WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

N-NITROSO COMPOUNDS: OCCURRENCE, BIOLOGICAL EFFECTS AND RELEVANCE TO HUMAN CANCER

Proceedings of the VIIIth International Symposium on N-Nitroso Compounds held in Banff, Canada, 5–9 September 1983

Co-sponsored by:

Agriculture Canada
Consumer and Corporate Affairs Canada
Environment Canada
Health and welfare Canada

Alberta Heritage Foundation for Medical Research
University of Alberta, Edmonton

EDITORS

I.K. O'NEILL, R.C. VON BORSTEL, C.T. MILLER, J. LONG & H. BARTSCH

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The International Agency for Research on Cancer (IARC) was established in 1965 by the World Health Assembly, as an independently financed organization within the framework of the World Health Organization. The headquarters of the Agency are at Lyon, France.

The Agency conducts a programme of research concentrating particularly on the epidemiology of cancer and the study of potential carcinogens in the human environment. Its field studies are supplemented by biological and chemical research carried out in the Agency's laboratories in Lyon and, through collaborative research agreements, in national research institutions in many countries. The Agency also conducts a programme for the education and training of personnel for cancer research.

The publications of the Agency are intended to contribute to the dissemination of authoritative information on different aspects of cancer research.

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FOREWORD

This volume, published as the proceedings of the Eighth International Meeting on *N*-Nitroso Compounds held in Banff, Alberta, Canada, focuses solely on *N*-nitroso compounds and their precursors, as did previous publications in this series. Many of the contributions confirm the widespread occurrence of *N*-nitroso compounds in the human environment and the fact that they can be formed endogenously in man from normal dietary constituents. Although they are clearly established as animal carcinogens, a causal relation between *N*-nitroso compounds and human cancer has not yet been rigorously established. At this meeting, for the first time, epidemiological evidence was presented to show such an association: oral cancer was linked with snuff dipping, and *N*-nitrosamines are the only carcinogens that have been detected in snuff, at levels exceeding by two orders of magnitude those in other consumer products. These data indicate that *N*-nitrosamine levels found in our environment may be sufficient to cause oral cancer in man and raise even more strongly the suspicion that they may be involved in other human cancers as well.

Because of their ubiquitous occurrence and their potent carcinogenicity (in 40 animal species), the endeavours of many research institutions, including this Agency, to identify sources of *N*-nitroso compounds in our environment and to find means for preventing exposure to them seem to be fully justified. In fact, there are many ways in which *N*-nitrosamine levels in food and in manufactured products can be lowered and many ways in which in-vivo nitrosation and potentiating risk factors, for example micronutrient deficiencies, may be reduced. Such preventive measures can clearly be implemented today, as pointed out in the closing remarks of Peter Magee who, together with M. Barnes, discovered in 1956 the carcinogenicity of *N*-nitrosodimethylamine in rats.

N-Nitroso compounds have also served as very valuable tools in basic cancer research, and many presentations in these proceedings contribute to our understanding of the cellular and molecular mechanisms by which *N*-nitroso compounds induce cancer.

I would like to thank the Programme Committee and all the members of the Canadian Executive Committee, in particular Drs R.C. Von Borstel, C.T. Miller and J.E. Long, who worked together to organize a meeting of such high quality. I express my deep gratitude to the co-sponsors of the meeting – the Alberta Heritage Foundation for Medical Research, the University of Alberta, Edmonton, and the four Departments of the Federal Canadian Government, Agriculture, Consumer and Corporate Affairs, Environment, Health and Welfare, as well as to the industrial companies that provided financial support. The highly efficient organization and management of the meeting at the Banff Conference Center by Mrs C. Hardie and her staff deserve particular acknowledgement.

