Technical Report

No 42

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

ISSN-0773-8072-42

Technical Report

N° 42

CRITICAL EVALUATION OF METHODS FOR THE DETERMINATION OF N-NITROSAMINES IN PERSONAL CARE AND HOUSEHOLD PRODUCTS

ISSN – 0773 – 8072 – 42

D-1991-3001-76

Brussels, february 1991

ECETOC Technical Report No 42

CONTENTS

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:

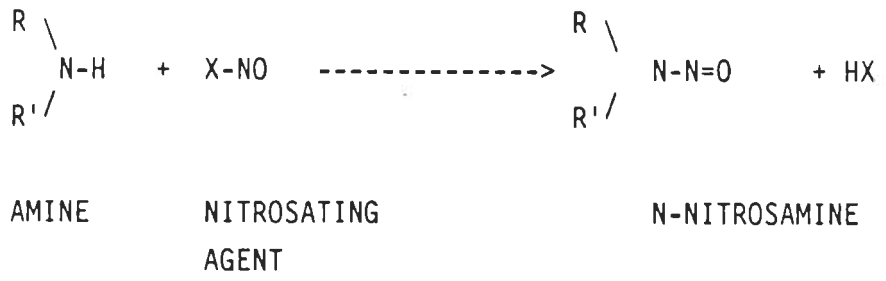
N-Nitroso-		Group *
-dimethylamine	(NDMA)	I
-diethylamine	(NDEA)	I
-morpholine	(NMOR)	I
-piperidine	(NPIP)	I
-pyrrolidine	(NPYR)	I
-dodecylmethylamine	(NDDMA)	II
-tetradecylmethylamine	(NTDMA)	II
-diethanolamine	(NDELA)	III
-diisopropanolamine	(NDIPLA)	III
-dibutanolamine	(NDBLA)	III

* 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

onto a column of silica gel 40 for further clean-up. After silylation, NDELA is analysed by GC-TEA.

It is often necessary to combine elements of the above methods depending on the specific problem under examination.

2.3.2. Chromatographic separation and determination

The main methods for determination use a chromatographic separation of NDELA from other contaminants followed by selective detection technique.

Gas chromatography (GC)

The advantages of gas chromatography over other chromatographic methods consist of greater separation efficiency and minimal problems with interfacing the GC-column with the most important nitrosamine detectors (ie. TEA or MS).

It is highly advisable to use purified extracts of samples for the gas chromatographic determination of NDELA. Direct injection of samples without clean-up can lead to artefactual formation of NDELA in the injector.

Gas chromatography without derivatisation (Spiegelhalder, 1983) is not recommended. NDELA is difficult to chromatograph because of adsorption effects. The presence of active sites in the column can also cause decomposition. Gas chromatography after trimethylsilylation is therefore normally preferred. Several column types can be used, eg. packed OV-225, packed OV-275 or capillaries coated with eg. poly-dimethylsiloxane (Brunemann and Hoffmann, 1983)

Several detectors with different specificities are in use:

The TEA detector linked with GC (Brunemann and Hoffmann, 1983) gives the best combination of selectivity and sensitivity. Nevertheless, the detection of positive signals needs confirmation by other methods.

The mass spectrometry detector (MS) is also very selective but usually less sensitive than TEA. More efficient clean-up is also required. MS-detection is mainly used for the confirmation of TEA results, (Webb, 1983; Wigfield and Lanouette, 1985; Wigfield et al, 1987). Recently, Schwarzenbach and Schmid (1989) reported the use of negative ion CI-mass spectrometry and claimed greater sensitivity than with the conventional EI-mode.

High Performance Liquid Chromatography (HPLC)

Unlike GC, HPLC does not require derivatisation but has several other drawbacks:

HPLC/TEA is only possible in "normal phase systems" (ie. water-free). Technical problems derive from large amounts of eluent which have to be condensed in cooling traps (Fine, 1983a). These interface problems may be overcome in the future by the use of micro columns. Nevertheless HPLC/TEA was successfully used for personal care products (Fine, 1983a), for cutting fluids (Fine, 1983b) and for pesticides (Wigfield et al, 1987). The limit of determination is of the same order of magnitude as with GC/TEA.

The combination of HPLC with ultraviolet (UV)-detection was used to analyse ethanolamines (Rosenberg et al, 1980, Dow Chemical, 1981), personal care products (Schwarzenbach and Schmid, 1989) and pesticides (Wigfield et al, 1985; Wigfield and Lanouette, 1987). The photo-conductivity detector, which uses a high intensity mercury lamp to photolyse compounds which contain halogens or nitrogen, appears to be more sensitive than UV detection (Bennett and Peterson, 1979). The combination of HPLC with electrochemical detection eg. polarography (Rappe and Samuelson, 1983) or amperometry (Righezza et al, 1987) have

been claimed to give a low limit of determination. Little is known about interferences in the latter methods.

HPLC-MS using a thermo-spray interface has been shown to be capable of determining underivatised NDELA in extracts produced by the Sommer's method (Sommer et al, 1989) at levels equal to those achieved by GC TEA-method (Sissons, 1989).

Other chromatographic separations

Supercritical fluid chromatography (SFC) (Smith, 1988) coupled with TEA or MS would appear to be a promising new approach which combines the advantages of GC (volatile mobile phase, CO_2) and HPLC (separation of non-volatile analytes). These advantages make the technique worthy of further investigation.

Thin-layer chromatography is not recommended because of problems with artefacts arising from the influence of air and of the active surface.

2.3.3. Direct total nitrosamine determination

NDELA can be determined by a "total nitrosamines" procedure after isolation from interfering materials (see Section 2.4). However, such an approach is generally considered to be not better than semi-quantitive.

2.3.4. Validation

It is particularly important to carry out a thorough validation programme on any methods devised or used for the analysis of Group III compounds. Examination of the literature shows that this has not always been the case.

