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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 TD_{50} 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 alkylarylnitrosamines 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 $\mu\text{g}/\text{kg}$ (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 µg/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 µg/kg) and NPYR (up to 79 µg/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 µg/kg (Spiegelhalder et al, 1980a,b) and 5.9 µg/kg (Scanlan et al, 1980) with peak levels of 47 µg/litre in dark beer and 68 µg/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 µg/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 µg/litre, averaging less than 1 µg/litre. Thus it can be stated that NDMA levels in beer are now generally well below the level of 5 µg/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 $\mu\text{g}/\text{kg}$ 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 Spiegelhalder, 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.