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**Human Exposure to N-Nitrosamines,
their Effects, and a Risk Assessment
for N-Nitrosodiethanolamine in
Personal Care Products**

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Summary and Conclusions

This report reviews the available data on the toxicology of N-nitrosodiethanolamine (NDELA) relative to other N-nitrosamines with particular reference to absorption, metabolism, mechanistic considerations and animal carcinogenicity. Data are presented that demonstrate the presence of nitrosamines in trace amounts in a wide range of foodstuffs, drinks, tobacco products, some occupational environments and in personal care products. The concentrations of the N-nitrosamine of concern in personal care products (NDELA) are shown to have reduced over the past few years. Data are also presented on the endogenous formation of N-nitrosamines following the ingestion of secondary amines and nitrosating agents (or their precursors).

It is concluded that all the N-nitrosamines are potent animal carcinogens but important differences exist within the group of N-nitrosamines studied. For instance, NDELA appears to be metabolised via a two stage process involving alcohol dehydrogenase with subsequent oxidation at the β -carbon. In general terms, the fraction of NDELA that is metabolised is small, with most being eliminated unchanged in urine. Other nitrosamines are metabolised to a much higher degree and appear to be oxidised at the α -carbon.

These factors, along with the existence of modulating factors, go some way to explaining the differences in the relative potency of nitrosamines in animal carcinogenicity studies. On the basis of TD₅₀ values, NDEA is the most potent nitrosamine within the group, with NDELA being the least potent. There are at least one and possibly two orders of magnitude difference between the potency of NDEA and NDELA in animal bioassays.

The significance of exposures to NDELA in personal care products is explored in this report. Indirect calculations based on N-nitrosodiethylamine (NDEA) are compared with calculations undertaken on NDELA data directly. It is concluded that, even when conservative assumptions are made on the amount of personal care products used and their duration of use, that current concentrations of NDELA in personal care products achieved with current high standards of manufacturing practice, do not pose a significant carcinogenic risk to man.

1. INTRODUCTION

N-Nitrosamines are compounds characterised by a nitrosyl group ($-N=O$) bound to the nitrogen atom of an amino group. In general, N-nitrosamines have no direct commercial applications, and there are very few examples where an N-nitrosamine is used in large quantities in industrial processes. Instead, they are usually formed unintentionally when amines and nitrosating agents come together. The yield of nitrosamine depends on the amine, its basicity, its steric requirements, on the nitrosating species and on the medium. Rates of reaction are faster for secondary amines than they are for primary or tertiary amines. In the case of ethanolamines, for example, the nitrosation rate is higher with diethanolamine (DEA) than with monoethanolamine (MEA) or triethanolamine (TEA) since MEA and TEA must first transform into DEA in order to produce nitrous derivatives (Kamp et al, 1989).

Human contact with nitrosamines is the result of two types of exposure: exogenous exposure to preformed nitrosamines in the environment, and endogenous exposure which occurs through their in vivo formation from nitrosable amino compounds in food, drugs, personal care products, etc. and from nitrosating agents such as nitrite and nitrous gases.

The carcinogenic potential of N-nitrosamines in animals has been recognised for approximately 30 years. Since Magee and Barnes (1956) first demonstrated the hepatocarcinogenic effects of N-nitrosodimethylamine (NDMA) in rodents, subsequent investigations have shown that many other alkyl or alkylaryl nitrosamines are potent and versatile animal carcinogens affecting over 30 species. Consistent with that fact, the importance of minimising human exposure to N-nitrosamines has been well recognised, even though to date there has been no clear demonstration that they contribute to the occurrence of cancer in man. Nevertheless, during the past 25 years, the analytical technology for N-nitrosamines has progressed enormously, and highly sensitive and specific techniques now exist which permit the reliable determination of N-nitrosamines at low $\mu\text{g/kg}$ (ppb) levels (ECETOC, 1990).

In recent years there has been substantial controversy concerning human exposure to N-nitrosamines, particularly N-nitrosodiethanolamine (NDELA) as a contaminant in personal care products. Therefore the objectives of this report are:

- to review information on the total human exposure to several important N-nitrosamines;
- to consider the implications of human exposure to N-nitrosamines and relate this to the risks arising from the exposure to NDELA in personal care products;
- to assess the risks arising from the exposure of large numbers of people to low levels of NDELA in personal care products.

For the purposes of this report, personal care products are defined as comprising of the following groups:

- Cosmetic products which are intended for placing in contact with the various external parts of the human body (epidermis, hair system, nails, lips, external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or principally to cleaning them, perfuming them or protecting them in order to keep them in good condition, change their appearance or correct body odours (EEC 1976)
- Preparations for minor medical conditions, mainly applied externally, e.g. antiseptics.

In this report major emphasis is given to the group of cosmetic products.

2. SOURCES OF EXPOSURE

During the past 25 years, there have been dramatic advances in N-nitrosamines analytical techniques. As a consequence, the limits of detection and reliability of measurements quoted in the older literature and used in this report may not be of an equivalent standard.

From an analytical point of view (ECETOC 1990), N-nitrosamines can be classified as follows (see Table 1):

Group I : steam volatile, short chain (e.g. NDMA) and heterocyclic compounds (e.g. NPIP),

Group II : other volatile, long-chain compounds (e.g. NDDMA),

Group III: non-volatile high polarity compounds (e.g. NDELA).

Methods for the determination of Group I-compounds were developed in the 1970's and are well established. The limit of determination for each product is in the range of 1 - 5 µg/kg if analyses are carried out by experienced analysts who carefully apply all measures necessary to prevent artefacts.

Group II-compounds are more difficult to analyse. A first method for hair care products was published in 1983 (Morrison et al, 1983) claiming a detection limit of 20 µg/kg.

The limits of determination discussed in the literature for Group III-products vary widely depending on the samples under discussion:

- about 10 - 100 µg/kg in the technical raw materials
- about 20-100 µg/kg in personal care products but may be as low as 10 µg/kg.

These limits can only be reached if a number of measures to avoid false negatives or false positives are carefully applied (ECETOC, 1990).

2.1 Food

N-Nitrosamines are formed principally by the reaction of naturally occurring secondary amines with nitrites that are added to foodstuffs or produced by bacterial reduction of nitrates. Their presence in food is a source of considerable concern.

Therefore, in the last decade extensive investigations of the volatile nitrosamine content of foodstuffs in various diets were carried out. Compilations of the results have been published by Havery et al (1978), Spiegelhalder et al (1980a,b), Gray (1981), Bogovski et al (1982), the British Ministry of Agriculture, Fisheries and Food (MAFF, 1987) and others, which are summarised in Table 2.

2.1.1 Meat and Meat Products

Many meat products (e.g. bacon, ham and various types of sausages) are cured with mixtures which contain nitrite or nitrate. This practice has led to an intensive search for nitrosamines in such products. It has been demonstrated that the use of nitrite containing curing-mixtures results in higher N-nitrosamine levels in meat compared to the curing with nitrate (Mirna, 1983). Significantly larger amounts of NPYR and to a lesser extent NDMA may be formed when nitrite containing bacon is cooked (Gray, 1981), but over recent years its nitrosamine content has been reduced from around 100 µg/kg of NPYR in 1971 to nearly 10 µg/kg in 1977 (Havery et al, 1978). This could partially be explained by decreasing the nitrite content and by addition of nitrosation inhibitors such as ascorbate. The amount of NPYR formed in cooked bacon is also influenced by the method of cooking, frying temperature, and cooking time (Gray, 1981).

2.1.2 Dairy Products

Nitrate is sometimes added to cheeses to prevent fermentation by certain strains of Clostridium. No correlation was observed between the levels of nitrate or nitrite and the presence of NDMA in the analysed cheese samples (Karlowski and Bojewski, 1987; Gough et al, 1977). The Danish State Food Institute (1981) indicated that comparable levels of nitrate (10 mg/kg) and nitrite (0.2 mg/kg) have been found in Danish cheeses regardless of whether or not nitrate had been added.

N-nitrosamine levels reported for cheese vary considerably. Whilst Havery et al (1976) failed to detect any of 14 nitrosamines in 17 samples of cheese, 10 of which had been processed with nitrate addition, Sen et al (1978) reported 31 samples of Dutch cheese imported into Canada to contain NDMA and NDEA at levels of up to 20 µg/kg. Most investigations showed, that N-nitrosamines could be detected in only 4 to 25% of the samples analysed at levels up to 2.5 µg/kg for NDMA and 2.3 µg/kg for NDEA (Table 2).

Whole milk, dried milk and milk products except yoghurt (Gough et al, 1977; Lakritz and Pensabene, 1981) have also been shown to contain small but detectable levels of NDMA (Table 2). Again, no correlation between the presence of nitrosamines and the levels of nitrate or nitrite was demonstrated (Karlowski and Bojewski, 1987).

2.1.3 Fish

Because of its relatively high amine content, fish is regarded as a likely source of nitrosamines. Several studies have been conducted during recent years with almost all investigators detecting volatile nitrosamines (Spiegelhalder, 1983). Their content tended to be higher after broiling (Yamamoto et al, 1984).

2.1.4 Fruit and Vegetables

Levels of N-nitrosamines detected in fruits and vegetables were $< 1 \mu\text{g/kg}$ (Webb and Gough, 1980).

2.1.5 Spices

Although the quantity of spices consumed is comparatively low, the concentrations of NDMA and NPYR in some are noteworthy. Pepper and pepper containing products have relatively high levels of NDMA (up to $51 \mu\text{g/kg}$) and NPYR (up to $79 \mu\text{g/kg}$) (Spiegelhalder, 1983).

2.1.6 Alcoholic Beverages

Over the past years, considerable attention has been focused on the presence of volatile nitrosamines in beer and other alcoholic beverages. Average NDMA levels reported were between $2.5 \mu\text{g/kg}$ (Spiegelhalder *et al.*, 1980a,b) and $5.9 \mu\text{g/kg}$ (Scanlan *et al.*, 1980) with peak levels of $47 \mu\text{g/litre}$ in dark beer and $68 \mu\text{g/litre}$ in one sample of a beer made with smoked malt ("Rauchbier") (Spiegelhalder *et al.*, 1980a,b).

To determine possible sources of nitrosamines in beer, several investigators have analysed all substances involved in the brewing process (Scanlan *et al.*, 1980; Spiegelhalder *et al.*, 1980a,b). The only significant source of NDMA was found to be the malt which had been processed by direct-fired drying. The use of a burner with reduced nitrogen oxide formation resulted in NDMA concentrations in the malt as low as 1 to $3 \mu\text{g/kg}$. This represents a 15- to 30-fold reduction. As a result, the NDMA levels in beer have also dropped considerably (Havery *et al.*, 1981; Preussmann *et al.* 1980). A study conducted by the FDA (Food and Drug Administration, 1980) showed that, following changes to the process, NDMA in 180 samples of domestic beer ranged from undetectable levels to $7 \mu\text{g/litre}$, averaging less than $1 \mu\text{g/litre}$. Thus it can be stated that NDMA levels in beer are now generally well below the level of $5 \mu\text{g/litre}$ at which the FDA would undertake regulatory action.

Goff and Fine (1979) failed to detect volatile nitrosamines in U.S. beverages other than beer and Scotch whisky. Walker *et al* (1979) reported that in 74 of 145 French Apple Brandy and Cognac samples volatile nitrosamines were found at concentrations generally ranging from 0.18 to 0.6 µg/kg. The highest level (10 µg/kg) was found in one sample of Apple Brandy.

2.1.7 Summary

Based on the data outlined above (see Table 2), ACS (1984) calculated the average uptake of total N-nitrosamines through the diet in various countries. The results vary between 0.3 µg/person/day for Sweden and 1.8 µg/person/day for Japan (Table 19).

2.2 Water

Drinking water has been shown to be generally uncontaminated with volatile N-nitrosamines (Fine *et al*, 1977b; Fan *et al*, 1978). In one instance, in well water with a high nitrate content, NDMA and NDEA were claimed to be present at levels below 0.01 µg/l (Fine and Rounbehler, 1976).

2.3 Air

2.3.1 Municipal Areas and Countryside.

A series of studies have been performed in the US, France and Austria to detect nitrosamines in municipal and rural air. Air-measurements in Los Angeles and the surrounding area (Gordon, 1979), New York, Boston, rural New Jersey (Fine *et al*, 1977a) and Paris (Chuong and Benarie, 1976) did not in general reveal any detectable N-nitrosamines at a detection limit of 10 ng/m³.

A recent study by Spiegelhalder and Preussmann (1987) measured nitrosamine concentrations in ambient air of a heavily industrialised area in Austria. Values between 0.01 - 0.04 µg/m³ of NDMA, NDEA and NMOR were found with only 6% of the samples containing >0.01 µg/m³. The authors concluded that

the presence of an amine producing chemical plant and high NO_x emissions does not necessarily lead to extensive nitrosamine formation in ambient air.

Measurements in Baltimore (Fine et al, 1977c) revealed levels of NDMA of 6 to $36 \mu\text{g}/\text{m}^3$. These high values were measured on the site of a chemical plant manufacturing 1,1-dimethylhydrazine for which NDMA was used as a precursor.

It can thus be concluded that airborne nitrosamines are not a general air pollution problem. One possible reason for low concentrations or absence of nitrosamines in the environmental air results from their rapid decomposition by light (Hanst et al, 1977).

2.3.2 Indoor Air

Brunnemann and Hoffmann (1978) studied several locations such as discotheques, bars and trains and reported concentrations of 0.1 - $0.24 \mu\text{g}/\text{m}^3$ NDMA in the air. The nitrosamine pollution was probably caused in the main by tobacco smoke. In ambient outdoor air no detectable amounts of NDMA were found.

Low concentrations of N-nitrosamines may occur in kitchens as a consequence of cooking (Sen et al, 1976).

2.3.3 Other Sites.

The air inside new automobiles has been shown to contain 0.07 - $0.83 \mu\text{g}/\text{m}^3$ NDMA, 0.04 - $0.4 \mu\text{g}/\text{m}^3$ NDEA and 0.07 - $2.5 \mu\text{g}/\text{m}^3$ NMOR (Fine et al, 1980). Their presence is thought to be due to rubber products in the cars.

2.4 Tobacco Use

2.4.1 Smoking

Tobacco use is a major source of exposure to N-nitrosamines for most people. At the time of harvesting tobacco is practically free of nitrosamines. Nitrosamine formation occurs during smoking and processing i.e. curing, ageing and fermentation. There is a great variation in the content and composition of nitrosamines in different types of tobacco. Contributory factors are the nature of the tobacco e.g. its alkaloid and nitrate contents, and the way of processing (Hoffmann et al, 1982a). More than 20 different nitroso compounds have been detected in tobacco smoke (Preussmann, 1989). They can be attributed to three different types: volatile N-nitrosamines, non-volatile N-nitroso compounds (mainly N-nitroso aminoacids), and tobacco-specific nitrosamines (TSNA) (Hill, 1988).

Volatile nitrosamines are mainly formed by pyrosynthesis during smoking, whereas TSNA are considered to be preformed (Neurath, 1983; Fischer et al, 1989a). Other investigators (Hoffmann et al, 1980) claim up to 70% of the TSNA in the main-stream smoke are formed by pyrosynthesis. Concentrations found in main-stream and side-stream tobacco smoke on list in Table 3. It can be seen that nitrosamine levels are elevated at high nitrate content. Concentrations of the TSNA in tobacco and smoke are given in Table 4. Up to 70% of volatile N-nitrosamines and TSNA can be removed by filters (Hoffmann et al, 1980).

NDELA was found in a range of 80 - 420 $\mu\text{g/kg}$ in unburned processed cigarette or cigar tobacco that had been treated with maleic hydrazide formulated as diethanolamine salt. In the main-stream smoke of these US cigarettes NDELA amounted to 10 - 68 ng/cigarette (Hoffmann et al, 1982b). After banning the use of this salt in tobacco management in 1981, NDELA was practically undetectable (Tricker and Preussmann, 1989a).

In vivo nitrosation (endogeneous nitrosation) has been demonstrated to occur in man (Fine et al, 1977b) as well as in laboratory animals (Rounbehler et al, 1977; Hoffmann et al, 1989). It could be demonstrated that inhaled cigarette main-stream smoke can N-nitrosate proline

endogeneously (Bartsch and Montesano, 1984). It was suggested that these findings may also apply to other N-nitrosatable amines and lead to endogeneous formation of carcinogenic nitrosamines (Hoffmann et al, 1989).

2.4.2 Smokeless Tobacco Use

2.4.2.1 Snuff (Nasal Use)

Snuff as used in the USA and in Europe was found to contain concentrations of total nitrosamines up to 20 mg/kg; NNN contributed 7.69 mg/kg, NNK 1.86 mg/kg, NAB/NAT 3.18 mg/kg, whereas the concentration of volatile N-nitrosamines was distinctly lower, e.g. for NDMA 0.025 mg/kg (Tricker and Preussmann, 1989b). NDELA was found in a concentration of 0.016 mg/kg which was probably due to aged tobacco treated with the previously mentioned diethanolamine salt. In 1987, Hoffmann et al (1987) found NDELA peaks values in snuff of 6.84 mg/kg.

2.4.2.2 Leaves for Chewing

Orally used tobaccos were found to contain 10 - 70 mg/kg total N-nitroso compounds, mostly TSNA, but with small amounts of NDELA (Table 5). Brunnemann et al (1987) found amounts of total nitrosamines in the range of 5 - 151 mg/kg. Hoffmann et al (1987) detected values between 9.6 and 289 mg/kg, with NDELA concentrations ranging from 0.03 to 1.1 mg/kg.

2.5 Occupational Exposure

Aliphatic nitrosamines are generally not produced intentionally or used in the chemical or other industries. There are two sources of N-nitrosamines in the work place (Preussmann and Spiegelhalter, 1984): exposure to preformed nitrosamines resulting from the reaction of amines with ubiquitous NO_x or other nitrosating chemicals (exogeneous exposure), or exposure to endogenously formed nitrosamines as a result of the uptake of amines by inhalation or dermal absorption.

The degree of nitrosamine formation in air depends on type and concentration of the respective amine and NO_x concentration, but also on temperature, humidity, acidity, light and other factors. Reaction rates in the gas phase are considerably faster than in the liquid phase. NO_x concentrations in work place air are usually below 1 ppm. Under certain unfavorable conditions (e.g. open heating systems or vehicle exhaust), high concentrations of NO_x may be present.

2.5.1 Rubber Industry

NDMA and NMOR were found in the air of the workplace of rubber factories at concentrations mostly in the range of $0.05 - 20 \mu\text{g}/\text{m}^3$ (Spiegelhalder and Preussmann, 1983; Fajen *et al*, 1979; McGlothlin *et al*, 1981). In extreme situations values as high as $90 \mu\text{g}/\text{m}^3$ (NDMA) and $380 \mu\text{g}/\text{m}^3$ (NMOR) were detected from personal monitoring samples. Values as high as $1,060 \mu\text{g}/\text{m}^3$ NDMA and $4,700 \mu\text{g}/\text{m}^3$ NMOR were found for static samples taken during the injection moulding and curing of conveyor belts. Further nitrosamines present in air during fabrication of rubber products were NDEA ($0.1 - 5 \mu\text{g}/\text{m}^3$), NPIP ($0.3 \mu\text{g}/\text{m}^3$) and NMPHA ($5 - 8 \mu\text{g}/\text{m}^3$). Other non-volatile nitrosamines have also been found (Spiegelhalder and Preussmann, 1983).

In rubber articles N-nitrosamine concentrations between 10 and $200 \mu\text{g}/\text{kg}$ and in rubber chemicals $4 - 3,500 \mu\text{g}/\text{kg}$ have been found. This led to concentrations up to $1.5 \mu\text{g}/\text{m}^3$ for NDMA and NMOR in tyre sales and store rooms (Spiegelhalder and Preussmann, 1983).

There have been attempts to reduce the formation and exposure of nitrosamines in the rubber industry by substituting certain chemicals. For example, nitroso-diphenylamine which might either transnitrosate or decompose to nitrogen oxides has been substituted by cyclohexylthiophthalimide thereby reducing nitrosamine levels (Spiegelhalder and Preussmann, 1983).

In a recent study in Germany 545 separate workplace measurements were performed using personal air samplers (Wolf, 1989). In 73% of the samples the sum of NDMA, NDEA, NDBA, NPIP, NMOR, NMPHA and NDPHA was $\leq 2.5 \mu\text{g}/\text{m}^3$. The range of the concentrations was 0.1 until $41 \mu\text{g}/\text{m}^3$.

2.5.2 Leather-Tanning Industry

Rounbehler et al (1979) were the first to detect NDMA in a tannery. They reported average levels of $19 \mu\text{g}/\text{m}^3$ and maximum levels of $47 \mu\text{g}/\text{m}^3$. After improving the factory conditions the values were reduced appreciably to $0.1 - 3.4 \mu\text{g}/\text{m}^3$. In a further study (Fajen et al, 1982) NDMA concentrations ranging from < 0.05 to $47 \mu\text{g}/\text{m}^3$ and NMOR concentrations of 0.05 to $2 \mu\text{g}/\text{m}^3$ were reported. In a recent study performed in Germany (Wolf, 1989) total N-nitrosamines (NDEA, NDMA and NMOR) were found to be $\leq 1 \mu\text{g}/\text{m}^3$ at each of 12 workplaces.

2.5.3 Metal Working Industry

In 1976 it was demonstrated by Zingmark and Rappe (1976) that NDELA was a contaminant in synthetic metal working fluids, which contained triethanolamine and nitrite as anticorrosion agents. Fan et al (1977) detected NDELA in synthetic cutting fluids at concentrations up to 3% of undiluted fluid. Efforts to reduce the formation of NDELA by using nitrite-free formulations have reduced N-nitrosamine concentrations remarkably. Spiegelhalder (1983) reported values between 0.5 to $250 \text{ mg}/\text{kg}$ with extreme values up to $750 \text{ mg}/\text{kg}$. The amounts of other nitrosamines such as NDMA were extremely low compared to NDELA (Hartung and Spiegelhalder, 1982).

Few air measurements have been reported. In one study approximately up to $1 \mu\text{g}/\text{m}^3$ NDELA was found in the air of workplaces where a fluid was used containing between $40 \mu\text{g}/\text{kg}$ and $1,6 \text{ mg}/\text{kg}$ NDELA (Wolf, 1989).

2.5.4 Chemical Industry

In the early seventies there were reports of substantial N-nitrosamine concentrations (up to $43 \mu\text{g}/\text{m}^3$) in the vicinity of amine production facilities (Bretschneider and Matz, 1974). In 1977, Fine et al (1977a) reported NDMA concentrations in the range of $0.01 - 1 \mu\text{g}/\text{m}^3$ in the air close to a methylamine factory. Recent investigations involving 4 aliphatic amine plants in Germany showed concentrations of total N-nitrosamines were $\leq 1 \mu\text{g}/\text{m}^3$ in 91% of the samples (Wolf, 1989).

2.5.5 Other Industries

In the fish processing industry NDMA values in the air were found to be between 0.01 and 0.06 $\mu\text{g}/\text{m}^3$ (Fajen et al, 1982). Certain non-industrial workplaces associated with high levels of tobacco smoking or cooking of food may also expose workers to N-nitrosamines.

2.6 Personal Care Products

NDELA was first reported to be present in personal care products by Fan et al (1977b). Its formation was almost certainly helped by the presence of diethanolamine (DEA) or impure triethanolamine (TEA) containing DEA as a contaminant. Other ingredients such as alkanolamides and nitrosating agents, an example of which is 2-bromo-2-nitro-1,3-propanediol (Bronopol), encouraged its formation. Amine containing compounds are used extensively in ingredients of personal care products including surfactants, detergents, foam boosters, protein additives and colouring agents.

Since the above 1977 report, a variety of personal care products from around the world have been analysed for NDELA content. The methodologies employed and their specific limits of detection have necessarily varied. The data are shown in Table 6. It is of interest that leave-on mucous membrane products generally have very low NDELA levels, whereas rinse-off preparations tend to be more variable. In the six personal care product formulations tested by Fan et al (1977b), the range of NDELA level was from a trace (5 - 10 $\mu\text{g}/\text{kg}$) to 49,000 $\mu\text{g}/\text{kg}$; one other of the cosmetics analysed also contained high levels (27,000 $\mu\text{g}/\text{kg}$) of NDELA with three of the seven samples containing 100 $\mu\text{g}/\text{kg}$ or less. In general, the lotions and shampoos they tested contained negligible (2 - 20 $\mu\text{g}/\text{kg}$) quantities of NDELA.

When leave-on products such as deodorants, skin lotions and sun screens were analysed more recently (IKW, 1989) only three out of 62 samples contained NDELA, the highest level being 53 $\mu\text{g}/\text{kg}$. In the same survey the highest level of NDELA in shampoos was 41 $\mu\text{g}/\text{kg}$. In the study reported by IKW (1986) there was a clear distinction between NDELA levels present in personal care products when tested either before or after 1983.

It appears that, having been alerted to the problem of NDELA contamination in personal care products, procedures have been introduced to minimise their formation. The major steps taken to minimise NDELA and other nitrosamine formations included:

- a) the avoidance of using DEA,
- b) the avoidance of using impure samples of TEA,
- c) the avoidance of coexistence of nitrosating agents and N-nitrosatable substances,
- d) minimisation of NO_x levels in the factory environment,
- e) the use of di- and monoethanolamides with low levels of free amines,
- f) the use of inhibitors of nitrosamine formation.

Inhibitors of nitrosamine formation are agents which act primarily as scavengers. Of these, ascorbic acid and sulphamic acid are considered particularly useful.

There are very few reported references to the presence of volatile nitrosamines in personal care products (ECETOC, 1990), however, Spiegelhalder and Preussmann (1984) reported the presence of NMOR but virtually no NDMA in some of the products they analysed.

For the purpose of this report and the following risk assessment it is assumed that personal care products currently contain 10 $\mu\text{g/kg}$ of NDELA.

2.7 Endogenous Formation

Following an evaluation of environmental sources of N-nitroso containing agents undertaken by the US National Academy of Sciences Panel (1978) it was concluded that the major source of human exposure to such agents was through nitrosation of secondary and tertiary amines in the stomach, i.e., endogenous formation of nitrosamines. As a result, it is necessary to consider the human exposure to nitrate, nitrite and to amines. Exposure to amines includes protein amides (100 g/day) and guanidines such as creatine and creatinine (1 g/day), primary amines (100 mg/day) and arylamines and urea (1-10 mg/day).

2.7.1 In vivo Demonstration of Endogenous Formation of Nitroso Compounds

The first demonstration that the formation of a nitroso compound in vivo could be sufficient to give rise to tumours in animals was that of Sander (1971). He fed methyl benzylamine and morpholine together with nitrite to rats and obtained tumours of the oesophagus and liver. In later experiments Taylor and Lijinsky (1975) investigated tumour induction in rats following administration of heptamethyleneimine and sodium nitrite in water at relatively high concentrations. They were able to demonstrate a dramatic increase in non-endocrine tumour formation in rats when given the combination regime but when each ingredient was given on its own no increase in tumours was observed. Telling et al (1976) investigated the ability to form nitrosamines following administration of nitrite and secondary amines to rats. In their experiment rats were given sodium nitrite at concentrations up to 3,000 mg/litre in the drinking water, whilst in the diet dimethylamine or pyrrolidine was given at various concentrations. After administration the animals were killed a few hours later and stomach content examined and analysed for NDMA and NPYR formation. Their findings indicated that there was no increase in nitrosamine levels over that in controls until the animals were given up to 1,000 mg/kg of amine in the diet concomitantly with 1,000 mg/litre of sodium nitrite in the drinking water. If the amine content in the diet was increased to 2,000 mg/kg then the sodium nitrite content of water could be reduced to 100 mg/litre and still enable the detection of nitrosamines.

Nitrosamine formation in the stomach depends on a number of factors including the concentration of amine, the pKa of amine, the concentration of nitrite, the gastric pH, the temperature and the amount of food in the stomach, the rate of emptying, the rate of removal of any nitrosamines which have been formed and the rate of removal of nitrite. It has been shown that nitrite is rapidly removed from the stomach of the fasting rat but not so rapidly in fed animals (Mirvish, 1975).

2.7.2 Ingested Levels of Nitrate and Nitrite

It has been estimated (White, 1975) that in the USA the average daily intake of nitrate is approximately 100 mg/day. In certain rural areas, where drinking water is taken from shallow wells this can increase to 2,200 mg/day. The average values quoted above agree with those from other sources (ECETOC, 1988). Many diets have been analysed and were found to provide an intake of nitrate of approximately 100 mg/day, with ranges of 31.4 for Norway to 130 for Poland. Nitrite levels are generally low at around 3 mg/person/day, the WHO recommended maximum being 8 mg/day (WHO, 1985). It has been demonstrated that nitrate can be reduced to nitrite by bacterial and mammalian pathways. In man, about 5% of dietary nitrate is converted to nitrite in saliva which is therefore a major site of nitrite formation in man (Spiegelhalter *et al*, 1976; Walters and Smith, 1981). This, together with nitrite from other sources such as diet contribute to the total nitrite burden in humans of around 10 mg/person/day. After a nitrate rich meal, 40-70 mg of nitrate could enter the stomach. It should be noted though that Spiegelhalter *et al* (1976) reported that ingestion of less than 54 mg of nitrate did not alter the salivary nitrate and nitrite levels, although this is not a consistent finding (Walters and Smith, 1981; Stephany and Schuller, 1980).

