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Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products

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SUMMARY AND CONCLUSIONS

Methods available for the analysis of N-nitrosamines in personal care and household products have been critically evaluated. The major classes of nitrosamines identified as being important in these products were, steam-volatile short chain and heterocyclic nitrosamines (Group I); other volatile, long chain nitrosamines (Group II) and non-volatile, high polarity nitrosamines (Group III). Methods have been presented for extraction, clean-up and detection of compounds in each group. In many cases several alternative approaches have been described and their relative merits considered.

The sensitivity and specificity of methods together with the sample matrices to which they apply is discussed.

Reliable analysis of N-nitrosamines present in personal care and household products is a technically demanding activity which is fraught with potential pit-falls for the unwary. Extraction and clean-up steps are often the most critical, particularly in the analysis of the Group III compounds. The avoidance of contamination and artefactual formation of nitrosamines in all stages of the analytical method including sampling, storage, clean-up and determination, must be closely monitored.

Long and complicated clean-up operations can lead to a partial loss of the analytes. Validated internal validated standards should be used to test the adequacy of recovery.

The highest levels of analytical sensitivity are achieved using gas chromatography coupled with a thermal energy analyser. It is prudent to confirm results generated in this manner using gas chromatography - mass spectrometry.

Generally applicable limits of determination can be given only for volatile N-nitrosamines (Group I compounds); collaborative studies have shown that limits in the range 1 - 5 µg/kg can be achieved, depending on the compound and the sample matrix.

For N-nitrosodiethanolamine (NDELA), the limits given in the literature are representative only of the specific formulation being analysed. Experience has shown that realistic limits of determination for NDELA in technical alkanolamines are in the range 10 - 100 µg/kg while those for personal care products are in the range 20 - 100 µg/kg.

Thorough validation of methods is particularly important where data are being used for regulatory purposes. Such validation should be carried out on an interlaboratory basis, should be to a statistically based protocol and must conform to internationally accepted standards for analytical methodology.

1. INTRODUCTION

1.1. Terms of Reference and Definitions

Although N-nitrosamines as a chemical class have been known for well over one hundred years, it was not until the early 1950's that the toxicity of this group of chemicals started to be recognised. Since then, many N-nitrosamines have been shown to be potent carcinogens in a variety of animal species. They are now known to occur in a range of materials where human exposure is likely. These include air (both urban and factory), tobacco and tobacco smoke, alcoholic beverages, cured meat products, pesticides, drugs and industrial chemicals. It has also been shown that N-nitrosamines may be present as contaminants in the raw materials used in the manufacture of personal care and household products. Additionally, they may be formed *in situ* during formulation, storage or use of these products. This document presents a critical review of the analytical methods available for the detection and measurement of N-nitrosamines in personal care and household products and their ingredients. The Terms of Reference used by the Task Force were as follows:

- Identify the reliability, scope and limitations for the analytical methods available for the determination of NDELA, volatile and total nitrosamines with particular reference to personal care and household products and their ingredients,

- recommend what further work is required to establish fully validated procedures.

For the purposes of this report the following definitions are used:

Personal care products:

- 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

clean, perfume or protect 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, eg. antiseptics.

Household products:

Products supplied to the general public through normal retail outlets for use within the home. These include materials supplied for cleaning, polishing, disinfecting and deodorising. Agrochemicals (pesticides) used within the home have been excluded from the scope of this definition.