Lorenzo Tomatis, M.D.
Director,
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for Research on Cancer,
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INTRODUCTION

The present volume, published as the Proceedings of the Eighth International Meeting on *N*-Nitroso Compounds[1], reflects in its title the increasing interest being generated to assess the relevance of these compounds to human cancer. The overview preceding the contributions to these proceedings was prepared from the reports of those chairing the sessions and is intended to highlight new and important developments and approaches. An attempt has been made to systematize the nomenclature of the *N*-nitroso compounds (p. 991) to ensure that chemists and biologists are referring to the same substances. In order to increase the usefulness of this large volume, *subject* and *author* indexes have been included at the end of this volume.

The editors wish to thank the chairpersons and the organizers for their contributions during the meeting, and the Programme and Executive Committee, whose guidance and assistance were invaluable.

The Editors

Lyon, 30 January 1984

[1] Ninth Meeting to be held on 1–5 September 1986 in Vienna, Austria, following the 14th International Cancer Congress in Budapest (21–27 August 1986)

N-NITROSO COMPOUNDS: OCCURRENCE, BIOLOGICAL EFFECTS AND RELEVANCE TO HUMAN CANCER - AN OVERVIEW [1]

In this review of the contributions presented in this volume, emphasis is placed on three aspects:

(i) new and important developments of methods and approaches that could help in understanding of basic mechanisms of carcinogenesis and suggestions for such studies on humans in situations in which exposure to *N*-nitroso compounds, or their precursors, is known to be or to have been high and in which methods exist for monitoring such exposure;

(ii) significant advances in laboratory methods that could be applied to an integrated laboratory and epidemiological approach; and

(iii) deficiencies in present knowledge and inadequacies of available methods, with suggestions for possible future directions of research to fill these gaps.

1. *Occurrence and formation of* N-*nitroso compounds*

New data on precursors of *N*-nitroso compounds (NOC) and on their occurrence were reported. The general population can be exposed *via* foodstuffs both to preformed NOC and to their precursors; the latter react with nitrosating agents to yield NOC and other reactive intermediates. As examples, tyramine and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, present in soya sauce, were mentioned as precursors that give rise to mutagenic compounds after nitrosation (p. 17). Elevated levels of both dimethylamine and *N*-nitroso-dimethylamine (NDMA) have been found in the intestines of patients with chronic renal failure (p. 161). *N*-Nitrosation of peptides has been studied in order to elucidate conditions under which polypeptides and proteins present in the gastric juice or mucosa might be converted to *N*-nitroso derivatives of biological significance (p. 7). Polyunsaturated lipids were found to be capable of serving as *N*-nitrosating agents in mouse skin after exposure to nitrogen dioxide, raising the possibility that similar reactions might be responsible for *N*-nitrosamine formation in other tissues (p. 283). Intravenous or oral administration to human subjects of nitrate (p. 193) including ammonium nitrate given to prevent formation of renal stones reproducibly increased endogenous formation of *N*-nitrosoproline (NPRO). A background of endogenously formed NPRO is unaffected by ingestion of ascorbate or α-tocopherol (p. 223), two known inhibitors of *N*-nitrosation reactions.

---

[1] This overview was prepared by a committee composed of M.C. Archer, H. Bartsch (Secretary), P. Bogovski, M. Börszönyi, B.C. Challis, P. Correa, E. Heseltine (Technical Editor), T. Kawabata, L. Keefer, P. Kleihues, W. Lijinsky, P.N. Magee, A.B. Miller, C.T. Miller, I.K. O'Neill (Rapporteur), A.E. Pegg, R. Preussmann, R.A. Scanlan, N. Sen, S.R. Tannenbaum and R.C. von Borstel

[2] Figures in parentheses refer to page numbers in this volume.

Thus, humans can synthesize NOC endogenously; good evidence for the occurrence of these reactions comes from studies in which proline (p. 223), piperazine (p. 171) and aminopyrine (p.179) were used as nitrosatable amines. The extent of the nitrosation reaction was shown to be linked to (dietary) intake of nitrite and nitrate; however, smoking (p. 811) and in-vivo oxidation of ammonia (p. 241) are also contributing factors; induced inflammation enhanced the rate of nitrate synthesis by oxidation of ammonia (p. 247) and related substrates. The role of bacteria-mediated nitrosation has been investigated further (p. 275), and an *Escherichia coli* strain has been shown to catalyse *N*-nitrosamine formation from nitrite and an amine.

