Fd Chem. Toxic. Vol. 25, No. 1, pp. 91–108, 1987
Printed in Great Britain

0278-6915/87 $3.00 + 0.00
Pergamon Journals Ltd

Review Section

ASSESSMENT OF THE RISK OF FORMATION OF CARCINOGENIC *N*-NITROSO COMPOUNDS FROM DIETARY PRECURSORS IN THE STOMACH

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(*Received* 20 *December* 1985; *revisions received* 23 *April* 1986)

Summary—A literature review has shown that the daily intakes of various *N*-nitroso-precursor classes in a typical European diet span five orders of magnitude. Amides in the form of protein, and guanidines in the form of creatine and creatinine, are the nitrosatable groups found most abundantly in the diet, approaching levels of 100 g/day and 1 g/day, respectively. Approximately 100 mg of primary amines and amino acids are consumed daily, whereas aryl amines, secondary amines and ureas appear to lie in the 1–10 mg range. The ease of nitrosation of each precursor was estimated, the reactivities being found to span seven orders of magnitude, with ureas at the top and amines at the bottom of the scale. From this information and an assessment of the carcinogenicity of the resulting *N*-nitroso derivatives, the potential health risk due to gastric *in vivo* nitrosation was calculated. The combined effects of these risk variables were analysed using a simple mathematical model: Risk = [daily intake of precursor] × [gastric concentration of nitrite]^n × [nitrosatability rate constant] × [carcinogenicity of derivative]. The risk estimates for the various dietary components spanned nine orders of magnitude. Dietary ureas and aromatic amines combined with a high nitrite burden could pose as great a risk as the intake of preformed dimethylnitrosamine in the diet. In contrast, the risk posed by the *in vivo* nitrosation of primary and secondary amines is probably negligibly small. The risk contribution by amides (including protein), guanidines and primary amino acids is intermediate between these two extremes. Thus three priorities for future work are a comprehensive study of the sources and levels of arylamines and ureas in the diet, determination of the carcinogenic potencies of key nitrosated products to replace the necessarily vague categories used so far, and the development of short-term *in situ* tests for studying the alkylating power or genotoxicity of *N*-nitroso compounds too unstable for inclusion in long-term studies.

Introduction

The consumption of nitrite and dietary nitrogen-containing compounds might be an aetiological factor in the development of some cancers, particularly of the gastro-intestinal tract, a possibility reviewed by Mirvish (1983), Sander (1971) and Tannenbaum *et al.* (1977). Under the acidic conditions found in the stomach, these compounds form nitrosamines and nitrosamides (Meier-Bratschi *et al.* 1983; Sander, 1971). The *N*-nitroso compounds (NOCs) produce reactive electrophiles which can bind to cellular nucleophiles (Fig. 1), among them DNA (Druckrey *et al.* 1967). The carcinogenic potency of NOCs is thought to rest primarily on their ability to form promutagenic DNA adducts (Lutz, 1979; Magee, 1977; Magee & Barnes, 1967; Magee & Farber, 1962).

The risk associated with the intake of *N*-nitroso precursors is a function of three variables: (1) the amount of precursor and nitrite ingested, (2) the rate of *in vivo* nitrosation and (3) the carcinogenic potency of the resulting NOC. Studies related to the first variable have concentrated mainly on the consumption of nitrite in the diet and have been reviewed by Ellen & Schuller (1983). Sodium nitrite, used primarily in preserving meats, is the best-known source of dietary nitrite. In Europe and North America the average daily intake of sodium nitrite lies around 4 mg/person. Nitrates may also play a role, as they can be reduced to nitrite in the human oral cavity. Nitrates are found in food in considerably higher quantities than nitrites; green leafy vegetables are particularly rich in nitrate (Ellen & Schuller, 1983). The average daily consumption of nitrate ions lies at approximately 100 mg/person/day in Western countries. According to Stephany & Schuller (1980), about 6% of ingested nitrate is reduced endogenously to nitrite; the 6 mg derived from nitrate would thus raise the daily nitrite burden to 10 mg/person.

Abbreviations: DMN = dimethylnitrosamine; MNNG = *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; NOC = *N*-nitroso compound; OPI = oncogenic potency index.

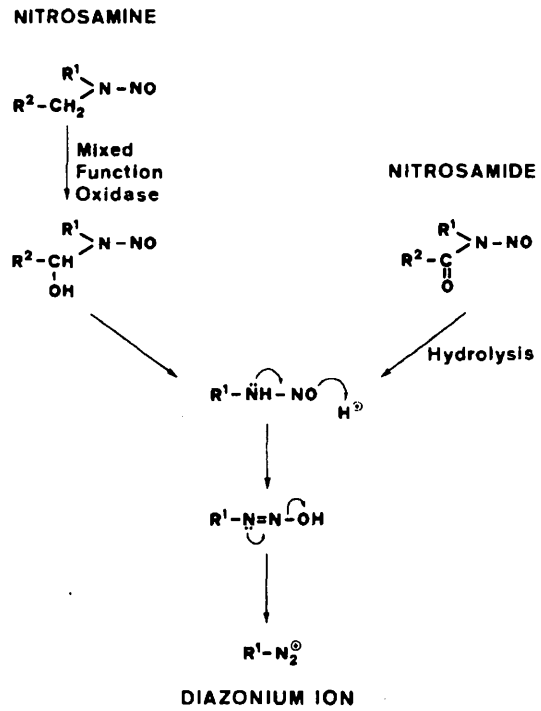

Fig. 1. Formation of electrophilic products by spontaneous or enzymatic degradation of *N*-nitroso compounds.

Drugs and other manufactured compounds contain groups that may react with nitrite to form NOCs (Coulston & Dunne, 1980; Lijinsky, 1981a). The contribution of amines and amides found in the natural diet has received less attention, with the notable exception of work by Mirvish (1971 & 1972), Möhler *et al.* (1972) and Walker (1981), although these compounds constitute an unavoidable source of *N*-nitroso precursors for the general population.

The measurement of *in vivo* nitrosation (the second variable) has been carried out using a variety of short-term tests, notably, examination of stomach contents after feeding nitrite and the test substance to rats (Lijinsky, 1981a), measurement of NOCs in human body fluids after ingestion of proline and nitrate (Ohshima & Bartsch, 1981), of amidopyrine (Spiegelhalder & Preussmann, 1984) or of piperazine (Bellander *et al.* 1984), isolation of DNA alkylated by metabolites or breakdown products of NOCs (Huber & Lutz, 1984; Meier-Bratschi *et al.*, 1983) or estimation of *in vivo* nitrosation through long-term carcinogenicity studies (Sander, 1971). Compounds tested include manufactured compounds, such as pesticides and drugs, and some food components. However, a systematic look at the *in vivo* nitrosatability of natural dietary *N*-nitroso precursors has not yet been undertaken. The kinetic work done to date suggests, however, that the rate of *in vivo* nitrosation correlates reasonably well with *in vitro* kinetic studies (Meier-Bratschi *et al.* 1983; Ohshima *et al.* 1983). Thus, estimates of the relative ease of nitrosation of the various classes of compound could be made on the basis of extensive *in vitro* kinetic work carried out by Mirvish (1975).

With respect to the third variable, Druckrey *et al.* (1967) have shown that NOCs, as a class, are extremely potent carcinogens. More recently, several studies have attempted to quantify and compare the strengths of strong carcinogens on the basis of an evaluation of long-term carcinogenicity data. Meselson & Russell (1977) developed a mathematical model describing the daily dose and duration of treatment necessary to induce a given number of tumours over the lifetime of rodents. Parodi *et al.* (1982) adapted the model, naming it the oncogenic potency index (OPI), and calculated the potencies of the best studied NOCs. No potency estimates, however, have as yet been calculated for many of the *N*-nitroso derivatives that could be formed *in vivo*.

In this review, the most widespread classes of naturally occurring NOC precursors are evaluated in detail with respect to each risk criterion: the most important dietary precursors of NOCs are listed; information is provided on the *in vitro* or *in vivo* nitrosation rate and an indication of the carcinogenic potency of the corresponding NOC is given. Furthermore, the combined effects of these risk variables are analysed using a simple mathematical model:

$$\text{Risk} = [\text{daily intake of precursor}] \times [\text{gastric concn of nitrite}]^n \times [\text{nitrosatability rate constant}] \times [\text{carcinogenicity of derivative}] \quad \ldots\ (1)$$

where the exponent 'n' equals 2 for amine nitrosation and 1 for amide-type nitrosation.

The quantitative risk assessments generated with this model are then used to identify the precursors (and precursor classes) and physiological and nutritional states where *in vivo* nitrosation poses the largest health risk.

Precursors of NOCs in the diet

Figure 2 lists the types of compounds that can form *N*-nitroso derivatives. Widely occurring dietary nitrosatable compounds are amines, amides, guanidines and ureas. Hydrazine derivatives could not be included in the study, first because no information on their kinetics of nitrosation was available and secondly because many hydrazine compounds are known to possess mutagenic and carcinogenic activity in their own right, without requiring prior nitrosation (Kimball, 1977; Parodi *et al.* 1981). The remaining compounds, while synthesized industrially, are not known to be natural dietary constituents and were therefore not included in this risk assessment. The study did not examine precursors of C- or S-nitroso compounds.

