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Original Article

Construction of an N-nitroso database for assessing dietary intake

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ABSTRACT

Dietary N-nitroso compounds are carcinogens synthesized during food processing from two main classes of precursors, oxides of nitrogen and amines or amides. Quantification of the dietary intake of N-nitroso compounds is significant to human cancers, including those of the stomach and upper gastro-intestinal tract, colon, and brain. Previous studies investigating these cancers primarily used proxy estimates of N-nitroso intake and not a full and complete database. In this report, we describe the development of a database to be used in conjunction with a food frequency questionnaire (FFQ) or 24 h dietary records. Published analytical data for N-nitroso compounds were compiled and evaluated for inclusion in the database. The final database consisted of 23 different N-nitroso compounds for 500 foods from 39 different food subgroups. Next, database foods were matched to foods in a standard FFQ by imputation, or calculated value, or assumed zero. Using the FFQ modified with N-nitroso values, we evaluated the ability to compute N-nitroso intakes for a sample of healthy control subjects of cancer epidemiological studies. N-nitroso content of food items ranged from <0.01 μg/100 g to 142 μg/100 g and the richest sources were sausage, smoked meats, bacon, and luncheon meats. The database is useful to quantify N-nitroso intake for observational and epidemiological studies.

1. Introduction

The mutagenic properties of N-nitroso compounds, recognized for many years, were first described in 1956 (Magee and Barnes, 1956) when rats fed N-nitroso compounds were observed to develop high rates of cancer. Approximately 300 N-nitroso compounds have been tested for carcinogenicity, 90% of those stimulated carcinogenesis in 40 animal species including higher primates, and at a variety of sites and organs (Forman, 1987; Lijinsky, 1990; Tricker and Preussmann, 1991). As a result, it was concluded that N-nitroso compounds are among the most potent dietary carcinogenic agents or chemicals.

Following the discovery of the carcinogenic potential of N-nitroso compounds in animals, analyses confirmed human exposure to N-nitroso compounds by identifying food sources with high concentrations in cured meats, bacon, smoked fish, and beer, summarized in Table 1. Processing conditions that promote formation of N-nitroso compounds include foods pickled, stored under humid conditions, smoked in air saturated with nitrogen, dried at high temperatures (i.e. protein-containing foods as beer ingredients, nonfat dry milk, cooked bacon, or dried meats), and cured with nitrate and/or nitrite (cured meat). Collectively, a wide array of foods and nearly all Western foods have since been assayed and shown to contain N-nitroso compounds. Currently, a growing interest in N-nitroso compounds and cancer is supported by scientifically sound reports that underscore the need for continuing surveillance and analysis of food products. For, example a comprehensive review on the advances of N-nitroso compounds within each scientific discipline was compiled by the European Cancer Prevention Organization (Reed, 1996a,b). Further, the possible role for N-nitroso compounds specific to pancreatic cancer risk (Risch, 2003), and the role of maternal intake of N-nitroso foods to pediatric brain cancer including a detailed background of N-nitroso compound chemistry and characteristics have been published (Dietrich et al., 2005).

The chemistry for the formation of N-nitroso compounds in foods is a complex process. In brief, the two main precursors of N-nitroso compounds are (1) oxides of nitrogen and (2) amino groups (either secondary amines or amides), together that react and form two major subgroups of N-nitroso compounds, N-nitrosamines and N-nitrosamides. The chemical properties and characteristics of N-nitroso compounds according to the two major subgroups have been described (Dietrich et al., 2005). Although efforts to reduce the amount of nitrite used to cure meat and other food sources has been instituted in the past two to three decades in most meats, naturally occurring precursors for N-nitroso compounds are present in foods and under appropriate conditions the precursors

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Table 1
N-nitroso compounds, examples of high food sources.

Compound abbreviation	Chemical nomenclature	High source foods
NDMA	N-nitrosodimethylamine	Beer, sausage, cured meats
NDEA	N-nitrosodiethylamine	Sausage, cheese
NPYR	N-nitrosopyrrolidine	Fried bacon, sausage, ham
NPIP	N-nitrosopiperidine	Bologna, sausages
NSAR	N-nitrosarcosine	Cured meats
NPRO	N-nitrosoproline	Fried bacon, cured meats
NTCA	N-nitrosotioazolidine-4-carboxylic acid	Smoked meats
NHMTCA	N-nitroso-2-hydroxymethylthiazolidine-4-carboxylic acid	Smoked meats
Nitrosating agents	Nitrate nitrite	Vegetables grains, additive to meats, other foods
Become nitrosated	Amines, amides	Protein foods