Specifically, older published methods often do not employ an internal standard to check recovery or use inhibitors to prevent the artefactual formation of NDELA. Sample clean-up, while tedious, is

frequently the most critical step in the analytical sequence. In practice, not all interfering impurities can be removed in the clean-up process. The efficiency of this process often limits the choice of the chromatographic separation/detection system. Long and complicated clean-up schemes can lead to partial loss of the analytes. These losses can be counteracted by the addition of an internal standard to determine recovery. It is important that the substance selected as internal standard has properties as similar as possible to those of NDELA. In some cases, for instance in the clean-up of personal care products of unknown composition, artefactual formation of NDELA (from nitrosating agents in the reagents or matrix) cannot be excluded.

Where NDELA has been detected in a sample its identity should always be confirmed. This can be done by a variety of methods including chromatography on a second column with different separation characteristics, by use of a different detection method (eg. MS instead of TEA), or application of an irradiation test and/or a chemical denitrosation test.

From the above discussions it is clear that a general limit of determination cannot be given for NDELA. Determination limits given in the literature are often over optimistic and normally apply only to the specific formulation being analysed. Often there are no published results from multicentre studies of proper statistical design which establish the validity of the methodology. It should be stressed that determination limits given in the literature for NDELA of 10 µg/kg may only be reached in special cases and have not been critically evaluated.

The method of Sommer et al (1988) has been validated in a collaborative trial organised by the IKW (see page 13). In this trial, NDELA was added to a specially prepared formulation at a level of 50 µg/kg. The details of this validation have been published by Sommer et al (1989).

Thus experience has shown that for technical alkanolamines the limit of determination for NDELA is in the range 10 - 100 µg/kg. For personal care products, the limit of determination has been claimed to be as low as 10 µg/kg (Sommer et al, 1989), however, this has been verified in only one collaborative study. More realistically, depending on the product and on the methodology applied, the limit of determination is often much higher (eg. 20 - 100 µg/kg or even more).

2.4. Total N-nitroso Compounds

Occasionally there is a need to monitor for nitrosated species (including N-nitrosamines) other than those of the 3 groups already described. Various methods have been described to achieve this. All are based on denitrosation techniques in which the N-N=O group is cleaved to liberate nitric oxide which is then measured.

Denitrosation can be achieved in both organic and aqueous media and measurement of liberated NO can be achieved by a variety of techniques. Some specificity can be built into the determination by the use of suitable pre-treatment prior to the denitrosation stage.

Many of the reported methods have been developed for the analysis of foodstuffs or biological fluids; their suitability for use with personal care and household products has not generally been evaluated. Eisenbrand and Preussman (1970) reported that hydrogen bromide in acetic acid was highly effective in cleaving the -N-N=O groups at room temperature and they used a colorimetric Griess-type reagent to measure the denitrosation products.

The great majority of subsequently reported methods have been based on denitrosation in a heated matrix and sparging the liberated NO into a chemiluminescence detector with an inert gas. This approach was first reported by Downes et al (1976).

Walters et al (1978) further developed these ideas to give a sequential method whereby the test substrate in ethyl acetate is heated and analysed

for its total N-nitroso content by sequential injections of glacial acetic acid and of hydrogen bromide-glacial acetic acid. Treatment with glacial acetic acid alone allows the determination of thermolabile, chemiluminescence forming compounds other than N-nitrosamines, prior to cleavage of the N-nitrosamine with glacial acetic acid/HBr.

It has been reported (Massey et al, 1984) that HBr is an efficient denitrosating reagent only under anhydrous conditions. Working in an aqueous system has the advantage that much larger sample aliquots can be used, thus giving lower limits of determination. Challis (1989) has estimated that up to 2% water can be tolerated in the reaction flask before the denitrosation reaction ceases.

Denitrosation in an aqueous medium can be achieved with sodium iodide in sulphuric acid/acetic acid or with Cu(I) chloride/Cu(II) chloride in sulphuric acid (Huels, 1982). These two approaches have been combined by Chou et al (1987) who used a methylene chloride extraction to separate polar and non-polar N-nitroso compounds. The former are then determined in the aqueous fraction using sodium iodide/sulphuric acid cleavage and the latter in the dried organic fractions using HBr/acetic acid cleavage.

Baptiste and Brown (1980) used a denitrosation system as described above but allowed the liberated NOBr to diffuse into Griess-type reagents. CTFA (1987) has described a technique based on denitrosation, capture of the liberated NO in a diazo reaction and measurement of the coloured derivative by HPLC.

Application of these techniques to the wide range of raw materials and products of interest is hampered by the interference of components other than N-nitrosamines which may be present in the matrix.

It has long been known that nitrate and nitrite both give rise to nitrogen oxides which produce signals in a chemiluminescence analyser. Different methods were applied to overcome these problems:

- In the majority of methods, nitrite is destroyed using ammonium sulphamate at pH values less than 2 or hydrazine sulphate at pH4. Downes et al (1976) reported that nitrite can also be destroyed by treatment with glacial acetic acid, but Massey (1988) has proposed that this reaction is due to the presence of traces of HBr. Pignatelli et al (1987) have shown that acetic acid fortified with 0.1% hydrochloric acid is a reliable reagent for the elimination of inorganic nitrite.

- Nitrite esters are important sources of positive interference. They can be destroyed by treatment with ammonium sulphamate, although the reaction is not always rapid.

- Prolonged treatment with ammonium sulphamate may cause decomposition of species such as nitroso ureas and nitrosopeptides.

- Inorganic nitrate gives about 1% theoretical yield of nitric oxide when treated with hydrogen bromide (Massey et al, 1984) and levels of up to about 100 mg/l can be nullified by the addition of sulphamic acid or sulphanilic acid to the denitrosation vessel (Massey, 1988). Higher levels of nitrate require pre-treatment by anion exchange chromatography (Cox et al, 1982).

- Other interfering compounds include S-nitroso compounds C-nitroso compounds and nitromusks.

- There is a lack of information as to whether other components of personal care and household products can give rise to false positive results.

False negative results can arise from premature denitrosation due to residual traces of HBr remaining in sequential denitrosation methods.