In the presentation of outline analytical methods, particular attention is paid to specificity, sensitivity, limits of determination and the range of substrates to which methods can be applied. Compounds discussed in this text are:

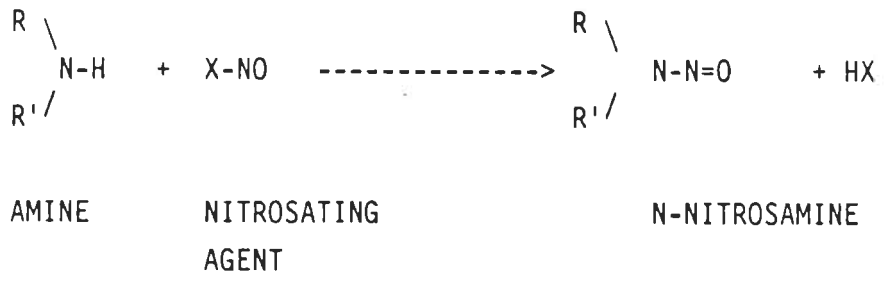
N-Nitroso-		Group *
-dimethylamine	(NDMA)	I
-diethylamine	(NDEA)	I
-morpholine	(NMOR)	I
-piperidine	(NPIP)	I
-pyrrolidine	(NPYR)	I
-dodecylmethylamine	(NDDMA)	II
-tetradecylmethylamine	(NTDMA)	II
-diethanolamine	(NDELA)	III
-diisopropanolamine	(NDIPLA)	III
-dibutanolamine	(NDBLA)	III

* see section 1.3.

Throughout the document concentrations are always given in µg of analyte per kg of sample.

1.2. Formation

The nitrosating species may be derived from oxides of nitrogen, nitrate, nitrite and some aromatic nitro compounds. The yield of nitrosamine depends on the amine, its basicity and stereochemistry, the nitrosating species and on the medium. Rates of reaction are faster for secondary amines than they are for primary or tertiary amines. The overall nitrosation reaction can be represented as follows:



Classically the first step in nitrosation is often considered to be reaction of a nitrite ion (NO_2^-) with protons (H^+ or H_3O^+) to give nitrous acid (HONO). Neither nitrite ion nor nitrous acid are themselves capable of N-nitrosation. The actual nitrosating species are believed to be the nitrous acidium ion (H_2O^+NO), dinitrogen trioxide (N_2O_3) or dinitrogen tetroxide (N_2O_4). Often a mixture of species is involved in the reaction depending on the acidity of the medium. Other factors which influence the formation of N-nitrosamines are: temperature (increasing temperature increases reaction rate), pH (formation favoured in acidic conditions), solubility of the amine substrate, the nature of the medium and the presence of catalysing agents (Williams, 1988; Hill, 1988).

1.3. Classification

Nitrosamines display a wide range of physical characteristics (e.g. volatility, polarity etc) and therefore it is necessary to apply some form of group classification to assist in the selection of isolation and determination procedures. Classification into 3 broad types is appropriate for personal care and household products.
These classes, are as follows:

Group I : steam-volatile short chain (eg. NDMA) and heterocyclic compounds, (eg. NPIP)
Group II : other volatile, long-chain compounds, (eg. NDDMA)
Group III : non-volatile high polarity compounds, (eg. NDELA).

1.4. Occurrence

Ingredients in personal care and household products which might in some circumstances give rise to N-nitrosamines are listed below:

Ingredients	N-nitrosamines expected
Alkanolamines (and their salts)) Alkanolamides)	N-nitrosoalkanolamines, eg. NDELA
Quaternary ammonium compounds	N-nitrosoalkylamines, eg. NDMA (from nitrosation of elimination products)
Betaines	NDMA (from nitrosation of elimination products)
Amine oxides	NDMA (from nitrosation of elimination products) and long-chain asymmetric N-nitrosodialkylamines, eg. NDDMA
Herb or spice extracts	Heterocyclic compounds eg. NPIP

2. METHODS FOR THE ANALYSIS OF N-NITROSAMINES

N-nitrosamines are compounds which are difficult to analyse. It is easy to generate false negative or positive results unless rigorous precautions are applied throughout. The most important are, the use of internal standards, the prevention of artefactual nitrosamine formation and the confirmation of results by different separation and/or detection techniques.