Primary amines

A wide variety of primary amines is found in foods, particularly in those that are produced by or subjected to microbial fermentation, such as cheese, meat and fish (Walker, 1981). Methylamine is the most widespread member of this class of compounds, and has been found in vegetables, stimulants, grains, cheeses, fish, pickles and meat (Neurath, 1977). For a complete list of these foods, see Appendix I.

Other amines arising from decarboxylated amino acids are found in substantial amounts, particularly

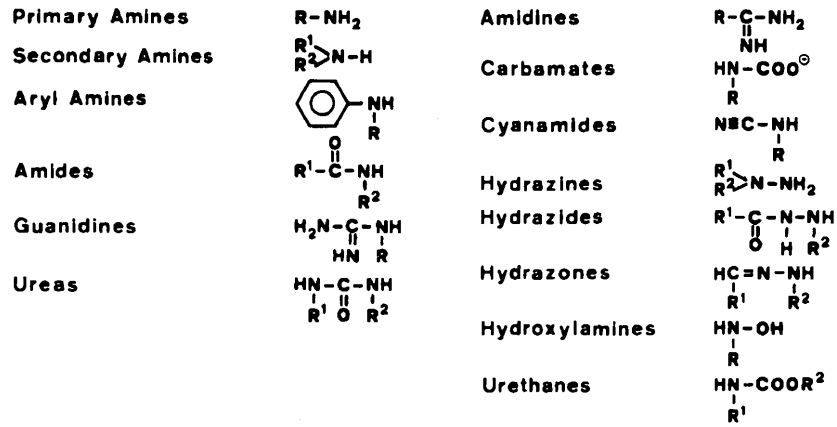

Fig. 2. Names and general formulae of *N*-nitroso-compound precursors.

in meat or fish that has begun to spoil, and in mature cheeses. The most important amines of this type are tyramine, cadaverine, putrescine, tryptamine and histamine (Belitz & Schormüller, 1965; Gangolli, 1981). Ethylamine, isopentylamine and ethanolamine are also found, although to a lesser extent than methylamine. Furthermore, the polyamines spermidine and spermine, along with serotonin, dopamine and sphingosine, have been reported in various types of food. Benzylamine and *n*-propyl- and *n*-pentylamine are minor components of vegetables (Neurath, 1977). A complete list of dietary sources of primary amines is found in Appendix I.

From the amounts of the various food items consumed per person and year in Switzerland, the average daily intake of each primary amine was estimated. Food consumption tables were drawn from *Produktion und Verbrauch von Nahrungsmitteln in der Schweiz 1969/70 bis 1980* (Schweizerisches Bauernsekretariat, 1983) using the 1980 figures. From the calculations compiled in Table 1, it is apparent that the so-called 'endogenous' amines and the polyamines spermidine, tyramine, cadaverine, putrescine and tryptamine are the major dietary amines.

Table 1. Approximate daily intake of primary amines and amino acids

Compound	Daily intake (mg/person)
Amines*	
Spermidine	35
Tyramine	21
Cadaverine	15
Putrescine	15
Tryptamine	9
Spermine	5
Methylamine	3
n-Propylamine	2
Histamine	2
Hexylamine	2
Serotonin	1
Ethanolamine	0.7
Ethylamine	0.6
Benzylamine	0.5
n-Butylamine	0.4
n-Pentylamine	0.3
Phenylethylamine	0.3
Isopentylamine	0.2
Dopamine	0.2
n-Dodecylamine	0.03
Isopropylamine	0.01
Isobutylamine	0.005
Amino acids†	
Glutamic acid	>3.2
Glycine	>1.3
Alanine	>0.4
Asparagine	>0.3
Serine	>0.3

*Intake values based on typical Swiss diet.
†Intake values based on amino acid content of milk.

Amino acids (see Appendix I) form an important subclass of primary amines. Meat contains relatively high concentrations of free aspartic acid, glutamic acid, alanine, glycine, serine and glutamine (Grau, 1968). Milk is also rich in all but the last of these particular amino acids. Of primary importance among the amino acids found in grains are glutamic acid, arginine and leucine (Rohrlich & Thomas, 1967). Cheese and eggs are also likely to contain fair amounts of free amino acids. In a comprehensive study on the free amino acid composition of mushrooms, Oka *et al.* (1981) found glutamic acid and alanine in the highest amounts (both 1.3 g/kg), followed by arginine (0.9 g/kg). Using the levels of free amino acids in milk as given by Kirchmeier (1968), a minimum daily intake of the major amino acids was calculated. The results, in Table 1, indicate that glutamic acid and glycine are consumed to the largest extent.

Secondary amines

Here again, the low-molecular-weight analogues, dimethyl-, methylethyl- and diethylamine, are the most widespread in foods (see Appendix I). They have been found in all the food classes examined. Also of importance are pyrrolidine, piperidine, *N*-methylbenzylamine, *N*-methylphenethylamine, chavicine and dipropyl- and dibutylamine (Neurath, 1977). Neurath (1977) found that the concentration of individual secondary amines rarely exceeded 10 ppm in vegetables, grains, stimulants, herring and four types of cheese.

The average daily intake of the major secondary amines was also calculated. As can be seen from Table 2, the quantities are, with the exception of dimethylamine, significantly smaller than those for the major primary amines.

Table 2. Approximate daily intake of secondary amines, secondary amino acids and aromatic amines

Compound	Daily intake* (mg/person)
Amines	
Dimethylamine	1.7
Pyrrolidine	0.6
N-Methylbenzylamine	0.6
N-Methylphenethylamine	0.3
Methylethylamine	0.3
Diethylamine	0.1
Piperidine	0.06
Pyrroline	0.03
Amino acids	
Proline	0.47
Sarcosine	1†
Aromatic amines	
N-Methylaniline	1.6
Aniline	1.0
Toluidines	0.2

*Values based on typical Swiss diet.
†Estimate for use in risk calculation (see text).

The secondary amino acids proline, 3-hydroxyproline and sarcosine are widely found. Proline, bound in proteins, is an important component of grains (Rohrlich & Thomas, 1967), and free proline has been reported at levels of 1.0 g/kg in mushrooms (Oka *et al.* 1981) and 1.5 ppm in milk (Kirchmeier, 1968). This last value would give a minimum daily intake of 0.47 mg proline per person (Table 2). Sarcosine has been reported in lobster and cartilaginous fish (Belitz & Schormüller, 1965).

Arylamines

The only two arylamines that seem to be widespread are aniline and *N*-methylaniline. According to Neurath (1977), aniline is found primarily in rapeseed cake (120 ppm) and carrots (31 ppm), and *N*-methylaniline in cheese (38 ppm). Other sources are listed in Appendix I and the daily intakes of these compounds, and of toluidines, are given in Table 2.

Amides

In the form of proteins, amides are consumed in large quantities in meat, milk, eggs, fish, poultry and grains. The daily intake amounts to 92 g/day (Table 3; Schweizerisches Bauernsekretariat, 1983). These are broken down to oligopeptides in the stomach by pepsin. Of the low-molecular-weight amides, asparagine and glutamine—as amino acids—are the most important (Appendix I). Carrot juice and asparagus are particularly rich in asparagine. Furthermore, glutamic acid, the most abundant amino acid, is easily converted by ring closure to pyrrolidone carboxylic acid, its amide. Glutathione is widely found in mammalian liver and muscle. Carnosine and anserine are also found in the muscles of mammals, fish and poultry, the former at values fluctuating between 90 and 4600 mg/kg meat (Belitz & Schormüller, 1965).

Table 3. Preliminary estimates of daily amide, guanidine and urea intakes

Compound	Daily intake* (mg/person)
Amides	
Protein	92,000
Carnosine	2000
Guanidines	
Creatine	800
Creatinine	300
Agmatine	0.7
Methylguanidine	0.2
Ureas	
Methylurea	1
N-Carbamoylputrescine	1†
Citrulline	1†

*Values based on typical Swiss diet.
†Estimate for use in risk calculation (see text).

Guanidines

Creatine and creatinine are important constituents of meat, comprising together 2.5% of the total protein (Grau, 1969). Arginine is an endogenous guanidine found in the urea cycle. Methylguanidine has been reported in beef and fish in concentrations up to 0.2% (Mirvish, 1972). Fresh abalone contains agmatine, the decarboxylation product of arginine (Kawabata *et al.* 1978; Appendix I) while arginine itself is found in mushrooms (0.9 g/kg; Oka *et al.* 1981) and in soy sauce. Canavanine (found in beans), arcaine and glycocyamine are the other major guanidines that have been reported in foods (Mirvish, 1972). From the few quantitative data on guanidines in food, crude estimates of the daily intake per person have been calculated (Table 3).