In an attempt to overcome these problems, Pignatelli et al (1987) have published a two stage technique based on the earlier work of Walters, et al, (1978). In this technique half the sample is injected into a

reaction flask containing acetic acid in refluxing ethyl acetate and the other half is injected into hydrogen bromide/acetic acid in refluxing ethyl acetate. The nitric oxide released in each determination is measured in an NOx chemiluminescence detector. The first determination is claimed to give a measure of thermo- and acetic acid labile responsive compounds (TAC). The second determination is claimed to measure both N-nitroso compounds and TAC. The difference between the two values is the response due to N-nitroso compounds. Challis (1989) has reported that it is beneficial to use a higher boiling solvent such as propyl acetate instead of ethyl acetate. The method as described by Pignatelli et al (1987) is for the determination of N-nitroso compounds in gastric juices and modifications will, almost certainly, be necessary if the method is to be applied to the wide range of products and raw materials of interest to the personal care and household products industry.

Challis (1989) has reported a method where the sample before and after treatment with ammonium sulphamate at pH2 is injected into a reaction flask containing hydrogen bromide in refluxing propyl acetate. The nitric oxide released in each determination is measured in an NOx chemiluminescence analyser. The first determination (without ammonium sulphamate) is claimed to give a measure of TAC and N-Nitroso compounds. The second determination (with ammonium sulphamate) is claimed to measure N-Nitroso compounds only.

2.4.1. Validation

The limits of determination claimed for this technique range from 50 µg/kg for non-aqueous systems (Chou et al, 1987) to 10 µg/kg for aqueous systems (Huels, 1982). The inclusion of internal standards and a nitrosatable amine are incompatible with this type of methodology and hence total reliance is placed on the avoidance of potential artefacts by sample pre-treatment.

As far as the Task Force were aware there are no published collaborative studies on the determination of NDELA in personal care and household products using a "total N-nitroso" method.

3. ARTEFACT FORMATION, CONTAMINATION AND THEIR PREVENTION

3.1. Introduction

Contamination and artefact formation during the analysis, can lead to a considerable uncertainty as to the validity of results. As can be seen from the number of literature references dealing with this topic, all stages of the analytical method including sampling, storage, clean-up and determination must be closely monitored if these problems are to be avoided.

3.2. Artefacts and contamination derived from facilities, apparatus and chemicals used for analysis

As Eisenbrand et al (1983) point out, it is advisable to use different rooms for sample storage, storage of standards, for work-up of the samples and for the preparation of the standard solutions to avoid cross contamination. Detergent residues on glassware used for the analysis can be a cause for false positives. The reagents used for sample preparation have to be carefully checked since they are known as a source of contamination. Deionised water (Fiddler et al, 1977) was found to be a source of nitrosamines since the ion exchange resins used in its preparation contained nitrosamines. Eisenbrand et al (1978) described the contamination of solvents, especially halogenated solvents, with nitrosamines. Contact of the samples with rubber, especially rubber stoppers must be avoided, since they may contain high levels of nitrosamines (Lakritz and Kimoto, 1980).

Sample storage can also cause false positives. Refrigerators are mentioned as a source of cross contamination (Eisenbrand et al, 1983). Freezing of samples in the absence of nitrosation inhibitor may be insufficient to prevent the formation of nitrosamines. Eisenbrand et al (1983) proposed alkalisation for this purpose. It is also reported that the presence of nitrosation inhibitors such as ascorbic acid, amidosulphonic acid (sulphamic acid) (Cox and Frank, 1982), sodium azide

and others (Wigfield and Lanouette, 1985) can prevent the artefactual production of nitrosamines.

3.3. Artefactual nitrosamine formation and contamination during sample preparation

Special care has to be used if samples containing large amounts of secondary amines have to be analysed. According to Sommer and Eisenbrand (1988) the most critical step is the extraction and purification on the kieselguhr column, since traces of NOx adsorbed on the column material can cause substantial nitrosamine formation. The problem is avoided if the kieselguhr is mixed with the nitrosation inhibitor sodium ascorbate. Rounbehler et al (1980) compared sampling techniques for airborne nitrosamines and claimed that amongst a big variety of different dry solid sorbents including activated charcoal, activated alumina, silica gel, Florisil^{R}, Tenax^{R} and ThermoSorb^{R}, only ThermoSorb^{R} was free of artefactual nitrosamine formation and at the same time capable of 100% retention of the preloaded nitrosamines.

Sommer and Eisenbrand (1988) describe the advantages of using ion-exchange removal of the amines in the sample matrix to avoid artefactual nitrosamine formation.

Krull et al (1978) discussed the formation of artefacts, the loss of analyte and the procedures to ensure reliable analysis. The procedures proposed to avoid the formation of nitrosamines during work-up include the use of a minimum number of analytical steps, addition of nitrosamine precursors, as well as addition of nitrosation inhibitors. No data are given to support these proposals. The same authors point out that false negatives can result if the samples are exposed to UV-light or heat.

Aitzetmueller and Thiele (1981) found that the sample clean-up on Extrelut^{R} has a tendency to convert volatile secondary amines to nitrosamines. They believe that the nitrosating agent is already present in the freshly delivered Extrelut^{R} in form of traces of nitrite. They were able to exclude the influence of NOx from the laboratory air.

According to these authors the reaction between secondary amines and nitrosating agents occurs mainly in acid medium and can therefore be avoided if the extraction scheme is inverted using the acid first followed by the basic extraction. Heating of the Extrelut^{R} overnight at 200^{o}C has proven useful in eliminating adsorbed NOx.

Additionally the authors propose the use of an excess of ascorbic acid in combination with the Extrelut^{R}. They found that sulphuric acid used for the acidification of the sample can contain traces of nitric and nitrous acid which can produce artefactual nitrosamines.

3.4. Artefact formation and false results during detection of N-Nitrosamines

The selectivity of the Thermal Energy Analyser has reduced the number of false positives (Fine and Rounbehler, 1975). In many cases the chromatographic analysis is possible without extensive sample clean-up. Care has to be taken in the gas chromatographic analysis, since the temperature of the injection-port (200^{o}C) is ideal for the formation of nitrosamines if precursors are not removed before injection (Fan and Fine, 1978). This problem was demonstrated by the injection of secondary amines onto a precolumn packed with potassium nitrite in a GC port (Freed and Mujsce, 1977). To prove the actual formation of nitrosamines in the injection port, Fan and Fine (1978) evaluated the effect of injection port temperature on the artefactual production of NDMA and showed a significant production between 150 and 230^{o}C. Even though the Thermal Energy Analyser is fairly selective for nitrosamines, false positives must also be considered. These can result from materials having labile NO groups, such as O-nitroso, C-nitroso, S-nitroso, O-nitro, or N-nitro compounds.

