Carcinogenic N-Nitroso Compounds
สารก่อมะเร็งเอ็น-ไนโตรโซ

Prasong Koonanuwatchaidet[1]

บทคัดย่อ : ประสงค์ คุณานุวัฒน์ชัยเดช. 2543. สารก่อมะเร็งเอ็น-ไนโตรโซ. วารสารวิจัยวิทยาศาสตร์การแพทย์ 14(2) : 101-118.

เอ็น-ไนโตรโซเป็นกลุ่มของสารประกอบที่พบได้ในสิ่งแวดล้อมทั่วไปรวมทั้งในอาหาร แม้ว่าจะพบในปริมาณที่ค่อนข้างน้อย แต่การศึกษาพบว่าสารกลุ่มนี้ออกฤทธิ์ทำให้เกิดมะเร็งในอวัยวะต่าง ๆของสัตว์ทดลองหลายชนิด และเมื่อนำไปทดสอบการก่อกลายพันธุ์ในบักเตรี ก็พบว่ามีฤทธิ์ก่อกลายพันธุ์ได้ ในปัจจุบันข้อมูลทางการแพทย์เกี่ยวกับความเป็นพิษของสารนี้ในคนยังมีไม่มากเพียงพอ แต่ก็บ่งชี้ว่าสารประกอบกลุ่มนี้สามารถมีผลต่อการก่อมะเร็งในคนได้ด้วย ดังนั้นจึงควรจะตระหนักถึงอันตรายของสารก่อมะเร็งประเภทนี้ และพึงระมัดระวังมิให้ได้รับสารพิษกลุ่มนี้เข้าสู่ร่างกาย บทความนี้ได้เรียบเรียงความรู้เกี่ยวกับสารกลุ่มเอ็น-ไนโตรโซในด้านต่าง ๆดังนี้คือ คุณสมบัติทางเคมี การสังเคราะห์ แหล่งที่พบ ความเป็นพิษ ปริมาณที่คาดว่าร่างกายคนเราอาจจะได้รับ และผลกระทบต่อสุขภาพพลานามัย

คำสำคัญ : สารประกอบเอ็น-ไนโตรโซ ความเป็นพิษ สารก่อมะเร็ง

[1] Department of Biochemistry, Faculty of Medicine, Chiang Mai University
ภาควิชาชีวเคมี คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

Abstract : Prasong Koonanuwatchaidet. 2000. Carcinogenic N-Nitroso Compounds. Thai J Hlth Resch 14(2) : 101-118.

N-nitroso compounds are present in environmental media including in food. Although the concentrations found are in notably low range, the carcinogenic action of these compounds in experimental animals have been shown to occur in many different organs. Most of them are positively mutagenic in bacterial test systems. There is still inadequate clinical data at present, but it is highly probable that N-nitroso compounds are carcinogenic in man. Human exposure to these carcinogens and their precursors should be kept as low as practically achievable. The information on their chemistry, formation, occurrence, toxic activity and possible evaluation of health risks to man is reviewed.

Key words : N-nitroso compound, toxic effect, carcinogen

Introduction

It is now over forty five years since one of N-nitroso compounds was firstly described to cause acute hepatic intoxication (Barnes and Magee, 1954). The subsequent historic experiments of Magee and Barnes (Magee, 1956 ; Magee and Barnes, 1962 ; Magee and Barnes, 1967; Magee, 1972) showed many cases of tumour induced by N-nitroso compounds in a variety of animal species. These carcinogens are widespread in various environmental media and frequently occur in industrial areas. The works on N-nitroso compounds have been mainly done on experimental animals or with bacterial test systems. Even though there is not much epidemiological evidence at present, it is markedly feasible that these compounds may also be carcinogenic in man. Human exposure to these potent carcinogens, and its precursors are related to health problems in all parts of the world. This article reviews the chemistry, synthesis, common sources, toxicity, mutagenic activity of these compounds, and briefly examines the effects and evaluation of health hazards to man.

Chemistry and Formation

The chemistry of N-nitroso compounds have been widely studied (Mirvish, 1975 ; Challis, 1982) . They have a general structure as shown below (1).

$$R_1 - \underset{\displaystyle R_2}{\overset{\displaystyle |}{N}} - N = O \qquad \text{.....(1)}$$

The N-nitroso compounds are divided into two classes with different chemical properties ; firstly, *nitrosamines*, derived from dialkyl, alkaryl, diaryl, or cyclic secondary amines. Where R_1 and R_2 are alkyl or aryl groups ; and secondly, *nitrosamides*, derived from N-alkylureas, N -alkylcarbamates, and simple N-alkylamides, where R_1 is an alkyl or aryl group and R_2 is an acyl group (Mirvish, 1975).

Nitrosamines are generally stable compounds which are slowly decomposed in light or in aqueous acid solutions. In contrast, nitrosamides are much less stable in aqueous acid and unstable in basic solutions. The physical properties of N-nitroso compounds widely vary depending upon the substituent groups in their molecular structures (Fieser and Fieser, 1967 ; Weast, 1976) . For instance, dimethylnitrosamine or N-nitrosodimethylamine (NDMA) is oily liquid miscible with polar solvents. Some are solids, e.g. diphenylnitrosamine or N-nitroso-N-phenylbenzenamine, is slightly soluble in ethanol and

insoluble in water. Nitrosamines show ultraviolet absorption peaks in aqueous solution at 230-350 nm, nitrosamides absorb in the long wavelenght region at 390-420 nm (Mirvish, 1975).