2.7.3 Conversion of Nitrite to form Nitrosamines

Nitrite reacts with secondary and tertiary amines to yield nitrosamines, with secondary and tertiary amides to form nitrosamides and with N-substituted ureas and carbamates to yield nitroso ureas and nitroso carbamates. The proportion of nitrite converted to nitrosamines remains a controversial area. The rate of formation of nitrosamines is proportional to the square of the nitrite concentration (Shepherd *et al*, 1987) but because of various factors such as pH, type and amount of amine, catalysts and inhibitors it is not possible to estimate precisely the extent to which ingested nitrite is used in *in vivo* nitrosation reactions. In 1978, the US National Research Council Panel on Nitrates assumed that the extent of the formation of nitroso compounds was approximately 5% of the nitrite ingested.

Urinary excretion of N-nitrosoproline (NPRO) has been used as predictor of endogenous nitrosamine formation. Estimates based on the NPRO method developed by Oshima and Bartsch (1981) suggest the earlier 5% figure could be too high an estimate and that 0.5% may be more realistic. The more recent work of Shepherd et al (1987) and of Tricker and Preussmann (1987) support this hypothesis.

Wagner et al (1984) have reviewed the value of the NPRO model for predicting the mechanism of endogenous nitrosation, and suggest the model may not reflect the extent of formation of other nitroso compounds. Licht and Deen (1988) on the basis of theoretical models have suggested that NPRO excretion is not an accurate index of gastric nitrosation under physiological conditions. Wagner et al (1984) investigated endogenous formation in human volunteers on a low-nitrate diet containing 150 μ mol nitrate/day. Under this regime, basal excretion of NPRO ranged from 5 - 49 nmol/day. Assuming a 5% utilisation of nitrate to nitrite this represents approximately 0.35% conversion of nitrite to nitrosamine when on a low-nitrate diet. Addition of ascorbic acid or α -tocopherol to the diet did not change the endogenous background level as shown by urinary NPRO measurements. When added with nitrite and proline there was a significant reduction in NPRO production with essentially complete inhibition of nitrate-induced NPRO synthesis (Wagner et al, 1984). It remains unclear therefore as to precisely where the endogenous synthesis of NPRO takes place. Although nitrate produced in the body is recirculated to the salivary glands and reduced to nitrite by bacteria in the oral cavity, the experiment mentioned above suggests that NPRO may also be generated as a result of non-gastric synthesis.

Tannenbaum (1987) considers there are at least 2 pools of NPRO in the body, one of these incorporating nitrogen and modulated by ascorbic acid whilst the other does not. The first of these pools probably represents gastric synthesis whilst the second represents mammalian cell mediated synthesis and/or nitrogen oxide mediated synthesis. Typically the non-gastric synthesis would be fairly uniform so that differences between individuals may be ascribed to gastric synthesis.

All these findings and speculations demonstrate the general lack of knowledge or agreement concerning the contribution of endogenous nitrosation to the total uptake of nitrosamines. However, Bartsch and Montesano (1984) suggest endogenous processes produce between 0.3 and 20 μg total nitrosamines per person per day.

3. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION

3.1 Absorption/Excretion

Due to technical complexities, it is extremely difficult to experimentally determine the rates of absorption of nitrosamines. NDELA, has been shown by various investigators to be largely eliminated unchanged in urine following all routes of exposure. Hence, urinary NDELA measurements provide at least an approximation of the degree of absorption of NDELA following an oral or dermal dose.

In rats, radiolabelled NDELA was apparently absorbed very slowly during the first 24 hours following a topical application of 0.5 or 50 mg NDELA in acetone; more than 60% of the dose remained at the application site, and only 3% of the radiolabel was recovered in urine within 24 hours after dosing (Lethco et al, 1982). Approximately 20% of the radiolabel was recovered as unchanged NDELA in urine of rats collected over a 96 hour period after dosing, and approximately 50% was found in urine during a one week period after dosing. In contrast to these results, Lijinsky et al (1981a) reported that rats excreted 20 - 30% (10 - 15 mg) of the dose in urine in 24 hours following topical administration of 50 mg NDELA (no vehicle). Similarly Airolidi et al (1984a) found that excretion of unchanged NDELA accounted for 25% of the dose during a 24 hour period after rats were given a 5 mg/kg unlabelled cutaneous dose as a mixture in water-acetone. Sansone et al (1980) also reported that about 20 - 30% of the dose was recovered unchanged in urine within 24 hours after rats were given 50 mg unlabelled NDELA (no vehicle), while only about 12% of the dose was recovered in urine of rats given 25 mg NDELA in cutting oil. Higher urinary excretion rates were reported by Preussmann et al (1981); approximately 70 - 80% of dose was excreted in urine within 24 hours after rats were given a single unlabelled cutaneous dose ranging from 28 to 184 mg NDELA per animal, using either water or acetone as a vehicle. The basis for the differences in the degree of skin absorption in these various studies remains unclear. Factors such as size of the application area, strain and age differences in thickness of stratum corneum, vehicle effects and analytical techniques may in part account for the

discrepancies observed. The balance of evidence seems to suggest the absorption rate for NDELA in rats is about 20 - 30%.

Substantial differences were noted in the disposition of unlabelled NMOR and NDELA after application to the skin of rats (Sansone et al, 1980; Lijinsky et al, 1981a). Less than 1% of the dose of NMOR was recovered unchanged in urine, while 20 - 30% of the NDELA dose was excreted unchanged in urine within 24 hours after dosing. The basis for this observed difference in urinary excretion is not clear, but it possible reflects differences in the rates of absorption, as well as in the rates and extent of metabolism.

Studies reported by Spiegelhalder et al (1982) showed that only a very small fraction of NMOR or NDMA doses were eliminated unchanged in urine of rats after oral, dermal, intravenous or intratracheal administration. By contrast, urinary excretion of unchanged parent compound represented the primary route of elimination of NDELA from rats.

Radiolabelled NDELA was applied in acetone or skin lotion for 24 hours to the skin of monkeys or pigs in four separate studies (Marzulli et al, 1981). The estimates of skin penetration in these studies were based on recovery of the radiolabel in urine over a 5-day period after dosing. Absorption was greater in monkeys than in pigs, and absorption was increased in both species when NDELA was applied in acetone as compared to skin lotion. Only 4% of the dose was absorbed in pigs while 23.4% was absorbed in monkeys when NDELA was applied in skin lotion. According to the authors, man and pig are generally more alike than man and monkey with regard to skin penetration, with monkey skin being more permeable than human skin.

In a study with a human volunteer, NDELA was detected in the urine after skin application of an experimental cosmetic formulation (12 - 13 g on 2090 cm² skin) containing 77 mg/kg NDELA (Edwards et al, 1979). The cosmetic formulation was removed from the skin 7 h 45 min after application; approximately 2% of the applied NDELA was recovered in urine within 20 hours after skin application. In vitro studies with excised human

abdominal skin indicated that NDELA penetrated slowly through the skin when applied either in water or propylene glycol vehicles. The permeability constants for water and propylene glycol were 5.5×10^{-6} and 3.2×10^{-6} cm/hr, respectively (Bronaugh et al, 1981). When NDELA was applied in a more lipoidal vehicle, isopropyl myristate, the rate was markedly enhanced (permeability constant 1.1×10^{-3} cm/hr).

In the risk assessment discussed later in this report it is assumed that the available pig and human data are most relevant and that the NDELA absorption rate is 5%.

Nitrosamines are well absorbed after oral administration. Lethco et al (1982) found that there was rapid absorption in rats after oral administration of an aqueous solution of radiolabelled NDELA at doses of 0.5 or 50 mg/kg. Urine was the major route of elimination; 67% of the low dose and 87% of the high dose was excreted as unchanged NDELA in urine within 24 hours after oral dosing. Sansone et al (1980) reported that absorption of NDELA and NMOR was rapid after the nitrosamines were given to rats by gavage in aqueous solutions. Only a small fraction of the NMOR oral dose was recovered in urine, but about 30% of the NDELA dose was excreted unchanged in urine within 24 hours. Higher urinary excretion rates were reported by Preussman et al (1978); about 70% of single NDELA oral doses ranging from 10 - 1000 mg/kg were excreted by rats as unchanged NDELA within 24 hours. There was no indication of dose-dependent differences in these studies. Similar results in rats were reported by Spiegelhalder et al (1982); 60 - 80% of oral NDELA doses ranging from 0.03 to 300 mg per animal were recovered in urine within 24 hours. In contrast, only a very small fraction (<1%) of an oral dose of NDMA was recovered in urine of rats given doses of NDMA ranging from 5 to 350 µg per animal. NMOR was likewise excreted to only a minimal extent (<1%) in urine after rats were given gavage doses ranging from 4 to 400 µg per animal. Hence it seems that there are differences in the disposition of NDELA in comparison to NDMA and NMOR after oral administration, although they may be due to the sensitivities of the assay rather than to the precise nature of the nitrosamines examined. Other studies have shown that uptake of NDMA is

slow from the stomach, but very rapid from the upper part of the small intestine (Hashimoto et al, 1976; Heading et al, 1974).

Biliary excretion has been shown to be a very minor route of elimination of NDELA in rats given oral or dermal doses (Lethco et al, 1982). Nevertheless, enterohepatic recycling might still be of significance with regard to the hepatic effects of the substance (Bonfanti et al, 1985).

Spiegelhalder et al (1982) evaluated NDMA urinary excretion in man given 10 - 100 µg NDMA in beer, orange juice, or orange juice with 6% ethanol. NDMA could be detected in urine only when it was given in combination with alcohol, and this was thought to be related to some type of metabolic interaction between alcohol and the nitrosamine.

3.2 Distribution

The distribution of radiolabelled NDELA has been evaluated in rats given a single 0.5 or 50 mg/kg dose via either the oral or dermal route (Lethco et al, 1982). The radiolabel was distributed throughout the organs and tissues of animals killed from 4 to 168 hours after dosing. There were no obvious differences in distribution of the radiolabel in animals dosed orally versus those dosed dermally. In general, the tissue content was low, and there was no evidence for retention of radiolabel by any organ, however, elevated levels of radiolabel were noted in the stomachs and livers of animals dosed orally during the first 24 hours. Airolidi et al (1984a) studied the kinetics and bioavailability of NDELA after intravenous and cutaneous administration to rats. A semi-logarithmic blood concentrationtime plot after iv injection showed a triphasic profile, indicating that a three-compartment model may adequately describe the kinetics of the compound. There was no apparent NDELA accumulation in liver after either iv or cutaneous administration.

Loefberg and Tjälve (1985) studied the distribution of NMOR by autoradiographic techniques. Low-temperature whole-body autoradiography of animals killed 1 min after an iv injection indicated that non-metabolised NMOR is able to pass freely through cellular membranes and distribute evenly in

most tissues of the body. Similar results were also obtained with NDEA and NPYR (Brittebo et al, 1981a,b; Loeffberg and Tjälve, 1984). Whole body autoradiography with dried tissue sections showed a high level of non-volatile NMOR metabolites in the liver and in the nasal mucosa at all time intervals ranging from 15 min to 24 hr after iv or sc dosing; the oesophageal mucosa accumulated NMOR metabolites to a lesser degree (Loeffberg and Tjälve, 1985). Tissues of the lymphomyeloid system and the gastro-intestinal mucosa also showed some accumulation of non-volatile NMOR metabolites; this observation was consistent with results from NDMA, NDEA and NPYR (Brittebo et al, 1981 a,b; Johansson and Tjälve, 1978).

3.3 Metabolism

Most dialkylnitrosamines studied have been shown to be oxidised at the carbon α to the nitroso group by tissue specific microsomal mixed function oxidases. In general, this oxidation results in an unstable product which collapses to a diazohydroxide, and ultimately to an electrophile. A carbonyl compound, also formed during oxidation of nitrosamines, has often been quantified to estimate the extent of metabolism. Thus, Farrelly et al (1984) determined that NDPA was oxidized at the α -carbon by rat liver microsomes in vitro, producing propionaldehyde plus propyldiazohydroxide. Similarly, Brouwers and Emmelot (1960) demonstrated that liver microsomes metabolise NDMA to formaldehyde, while Magour and Nievel (1971) and Arcos et al (1976) showed that acetaldehyde was a product of the microsomal oxidation of NDEA (Fig. 3).

It has also been well established that in rats NMOR is oxidized at the α -carbon (Hecht and Young, 1981; Manson et al, 1978). Following an intraperitoneal injection of NMOR, 1.5% of the dose was found unmetabolised in urine, together with 3 metabolites. In addition to (2-hydroxyethoxy)-acetic acid and N-nitroso-(2-hydroxyethyl)glycine, NDELA was also shown to be a urinary metabolite of NMOR (Hecht and Young, 1981; Brunnemann et al, 1983). The tissue specificity of NMOR metabolism has been studied in rats. Cytochrome P-450 dependent metabolism of NMOR was shown to occur in the nasal and oesophageal mucosa, as well as in the liver (Loeffberg and Tjälve, 1985).

NDELA is apparently not metabolized at the alpha carbon by rat liver microsomes in vitro. It has been shown to be oxidised to some extent at the β -carbon by non-microsomal enzymes to N-(2-hydroxyethyl)-N-carboxymethylnitrosamine and N-(2-hydroxyethyl)-N-(formylmethyl)-nitrosamine (Airoldi et al, 1984b; Farrelly et al, 1984; Lethco et al, 1982). This non-microsomal metabolic pathway has been demonstrated in rats, mice, hamsters and rabbits, and may possibly be a detoxification mechanism (Bonfanti et al, 1986) (Fig. 4).

A two-step mechanism has been proposed by which NDELA is first transformed by alcohol dehydrogenase into N-nitroso-2-hydroxymorpholine (NHMOR), the ring-closed hemiacetal form of N-(2-hydroxyethyl)-N-(formylmethyl)-nitrosamine. This cyclic β -hydroxynitrosamine appears to be a substrate for sulphotransferase. The resulting sulphate conjugate is suggested to be the ultimate genotoxic metabolite of NDELA (Sterzel and Eisenbrand, 1986) (Fig. 4).

The carcinogenic significance of the metabolites oxidated at the β -carbon of NDELA remains unclear, but ultimately the carcinogenicity of NDELA is thought to depend on its α hydroxylation or on the transnitrosation capacity of β -oxidated metabolites (Bonfanti et al, 1986).

3.4 Summary

Dialkylnitrosamines are absorbed after application to intact skin of laboratory animals. The rate of absorption is dependent on a variety of factors including physical properties of the nitrosamine, thickness of skin, area of application, vehicle effects and species based on results obtained from various rodent and primate studies (Table 7). Due to the complexities involved, exact skin penetration rates of dialkylnitrosamines are unknown. For NDELA approximately 20% of an applied dermal dose would be absorbed in 24 hr, based on results obtained in the various rodent studies (Table 7). In a single human experiment and from experiments in the pig, whose skin is more like man than rodent skin, it is more likely that 2 - 5% of the applied NDELA would be absorbed by man. In the later chapters of this report it is assumed that 5% of NDELA is absorbed.

Nitrosamines are also readily absorbed from the gastrointestinal tract. At least some studies have shown that NDELA is more readily absorbed via the gastrointestinal tract than via the dermal route. The various oral studies that have been conducted on NDELA (Table 8) suggest that approximately 70% of an oral dose could be absorbed within 24 hr.

After absorption, the nitrosamines are distributed throughout the body, but accumulation of non-volatile metabolites may occur in certain organs and tissues, e.g. liver. NDELA is primarily eliminated unchanged in urine, but a small fraction is believed to undergo oxidation at the β -carbon by non-microsomal enzymes. By contrast, other dialkyl nitrosamines are more extensively metabolised, primarily via oxidation at the carbon α to the nitroso group which results ultimately in the formation of an electrophile capable of reacting with cellular nucleophiles.

4. GENERAL TOXICITY

4.1 Acute Lethal Effects of Nitrosamines

The acute lethal effects of a number of selected nitrosamines are listed in Table 9. Of the nitrosamines for which data were obtained, the lower alkyl amines exhibited the highest order of acute lethal toxicity. Oxygenation appeared to have a large moderating effect on toxicity, NDELA exhibiting the lowest order of lethality with a reported oral LD₅₀ in rats of 7 g/kg body weight. Vinyl substitution on the other hand appear to have little modifying effect on the LD₅₀; thus, the reported oral LD₅₀ values of NDMA and NDEA in rats were approximately 50 and 88 mg/kg compared to LD₅₀ values of 24 and 102 mg/kg for methylvinylnitrosamine and ethylvinylnitrosamine respectively. The cyclic nitrosamines appeared to have a somewhat lower order of toxicity than the lower alkyl nitrosamines. Where data existed, LD₅₀ values for any one nitrosamine were relatively independent of the route of administration. Within a group of structurally related symmetrical nitrosamines, molecular weight appeared to play a major role in determining the acute lethal toxicity, the higher the molecular weight the lower the order of toxicity. Finally, where data were available on multiple species with the same chemical, no apparent trends appeared with regard to sensitivity to the lethal effects.

4.2 Descriptive Toxicology

The descriptive toxicity of nitrosamine compounds is almost exclusively confined to NDMA. A number of studies on the toxic properties of NDMA were initiated as a result of several case reports indicating toxic responses in humans exposed under laboratory conditions (Freund, 1937; Barnes and Magee, 1954; Jacobson *et al*, 1955). These studies identified the liver as the primary target organ affected. The toxicity to liver was demonstrated across a number of animal species including mice, dogs, rats, rabbits and guinea pigs. In general, lethality was produced by dosages of a similar order of magnitude when administered orally, by intraperitoneal injection, by intravenous injection or by subcutaneous injection. Barnes and Magee (1954), however, could not produce toxic effects by dermal application,

even when a dose of 100 mg/kg was applied to the uncovered, depilated skin of rats on four successive days. Freund (1937) and later Jacobson et al (1955) demonstrated that in spite of the low vapour pressure, inhalation of NDMA vapor could result in toxic responses. Single 4-hour LC₅₀ values of 50 to 75 ppm have been demonstrated in various animal species. After single acute exposures by any of the various routes, deaths were generally delayed for up to several days.

There have been a number of incidences of poisonings of livestock and domestically raised fur-bearing animals fed herring meal contaminated with NDMA due to improper processing. These incidences subsequently led to the investigations of toxicity of NDMA in cattle, sheep, poultry and mink (Koppang, 1974a, b; Carlton and Welser, 1968; Juskiewicz and Kowalski, 1976; Carter et al, 1969).

Mink appear to be exquisitely sensitive to the toxic effects of the chemical. Animals receiving a diet containing 5 mg/kg died in approximately four weeks, and those receiving 2.5 mg/kg died in the fifth week of the study (Carter et al, 1969). Both cattle and sheep appear to be sensitive to dosages in the range of 0.2 mg/kg/day and greater (Koppang, 1974a,b 1974b). Ducks receiving feed containing 0.005% NDMA died within two weeks (Carlton and Welser, 1968). Along with liver injury, kidney lesions were also noted as a major manifestation of the toxicity. Juskiewicz and Kowalski (1976) state that hens can tolerate doses of 3 mg/kg NDMA showing no immediate signs of intoxication and no changes in egg production. As in laboratory animals, in all these cases liver was identified as a major target organ.

4.3 Effects of NDMA on the Liver

Liver injury appears to be a common characteristic of acute poisoning from a wide variety of nitrosamine compounds including NDMA, NDEA (Wegiel et al, 1980), N-nitroso-n-butylmethylamine (Heath, 1962), N-nitrosovinylethylamine (Althoff et al, 1977), NPIP and NMOR (Althoff et al, 1974). Liver injury resulting from NDMA treatment has also been reported across a wide variety

of animal species. The injury produced by these nitrosamines appears to be consistent with haemorrhagic centrilobular necrosis.

McLean et al (1965) studied the time course of the lesion after single intraperitoneal injections of NDMA in the rat. Changes occurring in one day included extensive coagulative necrosis at the centre of the lobules with fatty accumulation in cells in the portal regions. After three days necrotic cells in the center of the lobules had disappeared and were replaced by "lakes" of blood with leucocytosis evident; the central veins were normal. Seven days after dosing, central vein occlusion appeared, endothelial proliferation was seen and collateral channels appeared in the vicinity of the blocked veins. By 10 days, fibrosis was evident along with frequent veno-occlusive lesions. The authors contended that these were not the result of direct toxic effect on the vein endothelium, but followed the obstruction to the liver circulation occurring early in the necrotic phase. Khanna and Puri (1966) also studied the time course of NDMA liver injury in rats; the effects of 75 mg/kg in feed being characterized as parenchymal necrosis, haemorrhage and regenerative activity.

Korsrud et al (1973) investigated the effect of NDMA in the Sprague-Dawley rat. Minimal histopathological changes were seen in the liver at a dose level of 1.9 mg/kg. Clumping and slight vacuolation of cells occurred in the central vein area. At higher dosage levels, injury was more severe with marked congestion and necrosis 100 mg/kg.

Reuber (1975) demonstrated a sex difference in the liver toxicity of NDMA in the Buffalo rat, females being more sensitive than males.

Wegiel et al (1980) investigated the effects of NDEA on the livers of male mice. Necrotic changes increased in severity over the first week after dosing, the resulting injury requiring two to three months to resolve to a condition approaching that of control animals. Electron microscopy revealed that NDEA caused a swelling of the mitochondria and severe degenerative changes in the rough endoplasmic reticulum which rapidly underwent vacuolisation and degranulation. There was also an increase in lipid droplet number and droplet volume. Changes observed by electron

microscopy were largely resolved within four days after dosing, but were still apparent in some animals after a month.

4.4 Possible Modifiers of Liver Toxicity

Investigations have been conducted on conditions and agents which modify the toxicity of NDMA in general, and which either increase or decrease the toxic effects on the liver in particular. Diets rich in protein, cystine and choline have been shown to offer some protection from the toxic effects of NDMA (Khanna and Puri, 1966), while at the same time protein-free diets also appear to protect against the liver toxicity (Swann and McLean, 1968; Hard and Butler, 1970). Complicating these results are those of Khanna and Puri (1966) and Pani et al (1977), who have shown that repeated administration of choline enhances the toxicity of NDMA and also increases the activity of N-nitrosodimethylamine demethylase. Indeed, there appears to be a great deal of evidence which suggests that treatments which enhance demethylase activity also enhance the toxic effects of NDMA on the liver. Pound and co-workers have conducted extensive studies which demonstrate that nonlethal doses of carbon tetrachloride protect the liver from the toxic effects of NDMA and also inhibit the demethylase activity (Pound et al, 1973; Pound and Lawson, 1974, 1975; Pound, 1975).

The fact that the toxic effects of NDMA on liver appear to be mediated through enzymatic N-demethylation may have important ramifications for other nitrosamines, particularly for NDELA where the major pathway of metabolism occurs through β -oxidation rather than α -oxidation and, thus, is not dependent on the demethylase enzyme (see Section 3.3).

4.5 Extrahepatic Target Organ Toxicity

Although the great majority of investigations on the target organ toxicity have focused on the liver, there are some suggestions of toxic effects on other organs and system. As mentioned above, the duck kidney appears to be uniquely sensitive to the toxic effects of NDMA (Carlton and Welser, 1968). There are reports of possible renal and cardiac toxicity in cattle, pigs and sheep (Koppang, 1974a, b). In addition Hard and Butler (1970) have

been able to produce damage to the testis of Porton rats with NDMA; this damage being accentuated in animals fed a protein-free diet.

Pielsticker et al (1967) demonstrated that NDEA did not produce teratogenic effects in golden hamsters when administered at various times during gestation.

Druckrey (1973) found NDMA to be highly foetotoxic but not teratogenic in rats after a single dose administered on either the tenth day or between the 13th and 15th day of gestation; NDEA was not teratogenic when doses of up to 70 mg/kg were administered in similar experimental design.

4.6 Toxic Effects in Man

Toxic effects of NDMA in man have been documented in a number of case reports (Freund, 1937; Barnes and Magee, 1954; Jacobson et al, 1955; Rouech, 1982). In all cases liver injury was a major manifestation of poisoning. When deaths occurred they were delayed; in the cases of a single dose homicidal poisoning, four days in an infant and five days in an adult (Rouech, 1982). Signs and symptoms experienced by victims included nausea accompanied by vomiting, loss of appetite, abdominal pain, ascites, diarrhoea, fever, haemorrhage and coma. In addition, elevated temperatures, malaise, evidence of decreased liver function with liver pathology of an undefined nature have also been reported (Jacobson et al, 1955). When necropsies or biopsies were performed, there was evidence of necrosis and cirrhosis.

4.7 Summary

Although most of the descriptive toxicology of acute nitrosamine poisoning is confined to NDMA, the information that does exist indicates that liver is a primary target organ. Little data appear to be available on the acute effects NDELA. The fact that NDELA is metabolized in a manner different from that of the lower alkyl nitrosamines (Section 3.3), and that toxic effects to the liver appear to be mediated through an active metabolite, suggest that NDMA and NDEA may not be an appropriate model for NDELA.

Clearly, the acute lethal effects of NDELA are of a lower order of magnitude than those of NDMA, NDEA and other aliphatic nitrosamine (Table 9). Nevertheless, the liver is the primary target organ of carcinogenic activity for most nitrosamines.

5. CARCINOGENICITY STUDIES IN ANIMALS

In 1937 it was reported that workers exposed to NDMA suffered quite moderate to severe liver damage, but it was not until the 1950's that Magee and Barnes (1956) demonstrated that NDMA was carcinogenic to the liver of mice and rats. Subsequent work by Druckrey (1975) showed that a whole range of nitrosamines possessed the potential to cause tumours in a variety of organs following oral administration. These studies have now been confirmed by many workers using this and other routes of administration (IARC, 1978, 1982, 1987).

A summary of the target organ toxicity associated with the major nitrosamines included in this survey are provided in Table 10. Malignant tumours have been found in many organs such as liver, lung, nasal cavity, brain and central nervous system, trachea, stomach, gut, bladder, pancreas and the haemopoietic system. The organotropic action is influenced by the chemical structure, the animal species under investigation, route of administration and dose. The main target organ in the rat for NDMA is the liver. For NDEA and for unsymmetrical agents such as NMEA, it is liver and oesophagus (Preussman and Wiessler, 1987). Lijinsky et al (1981b) demonstrated changes in target organ specificity associated with very small alterations in chemical structure such as odd or even alkyl chain length. They showed that for methyl alkyl nitrosamines with alkyl chain lengths of 7, 9 or 11 carbon atoms, liver and lung tumours in the rat occurred whereas the same chemical class with chain lengths of 8, 10, 12 and 14 carbon atoms selectively induced urinary tract tumours. In general, and where comparisons can be made, the main tumours in the mouse and hamster were of the lung, liver and respiratory tract, but not of the urinary tract.

The organotropic action of nitrosamines may be due to one or all of the following mechanisms:

1. different activation in different organs according to sub-classes of activating enzyme.
2. enzyme activation in the liver and transport of metabolites or conjugates to the target organ.

3. glucuronides or sulphate forming enzymatic reactions may have various capacities in different organs.
4. different repair capacities for alkylated bases in different organs or for different cell types in the same organ.

Table 11 refers specifically to the target organ specificity of NDELA. Two important papers include those of Lijinsky and Kovatch (1985) and Preussmann et al (1982a, b). Lijinsky and Kovatch (1985) administered NDELA in drinking water to 126 rats of both sexes at dose levels of 0, 24 and 64 mg/l for 100 weeks and at 64 and 160 mg/l for 50 weeks. Animals were examined histologically at death or when moribund or after 130 weeks. A statistically significant dose-response in the incidence of hepatocellular carcinomas and neoplastic nodules was seen in females and to a lesser extent in males at the higher dose levels. Low incidences of kidney tubular cell adenomas and carcinomas, which were not statistically significant were seen in most treatment groups. Preussmann et al (1982a, b) gave NDELA in drinking water to Sprague Dawley rats at 0, 1.5, 6, 25, 100 and 400 mg/kg/day for a lifetime. A statistically significant increased incidence of liver and nasal cavity tumours was seen after adjustment for early death. The increase was dose-dependent for liver tumours. In these studies tumours were observed at all dose levels.

Zerban et al (1988) investigated a dose-response relationship for the development of preneoplastic liver lesions in rats following administration of very low doses of NDELA. Dose levels ranging from 0.2 to 25 mg/kg/day in the drinking water were given for periods up to two years. Tumour formation along with the number of liver foci positive for glucose 6 phosphate dehydrogenase was examined. The results indicated that at dose levels of 0.2 mg/kg/day no tumour formation occurred. Evidence for glucose-6phosphate dehydrogenase positive foci was observed at all dose levels including the lowest of 0.2 mg/kg/day. The relevance of such foci to tumour formation continues to be debatable (EPA, 1986).

