EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Safety

SCCS

Opinion on Nitrosamines and Secondary Amines in Cosmetic Products

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at its 13th plenary
of 13-14 December 2011

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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All Declarations of working group members are available at the following webpage:
http://ec.europa.eu/health/scientific_committees/environmental_risks/members_wg/index_en.htm

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1. BACKGROUND

The possible health risks that may be associated with the presence of nitrosamines in cosmetic products are regulated under the Cosmetics Directive (76/768/EEC) under the following entries of Annexes II (total ban) and III (restricted use):

Annex II:

410. Nitrosamines
411. Secondary alkyl- and alkanolamines and their salts

Annex III:

60. Fatty acid dialkylamides and dialkanolamides. Maximum secondary amine content in the finished product: 0.5%. Maximum secondary amine content in the raw materials: 5% (applies). Maximum nitrosamine content 50 µg/kg. Should not be used with nitrosating systems and should be kept in nitrite-free environments.

61. Monoalkylamines, monoalkanolamines and their salts. Maximum secondary amine content in the finished product: 0.5%. Minimum raw material purity: 99%. Maximum secondary amine content in the raw materials: 0.5% (applies). Maximum nitrosamine content 50 µg/kg. Should not be used with nitrosating systems and should be kept in nitrite-free environments.

62. Trialkylamines, trialkanolamines and their salts. Maximum level in rinse off finished products: 2.5%; Minimum raw material purity: 99%. Maximum secondary amine content in the raw materials: 0.5% (applies). Maximum nitrosamine content 50 µg/kg. Should not be used with nitrosating systems and should be kept in nitrite-free environments.
(a) 2.5% (a) (b)

The scientific basis for the above provisions are the relevant opinions of the Scientific Committee on Cosmetology (SCC/023/92) and the Scientific Committee on Cosmetics and Non Food Consumer Products (SCCNFP/0110/99 and SCCNFP/0462/01).

The implementation of the above provisions in the market place has been difficult as the chemical terms used in the above entries are relatively generic and allow for different interpretations among economic operators and public authorities, ranging from those which consider that all chemical structures which contain a secondary amine group are covered by the above entries to those who take a more practical approach to consider only those secondary amines with unsubstituted or monosubstituted alkyl and/or alkanoyl groups.

According to the Cosmetics industry entry 411 of Annex II is to be interpreted in the following manner:

- A secondary alkylamine is one in which precisely two alkyl groups are attached to a nitrogen atom, with no further substitution of either the nitrogen or the alkyl groups. However, even this interpretation is ambiguous since a secondary alkylamine could be an amine carrying one or more secondary alkyl groups.

- A secondary alkanolamine is one in which the nitrogen atom carries precisely two monohydroxyalkyl groups, with no further substitution.

On the other hand, the Norwegian Competent Authority has asked the Commission for further assistance in the evaluation of the interpretation of entry 411 of Annex II concerning some polyamines like spermidine (CAS 334-50-9), Gerotine (CAS 71-44-3) and dipropylenetriamine (CAS 56-18-8) which are used as ingredients in cosmetic products

marketed by a Norwegian company. These are secondary aminoalkylamines meaning that strictly speaking they are neither alkylamines, nor are they secondary alkanolamines and hence should not be covered by entry 411 of Annex II although by definition (as secondary amines) can give rise to nitrosamines.
Dipropylenetriamine is a primary amine which contains a secondary group but chemically speaking it is neither a primary alkylamine nor a primary alkanolamine. Hence in the strict sense it is outside the scope of Annex III, part 1, entry 61, yet from the safety point of view this is rather illogical since it is a chemical which under appropriate conditions (nitrosating agents, heat etc) will most certainly give rise to its nitrosamine upon nitrosation of its secondary amine group. Other polyamines which potentially create similar interpretation challenges like Azamethonium (CAS 60-30-0), Pentamethonium (CAS 2365-25-5), and Hexamethonium (CAS 55-97-0) are banned under entries 121, 120 and 124 of Annex II for different reasons.

The situation is similar for the fatty acid dialkylamides and dialkanolamides. Here it is the fatty acids moiety that needs to be considered. They are normally understood to mean long-chain alkanoic or alkenoic acids, and would only include limited branching; stearic acid, isostearic acid, and oleic acid would all be considered fatty acids. When the alkyl chain is further substituted or if it is not linear, they do not 'fit' the standard definition of a fatty acid as understood in practice although chemically they are fatty acids. Thus cocoyl sarcosine is not considered to be fatty acid, and cocoyl sarcosinamide DEA is not considered to be a fatty acid dialkanolamide, and therefore not subject to the restrictions by Annex III, part 1, 60, yet they can both give rise to nitrosamines. Of course, even though these substances are not subject to any specific restrictions, they are still bound by annex II/410 and 411, and any secondary alkylamine or alkanolamine used in the manufacture must be absent from the finished product except as technically unavoidable levels, and as long as the finished product complies with Article 2 of the Cosmetics Directive.