N-nitroso compounds are formed by chemical reactions between nitrosating agent and nitrosatable amines. In most cases, the nitrosating agent which participates in N-nitroso compound formation is nitrous anhydride (N_2O_3). Nitrous anhydride readily forms from nitrite (NO_2) in aqueous acidic solutions as described in equation (2) to (4) as follows :

$$NO_2^- + H^+ \leftrightarrow HNO_2 \quad \text{..... (2)}$$

$$HNO_2 + H^+ \leftrightarrow H_2NO^+_2 \quad \text{..... (3)}$$

$$H_2NO^+_2 + NO_2^- \leftrightarrow N_2O_3 + H_2O \quad \text{..... (4)}$$

Nitrous anhydride reacts with the unshared pair of electrons on unprotonated nitrosatable amines, especially secondary amines to form N-nitroso compounds as indicated in equation (5), the reaction is called *N-nitrosation.*

$$N_2O_3 + R_1-\underset{\displaystyle R_2}{\overset{\displaystyle |}{N}}-H \leftrightarrow R_1-\underset{\displaystyle R_2}{\overset{\displaystyle |}{N}}-N=O + HNO_2 \quad \text{..... (5)}$$

The reaction rate is governed by the total amount of amine and nitrite as shown in equation (6) and it is also pH dependent. The optimal pH for nitrosation of most basic secondary amines is between 2.5 and 3.5. This is due to the counteracting effects of acidity on the concentration of unprotonated amine and of nitrous anhydride (Mirvish, 1975 ; Lane and Bailey, 1973).

$$\text{Rate} = k_1 [\text{Amine}] [\text{Nitrite}]^2 \quad \text{..... (6)}$$

Nitrosation can be influenced by a number of accelerators and inhibitors (Davies and McWeeny, 1977 ; Douglas *et al.*, 1978 ; Pignatelli *et al.*, 1980, Williams and Aldred, 1982). Several nucleophilic or anionic salts, for instances, nitrosyl iodide (I-NO) and nitrosyl thiocyanate (SCN-NO) can form effective nitrosating agents when they are present with nitrite in aqueous and acidic solutions. Enhanced nitrosation of lipophilic secondary amines had been demonstrated is aqueous systems containing micelles and some carbonyl compounds (Keefer and Roller, 1973 ; Okun and Archer, 1977). Ascorbic acid, sulfur dioxide, and gallic acid inhibited the N-nitroso compound formation in certain conditions (Mirvish *et al.*, 1975 ; Fiddler *et al.*, 1978 ; Gray *et al.*, 1982). It should be noted that ascorbic acid is required in the manufacture of bacon in many countries. The role of ascorbic acid is to reduce nitrous anhydride to nitric oxide (NO) in the absence of catalysts

as decribed in equation (7). Since nitric oxide, a non-nitrosating agent can be reoxidized to nitrogen dioxide, ascorbic acid has to be added into the system in excess amount (Scanlan, 1983).

$$N_2O_3 + \text{Ascorbic acid} \rightarrow 2\ NO + \text{Dehydroascorbic acid} + H_2O \quad \ldots\ldots (7)$$

$$NO + NO_2 \leftrightarrow N_2O_3 \quad \ldots\ldots (8)$$

Investigation of nitrosatable precursors or model compounds has shown that nitrosation reaction with nitrosating agents does not occur only *in vitro*. *In vivo* formation of N-nitroso compounds has been also found under similar conditions in both short-term and long-term experiments (Ziebarth, 1974 ; Mirvish, 1975 ; Scanlan, 1983 ; Scheunig and Ziebarth, 1976 ; Coulston and Olajos, 1982). Scheunig and Ziebarth (1976) reported that the quantity of amine drugs in the nitrosation in human stomach was equal to the maximally tolerated single dose and a nitrite level corresponding to the amount most likely to be ingested in a single day. It was observed that under conditions of very limited levels of amino drugs, yields of N-nitroso compounds and their derivatives are nondetectable. Essential information has been gained regarding the occurrence and identification of reaction kinetics, and influencing factors to N-nitrosation phenomena. In 1978, the World Health Organization(WHO) Study Group on carcinogenicity of nitrosatable drugs including other nitrosatable amino compounds established the standard conditions criteria as followings : concentrations of drugs and nitrite, reaction time, reaction temperature, and acid-base condition. These criteria were applied as a means of obtaining comparable nitrosation data, and of ranking of potentially nitrosatable compounds. With regard to the significance of nitrosatable amino compounds including drugs and nitrite-induced carcinogenesis in man, none of the drugs that are markedly nitrosated *in vivo* is known. Recently, studies related to the endogenous nitrosatable reactions were made in aminal and human experiments (Oshima and Bartsch, 1981 ; Oshima *et al.*, 1982; Oshima *et al.*, 1983 ; Hoffmann and Brunnemann, 1983). In a dose-response study on the *in vivo* formation in rats, estimations were carried out with a kinetic model using published data on nitrosation and carcinogenic activity of some selected N-nitroso compounds. In addition, the endogenous formation of carcinogenic N-nitroso compounds was readily monitored by measuring the amount of particular compounds, especially N-nitrosoproline (NPRO) excreted in urine and feces, since NPRO is almost completely excreted (Oshima and Bartsch, 1981 ; Oshima *et al.*, 1982).