Ureas

Citrulline is the most important of the naturally occurring urea compounds (listed in Appendix I) because of its endogenous role as a component of the urea cycle. Citrulline has also been found in watermelons, green peppers and soya sauce. Albizziin (reported in plants of the Mimosa family) and hydantoic acid are also constituents of foods. Ureas are important as intermediates in pyrimidine and purine metabolism and are also formed by enzymatic carbamoylation of amines and de-imidation of guanidines. Of these compounds, *N*-carbamoylputrescine, allantoic acid, hydantoic acid and *N*-carbamoylaspartic acid are the best known. The first three ureas are found in plants, and all but hydantoic acid have been detected in the urine of animals or man (Mirvish, 1972).

Cooking or frying creatine-containing foods appears to be a source of methylurea, produced by the breakdown of the intermediate creatinine. Mirvish *et al.* (1980b) have recently reported finding 25 ppm methylurea both in a Japanese fish product and in fried bacon. The same paper reports the finding of longer chain alkylureas in foods. Furthermore, Japanese papers have recently started to suggest that urea compounds may be important components of processed or cooked sea foods (Kodama *et al.* 1982). A rough estimate of methylurea intake was calculated from these data to be 1 mg/day (Table 3).

Comment

The intake of the various *N*-nitroso precursors spans five orders of magnitude. Amides, in the form of protein, and guanidines, in the form of creatine and creatinine, are the nitrosatable groups found most abundantly in the diet, approaching levels of

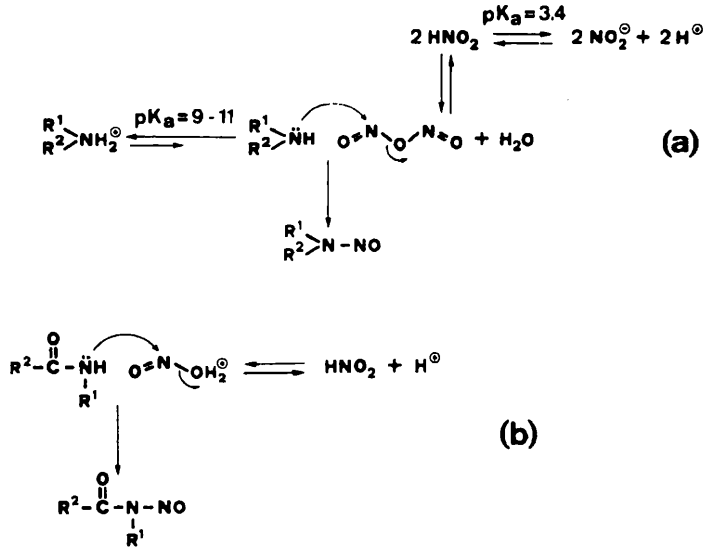

Fig. 3. Mechanisms of nitrosation of (a) amines and (b) amides.

100 and 1 g/day, respectively. Approximately 100 mg primary amines and free amino acids are consumed daily, whereas arylamines, secondary amines and ureas appear to lie in the 1–10 mg range. It should be noted, however, that sensitive analytical methods for the quantitative detection of non-volatile amides, guanidines and ureas in foods are still in the developmental stages. For this reason, daily intake estimates for these types of compounds should be regarded as preliminary, representing only the minimum ingested values.

Where no quantitative data on occurrence were available, an arbitrary minimum daily intake of 1 mg/person was assigned for the purpose of calculating a risk estimate. Such compounds are distinguished with a footnote in Tables 2 and 3.

Nitrosatability

Kinetic experiments on the *in vivo* nitrosation of dietary precursors in the human stomach have, for ethical reasons, only been carried out using proline and nitrate, since nitrosoproline is considered to be a non-carcinogen (Ohshima & Bartsch, 1981). The same authors also studied the kinetics of the *in vivo* nitrosation of proline, sarcosine and hydroxyproline by nitrite in rats (Ohshima *et al.* 1982 & 1983). They were able to show that the kinetic equation found *in vitro* (see below) was applicable to nitrosation in the rat stomach and was also compatible with the human data. Furthermore, they found that the relative nitrosation reactivity of the three amino acids *in vivo* matched the relative rates *in vitro* published by Mirvish (1975). Finally, the nitrosation yields of proline *in vivo* and *in vitro* at 37°C differed only by a factor of 4 (*in vitro* > *in vivo*). Iqbal *et al.* (1980) carried out *in vivo* nitrosation studies in mice using morpholine or dimethylamine plus nitrite and concluded that the *in vivo* and *in vitro* nitrosation rates corresponded well. These experiments suggest that, in the absence of *in vivo* data, knowledge of the *in vitro* nitrosation rate of the various precursors would provide a reasonable basis for predicting the relative nitrosatabilities *in vivo*.

Unfortunately, nitrosation rate constants have not been measured for most of the naturally occurring nitrosatable compounds, and the available *in vitro* studies either were not carried out under simulated gastric juice conditions (Nebelin *et al.* 1980) or used food mixtures rather than pure compounds (Groenen *et al.* 1982; Walters *et al.* 1976). Thus it was necessary to go one step further back and predict the nitrosation rate constants of the precursors, basing the estimates on the few data that have been published (Mirvish, 1975; Ridd, 1961) and on an understanding of the reaction mechanism and the general factors that influence reactivity (such as steric hindrance and resonance forms).

Amines

The nitrosation reaction sequence (Fig. 3) is an example of a nucleophilic substitution reaction (S_N2 reaction). The electron-rich amine nitrogen attacks the nitrogen of nitrous anhydride, replacing the nitrite group, which splits off. The reactants are the unprotonated amine R_1R_2NH and two molecules of nitrous acid, to give the following kinetic equation (Turney & Wright, 1959):

$$d[NOC]/dt = k_1[R_1R_2NH][HNO_2]^2 \ldots\ldots (2)$$

The most important factor determining the kinetics of amine nitrosation involves the acid–base equilibria of both amine and nitrous acid (Fig. 3a). The nitrous acid–nitrite equilibrium has a pK_a of 3.4; thus after a meal, at a stomach pH of approximately 3, about half the ingested 'nitrite' is present in the reactive, protonated form. However, at this pH, only a minute fraction of the basic amine is available as the unprotonated reactive species. The exact proportion depends on the basicity of the individual amine. The strongly basic (pK_a 9–11) simple primary and secondary amines have vanishingly small effective concentrations in the stomach (one billionth to one millionth

of the concentration of the protonated species), whereas the effective concentrations of the much less basic aryl amines are appreciable (Mirvish, 1975; Ridd, 1961).

The reactivity of the various amines is also affected by steric hindrance at the amine nitrogen. The primary amines with only one alkyl group would thus be expected to react more quickly than the secondary amines. Bulky alkyl groups (for example, isopropyl or benzyl groups) also decrease the reaction rate. However, when these groups are separated from the nitrogen by a short alkyl chain (as in serotonin or phenylethylamine), they should not affect the kinetics significantly.

Finally, intramolecular catalysis by the α-carboxyl group of amino acids could increase the nitrosation rate of these compounds over that of the simple amines. The mechanism probably involves removal of an amine proton by the carboxylate ion via a five-membered ring transition state.

The kinetic equation (2) can now be rewritten to take the acid–base equilibria into account by expressing the rate in terms of the total amount of 'amine' and 'nitrite' ingested:

$$d[NOC]/dt = k_2[\text{amine}][\text{nitrite}]^2. \quad \ldots. \quad (3)$$

Note that k_2 is pH dependent. In this form, the kinetic equation allows the various precursor types to be compared with one another with respect to nitrosatability. By comparing the structures and pK_a values of the dietary amines with amines for which k_2 values at optimal pH are known (pH range 2.5–3.4), using values taken from Mirvish (1975) and Ridd (1961), the maximal nitrosation rate constants were estimated. The k_2 values of the most important members of each class are listed in Table 4.

Amides, guanidines and ureas

The kinetics of amide-type nitrosation are different from those for the amines. The amide (and guanido and ureido) nitrogen is much less basic than an amine (pK_a 0 *v.* pK_a 10) and is normally unprotonated at stomach pH. The problem of effective concentration therefore involves only the nitrous acid–nitrite equilibrium, and thus reaction rates rise with increasing acidity of the solution. The mechanism of amide-type nitrosation (Fig. 3b) involves nucleophilic attack of the nitrogen on protonated nitrous acid. Water acts as the leaving group. The kinetic equations are as follows (Mirvish, 1971):

$$d[NOC]/dt = k_3[RNHCOR][HNO_2][H^+] \quad \ldots. \quad (4)$$

$$d[NOC]/dt = k_4[\text{amide}][\text{nitrite}][H^+]. \quad \ldots. \quad (5)$$

The reaction is first order with respect to nitrous acid, rather than second order as for the amines. This important difference means that the rate of amide-

Table 4. Approximate daily gastric *in vivo* nitrosation yields from the most abundant dietary precursors and nitrite