Many *N*-nitrosation inhibitors, such as ascorbic acid and α-tocopherol, have been characterized and used to advantage in lowering exposure to NOC (p. 223). Dietary phenolics (p. 213) appear to play a complex role, causing either increased or decreased endogenous formation of NOC such as NPRO, depending on factors such as pH and rate of production of saliva and the ratio of concentrations of precursor nitrite and amine.

Several new findings on the chemistry of formation and decomposition of NOC were described, including photolysis in non-aqueous media (p. 365). Formation and inhibition were also demonstrated to occur in emulsions (p. 347). Nitrite-ester mediated NOC formation from nitrite and amines was reported (pp. 311, 353). Progress has been made in the safe destruction of carcinogenic *N*-nitrosamides (p. 387).

2. *Analytical advances and identification of new NOC*

Significant progress has been made in developing high-performance liquid chromatographic (HPLC) and gas-liquid chromatographic (GLC) methods for analysing nitrosamides, by (i) post-column HPLC chemical denitrosation of the nitrosamides followed by chemiluminescence detection of the liberated NO, and (ii) modification of the Thermal Energy Analyzer pyrolysis conditions to enable detection of nitrosamides after separation by GLC (p. 121).

New developments and improvement of methods for the analysis of non-volatile NOC were presented (p. 138). Although progress has been made, more research will be required before the nature of most non-volatile NOC in foods and biological fluids can be elucidated.

For example, samples of canned cured meat and Chinese cabbage were found to contain apparently high concentrations of total NOC (p. 25), as determined by the method of Walters *et al.* Future work should be directed toward establishing that all NOC respond on a molar basis in this procedure, so that all NOC exposures can be quantified individually.

N-Nitrosothiazolidine 4-carboxylic acid (NTCA) and *N*-nitroso-2-methylthiazolidine 4-carboxylic acid (NMTCA) (*cis* and *trans* isomers) (pp. 77, 87) were isolated and identified in human urine for the first time. Future research should attempt to establish the origin and the biological significance of these two compounds. As the easily nitrosatable amino precursors, thiazolidine 4-carboxylic acid and its 2-methyl derivative, are formed readily by reaction of formaldehyde or acetaldehyde with cysteine *in vitro* and *in vivo* (p. 77), measurement of NTCA and NMTCA in urine may provide a further index for endogenous nitrosation in the human body and may also allow monitoring of exposure of human subjects to precursors like formaldehyde, acetaldehyde, nitrate and nitrite.

3. *DNA repair, macromolecular adducts and biological effects*

A DNA repair protein which removes alkyl groups from the O^6-position of guanine in DNA was described (p. 575); this protein was studied in human lymphocytes (p. 561), in various human organs and in rat liver and brain at various stages of fetal and post-natal development

(p. 571). Its concentration depends on both organ and species, the highest amounts being found in human liver (p. 575). Attempts have been made to relate low rate of DNA repair and high rate of cellular replication with cancer risk in rodent organs with different susceptibilities to induction of cancer by *N*-nitrosoalkylureas (p. 571).

The production of monoclonal antibodies (p. 589) of high specificity and affinity for alkylated deoxynucleosides and their use for quantitating these adducts (i.e., for monitoring human exposure to alkylating agents) by radioimmunoassay or immunofluorescence techniques was described. Another approach for exposure monitoring (p. 589) involved measurement of the formation of deuterated 7-methylguanine and *S*-methylcysteine in rats exposed to deuterated aminopyrine, a drug which yields NDMA *in vivo* upon nitrosation.

The results of a number of bioassays in which the same NOC were given to rats and hamsters were summarized (p. 617). The striking difference in target organs in which tumours occurred is difficult to explain on the basis of the known pathways for activation or DNA repair in these species and suggests an influence of other, presently unknown, factors possibly related to gene expression in cells at the time of interaction with the carcinogens.