6. MECHANISTIC CONSIDERATIONS

6.1 Initiation and Promotion

One of the main observations of the last decade concerning chemical carcinogenesis is the complexity of its biological nature. When this process is initiated there is almost always a long latency period before the tumour is expressed by a carcinogenic stimulus. The long latency appears due to stepwise, sequential transformation of normal into malignant cells via several intermediate cell populations during which they acquire increasing degrees of autonomy (Foulds, 1958; Medina, 1975; de Gerlache *et al*, 1984; Schulte-Hermann, 1985; Pitot and Campbell, 1987). In this multi-stage concept of carcinogenesis, formation and development of these intermediate cell populations and thus tumour formation results from at least three fundamentally different mechanisms: initiation, promotion and progression.

Tumour initiation refers to the earliest irreversible effect of exposure to a carcinogen which leads to a persistent and heritable alteration of a cell.

Promotion refers to a process that enhances the development of neoplasms by the selection and clonal proliferation of initiated cells. Tumour promoters are agents that increase the tumourigenic response to a genotoxic carcinogen when applied after the initiator (Weisburger and Williams, 1980).

It is well established that nitrosamines like NDMA, NDEA and NMOR are strong initiators and therefore widely used as positive controls in initiation-promotion studies. A high dose of the hepatocarcinogen NDEA produces approximately only one initiated foci (clone) per 10^6 hepatocytes in the liver of rats (Farber and Cameron, 1980; Pitot and Sirica, 1980). However, multistage carcinogenesis has been actively evaluated in a number of different organs including the skin and the lung of mice and the liver of rats (Boutwell, 1974; Uchida and Hirono, 1979; Pitot and Sirica, 1980; Pitot and Campbell, 1987). In these studies a strong quantitative

correlation occurred between carcinogen dose and the appearance of enzyme-altered hyperplastic foci (Schieferstein et al, 1974; Kunz et al, 1978; Emmelot and Scherer, 1980; Preat et al, 1986).

Twenty-four hours after being partially hepatectomised, adult rats were administered either a single dose of 10 mg NDEA/kg/body weight or, to study dose-response relationships, doses in the range of 0.3 to 30 mg NDEA/kg/body weight. After that, a progressive increase in the size of ATPase-deficient hepatocellular foci over time and a linear dose-response in the incidence of foci were noted (Scherer and Emmelot, 1976).

Kunz et al (1978) compared the number of gamma-glutamyltranspeptidase positive foci, average area of foci, and total foci areas induced by NDEA with the subsequent carcinogenicity of this compound. Total foci area correlated best with subsequent carcinogenicity, although it is recognised that not all foci will progress towards malignancy.

Zerban et al (1988) demonstrated that NDELA can induce the formation of liver foci. In the same study formation of neoplastic nodules and hepatocarcinoma was also demonstrated.

Preat et al (1986) initiated rats with single i.p. injections of NDEA or NMOR followed by treatment sequentially with 2-acetylaminofluorene (2-AAF) and phenobarbitone (PB). A necrogenic dose of CCl₄ was also applied during 2-AAF treatment. A dose-effect relationship between the dose of nitrosamine and the gamma-glutamyl transferase (GGT) positive foci was demonstrated with NDEA being more potent than NMOR. In the 44 week study only NDEA produced malignant tumours.

Comparable results have been reported by de Gerlache et al (1984) who have found that NDEA was more potent in inducing hepatocarcinogenesis than NMOR. A rapid increase of neoplastic nodules measured as GGT+ foci by a short treatment with 2-AAF coupled with partial hepatectomy after initiation by a single dose of NDEA was also reported by Ito et al (1982).

Lapis et al (1984) investigated promotion models with different stimulation of liver cell proliferation. Rats were treated with NDEA, and two weeks later promotion was effected by application of 2-AAF for 14 days. At midpoint of the promotion protocol, one group of rats was subjected to partial hepatectomy, the most potent stimulus of rapid liver growth. Two other groups of animals were treated either with carbon tetrachloride or with thioacetamide. The highest incidences of liver foci and of hepatocellular carcinomas were observed after partial hepatectomy and a good correlation with long-lasting elevated GGT-activity was found.

Recently placental glutathione S-transferase, which is hardly detectable in normal rat liver, was demonstrated as a new marker protein for preneoplastic liver foci in initiation-promotion assays (Sato et al, 1984; Tatematsu et al, 1985). More than 50 chemicals including hepatocarcinogens, non-hepatocarcinogens, hepatopromoters and others were investigated for their potential to modify GST-P positive foci development. In animal models, all hepatocarcinogens like NDEA and NDMA and all hepatopromoters like 2-AAF and PB clearly enhanced the induction of GST-P positive foci whereas non-hepatocarcinogens and non-hepatopromoters did not (Tatematsu et al, 1987). It is well established that they are strong initiators widely used as positive controls in initiation/promotion studies.

6.2 Mutagenicity and Genotoxicity

The mutagenicity and genotoxicity of N-nitrosamines has been widely studied in a variety of short-term in vitro and in vivo assays (IARC, 1978; Rao, 1984; Lijinski 1987).

In non-mammalian systems, N-nitrosamines are only mutagenic if metabolically activated by cytochrome P-450 linked mixed function oxidases. Most studies reported have been carried out with the Ames Salmonella-/microsome test (Ames et al, 1975). In general, liver homogenate fractions from hamsters are more effective than rat liver fractions in metabolising N-nitrosamines in this in vitro assay. This may explain why many

N-nitrosamines were poorly responsive when initially tested in the conventional plate incorporation assay (Tables 12 and 13).

6.2.1 NDMA

NDMA showed genotoxic activity in a variety of prokaryotes and non-mammalian eukaryotes after metabolic activation. In the host-mediated assay using mice or rats, mutagenicity was shown in S. typhimurium G46, C207 and C340 (Gabridge and Legator, 1969) and in Saccharomyces cerevisiae D-4 (Fahrig, 1975; Fahrig and Remmer, 1983). In the latter studies, high doses close to the LD₅₀ were necessary to produce any effect.

NDMA induced cross-linked recessive lethal mutations in Drosophila, 8-azaguanine-resistant mutants in Chinese hamster V79 cells, and chromosome aberrations and sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells, in the presence of a rat liver fraction (Fahmy et al, 1975; Kuroki et al, 1977; Natarajan et al, 1976). NDMA induced unscheduled DNA synthesis in isolated rat hepatocytes and in cultured human fibroblasts (Williams, 1977; Laishes and Stich, 1973). NDMA treatment of rat liver epithelial like cells maintained in culture resulted in transformed cells, which induced tumours at the site of injection in 80% of injected newborn rats (Montesano et al, 1975).

In vivo studies with NDMA showed induction of chromosome aberrations in liver cells of Chinese hamsters and in rat lymphocytes and, at high i.p. doses, induction of micronuclei in rat bone marrow cells (Brookes and Cregger, 1973; Lilly et al, 1975; Watanabe et al, 1982). A low incidence of gene mutations was observed in the mouse spot test after i.p. dosing (Fahrig et al, 1981). A dominant lethal test with NDMA in mice was negative (Epstein et al, 1972).

6.2.2 NDEA

NDEA produced reverse mutations in Salmonella typhimurium strains TA1535 and TA100 and in Escherichia coli, in the presence of a liver fraction from induced rats. In the host-mediated assay with mice, NDEA was mutagenic in

Salmonella typhimurium at doses of 50 mg/kg bw. NDEA also caused forward mutations in Saccharomyces cereviae and Neurospora crassa (IARC, 1978).

NDEA induced chromosome aberrations and SCEs in CHO cells in the presence of metabolic activation (Natarajan et al, 1976), and induced point mutations in the recessive lethal test with Drosophila melanogaster (Pasternak, 1963). Dominant lethal mutations were not seen in mice treated with 13.5 mg/kg bw NDEA (Propping et al, 1972). A low incidence of gene mutations was found in the mouse spot test after i.p. dosing (Fahrig et al, 1981). Transplacental treatment of hamster embryos led to transformation of the cells in primary culture (Evans and DiPaolo, 1975).

6.2.3 NMOR

NMOR was mutagenic in Salmonella typhimurium TA1530, TA1535, TA1537 and TA1538 in the presence of liver microsomal fractions from rats and human biopsies (Bartsch et al, 1976). NMOR induced 8-azaguanine resistant mutants in Chinese hamster V79 cells in the presence of rat liver S-9 (Kuroki et al, 1977). In BHK21 cells, NMOR induced 8-azaguanine resistant mutants as well as chromosomal aberrations (Kimble et al, 1973). Other studies showed mutagenic effects in a host-mediated assay with mice (dose 100 mg/kg bw) and induction of cross-linked recessive lethal mutations and translocations in Drosophila melanogaster (Henke et al, 1964; Zeiger and Legator, 1971). NMOR was negative in a dominant lethal assay in male mice carried out at doses of 50 and 100 mg/kg bw (Parkin et al, 1973).

6.2.4 NPIP

NPIP induced reverse mutations in Escherichia coli and in Salmonella typhimurium TA100, TA1530 and TA1535 (IARC, 1978; Dahl, 1985). It also induced 8-azaguanine resistant mutants in Chinese hamster V79 cells, in the presence of a metabolic activation system (Kuroki et al, 1977).

6.2.5 NNN

In the presence of a liver microsomal preparation of Aroclor-induced rats, NNN caused a dose-dependent increase in mutations in Salmonella typhimurium TA100 (highest doses tested 2.5 $\mu\text{mol/plate}$) and induced mutations in TA1530 at 5.7 $\mu\text{mol/plate}$. NNN induced unscheduled DNA synthesis in freshly isolated rat hepatocytes at 10^{-3} and 10^{-4} M concentrations (IARC, 1985).

6.2.6 NDELA

Although NDELA has been shown to produce an increase in mutations in bacterial tester strains, the results were not consistent. In particular, mutagenicity testing in the standard Ames assay, with rat liver S-9 mix or microsomal fraction used for activation has failed to indicate mutagenicity or has led to equivocal results. Mori et al (1987) reported that purified NDELA was positive in the presence of a liver activation system either from rats or hamsters, but that the mutagenicity was completely lost when DMSO was used as a solvent. In contrast, NDELA of purity of 93.8% showed mutagenicity without metabolic activation. NDELA was positive in a intrasanguineous host-mediated assay with E. coli K-12 343/113 as indicator strain (Kerklaan et al, 1981). A subcutaneous application of NDELA induced a dose-dependent increase of galactose fermenting mutants in cells recovered from the livers of mice exposed for 3 h to the compound (Knasmueller et al, 1986).

When incubated with alcohol dehydrogenase (ADH) from yeast or horse liver and NAD, NDELA is converted to a potent mutagen in Salmonella typhimurium TA98 and TA100. NDELA and its putative mutagenic metabolites N-nitroso-2-hydroxymorpholine (NHMOR), N-nitroso-(2-hydroxyethyl)-glycine (NHEG) and N-nitrosoiminodiacetic acid (NIDA) were also tested for induction of DNA single-strand breaks in primary rat hepatocytes. NDELA and NHMOR were clearly genotoxic in these cells whereas NHEG and NIDA were inactive (Denkel et al, 1986). NHMOR is also mutagenic in S. typhimurium TA 1535 with and without metabolic activation (Hecht, 1984). When assayed for DNA amplification, NDELA and its metabolites were not found to induce SV40 DNA synthesis in SV40-transformed Chinese hamster cells. NDELA induced SCEs,

chromosome aberrations and micronuclei in cultured human lymphocytes (Ditterberner et al, 1988).

NDELA, its metabolite N-nitroso-2-hydroxymorpholine (NHMOR) and other hydroxyxated N-nitrosoalkylamines induce single-strand breaks in rat liver after a single oral application (Denkel et al, 1987). After competitive inhibition of ADH by pretreatment with ethanol, induction of single-strand breaks by NDELA and N-nitroso-(2-hydroxyethyl)ethylamine (NHEEA) was completely suppressed, whereas breaks induced by NHMOR were only partially reduced. Moreover, pretreatment with the ADH inhibitor 3-butylthiolane-1-oxide considerably reduced the single-strand break potential of NDELA. Pretreatment with the sulphotransferase inhibitor 2,6-dichloro-4-nitrophenol (DCNP) completely suppressed the induction of single-strand breaks by NDELA. The data suggest that ADH and sulfotransferase are enzymes responsible for the in vivo activation of NDELA.

NDELA was negative in a micronucleus test and in a bone marrow metaphase assay in BALB/c mice (Gilbert et al, 1981).

6.3 DNA Binding

N-Nitrosamines like most genotoxic chemicals form covalent bonds with DNA or proteins thereby forming adducts. DNA adducts are likely to be of prime importance in tumour initiation and induction of mutation but their relevance to the later stages of carcinogenesis (promotion, progression) is unknown. The role of haemoglobin adducts is unclear. Two applications of the measurements of adducts have been discussed: exposure monitoring and health risk assessment. Whereas the use of DNA and protein adducts for exposure monitoring is very promising, the use for risk assessment is more of a qualitative nature. Evidence for DNA binding suggests an increased risk, but the magnitude of the increase may not be known (ECETOC, 1989).

Animal experiments have shown that the extent of DNA and protein adduct formation was directly proportional to the administered doses (ECETOC, 1989). However, a different ratio exists between DNA and haemoglobin

(protein) binding level. e.g. with NDEA the level of alkylation was found to be of about two orders of magnitude higher in liver of mice with DNA than in haemoglobin (Ostermann-Golkar and Bergmark, 1988).

At least 12 sites of alkylation in DNA have been identified, the major alkylation products are 7-me Gua, O⁶-me Gua, 3-me Ade and O⁴-me Thy (Montesano et al, 1988). Nevertheless, the level of adducts is also dependent on the degree of repair. The actual damage by DNA alkylation is the result of several factors such as distribution in tissues, metabolism and, as mentioned earlier, DNA repair. A comparison of the degree of alkylation of DNA by a number of methylating and ethylating nitrosamines showed that alkylation of O⁶ and N⁷ was much more pronounced by methylating than by the corresponding ethylating compounds, irrespective of their tumourigenic potencies. Thus the conclusion was drawn that reactions other than alkylation are also important in inducing tumours (Lijinsky, 1989).

The first study on the alkylation of DNA by N-nitrosamines was performed by Craddock and Magee (1965). By now, the group of N-nitroso compounds is very well studied for their DNA binding activity (Lutz, 1979; Hemminki, 1983). DNA adducts in cultured human tissue were detected, e.g. in bronchus, colon, oesophagus with NDMA and NDEA, whereas NPIP only reacted with bronchus cells (Harris, 1985).

The interaction of TSNA with DNA and haemoglobin in rats and the possibility of using such assays as potentially useful dosimeter for human exposure have been studied by Hecht et al (1988).

N-Nitroso-2-hydroxymorpholine, a mutagenic metabolite of NDELA, is believed to react with deoxyguanosine to form a cyclic 1, N²-glyoxaldehydeoxyguanosine (Chung and Hecht, 1985).

DNA hydroxyethylation was demonstrated in vivo in an immuno slot-blot assay with O⁶-2-hydroxyethyldeoxyguanosine (O⁶-HEdG) as reaction product; the degree of reaction by NDELA was considerably lower than with other N-nitroso-2-hydroxyalkylamines tested (Scherer et al, 1989).

6.4 Structure-Activity Relationships

Aliphatic nitrosamines like NDMA and NDEA are more potent mutagens in Salmonella typhimurium (with hamster liver S9 for metabolic activation) than cyclic nitrosamines. NDELA has failed to show clear positive results in the conventional Ames assay. Studies with aliphatic nitrosamines have shown a direct relationship between alkyl chain length and bacterial mutagenicity in contrast to the inverse relationship between chain length and carcinogenicity (Yahagi et al, 1977). This lack of congruence is due to the complexity of the metabolic processes leading to formation of proximate carcinogens. The deficiencies in the mutagenicity assays appear to arise from a lack of the necessary enzymes in the rat liver microsomal fractions normally used for activation. N-Nitrosamines bearing oxygen on the β -carbon of an alkyl chain are not oxidized by rat microsomal enzymes and hence are not converted to bacterial mutagens by rat liver microsomes. In contrast, the mutagenic activity of the cyclic nitrosamines (among these N-nitroso-piperidine and N-nitroso-morpholine) is more closely related to their carcinogenicity. In this group, which is less mutagenic in bacteria than the aliphatic nitrosamines, the bacterial mutagenicity increased with the size of the ring; their carcinogenic activities follow a similar pattern. Therefore it is concluded that, at least for aliphatic nitrosamines, there is no quantitative relationship between the bacterial mutagenicity and the carcinogenicity. Bacterial mutagenicity is not considered to be a relevant guide to the carcinogenic activity of aliphatic N-nitrosamines or to the mechanism by which these compounds induce cancer (Lijinsky et al, 1984b; Lijinsky, 1987).

Metabolism to a mutagenic intermediate is presumably only one of the critical factors determining the potency and the organ-specificity of carcinogenic N-nitrosamines. It is interesting to note that a better correlation between carcinogenicity and mutagenicity of N-nitrosamines was observed in mammalian cell-mediated mutagenesis assays (Jones and Huberman, 1984; Langenbach, 1986).

6.5 Possible Modulators of Genotoxic Effects

The organotropism of N-nitrosamines may, in some part, be due to the presence of modulators of their biological activities. For instance, there are indications that a number of dietary factors may help in either preventing the formation of endogenously produced nitrosamines (nitrosation inhibitors) or by exerting an anti-mutagenic or anticarcinogenic effect. Such agents include ascorbic acid, vitamin E and antioxidants such as BHA (Bartsch and Montesano, 1984; Bartsch, 1989; Bhide *et al*, 1989; Nair *et al*, 1989). These have also been implicated as possible inhibitors of nitrosation (Wang and Wu, 1989) as have polyphenols occurring in vegetables, fruits, beers and soft drinks (Pignatelli *et al*, 1982; Stich *et al*, 1983). In addition, the natural repair capacity in man may well confer an enhanced capability to withstand the possibly damaging effects of nitrosamines. It is known that there are species differences with respect to their repair capacity and in particular to levels of O⁶-alkylguanine DNA alkyltransferase. This class of enzyme is present in much greater quantities in the liver than in other tissues (Bartsch and Montesano, 1984). Furthermore, it is considered that human cells have a higher capacity to repair O⁶-methylguanine than do rodent cells (Bartsch and Montesano, 1984).

The liver clearly plays the major role in activation or detoxification of nitroso agents, particularly at low dose levels. In addition to the above mentioned factors, others such as protein restricted diet, ethanol, disulfiram and zinc deficiency may all lower the alkylation potential of the liver, resulting in increases in DNA alkylation in other tissues. Thus, modulation of activity through dietary factors could have positive and negative effects and emphasises the problems in attempting to determine whether there is a causal association between exposure to nitrosamines and some types of cancer.

6.6 Summary

Results obtained in initiation/promotion animal models strongly support the concept of an initiating role for various N-nitrosamines. This hypothesis

is firmly supported by results from various genotoxicity assays in which N-nitrosamines have demonstrated mutagenic and clastogenic activities. Some N-nitrosamines have been found to form covalently bound adducts with DNA, again lending support for initiating cellular events which could ultimately lead to tumour formation. It would appear that N-nitrosamines require metabolising to a reactive form in order to demonstrate their effects and dietary modulators may mitigate such effects.

7 EPIDEMIOLOGY

The epidemiological evidence implicating NDELA is rather limited. There are studies, that have involved exposures in part to nitrosamines including NDELA or their precursors. Comments on such studies are given below.

7.1 Nitrate in Drinking Water and Diet

Most epidemiological studies have concentrated on the relationship between the intake of nitrates and gastric cancer and to a lesser extent with oesophageal, liver or bladder cancer. The assumption is made in these studies that nitrate is reduced to nitrite in vivo which in turn reacts with naturally occurring amines to produce N-nitrosamines. N-nitrosamines are considered the proximate carcinogenic species in such studies.

There have been numerous studies that have either made geographical comparisons of high and low gastric cancer incidence or case-control studies in which the exposure to nitrate of gastric cancer control populations were compared. Reviews of the early evidence by the US National Academy of Sciences (1981) and WHO (1985) concluded that the evidence linking exposure of humans to high levels of nitrates and gastric or oesophageal cancer etc was, at best, circumstantial.

A recent review by Preussmann and Tricker (1989) considered the evidence from a number of the more significant studies (see Table 14). He found that when nitrate body burden was estimated by analysis of urine or saliva the relationship between body burden and cancer incidence was often conflicting. In Columbia Cuello et al (1976) showed a correlation between gastric cancer and urinary nitrate but not with salivary nitrate. In Chile (Armijo et al, 1981), Iran (Eisenbrand et al, 1980), China (Xu, 1981), Japan (Kamiyama et al, 1987) and UK (Foreman et al, 1985) high levels of urinary nitrate or salivary nitrate were found in areas of low gastric cancer incidence. Consequently, a simple relationship between nitrate body burden and cancer incidence does not appear to exist.

The importance of dietary factors in the incidence of gastric cancer have been considered by ECETOC (1988) and Preussmann and Tricker (1989). They considered that when a high nitrate intake was associated with the ingestion of vegetables, anti-carcinogenic agents such as vitamin C appeared to counteract any adverse effect of the nitrate (Mirvish, 1985, 1986; Tannenbaum and Correa, 1985; Foreman et al, 1985). On the other hand, populations with low gastric acidity appear to be more at risk of developing gastric cancer. It has been suggested that the higher pH allows bacteria to colonise the stomach and thus increase the amount of nitrate reduced to nitrite. The increased nitrite concentrations in turn could lead to higher N-nitrosamine formations. (Correa et al, 1975; Hill et al, 1973).

As a consequence of the dietary and health factors, the incidence of gastric cancer in developed countries is falling despite an increased nitrate intake. In developing countries where low gastric acidity, dietary deficiencies and high nitrate intake coexist, a higher incidence of gastric cancer remains (Fraser, 1985).

7.2 Occupational Studies

Occupational epidemiological studies can be divided into two groups. The first involves exposure to nitrates and, hence, are similar to dietary studies where an in vivo conversion of nitrates to N-nitrosamines is assumed. The second involves exposures in industries where N-nitrosamines are already formed or are produced during processing (metal working fluids, rubber industry).

The effect of non-dietary exposure to nitrates has been studied in fertiliser workers. Such workers are exposed to dusts that contain water soluble nitrates. Fraser et al (1982) in a census-based study compared fertiliser workers with the general population and found no evidence of elevated gastric cancer rates.

Al-Dabbagh et al (1986) studied fertiliser workers in the North East of England. He was able to demonstrate that such workers had higher nitrate

body burdens than non-fertiliser workers in the factory and also higher than the general population. The study found no excess of any cancer that could be associated with occupational exposure to nitrate.

There have been a number of epidemiological studies involving exposure to cutting fluids. In most studies there were mixed exposures to vegetable, mineral as well as synthetic oils. The degree that additives such as nitrites and ethanolamines were used is unknown. In general, these studies did not demonstrate any excess of cancer (Decoufle, 1976, 1978; Järvholm et al, 1981; Ely et al, 1970) despite the known association of mineral oils with skin cancer (Lee 1976). Even when studies were structured to emphasize the exposures to cutting fluids suspected of containing NDELA, no excess of cancer was found (Järvholm et al, 1986; Vincent et al, 1986). No attempt was made to measure NDELA exposures in these studies.

A further study involving exposures to an antirust oil may have demonstrated an excess of non-specific cancers (12 cases observed against 3.9 expected) but 7 different organs were involved. Suggestions by the authors that N-nitroso-N-phenyl-1-naphthylamine may be a causative agent are unsubstantiated (Järvholm and Lavenius, 1981).

A number of epidemiological studies have taken place within the Rubber Industry (IARC, 1982). Several authors have suggested that N-nitrosamines may have been associated with the excess of bladder, lung, stomach or other tumours found in the studies (Spiegelhalter and Preussmann, 1983). However, the complex nature of the exposures in the industry makes such a conclusion very speculative. For instance, other authors have implicated naphthylamines among the several hundred substances that have been involved in the Rubber Industry (IARC, 1982).

7.3 Tobacco Products

There is an increasing number of studies that suggest that N-nitrosamines in cigarette smoke, snuff or chewing tobacco contribute to the excess of cancer associated with the use of such products (Brunneman et al, 1987; Reichart and Mohr, 1987; Preston-Martin, 1987).

It has been pointed out that the complex nature of tobacco and tobacco smoke makes the interpretation of the role of any one component extremely difficult (Hecht and Hoffmann, 1989). For instance, apart from polynuclear aromatic hydrocarbons, phenols, carbon monoxide, hydrogen cyanide etc, over 20 different nitrosamines have been found in unburned cigarettes. Of these NDELA is reported to constitute only a minor fraction (Tricker and Preussmann, 1989a).

8. POTENCY RANKING

The nitrosamines discussed in this report are all potent genotoxic carcinogens. Nevertheless, they do display variations in the manner and degree that their carcinogenicity is expressed in animal studies. Variations also exist in their acute toxicity and their activity in mutagenicity screens. As carcinogenicity is the parameter of most concern with nitrosamines, only the carcinogenic potency of NDELA relative to other nitrosamines will be considered.

Comparisons of potency are notoriously difficult because of the many variables such as manner of dosing, range of doses, animal species and end-point of measurement. For instance, Berger *et al* (1989) suggest that NDELA is 40 times less potent than NDEA and 4 times less potent than NPYR based on time to death with tumour in the rat. Lijinsky (1987) suggests that hydroxylated nitrosamines are, in general, less potent than their parent unsubstituted nitrosamines. For instance, he suggests that NDELA is less potent than NDEA by up to 2 orders of magnitude based on total dose and median time-to-death. Dybing (1986) using TD₅₀ values (the daily dose rate in mg/kg which would halve the actuarially adjusted percentage of tumour-free animals at the end of a standard experiment) reported that NDEA was more potent than NDMA by up to a factor of 10 depending on species/sex/strain of animal and the site of tumour examined. Shepherd *et al* (1987) using an oncogenic potency index suggest NDEA is more potent than NDMA which in turn is more potent than NPYR. Rowland (1988) referring to a semiquantitative method of assessment suggests NDELA is much less potent than NDEA and NDMA. The EPA (1988) using Unit Risk Factors have calculated that NDMA and NDEA have very similar potency with NPYR being less potent by a factor of about 100.

8.1 Pair by Pair Comparison

In an attempt to understand the relative potency of the six nitrosamines of interest, different ways of displaying the data have been considered. For instance, the nitrosamines can be ranked by using the qualitative pair-by-pair comparison technique (CONCAWE, 1985). In this approach, the

data on each nitrosamine are compared with that on every other nitrosamine on a pair-by-pair basis. Thus, each of every possible pair of nitrosamines is examined in turn. In each case, the nitrosamine considered to be more active is given a score of 1 and the less active a score of zero. Where it is impossible to distinguish between two nitrosamines, each is given a score of 1/2. When all the pair comparisons are completed, the scores are added together to give the total score for each nitrosamine. A relative ranking is thus achieved based on the size of the score.

Most of the available data are in the rat using drinking water as the vehicle. Accordingly, it was considered that the most appropriate basis for comparison would be to concentrate on one species (rat) and the one route of dosing (drinking water). An inspection of the dataset in Table 10 shows that most nitrosamines were associated principally with liver tumours but NNN was associated only with nasal and oesophageal tumours. NDEA, on the other hand, was associated with both oesophageal and liver tumours. If the type of tumour is ignored, it is possible to rank the nitrosamines in a pair-by-pair comparison by using any one of the parameters, total dose, tumour incidence or time to death by tumour as the yardstick for comparison. (See Table 15 for relevant data). The result from such a pair-by-pair comparison on the six nitrosamines is given in Table 16. It can be seen that by using this simple technique the relative ranking is:

NDEA = NDMA > NMOR > NPYR > NNN > NDELA

8.2 TD₅₀ Calculations

An inspection of the literature summarised in Table 10 shows that some studies have sufficient data to allow a more rigorous comparison of potencies to be made. Appropriate data exist on NDEA, NDMA, NMOR, NPYR and NDELA to allow dose response curves to be modelled and TD₅₀ values to be calculated. Such appropriate data do not exist for NNN and, hence, NNN has been removed from this exercise.

As with the pair-by-pair comparison technique, it is possible to remove some confusing factors by concentrating on drinking water studies in the

rat. A further factor can be removed by concentrating on liver tumours only. NDEA is the only one of the nitrosamines being considered that is associated principally with oesophageal tumours as well as with liver tumours.

The bioassays in the literature are still subject to variability between laboratories, between sexes and strains of rats and between duration and size of dosing. In an attempt to standardise the combination of dose and duration of dose, the simple product "mg/kg/day x weeks" has been used in this exercise. This product is, of course, proportional to the total dose.