Compound	Daily intake (mg)	Approx. pKa	Rate constant estimate*	Yield of *N*-nitroso compound (pmol)	
				$[NO_2^-] = 1.7\ \mu M$	$[NO_2^-] = 72\ \mu M$
Primary amines					
Spermidine	35	10	0.005	12×10^{-3}	24
Tyramine	21	9.1	0.05	78×10^{-3}	144
Cadaverine	15	10	0.01	14×10^{-3}	28
Putrescine	15	9.7	0.01	18×10^{-3}	32
Methylamine	3	10.7	0.005	4×10^{-3}	8
Dopamine	0.2	9.1	0.01	0.7×10^{-3}	1
Primary amino acids					
Glutamic acid	>3.2	9.7	1	>0.2	>400
Glycine	>1.3	9.6	1	>0.2	>320
Alanine	>0.4	9.7	1	$>46 \times 10^{-3}$	>80
Secondary amines					
Dimethylamine	1.7	10.7	0.002†	0.7×10^{-3}	2
Methylbenzylamine	0.6	9.5	0.013†	0.7×10^{-3}	1
Pyrrolidine	0.6	11.2	0.005†	0.4×10^{-3}	0.8
Secondary amino acids					
Proline	>0.5	9.7	0.037†	$>2 \times 10^{-3}$	3.2
Sarcosine	1‡	9.7	0.23†	27×10^{-3}	48
Arylamines					
N-Methylaniline	1.6	4.85	250†	40	68×10^3
Aniline	1	4.6	500†	57	100×10^3
Amides					
In protein	92,000	0	0.001	800	32×10^3
Carnosine	2000	0	0.001	4	160
Guanidines					
Creatine	800	—	0.004	62	2.4×10^3
Creatinine	300	—	0.004	26	1×10^3
Agmatine	0.7	—	0.004	54×10^{-3}	2
Methylguanidine	0.2	—	0.004†	0.03	1
Ureas					
Methylurea	>1	0	10.5†	>400	$>14 \times 10^3$
N-Carbamoylputrescine	1‡	0	1	20	0.8×10^3
Citrulline	1‡	0	0.7†	10	0.4×10^3

*k_2 at optimal pH for amines, k_4 at pH 2 for amide-type compounds.
†Rate constants from Mirvish (1975).
‡Arbitrary daily intake of 1 mg assigned.

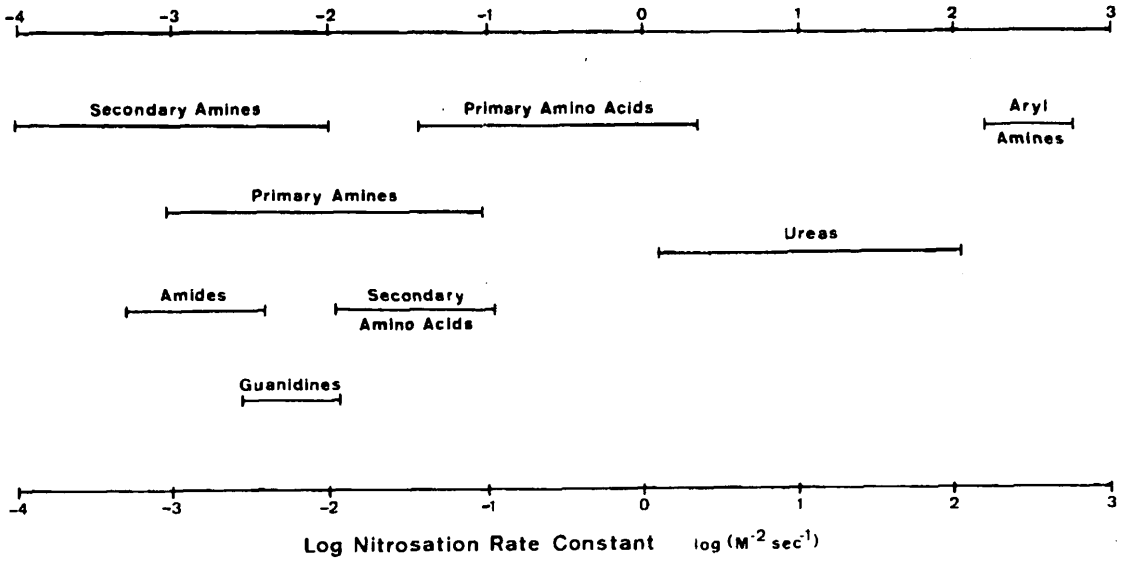

Fig. 4. Overview of nitrosation rate constant ranges of various precursor classes—k_2 ($M^{-2}\,sec^{-1}$) for amines at optimal nitrosation pH, k_4 ($M^{-2}\,sec^{-1}$) for amides at pH 2.

type nitrosation is less sensitive to the concentration of nitrite in the stomach.

The nucleophilicity of the different kinds of amide compound plays a decisive role in their reactivity. The carbonyl and protonated guanido groups are strongly electron-withdrawing. The nucleophilicity of the amide and guanidine N atoms is thus markedly reduced, and these types of compound nitrosate very slowly, with k_4 values between 10^{-3} and 10^{-2}. The presence of a second nitrogen in the urea compounds compensates for this electron-withdrawing effect, and the nucleophilicity remains strong. Reaction constants vary from 1 to 100 for these compounds. Within the reactivity range normal for each precursor type, the presence of other functional groups such as acids, amines or ring structures influence the reactivity of individual compounds. The k_4 values of the most important members of each class are listed in Table 4 (data from Mirvish, 1975).

Comment

An overview of the predicted reactivity ranges for all the precursor classes is provided in Fig. 4. The reactivities span seven orders of magnitude with arylamines and ureas highest and amines and amides lowest in the ranking order.

Nitrosation yields *in vivo*

The nitrosatability estimates from Fig. 4 were next used to calculate how much NOC would be formed in the stomach after a typical meal. No attempt was made to correct to 37°C the *in vitro* nitrosation rates determined at 25°C. The stomach volume was estimated to be 1 litre and reactants were assumed to remain at a constant concentration in the stomach for 1 hr. The total daily amount of each specific amine or amide-type compound was assumed to be eaten all at one meal. The gastric nitrite concentrations were taken from studies by Klein *et al.* (1978) and Tannenbaum *et al.* (1974). Calculations were carried out for a low nitrite burden, using [nitrite] = 1.7 μM and for a high nitrite burden, with [nitrite] = 72 μM. The pH in the stomach varies from <2 to >5, depending on the flow of gastric juice and the buffering capacity of its contents at any particular time (Walters *et al.* 1976). A pH of 2 was assumed for calculating amide-type nitrosation yields, and the optimum pH value for the nitrosation reaction was assumed in calculating nitrosamine yields (pH between 2.5 and 3.4). Given that the *in vivo* nitrosation yields span seven orders of magnitude, the errors introduced into the calculation through these assumptions are negligible.

The results of these calculations are found in Table 4. At low nitrite concentrations, the most significant yields of NOC come from protein and from methylurea (800 and 400 pmol, respectively), followed by the guanidines and arylamines (100 pmol). Because of greater sensitivity to the nitrite concentration, the *in vivo* nitrosation of arylamines becomes even more important than that of ureas plus protein at high nitrate concentrations (200 nmol versus 50 nmol). The amounts of nitrosamine produced *in vivo* from aliphatic amines are, in contrast, very small, comprising only picomole quantities even after consumption of large amounts of nitrite. The yields of *N*-nitrosamino acids lie between these two extremes, ranging from <1 pmol at low nitrite concentrations to >400 pmol at high nitrite levels.

The total gastric formation of NOCs is calculated to lie between 1 and 2 nmol/day at low and 200 nmol/day at high nitrite consumption. In comparison, the daily exposure to preformed volatile nitrosamines in food lies between 10 and 20 nmol/day (dimethylnitrosamine (DMN) 1 μg, *N*-nitrosopyrrolidine 0.1 μg; Spiegelhalder *et al.* 1980). The exposure to preformed non-volatile NOC in the diet appears to be 10–50 times higher (Kawabata *et al.* 1984), of the order of 100–1000 nmol/day. These

estimates can also be compared with the total concentration of NOCs that has been measured experimentally in human gastric fluid. Two studies on fasting subjects (normal and gastritis patients) gave mean values of <100 nmol NOCs/litre ($n = 50$; Bartsch *et al.* 1984) and 420 nmol/litre ($n = 455$, range 10–40,000 nmol/litre; Walters *et al.* 1982). Fasting normal subjects had a mean of 140 nmol/litre in a further study ($n = 50$; Reed *et al.* 1984). The gastric juice from four non-fasting normal subjects was sampled repeatedly over the course of a day, giving a mean value of 200–4000 nmol NOCs/litre with peaks up to 18,000 nmol/litre after a meal (Bavin *et al.* 1982). The estimated total daily exogenous and endogenous NOC burden correlates well with these measurements.

The relative contribution of endogenously formed NOCs to the total daily burden thus spans a range from 'negligible' to 'the same order of magnitude as exogenous NOC' depending on the status and eating habits of the individual. On the basis of quantity, therefore, the nitrosation of food precursors in the stomach could represent a potentially important source of NOCs, particularly nitrosamides, *N*-nitrosoureas, aromatic *N*-nitrosamines and *N*-nitrosoguanidines.