The organ specificity of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (p. 603) for the glandular cells of the stomach in rodents did correlate, however, with the high concentration of thiols in those cells, which facilitate decomposition of the carcinogen (p. 603). *N*-Nitrosomethylbenzylamine (NMBzA) was shown to be activated to a methylating agent in the target cells of rodents in which it induces tumours (p. 595), i.e., the oesophagus, lung and liver. NMBzA was also reported to be metabolized by oesophageal microsomes from humans (p. 473) but at a lower rate than by those from rats.

Data on the metabolism and genetic and carcinogenic activities of a variety of *N*-nitrosamines (p. 401) and *N*-nitramines (p. 485) were presented. Carcinogenic NOC previously reported to be non-mutagenic gave positive responses in a yeast test system (p. 721). Tumours were induced by *N*-nitrosodiethylamine (NDEA) in the liver, kidney, oral cavity and trachea of pythons, bringing the number of animal species in which *N*-nitroso compounds produce tumours up to 40 (p. 677).

The analysis of a large BIBRA/UK government study on dose and time relationships for *N*-nitrosamine carcinogenesis in rats was presented (p. 627). Two years of chronic treatment with NDMA and NDEA led to a clearly measurable cancer risk at a dose of 10 μg/kg body weight per day.

The underlying chemistry by which chemotherapeutic *N*-nitrosoalkylureas interact with DNA was described (p. 689); the formation of DNA adducts might be predicted on the basis of stereo-electronic control. It was shown in experimental systems that the antineoplastic activities of certain new nitrosoureas can be dissociated, at least partially, from their carcinogenicity (p. 695).

4. *Metabolism and modifying factors*

NDMA has been shown to be hydroxylated by a specific cytochrome P-450 isozyme which is inducible, for example, by pyrazole and acetone but not by phenobarbital (p. 423). Metabolic activation of *N*-nitrosamines can take place not only by oxidation at the carbon atom in the position α to the *N*-nitroso group, but also at the β- or ω-carbon atoms (p. 401); such reactions have important consequences for the metabolic fate and organotropic effects of *N*-nitrosodialkylamines.

A β-nitrosaminoaldehyde has been synthesized as a model compound, and the aldehyde group has been shown to be highly electrophilic (p. 429). As a consequence, such compounds can form reactive diazonium ions without metabolic activation. The nitrosaminoaldehyde was also active as a transnitrosating agent. These reactions may explain the biological activity of

N-nitrosamines such as *N*-nitrosodiethanolamine (NDELA), which can be oxidized to a monoaldehyde.

Various chemicals, e.g., ethanol, disulfiram (p. 519) (used as an anti-alcoholism drug in humans), certain isothiocyanates (p. 797), phenols (p. 213), coumarins and indoles of natural origin (p. 797), were shown to have marked effects on the pharmacokinetics and metabolism of *N*-nitrosamines, influencing in turn their carcinogenic effects in experimental animals, both qualitatively (target organ) and quantitatively (incidence). Similarly, in rats on a zinc-deficient diet, NDMA did not produce the expected tumours of the liver and kidneys, but tumours were induced in the forestomach (p. 543).

Ethanol, in small quantities, was shown to alter the distribution and metabolism of small oral doses of NDMA and NDEA in rats, increasing by several-fold the alkylation of DNA in organs that are particularly susceptible to their carcinogenic effect (p. 501) (i.e., the kidney for NDMA and the oesophagus for NDEA). As demonstrated with NDMA, this effect is the result of prevention of first-pass clearance in the liver of the *N*-nitrosamine. There is suggestive evidence that this also happens in humans, since a relatively high level of *N*-nitrosamines has been observed in human blood after ingestion of high nitrate meals with alcohol. Therefore, the influence of alcohol consumption on human cancer may be mediated through an effect on the pharmacokinetics of *N*-nitrosamines derived from diet, from tobacco smoke and from endogenous synthesis (pp. 501, 867).