2.1. Steam-volatile, short-chain and heterocyclic compounds (Group I Compounds)

Methods for the determination of steam-volatile N-nitrosamines are well-established, and are based on those developed in the 1970's for the determination of N-nitrosamines in food (Egan et al, 1978).

Isolation of this group is generally achieved by distillation or by direct extraction with dichloromethane. Determination is usually accomplished by gas chromatography, with the chromatograph being coupled either to a mass spectrometer (GC-MS) or to a Thermal Energy Analyser (GC-TEA).

2.1.1. Separation

There are two basic approaches for the isolation of Group I compounds from a range of matrices.

The most commonly used techniques are based on atmospheric, vacuum and steam distillation. These give isolates which, after partition into dichloromethane, are usually suitable for determination without further purification. When clean-up is necessary the use of a column of activated alumina as described by Telling (1972) can be recommended. Some matrices give problems with foaming but these can be overcome by the use of smaller sample sizes, antifoams or a pre-freezing technique (Spiegelhalder and Preussmann, 1983). Fine et al (1975) have described a vacuum distillation technique which

involves the addition of mineral oil and aqueous alkali to the sample at the start of the process. This latter approach overcomes foaming problems but tends to give distillates which are less pure than those obtained by the former techniques.

Extraction into dichloromethane without prior distillation has also been used by some workers. This can cause emulsion formation, but these problems can be overcome in many instances by absorption of the test sample onto an inert material such as celite or kieselguhr and subsequent elution with dichloromethane. When using such an extraction system, it is important to include an inhibitor of N-nitrosation with the dichloromethane. This technique tends to give eluates which require further clean-up (eg. on a silica column) before they can be determined by detectors other than the TEA (see below).

2.1.2. Determination

GC-MS is the only definitive method in common use for Group I compounds and generally requires a mass spectrometer with a resolution of at least 1 in 10,000. This requirement is particularly relevant in the analysis of products or raw materials based on amine oxides, where the presence of acetone oxime can cause interference in the determination of NDMA, which has a very similar spectrum (Telling, 1989).

GC-TEA is by far the most commonly used determinative system and it offers a higher throughput than GC-MS as well as better quantitation. This is counterbalanced by the need to confirm all positive GC-TEA findings, (eg. on a second chromatographic column of different polarity).

The principle of TEA detection is based on pyrolysis of N-nitrosamines to produce nitrosyl radicals, which are then reacted with ozone to produce excited state nitrogen dioxide. The decay of the latter species to ground state is accompanied by the emission of photons which are detected by photomultipliers and recorded by the TEA as a

positive response. This mode of operation is completely different from that of the mass spectrometer, and therefore its "specificity profile" is different. Thus, any results which indicate N-nitrosamines to be present by both GC-TEA and GC-MS are very unlikely to be in error. Unfortunately, because of the high cost of a medium resolution mass spectrometer, very few laboratories have the ability to confirm GC-TEA findings by GC-MS.

Other detectors have been used in conjunction with GC for the determination of volatile N-nitrosamines. Examples include the nitrogen selective alkali flame ionisation detector, the Coulson electroconductivity detector (Palframan et al, 1973) and the pyrolytic electrolytic conductivity Hall detector (Eisenbrand et al, 1976). In general these detectors show less sensitivity and selectivity than the TEA and mass spectrometer detectors (Scanlan, 1984).

With all of these systems chromatography is generally performed on packed columns. Superior limits of detection and improved chromatographic efficiency can however be achieved by employing capillary columns. High performance liquid chromatography (HPLC)-TEA conditions have been defined for the use of the determination of volatile N-nitrosamines (Goff, 1983) but this approach has seen much wider application in the determination of non-volatile nitrosamines.

2.1.3. Validation

There have been a number of collaborative studies on the determination of volatile N-nitrosamines using the above GC-based methods in a variety of substrates, but to the best of our knowledge not for personal care and household products.