Taking the references given in Table 17 and applying a logit model to the relationship between the proportion of rats with liver tumours and the total dose (expressed as the product "mg/kg/day x weeks"), the curves displayed in Figure 1 are obtained. The data from different studies were aggregated for each nitrosamine. The apparent differences in slopes can be explained by the quality of data. The slope for NDMA is based on only two points (the data from the Lijinski and Reuber (1981a) study were considered inconsistent with the more recent data). The slope of the NPYR curve is heavily influenced by one data point arising from the Lijinski and Reuber (1981b) paper. The slopes for NMOR and NDELA, appear well defined over a wide range of doses. Consequently, if it is assumed the slopes of the other nitrosamines would approach those for NMOR and NDELA if more data were available, all curves can be constrained to have the same slope. The result of such a treatment is given in Figure 2.

As mentioned earlier, TD_{50} values can be used to demonstrate the relative potencies of substances (Dybing 1986). The TD_{50} values that can be calculated from Figure 2 are as follows:

TD₅₀ VALUES DERIVED FROM LOGIT MODELS

<u>Nitrosamines</u>	<u>TD₅₀ (mg/kg/day x weeks)</u>
NDEA	34
NDMA	14
NMOR	43
NPYR	202
NDELA	974

These TD₅₀ values suggest that NDMA is the most potent liver carcinogen with NDELA being the least potent within the group.

However, the literature suggests that NDEA is more potent than NDMA (Dybing, 1986; Shepherd et al, 1987). The TD₅₀ value for NDMA in this exercise is probably unreliable because it is based on only two doses in one small study. The principle reason for the difference is almost certainly due to the inclusion of oesophageal tumours from NDEA in the treatments by Dybing (1986) and Shepherd et al (1987).

Peto et al (1984) in their large and very well conducted study considered the effects of NDEA and NDMA in drinking water of rats over a wide range of dose levels (15 steps from 0.033 ppm to 16.986 ppm plus controls). Animals were dosed for their full lifespan rather than the more normal 2 years. Consequently, at the lower doses, some animals were still being dosed after well over 3 years. The large number of animals involved (4080) and the long duration of the study allows the effects at very low doses to be estimated. As before, the authors found that both NDEA and NDMA were associated with liver tumours but only NDEA was associated significantly with oesophageal tumours. Oesophageal tumours were, less relevant at the low dose levels. In a related but smaller study, Peto et al (1984) also studied the effects of NPYR in the drinking water of rats. Ignoring tumour type they were able to display a relative potency based on TD₅₀ values (mg/kg/day) of NDEA > NDMA > NPYR. If their TD₅₀ values are recalculated in terms of mg/kg/day x weeks (assuming a median dosing period of 130 weeks) the following values are obtained:

	<u>TD₅₀ (mg/kg/day)</u>	<u>TD₅₀ (mg/kg/day x weeks)</u>
NDEA	0.06	8
NDMA	0.12	16
NPYR	0.5	65

It can be concluded, therefore, that NDEA is more potent than NDMA when all tumour types are considered.

Berger et al (1987) in their recent well conducted study have further demonstrated that NDEA is more potent than NPYR which in turn is more potent than NDELA.

Consequently, if we take all the observations in the literature, the pair-by-pair comparison, the calculated TD₅₀ values in this report and in the work by Peto et al (1984), it can be concluded that:

- (i) NDEA is the most potent nitrosamine within the group and,
- (ii) NDELA is less potent than NDEA by at least one and probably two orders of magnitude.

9. ESTIMATED DOSE OF NDELA FROM PERSONAL CARE PRODUCTS

9.1 Levels of NDELA in Personal Care Products

Subsequent to NDELA being identified as a contaminant in personal care products (Fan *et al*, 1977b), samples of various preparations have been analysed throughout the Western World using a variety of analytical procedures. A summary of the main findings are given in Table 6 where it can be seen that while analytical techniques have been improved the absolute levels of NDELA in personal care products and toiletries have decreased. As seen from Section 2.6, many products now contain no detectable levels of NDELA whilst others contain relatively small amounts. For the purpose of this risk assessment it is assumed, as a conservative approach, that all personal care products contain 10 µg/kg of NDELA.

9.2 Inhibitors of Nitrosamine Formation

There are two main types of agents which encourage the inhibition of N-nitrosamines formation in personal care products, these being sequestering and reducing agents.

The most effective agents are ascorbic acid, bisulphite, α -tocopherol, butylated hydroxytoluene (BHT) and ascorbic-palmitate.

As referred to in Section 9.3, it could be predicted that the addition of antioxidants and other scavengers in personal care products and toiletries may reduce the likelihood of N-nitrosamine formation. For personal care products which are frequently emulsions, lipids readily extract the nitrosating species from water (Douglass *et al*, 1978). Under such conditions nitrosation reactions are very fast. Since amines are also more soluble in the oil phase of emulsions it is prudent to add active inhibitors in this phase such as alpha-tocopherol, ascorbate or palmitate to minimise N-nitrosamine formation.

Dunnet and Telling (1984) examined the effectiveness of 0.05% BHT, α -tocopherol or ascorbate in shampoos and skin creams. They found that

α -tocopherol was the preferred inhibitor as ascorbate and BHT can both produce unacceptable darkening of the personal care products due to oxidation. However, Kabacoff et al (1983) have suggested that sodium bisulphite and ascorbyl palmitate were effective in inhibiting NDELA formation but the choice of inhibitor should be on the basis of their reactivity with nitrite and their oil or water solubility characteristics.

9.3 In situ Formation of N-Nitrosamines

In addition to possible exposure to pre-formed N-nitrosamines and endogenously formed N-nitrosamines, there has been speculation that in situ nitrosation could occur whereby additional nitrosamine formation could take place on the skin under certain environmental conditions. Studies into mechanisms of formation of N-nitrosamines in situ by Kamp et al (1989) and Powell (1987) showed that this was dependent on time, pH, nitrite or NO_x levels and type of amine present. Very large quantities of NDELA were produced at high pH of 10 - 11 and high levels of NO_x (5 ppm upwards) in the presence of DEA. Smaller quantities were also produced at pH values of 6.8 with high NO_x levels (5 ppm). The NO_x values found in urban air range from 0.002 - 0.5 ppm and those found in a smoke-filled room can be of the order of 1.0 ppm. Powell (1987) demonstrated in situ nitrosation can occur, although the NO_2 level needs to be very high at 600 ppb, compared with < 2 ppb present in normal clean air (Pool et al, 1988). Eisenbrand (1988) reported ambient levels of NO and NO_x of 2 and 12 ppb respectively. Even under the above extreme conditions, less than 3 ng/cm² NDELA was produced after a 24 hour exposure at high pH. Thus, whilst the theoretical probability of in situ nitrosation has been demonstrated, the likelihood of this phenomenon occurring, under normal usage and under normal environmental conditions, is exceedingly remote.

Furthermore, it has been demonstrated that the inclusion of antioxidants in personal care product formulations can reduce nitrosamine formation to below the limits of analytical detection. Since such agents prevent nitrosamine formation in personal care products it is likely they could also inhibit nitrosation in situ. This aspect of in situ nitrosation has not yet been fully evaluated.

9.4 Amount of Personal Care Products Used

It is difficult to obtain an accurate estimation of typical usage of personal care products and toiletries. The European trade association COLIPA conducted a survey in 1981 and the average amounts used per application are given in Table 18. It can be estimated that approximately 30 g of personal care products could be used daily. The figures refer to the amount of substance applied. Frequently products will be removed or discarded and therefore the quantities remaining on or in the skin (as a result of adsorption and metabolism) will be much lower than that initially applied. However, if the following conservative assumptions are made:

1. all of the 30 g of personal care products that are applied remain on the skin and are available for absorption,
2. all of these products contain as much as 10 $\mu\text{g}/\text{kg}$ of NDELA (even though all products are known not to contain NDELA),
3. based on the pig and human skin data (Bronaugh et al, 1981; Marzulli et al, 1981) that 5% of the NDELA is absorbed, then the internal dose would be 0.015 $\mu\text{g}/\text{day}$ (Section 3).

10. ESTIMATED TOTAL HUMAN DOSE

10.1 Uptake through the Diet

Average daily intakes of N-nitrosamines via the diet can be calculated using mean values of daily food consumption in combination with the N-nitrosamine content of various foodstuffs (Section 2.1). Such calculations have already been performed for different countries. Taking into account different eating habits, the estimated overall uptake of N-nitrosamines through the diet ranged from 0.3 µg/person/day for Sweden up to 1.8 µg/person/day for Japan (ACS, 1984). A compilation of all available estimates is given in Table 19. The different values for West Germany given in Table 19 are based on the considerable reduction of the N-nitrosamine content of beer between 1978 and 1981 (see also Section 2.1.6). The main N-nitrosamine contaminants of food were found to be NDMA and NPYR, and to a lesser extent NPIP, NDEA and NMOR. Based on the available results, NDELA was not detected in any of the foodstuffs analysed. Uptake of N-nitrosamines through drinking water is considered to be negligible (Section 2.2).

10.2 Uptake through Tobacco

The total exposure from tobacco use, expressed in µg/person/day, can be summarised as follows:

	Total NOC	NDELA
cigarette smoking	4 - 86	
snuff (nasal)	40	0.04
snuff (oral)	45 - 405	0.3

The figures for cigarette smoking were calculated on the basis of an average use of 20 cigarettes/day (Spiegelhalder, 1983; Bartsch and Montesano, 1984), nasal snuff consumption of 2 g/day and oral snuff use of 4.5 g/day of English snuff (total exposure: 310 µg), 14.3 g/day of Swedish snuff (total 152 µg), and 10 g/day of Indian zarda (total 405 µg) (Tricker and Preussmann, 1989b). These figures demonstrate that N-nitrosamine

uptake from snuff exceeds by far N-nitrosamine exposure from any other consumer product.

The calculations probably underestimate the actual exposure, as endogenous N-nitrosamine formation - especially of NNN - has not been considered (Tricker and Preussmann, 1989b).

10.3 Total Uptake

Using the above data and reference sources such as Bartsch and Montesano (1984) it is possible to estimate the dose to man of total nitrosamines and of NDELA from various exposure groups. These values cannot be precisely defined as the contribution from diet and from endogenous sources varies with individual lifestyle. However, for general assessment the ranges given below can provide a helpful guide.

Source	Total nitrosamine (excluding NDELA) in µg/person/day	NDELA
Diet	0.3 - 1.8	0
Tobacco (smoking)	4 - 40*	0
Tobacco (other uses)	4 - 400	10 - 30
Endogenous	0.3 - 20	0
Personal Care Products	0	0.015
(Workplace)	<5 - 180	<5 - 50

* For some brands up to 86

As a relatively small proportion of the population would be exposed to high levels of nitrosamine as a result of their workplace environment or indeed through use of snuff, these values will not be included for comparative estimates of dose, neither will the (considerable) contribution from cigarette smoking. Taking nitrosamine doses arising from diet or endogenous sources and comparing these with estimates of the possible dose

through use of personal care products, the contribution from these three sources covers the following ranges.

	Most favourable		Least favourable		Realistic	
	µg/day	(% of total)	µg/day	(% of total)	µg/day	(% of total)
Diet	0.6	65.6	1.6	6.5	1.0	24.9
Endogenous	0.3	32.8	20	93.4	3	74.7
Cosmetics	0.015	1.64	0.015	0.07	0.015	0.37
Total	0.915		21.415		4.015	

It can be seen that, from these three sources alone, the contribution from personal care products to the total nitrosamine body burden is generally less than 1%.

11. RISK ASSESSMENT BASED ON NDELA

The preceeding chapters of this report have demonstrated that all the nitrosamines of interest are potent genotoxic carcinogens. There are differences, in the manner and degree that they are metabolised. For instance, NDELA is metabolised to a much lower extent than other nitrosamines in the rat with most being excreted unchanged. NDELA also undergoes oxidation at the β -carbon rather than oxidation at the α -carbon in producing its prime metabolites. N-nitrosamines have also been shown to demonstrate different effects in different species both in terms of the target organs involved and the degree of effect displayed. Tumours have been induced in mammals, fish, birds, reptiles and amphibians indicating that N-nitrosamines have an unusually broad spectrum of target species (Rowland 1988). Within the rodents, there is evidence to show that rats are more susceptible than mice or hamsters (Lijinsky 1987).

It would seem prudent, therefore, in any risk assessment, to assume that N-nitrosamines are liable to be carcinogenic in man and that man is as susceptible as the most susceptible animal species (in this case, the rat). The data displayed in Section 10 show that the most realistic estimate of total nitrosamine in the non-smoking, non-occupationally exposed population is 4 $\mu\text{g}/\text{person}/\text{day}$ or about 0.06 $\mu\text{g}/\text{kg}/\text{day}$. As the major portion of this burden arises from varied endogenous sources, it is impossible to give a full breakdown of the contribution of each nitrosamine to the total. It is believed, that NDELA from personal care products, even with conservative estimates of usage and duration on the skin before rinsing, contributes less than 1% of the total (0.015 $\mu\text{g}/\text{day}$ or about 0.0002 $\mu\text{g}/\text{kg}/\text{day}$).

The most appropriate animal study for estimating the risk from very low levels of exposure to nitrosamines is that by Peto et al (1984).

The very large number of animals involved allowed Peto to calculate effects from very low levels of NDEA in drinking water. It was demonstrated that at such very low doses oesophageal tumours were much less important than liver tumours and could be ignored. Peto applied a "double Weibull" statistical model to the data as this was considered the most appropriate method for

dealing with large amounts of data. The approach is also believed to better represent the rates at which cellular processes of carcinogenesis operate. Such models assume that the cumulative tumour incidence is proportional to the duration of dosing in years raised to the power of 7. Under such circumstances, the liver tumour risk (in the absence of other causes of death) in a two year experiment starting with rats at 6 weeks of age was found to be about 0.8×10^{-3} per $\mu\text{g/kg/day}$. Assuming a straight line relationship at very low doses, the risk from exposure to $0.0002 \mu\text{g/kg/day}$ of NDEA would be 0.16×10^{-6} .

If it is assumed that:

- i) the data on NDELA would follow a similar dose-response relationship to that for NDEA and
- ii) NDELA is less potent than NDEA by at least one and possibly two orders of magnitude,

then the liver tumour risk from exposure to $0.0002 \mu\text{g/kg/day}$ of NDELA from personal care products becomes 0.16×10^{-7} to 0.16×10^{-8} .

Peto et al (1984) also demonstrated an increased risk among animals allowed to live out their normal lifespan. (Some of these animals died before completing two years of treatment while others survived substantially longer and therefore suffered higher tumour-onset rates). Under such circumstances, the absolute risk from the treatment was about 7 times higher than the liver tumour risk from a two year exposure. Consequently, if the very conservative assumption is made to use lifelong exposure risks rather than risks resulting from the usual two year exposure models, then the risk from exposure to $0.0002 \mu\text{g/kg/day}$ of NDELA becomes 1.1×10^{-7} to 1.1×10^{-8} . The US-EPA have also undertaken a formal risk assessment on NDELA data directly. Using drinking water carcinogenicity studies in the rat, they have determined that the excess cancer risk to man following daily exposure to $1 \mu\text{g/kg}$ for a lifetime would be 1×10^{-4} (EPA, 1988). Thus, assuming a straight line relationship at very low doses, the risk from exposure to $0.0002 \mu\text{g/kg/day}$ would be in the order of 0.2×10^{-7} . This value agrees well with the risk level calculated in this report by extrapolation from the NDEA data.

It must be stressed that these values are based on very conservative assumptions and clearly reflect "worst-case"-situations. Nevertheless, these risks are much smaller than the lifetime risk of 10^{-5} chosen by the Californian Health and Welfare Agency as the definition of no significant risk (California Department of Health Services, 1985).

Given assumptions made above and taking due cognizance of the contributions of modulating factors (Section 6.5), it is considered that the carcinogenic risk from exposure to NDELA in personal care products is not significant and poses no threat to human health.

BIBLIOGRAPHY

- ACS (1984). N-nitroso carcinogens in the environment. Monograph No. 182, American Chemical Society.
- AIDJANOV, M.M., SHARMANOV, T.S. (1982). Nitrosamines and their precursors in some Kazakh foodstuffs. In: N-nitroso compounds: Occurrence and biological effects, IARC Sci. Publ. 41, 267-276.
- AIROLDI, L., MACRI, A., BONFANTI, M., BONATI, M., FANELLI, R. (1984a). Kinetics and bioavailability of N-nitrosodiethanolamine after intravenous and cutaneous administration to rats. *Fd. Chem. Toxicol.* **22**, 133-138.
- AIROLDI, L., BONFANTI, M., FANELLI, R., BOVE, B., BEFENATI, E., GARIBOLDI, P. (1984b). Identification of a nitrosamino aldehyde and a nitrosamino acid resulting from beta-oxidation of N-nitrosodiethanolamine. *Chem. Biol. Interactions* **51**, 103-113.
- AL-DABBAGH, S., FORMAN, D. and BRYSON, D. (1986). Mortality of nitrate fertilizer workers. *Br. J. Ind. Med.* **43**, 507-515.
- ALTHOFF, J., WILSON, R., CARDESA, A. and POUR, P. (1974). Comparative Studies of neoplastic response to a single dose of nitroso compounds 3. The effect of N-nitrosopiperidine and N-nitrosomorpholine in Syrian golden hamsters. *Z. Krebsforsch.* **81**, 251-259.
- ALTHOFF, J., GRANDJEAN, C., RUSSELL, L. and POUR, P. (1977). Vinylethyl nitrosamine: A potent respiratory carcinogen in Syrian hamsters. *J. Natl. Cancer Inst.* **58**, 439-441.
- ALTHOFF, J., FEHST, H.-J. and MOHR, U. (1985). The influence of metabolic liver defects on diethylnitrosamine (NDEA)-carcinogenesis in Gunn rats. *Exp. Path.* **27**, 171-178.
- AMADORI, D., RAVAIOLI, A., GORDINI, A., LIVERANI, M., ZOLI, W., TONELLI, B., RIDOLFE, R. and GENTILINI, P. (1980). *Tumori* **66**, 145-152.
- AMES, B.N., McCANN, J., YAMASAKI, E. (1975). Methods for detecting carcinogens and mutagens with Salmonella/mammalian microsome mutagenicity test. *Mutation Res.* **31**, 347-363.
- ARCOS, J.C., BRYANT, G.M., PASTOR, K.M., and ARGUS, M.F. (1976). Structural limits of specificity of methylcholanthrene-repressible nitrosamine N-dealkylases. Inhibition of analog substrates. *Z. Krebsforsch.* **86**, 171-183.
- ARMIJO, R. and COULSON, A.H. (1975). Epidemiology of stomach cancer in Chile. The role of nitrogen fertilizers. *Int. J. Epidemiol.* **4**, 301-309.
- ARMIJO, R., GONZALES, A., ORELLANA, M., COULSON, A.H., SAYRE, J.W. and DETELS, R. (1981). Epidemiology of gastric cancer in Chile: II. Nitrate exposures and stomach cancer frequency. *Int. J. Epidemiol.* **10**, 57-62.
- BANNASCH, P. and MUELLER, H.A. (1964). Lichtmikroskopische Untersuchungen xber die Wirkung von N-Nitrosomorpholin auf die Leber von Ratte und Maus. *Arzneimittel-Forsch.* **14**, 805-814.
- BARNES, J. M. and MAGEE, P. M. (1954). Some toxic properties of dimethylnitrosamine. *Brit. J. Ind. Med.* **11**, 167-174.
- BARTSCH, H., CAMUS, A., MALVEILLE, C. (1976). Comparative mutagenicity of N-nitrosamines in a semi-solid and in a liquid incubation system in the presence of rat or human tissue fractions. *Mutation Res.* **37**, 149-162.
- BARTSCH, H., MONTESANO, R. (1984). Relevance of nitrosamines to human cancer. *Carcinogenesis* **5**, 1381-1393.
- BARTSCH, H. (1989). Nitrosamines and human cancer - where do we stand. IARC 10th International meeting on N-nitroso compounds, mycotoxins and tobacco smoke. Relevance to human cancer. Lyon, 25-28. Sept. 1989.
- BERESFORD, S.A. (1981). *Int. J. Epidemiol.* **10** 103-115.
- BERGER, M.R., SCHMÄHL, D. and ZERBAN, H. (1987). Combination experiments with very low doses of 3 genotoxic N-nitrosamines with similar organotropic carcinogenicity in rats. *Carcinogenesis* **8**, 1635-1643.
- BERGER, M.R., SCHMÄHL, D. and EDLER, L. (1989). On the relation between dose and risk reduction: sublethal evaluation of a rat combination experiment with 3 hepatocarcinogenic N-nitrosamines. IARC 10th International meeting on N-nitroso compounds, mycotoxins and tobacco smoke. Relevance to human cancer. Lyon, 25-28. Sept. 1989.
- BHIDE, S. V., PADMA, P. R., and AMONKAR, A. J. (1989). Antimutagenic and anticarcinogenic effects of betel leaf extract against tobacco specific nitrosamines. IARC 10th International meeting on N-nitroso compounds, mycotoxins and tobacco smoke. Relevance to human cancer. Lyon, 25-28. Sept. 1989.

- BOGOVSKI, P., ROOMA, M., UIBU, J., KAHN, J., TAUTS, O. (1982). Research on environmental N-nitroso compounds in the USSR. IARC Sci. Publ. 41, 259-266.
- BONFANTI, M., CASTELLI, M.G., FANELLI, R., AIROLDI, L. (1985). Enterohepatic recycling of N-nitrosodiethanolamine in rats. *Fd. Chem. Toxicol.* 23, 1011-1013.
- BONFANTI, M., MAGAGNOLI, C., FANELLI, R., AIROLDI, L. (1986). Comparative in vitro and in vivo β -oxidation of N-nitrosodiethanolamine in different animal species. *Chem. Biol. Interact.* 59, 203-210.
- BOUTWELL, R.K. (1974). The function and mechanism of promoters of carcinogenesis. *CRC Crit. Rev. Toxicol.* 2, 419-443.
- BRAMBILLA, G., CARLO, P., FINOLLO, R., SCIABA L. (1987). Dose-response curves for liver DNA fragmentation induced in rats by sixteen N-nitroso compounds as measured by visometric and alkaline elution analyses. *Cancer Research* 47 (13), 3485-3491.
- BRETSCHNEIDER, K. and MATZ, J. (1974). Nitrosamine in the atmospheric air and in the air at places of employment. *Arch. Geschwulstforsch.* 43, 36-41.
- BRITTEBO, E.B., LÖFBERG, B., and TJÄLVE, H. (1981a). Sites of metabolism of N-nitrosodiethylamine in mice. *Chemico-Biol. Interactions* 34, 209-221.
- BRITTEBO, E.B., LÖFBERG, B., and TJÄLVE, H. (1981b). Extrahepatic sites of metabolism of N-nitrosopyrrolidine in mice and rats. *Xenobiotica*, 11, 619-625.
- BRONAUGH, R.L., CONGDON, E.R., SCHEUPLEIN, R.J. (1981). The effect of personal care product vehicles on the penetration of N-nitrosodiethanolamine through excised human skin. *J. Invest. Dermatol.* 76, 94-96.
- BROOKES, A.L. and CREGGER, V. (1973). Production of chromosome type aberrations in the liver cells of the Chinese hamster by dimethylnitrosamine (DMN). *Mutation Res.* 21, 214.
- BROWERS, J.A.J., and EMELOT, P. (1960). Microsomal N-demethylation and the effect of the hepatic carcinogen dimethylnitrosamine on amino acid incorporation into the proteins of rat livers and hepatomas. *Exp. Cell Res.* 19, 467-474.
- BRUNNEMANN, K.D. and HOFFMANN, D. (1978). Chemical studies on tobacco smoke. LIX. Analysis of volatile nitrosamines in tobacco smoke and polluted indoor environments. in: IARC Sci. Publ. 19, 343-356.
- BRUNNEMANN, K.D., HECHT, S.S., HOFFMANN, D. (1983). N-Nitrosamines: environmental occurrence in vivo formation and metabolism. *J. Clin. Toxicol.* 19, 661-688.
- BRUNNEMANN, K.D., RIVENSON A., ADAMS, J.D., HECHT, S.S. and HOFFMAN, D. (1987). A study of snuff carcinogenesis. IARC Sci. Publ. 84, 456-459.
- BUDEVSKA, B., RIZOV, N., GEORGIEV, G. (1988). Content of volatile nitrosamines in some Bulgarian foods. *Khig. Zdravopaz.* 31, 52-57.
- CALIFORNIA DEPARTMENT OF HEALTH SERVICES (1985). Guidelines for chemical carcinogen risk assessments and their scientific rationale. California Department of Health Services, Health and Welfare Agency, Sacramento, CA, USA.
- CARDESA, A., POUR, P., ALTHOFF, J. and MOHR, U. (1974). Comparative studies of neoplastic response to a single dose of nitroso compounds. IV. The effect of dimethyl and diethyl nitrosamine in Swiss mice. *Z.Krebsforsch.* 81, 229-233.
- CARLTON, W. W. and WELSER, J.R. (1968). Glomerular lesions induced in peking ducks by dietary administration of dimethylnitrosamine. *Toxicol. Appl. Pharmacol.* 13, 404-411.
- CARTER, R.L. PERCIVAL, W.H. and ROE, F.J.C. (1969). Exceptional sensitivity of mink to the hepatotoxic effects of dimethylnitrosamine. *J. Path.* 97, 79-88.
- CATER, K.C., GANDOLFI, A.J., SIPES, I.G. (1985). Characterization of dimethylnitrosamine-induced focal and nodular lesions in the livers of newborn mice. *Toxicol. Pathol.* 13, 3-9.
- CHUNG, F.-L. and HECHT, S.S. (1985). Formation of the cyclic 1,N²-glyoxal desoxyguanosine adduct upon reaction of N-nitroso-2-hydroxymorpholine with desoxyguanosine. *Carcinogenesis* 6, 1671-1673.
- CHUONG, B.T. and BENARIE, M. (1976). Recherche d'une methode pour le dosage de la N-nitrosamine dans l'atmosphere. *The Science of the Environment*, 6, 181-193.
- CLAPP, M.K. and TOYA, R.E. (1970). Effects of cumulated dose and dose rate on dimethylnitrosamine oncogenesis in RF mice. *J. Nat. Cancer Inst.* 45, 495-498.
- CLAPP M.K., CRAIG, A.W. and TOYA, R.E. (1970). Diethylnitrosamine on cogenesis in RF mice as influenced by variations in cumulative dose. *Int. J. Cancer* 5, 119-123.
- COLIPA (1986). NDELA content of cosmetics in 1979. Survey Document 86/244.