Environmental Occurrence

The occurrence of N-nitroso compounds in urban air was reported twenty years ago at a wide range of concentration 0.02-0.96 ppb (Bretschneider and Matz, 1976 ; Fine *et al.*, 1976). The compounds are present in air presumably either due to industrial omissions or due to their formation from secondary amines and nitrogenous oxides. There are very few reports on the occurrence of N-nitroso compounds in water. In 1976, Fine *et al.* (1976) analyzed water samples from the Mississippi River and from water treatment plants by using the new techniques. The estimated concentrations were about 0.1 μg/kg on average, and these levels should belong to N-nitroso derivatives of some pesticides. Since precursors for production of N-nitroso compounds occur in tobacco, it was thought that tobacco and tobacco smoke might contain trace amount of these compounds (Neurath, 1972 ; Hoffman *et al.*, 1974).

Numerous determinations of N-nitroso compounds have been made in a variety of foods from various countries (Crosby *et al.*, 1972 ; Fong and Chan, 1973 ; Sen, 1974 ; Panalaks *et al.*, 1974 ; Scanlan, 1975 ; Stephany *et al.*, 1976 ; Gough *et al.*, 1977 ; Scanlan *et al.*, 1980 ; Sen *et al.*, 1980a ; Sen *et al.*, 1980b ; Gray, 1981; Scanlan, 1983). The great majority of the investigations list volatile nitrosamine levels in cured meats, dairy products, fish, whiskey, and beer. The methods employed for analysis mainly involved gas chromatography. Some results were confirmed by mass-spectroscopic techniques, which are now increasingly employed. A summary of reported occurrences of N-nitroso compounds in foods, adapted from Sen (1974) and Scanlan (1983) is presented in Table 1. The nitrosation is readily affected under the conditions in which various amines, amino acids, proteins and other food constituents can react with nitrite in food systems (Sen *et al.*, 1973; Scanlan, 1983) . The accelerators and inhibitors which may be present in certain foods possibly play an important role in this particular reaction (Davies and McWeeny, 1977, Douglas *et al.*, 1978 ; Scanlan, 1983) Obviously, the formation of N-nitroso compounds in foods is influenced by a storing of foods, thus making it difficult to predict the extent to which these compounds might be produced (Pensabene, 1974 ; Fong *et al.*, 1980; Scanlan, 1983).

Moreover, since a number of drugs and some pesticides are tertiary amines, volatile nitrosamines have been produced by nitrosation reaction under defined conditions (Lijinsky, 1974 ; Linjinsky and Singer, 1974; Mirvish, 1975 ; Coulston and Olajos, 1982). N-nitroso compounds, especially nitrosamines, have been also determined in industrial

environments, dish washer, cutting fluids including rubber baby bottle, rubber nipples and pacifiers (Craddock, 1981 ; Craddock, 1983 ; Loeppky, 1983 ; Harvey *et al.*, 1983 ; Osterdahl, 1983). It was reported that a portion of those volatile nitrosamines could migrate from the rubber nipples into the baby's milk in the bottle upon overnight storage.

Experimental Studies on Toxicity, Carcinogenicity and Mutagenicity

Acute toxicity study

The toxicity of this class of compounds is not of great significance (Magee and Barnes, 1967 ; Shank, 1975 ; Coulston and Olajos, 1982) , and there is no good correlation between acute toxicity and carcinogenic potential of N-nitroso compounds. It was firsty reported that N-nitrosodimethylamine at 20-40 mg/kg body weight given to rats, dogs rabbits, and guinea pigs produced severe hepatic damge (Barnes and Magee, 1954). A single dose of this compound orally administered to rats, or by intravenous, intraperitoneal or subcutaneous injection, produced centrilobular necrosis accompanied by haemorrhages in the liver. The centrilobular and midzonal regions of liver cells became pale, and the cytoplasm then became amorphous and vacuolated ; the nuclei were pale and irregular in outline. The cells were necrotic and influential areas became haemorrhagic by 24 hours, it was generally enhanced after 48 hours but after 72 hours the recovery process had started and was almost complete in 3 weeks (Barnes and Magee, 1954 ; Magee and Barnes, 1962). Pathological and biochemical changes in the liver of a number of animal species have been observed ; the main effect is to inhibit protein synthesis which might be a result of an accelerated degradation of messenger ribonucleic acid (mRNA) (Magee, 1956; Magee and Barnes, 1967). However, the acute toxicity of N-nitroso compounds is widely varied ; some were mildly toxic while others pronounced highly destructive lesions of target organs (Magee and Barnes, 1967 ; Magee *et al.*, 1976; Coulston and Olajos, 1982; Shank, 1975).