It is known (Fine et al, 1977) that substituted olefins which exhibit chemiluminescence with ozone, can also lead to false positive results. False negative results may occur if the nitrosamine under study suffers decomposition when exposed to sunlight or fluorescent light (Fine et al, 1976). Gough et al (1977) reported that the coelution of excess amine

with nitrosamines can result in recombination of the NO radical with the amine and thus can diminish the signal.

There are several proposed ways to avoid artefact formation or to determine whether or not artefacts are formed during the analysis. Several sources propose the use of inhibitors such as ammonium sulphamate, ascorbic acid, sodium ascorbate, glutathione and urea (Sen and Donaldson, 1975; Fine and Rounbehler, 1975; Ziebarth, 1981; Osterdahl and Bellander, 1983) in aqueous samples and tocopherol in non-aqueous samples (Ragelis et al, 1983). It has been shown that the inhibition may be pH dependent (Osterdahl and Bellander, 1983). Under certain conditions ammonium sulphamate (Osterdahl and Bellander, 1983) was shown to increase the formation of N-N'-dinitroso-piperazine in the analysis of gastric juices, and formation of mono-nitroso-piperazine in urine. The reason for this is most likely the change of pH.

To show that a method avoids the formation of artefacts it is necessary to demonstrate the effect of the inhibition. Some sources propose the addition of an easily nitrosatable amine such as morpholine (Walker et al, 1980; Sen et al, 1987). It is claimed that any formation of N-nitroso morpholine would suggest that the method used is prone to artefact formation.

Some authors (Krull et al, 1978) propose that the samples be irradiated with UV-light to destroy nitrosamines present and reanalyzed to prove that the positive results originate from nitrosamines.

Of course in all cases it is necessary to run reagent blanks and verify recovery by means of added internal standards, to validate the results of the analytical procedure used (Eisenbrand et al, 1983).

3.5. Conclusions

Positive findings of N-nitrosamines can be trusted only if appropriate steps have been taken to avoid artefact formation and an internal standard has been added. Reagent blanks must be run under identical

conditions to demonstrate that no contamination source is present. Furthermore it is necessary to verify positive results with a second detection technique (eg. mass spectroscopy where TEA has first been used).

4. N-NITROSAMINE STANDARDS

N-nitrosamines decompose during storage particularily as dilute solutions. Data on shelf life are often lacking. Therefore it is imperative to check the identity and concentration standards prior to each series of analyses. For commercially available standards the analytical method used for their validation is often not specified. Identity and purity have to be determined. These can best be established by a combination of chromatography, spectroscopy and electrochemical methods, eg. gas chromatography, mass spectroscopy, ultraviolet spectrometry and polarography. Standards must be stored in the dark at low temperature (below $4^{o}C$).

5. RECOMMENDATIONS

The widespread occurrence of nitrosoamine precursors and the inherent instability of N-Nitroso compounds causes particular problems in their determination.

Any validated method for the analysis of N-nitrosamines in Groups I-III must be shown to have taken the following points into consideration:

- use of a suitable internal standard to check for recovery,

- inclusion of a suitable nitrosatable amine to check for artefact formation,

- the use of appropriate techniques to confirm positive results.

There already are general criteria laid down for the validation of analytical methods to internationally accepted standards (eg. AOAC, 1989). We therefore recommend that, before any rules to regulate N-nitrosamines levels are enacted, suitable methods meeting these criteria are available and published in the open literature.

All published methods should include performance data and proof of validation in the text.

Before official acceptance of such methodology further validation by inter-laboratory study followed by publication in the open literature is necessary. The publication should include the statistical model and the raw data. It must be recognised, that such a collaborative study validates a method only for the substrate analysed and the levels determined.

If these criteria are applied to the literature methods reviewed in this document then it is clear that many are not acceptable.

More specifically, methods for steam-volatile N-Nitrosamines seem to be adequate but published collaborative studies for personal care and household products are lacking.

Methods for other volatile long chain N-Nitrosamines are still in a very early stage of investigation. There is a need for much more work on methods before any recommendation can be made. Methods for non-volatile, high polarity N-Nitrosamines have been extensively published but most of them have not been adequately validated with respect to their application and performance. There is a need to identify those methods which are worthy of further validation and organise the necessary collaborative studies.

Methods for total N-Nitrosamines determination as presently published are only suitable for screening purposes. There is a need for the development of robust quantitative methods before these could be recommended for more general application.