5. *NOC in tobacco carcinogenesis*

(a) Formation and analysis of NOC in tobacco products

That NOC occur in fermented tobacco and tobacco smoke is now established (p. 743), and this is the greatest and most widespread source of human exposure presently known (except for some occupational exposures). Eleven volatile and two non-volatile *N*-nitrosamines, including four volatile tobacco-specific nitrosamines (TSNA), have been detected (p. 743). The concentrations of TSNA are high in tobacco smoke and even higher in snuff and chewing tobacco (p. 743). The concentration of NOC, especially TSNA, was shown to be related to the nitrate content of tobacco products (p. 878). Levels of volatile *N*-nitrosamines are considerably higher in sidestream smoke than in mainstream smoke (p. 743). There was greater endogenous nitrosation in smokers than in non-smokers (pp. 811, 819).

(b) Carcinogenicity and metabolic activation of TSNA

4-(*N*-Methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) has been shown to be a potent carcinogen (p. 763) which can cross the placental barrier in animals (p. 787). A metabolite of NNK can be reconverted to the parent compound, thus leading to prolonged exposure to NNK (p. 805). In metabolic studies *in vitro* and in experimental animals, NNK and *N*-nitrosonornicotine (NNN) were shown to be readily converted into electrophiles that can interact with DNA (p. 805).

(c) Epidemiological studies and tobacco NOC

Epidemiological data on types of tobacco that determine the risk of developing cancer at sites such as the lung, larynx and bladder were reviewed. No important difference in risk due to the method of curing tobacco was noted (p. 867).

The correlation between oral cancer and chewing of betel quid (often containing tobacco) observed in India and other south-east Asian countries is well established (p. 851). In studies

to identify the etiological agents involved, it was shown that nitrosation of arecoline, a betel-nut alkaloid, leads to the formation of three NOC, of which *N*-nitroso-*N*-methylpropionitrile was carcinogenic to experimental animals (p. 859). This carcinogen and other products arising from nitrosation of betel-nut constituents should be investigated further to establish whether they play a role in oral cancer produced in betel-quid chewers.

An association between human cancer and tobacco chewing and snuff dipping has been confirmed in some southern states of the USA (p. 837); tumours usually arise at the site in the mouth where the tobacco product is retained. Since these non-combusted tobacco products have not yet been shown to contain carcinogens other than TSNA (in relatively high concentrations), a direct correlation between *N*-nitrosamine exposure and human cancer must be assumed in this specific case, although the role of alcohol has not been fully examined.

6. *Epidemiological studies and combined laboratory/epidemiology investigations to link NOC and their precursors with human cancers*

A number of epidemiological studies were performed to investigate possible links between adverse biological effects in humans and presumed exposure to NOC and their precursors; however, in no case were NOC clearly identified as the agents responsible, nor was the exposure quantified. Such data are still suggestive and indicate that further investigations must be performed using approaches that allow a more precise estimate of human exposure to NOC. Some evidence was presented linking presumed exposure to NOC or precursors with the development of cerebral tumours (p. 887). The consumption by parents of large amounts of Icelandic smoked mutton was suggested to induce diabetes in their male progeny (p. 911). This diabetogenic effect was also produced experimentally in male offspring of mice fed with Icelandic smoked mutton after mating. High (mg/kg) levels of *N*-nitrosothiazolidine and NTCA were detected in smoked meat products, including Icelandic mutton (p. 911). In a follow-up study on patients who had undergone gastric surgery or who had been treated for pernicious anaemia (situations in which it has been hypothesized that abnormally large amounts of NOC are produced), an excess incidence of gastric cancer over that expected was found (p. 895). A higher concentration of nitrate in saliva was found (p. 921) in subjects living in a high-risk area for cholangiocarcinoma in Thailand than in those living in a low-risk area. However, these findings need further confirmation.

Several industrial processes were described in which occupational exposure to high concentrations of volatile *N*-nitrosamines may occur (p. 938). Exposure to NDELA can be monitored biologically in the urine of workers (p. 943). Only a small number of studies are in progress in which the role of occupational exposure to *N*-nitrosamines is being assessed; more such epidemiological investigations are warranted.