Carcinogenicity

Finally, it was necessary to estimate the carcinogenic potency of the *N*-nitroso derivatives that could form in the stomach. Lifetime rodent bioassays on carcinogenicity provide the largest source of data for this purpose. Studies were selected in which the NOC was administered orally to rats. The oncogenic potency model defined by Meselson & Russell (1977) was used to convert the variables of daily dose D (mmol/kg body weight/day), treatment period t (in 2-yr units) and fraction of tumour-bearing animals I into a numerical estimate of carcinogenic potency, the OPI:

$$\text{OPI} = \frac{-\ln(1 - I)}{D \times t^n} \quad \ldots\ (6)$$

The variable n was assigned a value of 3, in accordance with results from Schmähl (1979) and Meselson & Russell (1977).

It must be stressed that the criteria used here to select carcinogenicity data were far less stringent than those applied by Parodi *et al.* (1982) or by Meselson & Russell (1977); consequently, the OPI values summarized in Table 5 are to be considered only as a rough guide to carcinogenic strength. The various estimates for each compound allow the substances to be divided into five categories (Table 6): 10^3, very strong; 100, strong; 10, moderate; 1, weak; <0.1, 'non-carcinogenic'.

Even with relaxed requirements there were many compounds for which no quantitative data were available. In these cases, extrapolations had to be made using the following general toxicological principles and structure–reactivity relationships:

An increase in side-chain length (R groups in Fig. 2) beyond two carbon atoms has been shown empirically to decrease carcinogenic potency (Druckrey *et al.* 1967). This may be because the larger diazonium ions are sterically hindered from reaching the active sites on the DNA, or because the resulting adducts are less stable or more easily repaired than methylated or ethylated bases. Furthermore, the addition of a charged or polar side group, such as a carboxyl, hydroxyl or amino group, also results in decreased carcinogenicity. Such structure–reactivity phenomena have been extensively studied by Lijinsky (1981b). The reason is probably that polar hydrophilic compounds diffuse only with difficulty through membranes and tend to be excreted rapidly in the urine.

Primary amines

Primary nitrosamines are intermediates in the breakdown pathway of secondary nitrosamines (see Fig. 1). They are unstable in aqueous solution and, not surprisingly, no long-term studies have been attempted on these compounds. Their behaviour in biological matrices has not been characterized. The *in vivo* nitrosation of radioactive methylamine with nitrite in rats led to measurable amounts of 7-[^{14}C]methylguanine in DNA isolated from the gastro-intestinal tract (Huber & Lutz, 1984). It thus appears that primary nitrosamines are stable enough to penetrate the cell membrane. Overall, their carcinogenic potencies may be somewhat smaller than those of the secondary amine analogues in the respective target organs. Because the effect of the chemical instability could not be quantified, OPI estimates were conservative and were based on the potency of the secondary nitrosamines. Thus, from studies on DMN and dibutylnitrosamine and on *N*-nitrosopiperidine, *N*-nitrosopyrrolidine and alkylarylnitrosamines (Druckrey *et al.* 1967), methylnitrosamine was ascribed a potency of 10^3 and the larger compounds a potency of 100.

Primary amino acids

These *N*-nitroso derivatives are also unstable and, again, no long-term studies were found. As direct-acting carcinogens in the stomach, nitrosamino acids might be predicted to have potencies similar to those of the primary nitrosamines. Nitrosoproline is here inappropriate as a reference substance because its *N*-nitroso derivative is stable and not metabolized in the body (Ohshima *et al.* 1982). *N*-Nitrosoglycine and *N*-nitrosoalanine were assigned a potency of 100, whereas *N*-nitrosoglutamic acid was assigned a potency of 10 because of its second acid group. As these are derivatives of endogenous compounds, there is also the possibility that membrane carrier systems might help the compound to gain access to cell components. The importance of this factor is difficult to assess.

Secondary amines

The secondary nitrosamines have been extensively studied. Pioneer work was carried out by Druckrey *et al.* (1967) and various members of this class were considered in the IARC Monograph series on the evaluation of carcinogenic risk to man (IARC Working Group, 1978). Hence, OPI values could be calculated for most of the compounds (Table 5). It is notable that in spite of the rather large variations in estimates from one study to the next, the OPI value

Table 5. Oncogenic potency index (OPI) estimates of *N*-nitroso compounds calculated using published data

Compound*	Daily dose (mg/kg)	Length of experiment (days)	Tumour-bearing animals (%)	Potency (OPI)	Reference
Secondary *N*-nitrosamines:					
diethyl	1–10	730	100	61	Druckrey *et al.* 1963
	0.15–0.6	730	high		Druckrey *et al.* 1963
	0.075	840	71	802	Schmähl, 1979
	14.2	68	100		Schmähl, 1979
	>0.15		100		Druckrey *et al.* 1963
dimethyl	4	730	high		Argus & Hoch-Ligeti, 1961
	4	270	65	384	Druckrey *et al.* 1967
	0.04	<840	2.7	>33	Terracini *et al.* 1967
	0.1	<840	7.4	>37	Terracini *et al.* 1967
	0.2	<840	40	>124	Terracini *et al.* 1967
	0.4	<840	65	>127	Terracini *et al.* 1967
	1	<840	83	>86	Terracini *et al.* 1967
methylbenzyl	0.25	500	50	860	Druckrey *et al.* 1967
	1	250	95		Druckrey *et al.* 1967
methylethyl	1	500	60	291	Druckrey *et al.* 1967
piperidine	5	280	90		Druckrey *et al.* 1967
	20	730	100	17	Druckrey *et al.* 1967
	3	392	82		Eisenbrand *et al.* 1980
	0.6	795	41	77	Eisenbrand *et al.* 1980
	0.12	746	8	93	Eisenbrand *et al.* 1980
pyrrolidine	5–20	290–470	92		Druckrey *et al.* 1967
	4	45–105	90		Lijinsky & Taylor, 1976
	10	444	46	27	Preussmann *et al.* 1977
	3	535	84	155	Preussmann *et al.* 1977
	1	685	32	47	Preussmann *et al.* 1977
	0.3	664	20	97	Preussmann *et al.* 1976
Secondary *N*-nitrosamino acids:					
proline	50–100	730	<5	<0.1	Druckrey *et al.* 1967
		365	0		Mirvish *et al.* 1980b
sarcosine	100	28	36	1.2	Druckrey *et al.* 1967
	200	57	50	1.1	Druckrey *et al.* 1967
Aromatic *N*-nitrosamines:					
methylaniline	10	450	80	73	Druckrey *et al.* 1967
N-Nitrosamides:					
methylbenzamide	4.4	730	88	65	Bulay *et al.* 1979
methylacetamide	1	500	100	952	Druckrey *et al.* 1967
N-Nitrosoguanidines:					
MNNG	6.6	365	73	232	Bralow *et al.* 1973
	6.6	365	10	19	Bralow *et al.* 1973
	5.9	730	45	>15	Lijinsky & Reuber, 1984
cimetidine	3	742	0	<3	Lijinsky & Reuber, 1984
N-Nitrosoureas:					
carboxymethyl†	5.2	730	60	20	Bulay *et al.* 1979
dihydrouracil	0.9	365	95	3000	Bulay *et al.* 1979
ethyl	0.4	365	62	1700	Pelfrene *et al.* 1975
	0.8–7	730	84	53	Ogiu *et al.* 1974
	2	180	95		Bulay *et al.* 1979
hydantoin	11.8	730	84	15	Bulay *et al.* 1979
hydroxyethyl	1.5	365	90	1275	Bulay *et al.* 1979
methyl	4–8	240–300	90	560	Druckrey *et al.* 1967
butyl	5	350	90	427	Takeuchi *et al.* 1984

MNNG = *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine

*Primary *N*-nitrosamines and primary *N*-nitrosamino acids are not included because no relevant data were traced in the literature.

†Hydantoic acid.

of each compound seems to be predictable to within a factor of ten. The major sources of error arose from making use of studies that lasted less than 2 years, and from the various authors' different definitions of tumour incidence. Most potencies lay between 10 and 100, with the exceptions of DMN, diethylnitrosamine and methylbenzylnitrosamine (all 10^3).

Secondary amino acids

The work on *N*-nitrosoproline (Mirvish *et al.* 1980a) and *N*-nitrososarcosine (Druckrey *et al.* 1967) showed that the presence of the carboxylic acid group greatly reduced carcinogenicity. Nitrososarcosine has a potency of about 1, and nitrosoproline appears to be virtually non-carcinogenic with a potency of <0.1.