BIBLIOGRAPHY

- AITZETMEULLER,K. and THIELE,E. (1981), Artifact formation in Nitrosamine Analysis. Analysis of N-Nitrosodimethylamine in Beer. Z. fur Lebensm.- Unters. und Forsch. 173, (2), 117-120.
- AOAC-ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (1989), Guidelines for collaborative study procedure to Validate Characteristics of a Method of Analysis. J. Assoc. Off. Anal. Chem. 72, (4), 694-704.
- BAPTISTE, V.H. and BROWN, R. (1980), Nitrosamines determination by the use of conventional Equipment. J. Soc. Cosm. Chem. 31, 219.
- BASF (1988), unpublished personal communication, BASF AG, W. Germany.
- BENNETT, R.H. and PETERSON, E.S. (1979), Determination of Nitrosamines by liquid Chromatography using a Photoconductivity Detector. Texaco Paper No. 2323 presented in August 1979. 21st Rocky Mountain conference on Anal. Chem. Denver, Colarado.
- BRUNEMANN, K.D. and HOFFMANN D. (1983), GC-TEA of NDELA from tobacco products In: Environmental carcinogens - selected methods of analysis, volume 6 - N-Nitroso Compounds, Ed. EGAN, H, IARC Scientific Publications, Lyon No 45, 399-402.
- CHALLIS, B. (1989), Open University, Milton Keynes, UK. Personal Communication.
- CHOU, H.J., YATES, R.L. and WENNIGER, J.A. (1987), Screening Cosmetic Products for N-Nitroso Compounds by Chemiluminescent Determination of Nitric Oxide. J. Assoc. Off. Anal. Chem. 70, (6), 960-963.
- COX, R.D. and FRANK, C.W. (1982), Determination of total N-Nitroso content in Cutting Fluids. Analytical Chemistry, 54,(3), 557-559.
- COX, R.D., FRANK, C.W., NIKOLALSEN, C.D. and CAPUTO, R.E. (1982), Screening Procedure for Determination of Total N-Nitroso Content of Urine. Anal. Chem., 54, 253-256.
- CTFA, (1987), Recommended procedures for conducting total Nitrosamines analysis, cosmetics, toiletry and fragrances Association. 1110 Vermont Av. NW, Washington DC 2005.
- DOW CHEMICAL (1981), DOW Europe, 8810 Horgen, Switzerland, personal communication.
- DOWNES, M.J., EDWARDS, M.W., ELSEY, T.S. and WALTERS, C.L. (1976), Determination of a non-volatile nirosamine by using denitrosation and a chemiluminescence analyser. Analyst, 101, 742-748.
- EARL, L.G., SMITH, W.J., MALANOSKY, A.J. (1982), Mineral oil vacuum destillation method for Nitrosmines in fried bacon with thermal energy analyzer: Collaborative Study; J. Assoc. Off. Anal. Chem. 65 (6) 1319.
- EGAN, H., PREUSSMANN, R., CASTEGNARO, M., WALKER, E.A., WASSERMANN, A.E. Eds (1978), In: Environmental carcinogens selected method of analysis. Volume 1. Analysis of Volatile Nitrosamins in Food. IARC, Scientific Publications. Lyon. No 18.
- EISENBRAND, G. and PREUSSMAN, R. (1970), Eine neue Methode zur Kolorimetrischen Bestimmung von Nitrosaminen nach Spaltung der N-nitroso-gruppe mit Bromwasserstoff in Eisesig. Arzneimittel. Forsch., 20, 1513-1517.
- EISENBRAND, G., VON RAPPARDT, E., ZAPPE, R. and PREUSSMAN, R. (1976), Trace analysis of volatile nitrosamines by modified nitrogenspecific detector in pyrolytic mode and by ion-specific determination of heptafluorobutyramides in a gas chromatography-mass spectrometry system. In: Environmental Aspects of N-Nitroso compounds. Eds. Walker, E.A., Bogovski, P and Griciute, L., IARC Scientific Publication, Lyon No. 14, 65-75.
- EISENBRAND, G., SPIEGELHALTER, B., JANZOWSKI, C., KANN, J., PREUSSMANN, R. (1978), Volatile and non-volatile N-Nitroso compounds in Food and other Environment Media In: Environmental Aspects of N-Nitroso Compounds. Eds. Walker E.A., Castegnero, M., Griciute, L., and Lyle, R. IARC Scientific Publications, Lyon, No 19, 311-24.
- EISENBRAND, G., ARCHER, M., BRUNNEMANN, K.D., FINE, D.H., HECHT, S.S., HOFFMANN, D.,KRULL, J., WEBB, K.S. (1983), Problems of Contamination and Artefact formation in Nitrosamine Sampling and Analysis In: Environmental Carginogens selected methods of analysis. Vol 6-N-Nitroso compounds. Ed. Egan, H. IARC Scientific Publications, Lyon, No 45, 25-34.
- FAN, T.Y., FINE, D.H. (1978), Formation of N-Nitrosodimethylamine in injection Port of a Gas Chromatograph-Artifact in Nitrosamine Analysis. J. Agric. Food Chem., 26, (6), 1471-1472.
- FIDDLER, W., PENSABENE, J.W., DOERR, R.C., DOOLEY, C.J. (1977), Presence of Dimethyl-Nitrosamines and Diethyl-Nitrosamines in deionized Water. Food Cosmet. Toxicol., 15,(5), 441-443.