The distillation/extraction method can be applied to all types of products and raw materials and the limit of determination for each volatile N-nitrosamine is 1 - 5 µg/kg, depending on the compound and the sample matrix. When artefact formation is a problem, the limit may be higher. Most published methods required the use of an internal

standard, distillation from alkaline medium and the presence of a suitable inhibitor to prevent artefact formation. The general indication is that there are no problems when these methods are used by experienced analysts (Earl et al, 1982; Sen et al, 1984; Gray and Stachiw, 1987).

2.2. Other volatile long-chain compounds (Group II Compounds)

Long chain N-alkyl-N-methyl-nitrosamines (eg. NDDMA) have been reported to be present in amine oxides used in personal care and household products.

There are only a few literature references to appropriate analytical methods for N-nitrosamines in Group II. According to Morrison et al (1983) a sample of a hair-care product, after addition of ascorbic acid as an inhibitor, was stirred with ether and the mixture dried over anhydrous sodium sulphate. The ether solution was concentrated under reduced pressure and the residue applied to a short silica-gel column. The compounds of interest were eluted with chloroform and the concentrated eluate was analysed by GC-TEA. The authors also discussed the use of a more powerful inhibition system with ammonium amidosulphonate (ammonium sulphamate) and α-tocopherol. The limit of detection was claimed to be 20 µg/kg. A ^{14}C-nitrosamine was used as internal standard (recovery 70 - 90 %). Artefact formation was tested by adding sodium nitrite and the corresponding free amine. Identity of peaks was confirmed by GC-MS.

Ruehl (1989) mixed samples with silica-gel to give a free-flowing powder, ammonium amidosulphonate (ammonium sulphamate) was added as inhibitor and the mixture was transferred onto the top of a silica-gel column. The nitrosamines were eluted with hexane/acetone, this eluate was concentrated and then passed through a Sep-Pak^{R} Alumina B-cartridge. The eluate was discarded. The N-nitrosamine was eluted with acetone, concentrated and then analysed by GC-TEA. Positive results were confirmed by photolysis and by GC on a column with different polarity. The limit of detection was claimed to be 10 µg/kg. After adding 50 µg/kg

nitrosamine to a nitrosamine free sample, the recovery was found to be in the range of 70 - 80 %. Recovery data at or around the claimed limit of detection are not available.

Members of the Task Force are not aware of any published validation of the Morrison et al (1983) method and were unable to comment on the method of Ruehl.

2.3. Non-Volatile high polarity compounds (Group III Compounds)

The physical properties of the Group III compounds are very different from those of the Group I and II compounds: Group III are polar, and hydrophilic. This requires a completely different approach in their isolation and determination.

NDELA is a very important member of this Group of non-volatile, high polarity compounds. Methods which have been described for NDELA should be applicable to other compounds of this Group (eg. NDIPLA and NDBLA). In order to reach the required limits of determination the steps of the analytical procedure (clean-up, separation, determination) have to be carefully selected and optimised.

To avoid false negatives/positives, the following measures should be applied where possible:

- the addition of an internal standard with properties similar to NDELA to check for the recovery (to avoid false negatives). Suitable compounds are NDIPLA, N-nitroso-(2-hydroxyethyl)-(2-hydroxypropyl)-amine (NEHPA) (Sommer et al, 1988) or ^{14}C-NDELA.

- the addition of a nitrosatable amine with similar properties to NDELA (eg. 2-hydroxyethyl-2-hydroxypropylamine) to check for the formation of N-nitrosamines during clean-up.

- the use of two independent methods for separation and determination (eg. two GC columns of different polarities). Where positive

results are found, the use of a second detector with different selectivity, (eg. MS instead of TEA), should be employed.