Carcinogenicity

The carcinogenic activity of N-nitroso compounds has been studied by Magee and Barnes (1967) . Several animal species including mammals, amphibians, avians, and fishes have been demonstrated to be susceptable to the carcinogenic action of nitrosamines. These compounds have been introduced in rats, not less than 80 percent of them have proved to be carcinogenic (Montesano and Bartsch, 1976). N-nitroso compounds show an obvious

Table 1 Levels of n-nitroso compounds in various foods

Foods	Country	N-nitroso compounds found	Level (μg/kg)
Dry sausage	Canada	NDMA	10-20
Salami sausage (uncooked)	Canada	NDMA	20-80
Bacon	Canada	NPYR	4-25
Bacon	Canada	NPYR	25-40
Bacon	Netherlands	NDMA	0.8
Bacon	Netherlands	NPYR	0.4
Bacon	Netherlands	NPIP	0.6
Smoked meat	Netherlands	NDMA	3.0
Smoked meat	Netherlands	NDEA	7.91
Cooked ham	Netherlands	NDMA	6.0
Bologna sausage	Canada	NDEA	25.0
Bologna sausage	Canada	NPYR	20-105
Fish meal	Canada	NDMA	0.35-0.50
Smoked or salted fish (uncooked)	UK	NDMA	1-9
Salted white herring	Hong Kong	NDMA	40-100
Salted yellow croakers	Hong Kong	NDMA	10-60
Beer	USA	NDMA	1.5
Beer	Canada	NDMA	1.5
Nonfat dry milk	USA	NDMA	0.3-0.7

NDMA : N-nirosodimethylamine NPYR : N-nitrosopyrrolidine NPIP : N-nitrosopiperidine NDEA : N-nitrosodiethylamine

Table 2 Localization of cancers induced by n-nitroso compounds in rats [a]

Number of N-nitroso compounds affecting target organ			Number of N-nitroso compounds affecting target organ		
Target organ	Nitrosamines	Nitrosamides	Target organ	Nitrosamines	Nitrosamides
Liver	35	2	Testis	1	1
Oesophagus-pharynx	32	3	Ovary	1	2
Nasal cavities	18	-	Mamary gland	1	1
Respiratory tract	10	1	Intestine	-	7
Kidney	8	9	Glandular stomach	-	6
Tongue	8	-	Skin	-	3
Forestomach	7	11	Jaw	-	1
Bladder	4	1	Uterus	-	2
Nervous system [b]	2	9	Vagina	-	1
Ear duct	2	1	Haemopoietic system	-	2

[a] Adapted from Montesano and Bartsch (1976) [b] Central and peripheral

organ specificity as shown in Table 2. Nitrosamines produce a carinogenic effect in the liver, oesophagus, kidney , and respiratory system, whereas nitrosamides mainly affect the gastro-intestinal tract organs, and central and peripheral nervous systems. In addition, nitrosamines exert their adverse biological activities after being metabolically activated by microsomal mixed function oxidases to form reactive intermediates. The importance of hydroxylation at the α-position of nitrosamines is demonstrated by study with diphenylnitrosamines (Magee *et al.*, 1976).

The biochemical mechanisms of carcinogenesis produced by N-nitroso compounds have been extensively studied by a number of investigators (Magee and Barnes, 1967 ; Swann and Magee, 1971 ; Lijinsky *et al.*, 1973 ; O' Conner *et al.*, 1973; Magee *et al.*, 1976). In early studies, it was suggested that the mechanism of carcinogenic action involved the alkylation of the N, 7- position of guanine base in nucleic acid. An important further finding of O'Conner *et al.* (1973) was that O, 6-alkylation of deoxyguanosine was the significant site reaction. The amount of methylation at 0, 6-position of guanine DNA isolated from animals treated with N-nitrosodimethylamine (NDMA) was estimated at approximately 4-6 percent of methylation. The 0, 6-methylguanine was lost from DNA with a half life of about 13 hours. It was demonstrated that the excision of the abnormal components of DNA, and the unstable acid-labile products might be important in hepatic carcinogenesis. Events leading to the cancer development were presumably related to the efficiency of the cellular excision system for such certain alkylation products rather than to the level of alkylation at a particular site.

Mutagenicity

The early investigation of mutagenic activity of N-nitroso compounds was made by using *Neurospora crassa* as a genetic indicator (Malling, 1966). More recently, the mutagenic effect of this class of compounds was assayed by mutation in *Salmonella typhimurium*. It was found that the metabolic activation would be essentially required for the system (Magee and Barnes, 1967 ; Couch and Friedman, 1975; Osterdahl, 1983). The development of this screening test system was established with many bacterial tester strains since last three decades by Ames's group (Ames *et al.*, 1972 ; Ames *et al.*, 1975 ; McCann *et al.*, 1975a ; McCann *et al.*, 1975b ; McCann and Ames, 1976; Ames and McCann, 1976). Ames's method has been widely introduced in a great number of laboratories for detection the carcinogens as mutagens (Ho *et al.*, 1976 ; Fong and Chan,

1977 ; De Serres and Shelby, 1979). The mutagenicity of nitrosamines could not be tested with bacteria and liver preparation in an over-layer agar, eventhough those substances could be detected in a liquid system (Nakajima and Iwahara, 1973 ; Nakajima *et al.*, 1974; Bartsch *et al.*, 1975 ; Bartsch *et al.*, 1976; Fong and Chan, 1977). Yahagi and his associates (Yahagi *et al.*, 1977) therefore developed a new technique by combining the Ames's standard test with the liquid method. In this modification, The specific strains of *Salmonella typhimurium* and test chemicals were preincubated with microsomes at 25 ° C for 20 minutes, and then the mixture poured onto a plate containing a limited amount of L-histidine. Yahagi's technique is simple, economical, reliable and more sensitive for detecting nitrosamines, and has been applied by a large group of investigators (Fong and Chan, 1977; Ho *et al.*, 1976 ; Andrew *et al.*, 1978 ; Zeiger and Sheldon, 1978 ; Rao *et al.*, 1979 ; Guttenplan, 1979 ; Guttenplan, 1980 ; Bartsch *et al.*, 1980 ; Andrew and Lijinsky, 1980 ; Lee and Guttenplan, 1980 ; Sugimura and Sato, 1983) .