- CONCAWE (1985). Investigations into the relationship between the chemical composition of mineral oils and their ability to cause skin cancer in mice. Report No. 4/85 Den Haag.
- CORREA, P., HAENSZEL, W. and CUELLO, C. (1975). A model for gastric cancer epidemiology. *Lancet* 2, 58-60.
- CRADDOCK, V.W. and MAGEE, P.W. (1965). Methylation of liver DNA in the intact animal by the carcinogen dimethylnitrosamine during carcinogenesis. *Biochem. Biophys. Acta*, 95, 677-678.
- CTFA (1984). Progress report on the analysis of cosmetic products for nitrosamines, Sept. 28, 1984.
- CUELLO, C., CORREA, P., HAENSZEL, W., GORDELLO, G., BROWN, C., ARCHER, M. and TANNENBAUM, S. (1976). Gastric cancer in Columbia. I. Cancer risk and suspect environmental agents. *J. Natl. Cancer Inst.* 57, 1015-1020.
- DAHL, A.R. (1985). Mutagenicity of some dialkylnitrosamines, cyclic nitrosamines and N-nitrosodiethanolamine in *Salmonella typhimurium* with rat and rabbit nasal, lung and liver S9 homogenates. *Mutation Res.* 158 (3), 141-147.
- DANISH STATE FOOD INSTITUTE (1981). Nitrat og Nitrit i Kodvarer. Rapport fra in Intern Arbejdsgræppe. Søborg, Denmark.
- DAVIES, J.M. (1980). Stomach Cancer mortality in Worksop and other Nottinghamshire mining towns. *Br.J. Cancer*, 41, 438-445.
- DECOUFLE, P. (1976). Cancer mortality among workers exposed to cutting oil mist. *Ann. N.Y. Acad. Sci.* 271 94-101.
- DECOUFLE, P. (1978). Further analysis of cancer mortality patterns among workers exposed to cutting oil mist. *J. Nat. Cancer Inst.* 61, 1025-1030.
- De GERLACHE, J., LANS, M., PREAT, V., TAPER, H., ROBERFROID, M. (1984). Comparison of different models of rat liver carcinogenesis: Conclusions from a systematic analysis. *Toxicol. Pathol.* 12, 374-382.
- DENKEL, E., POOL, B.R., SCHLEHOFER, J.R., EISENBRAND, G. (1986). Biological activity of N-nitrosodiethanolamine and of potential metabolites which may arise after activation by alcohol dehydrogenase in *Salmonella typhimurium*, in mammalian cells, and *in vivo*. *J. Cancer Res. Clin. Oncol.* 111, 149-153.
- DENKEL, E., STERZEL, W., and EISENBRAND, G. (1987). *In vivo* activation of NDELA and other N-nitroso-2-hydroxyalkylamines by alcohol dehydrogenase and sulfotransferase, *IARC Sci. Publ.* 84, 83-86.
- DITTBERNER, U., EISENBRAND, G., ZANKL, H. (1988). Cytogenetic effects of N-nitrosodiethanolamine (NDELA) and NDELA-monoacetate in human lymphocytes. *J. Cancer Res. Clin. Oncology*, 114 (6), 575-578.
- DIPALO, J.A., NELSON, R.L., DONOVAN, P.J. (1972). *In vitro* transformation of Syrian hamster embryo cells by diverse chemical carcinogens. *Nature*, 235, 278-280.
- DOUGLASS, M.L., KABAKOFF, G.L., ANDERSON, G.A. and CHENG, M.C. (1978). The chemistry of nitrosamine formation, inhibition and destruction. *J. Soc. Cosmet. Chem.* 29, 581-606.
- DRUCKREY, H. and PREUSSMANN, R. (1962). Zur Entstehung karzinogener Nitrosamine am Beispiel des Tabakrauches, *Naturwissenschaften* 49, 498-499.
- DRUCKREY, H., SCHILDBACH, A., SCHMAEHL, D., PREUSSMANN, R. and IVANKOVIC, S. (1963). Quantitative Analyse der carcinogenen Wirkung von Diethylnitrosamin. *Arzneim.-Forsch.* 13, 841-851.
- DRUCKREY, H., PREUSSMANN, R., IVANKOVIC, S. and SCHMAEHL, D. (1967). Organotrope carcinogene Wirkungen bei 65 verschiedenen N-Nitrosoverbindungen an BD-Ratten. *Z. Krebsforsch.* 69, 103-201.
- DRUCKREY, H. (1973). Specific carcinogenic and teratogenic effects of 'indirect' alkylating methyl and ethyl compounds and their dependency on stages of oncogenic developments. *Xenobiotica* 3, 271-303.
- DRUCKREY, H. (1975). Chemical carcinogenesis of N-nitroso derivatives, in : Recent topics on chemical carcinogenesis. *GANN Monogr. on Cancer Res.* 17, 107-132.
- DUNNETT, P.C. and TELLING, G.M. (1984). Study on the fate of Bronopol and the effects of antioxidants on N-nitrosamine formation in shampoos and skin creams. *Int. J. Cosmet. Science* 6, 241-247.
- DYBING, E. (1986). Predictability of human carcinogenicity from animal studies. *Reg. Toxicol. and Pharmacol.* 6, 399-415
- ECETOC (1988). Nitrate and Drinking Water. Technical Report No 27.
- ECETOC (1989). DNA and protein adducts: evaluation of their use in exposure monitoring and risk assessment. Monograph No. 13.
- ECETOC (1990). Critical evaluation of methods for determination of N-nitrosamines in personal care products and household products. Technical Report No 42.

- EEC (1976). Council directive of 27 July 1976 on the approximation of the laws of the member states relating to cosmetic products (76/768/EEC).
- EDWARDS, G.S., PENG, M., FINE, D., SPIEGELHALDER, B. and KANN, J. (1979). Detection of N-nitrosodiethanolamine in human urine following application of a contaminated cosmetic. *Toxicol. Lett.* 4, 217-222.
- EISENBRAND, G., SPIEGELHALDER, B. and PREUSSMAN, R. (1980). Nitrate and nitrite in saliva. *Oncology*, 37, 227-231.
- EISENBRAND, G. (1988). Study of formation of nitrosamines and inhibition of N-nitrosation in cosmetics. Report to the EEC concerning Cosmetics Directive 76/768/EEC, 27.3.1988.
- ELLEN, G., SCHULLER, P.L. (1983). N-Nitrosamine investigations in the Netherlands: Highlights from the last ten years. In: *Das Nitrosamin-Problem*, Ed. R. Preussmann, Verlag Chemie, Weinheim, 81-92.
- ELY, T.S., PEDLEY, S.F., HEARNE, F.T. and STILLE, W.T. (1970). A study of mortality, symptoms and respiratory functions in humans occupationally exposed to oil mist. *J. Occ. Med.* 12 253-61.
- EMMELOT, P., SCHERER, E. (1980). The first relevant cell stage in rat liver carcinogenesis: A quantitative approach. *Biochem. Biophys. Acta* 605, 247-304.
- EPA (1986). Proliferative hepatocellular lesions of the rat. Review and future use in risk assessment. Risk Assessment Forum. US-EPA, February 1986.
- EPA (1988). Risk Assessment of MOELA, (03/01/88); attachment 88-PT-20.
- EPSTEIN, S.S., ARNOLD, E., ANDREA, J., BASS, W., BISHOP, Y. (1972). Detection of dominant lethal mutations in the mouse. *Toxicol. Appl. Pharmacol.* 23, 288-325.
- EVANS, C.H., DIPALO, J.A. (1975). Neoplastic transformation of guinea pig fetal cells in culture induced by chemical carcinogens. *Cancer Res.* 35, 1035-1044.
- FAHMY, D.G., FAHMY, M.S., and WIESSLER, M. (1975). 2-acetoxy-dimethylnitrosamine: a proximate metabolite of the carcinogenic amine. *Biochem Pharmacol.* 24, 1145-1148.
- FAHRIG, R. (1975). Development of host-mediated mutagenicity tests - yeast systems. II. Recovery of yeast cells out of testes, liver, lung and peritoneum of rats. *Mutation Res.* 31, 381-394.
- FAHRIG, R., DAWSON, G.W.R., RUSSELL, L.B. (1981). Mutagenicity of selected chemicals in the mammalian spot test. *Environ. Sci. Res.* 24, 709-727.
- FAHRIG, R. and REMMER, H. (1983). The organo-specific activity of six N-nitroso compounds in the host-mediated assay with yeasts and rats. *Teratogen. Carcinogen. Mutagen.* 3, 41-49.
- FAJEN, J.M., CARSON, J.A., ROUNBEHLER, D.P., FAN, T.Y., VITA, R., GOFF, U.E., WOLF, M.H., EDWARDS, G.S., FINE, D.H. (1979). N-nitrosamines in the rubber and tire industry. *Science* 205, 1262-1264.
- FAJEN, J.M., ROUNBEHLER, D.P., FINE, D.H. (1982). Summary report on N-nitrosamines in the factory environment. *IARC Sci. Publ.* 41, 223-229.
- FAN, T.Y., MORRISON, J., ROUNBEHLER, D.P., ROSS, R., FINE, D.H., MILES, W., SEN, N.P. (1977a). N-nitrosodiethanolamine in synthetic cutting fluids: a part-per-hundred impurity. *Science* 196, 70-71.
- FAN, T.Y., GOFF, U., SONG, L., FINE, D.H., ARSENAULT, G.P. and BIEMANN, K. (1977b). N-nitrosodiethanolamine in cosmetics, lotions and shampoos. *Fd. Cosmet. Toxicol.* 15, 423-430.
- FAN, T.Y., ROSS, R., FINE, D.H., KEITH, L.H., GARRISON, A.W. (1978). Isolation and identification of some thermal energy analyzer (TEA) responsive substances in drinking water. *Environ. Sci. Technol.* 12, 692-695.
- FARBER, E., CAMERON, R. (1980). The sequential analysis of cancer development. *Cancer Res.* 31, 125-226.
- FARRELLY, J.G., STEWART, M.L., LIJINSKY, W. (1984). The metabolism of nitrosodi-n-propylamine, nitrosodiallamine and nitrosodiethanolamine. *Carcinogenesis* 5, 1015-1019.
- FINE, D.H., ROUNBEHLER, D.P. (1976). N-nitroso compounds in water, in: *Identification and analysis of organic pollutants in water*. Ann Arbor Science, Ann Arbor, pp. 255-263.
- FINE, D.H., MORRISON, J., ROUNBEHLER, D.P., SILVERGLEID, A., SONG, L. (1977a). in: "Toxic Substances in the Air Environment", Air Pollution Control Association, Pittsburg, 168-181.
- FINE, D.H., ROSS, R., ROUNBEHLER, D.P., SILVERGLEID, A.S. and SONG, L. (1977b). *In vivo* formation of volatile n-nitrosamines in man following ingestion of cooked bacon and spinach. *Nature* 265, 753-754.
- FINE, D.H., ROUNBEHLER, D.P., ROUNBEHLER, A., SILVERGLEID, A., SAWICKI, E., KROST, K., DEMARRAIS, G.A. (1977c). Determination of dimethylnitrosamine in air, water, and soil by thermal energy analysis measurements in Baltimore, Md. *Environ. Sci. Technol.* 11 581-584.

- FINE, D.H., REISCH, J. and ROUNBEHLER, D.P. (1980). Nitrosamines in new automobiles. IARC Sci. Publ. 31, 541-554.
- FISCHER, S., SPIEGELHALDER, B. and PREUSSMANN, R. (1989a). Tobacco-specific nitrosamines in European cigarettes. Possibilities for exposure reduction. IARC 10th International meeting on N-nitroso compounds, mycotoxins and tobacco smoke. Relevance to human cancer. Lyon, 25.-28. Sept. 1989.
- FISCHER, S., SPIEGELHALDER, B. and PREUSSMANN, R. (1989b). Tobacco-specific nitrosamines in mainstream smoke of West German cigarettes - tar alone is not a sufficient index for the carcinogenic potential of cigarette smoke. Carcinogenesis 10, 169-173.
- FISCHER, S., SPIEGELHALDER, B. and PREUSSMANN, R. (1989c). Preformed tobacco-specific nitrosamines in tobacco - role of nitrate and influence of tobacco type. Carcinogenesis 10, 1511-1517.
- FOREMAN, D., AL-DABBAGH, S. and DOLL, R. (1985). Nitrates, nitrites and gastric cancer in Great Britain. Nature 313, 620-625.
- FOULDS, L. (1958). The natural history of cancer. J. Chronic Dis. 8, 2-37.
- FRASER, P., CHILVERS, C. (1981). Health aspects of nitrate in drinking water. Sci. Total Environ. 18, 103-116.
- FRASER, P., CHILVERS, C. and GOLDBLATT, P. (1982). Census-based mortality study of fertiliser manufacturers. Br. J. Ind. Med. 39, 323-329.
- FRASER, P. (1985). Nitrates: epidemiological evidence. IARC Sci. Publ. 65, 183-194.
- FOOD and DRUG ADMINISTRATION (1980). Dimethylnitrosamine in malt beverages; Availability of guide. Fed. Regist. 45, 39341-39342.
- FREUND, H. A. (1937). Clinical manifestations and studies in parenchymatous hepatitis. Ann. Int. Med. 10, 1144-1154.
- FROMMBERGER, R., ALLMANN, H. (1983). Ergebnisse der Lebensmittelüberwachung in der Bundesrepublik Deutschland. In: Das Nitrosamin-Problem, Ed. R. Preussmann, Verlag Chemie, Weinheim, pp. 57-63.
- GABRIDGE, M.G., LEGATOR, M.S. (1969). A host-mediated microbial assay for the detection of mutagenic compounds. Proc. Soc. Exp. Biol. (N.Y.), 130, 831-834.
- GILBERT, P., FABRY, L., ROLLMANN, B., LOMBART, P., RONDELET, J., PONCELET, F., LEONARD, A., MERCIER, M. (1981). Mutagenicity of N-nitrosodiethanolamine and its acetyl derivatives. Mutation Res. 89 (3), 217-228.
- GOFF, E.U., FINE, D.H. (1979). Analysis of volatile N-nitrosamines in alcoholic beverages. Food Cosmet. Toxicol. 17, 569-573.
- GORDON, R.J. (1979). Survey for airborne nitrosamines in two Californian countries. Proc. Ann. Meet. Air Pollut. Control Assoc. 1979, 72nd meeting, paper 79-95.6.
- GOUGH, T.A., McPHAIL, M.F., WEBB, K.S., WOOD, B.J., COLEMAN, R.F. (1977). An examination of some foodstuffs for the presence of volatile nitrosamines. J. Sci. Food Agric. 28, 345-351.
- GOUGH, T.A., WEBB, K.S., COLEMAN, R.F. (1978). Estimate of the volatile nitrosamine content of UK food. Nature 272, 161-163.
- GRAY, J.J. (1981). Formation of N-nitroso compounds in foods. In: N-Nitroso Compounds, Eds. R.A. Scanlan and S.R. Tannerbaum, ACS Symp. Series, pp. 165-180.
- GREENBLATT, M. and LIJINSKY, W. (1972). Failure to induce tumors in Swiss mice after concurrent administration of amino acids and sodium nitrite. J. Nat. Cancer Inst. 48, 1389-1392.
- HAAS, H., MOHR, U. and KRUGER, F.W. (1973). Comparative studies with different doses of N-nitrosomorpholine, N-nitrosopiperidine, N-nitrosomethylurea and dimethylnitrosamine in Syrian golden hamsters. J. Nat. Cancer Inst. 51, 1295-1301.
- HADJIOLOV, D. (1972). Hemangiothelial sarcomas of the liver in rats induced by diethylnitrosamine. Neoplasms 19, 111-114.
- HANST, P.L., SPENCE, J.W., MILLER, M. (1977). Atmospheric chemistry of N-nitrosodimethylamine. Environ. Sci. Technol. 11, 403-405.
- HARD, G.C. and BUTLER, W.H. (1970). Toxicity of dimethylnitrosamine for the rat testis. J. Path. 102, 201-207.
- HARRIS, C. (1985). Future directions in the use of DNA adducts as internal dosimeters for monitoring human exposure to environmental mutagens and teratogens. Environ. Health Perspectives 62, 195-91.
- HARTUNG and SPIEGELHALDER, B. (1982). On external and internal nitrosamine contamination caused by hand metal grinders. Arbeitsmed., Sozialmed., Präventivmed. 11, 273-275.

- HASHIMOTO, S., YOKOKURA, T., KAWAI, Y. and MUTAI, M. (1976). Dimethylnitrosamine formation in the gastrointestinal tract of rats. *Fd. Cosmet. Toxicol.* 14, 553-556.
- HAVERY, D.C., KLINE, D.A., MILETTA, E.M., JOE, F.L., FAZIO, T. (1976). Survey of food products for volatile N-nitrosamines. *J. Assoc. Off. Anal. Chem.* 59, 540-546.
- HAVERY, D.C., FACIO, T., HOWARD, J.W. (1978). Trends in levels of N-nitrosopyrrolidine in fried bacon. *J. Assoc. Off. Anal. Chem.* 61, 1379-1382.
- HAVERY, D.C., HOTCHKISS, J.H., FAZIO, T. (1981). Nitrosamines in malt and malt beverages. *J. Food Sci.* 46, 501-505.
- HEADING, C.E., PHILLIPS, J.C., LAKE, B.G., GANGOLLI, S.D., and LLOYD, A.G. (1974). Some aspects of the metabolism of dimethylnitrosamine in the rat. *Biochem. Soc. Trans.* 2, 607-610.
- HEATH, D.F. (1962). The decomposition and toxicity of dialkylnitrosamines in rats. *Biochem. J.* 85, 72-91.
- HECHT, S.S. and YOUNG, R. (1981). Metabolic alpha-hydroxylation of N-nitrosomorpholine and 3,3,5,5-tetradeutero-N-nitrosomorpholine in the F344 rat. *Cancer Res.* 41, 5039-5043.
- HECHT, S.S., YOUNG, R. and MAEURA, Y. (1983). Comparative carcinogenicity in F344 rats and Syrian golden hamsters of N-nitrosomorpholine and N-nitrosomorpholine-1-N-oxide. *Cancer Letters* 20, 333-340.
- HECHT, S.S. (1984). N-nitroso-2-hydroxymorpholine. A mutagenic metabolite of N-nitrosodiethanolamine. *Carcinogenesis*, 5 (12), 1745-1747.
- HECHT, S.S., CARMELLA, S.G., TRUSHIN, N., SPRATT, T.E., FOILES, P.G. and HOFFMANN, D. (1988). Approaches to the development of assays for interaction of tobacco-specific nitrosamines with haemoglobin and DNA. *IARC Sci. Publ.* 89, 121-128.
- HECHT, S.S. and HOFFMANN, D. (1989). N-Nitroso compounds and tobacco-induced cancers in man. in: *IARC 10th International Meeting on N-nitroso compounds, mycotoxins and tobacco smoke: Relevance to human cancer.* Lyon, 25-28. Sept. 1989.
- HECHT, S.S., LIJINSKY, W., KOVATCH, R.M., CHUNG, F.L. and SAAVEDRA, J.E. (1989). Comparative tumorigenicity of N-nitroso-2-hydroxymorpholine, N-nitrosodiethanolamine and N-nitrosomorpholine in A/J mice and F344 rats. *Carcinogenesis* 10, 1475-1477.
- HEMMINKI, K. (1983). Nucleic acid adducts of chemical carcinogens and mutagens. *Arch. Toxicol.* 52, 249-285.
- HENKE, H., HOEHNE, G., KUENKEL, H.A. (1964). ber die die mutagene Wirkung von Roentgenstrahlen, N-nitroso-N-methyl-urethan und N-nitroso-morpholin bei Drosophila melanogaster. *Biophysik* 1, 418-421.
- HERROLD, K.M. (1967). Histogenesis of malignant liver tumours induced by dimethylnitrosamine. An experimental study in Syrian hamsters. *J. Nat. Cancer Inst.* 39, 1099-1111.
- HESBERT, A., LEMONNIER, M. CAVALIER, C. (1979). Mutagenicity of N-nitrosodiethanolamine on *Salmonella typhimurium*. *Mutation Res.* 68, 207-210.
- HILFRICH, J., ALTHOFF, J. and MOHR, U. (1971). Untersuchungen zur Stimulation der Lungentumorrates durch Diethylnitrosamin bei O-20-Mäusen. *Z. Krebsforsch.* 75, 240-242.
- HILFRICH, J., SCHMELTZ, I. and HOFFMANN, D. (1977). Effects of N-nitrosodiethanolamine and 1,1-diethanolhydrazine in Syrian golden hamsters. *Cancer Lett.* 4, 55-60.
- HILL, M.J. (1988). Nitrosamines. Toxicology and microbiology. Chichester, UK.
- HILL, M.J., HAWKSWORTH, G. and TATTERSALL, G. (1973). Bacteria nitrosamines and cancer of the stomach. *Br. J. Cancer* 28, 562-567.
- HOFFMANN, D., RAINERI, R., HECHT, S.S., MARONPOT, R. and WINDER, E.L. (1975). A study of tobacco carcinogenesis. XIV. Effects of N-nitroso nicotine and N-nitroso anabasine in rats. *J. Nat. Cancer Inst.* 55, 977-981.
- HOFFMANN, D., HECHT, S.S., ORNAF, R.M., WINDER, E.L. and TSO, T.C. (1976). Chemical studies on tobacco smoke nitroso-nicotine: presence in tobacco formation and carcinogenicity. *IARC Sci. Publ.* 14, 307-320.
- HOFFMANN, D., ADAMS, J.D., PIADÉ, J.J. and HECHT, S.S. (1980). Chemical studies on tobacco smoke. LXVIII. Analysis of volatile and tobacco-specific nitrosamines in tobacco products. *IARC Sci. Publ.* 31, 507-516.
- HOFFMANN, D., ADAMS, J.D., BRUNNEMANN, K.D., HECHT, S.S. (1981). Formation, occurrence and carcinogenicity of N-nitrosamines in tobacco products. In: *N-nitroso compounds.* Scanlan, P.; Tannerbaum, S., eds: ACS Symposium Series No. 174, Washington, D.C., pp. 247-273.

- HOFFMANN, D., ADAMS, J.D., BRUNNEMANN, K.D., HECHT, S.S. (1982a). in: N-nitroso compounds. Scanlan, P., Tannenbaum, S., eds: ACS Symposium Series No. 174, ACS: Washington, D.C.
- HOFFMANN, D., BRUNNEMANN, K.D., RIVENSON, A., HECHT, S.S. (1982b). N-nitrosodiethanolamine: Analysis, formation in tobacco products and carcinogenicity in Syrian golden hamsters. IARC Sci. Publ. 41, 299-308.
- HOFFMANN, D., RIVENSON, A., ADAMS, J.D., JUCHATZ, A., VINCHKOSKI, M., HECHT, S.S. (1983). Effects of route of administration and dose on the carcinogenicity of N-nitrosodiethanolamine in the Syrian golden hamster. Cancer Res. 43, 2521-2524.
- HOFFMANN, D., ADAMS, J.D., LISK, D., FISENNE, I. and BRUNNEMANN, K.D. (1987). Toxic and carcinogenic agents in dry and moist snuff. JNCI 79, 1281.
- HOFFMANN, D., MELIKIAN, A.A., BRUNNEMANN, K.D. (1989). Studies in tobacco carcinogenesis. 10th Intl. Meeting on N-nitroso compounds, mycotoxins and tobacco smoke. Relevance to human cancer. IARC, Lyon, 25-28 Sept. 1989.
- HU, J., SONG, P. (1985). Determination of volatile nitrosamines in food. Zhonghua Yufangyixue Zazhi 19, 378-381.
- IARC (1978). Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Volume 17, Some N-nitroso compounds. Intl. Agency Res. on Cancer, Lyon, France.
- IARC (1982). N-nitroso compounds: Occurrence and biological effects. Scientific Publication 41.
- IARC (1985). Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Volume 37, Tobacco Habits Other than Smoking: Betel-Quid and Areca-Nut Chewing; and some related Nitrosamines, Intl. Natl. Agency Res. on Cancer, Lyon, France.
- IARC (1987). The relevance of N-nitroso compounds to human cancer: exposures and mechanisms. Scientific Publication 84.
- IKW (1986). Empfehlung zur Vermeidung von Nitrosaminen in kosmetischen Mitteln. Industrieverband Koerperpflege und Waschmittel e.V., Frankfurt a.M.
- IKW (1989). Empfehlung zur Vermeidung von Nitrosaminen in kosmetischen Mitteln. Industrieverband Koerperpflege und Waschmittel e.V., Frankfurt a.M.
- ITO, M., TSUDA, M., HASEGAWA, R., IMAIDA, K. (1982). Sequential observation of pathomorphologic alterations in preneoplastic lesions during the promoting stage of hepatocarcinogenesis and the development of short-term test system for hepatopromoters and hepatocarcinogens. Toxicol. Pathol. 10, 37-49.
- JACOBSON, K.H., WHEELWRIGHT, M.J., CLEM, J.H. and SHANNON, R.N. (1955). Studies on the toxicity of N-nitrosodimethylamine vapor. A.M.A. Arch. Ind. Med. 121, 617-622.
- JÄRVHOLM, B., LILLIENBERGH, L., SÄLLSTEN, G., THIRINGER, G., AXELSON, O. (1981). Cancer morbidity among men exposed to oil mist in the metal industry. J. Occ. Med. 23, 333-337.
- JÄRVHOLM, B., LAVENIUS, B. (1981). A cohort study on cancer among workers exposed to an anti-rust oil. Scand. J. Work. Environ. Health 7, 178-184.
- JÄRVHOLM, B., LAVENIUS, B. and SÄLLSTEN, G. (1986). Cancer morbidity in workers exposed to cutting fluids containing nitrite and amines. Brit. J. Ind. Med. 43, 563-565.
- JENSEN, O.M. (1982). Nitrate in drinking water and cancer in Northern Jutland, Denmark, with special reference to stomach cancer. Ecotoxicol. Environ. Saf. 6, 258-267.
- JOHANSSON, E.B., and TJÄLVE, H. (1978). The distribution of [¹⁴C] dimethylnitrosamine in mice. Autoradiographic studies in mice with inhibited and non-inhibited dimethylnitrosamine metabolism and a comparison with the distribution of [¹⁴C] formaldehyde. Toxicol. Appl. Pharmac. 45, 565-575.
- JONES, C.A., HUBERMAN, E. (1984). The relationship between the carcinogenicity and mutagenicity of Nitrosamines in a Hepatocyte-mediated Mutagenicity Assay. In: T.K. Rao, W. Lijinsky and J.L. Epler (eds), Genotoxicology of N-nitroso compounds, Plenum Press, New York, pp. 119-127.
- JUHAZ, L., HILL, M.J., NAGY, G. (1980). IARC Sci. Publ. 31, 619-623.
- JUSZKIEWICZ, T. and KOWALSKI, B. (1976). Dietary nitrosamines. Fd. Cosmet. Toxicol. 14, 653-654.
- KABACOFF, B.L., DOUGLASS, M.L., ROSENBERG, I.E., LEVAN, L.W., PUNWAR, J.K., VIELHUBER, S.F. and LECHNER, R.J. (1983). Formation of nitrosamines in non-ionic and ionic emulsions in the presence and absence of inhibitors. IARC Sci. Publ. 57, 347-351.
- KAMP, E., BLANKART, M. and EISENBRAND, G. (1989). Occurrence and formation of nitrosamines in cosmetics (in German). GIT Supplement 2/89 - Lebensmittel 47-51.

- KAMIYAMA, S., OHSHIMA, H., SHIMADA, A., SAITO, N., BOURGADA, M.-C., ZIEGLER, P. and BARTSCH, H. (1987). Urinary excretion of N-nitrosamino acids and Nitrate by inhabitants in high and low risk areas for stomach cancer in Northern Japan. IARC Sci. Publ. 84, 497-502.
- KARLOWSKI, K., BOJEWSKI, J. (1987). Investigations of Polish cheeses for the presence of nitrates, nitrites and volatile N-nitrosamines. Roczn. Panstw. Zakl. Hig. 38, 199-205.
- KARHYSHEVA, L.F., ZHUKOVA, G.F., SAFRONOVA, G.A., SOMINA, S.I., PETRAKOVA, A.N., KALININA, L.A. (1981). Investigation of contents and formation of volatile N-nitrosamines in sausages. In: Carcinogenic N-nitroso compounds and their precursors - Formation and analysis in the environment, Eds. S. Bogovski, G. Loogna and A. Itra, Abstr. 4th All-Union Symp., Tallin, Inst. Exp. Clin. Med., 9-10.
- KELLY, M.G., O'GARA, R.W., ADAMSON, R.H., GADEKER, K., BOTKIN, C.C., REESE, W.H. and KERBER, W.T. (1966). Induction of hepatic cell carcinomas in monkeys with N-nitrosodiethylamine. J. Nat. Cancer Inst. 36, 323-351.
- KERKLAAN, P., MOHN, G., BOUTER, S. (1981). Comparison of the mutagenic activity of dialkyl nitrosamines in animal-mediated and in vitro assays using an Escherichia coli indicator. Carcinogenesis, 2, 909-914.
- KETKAR, M.B., SCHNEIDER, P., PREUSSMANN, R. and MOHR, U. (1982). Carcinogenic effect of low doses of nitrosopyrrolidine administered in drinking water to Syrian golden hamsters. J. Cancer Res. Clin. Oncol. 104, 75-79.
- KETKAR, M.B., FUHST, R., PREUSSMANN, R. and MOHR, U. (1983a). The carcinogenic effect of nitrosopiperidine administered in the drinking water of Syrian golden hamsters. Cancer Lett. 21, 219-224.
- KETKAR, M.B., HOLSTE, J., PREUSSMANN, R. and ALTHOFF, J. (1983b). Carcinogenic effect of nitrosomorpholine administered in drinking water to Syrian golden hamsters. Cancer Lett. 17, 333-338.
- KHANNA, S.D. and PURI, D. (1966). The hepatotoxic effects of dimethylnitrosamine in the rat. J. Path. Bact. 91, 605-608.
- KIMBLE, C.E., SINSKY, A.J., GORCZYCA, P. (1973). Mutagenesis of BHK-21 cells by N-nitroso compounds and ultraviolet light. In Vitro 8, 442-443.
- KLEIN, D., GIRARD, A.N., DESMEDI, J., FELLION, Y. and DEBRY, G. (1981). Analysis of nitrosamines in industrial cosmetic products. J. Cosmet. Toxicol. 19, 223-235.
- KNASHMUELLER, S., STEHLIK, G., MOHN, G. (1986). Studies on the metabolic activation of diethanol nitrosamine in animal-mediated and in vitro assays using Escherichia coli K-12 343/113 as an indicator. J. Cancer Clin. Oncol. 112 (3), 266-271.
- KOPPANG, N. (1974a). Toxic effect of dimethylnitrosamine in cows. J. Natl. Can. Inst. 52, 523-531.
- KOPPANG, N. (1974b). The toxic effects of dimethylnitrosamine in sheep. Acta Vet. Scand. 15, 533-543.
- KORSLUD, G.O., GRICE, H.G., GOODMAN, T.K., KNIPFEL, J.E. and McLAUGHLAN, J.M. (1973). Sensitivity of several serum enzymes for the detection of thioacetamide-, dimethylnitrosamine- and diethanolamine-induced liver damage in rats. Toxicol. Appl. Pharmacol. 26, 299-313.
- KUNZ, W., APPEL, K.G., SCHWARZ, M., STOECKLE, G. (1978). Enhancement and inhibition of carcinogenic effectiveness of nitrosamines. In: Primary liver tumours, Eds. H. Remmer, H.M. Bolt. P. Bannasch, H. Popper, MTP Press, Lancaster, pp. 261-284.
- KUROKI, T., DREVON, C., and MONTESANO, R. (1977). Microsome-mediated mutagenesis in V 79 chinese hamster cells by various nitrosamines. Cancer Res. 37, 1044-1050.
- LAISHES, B.A. and STICH, H.F. (1973). Repair synthesis and sedimentation analysis of DNA of human cells exposed to dimethylnitrosamine and activated dimethylnitrosamine. Biochem. Biophys. Res. Commun. 52, 827-833.
- LAKRITZ, L., PENSABENE, J.W. (1981). Survey of fluid and nonfat dry milks for N-nitrosamines. J. Dairy Sci. 64, 371-374.
- LANGENBACH, R. (1986). Mutagenic activity and structure-activity relationship of short-chain dialkyl N-nitrosamines in a hamster hepatocyte V79 cell-mediated system. Mutation Res. 163, 303-311.
- LAPIS, K., UJHELYI, E., GYENES, M., JENEY, A. (1984). Alterations of markers during hepatocarcinogenesis in rats. In: Models, Mechanisms and Etiology of Tumour Promotion, Eds. M. Boerzsoenyi, K. Lapis, M.F. Day, H. Yamasaki, IARC Sci., Publ. 56, Lyon, pp. 25-36.
- LEE, W.R. (1976). Occupational aspects of scrotal cancer and epithelioma. Ann. N.Y. Acad. Sci. 271, 138-142.