- FINE, D.H. (1983a), HPLC-TEA Determination of NDELA and similar compounds in Cosmetics. In: Environmental carcinogens - selected methods of analysis, volume 6 - N-Nitroso Compounds, Ed. EGAN, H., IARC Scientific Publications, Lyon, No 45, 309-317.
- FINE, D.H. (1983b), HPLC-TEA Determination of NDELA in Cutting-fluids In: Environmental carcinogens - selected methods of analysis, volume 6 - N-Nitroso Compounds, Ed. EGAN, H., IARC Scientific Publications Lyon, No 45, 319-323.
- FINE, D.H. and ROUNBEHLER, D.P., (1975), Trace Analysis of volatile N-Nitroso compounds by combined Gas-Chromatography and Thermal Energy Analysis. J. Chromat., 109, (2), 271-279.
- FINE, D.H., ROUNDBEHLER, D.P. and OETTINGER, P.E. (1975), A rapid method for the determination of sub-part per billion amounts of N-nitroso-compounds in foodstuffs. Anal. Chem. Acta., 78, 383-389
- FINE, D.H., ROSS, R., ROUNBEHLER, D.P., SILVERGLEID, A. and SONG, L. (1976), Analysis of nonionic nonvolatile N-Nitroso compounds in Foodstuffs. J. Agric. Food Chem. 24, (5), 1069-1071.
- FINE, D.H., ROUNBEHLER, D.P., SILVERGLEID, A. and ROSS, R. (1977), Trace Analysis of polar and apolar N-Nitroso compounds by combined High-perfomance Liquid Chromatogrphy and Thermal Energy Analysis. In: Proceedings of the Second International Symposium on Nitrite in Meat Products. FSTA, 9, 12S2047.
- FREED, D.J. and MUJSCE, A.M (1977), Generation of Nitrosamines for GC -Analysis via direct injection. Anal. Chem., 49, (11), 1544-1545.
- FUKUDA, Y., MORIKAWA, Y. and MATSUMOTO, I. (1981), Ion-Exchange Chromatographic Separation of N-Nitrosodiethanolamine in Cosmetics. Anal. Chem., 53, 2000-2003.
- GOFF U. (1983), High Performance Liquid Chromatography of Volatile Nitrosamines. In: Environmental carcinogens - selected method of analysis volume 6-N-Nitroso compounds. Ed. Egan, H. IARC Scientific Publication, Lyon, No 45, 389-394.
- GOUGH, T.A., WEBB, K.S., MCPHAIL, M.F. (1977), Volatile Nitrosamines from Ion-Exchange Resins. Food Cosmet. Toxicol, 15, (5), 437-440.
- GRAY, J.I. and STACHIW, M.A (1987), Gas chromatographic - Thermal Energy Analysis Method for determination of volatile N-Nitrosamines in Baby Bottle Rubber Nipples: Collaborative Study. J. Assoc. Off. Anal. Chem., 70, (1), 64.
- GREENFIELD, E.L., SMITH, W.J. and MALANOSKY, A.J. (1982), Mineral oil vacuum distillation method for Nitrosamines in fried bacon, with thermal energy analyzer: collaborative study. J. Assoc. Off. Anal. Chem. 65, (6), 1319.
- HILL, M.J. (1988), The Chemistry of N-Nitrosation In: Nitrosamines, Toxicology and Microbiology. Ed. Hill. MJ. Ellis Horwood Ltd UK, 48-68.
- HUELS (1982), unpublished personal communication, HUELS AG, 4370 Marl, W. Germany.
- KRULL, I.S., FINE, D.H. and FAN,T.Y. (1978), Problem of Artifacts in Analysis of Nitroso compounds. Analytical Chemistry, 50, (6), 698-701.
- KRULL, J.S., GOFF, E.U., HOFFMAN, G.G., FINE, D.H. (1979), Conformatory Methods for the Thermal Energy Determination of N-nitroso Compounds at Trace Levels. Anal. Chem, 51, 1706.
- LAKRITZ, L. and KIMOTO, W. (1980), N-Nitrosamines contaminants in Blood-Collection Tubes. Food Cosmet. Toxicol, 18, (1), 31-34.
- MASSEY, R.C., (1988), Analysis of N-Nitroso Compounds in Foods and Human Body Fluids In Nitrosamines, Toxicology and Microbiology. Ed. M.J. Hill Ellis Horwood Ltd U.K.
- MASSEY, R.C., BAYLY, J.M., KEY, P.E., MCWEENG, D.J. and KNOWLES, M.E. (1984), Total N-nitroso group analysis in foods II. Further studies on their precision and sensitivity of the assay. Fd. Add. Contam., 1, 237-244.
- MORRISON, J.B., HECHT, S.S., WENNINGER J.A. (1983), N-Nitroso-N-Methyloctadecylamine in Hair-Care Products. Fd. Chem. Toxic, 21, (1), 69-73.
- OSTERDAHL, B.G., BELLANDER, B.T.D. (1983), Determination of N-Mononitrosopiperazine and N,N-Dinitroso-piperazine in Human-Urine, Gastric-Juice and Blood. J. Chrom., 278, (1), 71-80.
- PALFRAMAN, J.F., MACNAB, J. and CROSBY, N.T. (1973), An evaluation of the alkali flame ionisation detector and the Coulson electrolytic conductivity detector in the analysis of N-nitrosamines in foods. J. Chromat., 76, 307-319.
- PIGNATELLI, B., RICHARD, I., BOURGADE, M.C. and BARTSCH, H. (1987), Improved Group Determination of Total N-Nitroso Compounds in Human Gastric Juice by Chemical Denitrosation and Thermal Energy Analysis. Analyst, 112, 945-949.