In general, N-nitroso compounds have proved relatively active in liquid pre-incubation assay system (Yahagi *et al.*, 1977), and high concentration is required for some certain nitrosamines. Their mutagenic activity is dependent on metabolic activation, an acid-base condition, being enhanced in weakly acidic mixture solution (Guttenplan, 1980 ; Negishi and Hayatsu , 1980). Even thought many N-nitrosamines show a good qualitative correlation between mutagenicity and carcinogenicity, there are several important exceptions. Potency association between mutagenesis and carcinogenesis of these particular carcinogens are also dependent on their chemicical structure (Andrew and Lijinsky, 1980 ; Lee and Guttenplan, 1980 ; Sugimura and Sato, 1983). Thus, the activity correlations may only be used the semi-quantitative indication for N-nitroso compounds.

Exposure of Humans and Evaluation fo Health Risks

The levels of N-nitroso compounds in the environment including in diet, and determination of the risk of human exposure to these carcinogenic substances have been investigated in several countries (Day, 1975 ; Harmozdian, *et al.*, 1975 ; Ho *et al.*, 1976 ; Purchase *et al.*, 1976 ; Eisenbrand *et al.*, 1976 ; Spiegelhalder *et al.*, 1980 ; Stephany and Schuller, 1980). Even though the epidemiology of cancer in various target organs has been reported, the availability of clinical data is not adequate to illustrate the relationship with exposure to N-nitroso compounds or their possible precursors such as nitrites, nitrates and nitrosatable amines occurring as environmental and food components (Ho *et al.*, 1976 ;

Day, 1975; Haenzel and Correa, 1975; Correa *et al.*, 1975; Scanlan, 1983 ; Lijinsky, 1983). In addition, the US National Academy of Science had demonstrated currently the assessment of human health effects of N-nitroso compounds and their precursors (anonymous, 1981).

Undoubtedly, more than 80 percent of over one hundred N-nitroso compounds have been proved to be mutagenic in test systems, and carcinogenic in a wide range of animal species, producing cancers in many organs. A dose-response relationship has been established in different experimental animals for these carcinogenic substances. As the dose is reduced, the cancer incidence decreases, the induction time for cancer increases and the life span of the animals is prolonged. From available results, it is obviously feasible that N-nitroso compounds are carcinogenic to man. The related precursors precursors which can be found in the environment may also be classified as potential cancer-inducing factors.

Additional research on N-nitroso compounds-induced human carcinogenesis will be further required in order to clarify the role of these carcinogens in the etiology of human cancers. However, present knowledge of N-nitroso compounds and an understanding of their mechanism of action suffice to warrant precaution toward their potential effects.

References

Ames BN., Durston WE., Yamasaki E. and Lee FD. 1972. Carcinogens are mutagens : A simple test system combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci USA 70 : 2281-2285.

Ames BN. and McCann J. 1976. Carcinogens are mutagens : A simple test system. In : Screening Test in Chemical Carcinogenesis. Montesano T., Bartsch H. and Tomatis L. (Eds.) IARC Sci Publ No. 12, Lyon, pp. 493-504.

Ames BN., McCann J. and Yamasaki E. 1975. Methods for detecting carcinogens and mutagens with Salmonella/mammalian - microsome mutagenicity test. Mutation Res 31 : 347-364.

Andrew AE. and Lijinsky W. 1980. The mutagenicity of 45 nitrosamines in Salmonella *typhimurium*. Carcinogenesis and Mutagenesis 1 : 295-303.

Andrew AE., Thibalt LH. and Lijinsky W. 1978. The relationship between mutagenicity and carcinogenicity of some nitrosamines. Mutation Res 67 : 21-26.

Anonymous. 1981. The Heath Effects of Nitrate, Nitrite, and N-Nitroso Compounds. National Academy of Sciences. National Academy Press, Washington D.C., pp.7-36.

Barnes JM. and Magee PN. 1954 Some toxic properties of dimethylnitrosamine. Br J Ind Med 11 : 167-174.

Bartsch H., Malaveille C., Camus AM., *et al.* 1980. Validation and comparative studies on 180 chemicals with S. *typhimurium* stains and V79 Chinese hamster cells in the presence of various metabolising systems. Mutation Res 76 : 1-50.

Bartsch H., Malavelille C. and Montesano R. 1975. *In vitro* metabolism and microsome mediated mutagenicity of dialkylnitrosamines in rat, hamster, and mouse tissues. Cancer Res 35 : 644-651.

Bartsch H., Malaveille C. and Montesano R. 1976. The predictive value of tissue mediated mutagenicity assays to assess the carcinogenic risk of chemicals. In : Screening tests in Chemical Carcinogenesis. Montesanno R., Bartsch H. and Tomatis L (Eds.) IARC Sci Publ No. 12. Lyon, pp. 457-491.