- LETHCO, E.J., WALLACE, W.C., BROWER, E. (1982). The fate of N-nitrosodiethanolamine after oral and topical administration to rats. *Fd. Chem. Toxicol.* 20, 401-406.
- LIBBEY, L.M., SCANLAN, A.R., BARBOUR, J.F. (1980). N-Nitrosodimethylamine in dried dairy products. *Food Cosmet. Toxicol.* 18, 459-461.
- LICHT, W.R., DEEN, W.M. (1988). Theoretical model for predicting rates of nitrosamine and nitrosamide formation in the human stomach. *Carcinogenesis*, 9, 2227-2237.
- LIJINSKY, W., REUBER, M.D., MANNING, W.B. (1980). Potent carcinogenicity of nitrosodiethanolamine in rats. *Nature* 288, 589-590.
- LIJINSKY, W. and REUBER, M.D. (1981a). Comparative carcinogenesis by some aliphatic nitrosamines in Fischer rats. *Cancer Letters* 14, 297-302.
- LIJINSKY, W. and REUBER, M.D. (1981b). Carcinogenic effect of nitrosopyrrolidine, nitrosopiperidine and nitrosohexamethyleneamine in Fischer rats. *Cancer Letters* 12, 99-103.
- LIJINSKY, W., LOSIKOFF, A.M. and SANSONE, E.B. (1981a). Penetration of rat skin by N-nitrosodiethanolamine and N-nitrosomorpholine. *J. Natl. Cancer Inst.* 66, 125-127.
- LIJINSKY, W., SAAVEDRA, J.E., REUBER, M.D. (1981b). Induction of carcinogenesis in Fischer rats by methylalkylnitrosamines. *Cancer Res.* 41, 1288-1292.
- LIJINSKY, W., REUBER, M.D. and RIGGS, C.W. (1981c). Dose response studies of carcinogenesis in rats by nitrosodiethylamine. *Cancer Res.* 41, 4997-5003.
- LIJINSKY, W., REUBER, M.D. (1984a). Dose-response study with N-nitrosodiethanolamine in F344 rats. *Fd. Chem. Toxicol.* 22, 23-26.
- LIJINSKY, W. and REUBER, M.D. (1984b). Carcinogenesis in rats by nitrosodimethylamine and other nitrosomethylalkylamines at low doses. *Cancer Letters* 22, 83-88.
- LIJINSKY, W., EPLER, J.L., RAO, T.K. (1984). Comparison of mutagenic and carcinogenic properties. In: T.K. Rao, W. Lijinsky and J.L. Epler (eds), *Genotoxicology of N-nitroso compounds*, Plenum Press, New York, pp. 233-263.
- LIJINSKY, W., KOVATCH, R.M. (1985). Induction of liver tumors in rats by nitrosodiethanolamine at low doses. *Carcinogenesis* 6, 1679-1681.
- LIJINSKY, W. (1987). Structure-activity relations in carcinogenesis by N-nitroso compounds. *Cancer Metastasis Rev.* 6, 301-356.
- LIJINSKY, W., KOVATCH, R.M., RIGGS, C.W. and WALTERS, P.T. (1988). Dose response study with N-nitrosomorpholine in drinking water of F344 rats. *Cancer Res.* 48, 2089-2095.
- LIJINSKY, W. (1989). Alkylation of DNA related to organ-specific carcinogenesis by N-nitroso compounds. IARC 10th International meeting on N-nitroso compounds, mycotoxins and tobacco smoke. Relevance to human cancer. Lyon, 25-28. Sept. 1989.
- LILLY, L.J., BAHNER, B., MAGEE, P.N. (1975). Chromosome aberrations induced in rat lymphocytes by N-nitroso compounds as a possible basis for carcinogen screening. *Nature* 258, 611-612.
- LÖFBERG, B. and TJÄLVE, H. (1984). The disposition and metabolism of N-nitrosodiethylamine in adult, infant and foetal tissues of the Syrian golden hamster. *Acta Pharmac. Tox.* 54, 104.
- LÖFBERG, B., TJÄLVE, H. (1985). Tissue specificity of N-nitrosomorpholine metabolism in Sprague-Dawley rats. *Fd. Chem. Toxicol.* 23, 647-654.
- LU, F., LI, M. (1986). Determination of volatile nitrosamines in marine fish and shrimp from Guangdong Province. *Zhongguo Yixue Kexueyuan Xuebao* 8, 317-319.
- LUTZ, W.K. (1979). In vivo covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. *Mut. Res.* 65, 289-356.
- MAFF (1987). Nitrate, nitrite and N-nitroso compounds in food. Food Surveillance Paper No. 20, Ministry of Agriculture, Fisheries and Food, London. ISBN 0 11 242811 8.
- MAGEE, R.N., BARNES, J.M. (1956). The production of malignant primary hepatic tumours in the rat by feeding DMNA. *B. J. Cancer* 10, 114-122.
- MAGOUR, S., and NIEVEL, J.G. (1971). Effect of inducers of drug metabolizing enzymes on diethylnitrosamine metabolism and toxicity. *Biochem. J.* 123, 8P-9P.
- MANSON, D., COX, P.J., and JARMAN, M. (1978). Metabolism of N-nitrosomorpholine by the rat *in vivo* and by rat liver microsomes and its oxidation by the Fenton System. *Chemico-Biol. Interactions* 20, 341-354.

- MARZULLI, F.N., ANJO, D.M., MAIBACH, H.I. (1981). *In vivo* skin penetration studies of 2,4-toluenediamine, 2,4-diaminoanisole, 2-nitro-p-phenylenediamine, p-dioxane and N-nitrosodiethanolamine in cosmetics. *Fd. Cosmet. Toxicol.* 19, 743-747.
- HASSEY, R.C., Key, P.E., McKeeney, D.J., Tricker, A.R. (1988). Non-volatile N-nitroso compounds in cured meats. In: *Living in a chemical world*, Eds. C. Maltoni and I.J. Selikoff, Ann. New York Acad. Sci. 534, 73-81.
- MCGLOTHLIN, J.D., WILCOX, T.C., FAJEN, J.M., EDWARDS, G.S. (1981). A health hazard evaluation of nitrosamines in a tire manufacturing plant. In: *Chemical Hazards in the Workplace*, Choudkary, G., Ed.; ACS Symposium Series No. 149, ACS: Washington D.C., pp. 283-299.
- McLEAN, E., BRAS, G. and McLEAN, A.E.M. (1965). Venous occlusion in the liver following dimethylnitrosamine. *Brit. J. Exptl. Path.* 46, 367-369.
- MEDINA D. (1975). Tumour progression. In: *Cancer - a comprehensive treatise*, Vol. 3. Biology of tumours: Cellular Biology and Growth, Ed. F.F. Becker, Plenum Press, New York, pp. 99-119.
- MIRNA, A. (1983). Fluchtige N-Nitrosamine in Fleischwaren. In: *Das Nitrosamin-Problem*, Ed. R. Preussmann, Verlag Chemie, Weinheim, 41-47.
- MIRVISH, S.S. (1975). Formation of N-nitroso compounds: Chemistry, kinetics and in vivo occurrence. *Toxicol. Appl. Pharmacol.* 31, 325-351.
- MIRVISH, S.S. (1985). Gastric cancer and salivary nitrate and nitrite. *Nature* 315, 461-462.
- MIRVISH, S.S. (1986). Effects of vitamin C and E on N-nitroso compound formation, carcinogenesis and cancer. *Cancer* 58, 1842-1850.
- MOHR, U., ALTHOFF, J. and PAGE, W. (1972). Tumors of the respiratory system induced in the common European hamster by N-diethylnitrosamine. *J. Natl. Cancer Inst.* 49, 595-597.
- MOISEEV, G.E. and BENEMANSKY, V.D. (1975). On carcinogenic activity of low concentrations of nitrosodimethylamine in inhalation. *Vop. Onkol.* 21, 107-109.
- MONTESANO, R., SAINT-VINCENT, L., DREVON, C., and TOMATIS, L. (1975). Production of epithelial and mesenchymal tumours with rat liver cells transformed in vitro. *Int. J. Cancer* 16, 550-558.
- MONTESANO, R. and BARTSCH, H. (1976). Mutagenic and cancerogenic N-nitroso compounds: possible environmental hazards. *Mutat. Res.* 32, 179-228.
- MONTESANO, R., BRESIL, H., DEGAN, P., MARTEL-PLANCHE, G., SERRES, M. and WILD, C.P. (1988). Detection in human cells of alkylated macromolecules attributable to exposure to nitrosamines. *IARC Sci. Publ.* 89, 75.
- MORI, Y., YAMAZAKI, H., KONISHI, Y. (1987). Mutagenicity of N-nitrosodiethanolamine in the Salmonella/microsome test. *Mutat. Res. Letters* 192, 91-94.
- MORRISON, J.B., HECHT, S.S. and WENNINGER, J.A. (1983). N-nitroso-N-methyl octadecylamine in hair-care products. *Fd. Chem. Toxic.* 21, 69-73.
- NAIR, U. J., AMMIGAN, N., NAGABHUSHAN, M., AMONKAR, A. J. and BHIDE, S. V. (1989). Modulatory effect of Vitamin A status on exposure to tobacco extract or N-nitrosomornicotine in Sprague-Dawley rats. *IARC 10th International meeting on N-nitroso compounds, mycotoxins and tobacco smoke. Relevance to human cancer.* Lyon, 25-28. Sept. 1989.
- NATARAJAN, A.T., TATES, A.D., van BUUL, P.P.W., MEIJERS, M. and DE VOGEL, N. (1976). Cytogenetic effects of mutagens/carcinogens after activation in a microsomal system in vitro. I. Induction of chromosome aberrations and sister chromatid exchanges by diethylnitrosamine (NDEA) and dimethylnitrosamine (DMN) in CHO cells in the presence of rat-liver microsomes. *Mutation Res.* 37, 83-90.
- NATIONAL ACADEMY OF SCIENCES (1978). Nitrates - an environmental assessment. Commission on Natural resources, Washington DC, National Academy of Sciences.
- NATIONAL ACADEMY OF SCIENCES (1981). The health effects of nitrate, nitrite and N-nitroso compounds. National Academy of Sciences, Washington.
- NEURATH, G. (1983). N-Nitrosoverbindungen in Tabak und Tabakrauch. in: Preussmann, R. (ed.), *Das Nitrosaminproblem*. Weinheim, pp. 65-71.
- NITRITE SAFETY COUNCIL (1980). A survey of nitrosamines in sausages and dry-cured meat products. *Food Technol.* 34, 45-51.
- 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.
- OSTERMANN-GOLKAR, S. and BERGMARK, E. (1988). Alkylation of haemoglobin, plasma proteins and DNA in the mouse by diethylnitrosamine. *Carcinogenesis* 9, 1915-1917.

- PANI, P., COLUMBANO, A., DESSI, S., PORCU, M., LEDDA, G. M. and DIAZ, G. (1977). Effect of choline administration on the toxicity of N-nitrosodimethylamine in female rats. *Toxicol. Appl. Pharmacol.* **42**, 613-616.
- PARKIN, R., WAYNFORTH, H.B., MAGEE, P.W. (1973). The activity of some nitroso compounds in the mouse dominant-lethal mutation assay. I. Activity of N-nitroso-N-methylurea, N-methyl-N-nitroso-N'-nitroso-guandine and N-nitrosomethylmorpholine. *Mutation Res.* **21**, 155-161.
- PASTERNAK, L. (1963). Untersuchungen über die mutagene Wirkung von Nitrosaminen und Nitrosomethylharnstoff. *Acta biol.med. germ.* **10**, 436-438.
- PETO, R., GRAY, R., BRANTON, P. and GRASSO, P. (1984). Nitrosamine carcinogenesis in 5120 rodents: chronic administration of 16 different concentrations of NDEA, NDMA, NPYR and NPIP in the water of 4440 inbred rats, with parallel studies on NDEA alone of the effect of age of starting (3,6 or 20 weeks) and of species (rats, mice or hamsters). *IARC Sci. Publ.* **57**, 627-665.
- PIELSTICKER, K., MOHR, U. and KLEMM, J. (1967). Absence of malformations following dimethylnitrosamine in the golden hamster. *Naturwissenschaften* **13**, 340-341.
- PIGNATELLI, B., BEREZIAT, J. C., DESCOTES, G., and BARTSCH, H. (1982). Catalysis of nitrosation *in vitro* and *in vivo* in rats by catechin and resorcinol and inhibition by chlorogenic acid. *Carcinogenesis* **3**, 1045-1049.
- PITOT, H.C. and SIRICA, A.E. (1980). The stages of initiation and promotion in hepatocarcinogenesis. *Biochem. Biophys. Acta* **605**, 191-215.
- PITOT, H.C., CAMPBELL, H.A. (1987). An approach to the determination of the relative potencies of chemical agents during the stages of initiation and promotion in multistage hepatocarcinogenesis in the rat. *Environ. Health Perspect.* **76**, 49-56.
- POOL, B.L., JANAVSKY, I., KLEIN, P., KLEIN, R.G., SCHMEZER, P., VOGT-LEUCHT, G. and ZELLER, W.J. (1988). Effects of SO₂ or NO_x on toxic and genotoxic properties of chemical carcinogens. *In vitro* studies. *Carcinogenesis* **9**, 1237-1245.
- POUND, A.W., HORNE, L. and LAWSON, T.A. (1973). Decreased toxicity of dimethylnitrosamine in rats after treatment with carbon tetrachloride. *Path.* **5**, 233-242.
- POUND, A.W. and LAWSON, T.A. (1974). Protection by a small dose of carbon tetrachloride against the toxic effects of dimethylnitrosamine in rats. *Br. J. Exp. Path.* **55**, 203-212.
- POUND, A.W. (1975) The effect of a dose of dimethylnitrosamine on the toxicity of a subsequent dose and on the toxicity of carbon tetrachloride in mice. *Br. J. Exp. Path.* **56**, 271-275.
- POUND, A.W. and LAWSON T.A. (1975). Protection by carbon tetrachloride against the toxic effects of dimethylnitrosamine in mice. *Br. J. Exp. Path.* **56**, 77-82.
- POURIER, S., HUBERT, A., DE-THE, G., OHSHIMA, H., BOURGADE, M.-C., BARTSCH, H. (1987). Occurrence of volatile nitrosamines in food samples collected in three high-risk areas for nasopharyngeal carcinoma. *IARC Sci. Publ.* **84**, 415-422.
- POWELL, J.B. (1987). Minimising NDELA formation from nitrite and NO₂ in non-aqueous triethanolamine systems. *J. Soc. Cosmet. Chem.* **38**, 29-42.
- PREAT, V., DeGERLACHE, J., LANS, M., TAPER, H., ROBERFROID, M. (1986). Influence of the nature and the dose of the initiator on the development of premalignant and malignant lesions in rat hepatocarcinogenesis. *Teratog. Carcinog. Mutag.* **6**, 165-172.
- PRESTON-MARTIN, S. (1987). N-nitroso compounds as a cause of human cancer. *IARC Sci. Publ.* **84**, 477-484.
- PREUSSMANN, R., WURTELE, G., EISENBRAND, G., SPIEGELHALDER, B. (1978) Urinary excretion of N-nitrosodiethanolamine administered orally to rats. *Cancer Lett.* **4**, 207-209.
- PREUSSMANN, R., SPIEGELHALDER, B., EISENBRAND, G. (1980). Reduction of human exposure to environmental N-nitroso-carcinogens. Examples of possibilities for cancer prevention. In: *Carcinogenesis: Fundamental Mechanisms and Environmental Effects*, Eds. B. Pullman, P.O.P. Ts'o, and M. Gelboin, D. Reidel Publ. Co., Netherland, 273-285.
- PREUSSMANN, R., SPIEGELHALDER, B., EISENBRAND, G., WURTELE, G., HOFMANN, I. (1981). Urinary excretion of N-nitrosodiethanolamine in rats following its epicutaneous and intratracheal administration and its formation *in vivo* following skin application of diethanolamine. *Cancer Lett.* **13**, 227-232.
- PREUSSMANN, R., HABBS, M., SCHMÄHL, D., EISENBRAND, G. (1982a). Dose-response study on the carcinogenicity of N-nitrosodiethanolamine in male Sprague-Dawley rats. *IARC Sci. Publ.* **41**, 591-596.

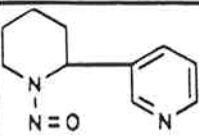
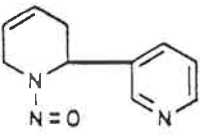
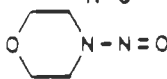
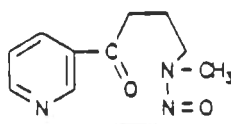
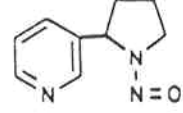
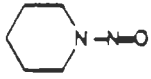
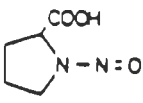
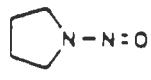
- PREUSSMANN, R., HABS, M., HABS, M., SCHMÄHL, D. (1982b). Carcinogenicity of N-nitrosodiethanolamine in rats at five different dose levels. *Cancer Res.* 42, 5167-5171.
- PREUSSMANN, R. and SPIEGELHALDER (1984). In: ACS Monograph Series No.182: Chemical Carcinogens. Ed.: C.E Searle.
- PREUSSMANN, R., WIESSLER, M. (1987). The enigma of the organ-specificity of carcinogenic nitrosamines. *TIPS* 8, 185-189.
- PREUSSMANN, R. (1989). N-Nitroso compounds and tobacco-related cancers. Concluding remarks. in: Maskens, A.P. *et al* (eds): Tobacco and cancer, perspectives in preventive research, Elsevier Sci. Publ., p. 59.
- PREUSSMANN, R. and TRICKER, A.R. (1989). Endogenous nitrosamine formation and nitrate burden in relation to gastric cancer epidemiology. In: Reed, P.I. and Hall, M.J. (eds): Gastric Carcinogenesis. Elsevier Sci., 147-160.
- PROPPING, P., ROEHRBORN, G. and BUSELMAIER, W. (1972). Comparative investigations on the chemical induction of point mutations and dominant lethal mutations in mice. *Mol. Gen. Genet.* 117, 197-209.
- RAO, T.K. (1984). Structural basis for mutagenic activity of N-nitrosamines in the Salmonella Histidine Reversion Assay. In: T.K. Rao, W. Lijinsky and J.L. Epler (eds) Genotoxicology of N-nitroso compounds, Plenum Press, New York, pg. 45-58.
- REICHART, P. and MOHR, U. (1987). Correlation between chewing and smoking habits and precancerous lesions in hill tribes of Northern Thailand. *IARC Sci. Publ.* 84, 463-464.
- REUBER M.D. (1975). Hepatic vein thrombosis in buffalo strain female rats ingesting dimethylnitrosamine. *Path. Europ.* 10, 241-244.
- ROLLMANN, B., LOMBART, P., ROULELET, J. and MERCIER, M. (1981). Determination of N-nitrosodiethanolamine in cosmetics by gas chromatography with electron capture detection. *J. Chromat.* 206, 153-163.
- ROJECH, B. (1982). Annals of medicine: the prognosis for this patient is horrible. *New Yorker Mag.* Jan. 25, 57-71.
- ROUNBEHLER, D.P., ROSS, R., FINE, D.H., IQBAL, Z.M., EPSTEIN, S.S. (1977). Quantitation of dimethylnitrosamine in the whole mouse after biosynthesis in vivo from trace levels of precursors. *Science* 197, 917-918.
- ROUNBEHLER, D.P., KRULL, I.S., GOFF, E.U., MILLS, K.M., MORRISON, J., EDWARDS, G.S., FINE, D.H., FAJEN, J.M., CARSON, G.A., RHEINHOLD, V. (1979). Exposure to N-nitrosodimethylamine in a leather tannery. *Fd. Cosmet. Toxicol.* 17, 487-491.
- ROWLAND, I.R. (1988). The toxicology of N-nitroso compounds. In: Nitrosamines. Toxicology and Microbiology. Hill, M.J. (Ed.), VCH Publishers Ltd., UK.
- RUTCHKOVSKY, B.S., TITKIN, L.A., DRAGA, N.V., GLUSHKOV, P.V. (1981). Dimethylnitrosamin and sodium nitrite in some types of sausages produced in Kiev. In: Carcinogenic N-nitroso compounds and their precursors - Formation and analysis in the environment, Eds. S. Bogovski, G. Loogna and A. Itra. Abstr. 4th All-Union Symp., Tallin, Inst. Exp. Clin. Med., pp. 10-11.
- SANDER, J. (1971). Untersuchungen über die Entstehung cancerogener Nitrosoverbindungen im Magen von Versuchstieren und ihre Bedeutung für den Menschen. *Arzneimittel-Forsch.* 21, 1572-1580.
- SANSONE, E.B., LOSIKOFF, A.M., LIJINSKY, W. (1980). The penetration of rat skin by some nitrosamines of environmental importance. In: N-nitroso compounds analysis formation and occurrence, IARC Sci. Publ 31, 705-716.
- SATO, K., KITAHARA, A., SATOH, K., ISHIKAWA, T., TATEMATSU, M., ITO, N. (1984). The placental form of glutathione S-transferase as a new marker protein for preneoplasia in rat chemical hepatocarcinogenesis. *Gann.* 75, 199-202.
- SCANLAN, R.A., BARDOUR, J.F., HOTCHKISS, J.H., LIBBEY, L.M. (1980). N-Nitrosodimethylamine in beer. *Food Cosmet. Toxicol.* 18, 27-29.
- SCHERER, E., EMMELOT, P. (1976). Kinetics of induction and growth of enzyme-deficient islands involved in hepatocarcinogenesis. *Cancer Res.* 36, 2544-2554.
- SCHERER, G., LUDEKA, B., KLEIHUES, P., LOEPPKY, R.N. and EISENBRAND, G. (1989). Mutagenicity, DNA damage and DNA adduct formation by N-nitroso-2-hydroxyalkylamines and corresponding aldehydes. 10th International Meeting on N-nitroso Compounds, Mycotoxins and Tobacco Smoke: Relevance to Human Cancer, IARC, Lyon, 25.-28. Sept.1989, Abstr. No. P-43.

- SCHIEFERSTEIN, G., PIRSCHEL, S., FRANK, W., FRIEDRICH-FREKSA, H. (1974). Quantitative Untersuchungen über den irreversiblen Verlust zweier Enzymaktivitäten in der Rattenleber nach Verfütterung von Diethylnitrosamin. *Z. Krebsforsch.* **82**, 191-208.
- SCHULTE-HERMANN, R. (1985). Untersuchungen zur Tumorpromotion an mutmaßlichen Tumor-Vorstadien in der Leber. *BGA-Schriften* **6/1985**, 108-118.
- SEN, M.P., SEAMAN, S. and MILES, W.F. (1976). Dimethylnitrosamine and nitrospyrrolidine in fumes produced during the frying of bacon. *Food Cosmet. Toxicol.* **14**, 67-170.
- SEN, M.P., DONALDSON, B.A., SEAMAN, S., IYENGAR, J.R., MILES, W.F. (1978). Recent studies in Canada on the analysis and occurrence of volatile and non-volatile N-nitroso compounds in food. *IARC Sci. Publ.* **19**, 373-393.
- SEN, M.P., SEAMAN, S., MILES, W.F. (1979). Volatile nitrosamines in various cured meat products: Effect of cooking and recent trends. *J. Agric. Food Chem.* **27**, 1354-1360.
- SHEPHERD, S.E., SCHLATTER, CH., LUTZ, W.K. (1987). Assessment of the risk of formation of carcinogenic N-nitroso compounds from dietary precursors in the stomach. *Fd. Chem. Toxicol.* **25**, 91-108.
- SONG, P.J., HU, J.F. (1988). N-Nitrosamines in Chinese foods. *Fd. Chem. Toxic.* **26**, 205-208.
- SPIEGELHALDER, B., EISENBRAND, G., PREUSSMANN, R. (1976). Influence of dietary nitrate on nitrite content of human saliva: possible relevance to *in vivo* formation of N-nitroso compounds. *Fd. Cosmet. Toxicol.* **14**, 545-548.
- SPIEGELHALDER, B., EISENBRAND, G., PREUSSMANN, R. (1979). Contamination of beer with trace quantities of N-nitrosodimethylamine. *Food Cosmet. Toxicol.* **17**, 29-31.
- SPIEGELHALDER, B., EISENBRAND, G., PREUSSMANN, R. (1980a). Volatile nitrosamines in food. *Oncology* **37**, 211-216.
- SPIEGELHALDER, B., EISENBRAND, G., PREUSSMANN, R. (1980b). Occurrence of volatile nitrosamines in food: A survey of the West German market. *IARC Sci. Publ.* **31**, 467-479.
- SPIEGELHALDER, B., EISENBRAND, G., PREUSSMANN, R. (1982). Urinary excretion of N-nitrosamines in rats and humans. *IARC Sci. Publ.* **41**.
- SPIEGELHALDER, B. (1983). Vorkommen von N-Nitroso-Verbindungen. In: *Das Nitrosamin-Problem*, Ed. R. Preussmann, Verlag Chemie, Weinheim, pp. 27-40.
- SPIEGELHALDER, B., PREUSSMANN, R. (1983). Occupational nitrosamine exposure. 1. Rubber and tyre industry. *Carcinogenesis* **4**, 1147-1152.
- SPIEGELHALDER, B., PREUSSMANN, R. (1984). Contamination of toiletries and cosmetic products with volatile and non-volatile N-nitroso carcinogens. *J. Cancer Res. Clin. Oncol.* **108**, 160-163.
- SPIEGELHALDER and PREUSSMANN (1987). Nitrosamine measurements in ambient air of an industrial area in Austria. *IARC Sci. Publ.* **84**, 411-414.
- STEPHANY, R.W., SCHULLER, P.L. (1980). Daily dietary intakes of nitrate, nitrite and volatile N-nitrosamines in the Netherlands using the duplicate portion sampling technique. *Oncology* **37**, 203-210.
- STERZEL, W. and EISENBRAND, G. (1986). N-nitrosodiethanolamine is activated in the rat to an ultimate genotoxic metabolite by sulfotransferase. *J. Cancer Res. Clin. Oncology* **11**, 20-24.
- STICH, H. F., OSHIMA, H., PIGNATELLI, B., MICHELON, J., and BARTSCH, H. (1983). Inhibitory effect of betel nut extracts on endogenous nitrosation in humans. *J. Nat. Cancer Inst.* **70**, 1047-1050.
- SWANN, P.F. and McLEAN, A.E.M. (1968). The effect of diet on the toxic and carcinogenic action of dimethylnitrosamine. *Biochem. J.* **107**, 14P.
- TANNENBAUM, S.R. and CORREA, P. (1985). Nitrate and gastric cancer risks. *Nature* **317**, 675-676.
- TANNENBAUM, S.R. (1987). Endogenous formation of N-nitroso compounds: a current perspective. *IARC Sci. Publ.* **84**, 292-296.
- TATEMATSU, M., MERA, Y., ITO, N., SATOH, K., SATO, K. (1985). Relative merits of immunohistochemical demonstrations of placental A, B and C forms of glutathione S-transferase and histochemical demonstration of gamma-glutamyl-transferase as markers of altered foci during liver carcinogenesis in rats. *Carcinogenesis* **6**, 1621-1626.
- TATEMATSU, M., TSUDA, H., SHIRAI, T., MASUI, T., ITO, N. (1987). Placental glutathione S-transferase (GST-P) as new marker for hepatocarcinogenesis: In vivo short-term screening for hepatocarcinogens. *Toxicol. Pathol.* **15**, 60-68.