The excretion of nitrosated amino acids was compared in subjects suffering from chronic atrophic gastritis (at high risk for gastric cancer) and in healthy controls (p. 957). Following ingestion of nitrate and proline, urinary NPRO levels in the patients were dependent on gastric pH, showing maximal yields at around pH 2. Healthy controls excreted no apparent excess of NPRO. Urinary NPRO was not correlated with total intragastric NOC in any study subject, but smokers excreted more total *N*-nitrosamino acids in their urine than non-smokers. These data indicate that endogenous nitrosation does occur in the human stomach; however, its relation to the induction of upper gastrointestinal cancer remains to be proven.

The effects of H2 blockers on intragastric nitrosation was examined in healthy volunteers and in duodenal ulcer patients who ingested proline. The concentration of NPRO excreted in the urine was not affected by treatment with ranitidine, but that of NTCA was significantly increased (p. 971).

Hypochlorhydric subjects showed a significant reduction in mean total NOC concentration in gastric juice after four weeks' treatment with ascorbic acid; the total rose again one month

after discontinuing treatment. Mean gastric concentrations of nitrite and of nitrate-reducing organisms were also lowered by ascorbic acid treatment. There was a significant reduction in total NOC during ascorbic acid treatment in patients with partial gastrectomy but not in those suffering from pernicious anaemia or atrophic gastritis.

A high concentration of nitrite was detectable, especially one hour after ingestion of nitrate, in the gastric juice of subjects with chronic atrophic gastritis and in those who had undergone partial gastrectomy.

The etiological factors that may be involved in the causation of oesophageal cancer in certain provinces of Northern China were summarized (p. 948), providing evidence that NOC and their precursors are probably involved. The excretion of urinary *N*-nitrosamino acids by inhabitants living in high-risk (Linxian) and low-risk (Fanxian) areas for oesophageal cancer was compared. Linxian subjects excreted significantly more nitrate and *N*-nitrosamino acids (NPRO, NTCA, NMTCA) than those living in Fanxian. When Linxian subjects were given ascorbic acid (3 × 100 mg after each meal), the level of urinary *N*-nitrosamino acids was reduced to those found in Fanxian. Ascorbic acid, an efficient inhibitor of endogenous nitrosation, should now be examined in intervention trials.

7. *Conclusions*

In view of the evidence that has been accumulated and presented at this meeting on the possible role of NOC in the causation of human cancer, a causal association, although not yet rigorously established, must be assumed. On the grounds of biochemical and histopathological data, there is also little reason to believe that humans are resistant to the carcinogenic action of NOC. Therefore, preventive measures against the induction of cancer in humans by NOC should be devised and implemented (p. 987): (i) a reduction in exposure to NOC, e.g., by limiting the use of tobacco products; (ii) use of inhibitors of the nitrosation reaction, like ascorbic acid, to reduce exposure to NOC, in particular those formed in the mammalian body; and (iii) selective inhibition of the metabolic activation of *N*-nitrosamines, although this difficult approach has not yet been fully explored.

CONTENTS

Members of organizing committees i
Foreword iii
Introduction v
Overview vii
List of presentations xiii
Presentations 1–990
Nomenclature and abbreviations 991
List of participants 993
Author index 1001
Subject index 1005

LIST OF PRESENTATIONS

ENVIRONMENTAL OCCURRENCE OF *N*-NITROSO COMPOUNDS AND NITROSATABLE PRECURSORS

Occurrence and exposure to *N*-nitroso compounds and precursors
R. Preussmann 3

Presence of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids and tyramine as precursors of mutagens in soya sauce after nitrite treatment
K. Wakabayashi, M. Nagao, M. Ochiai, M. Tsuda, Z. Yamaizumi, H. Saitô & T. Sugimura 17

Analysis and occurrence of total *N*-nitroso compounds in the Japanese diet
T. Kawabata, M. Matsui, T. Ishibashi & M. Hamano 25

