some pharmaceuticals. Perhaps most importantly, he was a passionate advocate for chemical approaches to cancer prevention and his voice will be greatly missed.

He also provided inspiration at the outset to my (SPM) interest as an epidemiologist in investigating a possible association between exposure to *N*-nitroso compounds (NOC) and the development of brain neoplasms in humans and encouraged my decision to focus this quest on transplacental exposure to NOC and the subsequent development of childhood brain tumors (CBT) in the offspring of exposed pregnant women. After Willie retired we (SPM and JMP) approached him to see if he would be willing to be a consultant to help us to analyze in a more appropriate way our complex epidemiologic data from both the US West Coast CBT and the international collaborative CBT studies. Willie's input was essential to enable us to understand not only

what chemical compounds might be formed endogenously when pregnant women ate various food and took various medications, but more importantly how potentially carcinogenic these various compounds might be to the brain of the developing fetus.

Willie, thank you for your guidance and inspiration. It was indeed a pleasure working with you and getting to know you personally. You were an outstanding colleague, and we feel honored to have known you.

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