- TAYLOR, H.W. and LIJINSKY, W. (1975). Tumour induction in rats by feeding heptamethyleneimine and nitrate in water. *Cancer Res.* 35, 812-815.
- TELLING, G.M., HOAR, D., CASWELL, D. and COLLINGS, A.J. (1976). Studies on the effect of feeding nitrate and secondary amines to Wistar rats. *IARC Sci. Publ.* 14, 247-254.
- TERRACINI, B., PALESTRO, G., RAMELLA, GIGLIARDI, M. and MONTESANO, R. (1966). Carcinogenicity of dimethylnitrosamine in Swiss mice. *Brit. J. Cancer* 20, 871-876.
- TERRACINI, B., MAGEE, P.W. and BARNES, J.M. (1967). Hepatic pathology in rats on low dietary levels of dimethylnitrosamine. *Brit. J. Cancer* 21, 559-565.
- TRGS (1988). Technische Regeln für Gefahrstoffe: Nitrosamine. No 552. Carl Heymanns Verlag, Koeln.
- TRICKER, A.R., PREUSSMANN, R. (1987) Influence of cysteine and nitrate on the endogenous formation of N-nitrosamine acids. *Cancer Lett.* 34, 39-47.
- TRICKER, A.R., PREUSSMANN, R. (1989a). Occurrence and exposure to N-nitroso compounds in tobacco. in: IARC 10th International Meeting on N-nitroso compounds, mycotoxins and tobacco smoke: Relevance to human cancer. Lyon, 25-28 Sept. 1989.
- TRICKER, A.R., PREUSSMANN, R. (1989b). Preformed nitrosamines in smokeless tobacco. in: A.P. Maskens (ed), Tobacco and cancer. Perspectives in preventive research. Elsevier Publ. p. 35-47.
- UCHIDA, E., HIROMO, I. (1979). Effect on phenobarbital on induction of liver and lung tumours by dimethylnitrosamine in newborn mice. *Gann* 70, 639-644.
- VINCENT, F., DAVID, R.J., KRUGER, R.A., NELSON, R.L., LOEPPKY, R. and HARKINS, M.E. (1986). Integration of laboratory and epidemiological studies to evaluate genotoxic exposure in tool and die workers. *Prog. Clin. Biol. Res.* 27, 183-193.
- WAGNER, D.A., SHUBER, D.E.G., BILMAZES, C., OBIEDZINSKI, M., YOUNG, V.R. and TANNENBAUM, S.R. (1984). Modulation of endogenous synthesis of N-nitrosoamine acids in humans. *IARC Sci. Publ.* 57, 223-229.
- WALKER, E.A., CASTEGNARO, M., GARREN, L., TOUSSAINT, G., KOWALSKI, B. (1979). Intake of volatile nitrosamines from consumption of alcohols. *J. Natl. Cancer Inst.* 63, 947-951.
- WALLCAVE, L., POUR, P. (1981). The carcinogenicity of N-nitrosodiethanolamine: an environmental pollutant in Syrian hamsters. *Canc. Lett.* 14, 23-27.
- WALTERS, C.L. and SMITH, P.L.R. (1981). The effect of water-borne nitrate on salivary nitrite. *Fd.Cosmet. Toxicol.* 19, 297-302.
- WANG, H. and WU, Y. (1989). The blocking effect of Chinese Tea on N-nitrosation in vitro and in vivo. IARC 10th International meeting on N-nitroso compounds, mycotoxins and tobacco smoke. Relevance to human cancer. Lyon, 25-28. Sept. 1989.
- WATANABE, M., HONDA, S., HAYASHI, M., MATSUDA, T. (1982). Mutagenic effects of combinations of chemical carcinogens and environmental pollutants in mice as shown by the micronucleus test. *Mutation Res.* 97, 43-48.
- WEBB, K.S., GOUGH, T.A. (1980). Human exposure to preformed environmental N-nitroso compounds in the U.K.. *Oncology* 37, 195-198.
- WEGIEL, J., WANIEWSKI, E., SZCZESNIAK, E., MITEK C., KRAWCZYNSKA, M., KARPINSKA, E., OBIEDZINSKI, M. and SZMIGIELSKI, S. (1980). Morphometric methods used for assessing the dynamics of ultrastructural changes in mouse liver in acute poisoning with diethylnitrosamine. *Acta Med. Pol.* 21, 405-407.
- WEISSBURGER, J.H., WILLIAMS, G.M. (1980). Chemical carcinogens. In: Toxicology - The basic science of poisons, Eds. J. Doull, C.D. Klaasen, M.O. Andur, Macmillan Publishing Co. New York, pp. 84-138.
- WHITE, J.W. (1975). Relative significance of dietary sources of nitrate and nitrite. *J. Agric. Fd. Chem.* 23, 886-891.
- WHO (1985). Health hazards from nitrates in drinking-water. Report on a WHO Meeting. Copenhagen, 5-9 March 1984.
- WILLIAMS, G.M. (1977). Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. *Cancer Res.* 33, 602-612.
- WOLF, D. (1989). N-Nitrosamine am Arbeitsplatz. *Staub-Reinh.d.Luft* 49, 183-186.
- XU, G.-W. (1981). Gastric Cancer in China. A Review. *J.R. Soc. Med.* 74, 210-211.
- YAHAGI, T., NAGAO, M., SEIMO, Y., MATSUSHIMA, T., SUGIMURA, T., OKADA, M. (1977). Mutagenicities of N-nitrosamines on *Salmonella*, *Mutation Res.* 48, 121-130.
- YAMAMOTO, M., IWATA, R., ISHIWATA, H., YAMADA, T., TANIMURA, A. (1984). Determination of volatile nitrosamine levels in foods and estimation of their daily intake in Japan. *Fd. Chem. Toxic.* 22, 61-64.

- ZALDIVAR, R. and ROBINSON, H. (1973). Epidemiological investigation on stomach cancer mortality in Chileans: Association with nitrate fertilizer. Z. Krebsforsch. 80 289-295.
- ZALDIVAR, R. (1977). Nitrate fertilizers as environmental pollutants: Positive correlation between nitrates (NaNO_3 and KNO_3) used per unit area and stomach cancer mortality rates. Experimentia 33, 264-265.
- ZALDIVAR, R. and WETTERSTRAND, W.H. (1978). Nitrogen levels in drinking water of urban areas with high and low risk populations for stomach cancer: an environmental epidemiological study. Z. Krebsforsch. 92, 227-234.
- ZEIGER, E., LEGATOR, M.S. (1971). Mutagenicity of N-nitrosomorpholine in the host-mediated assay. Mutation Res. 12, 413-429.
- ZERBAN, H., PREUSSMANN, R., BANNASCH, P. (1988). Dose time relationship of the development of pre-neoplastic liver lesions induced in rats with low doses of N-nitrosodiethanolamine. Carcinogenesis 9, 607-610.
- ZINGMARK and RAPPE (1976). On the formation of N-nitrosodiethanolamine from a grinding fluid under simulated gastric conditions. Ambio, 5, 80-81.

Table 1. Chemical Names of N-nitrosamines, Abbreviations Used in the Text and their Allocation to Analytical Groups (see text p. 4)

Abbrev.	Chemical Name	Group	Structural Formula
NAB*	N-nitrosoanabasine	II	
NAT*	N-nitrosoanatabine	II	
NDBA	N-nitrosodi-n-butylamine	I	$(n-C_4H_9)_2N-N=O$
NDDMA	N-nitrosododecylmethylamine	II	$C_{12}H_{25}-N(CH_3)-N=O$
NDEA	N-nitrosodiethylamine	I	$(C_2H_5)_2N-N=O$
NDELA	N-nitrosodiethanolamine	III	$(HOCH_2CH_2)_2N-N=O$
NDMA	N-nitrosodimethylamine	I	$(CH_3)_2N-N=O$
NDPA	N-nitrosodi-n-propylamine	I	$(n-C_3H_7)_2N-N=O$
NDPHA	N-nitrosodiphenylamine	I	$(C_6H_5)_2N-N=O$
NMEA	N-nitrosomethylethylamine	I	$C_2H_5-N(CH_3)-N=O$
NMOR	N-nitrosomorpholine	I	
NNK*	4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone	II	
NNN*	N-nitrosornornicotine	II	
NPIP	N-nitrosopiperidine	I	
NPRO	N-nitrosoproline	n.c.	
NPYR	N-nitrosopyrrolidine	I	
TSNA	tobacco-specific nitrosamines	II	

* = belonging to the group of tobacco-specific nitrosamines.
n.c. = not classifiable under this scheme.

Table 2. Nitrosamine Content of Food Stuffs (ug/kg)

FOOD	NDMA	NDGA	NPYR	REFERENCE
Meat and Meat Products				
Cured bacon and Ham	≤ 5 0.3-1.5 (mean) 3.5 (mean)		1-20* 0.1-5.3 (mean) 7.4 (mean)	Gough et al, 1978 Spiegelhalder, 1983 Massey et al, 1988
(raw)	1.6 (mean) 1.8 (mean)	0.7 (mean) 2.4 (mean)	n.d. n.d.	Ellen and Schuller, 1983 Ellen and Schuller, 1983
Other Cured Meat	n.d. (<1)		n.d. (<1)	Massey et al, 1988; Sen et al, 1979; Gough et al, 1978; Nitrite Safety Council, 1980
(air dried)	≤ 54			
(smoke-dried)	3.8 (mean)	9.1 (mean)	n.d.-12 n.d.	Aidjanov and Sharmanov, 1982 Ellen and Schuller, 1983
Sausages (various)	0.2-10.0 9.7 (mean)	n.d.-10.0	n.d.-10.0	Karmysheva et al, 1981 Hu and Song, 1984
(smoked)	2.5-9.7			
(boiled)	n.d.-0.1			Rutchkovsky et al, 1981
Dairy Products				
Cheese	n.d.-20 1-5* n.d.-1.2	n.d.-20		Sen et al, 1978 Danish Food Institute, 1981 Fromberger and Almann, 1983
	n.d.-2.5	n.d.-2.3 n.d.-0.2		Yamanoto et al, 1984 Karlowski and Bojewski, 1987

* occasionally higher

Table 2. Nitrosamine Content of Food Stuffs (ug/kg) (cont/d)

FOOD	NDMA	NDEA	NPYR	REFERENCE
Dairy Products				
Milk	0.05-0.17			Lakritz and Pensabene, 1981
Milk Products	<0.1-0.7			Song and Hu, 1988
Dried Milk	n.d.-4.9*			Fromberger and Allmann, 1983
	<0.1-0.8			Karlowski and Bojewski, 1987
Fish				
	n.d.-10			Gough et al, 1978; Webb and Gough, 1980
	n.d.-9.2			Budevska et al, 1988
(dried)	10.8-14.3	11.4-14.6		Yamamoto et al, 1984
	0.1-36	0.1-36		Lu and Li, 1986
	n.d.-14*			Pourier et al, 1987
(dried, broiled)	9.6-29.7	20.8-36.6		Yamamoto et al, 1984
(smoked)	n.d.-10.5			Karlowski and Bojewski, 1987
Fish Products				
(dried)	5.9-34.3*			Yamamoto et al, 1984
	0.1-36	0.1-36		Lu and Li, 1986
Beer				
	0.4-7.0			Goff and Fine, 1979
	2.5-2.7 (mean)			Spiegelhalder et al, 1979;
				1980 a,b
	n.d.-7			FDA, 1980
	5.9 (mean)			Scanlan et al, 1980
	n.d.-5.7			Stephany and Schuller, 1980
	n.d.-13			Harvery et al, 1981
	n.d.-1.0			Bogovski et al, 1982
	0.44 (mean)			Spiegelhalder, 1983
	0.1-7.4			Song and Hu, 1988
Dark beer, "Rauchbier"	up to 63			Spiegelhalder, 1980 a,b
Spices	n.d. -51		n.d.-79	Spiegelhalder, 1983

* occasionally higher

Table 3. Volatile N-Nitrosamines in Tobacco Smoke (ng/cigarette)

Source of Smoke	NDMA		NMEA		NDEA		NPYR	
	MS	SS	MS	SS	MS	SS	MS	SS
U.S. commercial cigarettes	6-65	680-1040	0.4-8	9-30	1-8	8-73	5-33	205-390
French cigarettes with cellulose-acetate filter	4	-	0.5	-	0.1	-	11	-
French cigarettes without filter	29	-	3	-	0.6	-	25	-
Low-nitrate experimental cigarettes	10-20	-	0.1-1.2	-	2-3	-	3-6	-
High-nitrate experimental cigarettes	76-97	-	5-9	-	3-5	-	32-52	-
Small cigars	43	1770	0.4	75	1	29	5	612

Values according to Brunnemann and Hoffmann, 1977; Hoffmann et al, 1980 and 1981.

MS = Main Stream

SS = Side Stream

Table 4. Tobacco-Specific Nitrosamines in Tobacco and Tobacco Smoke

Source	Tobacco (mg/kg)			Main-stream Smoke ($\mu\text{g}/\text{cigarette}$)				Source
	NNN	NNK	NAT	NNN	NNK	NAT		
U.S. commercial cigarettes	1.4-1.7	0.7	1.3-1.6	0.24-0.31	0.1-0.15	0.3-0.4		a
French cigarettes	2.7-11.9	0.4-1.1	1.5-2.0	0.5-3.2	0.1-0.4	0.2-0.6		a
German cigarettes	0.02-8.85	<0.05-1.4		0.005-0.625	<0.004-0.43			b
Low-nitrate experimental cigarette	0.2-0.6	0.1-0.4	0.4-0.6	0.4-0.6	0.16-0.4	0.4-0.5		a
High-nitrate experimental cigarette	7	n.d.	3.2	3.7	0.32	4.6		a
Little cigar	45	35	13	5.5	4.2	1.7		a
Columbia cigar (5.7 g)	11	1	3	3.2	1.9	1.9		a

Sources: a: Hoffmann et al (1980)

b: Fischer et al (1989b,c)

Table 5: N-Nitroso Compounds in Oral Tobaccos

Nitrosamine	Mean Concentration (µg/kg)		
	English moist snuff	Swedish moist snuff	Indian zarda
NDMA	40	1.5	11
NEMA	1.5	n.d.	1.0
NPIP	20	n.d.	0.3
NPYR	270	5.0	100
NMOR	0.5	1.0	n.d.
NDLA	230	19	9.5
NAB/NAT	27,620	2,640	16,030
NNN	22,670	3,360	13,420 a)
HNK	4,900	790	13,420 a)
others	12,985	2,753	6,934
TOTAL	68,900	9,570	40,500

According to Tricker and Preussmann (1989b).

n.d. = not detected

a) This value given in the original paper probably must read: 3994.

Table 6. MDELA CONTENT IN PERSONAL CARE PRODUCTS (1976-1988)

Product	No. Positive samples	MDELA content ug/kg	Analytical method	Detection Limit ug/kg	Time of sample	References
Cosmetic Lotion	6/7	41-47000	TEA/HPLC	10	1976/7	Fan et al, 1977
Shampoo	7/13	5-140			1976/7	
	7/9	5-260			1976/7	
Face cream	8/10	100-350	GLC		1981	Rollmann et al, 1981
Shampoo	3/7	46-4113	TEA/HPLC	10	1980/81	Klein et al, 1981
Bath mousse	1/1	26.5				
Liquid soaps	0/1	-				
Creams	0/1	-				
Shampoo	14/5	150 (Average)	TEA/GLC	1-3	1981/82	Spiegelhalder and Preussmann, 1984
Colour toners	2/7	4				
Foam bath	2/7	90				
Cream bath	1/8	43				
Shower gel	2/9	80				
Body lotions	1/6	50				
Cosmetics	1/29	200				

Table 6. NDELA CONTENT IN PERSONAL CARE PRODUCTS (1976-1988) (cont/d)

Product	No. Positive samples	NDELA content ug/kg	Analytical method	Detection Limit ug/kg	Time of sample	References
Bubble bath	5/5	1280-41800	TEA/HPLC		1979/83	CTFA, 1984
Shampoo	0/3	-				
Cosmetic creams	1	140				
Shampoo	7/7	93-650	-		before 1983	IKW, 1986
Shampoo	3/4	12-50	-		after 1983	
Bath lotions	3/3	78-1334	-		before 1983	
Bath lotions	1/2	20	-		after 1983	
Body lotion	1/1	200	-		before 1983	
Body lotion	1/1	50	-		after 1983	
Cosmetics	2/2	153-3730	-		before 1983	
Cosmetics	0/2	-	-		after 1983	
Contact mucous membrane	0/9	-	-	10	1979	COLIPA, 1986
Non rinse	0/5	-	-			
Rinse off shampoo	3/5	75-700	-			
Soap	0/5	-	-			
Hair dyes	0/3	-	-			
Shower gel	3/5	16-126	-			
Haircare	0/10	-	-			
Shampoos	7/15	8-41	TEA/HPLC	5	1988	IKW, 1989
Skin lotions	2/35	10-53				
Shower gel	5/16	5-13				
Hair care	1/12	22				
Sunscreens	1/25	10				
Deodorants	0/7	-				

Table 7. Percentage (of dose) found in urine after dermal application of NDELA

Species	Dose (1)	% (of dose) found in urine	Reference
Pig	about 12-60 ug (2) (in skin lotion)	4% within 5 days	Marzulli et al (1981) (3)
Monkey	about 12-60 ug (2) (in skin lotion)	23.4% within 5 days	
Rat	0.5 or 50 mg(2) (in acetone)	3 % within 24 h 20 % within 96 h 50 % within one week	
Rat	50 mg (no vehicle)	20-30% within 24 h	Lijinsky et al (1981a)
Rat	5 mg/kg b.w. (in water-acetone) mixture	25 % within 24 h	Airolidi et al (1984a)
Rat	50 mg (no vehicle) 25 mg (in cutting oil)	20-30% within 24 h 12 % within 24 h	Sansone et al (1980)
Rat	28-184 μ g (in water or acetone)	70-80% within 24 h	Preussmann et al (1981)
Man	980 μ g (via cosmetic formulation)	2% within 20 h	Edwards et al (1979)

1: per animal, if not indicated otherwise

2: radiolabelled NDELA

3: monkey treated at abdominal skin which is more permeable than back skin

Table 8. Percentage (of dose) found in urine after oral administration of NDELA

Species	Dose	% (of dose) found in urine	Reference
Rat	0.5 mg/kg bw* 50 mg/kg/bw*	67 % within 24 h 87 % within 24 h	Lethco <u>et al</u> (1982)
Rat	50 mg/rat	30 % within 24 h	Sansone <u>et al</u> (1980)
Rat	10-1000 mg/kg bw	70 % within 24 h	Preussmann <u>et al</u> (1978)
Rat	0.03-300 µg/kg bw	60-80% within 24 h	Spiegelhalder <u>et al</u> (1982)

* radiolabelled NDELA

Table 9. Acute lethality of a number of N-nitrosamines

N-Nitroso Derivative	MW	Species	Route	LD50 (mg/kg)	Reference
NDELA	134	Rat	Oral	7500	Druckrey <u>et al</u> (1967)
NDMA	74	Rat	IP	26.5	Barnes & Magee (1954)
		Rat	Oral	<50	Barnes & Magee (1954)
		Rat	Inh.	78*	Jacobson <u>et al</u> (1955)
		Mouse	Inh.	57*	Jacobson <u>et al</u> (1955)
NDEA	102	Rat	IV	280	Druckrey <u>et al</u> (1967)
		Rat	IP	216	Heath (1962)
		Rat	Oral	280	Druckrey <u>et al</u> (1963)
		Rat	Sc	204	Althoff <u>et al</u> (1985)
		Guinea Pig	Oral	250	Druckrey <u>et al</u> (1967)
		Hamster	Sc	246	Mohr <u>et al</u> (1972)
Nitrosos-isopropylamine	130	Rat	Oral	850	Druckrey <u>et al</u> (1967)
Nitrosomethyl-vinylamine	86	Rat	Oral	24	Druckrey <u>et al</u> (1967)
Nitrosoethyl-vinylamine	100	Rat	Oral	88	Druckrey <u>et al</u> (1967)
		Hamster	Sc	109	Althoff <u>et al</u> (1977)
NPYR	100	Rat	Oral	900	Druckrey <u>et al</u> (1967)
		Hamster	Oral	1023	Ketkar <u>et al</u> (1982)
NPIP	114	Rat	Oral	200	Druckrey <u>et al</u> (1967)
		Rat	Sc	100	Druckrey <u>et al</u> (1967)
		Rat	IV	60	Druckrey <u>et al</u> (1967)
		Hamster	Oral	617	Ketkar <u>et al</u> (1983a)
		Hamster	Sc	324	Althoff <u>et al</u> (1974)
NMOR	116	Rat	Oral	320	Druckrey <u>et al</u> (1967)
		Rat	IV	98	Druckrey <u>et al</u> (1967)
		Hamster	Oral	956	Ketkar <u>et al</u> (1983b)
		Hamster	Sc	491	Althoff <u>et al</u> (1974)

*4-hour LC50, ppm

Table 10. Tumorigenic Responses of Various N-Nitrosamines in Animal Studies
(* for calculation of dose level see Appendix 1)

SPECIES	ROUTE	DOSE LEVEL	DOSE LEVEL* mg/kg/day	DOSE DURATION	TARGET ORGAN/TUMOUR	STUDY DURATION TIME TO TUMOUR	REFERENCE
NDMA							
Mouse	DW	50 mg/L	1.25	1 week	kidney; lung	-/30-60 wks	Terracini et al, 1966
Mouse	DW	0.4mg/kg/d 0.91 1.8	0.4 0.91 1.8	400 d 266 d 49 d	13/17 lung; 2/10 liver 91/92 lung; 89/93 liver 82/83 lung; 9/86 liver	400 d/- 266 d/- 49 d/-	Clapp & Ioya, 1970
Rat	diet/DW	50-100mg/kg	4	long-term	liver		IARC 17, 1978
Rat	diet	100-500mg/kg	30		kidney		IARC 17, 1978
Rat	diet	2mg/kg 5 10 20 50	0.144 0.36 0.72 1.44 3.60	52 weeks	2.7% liver 7.4% liver 40% liver 65% liver 83% liver	104/above 60 wks 104/above 60 wks 104/above 60 wks 104/under 60 wks 104/under 60 wks	Terracini et al, 1967
Rat	DW	45mg/kg	3	5d/week x30	27% liver	104/-	TRGS 552; 1988
Rat	DW	1.3 mmol/L	0.38	31 weeks	100% liver	long-term/31 wks	Lijinsky, 1987
Rat	DW	13 mg/L	0.32	30 weeks	17/20 liver 20/20 lung 1/20 Oesoph 1/20 forestomach	30/80 wks	Lijinsky & Reuber 1984b
		5.5mg/L	0.14	30 weeks	14/20 liver 1/20 lung	30/100 wks	

Table 10. Tumourigenic Responses of Various N-Nitrosamines in Animal Studies (cont/d)

SPECIES	ROUTE	DOSE LEVEL	DOSE LEVEL mg/kg/day	DOSE DURATION	TARGET ORGAN/TUMOUR	STUDY DURATION TIME TO TUMOUR	REFERENCE
MDMA							
Rat	DW	33mg/L	0.82	30 weeks	18/20 liver 17/20 lung 5/20 leukaemia	30/40 wks	Lijinsky & Reuber, 1981a
Rat	Gavage	1.6 nmoles	0.33	45 weeks	80% lung; 50% kidney, liver and nasal	long-term/45 wks	Lijinsky, 1987
Hamster	DW	25mg/L	2.5	11 weeks	liver	-	IARC 17, 1978
Mouse	inh	0.2 mg/m ³ 0.005 mg/m ³	-	Daily for 17 months	lung; liver; kidney no effect	17 months 17 months	Moiseev & Benemansky, 1975
Rat	inh	4mg/kg	-	30 mins 2x/week	70% nasal	-	Druckrey et al, 1967
Mouse	sc	1 mg/kg 2 mg/kg 4 mg/kg 8 mg/kg	-	single	29% lung 35% lung 39% lung 67% lung	-	Cardesa et al, 1974
Rat	lm	10-30mg/rat	-	286-369 d	38% kidney	Single/286-369 days	IARC 17, 1978
Hamster	sc	0.5-1.0	-	6-20 weeks	liver	-	Herrold, 1967
Mouse	ip	7 or 14 mg/kg	-	single	lung	-	IARC 17, 1978

Table 10. Tumorigenic Responses of Various N-Nitrosamines in Animal Studies (cont/d)

SPECIES	ROUTE	DOSE LEVEL	DOSE LEVEL mg/kg/day	DOSE DURATION	TARGET ORGAN/TUMOUR	STUDY DURATION TIME TO TUMOUR	REFERENCE
NDMA							
Mouse	ip	6 mg/kg/week	-	weekly x10	vascular	-	IARC 17, 1978
NDEA							
Mouse	oral	2-13	2-13	long-term	100% liver		Clapp & Taya, 1970
Rat	DW	1 mg/kg/day	-	134 days	23/25 liver		Hadjilolow, 1972
Rat	DW	0.1mg/kg/day	-	lifetime	45% liver 31% GI 1.3% urinary	LT/900 d	Berger et al, 1987
		0.032mg/kg/d	-	lifetime	3.8% liver 8.8% GI 13.8% neurogenic 1.3% urinary	LT/900 d	
		0.01mg/kg/d	-	lifetime	2.5% liver 11.3% GI 12.5% neurogenic 2.5% urinary	LT/900 d	
Hamster	gavage	0.08 mmole	0.12	43 weeks	100% liver 23% nasal	43/43	Lijinsky, 1987
Rat	Diet	0.0114%		26 weeks	24% Oesophagus	28 weeks	Reuber, 1975

Table 10. Tumourigenic Responses of Various N-Nitrosamines in Animal Studies (cont/d)

SPECIES	ROUTE	DOSE LEVEL	DOSE LEVEL mg/kg/day	DOSE DURATION	TARGET ORGAN/TUMOUR	STUDY DURATION TIME TO TUMOUR	REFERENCE
NDEA							
Rat	DW	1.0 mmole	2	26 weeks	65% liver 95% oesophagus	26/26	Lijinsky, 1987
Rat	DW	0.45mg/L	1.35mg/rat	30 weeks	1/20 oesophagus 3/20 GI 1/20 liver	30/120 weeks	Lijinsky et al, 1981c
Rat	DW	0.45mg/L	2.7mg/rat	60 weeks	3/20 oesophagus 10/20 GI 6/20 liver	60/120 weeks	
Rat	DW	0.45mg/L	4.6mg/rat	104 weeks	13/20 oesophagus 20/20 GI 4/20 liver	104/120 weeks	
Rat	Diet		1-10mg/kg/d	life-time	100% tumours, liver and bile		IARC 17, 1978
Rat	DW	0.15mg/kg/d		life-time	90% liver; 7% lung 75% oesophagus		Druckrey et al, 1963
Monkey	oral	2-30mg/kg/d		long-term	3/15 liver;	14-24 months	Kelly et al, 1966
Mouse	sc	50mg/kg		2x/week; 4-8 times	18/33 trachea or lungs		Mittrich et al, 1971

Table 10. Tumorigenic Responses of Various N-Nitrosamines in Animal Studies (cont/d)

SPECIES	ROUTE	DOSE LEVEL	DOSE LEVEL mg/kg/day	DOSE DURATION	TARGET ORGAN/TUMOUR	STUDY DURATION TIME TO TUMOUR	REFERENCE
NPYR							
Rat	DW	200mg/kg	0.3	long-term	5% liver		TRGS 552, 1988
Rat	DW	0.4mg/kg/d	-	lifetime	21.3% liver 7.5% GI 12.5% neurogenic	LT/900 d	Burger et al, 1987
		0.13mg/kg/d	-	lifetime	5% liver 8.8% GI 6.3% lymphatic 1.3% urinary	LT/900 d	
		0.04mg/kg/d	-	lifetime	1.3% liver 7.5% GI 7.5% lymphatic 2.5% urinary	LT/900 d	
Hamster	DW	100mg/kg 400mg/kg	2mg/kg, wk 8mg/kg/wk	50 weeks 50 weeks	2% liver 5% liver		TRGS 552, 1988
Rat	DW	4.5 amoles	2.3	101 weeks	100% liver	101 weeks 12/12	Lijinsky, 1987
Mouse	DW	0.01%	12.5	5x/week x12	12% lung	12 weeks	Greenblatt & Lijinsky, 1972
Rat	DW		5 mg/kg/d 10mg/kg/d	lifetime	92% liver	290 d 470 d	Druckrey et al, 1967