- the use of the UV irradiation test for confirmation of the presence of NDELA (Krull et al, 1979)

2.3.1. Clean-up procedures

Clean-up is necessary in almost all cases to avoid interferences with the determination of NDELA. Selection of the clean-up procedure often depends both on the type of matrix and on the determination techniques to be used. Therefore both stages need to be considered together. The following clean-up steps are possible: extraction, chromatography on ion exchange resins, on reversed phase or normal phase silica gel, or on other materials. Clean-up in combination with a concentration step is often the only way to reach the required limit of determination. Some important clean-up approaches described in the literature are given below as examples. These special clean-up steps can be varied or combined according to the problem under investigation.

Clean-up of Technical alkanolamines

The presence of large amounts of amines especially secondary amines, gives rise to a high risk of artefact formation during clean-up and analysis. The removal of amine by cation exchange resin (acidic form) helps to circumvent this problem. Some examples of this approach are described below:

1) The alkanolamine is diluted with water/methanol and bound on cation exchange resin in acidic form. The water/methanol eluate is evaporated and the residual NDELA is transferred into a small volume of organic solvent. The residue can be directly silylated (BASF, 1988, unpublished).

In a modification, the extract is further purified by chromatography on silica gel before silylation and GC/TEA analysis (Sommer et al, 1988). This procedure was tested in a collaborative study of the German Federation of Cosmetics and Detergents Manufacturers (IKW = Industrieverband Koerperpflege und Waschmittel ev./Franfurt) and is recommended by the IKW because satisfactory results were obtained with samples of triethanolamine to which NDELA has been added at a level of 50 µg/kg and with corresponding control samples (Sommer et al, 1989).

2) In another approach a cation exchange column is placed in tandem with a normal separatory column within an HPLC system. This procedure allows direct injection of the test sample. In the case of triethanolamine samples, UV detection is used whereas for diethanolamine samples TEA-detection is employed (Dow Chemical, 1981).

Clean-up of other Raw Materials

It is likely that other raw materials will contain traces of alkanolamines. Here clean-up by an ion exchange column would not be appropriate. It is probable that use of the Extrelut^{R}/Silica gel system described by Sommer et al (1988) would be a suitable approach.

Clean-up of Personal care and household products

There are several problems encountered in the analysis of NDELA in personal care and household products these are as follows:

- the exact composition of these materials is often not known, so that the influence of interfering components cannot be taken into account.

- it is possible that some preservatives present in the formulation are able to nitrosate diethanolamine to NDELA. This can also happen under the conditions of the clean-up (artefactual NDELA formation) (Schmeltz and Wenger, 1979).

- emulsions formed after addition of water can impede the extraction of NDELA.

- problems can arise during extraction due to the presence of thickeners.

Fine (1983a) gave an overview of the different possibilities for clean-up:

- Solids are extracted with acetone after addition of sulphamic acid to avoid artefactual NDELA formation, ultrasonification and centrifugation are proposed.

- Aqueous samples are extracted with ethyl acetate/acetone.

- Non-aqueous samples are diluted with acetone before analysis.

The solutions obtained by these procedures were analysed by HPLC/TEA. Silica gel and LiChrosorb^{R} NH_2 -phase respectively, were proposed for analysis and confirmation.

Cationic components are removed on a suitable ion-exchange resin (Fukuda et al, 1981). NDELA is specifically adsorbed on a strongly basic anionic exchange resin (OH-form) in 80% ethanol and quantitatively recovered by elution with 10% acetic acid/ethanol. Under these conditions anionic surfactants adsorbed on the anion exchanger resin are not eluted. NDELA is finally determined by HPLC (silica gel)/TEA.

Sommer and Eisenbrand (1988) added ammonium sulphamate (for removal of nitrite), together with an internal standard, water and sodium chloride (to break the emulsion) to the sample and applied the mixture on an Extrelut^{R} column (containing 50% sodium ascorbate to prevent artefactual formation of nitrosamines). Interfering compounds are first removed by washing with cyclohexane:dichloromethane (1:1v/v), NDELA is then eluted with n-butanol. The NDELA extract is transferred