The above examples clearly demonstrate the need for a proper definition of the relevant entries in Annexes II and III taking into account the scientific basis of the previous SCC and SCCNFP opinions and the evolving scientific knowledge. In those opinions and as documented in the scientific literature available in the public domain, the scientific basis for the entries into the Annexes II and III of the Directive was the relative genotoxic carcinogenicity potential of certain well studied nitrosamines which are (or were at the time) most commonly found as contaminants in cosmetic and consumer products containing mono-, di- and trialkyl amines. At the same time, the SCC and SCCNFP opinions and the evolving scientific knowledge make it absolutely clear that no blanket statements and generalisations should be used to manage the potential risks of nitrosamines because 1) there is to date no evidence that all nitrosamines that can be formed when secondary amine group(s) present in a given chemical have carcinogenic potential. Besides the fact that for most of them there is simply no data, other factors may play a role in this such as the chemical stability of the nitrosamine, the special arrangements of the molecules which may hinder its genotoxic potential, etc; and 2) not all nitrosamines which have been studies to date exhibit the same inherent genotoxic and carcinogenic potential.

Finally, a definition of the wording used in annex III entries 60-62 of "nitrosating systems" is needed as this has also led to different interpretations.

2. TERMS OF REFERENCE

Taking the above together and in light of the previous SCC, SCCNFP opinions on the subject and the currently available data on the genotoxicity/carcinogenicity of nitrosamines, the SCCS is asked to:

Elaborate an opinion on the potential risks to human health by the presence in cosmetics of nitrosamines or of chemicals with secondary amine groups which may give rise to N-nitroso compounds, to provide guidance to the Commission in revising the relevant entries of Annexes II and III of the Cosmetics directive (76/768/EEC). To this end, the SCCS should:

1) Identify chemical classes that can give rise to nitrosamines.

2) Provide a definition (or provide a generic definition) of the substances regulated in Annex II 411 and Annex III 60-62, i.e. secondary alkylamine and secondary alkanolamine, fatty acid dialkylamides and dialkanolamides and mono- and tri-alkylamines and alkanolamines.

3) Comment on the possibility to group chemicals and/or chemical classes with respect to their reactivity towards nitrosating agents and their propensity to give rise to nitrosamines. Identify chemicals or groups/classes for which such grouping with respect to nitrosation may not be possible and case-by-case assessments need to be made.

4) Identify the factors/conditions that may influence/enhance /inhibit the formation of nitrosamines i.e. N-nitroso compounds (e.g. N-Nitroso-oxazolidines), such as nitrogen oxides, nitrite, preservatives, catalysts (e.g. formaldehyde) or others. Provide a clear definition for nitrosating systems. Clarification is required to address whether a nitrosating agent or a nitrosating system should be basis for the regulation of nitrosamine formation in cosmetic ingredients and cosmetic formulations.

5) List the nitrosamines found in cosmetics and advise the Commission of approaches to rank nitrosamines that may occur in cosmetics with respect to their carcinogenic potency.

6) Is there a way to identify chemical classes, and ranking them in terms of their propensity to give rise to carcinogenic nitrosamines and their potency? Inversely, is there a way to relate the carcinogenic potential of nitrosamines formed with the parent chemical class?

7) Comment on the levels of 50 µg nitrosamine/ kg *as set out currently in the Annexes of Directive 76/768/EEC*. Should it apply to finished products or to raw materials? Should it be considered for all nitrosamines potentially formed? Should it be modified, following the ranking of carcinogenic potency of nitrosamines in question? Comment on the "maximum secondary amine content (5% in raw materials and 0.5% in finished products)".

8) On the basis of the answers above SCCS to pronounce itself
   - on the specific cases of spermidine (CAS 334-50-9), gerotine (CAS 71-44-3) and dipropylenetriamine (CAS 56-18-8);
   - on the "Maximum secondary amine content: 5% (applies to raw materials)" and that "Maximum secondary amine content: 0.5%" in the finished cosmetic products" for the Fatty acid dialkylamides and dialkanolamines listed in entry 60 of Annex III, part I.

3. INTRODUCTION

N-Nitroso compounds (NOC) are amongst the most potent carcinogens. More than 300 of these compounds have been tested in about 40 mammalian animal species, including subhuman primates. No species has been found to be resistant against the carcinogenic efficacy of these compounds. It has been also shown that their metabolism in animals is

similar to the metabolism in human tissues. The probability therefore is high that N-Nitroso compounds (NOCs) may be carcinogenic in humans as well. Moreover, the likelihood of a new NOC of as yet unknown biological activity to be genotoxic/ mutagenic/ carcinogenic is very high. NOCs comprise nitrosamines and nitrosamides. Whereas nitrosamines are to be metabolically activated, mostly by cytochrome P450 dependent enzymes, nitrosamides do not require metabolic activation for genotoxic/ carcinogenic activity. In cosmetics only few NOCs have been identified. Mainly two nitrosamines, N-nitrosodiethanolamine (NDELA) and N-nitrosobis (2-hydroxypropyl)amine (NBHPA) have been found.

