MODEL RISK ANALYSIS OF NITROSATABLE COMPOUNDS IN THE DIET AS PRECURSORS OF POTENTIAL ENDOGENOUS CARCINOGENS

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The potential health risk posed by the endogenous formation of *N*-nitroso compounds (NOC) from nitrosation of dietary ureas, guanidines, amides, amino acids and amines (primary, secondary and aromatic) was estimated according to the model:

Risk = [daily intake of precursor] × [gastric concentration of nitrite]^n × [nitrosatability rate constant] × [carcinogenicity of derivative].

The daily intakes of these compound classes span five orders of magnitude (100 g/day amides, top; 1-10 mg/day secondary amines, ureas, bottom); the nitrosation rate constants span seven orders of magnitude (aryl amines, ureas, top; amides, secondary amines, bottom); and the carcinogenicity estimates span a 10 000-fold range from 'very strong' to 'virtually noncarcinogenic'. The resulting risk estimates likewise span an enormous range (nine orders of magnitude): dietary ureas and aromatic amines combined with high nitrite concentration could pose as great a risk as the intake of preformed *N*-nitrosodimethylamine in the diet. In contrast, the risk posed by the in-vivo nitrosation of primary and secondary amines is probably negligible. The risk contributed by amides (including protein), guanidines and primary amino acids is intermediate between these two extremes.

The human diet contains a variety of nitrosatable precursors, which differ markedly with respect to (i) daily intake, (ii) rate of nitrosation, and (iii) carcinogenicity of the nitroso derivative. After combination of all three variables, the different precursor classes were evaluated for their relative importance as a potential source of endogenous carcinogens (Shephard *et al.*, 1987).

Precursors of *N*-nitroso compounds in the diet

The substances of interest in this model risk assessment were those nitrosatable compounds widely found in the natural human diet: amines, amino acids, amides, guanidines and ureas. Unlike industrial or pharmaceutical NOC precursors, these substances constitute an unavoidable source of nitrosatable substances for the general population. The average daily intake of each precursor, *C*, was then calculated as shown in Equation 1 on the basis of average eating habits in Europe, using 1980 food consumption statistics (Schweizerisches Bauernsekretariat, 1983).

$$\text{Daily intake}_C = \sum_i [\text{amount of } C \text{ in food item}_i] \times [\text{daily intake of food item}_i]. \quad (1)$$

Not surprisingly, those compounds of nutritional or biological importance, and their derivatives, are the precursors consumed in the largest amounts (Table 1). Amides are the most important precursor class in the diet: in the form of proteins, they are consumed at a level of almost 100 000 mg/day from meat, poultry, fish, milk, eggs, cheese and grain products (Schweizerisches Bauernsekretariat, 1983). Guanidines form the second most important group: creatine and creatinine are important constituents of meat, comprising about 2 % of the total protein; daily consumption of guanidines amounts to 1000 mg. Free amino acids are also found in all protein-rich foods: glutamic acid, glycine and alanine predominate, and total daily intake amounts to 10-50 mg. Proline (found in collagen fibres) is the major secondary amino acid; intake of free proline is about 0.5 mg/day. Primary amines, formed by decarboxylation of amino acids during microbial fermentation of foods, are eaten at a level of 100 mg/day; cheese and preserved meats are the major sources of biogenic amines. The ureas citrulline and ornithine are also of biological importance, but analytical methods for their detection are lacking; a preliminary estimate of daily intake is >1 mg/day. Secondary amines and aryl amines are not nutritionally important classes; they are found in trace amounts in most foods, and daily intakes amount to approximately 5 and 2 mg, respectively.

Nitrosation rate

The chemistry and kinetics of the nitrosation of amine- and amide-type compounds are well understood (reviewed by Mirvish, 1975). The most important factor governing the nitrosation rate of aliphatic and aromatic amines is the pK of the amine group (lower $pK_a \propto$ faster rate). The amino acids have the additional possibility of intramolecular catalysis by the carboxyl group, which increases their nitrosation rate approximately 100 fold over that of the simple amines. Resonance forms that donate electron density to the *N* atom determine the relative nitrosation speeds of the amide-type compounds: ureas > amides = guanidines.