- RAGELIS, E.P., EDWARDS, G.S., COOMBS, J.R. and REISCH, J.W. (1983), Determination of Nitrite and Volatile Nitrosamines in Animal Diets. J. Agric. Food Chem., 31, (5), 1026-1030.
- RAPPE, C. and SAMUELSON, R. (1983), Voltametry and Polarography. In: Environmental carcinogens - selected methods of analysis, volume 6 - N-nitroso Compounds, Ed. EGAN H., IARC Scientific Publications, Lyon, No 45, 463.
- RIGHEZZA, M., MURELLO, M.H. and SIOUFFI, A.M. (1987), Determination of Nitrosamines by liquid chromatography with Post-Column Photolysis and Electrochemcial Detection. J. Chromat, 410, 145-155.
- ROSENBERG, I.E., GROSS, J., SPEAR, T., RAHN, P. (1980), Analysis of Nitrosamines in Cosmetic Raw Materials and Finished Product by High Pressure Liquid Chromatography and UV Detection. J. Soc. Cosmet. Chem, 31, 237-252.
- ROUNBEHLER, P., REISCH, J.W., COOMBS, J.R. and FINE, D.H. (1980), Nitrosamine Air Sampling Sorbents compared for Quantitative Collection and Artifact Formation. Anal.Chem, 52, 2, (273-276).
- RUEHL, C. (1989), BGA, Berlin 33. Bestimmung von Methyl-n-alkyl-nitrosaminen in kosmetischen. Erzeugnissen und in Spuelmitteln, personal communication. BGA, Berlin 33.
- SCANLAN, R.A. (1984), Chemiluminescence for measurement of N-nitrosamines in foods. Analysis of Food Contaminants. Elsevier Applied Science: London 321-376.
- SCHMELTZ, I. and WENGER, A. (1979), 2-Bromo-2-Nitropropane-1,3-Diol as a Nitrosating Agent for Diethanolamine: A Model Study. Food Cosmet. Toxicol, 17, 105-109.
- SCHWARZENBACH, R. and SCHMID, J.P. (1989), Determination of N-Nitrosodiethanolamine in Cosmetics. HPLC and GC-MS as Alternative Methods to Chemiluminescence Detection. J. Chromat, 472, 231-242.
- SEN, N.P. and DONALDSON, B. (1975), Effect of Ascorbic Acid and Glutathione on the formation of Nitrosopiperazines from Piperazine Adipate and Nitrite. In: N-Nitroso compounds in Environment. IARC, Scientific Publications, Lyon, 9, 103-6.
- SEN, N.P., SEAMAN, S.W. and KARPINSKY, K. (1984), Determination of N-Nitrosodimethylanine in non fat dry milk: collaborative study. J. Assoc. Off. Anal. Chem., 70 (1), 64.
- SEN, N.P., SEAMAN, S.W. and KUSHWAHA, S.C. (1987), Improved Method for determination of volatile Nitrosamines in Baby Bottle Rubber Nipples and Pacifiers. J. Assoc. Off. Anal. Chem., 70, (3), 434-8.
- SISSONS, T.G. (1989), Unilever Research, Sharnbrook, Bedford, UK. Unpublished, personal communication.
- SMITH, R.M. (1988), Supercritical Fluid Chromatography, RSC Chromatography Monographs, Royal Society of Chemistry, London.
- SOMMER, H. and EISENBRAND, G. (1988), A Method for determination of N-Nitrosoalkanolamines in cosmetics. Z. fur Lebensm.-Unters. und Forsch., 186, (3), 235-238.
- SOMMER, H., LOEFFLER, H. and EISENBRAND, G. (1988), A Method to determine N-nitrosoalkanolamines in alkanolamines. J. Soc. Cosm. Chem., 39, 133-137.
- SOMMER, H., BLANKERT, M. and EISENBRAND, G. (1989), Determination of N-Nitrosodiethanolamine in cosmetics and in alkanolamines: results of collaborative studies. Z. Lebensm. Unters. Forsch., 189, 144.
- SPIEGELHALDER, B. (1983), General Remarks and aspects of the GC of Specific non-volatile Nitrosamines. Environmental carcinogens - selected methods of analysis, volume 6 - N-Nitroso Compounds. EGAN H., IARC Scientific Publications, No 45, 395.
- SPIEGELHALDER, B. and PREUSSMAN, R. (1983), Analysis of cosmetics, cutting fluids and other products containing detergents surfactants and amines. In: Environmental carcinogens - selected methods of analysis, volume 6 N-Nitroso compounds. Ed. EGAN H., IARC Scientific Publications, 45, 259-263.
- TELLING, G.M. (1972), A gas liquid chromatography procedure for the detection of volatile N-Nitrosamines at the ten parts per bilion level in foodstuffs after conversion to their corresponding nitrosamines. J. Chromat, 73, 79-87.
- TELLING, G.M. (1989), Unilever Research, Bedford, U.K. private communication.
- WALKER, E.A., GRICIUTE, L., CASTEGNARO, M., BOERSOENYI, M., DAVIS, W. Eds (1980), N-Nitroso compounds: analysis, formation and occurrence. IARC Scientific Publication, Lyon, 31, 831i.
- WALTERS, C.L., DOWNES, M.J., EDWARDS, M.W. and SMITH, P.R. (1978), Determination of a non volatile N-nitrosamine on a food matrix. Analyst, 103, 1127-1133.
- WEBB K.S. (1983), Mass Spectrometric Analysis of N-Nitrosocompounds and Derivates. In: Environmental carcinogens - selected methods of analysis, volume 6 - N-Nitroso Compounds, Ed. EGAN H. IARC Scientific Publications, Lyon, No 45, 449.

- WIGFIELD, Y.Y., LANOUETTE, M. (1985), Liquid Chromatographic and Gas Chromatographic Determination of N-Nitrosodiethanolamine in 2,4-Diethanolamine Pesticide Formulations. J. Ass. Off. Anal. Chem., 68, (6), 1142-1148.
- WIGFIELD, Y.Y., GURPRASAD, N.P., LANOUETTE, M. and RIPLEY, S. (1987), Determination of N-Nitroso-diethanolamine in Dinoseb Formulations by Mass Spectrometry and Thermal Energy Analyzer Detection. J. Ass. Off. Anal. Chem., 70, (5), 792.
- WILLIAMS, D.L.H. (1988) In: Nitrosation. Ed. Williams, D.L.H. Cambridge University Press, N.Y.
- ZIEBARTH, D. (1981), Nitrosatable Drugs. Archiv fur Geschwulstforschung. 51, (7), 587-595.

APPENDICES

Appendix 1: Members of ECETOC Task Force

Dr. G.T. STEEL	ICI, Cheshire GB
Mr. G.M. TELLING	UNILEVER, Beford GB
Dr. W. KIMPENHAUS	HUELS, Marl D
Dr. H. KLOTZ	HOECHST, Frankfurt D
Dr. G. SCHULZ	BASF, Ludwigshafen D
Mr. D. FALGOUX	BP CHEMIE, Lavera F
Dr. W. GRAF	DOW EUROPE, Horgen CH
Mr. J. FLETCHER	UNION CARBIDE, South Charleston USA
Dr. W. HAEBLER	ECETOC, Brussels B

APPENDIX 3

Members of ECETOC Scientific Committee
(Peer Review Committee)

I.F.H PURCHASE (Chairman), Director, Central Toxicology Laboratory	ICI UK - Alderley Park
M. SHARRATT, (Vice-Chairman), Group Toxicology Advisor	BP UK - Guildford
H. DE HENAU, European Technical Centre Professional and Regulatory Services	PROCTER AND GAMBLE B - Brussels
H.O. ESSER, Vice-Director Central Function - Product Safety	CIBA-GEIGY CH - Basel
P.A. GILBERT*, Head, Environmental Relations	UNILEVER UK - Port Sunlight
I.J. GRAHAM-BRYCE, Head of Environmental Affairs	SHELL NL - Den Haag
B. HILDEBRAND, Head, Department of Toxicology	BASF AG D - Ludwigshafen
J.R. JACKSON*, Director Medicine and Health Science	MONSANTO EUROPE B - Brussels
K. KUENSTLER, Head of Toxicology Department	HENKEL D - Duesseldorf
E. LOESER, Head of Institute of Industrial Toxicology	BAYER D - Wuppertal
R. MILLISCHER, Chief Toxicologist	ATOCHEM F - Paris
W.F. TORDOIR, Head of Occupational Health and Toxicology Division	SHELL NL - Den Haag
H. VERSCHUUREN, Head of Toxicology Department	DOW CHEMICAL CH - Horgen

* Stewards, - responsible for primary peer review.