are synthesized to form N-nitroso compounds. Moreover, regulatory efforts to curtail nitrite and nitrosamines have not reduced the incidence of cancers related to their intake. Recent experiments investigated chemical conditions enhancing formation of *N-nitroso-N*-methylurea (NMU). In these experiments, a more sensitive assay was used including a specific analytical HPLC technique and a GC–MS confirmation technique, Results showed that 3 of twenty-four samples of meat with no added nitrites, formed 2–26 ng of NMU/10 g of meat, and later samples with added nitrite increased detection (Sen et al., 2000). Although the amounts of NMU formed with no added nitrite were extremely small, this report demonstrates the formation of NMU from cured meats with and without additional nitrite. From an international perspective it is important for food scientists and nutritional database specialists to maintain surveillance and awareness of N-nitroso compounds. For example, in parts of Asia, a very high incidence of esophageal, stomach and gastro-esophageal cancer occurs. Because of the frequent consumption in these cultures of aged and smoked fish sauces, smoked and aged vegetables, and vegetable sauces that are sources of high levels of N-nitroso compounds, these compounds are suspected to be related to these cancers.

Coinciding with the carcinogenic potential of the N-nitroso compounds, biologically plausible mechanisms responsible for their action have been identified. These investigations pinpoint three mechanisms for a genotoxic role of N-nitroso compounds in the promotion of cancer that include: (1) the formation of N-nitroso-DNA adducts, (2) loss of the ability to repair DNA damage and (3) base exchange of the K-ras oncogene (Bos, 1989; Palli et al., 2001; Silber et al., 1996; Souliotis et al., 2002). The first epidemiological study investigating the N-nitroso hypothesis was conducted for brain cancer in children in the late 1970s (Preston-Martin et al., 1982). Subsequent epidemiological studies examined the risk of N-nitroso compounds based on a proxy exposure (i.e. estimates of selected high food sources) to brain (Preston-Martin et al., 1989), stomach (La Vecchia et al., 1995), esophageal (Gao et al., 1994), and nasopharynx cancers. In a review on findings from 50 case control studies on the N-nitroso hypothesis and specific cancers (Eichholzer and Gutzwiller, 1998) one-third of the studies had statistical significance for detecting an association between a food group and cancer, with odds ratios ranging from 1.3 to 7.0. Despite these studies, human risk assessment of N-nitroso compounds for epidemiological studies or among racial/ethnic groups has been severely impeded by lack of a reliable N-nitroso database, lack of clarity in N-nitroso compounds in foods, and confusion in relation to the two classes of compounds, nitrosamines and nitrosamides (Dietrich et al., 2005). Accordingly, to address these gaps we report our research that created a comprehensive database of N-nitroso values in foods that can be used in conjunction with an existing food frequency questionnaire or with dietary food records.

2. Methods

2.1. Sources of N-nitroso database information

N-nitroso compounds constitute a family of compounds formed by the reaction of nitrogen oxides with secondary or tertiary amines or amides. Assays reported for N-nitroso compounds were identified by Medline and internet searches. The search terms most often used were N-nitroso, nitrates, nitrosamines, nitrosamides, nitrites, cured meats, ham, bacon, and combinations of these terms. Other publications on food composition and food assays were thoroughly searched including data of N-nitroso compound content of foodstuffs reported in epidemiological studies. Finally, reports from government agencies that investigated the N-nitroso compounds were incorporated in the database.

Analyses on N-nitroso compounds in foods had a peak of research conducted during 1970–1980. In most cases each reference reported analyses devoted to a specific food component and one or more N-nitroso compounds. More recent assays and reports were conducted in the 2000s (Groves et al., 2002; Mirvish et al., 2002, 2003; Sanches Filho et al., 2003; Zhou et al., 2006). Sen et al. have focused on N-nitroso food analyses, advancing our understanding with improvements in methodology and experimental procedures (Sen et al., 1990, 1997, 2000, 2001; Sen, 1991; Zhou et al., 2006). Several reviews have made substantial contributions to information on N-nitroso perspective of foods (Hecht, 1998; Lijinsky, 1988, 1992; Tricker, 1997; Walker, 1990).

2.2. Database product

Values for N-nitroso compounds in foods reported from references in tables or text were entered into a Microsoft Office Access 2003 database. Sources were documented with information on their sampling and handling plans for foods and beverages, the laboratory methods used to determine N-nitroso content, analytical quality control, cooking method, food descriptor and name, country of assay, number of samples used in the assay determination, unit of assay, and reference citation.

Because of the complexities of assays for the vast number of N-nitroso compounds, analysis by one laboratory (reference) on a food product was usually restricted to a few N-nitroso compounds, many foods did not have values reported for all compounds, as shown in Table 2. The final research core database consisted of 4301 entries for 23 N-nitroso compounds, for 500 foods, from 47 references. When similar foods were aggregated on the basis of common usage and nutrient composition 39 food subgroups were formed. Food subgroups consisted of those with high concentrations of N-nitroso compounds (sausage, ham, bacon, cured lunch meat, hotdog, organ meats, and beer), and foods with lower concentrations (vegetables, grains, fresh dairy, etc.).