Kinetic studies have been carried out on only a few of the compounds considered in this model risk assessment, and estimates for the primary amines and primary amino acids had to be based on data from their secondary analogues. Estimates of rate constants k_2 for amines at optimal pH and k_6 for amides at pH 2 (nomenclature from Mirvish, 1975) were made by comparison with the in-vitro values reported by Ridd (1961) and Mirvish (1975), using the above guidelines and the general chemical principle that bulky compounds sterically hinder a reaction. The individual estimates are given in Table 1. As classes, the precursors can be ranked as follows: aryl amines > ureas > primary amino acids > secondary amino acids > primary amines > secondary amines = guanidines > amides, with respect to ease of nitrosation. The estimates span seven orders of magnitude.

In-vivo yield of *N*-nitroso compounds

The in-vitro rate constants at 25°C correspond reasonably well to in-vivo rates at 37°C (reviewed by Shephard *et al.*, 1987). The in-vivo yields of NOC were calculated assuming a stomach volume of 1 litre, a reaction time of 1 h, a pH optimal for the reaction to proceed (2.5-3.4 for the amines, and 2.0 for the amide-type compounds), and two realistic gastric nitrite concentrations taken from the literature: 'low nitrite' = 1.7 μM (Klein *et al.*, 1978) and 'high nitrite' = 72 μM (Tannenbaum *et al.*, 1974). At the low nitrite concentration, the most significant yields of NOC come from protein, 800 pmol, and from methylurea, 400 pmol, followed by the guanidines and aryl amines (50 pmol each). Because of greater sensitivity to nitrite concentration, the in-vivo nitrosation of aryl amines becomes even more

Table 1. Estimates of health risks posed by gastric in-vivo nitrosation of food precursors relative to consumption of preformed NDMA

Precursor	Daily intake (mg)	Rate constant estimate[a]	Carcinogenic potency (OPI)	Health risk relative to NDMA (risk from NDMA = 1)	
				[nitrite]=1.7 μM	[nitrite]=72 μM
PRIMARY AMINES					
Spermidine	35	0.005	100	1×10^{-7}	2×10^{-4}
Tyramine	21	0.05	100	7×10^{-7}	1×10^{-3}
Cadaverine	15	0.01	100	1×10^{-7}	3×10^{-4}
Putrescine	15	0.01	100	2×10^{-7}	3×10^{-4}
Methylamine	3	0.005	10^3	4×10^{-7}	8×10^{-4}
Total	100			10^{-6}	10^{-3}
PRIMARY AMINO ACIDS					
Glutamic acid	> 3.2	1	10	1×10^{-6}	2×10^{-3}
Glycine	> 1.3	1	100	1×10^{-5}	1×10^{-2}
Alanine	> 0.4	1	100	3×10^{-6}	8×10^{-3}
Total	> 10			10^{-5}	10^{-2}
SECONDARY AMINES					
Dimethylamine	1.7	0.002[b]	10^3	7×10^{-8}	2×10^{-4}
N-Methylbenzylamine	0.6	0.013[b]	10^3	7×10^{-7}	1×10^{-3}
Pyrrolidine	0.6	0.005[b]	100	3×10^{-9}	8×10^{-6}
Total	5			$< 10^{-6}$	10^{-3}
SECONDARY AMINO ACIDS					
Proline	> 0.5	0.037[b]	< 0.1	$< 1 \times 10^{-10}$	$< 1 \times 10^{-7}$
Sarcosine	1	0.23[b]	1	3×10^{-9}	5×10^{-6}
Total	> 1			10^{-9}	$< 10^{-5}$
ARYL AMINES					
N-Methylaniline	1.6	250[b]	100	4×10^{-4}	0.8
Aniline	1	500[b]	100	6×10^{-4}	1
Total	2			10^{-3}	> 1
AMIDES					
Protein	92 000	0.001	10	8×10^{-4}	4×10^{-2}
Carnosine	2000	0.001	1	2×10^{-7}	1×10^{-5}
Total	10^5			$< 10^{-3}$	10^{-2}
GUANIDINES					
Creatine	800	0.004	1	6×10^{-6}	2×10^{-4}
Creatinine	300	0.004	10	2×10^{-5}	1×10^{-3}
Methyl guanidine	0.2	0.004[b]	10^3	1×10^{-5}	6×10^{-4}
Total	10^3			$< 10^{-4}$	$< 10^{-2}$
UREAS					
Methyl urea	> 1	10.5[b]	10^3	$> 5 \times 10^{-2}$	> 1
N-Carbamoyl putrescine	1[c]	1	100	2×10^{-5}	1×10^{-3}
Citrulline	1[c]	0.7[b]	10	1×10^{-5}	4×10^{-4}
Total	> 1			$> 5 \times 10^{-2}$	> 1