Bretschneider K. and Marz T. 1976. Occurrence and analysis of nitrosamines in air. IARC Sci Publ No. 14. Lyon, pp - 401-408.

Challis BC. 1982. The chemistry and formation of N-nitroso compounds. In : Safety Evaluation of Ntirosatable Drugs and Chemicals. Gibbson GG. and Ioannides C. (Eds). Taylor and Francis Ltd. London . pp. 16-55.

Correa P., Haenszel W., Cuello C., *et al.* 1975. A model for gastric cancer epidemiology. Lancet. July 12 : 58-59.

Couch DB. and Friedman MA. 1975. Interactive mutagenicity of sodium, dimethyl-nitrosamine, methylurea and ethylurea. Mutation Res 31: 109-114.

Coulston F. and Olajos El. 1982. Toxicology of N-nitroso compounds. Ecotoxicol Environ Safety 6 : 89-96.

Craddock VM. 1981. Environmental nitrosamines in cancer Nature (London) 294 : 694-695.

Craddock VM. 1983. Nitrosamines and human cancer. Nature (London) 306 : 638-639.

Crosby NT., Foreman KK., Palframan JF., *et al.* 1972. Determination of volatile nitrosamines in food products at the μg/kg level. IARC Sci Publ No. 3. Lyon , pp. 38-42.

Davies R. and McWeeny DJ. 1977. Catalytic effects of nitrosophenols and N-nitrosamine Formation. Nature (London) 266 : 657-658.

Day NE. 1975. Some aspects of the epidemiology of esophageal cancer. Cancer Res 35 : 3304-3307.

De Serres FJ. and Shelby MD. 1979. The Salmonella mutagenicity assay. Science (Washington D.C.) 203 : 563-565.

Douglass ML., Kabacoff BL., Anderson GA., *et al.* 1978. The chemistry of nitrosamine, formation, inhibition and destruction . J Soc Cosmet Chem 29 : 581-606.

Eisenbrand G., von Rappard E., Zappe R., *et al.* 1976. Trace analysis of volatile nitrosamines by modified nitrogen specific detector in pyrolytic mode and by ion-specific determination of heptafluorobutyramide in a GC/MS system. IARC Sci Publ No. 14. Lyon, pp. 65-75.

Fiddler W., Pensabene JW., Piotrowsaki EG., *et al.* 1978. Inhibition and formation of volatile nitrosamines in fried bacon by the use of cure solubilized α - tocopherol. J. Agric Food Chem 26 : 653-656.

Fieser LF. and Fieser M. 1967. Reagents for Organic Synthesis. Wiley and Sons Company, New York. pp. 747-749.

Fine DH., Rounbehler DP., Belcher NM., *et al.* 1976. N-Nitroso Compounds in Air and Water. IARC Sci Publ No. 14. Lyon, pp. 401-408.

Fong YY. and Chan WC. 1973. Dimethylnitrosamine in chinese marine salt fish. Food Cosmet Toxicol 11 : 841-845.

Fong YY., and Chan WC. 1977. Nitrate, nitrite, dimethylnitrosamine and N-Nitrosopyrrolidine in some chinese food products. Food Cosmet Toxicol 15 : 143-145.

Fong YY., Ton CT., Koonanuwatchaidet P., *et al.* 1980. Mutagenicity of peanut oils and effect of repeated cooking. Food Cosmet Toxicol 18 : 467-470.

Gough TA., McPhal MF., Webb KS., *et al.* 1977. An examination of some foodstuffs for the presence of volatile nitrosamines. J Sci Food Agric 28 : 345-351.

Gray JI. 1981. Formation of N-Nitroso Compounds in Foods. In : N-Nitroso Compounds. Scanlan RA., and Tennabaum SR. (Eds). American Chemical Society. Washington, D.C.

Gray JI., Reddy SD., Price JF., *et al.* 1982. Inhibition of N-nitrosamines in bacon. Food Technol 36 : 39-45.

Guttenplan JB. 1979. Detection of trace amount of Dimethylnitrosamine with a modified Salmonella microsome mutagenicity assay. Mutation Res 64 : 91-94.

Guttenplan JB. 1980. Enhanced mutagenic activities of N-nitroso compounds in weakly acidic media. Carcinogenesis 1: 439-444.

Haenszel W. and Carrea P. 1975. Developments in epidemiology of stomach cancer over the past decade. Cancer Res 35 : 3452-3459.

Harmozdian H., Day NE., Aramesh B., *et al.* 1975. Dietary Factors and esophageal cancer in Caspian littoral of Iran. Cancer Res 35 : 3493-3498.

Havery DC., Hotchkiss JH., Fagio T., *et al.* 1983. Survey of Baby bottle rubber nipples for volatile nitrosamines. J Assoc Off Anal Chem 66 : 1500-1503.

Hoffman D. and Brunnemann KD. 1983. Endogenous formation of N-nitrosoproline in cigarette smokers. Cancer Res 43 : 5570-5574.

Hoffman D., Hecht SS., Ornaf RM., *et al.* 1974. N-nitrosonicotine in tobacco. Science (Washington D.C.) 186 : 265-267.

Ho JHC., Huang DP. and Fong YY. 1976. Salted fish and nasopharyngeal carcinoma in southern chinese. Lancet September 16 : 626.

Keefer LK. and Roller PP. 1973. N-nitrosation by nitrite ion in neutral and basic medium. Science (Washington D.C.) 181 : 1245-1247.