Mutagenicity of various Japanese foodstuffs treated with nitrite. II. Directly-acting mutagens produced from *N*-containing compounds in foodstuffs
I. Tomita, N. Kinae, Y. Nakamura, H. Takenaka, H. Kanamori, H. Hashizume & T. Yokoyama 33

Determination of *N*-nitrosobis(2-hydroxypropyl)amine in environmental samples
P. Issenberg, E.E. Conrad, J.W. Nielson, D.A. Klein & S.E. Miller 43

Volatile *N*-nitrosamines in baby bottle rubber nipples and pacifiers. Analysis, occurrence and migration
N.P. Sen, S. Seaman, S. Clarkson, F. Garrod & P. Lalonde 51

NEW *N*-NITROSO COMPOUNDS AND IMPROVED METHODS OF ANALYSIS

Nitrosation of peptides
B.C. Challis, A.R. Hopkins, J.R. Milligan, R.C. Mitchell & R.C. Massey 61

Production of *N*-nitrosoiminodialkanoic acids by nitrite in gastric juice
J.R. Outram & J.R.A. Pollock 71

Presence in human urine of new sulfur-containing *N*-nitrosamino acids: *N*-nitroso-thiazolidine 4-carboxylic acid and *N*-nitroso 2-methylthiazolidine 4-carboxylic acid
H. Ohshima, I.K. O'Neill, M. Friesen, B. Pignatelli & H. Bartsch 77

A new type of *N*-nitrosamino acid, *N*-nitroso-L-thioproline and *N*-nitroso-L-methylthio-prolines, found in human urine as major *N*-nitroso compounds
M. Tsuda, T. Kakizoe, T. Hirayama & T. Sugimura 87

Investigation on the mutagenicity of *N*-nitrosothiazolidine using the Ames *Salmonella* test
W. Fiddler, A.J. Miller, J.W. Pensabene & R.C. Doerr 95

Amadori- and *N*-nitroso-Amadori compounds and their pyrolysis products. Chemical, analytical and biological aspects
H. Röper, S. Röper & B. Meyer 101

Pitfalls to avoid in determining *N*-nitroso compounds as a group
C.L. Walters, P.L.R. Smith & P.I. Reed 113
A new Thermal Energy Analyzer for direct high-performance liquid chromatographic and gas chromatographic analysis of *N*-nitrosamides
D.H. Fine, D.P. Rounbehler, W.C. Yu & E.U. Goff 121
N-Nitrosamine analysis in foods: *N*-nitrosoamino acids by high-performance liquid chromatography/thermal energy analysis and total *N*-nitroso compounds by chemical denitrosation/thermal energy analysis
R.C. Massey, P.E. Key, D.J. McWeeny & M.E. Knowles 131
On-line combination of high-performance liquid chromatography and total *N*-nitroso determination apparatus for the determination of *N*-nitrosamides and other *N*-nitroso compounds, and some recent data on the levels of *N*-nitrosoproline in foods and beverages
N.P. Sen & S. Seaman 137
Nonvolatile *N*-nitrosamine investigations: Methods for the determination of *N*-nitrosoamino acids and preliminary results of the development of a method for the determination of *N*-nitrosodipeptides *N*-terminal in proline
S.J. Kubacki, D.C. Havery & T. Fazio 145