Table 10. Tumorigenic Responses of Various N-Nitrosamines in Animal Studies (cont/d)

SPECIES	ROUTE	DOSE LEVEL	DOSE LEVEL mg/kg/day	DOSE DURATION	TARGET ORGAN/TUMOUR	STUDY DURATION TIME TO TUMOUR	REFERENCE
MMOR							
Rat	DW	1.7 nmoles	2.16	50 wks	100% liver 60% oesophagus 20% nasal	52 weeks	Lijinsky, 1987
Rat	DW	0.45mg/L 0.45mg/L 6.4 mg/L 6.4 mg/L	2.25mg/rat 4.5mg/rat 32 mg/rat 64 mg/rat	150 wks 100 wks 50 wks 100 wks	6/48 liver 7/47 liver 14/24 liver 23/24 liver	50/109 weeks 100/110 weeks 50/97 weeks 100/97 weeks	Lijinsky et al, 1988
Hamster	Gavage	1.2 nmoles	3.8	26 wks	75% nasal 30% trachea	35 weeks	Lijinsky, 1987
Mouse	DW	16mg/kg/d	16	lifetime	27% liver lung	lifetime	Bennasch and Mueller, 1964
Mouse	DW	0.2umole	2.16	10 weeks	100% liver lung		Hecht et al, 1989
Hamster	SC	25mg/kg 50 100	-	weekly for life	69-100% nasal lung	lifetime	Haas et al, 1973
Rat	DW	1.1 mmole	1.1	50 weeks	100% liver lung		

Table 10. Tumorigenic Responses of Various N-Nitrosamines in Animal Studies (cont/d)

SPECIES	ROUTE	DOSE LEVEL	DOSE LEVEL mg/kg/day	DOSE DURATION	TARGET ORGAN/TUMOUR	STUDY DURATION TIME TO TUMOUR	REFERENCE
HHN							
Rat	DW	9 nmoles	21	43 weeks	100% nasal	43 weeks	Lijinsky, 1987
Rat	DW	9.6mg/kg/d	-	36 weeks	100% oesophagus 100% nasal	36/4wks	Wecht et al, 1983
Rat	DW	0.02%	17	5d/wk x 30wk	oesophagus 100% 1/12 lung 3/12 nasal no tumours	44 weeks	Hoffmann et al, 1975
Mouse	Topical	30ug/ application		3d/wk x 50		-	Hoffmann et al, 1976
Hamster	SC	5mg/animal	25	3d/wk x 25	12/15 trachea	38 weeks	Wittlich et al, 1977
				total = 375mg/ animal	1/19 nasal		

Table 11. Tumorigenic Responses of NDELA in Animal Studies

SPECIES	ROUTE	DOSE LEVEL	DOSE LEVEL mg/kg/day	DOSE DURATION	TARGET ORGAN/TUMOUR	STUDY DURATION TIME TO TUMOUR	REFERENCE
NDELA							
Rat	DW	400 mg/L; 2g/rat	24	5d/wk/50	100% liver, 53% nasal	lifetime/-	Lijinsky and Reuber, 1984a
		400 mg/L; 3g/rat	24	5d/wk/75	100% liver, 50% nasal	lifetime/-	
		1000 mg/L; 5g/rat	60	5d/wk/50	100% liver, 28% nasal	lifetime/-	
		2500 mg/L; 11g/rat	120	5d/wk/45	100% liver, 55% nasal	lifetime/-	
Rat	DW	860 mg/kg 3740 mg/kg	1.5 6	82 weeks 82 weeks	10% liver 60% liver, 3% nasal	long-term/-	TRGS 558; 1988
Rat	DW	22 nmoles	15	long-term	100% liver; 56% nasal	-/81 weeks	Lijinsky, 1987
Rat	DW	2mg/kg/d		lifetime	7.5% liver 7.5% GI tract 13.8% neurogenic 8.8% lymphatic	LT/1000 d	Berger et al, 1987
		0.63mg/kg/d		lifetime	1.3% liver 20% neurogenic 6.3% lymphatic	LT/1000 d	
		0.2mg/kg/d		lifetime	2.5% liver 8.8% GI 10% lymphatic	LT/1000 d	

Table 11. Tumorigenic Responses of NDELA in Animal Studies (cont/d)

SPECIES	ROUTE	DOSE LEVEL	DOSE LEVEL mg/kg/day	DOSE DURATION	TARGET ORGAN/TUMOUR	STUDY DURATION TIME TO TUMOUR	REFERENCE
NDELA							
Rat	DW	3900ppm 31250ppm	3900 31250	34 weeks	100% liver 100% liver	34/34 wk	Lijinsky et al, 1980
Rat	Gavage	22 mmols	32	long-term	60% liver, 100% nasal, 50% oesophagus, 20% tongue	-/40 weeks	Lijinsky, 1987
Rat	DW	3900 ppm 7800 ppm 15,600 ppm 31,250 ppm 62,500 ppm		5d/wk x 34	100% liver 100% liver 100% liver 100% liver Dead	40/30-40 wks 30-34 wks	Lijinsky et al, 1980
Hamster	SC	58 mg/kg 170 mg/kg 500 mg/kg		1x weekly x27	0% nasal; 7% trachea; 0% larynx 24% nasal; 14% trachea; 0% larynx 63% nasal; 23% trachea; 7% larynx	20 months/-	Hoffmann et al, 1983
Hamster	SC Dermal	2.5 mg 8 mg 25 mg 20 mg		3x weekly x36	0% nasal; 7% trachea; 0% larynx 24% nasal; 3% trachea; 0% larynx 17% nasal; 13% trachea; 3% larynx 44% nasal; 16% trachea; 3% larynx	20 months/-	Hoffmann et al, 1983
	Oral cavity			3x weekly x45			

Table 11. Tumourigenic Responses of MDELA in Animal Studies (cont/d)

SPECIES	ROUTE	DOSE LEVEL	DOSE LEVEL mg/kg/day	DOSE DURATION	TARGET ORGAN/TUMOUR	STUDY DURATION TIME TO TUMOUR	REFERENCE
MDELA							
Hamster	Gavage	14 mmoles	23	life-time	90% nasal	-/35 weeks	Lijinsky, 1987
Hamster	SC	250 mg/kg	-	1 x weekly	48% nasal; 7% trachea 11% larynx	lifetime/-	Mallcave & Pour, 1981
		500 mg/kg			50% nasal; 16% trachea 7% larynx	lifetime/-	
		1000 mg/kg			73% nasal; 31% trachea; 32% larynx	lifetime/-	
Rat	DW	160 mg/L	12.8	50 wks	85% liver; 9% kidney		
		64 mg/L	5.1	50 wks	17.5% liver; 2.5% kidney	lifetime/100wk	Lijinsky and Kovatch, 1985
		64 mg/L	5.1	100 wks	62.5% liver; 7.5% kidney		
		28 mg/L	2.2	100 wks	20.5% liver; 1.3% kidney		
Rat	DW	1.5 mg/kg/d	1.5	lifetime	10% liver; 3% nasal	lifetime/801 d	Preussmann et al, 1982b
		6	6		60% liver; 0% nasal	lifetime/809 d	
		25	25		42% liver; 17% nasal	lifetime/624 d	
		100	100		89% liver; 17% nasal	lifetime/465 d	
		400	400		86% liver; 3% nasal	lifetime/351 d	
Rat	DW	0.2mg/kg/d	1.2	24 month	> effects at higher dosages	24 month/21 month	Zerban et al, 1988
		0.63	0.33		> Due to the design of the		
		1.5	1.5		> study the incidence		
		6	6		> cannot be calculated		
		25	25				
Mouse	DW	0.2um/ml	5.4	10 weeks	70% lung other organs not examined		Hecht et al, 1989
Rat	DW	5.6mmole	6.4	50 weeks	75% liver 5% lung		Hecht et al, 1989

Table 12. Mutagenicity of NDMA, NDEA, NMOR, NPIP and NNN

Test system	NDMA	NDEA	NMOR	NPIP	NNN
Standard bacterial mutation	+	+	+	+	+
In vitro cytogenetics	+	+	+	n.d.	n.d.
Gene mutation in mamm. cells	+	+	+	+	n.d.
Unscheduled DNA synthesis	+	n.d.	n.d.	n.d.	+
DNA strand breaks	n.d.	n.d.	n.d.	n.d.	n.d.
Host-mediated assay	+	+	+	n.d.	n.d.
Metaphase analysis in vivo	+	n.d.	n.d.	n.d.	n.d.
Micronucleus test	+	n.d.	n.d.	n.d.	n.d.
Dominant lethal test	-	-	-	n.d.	n.d.
Mouse spot test	(+)	(+)	n.d.	n.d.	n.d.

Table 13. Mutagenicity of NDELA and its metabolites

Test system	NDELA	Metabolites
Standard bacterial mutation	+/-	+/-
Bacterial mutation + microsomes and ADH/NAD	+	+
In vitro cytogenetics	+	+
Gene mutation in mamm. cells	n.d.	n.d.
Unscheduled DNA synthesis	n.d.	n.d.
DNA strand breaks	+	+
Metaphase analysis in vivo	-	n.d.
Host-mediated assay	+	n.d.
Micronucleus test	-	n.d./
DNA strand breaks	+	+
Dominant lethal test	n.d.	n.d.
Mouse spot test	n.d.	n.d.

n.d. = no data available

Table 14. Correlation Between High Nitrate Burden and Gastric Cancer Risk
(As modified from Preussmann and Tricker, 1989)

Country	High Nitrate Source	Correlation	Reference
CHILE	Vegetables and drinking water due to high use of nitrate fertilizers	Positive	Zaldivar and Robinson (1973)
		Positive	Zaldivar (1977)
		Positive	Armijo a.Coulson(1975)
		Negative	Zaldivar and Wetterstrand (1978)
		Negative	Armijo <u>et al</u> (1981)
CHINA	Vegetables due to low molybdenum content of soil. Drinking water	Positive	Xu (1981)
COLUMBIA	Vegetables and drinking water due to high nitrate content of soil	Positive	Cuello <u>et al</u> (1976)
DENMARK	Drinking water	* Inconsistent	Jensen (1982)
ENGLAND	Drinking water	Negative	Davies (1980)
		Negative	Beresford (1981)
		Negative	Foreman <u>et al</u> (1985)
		* Inconsistent	Hill <u>et al</u> (1973)
		* Inconsistent	Fraser and Chilvers (1981)
HUNGARY	Drinking Water	* Inconsistent	Juhaz <u>et al</u> (1980)
ITALY	Drinking water	* Inconsistent	Amadori <u>et al</u> (1980)
JAPAN	Vegetables due to high nitrate content of soil	Negative	Kamiyama <u>et al</u> (1987)

* Inconsistent is defined as being unable to reach a conclusion because of

- a) varying incidence in men and women
- or b) varying incidence with time
- or c) the data set was limited.

Table 15. Summary of Rat Carcinogenicity Data Used in the Pair-by-Pair Comparison Ranking Exercise

N-NITROSAMINE	DOSE LEVEL (MG/KG/DAY)	DOSE DURATION (WEEKS)	AVERAGE TIME TO DEATH WITH TUMOUR (WEEKS)	% OF ANIMALS WITH TUMOURS	REFERENCE
NDMA	2.6	30	31	Liver 90	A
	1.0	30	75	Liver 90	B
	0.44	30	95	Liver 65	B
NDEA	3.6	22	26	Oesophagus 95/ Liver 65	A
	1.4	30	35	Oesophagus 95/ Liver 5	C
	0.56	30	50	Oesophagus 85/ Liver 5	C
NPYR	7.2	50	101	Liver 95	D
	0.4	170	135	Liver 95	E
NMOR	3.2	40	52	Liver 96	F
	0.5	50	97	Liver 58	F
	0.5	100	97	Liver 96	F
NNN	9.6	36	41	Oesophagus 100 /Nasal 100	G
NDELA	12.8	50	110	Liver 85	H
	5.1	50	105	Liver 17.5	H
	2.2	100	105	Liver 20.5	H

A Lijinsky and Reuber 1981a
 B Lijinsky et al, 1981b
 C Lijinsky et al, 1981c
 D Berger et al, 1987
 E Lijinsky and Reuber 1984b
 F Lijinsky and Reuber 1981b
 G Lijinsky et al, 1988

Table 16. Ranking of N-Nitrosamines in Order of Carcinogenic Activity: Pair-by-Pair Comparison

COMPARISON OF	COMPARISON WITH:						SUMMATION OF SCORES
	NDELA	NMOR	NDMA	NDEA	NNN	NPYR	
NDELA	-	0	0	0	0	0	0
NMOR	1	-	0	0	1	1	3
NDMA	1	1	-	$\frac{1}{2}$	1	1	$4\frac{1}{2}$
NDEA	1	1	$\frac{1}{2}$	-	1	1	$4\frac{1}{2}$
NNN	1	0	0	0	-	0	1
NPYR	1	0	0	0	1	-	2

Relative Ranking: NDEA = NDMA > NMOR > NPYR > NNN > NDELA

Table 17. Literature Used in Potency Ranking Exercise (TD₅₀ Values)

COMPOUND	REFERENCE
Nitrosodiethylamine	Lijinsky <u>et al</u> , 1981c Berger <u>et al</u> , 1987
Nitrosodimethylamine	Lijinsky and Reuber 1984b Lijinsky and Reuber 1981a
Nitrosopyrrolidine	Lijinsky and Reuber 1981b Berger <u>et al</u> , 1987
Nitrosomorpholine	Lijinsky <u>et al</u> , 1988
Nitrosodiethanolamine	Lijinsky and Kovatch 1985 Preussman <u>et al</u> , 1982b Zerban <u>et al</u> , 1988 Berger <u>et al</u> , 1987

Table 18. Use of personal care products and Toiletries*

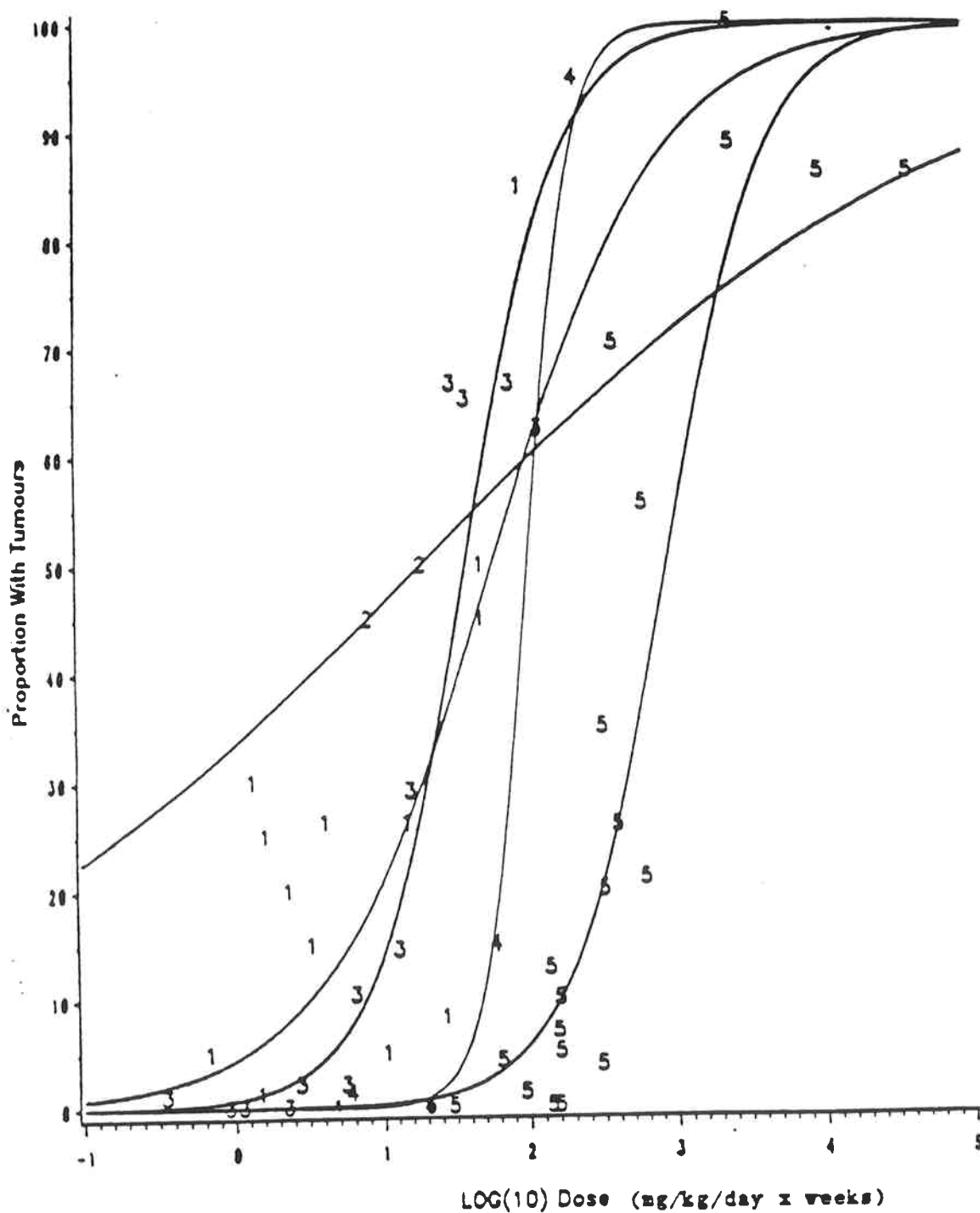
PRODUCT TYPE	AVERAGE QUANTITY PER APPLICATION (grams)	FREQUENCY OF APPLICATION
I. MUCOUS MEMBRANE CONTACT		
Toothpaste	1.5	1-2/day
Mouthwash (ready to use)	12	1-3/day
Eye make-up: powder	0.01	1-3/day
mascara	0.025	1/day
liner	0.005	1/day
Eye make-up remover (wiped off)	0.5	1-2/day
Lipstick	0.01	2-6/day
II. NON-RINSE PRODUCTS		
Face cream	0.8	2/day
After-shave	1.2	1-2/day
General purpose cream	1mg/cm2	1-2/day
Body lotion	7.5	1-2/day
Setting product	12	1-2/week
Hairspray (as sprayed)	10	1-2/day
Temporary hair dye	12	1-2/week
Toilet water	0.75	1-5/day
Talcum powder	2.5	1-2/day
Anti-perspirant	3	1/day
deodorant spray (as sprayed)		
Make-up remover	2.5	1-2/day
Anti-perspirant/deodorant: others	0.5	1-3/day
Nail product	0.25	2-3/week
Sun cream	8}	{2-3/day for 2 weeks/year+ 1 week in winter, on face
Sun lotion	10}	
III. RINSE-OFF PRODUCTS		
Shaving cream	2	1/day
Soap bar	0.8	3-6/day
Foam bath (undiluted)	17	1-2/week
Shower gel	10	1-4/week
Shampoo	12	1-7/week
Hair conditioner	14	1-3/week
Semi-permanent hair dye	30	8-18/year
Permanent hair dye (ready to use)	50	8-12/year

* Source COLIPA

Table 19. Average Uptake of Total N-nitrosamines Through the Diet in Various Countries (ACS, 1984)

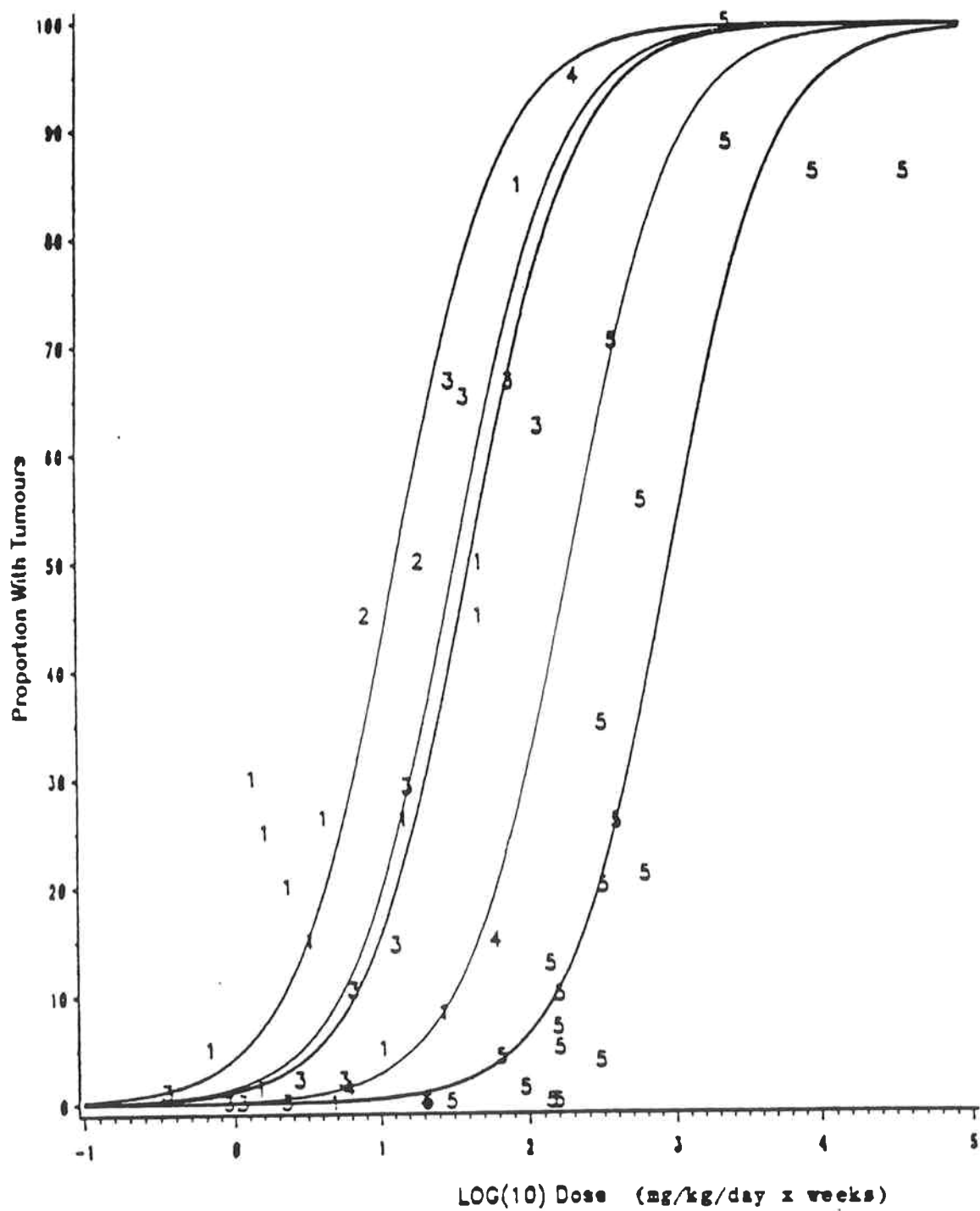
Country	Uptake ($\mu\text{g}/\text{person}/\text{day}$)
Sweden	0.3
Great Britain	0.5 (excluding beer)
The Netherlands	1.1
Germany	1.1 (estimation 1978/79)
Germany	0.6 (estimation 1981)
Japan	1.8

Fig 1 DOSE RESPONSE RELATIONSHIPS FOR LIVER CARCINOGENICITY OF N-NITROSAMINES (UNEQUAL SLOPES)



KEY: NDEA : 1
 NDMA : 2
 NMOR : 3
 NPYR : 4
 NDELA : 5

Fig 2 DOSE RESPONSE RELATIONSHIPS FOR LIVER CARCINOGENICITY OF N-NITROSAMINES
(SLOPES ASSUMED EQUAL)



KEY: NDEA : 1
NDMA : 2
NMOR : 3
NPYR : 4
NDELA : 5

Fig. 3. Metabolism of NDEA as an Example for Most N-Nitrosodialkylamines

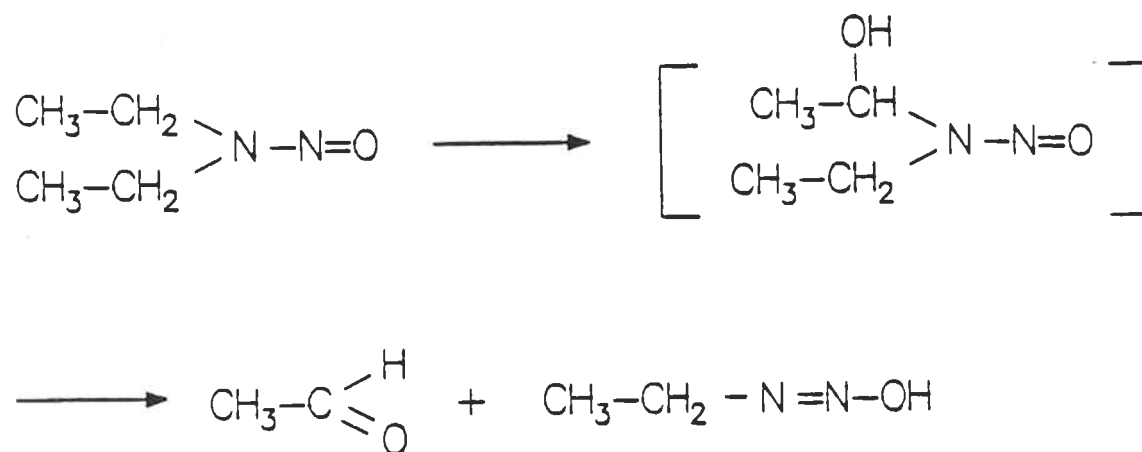
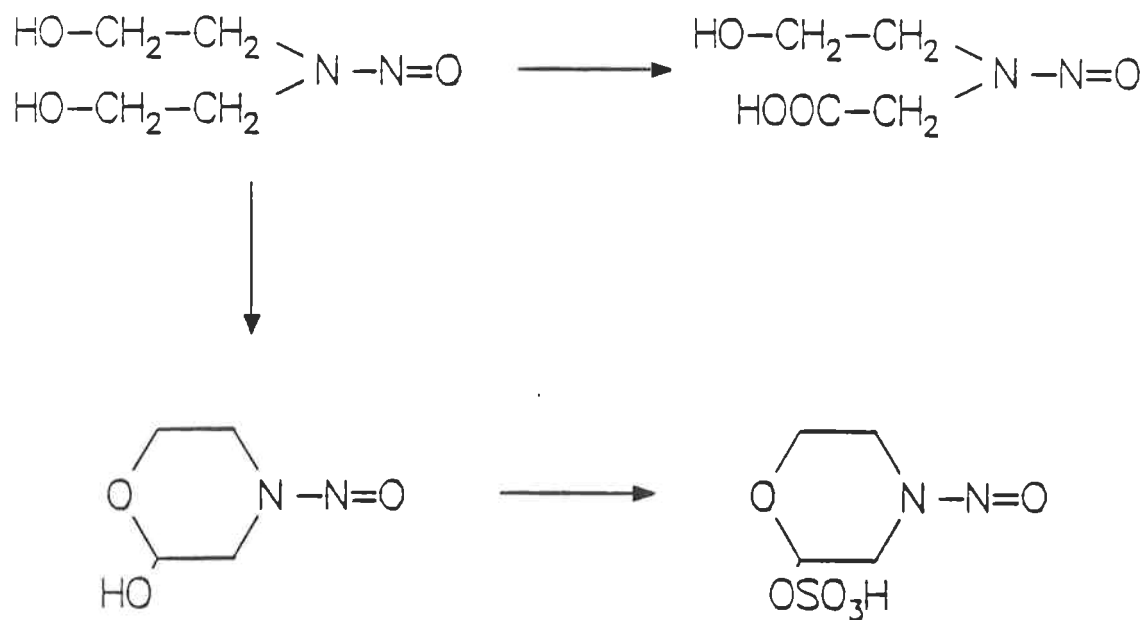


Fig. 4. Metabolism of NDELA



APPENDIX 1

Estimation of Daily Intake from Percentage Active in Diet or Drinking Water.
Basis of calculation of Standardised dose in Table 10.

A 250 g rat will eat about 18 g of food a day, so food intake is 72 g/kg bw/d.

A substance present at 1% in the diet will give a dose equivalent to 720 mg/kg bw/d.

A 250 g rat will drink 20 ml water a day, so water intake is 80 ml/kg bw/d.

A substance present at 1% in the drinking water will give a dose equivalent to 800 mg/kg bw/d.

A 40 g mouse will eat 5 g food a day, so food intake is 125 g/kg bw/d.

A substance present at 1% in the diet will be equivalent to 1,250 mg/kg bw/d.

A 40 g mouse will drink 5 ml water a day, so water intake is 125 g/kg bw/d.

A substance present at 1% in the drinking water will be equivalent to 1,250 mg/kg bw/d.

APPENDIX 2

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