Lane RP. and Bailey ME. 1973. Effect of pH on dimethylnitrosamine formation in human gastric juice. Food Cosmet Toxicol 11 : 851-854.

Lee SY. and Guttenplan JE. 1980. A correlation between mutagenic and carcinogenic N-nitrosamines. Carcinogenesis 2 : 1339-1344.

Lijinsky W. 1974. Reaction of drugs with nitrous acid as a source of carcinogenic nitrosamine. Cancer Res 34 : 255-258.

Lijinsky W. 1983. Summation and new approaches to diet and cancer. Cancer Res (Suppl.) 43 : 2441s-2445s.

Lijinsky W., Keefer L., Loo J., *et al.* 1973. Studies of alkylation of nucleic acids in rats by cyclic nitrosamines. Cancer Res 33 : 1634-1641.

Lijinsky W. and Singer GM. 1974. Formation of nitrosamines from tertiary amines and nitrous acid. In : N-Nitroso Compounds in Environment. Bogovski P. and Walker EA. (Eds). IARC Sci Publ No 9. Lyon, pp. 111-113.

Loeppky RN. 1983. Reducing nitrosamine contamination in cutting fluid. Food Chem Toxicol 21 : 607-613.

Magee PN. 1956. Toxic liver injury : The metabolism of dimethylnitrosamine. Biochem J 64 : 676-682.

Magee PN. 1972. Possibilities of hazard from nitrosamines in industry. Ann Occup Hyg 15 : 19-22.

Magee PN. and Barnes JM. 1962. Induction of kidney tumours in the rat with dimethyl-(N-nitrosodimethylamine) nitrosamine. J Path Bacteriol 84 : 19-21.

Magee PN. and Barnes JM. 1967. Carcinogenic nitroso compounds. Adv Cancer Res 10 : 163-166.

Magee PN., Montesano R. and Preussmann R. 1976. N-nitroso compounds and related carcinogens. In : Chemical Carcinogens. Searle E. (Ed.) American Chemical Sociely. Monogroph No. 173, pp.491-625.

Malling HV. 1966. Mutagenicity of two potent carcinogens : Dimethylnitrosamine and diethylnitrosamine in Neurospora crassa. Mutation Res 3 : 539-540.

McCann J. and Ames BN. 1976. Detection of carcinogens as mutagens in the Salmonella/microsome test. Assay of 300 chemicals : Discussion. Proc Natl Acad Sci 73 : 950-954.

McCann J., Choi E., Yamasaki E., *et al.* 1975. Detection of carcinogens as mutagens in the Salmonella / microsome test. Assay of 300 chemicals. Proc Natl Acad Sci 73 : 955-957.

McCann J., Spingarn NE., Kobori J., *et al.* 1975. Detection of carcinogens as mutagens : Bacterial tester strains with R factor plasmid. Proc Natl Acad Sci 73 : 5135-5139.

Mirvish SS. 1975. Formation of N-nitroso compounds : Chemistry, kinetics and in vivo occurrence. Toxicol Appl Pharmacol 31 : 325-351.

Mirvish SS., Cardesa A., Wallcave L., *et al.* 1975. Induction of mouse lung adenomas by amines or urea plus nitrite and by N-nitroso compounds. J Natl Cancer Inst 55 : 633-636.

Montesano R. and Bartsch H. 1976. Mutagenic and carcinogenic N-nitroso compounds : Possible environment hazards. Mutation Res 32 : 179-208.

Nakajima T. and Iwahara S. 1973. Mutagenicity of dimethylnitrosaine in the metabolic process by rat liver chromosomes. Mutation Res 18 : 121-127.

Nakajima T., Tanaka A. and Tojyo K. 1974. The effect of metabolic activation with rat liver preparations on the mutagenicity of N-nitrosamines on a streptomycin-dependent strain of Escherichia coli. Mutation Res 18 : 121-127.

Negishi T. and Hayatsu H. 1980. The pH-dependent response of Salmonella *typhimurium* TA 100 to mutagenic N-nitrosamines. Mutation Res 79 : 223-230.

Neurath H. 1972. Nitrosamine Formation from Precursors In Tobacco Smoke. IARC Sci Publ No. 3 Lyon, pp. 134-136.

O' Conner PJ., Capps MJ. and Craig AW. 1973. Comparative studies of the hepatocarcinogens N, N-dimethylnitrosamine in vivo. Br J Cancer 27 : 153-166.

Okun JD. and Archer MC. 1977. Kinetics of nitrosamine on the presence of micelle - forming surfactant. J Natl Cancer Inst 58 : 409-411.

Oshima H. and Bartsch H. 1981. Quantitative estimation of endogenous nitrosation in humans by monitoring N-nitrosoproline excreted in the urine. Cancer Res 41 : 3658-3662.

Oshima H., Bereziat JC. and Bartsch H. 1982. Monitoring N-nitrosamino acids excreted in urine and fece of rats as an index for endogenons nitrosation. Carcinogenesis 3 : 115-120.

Oshima H., Mahon GAT., Wahrendorf J., *et al.* 1983. Dose-response study of N-nitrosoproline fomation in rats and a deduced kinetic model for prodicting carcinogenic effects caused by endogenous nitrosation. Cancer Res 43 : 5072-5076.