Aromatic amines

The breakdown of aromatic nitrosamines leads to the formation of diazo compounds. Such substances are well-known reactive intermediates in organic syntheses and would be expected to react efficiently with cell components. From the one study traced, *N*-nitrosomethylaniline was calculated to have an OPI of about 100 (Druckrey *et al.* 1967). The *N*-nitroso

Table 6. Estimate of health risk posed by gastric *in vivo* nitrosation of food precursors relative to consumption of preformed dimethylnitrosamine (DMN)

Compound	Carcinogenic potency*	Health risk relative to DMN†	
		$[NO_2^-] = 1.7\ \mu M$	$[NO_2^-] = 72\ \mu M$
Primary amines			
Spermidine	100	1×10^{-7}	2×10^{-4}
Tyramine	100	7×10^{-7}	1×10^{-3}
Cadaverine	100	1×10^{-7}	3×10^{-4}
Putrescine	100	2×10^{-7}	3×10^{-4}
Methylamine	10^3	4×10^{-7}	8×10^{-4}
Spermine	100	3×10^{-8}	3×10^{-5}
		Total... 10^{-6}	10^{-3}
Primary amino acids			
Glutamic acid	10	1×10^{-6}	2×10^{-3}
Glycine	100	1×10^{-5}	1×10^{-2}
Alanine	100	3×10^{-6}	8×10^{-3}
		Total... 10^{-5}	10^{-2}
Secondary amines			
Dimethylamine	10^3	7×10^{-8}	2×10^{-4}
N-Methylbenzylamine	10^3	7×10^{-7}	1×10^{-3}
Pyrrolidine	100	3×10^{-9}	8×10^{-6}
		Total... $<10^{-6}$	10^{-3}
Secondary amino acids			
Proline	<0.1	$<1 \times 10^{-10}$	$<1 \times 10^{-7}$
Sarcosine	1	3×10^{-9}	5×10^{-6}
		Total... 10^{-9}	$<10^{-5}$
Arylamines			
N-Methyl aniline	100	4×10^{-4}	0.8
Aniline	100	6×10^{-4}	1
Toluidine	100	3×10^{-5}	3×10^{-2}
		Total... 10^{-3}	>1
Amides			
Protein	10	8×10^{-4}	4×10^{-2}
Carnosine	1	2×10^{-7}	1×10^{-5}
		Total... $<10^{-3}$	10^{-2}
Guanidines			
Creatine	1	6×10^{-6}	2×10^{-4}
Creatinine	10	2×10^{-5}	1×10^{-3}
Agmatine	10	6×10^{-8}	2×10^{-6}
Methyl guanidine	10^3	1×10^{-5}	6×10^{-4}
		Total... $<10^{-4}$	$<10^{-2}$
Ureas			
Methyl urea	10^3	$>5 \times 10^{-2}$	>1
N-Carbamoyl putrescine	100	2×10^{-5}	1×10^{-3}
Citrulline	10	1×10^{-5}	4×10^{-4}
		Total... $>5 \times 10^{-2}$	>1

*Approximate OPI of nitroso derivative.
†Risk from DMN = 1.

derivatives of the two other dietary aromatic compounds, *N*-nitrosotoluidine and *N*-nitrosoaniline, were also assigned potencies of 100, on the basis of their very similar structures.

Amides

Here again the smaller analogue, methylnitrosoacetamide (OPI about 1000) is a more powerful carcinogen than the larger methyl-*N*-nitrosobenzamide (OPI 100; Bulay *et al.* 1979; Druckrey *et al.* 1967). The potencies are analogous to those of the secondary nitrosamines. Nitrosocarnosine was assigned a potency of 1, due to its size and the presence of an acid group.

Nitrosated peptides have apparently never been tested for their carcinogenicity. One would expect the potency to be moderately low (here estimated at 10) because of their size and polarity, but to be higher if they were broken down to constituent amino acids (OPI approximately 100; see discussion of primary amino acids above). Peptides could, however, provide a relatively stable transport form of nitrosated amino acids.

Guanidines

While many studies have been conducted on *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and related compounds, no data on the *N*-nitroso derivatives of dietary guanidines are available. MNNG has a potency of 10–100, whereas *N*-nitrosocimetidine has an OPI of <3 (Lijinsky & Reuber, 1984). From this meagre information, it was assumed that a nitrosated guanidine would not be more potent than the analogous nitrosated amine or amide. Methylnitrosoguanidine was thus assigned an OPI of 10^3. Nitrosoagmatine and nitrosocreatinine were assumed to be intermediate in strength between *N*-nitrosocadaverine and *N*-nitrososarcosine and given an OPI of 10. Nitrosocreatine was considered to be equivalent to *N*-nitrososarcosine (OPI 1).

Ureas

Nitrosoureas have been fairly well studied, and from Table 5 it is clear that the potencies follow the same pattern as seen with the other classes of NOCs (small alkyl > large alkyl > polar substituted). Quantitatively, however, this class appears to be more potent than the others (compare *N*-nitrosobutylurea, OPI 400, with *N*-nitrosopyrrolidine, OPI 70, for example, or *N*-nitrosohydantoic acid, OPI 20, with *N*-nitrososarcosine, OPI 1). The one compound that does not follow this pattern is *N*-nitrosodihydrouracil. Its structural similarity to thymine perhaps allows it to act either as an NOC or as a base analogue to produce DNA lesions, hence the increased oncogenic potency. The biologically important *N*-nitrosoureas, *N*-nitrosocarbamoylputrescine and *N*-nitrosocitrulline, were assigned potencies of 100 and 10, respectively.

Calculation of health risk

The health risk posed by *in vivo* nitrosation of food components was compared to that posed by the presence of preformed DMN in foods. Estimates of the health risks due to particular NOCs were calculated using equation 1:

$$\begin{aligned}\text{Risk}_{\text{NOC}} = {} & \text{daily intake of precursor C (mol/day)} \\ & \times \text{gastric concentration of nitrite}^n \\ & (1.7 \text{ or } 72 \times 10^{-6}\,\text{M})^n \\ & \times \text{nitrosatability rate constant} \\ & k_2\,(\text{sec}^{-1}\,\text{M}^{-2}) \text{ or } k_4\,(\text{sec}^{-1}\,\text{M}^{-2}) \\ & \times \text{carcinogenicity of derivative OPI} \\ & (\text{kg mmol}^{-1}\,\text{day}^{-1}\,\text{year}^{-3}) \qquad \ldots\ (7)\end{aligned}$$

The OPI categories in Table 6 were used as estimates of the carcinogenicity. The model assumes that health risk is linearly related to both the carcinogenicity and the daily endogenous yield of each NOC. Similarly, the risk due to preformed DMN (intake 10 nmol/day; Spiegelhalder *et al.* 1980) was calculated as follows, using the best OPI estimate available (Parodi *et al.* 1982):

$$\begin{aligned}\text{Risk}_{\text{DMN}} &= \text{daily exposure to DMN} \times \text{OPI} \\ &= 10\,\text{nmol} \times 3000 = 30{,}000. \qquad \ldots\ (8)\end{aligned}$$

The relative risk can be expressed as follows:

$$\text{Relative risk} = \text{Risk}_{\text{NOC}}/\text{Risk}_{\text{DMN}}. \qquad \ldots\ (9)$$

Results of these calculations may be found in Table 6. Risk contributions from individual members of each compound class are listed to demonstrate the span of risk estimates within each class (up to three orders of magnitude). Tyramine, glycine, *N*-methylbenzylamine, sarcosine and aniline are the compounds with the largest individual risk contributions in each of the amine categories. Similarly, risk estimates for protein, creatinine and methylurea were calculated to be the largest within the different amide-type categories. It must be noted that this risk analysis compares isolated precursors to DMN, and whereas the total contribution of precursors is probably greater than the sums given here, the total contribution of preformed NOCs in the diet may also prove to be larger than at present imagined. A detailed picture of the types and amounts of non-volatile NOCs in the diet is lacking at present.

Figure 5 gives an overview of the relative health risks posed by *classes* of dietary *N*-nitroso precursors. The results span nine orders of magnitude with ureas and aryl amines at the top and secondary

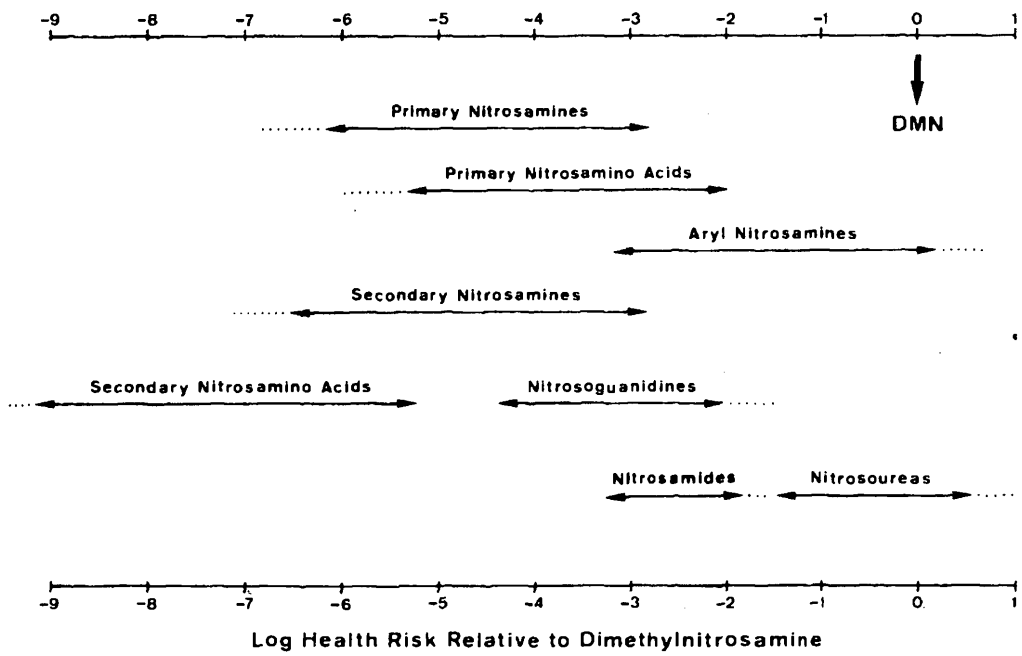

Fig. 5. Relative risk posed by dietary intake of nitrosatable precursors as compared to risk from dietary intake of dimethylnitrosamine: ▶ high nitrite concentration; ◀ low nitrite concentration; ... low pH.

amino acids at the bottom of the scale. Each line represents the risk arising from the endogenous nitrosation of a particular precursor class over a span of gastric nitrite concentrations. The risk from amine precursors is more sensitive to gastric nitrite concentration, covering three orders of magnitude, as compared to one or two orders of magnitude with the amide-type compounds. The arrows on either end of the line indicate how the risk estimate is affected by gastric nitrite concentrations below 1.7 μM (left arrow) or above 72 μM (right arrow). In addition, the effect of gastric pH below 2 is indicated by the dotted line. A low gastric pH represents an additional risk factor for amide-type compounds and arylamines, whereas it is a mitigating factor in the risk posed by alkylamines and amino acids. It is noteworthy that the risks calculated in this study are all equal to or below the risk from preformed dietary DMN.