Responsible editor : D.A. Stringer, ECETOC
Avenue Louise 250, Bte 63
B - 1050 Brussels

D - 1990-3001-76

LIST OF ECETOC PUBLICATIONS

MONOGRAPHS

No.	Title
No.1	Good Laboratory Practice
No.2	Contribution to Strategy for Identification and Control of Occupational Carcinogens
No.2	Definition of a Mutagen, for 6th Amendment
No.3	Risk Assessment of Occupational Chemical Carcinogens
No.4	Hepatocarcinogenesis in Laboratory Rodents : Relevance for Man
No.5	Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology)
No.6	Acute Toxicity Tests, LD_{50}(LC_{50}) Determinations and Alternatives
No.7	Recommendations for the Harmonisation of International Guidelines for Toxicity Studies
No.8	Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment
No.9	Assessment of Mutagenicity of Industrial and Plant Protection Chemicals
No.10	Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man
No.11	Eye Irritation Testing
No.12	Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity/teratogenicity)
No.13	DNA and Protein Adducts: Evaluation of their Use in exposure Monitoring and Risk Assessment
No.14	Skin Sensitisation Testing
No.15	Skin Irritation

TECHNICAL REPORTS

No.	Title
No.1	Assessment of Data on the Effects of Formaldehyde on Humans
No.2	The Mutagenic and Carcinogenic Potential of Formaldehyde
No.3	Assessment of Test Methods for Photodegradation of Chemicals in the Environment
No.4	The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man
No.5	Toxicity of Ethylene Oxide and its Relevance to Man
No.6	Formaldehyde Toxicology : an Up-Dating of the ECETOC Technical reports 1 and 2
No.7	Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere
No.8	Biodegradation Testing: An Assessment of the Present Status
No.9	Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients
No.10	Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits
No.11	Ethylene Oxide Toxicology and its Relevance to Man : An Up-Dating of ECETOC Technical Report n°5
No.12	The Phototransformation of Chemicals in Water : Results of a Ring-Test
No.13	The EEC 6th Amendment : A Guide to Risk Evaluation for Effects on the Environment
No.14	The EEC 6th Amendment : A Guide to Risk Evaluation for Effects on Human Health
No.15	The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision. Accuracy and Limiting Values Precision, Accuracy and Limiting Values
No.16	A review of Recent Literature on the Toxicology of Benzene
No.17	The Toxicology of Glycol Ethers and its Relevance to Man : An Up-Dating of ECETOC Technical Report n°4

No.18	Harmonisation of Ready Biodegradability Tests
No.19	An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment
No.20	Biodegradation Tests for Poorly-Soluble Compounds
No.21	Guide to the Classification of Carcinogens, Mutagens and Teratogens Under the 6th Amendment
No.22	Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity
No.23	Evaluation of the Toxicity of Substances to be Assessed for Biodegradability
No.24	The EEC 6th Amendment : Prolonged Fish Toxicity Tests
No.25	Evaluation of Fish Tainting
No.26	The Assessment of Carcinogenic Hazard for Human Beings Exposed to Methylene Chloride
No.27	Nitrate and Drinking Water
No.28	Evaluation of Anaerobic Biodegradation
No.29	Concentrations of Industrial Organic Chemicals Measured in the Environment : The Influence of Physico-Chemical Properties, Tonnage and Use Pattern
No.30(3)	Existing Chemicals : Literature Reviews and Evaluations
No.31	The Mutagenicity and Carcinogenicity of Vinyl Chloride : A Historical Review and Assessment
No.32	Methylene Chloride (Dichloromethane) : Human Risk Assessment Using Experimental Animal Data
No.33	Nickel and Nickel Compounds : Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis
No.34	Methylene Chloride (Dichloromethane) : An Overview of Experimental Work Investigating Species, Differences in Carcinogenicity and their Relevance to Man
No.35	Fate, Behaviour and Toxicity of Organic Chemicals Associated with Sediments
No.36	Biomonitoring of Industrial Effluents
No.37	Tetrachloroethylene : Assessment of Human Carcinogenic Hazard
No.38	A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens
No.39	Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea
No.40	Hazard Assessment of Chemical Contaminents in Soil
No.41	Human Exposure to N-Nitrosmaines, Their Effects and a Risk Assessment for n-Nitrosodiethanolamine in Personal Care Products

JACC REPORTS

No.	Title
No.1	Joint Assessment of Commodity Chemicals, Melamine
No.2	Joint Assessment of Commodity Chemicals, 1,4-Dioxane
No.3	Joint Assessment of Commodity Chemicals, Methyl Ethyl Ketone
No.4	Joint Assessment of Commodity Chemicals, Methylene Chloride
No.5	Joint Assessment of Commodity Chemicals, Vinylidene Chloride
No.6	Joint Assessment of Commodity Chemicals, Xylenes
No.7	Joint Assessment of Commodity Chemicals, Ethylbenzene
No.8	Joint Assessment of Commodity Chemicals, Methyl Isobutyl Ketone
No.9	Joint Assessment of Commodity Chemicals, Chlorodifluoromethane
No.10	Joint Assessment of Commodity Chemicals, Isophorone
No.11	Joint Assessment of Commodity Chemicals, (HFA-132b) 1,2-Dichloro-1,1-Difluoroethane
No.12	Joint Assessment of Commodity Chemicals, (HFA-124) 1-Chloro-1,2,2,2-Tetrafluoroethane
No.13	Joint Assessment of Commodity Chemicals, (HFA-123) 1,1-Dichloro-2,2,2-Trifluoroethane
No.14	Joint Assessment of Commodity Chemicals, (HFA-133a) 1-Chloro-2,2,2-Trifluoromethane
No.15	Joint Assessment of Commodity Chemicals, (HFA-141B) 1-Fluoro 1,1-Dichloroethane
No.16	Joint Assessment of Commodity Chemicals, (HCFC-21) Dichlorofluoromethane Dichlorofluoromethane