Osterdahl BG. 1983. N-nitrosamines and nitrosatable compounds in rubber nipples and pacifiers. Food Chem Toxicol 21:755-757.

Panalaks T., Iyengar JR., Donalson BA., *et al.* 1974. Further survey of cured meat products for volatile N-nitrosamines. J Assoc Off Anal Chem 57 : 806-812.

Pensabene JW., Fiddler W., Gates RA., *et al.* 1974. Effect of frying and other cooking conditions on nitrosopyrrolidine formation in bacon. J Food Sci 39 : 314-316.

Pignatelli B., Friesen M. and Walker EA. 1980. The role of phenols in catalysis of nitrosamine formation. In : N-Nitroso Compounds : Analysis, Formation and Occurrence. Walker EA., Gricuite L., Castegnaro M., *et al.* (Eds.). IARC Sci Publ No. 31. Lyon, pp.95-109.

Purchase IFH., Tsutin RC. and Rensberg SJ. 1976. Biological testing of food grown in Transkei. Food Cosmet Toxicol 13 : 639-647.

Rao TK., Ramey DW., Lijinsky W., *et al.* 1979a. Mutagenicity of cyclic nitrosamines in Salmonella : Effect of ring size. Mutation Res 67 : 21-26.

Rao Tk., Young JA., Lijinsky W., *et al.* 1979b. Mutagenicity of aliphatic nitrosamines in Salmonella typhimurium. Mutation Res 66 : 1-9.

Scanlan RA. 1975. N-nitrosamines in foods. Crit Rev Food Technol 5 : 37-402.

Scanlan RA. 1983. Formation and occurrence of nitrosamines in food. Cancer Res (suppl) 43 : 2435s-2440s.

Scanlan RA., Barbour JF., Hotchkiss JH., *et al.* 1980. N-nitrosodimethylamine in beer. Food Cosmet Toxicol 18 : 27-29.

Scheunig G. ard Ziebarth D. 1976. Formation of nitrosamine by interaction of some drugs with nitrite in human gastric juice. In : Environmental N-Nitroso Compounds : Analysis and Formation. Walker EA, Bokovski R, and Gricuite L. (Eds.) IARC Sci Publ No. 14. Lyon, pp269-277.

Sen NP. 1974. Nirosamines in Toxic Constituents of Animal Foodstuffs. Academic Press Inc. New York, pp. 131-138.

Sen NP., Miles WF., Donalson B., *et al.* 1973. Formation of nitrosamines in meat curing mixture. Nature (London) 245 : 104-105.

Sen NP., Seaman S. and McPherson M. 1980a. Nitrosamines in alcoholic beverages. J Food Safety 2 : 13-18.

Sen NP., Seaman S. and McPherson. 1980b. Further studies on the occurrence of volatile and non-volatile nitrosamines in foods. In : N-Nitroso Compounds : Analysis, Formation and Occurrence. Walker BA, Gricuite M, Castegnaro M, *et al.* (Eds.) IARC Sci Publ No. 31. Lyon, pp. 457-463.

Shank RC. 1975. Toxicology of N-nitroso compounds. J Toxicol Appl Pharmacol 31 : 361-368.

Spiegelhalder B., Eisenbrand G. and Preusmann R. 1980. Occurrence of volatile nitrosamines in food : A survey of the West German Markets. In : N-Nitroso Compounds : Analysis, Formation, and Occurrence. Walker EA., Gricuite L., Castegnaro M., *et al.* (Eds.) IARC Sci Publ No. 31. Lyon, pp. 467-479.

Stephany RW., Freudenthal J. and Schuller PL. 1976. Quantitative and Qualitative Determination of Some Volatile Nitrosamines in Various Meat Products. IARC Sci Publ No. 14. Lyon, pp. 343-354.

Stephany RW. and Schuller PL. 1980. Daily dietary intakes of nitrate, nitrite, and volatile Nitrosamines in the Netherlands using the duplicate portion sampling technique. Oncology (Basel) 37 : 203-210.

Sugimura T. and Sato S. 1983. Mutagen-carcinogens in foods. Cancer Res (Suppl) 43 : 2415s-2421s.

Swann PE. and Magee PN. 1971. Nitrosamine-induced carcinogenesis : The alkylation of N-7 guanine of nucleic acids of the rat by diethylnitrosamine, N-ethyl-N-nitroso urea and ethylmethane sulphonate. Biochem J 125 : 841-847.

Weast RC. 1976. Handbook of Chemistry and Physics. 57th Edition. CRC Press, Cleveland, pp. C81-C85.

Williams DLH. and Aldred SE. 1982. Inhibition of nitrosation of amines by thiols, alcohols, and carbolydrates. Food Chem Toxicol 20 : 79-81.

Yahagi T., Nagao M., Seino Y., *et al.* 1977. Mutagenicities of N-nitrosamines on Salmonella. Mutation Res 48 : 121-130.

Ziebarth D. 1974. N-nitrosation of secondary amines and particular of drugs in buffer solutions and human gastric juice. In : N-Nitroso compounds in the Environment. Bogovski P. and Walker EA. (Eds.) IARC Sci Publ No. 9, Lyon, pp. 137-140.

Zieger E. and Sheldon AE. 1978. The mutagenicity of Heterocyclic N-nitrosamines for Salmonella *typhimurium*. Mutation Res 66 : 1-9.