[a] k_2 at optimal pH for amines; k_6 at pH 2 for amide-type compounds

[b] Rate constants from Mirvish (1975)

[c] Preliminary estimate of 1 mg/day assigned

important than that of ureas or protein after a nitrite-rich meal (100 nmol *versus* 30 nmol). The amounts of nitrosamine produced *in vivo* from aliphatic amines are, in contrast, very small, comprising only picomole quantities even in the presence 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.

Carcinogenicity of nitroso derivatives

The carcinogenic potency of the nitroso derivatives of each compound listed in Table 1 was estimated. The results of chronic feeding studies in rats were used as the data base, and the data were normalized using the Oncogenic Potency Index (OPI, adapted from Meselson & Russell, 1977):

$$\text{OPI} = \ln (1 - \text{tumour incidence}) / \text{daily dose} / (\text{time})^3. \quad (2)$$

Quite precise estimates of the OPI could be made for the secondary amines, secondary amino acids and ureas. No study was available on nitrosopeptides, the nitrosoguanidines of interest, or the unstable primary nitrosamine classes. Rough OPI estimates were made for these latter compound classes, using the following empirical guidelines (Druckrey *et al.*, 1967):

(i) The short-chain alkyl substituted NOC are very potent: OPI 10^3.

(ii) The larger NOC become less potent: OPI 10-100.

(iii) A stable compound with a polar or charged substituent is a weak or noncarcinogen: OPI < 0.1-1.

The potency of the unstable NOC depends on their half-life within the cell. *para*-Hydroxymethylbenzene diazonium ion, given orally to mice, and *N*-nitrosomethylamine, generated *in situ* in rat stomach, both produced covalently bound DNA adducts (Huber & Lutz, 1984; Shephard, Fischer & Lutz, in preparation). On the basis of this information, conservative potency estimates were made for the primary nitrosamines, assuming long enough lifetimes to reach the DNA.

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 *N*-nitrosodimethylamine (NDMA) in foods. Estimates of the health risks due to particular NOC were calculated using equation 3:

$$\begin{aligned}\text{Risk}_{\text{NOC}} = {} & \text{daily intake of precursor } C \text{ (mol/day)} \\ & \times \text{gastric concentration of nitrite}^n \text{ (1.7 or } 72 \times 10^{-6} \text{ M)}^n \\ & \times \text{nitrosatability rate constant } k_2 \text{ (s}^{-1}M^{-2}\text{) or } k_6 \text{ (s}^{-1}M^{-2}\text{)} \\ & \times \text{carcinogenicity of derivative OPI (kg mmol}^{-1} \text{ day}^{-1} \text{ year}^{-3}\text{).}\end{aligned} \quad (3)$$

The parameter n is 2 for amines and 1 for amide-type precursors. The model assumes that health risk is linearly related to both the carcinogenicity and to the daily endogenous yield of each NOC. Similarly, the risk due to preformed NDMA (intake 10 nmol/day, Spiegelhalder *et al.*, 1980b; OPI value 3000, Parodi *et al.*, 1982) is 10 nmol × 3000 = 30 000. The relative risk can be expressed as $\text{Risk}_{\text{NOC}}/\text{Risk}_{\text{NDMA}}$.

Results of these calculations can also be found in Table 1. The risk estimate totals of the various precursor classes span a range of nine orders of magnitude. According to these calculations, the aryl amines and ureas are the most important precursor classes. 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 NDMA in the diet. Guanidines, amides and primary amino acids fall into a medium-risk category. Under normal conditions, they constitute a risk of 0.1-1% that of NDMA, but under extreme conditions of high nitrite or low pH (guanidines and amides), they could also become important. The primary amines, secondary amines and secondary amino acids fall into the lowest class, and the risk posed by their nitrosation is negligible, which agrees with the conclusions reached by Fine *et al.* (1982).

Two priorities for future investigation emerge from this model risk analysis. Firstly, 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 aryl amines 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 (Shephard *et al.*, this volume) that will allow us to characterize the overall reactivity (nitrosatability of precursor and alkylating power) or genotoxicity of dietary components that form unstable NOC.