Discussion

Attention was first given to the issue of *in vivo* nitrosation because of the extremely potent carcinogenicity of the secondary nitrosamines. It was thus natural that the environmental secondary amines were first examined as potential precursors for endogenous nitrosation (Lijinsky & Epstein, 1970; Walker, 1981). These simple amines did not cause tumours when fed concurrently with nitrite (Sander, 1971; Telling *et al.* 1976). Emphasis thus gradually shifted to the weakly basic secondary amines (synthetic compounds and drugs) and to nitrosamide-type compounds. Mirvish (1971, 1977 & 1983) has long maintained that *in vivo* nitrosation would only be a problem for rapidly nitrosatable compounds, and has thus emphasized the role of ureas as precursors. Interest has also been growing in the possible nitrosation of natural amines, peptides and proteins because of their assumed or known high concentrations in the stomach (Challis *et al.* 1984; Outram & Pollock, 1984; Piacek-Llanes *et al.* 1982; Preussmann, 1984; Walters *et al.* 1983). *In vitro* nitrosation experiments on food mixtures have also suggested that unidentified non-volatile NOC are synthesized in much larger amounts than the well-known volatile nitrosamines (Walters *et al.* 1974). Model calculations by Fine *et al.* (1982) also suggested that compound classes other than secondary amines must be more important as *in vivo* nitrosation precursors, at least as far as gastric cancer is concerned. Mutagenicity and carcinogenicity studies of nitrosamide-type compounds have revealed these substances to be activation-independent agents with a range of potencies similar to those found in nitrosamines (Bulay *et al.* 1979; Piacek-Llanes *et al.* 1982).

In our risk assessment, an attempt has been made to pinpoint areas where further research is warranted; conclusions are based not just on one criterion, such as 'high concentration in gastric juice', 'rapid nitrosation' or 'highly carcinogenic', but on the likely interactions of these several factors. The risk ranges presented in Fig. 5 show an enormous span between the highest and lowest classes of compound. Whereas the food contents of these compounds had a span of five or six, the nitrosatability rate constants seven and the carcinogenicity five orders of magnitude, the risk estimates cover nine orders of magnitude. This suggests that errors involved in the primary assumptions, even up to a factor of 10 or 100, would not seriously sway the conclusions.

This preliminary study suggests that ureas, aromatic amines and amides are the most important precursors of endogenously formed NOC. Under conditions of high gastric nitrite concentration, endogenously formed *N*-nitrosoureas and aromatic nitrosamines could pose a risk equal to or greater than that of unavoidable DMN in the diet.

Work on developing methods to identify ureas in foods (Kawabata *et al.* 1980; Mirvish *et al.* 1980b), synthesizing new *N*-nitroso derivatives of naturally occurring ureas, and testing the carcinogenicity of such compounds (Bulay *et al.* 1979) deserves to be intensified. Far too little is known about dietary ureas at present.

In contrast, the aromatic amines are relatively well known to toxicologists, because they induce methaemoglobin formation and some are established carcinogens for the human bladder. It is therefore hardly surprising that additional mechanisms of toxicity, such as the aspects discussed here, have not yet been studied in depth. On the other hand, diazo-coupling reactions are well known in the dye industry, so that one might anticipate the carcinogenic potential of nitrosated aromatic amines postulated from this study.

Nitrosated proteins and guanidines from food precursors only approach the risk levels of DMN under extreme conditions (very low stomach pH and high nitrite concentration). Normally, they constitute a risk of approximately 1% of preformed DMN. Work underway to examine the mutagenicity of *N*-nitroso derivatives of natural amides (Piacek-Llanes *et al.* 1982) should be expanded, as should kinetic studies designed to determine which side groups influence nitrosatability positively or negatively (Walters *et al.* 1974; Shephard *et al.* 1987). The stability of nitrosated peptides should be examined, along with their possible role as storage forms of nitrosated amino acids (see below).

Guanidines fall into the medium priority category. Mirvish (1971) suggested that they might be of importance because they could form *N*-nitrosoureas via an oxidative nitrosation reaction. However, in the light of the potency of MNNG and related compounds, it would seem justified to examine the contribution of *N*-nitrosoguanidines in their own right. Aside from creatine and creatinine, very little is known about the sources or reactivities of dietary guanidines.

The primary amines and amino acids have been largely ignored as nitrosatable precursors because of the instability of primary nitrosamines. This appears justified in the case of alkylamines, where the calculated risk is low in comparison to exogenous DMN; our risk estimate correlates well with a risk assessment made by Huber & Lutz (1984). Primary *N*-nitrosamino acids present a somewhat higher risk, and if nitrosated peptides prove to be a storage form of nitrosamino acids, capable of transporting *N*-nitrosamino acids into the cell, their potency as possible activation-independent carcinogens would be worth further study. The main question is the

stability/reactivity of the *N*-nitroso derivatives and how this is influenced by the side chains of the various amino acids.

Weakly basic secondary amines do not appear to be found in a normal human diet; it is thus possible that secondary amines are a negligible risk factor in comparison to other precursor classes. This hypothesis correlates with *in vitro* food nitrosation studies, where resulting volatile nitrosamines (DMN, diethylnitrosamine, *N*-nitrosopiperidine, *N*-nitrosopyrrolidine) were rarely found under conditions comparable to those observed in gastric fluid (Groenen *et al.* 1982).

Finally, the secondary *N*-nitrosamino acid received a very low ranking in the risk estimates. Nitrososarcosine is weakly carcinogenic, while *N*-nitrosoproline has no carcinogenic potency (Oshshima & Bartsch, 1981). If the other secondary *N*-nitrosamino acids also prove to be excreted unchanged in the urine, their low risk ranking would appear to be justified.

Conclusions

Dietary precursors of NOC can be grouped, as follows, into classes according to the risk that arises from their endogenous nitrosation:

(i) Ureas, aromatic amines: potentially important risk factors in gastric cancer.
(ii) Amides (including protein), guanidines, primary amino acids: risk contribution uncertain.
(iii) Alkylamines (primary and secondary), secondary amino acids: most probably negligible risk factors.

Two priorities for future investigation emerge from this risk analysis. First, the sources and levels of arylamines and ureas in the diet should be studied comprehensively. This would allow a more realistic estimate of the *total* risk contributed by arylamines and ureas. Secondly, the carcinogenic potencies of key nitrosated products should be determined more precisely than the necessarily vague categories presented here. Unfortunately, the instability of some *N*-nitroso derivatives precludes their testing in long-term studies. Work is currently in progress in our laboratory to develop short-term tests; these will allow us to characterize the overall reactivity (nitrosatability of precursor and alkylating power) or genotoxicity of dietary components that form unstable NOC.

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Appendix I: Food sources of *N*-nitroso precursors

Quantities, where given, are means or ranges of the levels found—in mg of the given compound per kg of the specified food.