ENDOGENOUS FORMATION OF *N*-NITROSO COMPOUNDS

Analysis for and intestinal metabolism of precursor nitroso compounds in normal subjects and in patients with chronic renal failure
M.L. Simenhoff, S.R. Dunn & P.S. Lele 161
Nitrosation of piperazine in man
B.T.D. Bellander, B.-G. Österdahl & L. Hagmar 171
In-vivo formation of *N*-nitrosodimethylamine in humans after amidopyrine intake
B. Spiegelhalder & R. Preussmann 179
A sensitive new method for the detection of *N*-nitrosomorpholine formation *in vivo*
J.B. Morrison & S.S. Hecht 185
N-Nitrosoproline in urine from patients and healthy volunteers after administration of large amounts of nitrate
G. Ellen & P.L. Schuller 193
Studies on the excretion of endogenously-formed *N*-nitrosoproline. I. Percutaneous excretion of *N*-nitrosoproline in humans
P.A. Bogovski, J.M. Kann & M.A. Rooma 199
N-Nitrosoproline excretion in urine, faeces and milk from cows in relation to feed composition
L.W. van Broekhoven, J.A.R. Davies & J.H. Geurink 205
Dietary phenolics and betel nut extracts as modifiers of *N*-nitrosation in rat and man
H.F. Stich, B.P. Dunn, B. Pignatelli, H. Ohshima & H. Bartsch 213
Modulation of endogenous synthesis of *N*-nitrosamino acids in humans
D.A. Wagner, D.E.G. Shuker, C. Bilmazes, M. Obiedzinski, V.R. Young & S.R. Tannenbaum 223
The blocking effects of Chinese *Actinidia sinensis* juice on *N*-nitrosamine formation *in vitro* and *in vivo*
P. Song, Z. Lin, L. Yinzeng, D. Lan, S.R. Tannenbaum & J.S. Wishnok 231

Experimental model for evaluating animal exposure to endogenous *N*-nitrosodi-*n*-butylamine by measuring its urinary metabolites *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine and *N*-butyl-*N*-(3-carboxypropyl)nitrosamine
L. Airoldi, C. Spagone, A. Macri & R. Fanelli 237
Oxidation of ammonia and hydroxylamine to nitrate in the rat
R.L. Saul & M.C. Archer 241
Mammalian nitrate biochemistry: metabolism and endogenous synthesis
D.A. Wagner, D.S. Schultz, W.M. Deen, V.R. Young & S.R. Tannenbaum 247
Absorption, secretion and excretion of dimethylamine in rats
H. Ishiwata, R. Iwata & A. Tanimura 255

FORMATION OF *N*-NITROSO COMPOUNDS. CATALYSIS, INHIBITION AND MECHANISMS

Catalysis and inhibition of *N*-nitrosation reactions
M.C. Archer 263
N-Nitrosamine formation by intestinal bacteria
K. Suzuki & T. Mitsuoka 275
A nitrosating agent from the reaction of atmospheric nitrogen dioxide (NO_2) with methyl linoleate: comparison with a product from the skins of NO_2-exposed mice
S.S. Mirvish & J.P. Sams 283
In-vivo nitrosation of amines in mice by inhaled nitrogen dioxide and inhibition of biosynthesis of *N*-nitrosamines
Z.M. Iqbal 291
Further factors influencing *N*-nitrosamine formation in bacon
J.I. Gray, D.J. Skrypec, A.K. Mandagere, A.M. Booren & A.M. Pearson 301
Nitrosation by alkyl nitrites. Catalysis by inorganic salts
R. Dabora, M. Molina, V. Ng, J.S. Wishnok & S.R. Tannenbaum 311
Nitrosamide carcinogenesis: nitrosation of amide linkages and facile decomposition of resulting nitrosamides
Y.L. Chow, S.S. Dhaliwal & J. Polo 317
Nitrosating properties of bis-methylthio-diiron-tetranitrosyl (Roussin's red methyl ester), a nitroso compound isolated from pickled vegetables consumed in Northern China
A. Croisy, H. Ohshima & H. Bartsch 327
Rapid formation of *N*-nitrosodimethylamine from gramine, a naturally occurring precursor in barley malt
M.M. Mangino & R.A. Scanlan 337
Formation of nitrosamines in non-ionic and anionic emulsions in the presence and absence of inhibitors
B.L. Kabacoff, M.L. Douglass, I.E. Rosenberg, L.W. LeVan, J.K. Punwar, S.F. Vielhuber & R. J. Lechner 347
Ester-mediated nitrosamine formation from nitrite and secondary or tertiary amines
R.N. Loeppky, W. Tomasik & T.G. Millard 353
Photochemistry of *N*-nitrosamines in neutral media
C.J. Michejda & T.M. Rydstrom 365