Primary amines

Methylamine
Spinach (12)
Cabbage (3.4)
Kale (16.6)
Carrot (3.8)
Radish (42)
Maize (26.8)
Apple (5.6)
Cod roe (10.3)
Freeze-dried coffee (16–80)
Barley (4.5)
Soya bean (50)
Spoiled sausage
Fresh pork (1.5)
Pickles (1–26)
Red cabbage (22.7)
Cauliflower (65)
White beet (17.6)
Red beet (30)
Celery (6.4)
Lettuce (37.5)
Herring (2–7)
Cheese (3–12)
Cocoa (60)
Tea (50)
Hops (3.7)
Rapeseed cake (550)
Green algae
Bacon (0.7)

Ethylamine
Spinach (8.4)
White beet (4.3)
Radish (10)
Maize (2.4)
Apple (3)
Freeze-dried coffee (1.5–2)
Wine (0.5–1.7)
Red cabbage (1.3)
Carrots (1)
Red radish (40)
Lettuce (3.3)
Cheese (1–4)
Barley (3.4)
Hops (5.2)

n-*Propylamine*
Swede (5)
Pickled cucumber (7.5)
Pickled onions (1.8)
Cheese (2–8.7)
Milk (3–10)
Pickled paprika (2.3–10.6)
Pickled pepperoni (1.4)
Pickled celery (2.7)
Brown bread (1.6)

Isopropylamine
Maize (2.3)

n-*Butylamine*
Kale (7)
Cheese (3.7)
Brown bread (1.1)
Pickled cucumber with mustard (5.3)
Soya-bean oilseed (1)

Isobutylamine
Freeze-dried coffee (1)
Cocoa (6)

n-*Pentylamine*
Cabbage (1.4)
Swede (1)
Lettuce (3)
Pickled cucumber (1.7)
Herring (17)
Freeze-dried coffee (10–15)
Cauliflower (3.3)
Red radish (6.9)
Pickled paprika (1.6–3)
Pickled cucumber with mustard (2.8)
Cheese (1.2)

Isopentylamine
Spinach (3.8)
Swede (5)
Freeze-dried coffee (1)
Rapeseed cake (140)
White beet (7.6)
Rhubarb (3.9)
Cocoa (10)
White wine (2–3)

Benzylamine
Spinach (6.1)
Cabbage (2.8)
Kale (3.8)
Carrots (2.8)
Red radish (4.8)
Maize (3.4)
Rhubarb (2.9)
Red cabbage (3.3)
Cauliflower (1.4)
White beet (5.3)
Radish (1.8)
Celery (3.4)
Lettuce (11.5)

Phenethylamine
Spinach (1.1)
Cabbage (2.1)
Kale (3)
Swede (40)
Rhubarb (3.2)
Rapeseed cake (90)
Red cabbage (8.6)
Cauliflower (1.8)
White beet (1.3)
Carrots (2)
White wine (2)
Mature sausage (5)

n-*Dodecylamine*
Pickled cucumber (2.7)
Pickled onions (3.6)
Pickled pepperoni (6.4)

Tyramine
Cheese (27–1500)
Spoiled meat
Tomatoes (4)
Smoked/pickled fish (470–3000)
Milk (1.1)
Sauerkraut (20–95)
Raspberries (13–98)

Appendix I: Food sources of *N*-nitroso precursors

Quantities, where given, are means or ranges of the levels found—in mg of the given compound per kg of the specified food.

	Ham (2–8)
	Spoiled fish
	Bananas (7)
	Beer (2–11)
	Meat (0–1200)
	Octopus
	Mature sausage (60–564)
	Wine (3)
	Pork (11)
	Avocados (23)
Histamine	Cheese (1)
	Fish (1–30)
	Smoked pork (4–5)
	Spinach (38)
	Sausage (60–140)
	Milk (0.4)
	Spoiled fish
	Pork (6)
	Sauerkraut (6–100)
	Tomato (22)
	Wine (7)
	Bacon (1–15)
Cadaverine	Cheese
	Peas (6–7)
	Smoked pork (14–630)
	Sausage (15–174)
	Spoiled fish
	Soy sauce (200)
	Pork (170)
	Sauerkraut (3–30)
Putrescine	Cheese
	Soy sauce (80)
	Mature sausage (90)
	Pork (170)
	Spoiled fish
	Smoked pork (11–500)
	Bacon (2–36)
	Sauerkraut (1–40)
Spermidine	Pork (420)
	Soya beans
	Endogenous in mammalian tissue
	Smoked pork (150–1270)
	Bacon (3)
	Ham (4)
Dopamine	Bananas (8)
Ethanolamine	Ham (4–6)
	Bacon (10–22)
	Pork (8)
	Sauerkraut (2–7)
Tryptamine	Tomatoes (4)
	Pork (14)
	Bacon (3–13)
	Cheese (0–1100)
	Sausage (10)
	Ham (8–67)
Serotonin	Bananas (28)
	Pineapple (17–65)
	Tomatoes (12)
Hexylamine	Milk (5–17)
Spermine	Pork (61)
	Smoked pork (6–800)
	Bacon (1–37)
	Ham (51)
	Amino acids
Glutamic acid	Milk
	Meat (large amount)
	Cheese
	Grain
	Eggs
	Mushrooms (1300)
Glycine	Milk
	Meat (large amount)
	Mushrooms (200)
Aspartic acid	Milk
	Meat (moderate amount)
	Mushrooms (200)
Alanine	Milk
	Pork (large amount)
	Mushrooms (1300)
Serine	Milk
	Meat (large amount)
	Mushrooms (300)
Glutamine	Meat
	Mushrooms (400)
Arginine	Grain
	Mushrooms (900)
Leucine	Grain
	Mushrooms (400)
Proline	Milk (1.5)
	Mushrooms (1000)
Sarcosine	Lobster
	Cartilaginous fish
	Secondary amines
Dimethylamine	Red cabbage (2.8)
	Cauliflower (14)
	Red radish (1.1)
	Maize (26.8)
	Pickled cucumber (15.4)
	Herring (3.4–7.8)
	Freeze-dried coffee (3–6)
	Soya-beans (8)
	Pork (0.1–0.2)
	Octopus with soya (369)
	Cabbage (2)
	Kale (5.5)
	Celery (5.1)
	Lettuce (7.2)
	Pickled onions (1)
	Brown bread (3.1)
	Barley (1.6)
	Hops (1.4)
	Sardines in oil (180)
	Salmon (48)
	Mackerel (26)
Diethylamine	Spinach (15)
	Pickled cucumber (1.4)
	Herring (1.9–5.2)
	Barley (5.7)
	Beef (2×10^{-4})
	Apple (3)
	Pickled onion (3.2)

Appendix I: Food sources of *N*-nitroso precursors

Quantities, where given, are means or ranges of the levels found—in mg of the given compound per kg of the specified food.

	Cod roe (5.2)
	Hops (3.1)
	Spoiled fish
Methylethylamine	White beet (7.6)
	Maize
	Freeze-dried coffee (1–2)
	Carrots (7)
	Lettuce
	Herring (1)
	Hops (3.7)
Pyrrolidine	Spinach (2.5)
	Maize (3.5)
	Pickled cucumber (5.6)
	Pickled onions (8.4)
	Cheese (1–20)
	Hops (1)
	Malt (1.5)
	Red radish (38)
	Pickled paprika (1.4)
	Pickled pepperoni (1.8)
	Pickled celery (2.6)
	Freeze-dried coffee (7–10)
Pyrroline	Red radish (20)
Piperidine	Pickled paprika (5.2)
	Pickled celery (1)
	Cocoa (9)
	Barley (1)
	Beef (1×10^{-3})
	Pickled pepperoni (3.4)
	Freeze-dried coffee (1–2)
	Hops (2.5)
	Pepper
N-*Methylbenzylamine*	Lettuce (10)
	Carrots (16)
	Herring (2)
N-*Methylphenethylamine*	Cauliflower (1.6)
	White beet (1.6)
	Carrots (2)
	Red radish (5.4)
	Rhubarb (2.6)
	Pickled cucumber (2.2)
	Pickled onions (6.5)
	Cheese (2.6)
	Spinach (2.4)
	Kale (2)
	Swede (2)
	Radish (6.6)
	Maize (1.1)
	Apple (1.2)
	Pickled cucumber with mustard (7.3)
	Red cabbage (3.7)
(*Morpholine*	Solvent in food industry)
Chavicine	Pepper
	Arylamines
Aniline	Red cabbage (1)
	Cauliflower (22)
	Carrots (30.9)
	Red radish (4.6)
	Apple (1.5)
	Cabbage (4)
	White beet (1.2)
	Radish (2.8)
	Rhubarb (5)
	Rapeseed cake (120)
Toluidines	Kale (1.1)
	Celery (1.1)
	Carrots (7.2)
N-*Methylaniline*	Spinach (3.4)
	Pickled cucumber (13.8)
	Pickled celery (7)
	Pickled paprika (13.1)
	Pickled onions (6.8)
	Cheese (37.9)
	Amides
Asparagine	Carrot juice
	Asparagus
Glutamine	Meat
Pyrrolidone carboxylic acid	From glutamate
Glutathione	Liver
	Muscle
Carnosine	Muscle (90–4600)
	Fish
Anserine	Muscle
	Poultry
	Guanidines
Guanidineacetic acid	Mammalian blood
Creatine	Vertebrate muscle (5000)
	Fish muscle (7000)
Methylguanidine	Dried fish products (20–180)
	Sardines (60–1900)
	Cod
	Milk
	Liver
	Shark
	Beef
Agmatine	Shellfish (<650)
	Cheese
	Abalone
Creatinine	Dried fish (4100)
	Bacon (3300)
	Fried beef (540)
	Ureas
Citrulline	Watermelon
	Green peppers
	Soy sauce
	Mushrooms
Albizziin	Mimosa
(*β*-(p-*Nitrophenyl*)-*ureidopropionic acid*	Sweetener)
N-*Carbamoylputrescine*	Grains
Allantoic acid	Plants
Hydantoic acid	Sugar-beet sprouts
N-*Carbamoylaspartic acid*	Intermediate in pyrimidine biosynthesis