4. OPINION

4.1. Chemical classes that can give rise to nitrosamines

Compounds / cosmetic constituents considered precursors of relevance for generation of N-Nitroso compounds, i.e. nitrosamines in raw materials and finished cosmetics.

Primary, secondary and tertiary amines can all be nitrosated to generate nitrosamines. The secondary amines in general are the most reactive compounds towards nitrosating agents, generating nitrosamines.

Primary alkyl amines react with nitrosating agents to give short-lived, highly reactive diazonium ions. These reactive intermediates decompose to give molecular nitrogen by substitution, elimination, and molecular rearrangement pathways. However, nitrosamines occasionally arise from secondary processes (see section 4.3.1. below).

Any compound containing the secondary amine functional group is expected to react with nitrosating agents to produce a NOC. The extent of nitrosamine formation will depend upon the structural features of the amine that influence the rates of these transformations and the concentration of the nitrosating agent.

Nitrosamines may also be formed from tertiary amines [1, 3, 9]. The transformation requires the cleavage of the carbon-nitrogen bond of one of the alkyl groups attached to the nitrogen atom. The rates and the nature of these processes depend significantly on the structure of the tertiary amine [9]. Some tertiary amines have unusually high reactivity towards nitrosation, related to the presence of special structural features [9].
Amides and related carbonyl derivatives of amines and of ammonia can react with nitrosating agents to generate NOCs [1, 3]. Because of the electron withdrawing properties of the carbonyl group attached to the nitrogen, the reactivity of amides toward common nitrosating agents is low. Primary amides react with nitrous acid to give the parent acid of the amide and N_2, and can be used as nitrous acid traps. Amides of dialkanolamines can give rise to the corresponding nitrosamines by reaction with ionic nitrites [17, 18].

4.2. Definitions of substances regulated

Amines are classified as primary, secondary or tertiary, corresponding to one, two, or three alkyl or aryl groups bonded to nitrogen (Wade, L.G., Jr. Organic Chemistry, 6th edition 2006, Pearson Prentice Hall, ISBN 0-13-147871-0). A secondary amine may also be regarded as a derivative of ammonia in which two of the hydrogen atoms have been replaced by two organic groups (which may be equal or different from each other and may carry further substituents), linked to the nitrogen by single bonds to the carbon atoms. These carbon atoms cannot be doubly or triply bonded, or bound to other hetero atoms, such as oxygen or nitrogen.

A secondary alkylamine is a derivative of ammonia in which two of the hydrogen atoms have been replaced by two alkyl groups linked to the nitrogen by single bonds to the carbon atoms.

A secondary alkanolamine is a derivative of ammonia where two of the hydrogen atoms are replaced by alkanol groups. The hydroxyl groups should not be substituted.

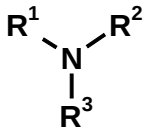
A fatty acid dialkylamide is a derivative of a fatty acid amide, where the alkyl fatty acid moieties could be a branched chain or unsaturated.

A fatty acid dialkanolamide is a fatty acid derivative of a dialkanolamide, where the alkyl fatty acid moiety could be a branched chain or unsaturated.

A monoalkylamine (primary amine) is a derivative of ammonia, where one hydrogen atom is replaced by an alkyl group. A monoalkanolamine is a derivative of a monoalkylamine, where the alkyl group is replaced by an alkanol group.

A trialkylamine (tertiary amine) is a derivative of ammonia, where hydrogen atoms are replaced by alkyl groups. A trialkanolamine is a derivative of trialkylamine, where three of the alkyl groups are replaced by an alkanol group.

Structures



Amines

R^1, R^2, R^3	= H	ammonia
R^1	= organic group	primary amine
R^1, R^2	= organic group	secondary amine
R^1, R^2, R^3	= organic group	tertiary amine

Organic group: alkyl, alkanol, alkylamine, aryl, aryl-alkyl

Amides: R^1 = organic acid residue

R^2, R^3	= H	primary amide
R^2	= organic group	} secondary amide
R^3	= H	
R^2, R^3	= organic group	tertiary amide

4.3. Grouping of chemicals with respect their nitrosating reactivity

4.3.1. Grouping of chemicals

Comment on the possibility to group chemicals and/or chemical classes with respect to their reactivity towards nitrosating agents and their propensity to give rise to nitrosamines.

General considerations:

Nitrosamines may inadvertently be formed during the process of manufacture of commercial preparations, often during the formulation process through the action of unsuspected added ingredients or environmental conditions. While in the case of cosmetics and personal care items, these transformations rarely involve acidic nitrosation, the classical method for preparation of nitrosamines; it is instructive to review some aspects of the acidic nitrosation process in order to reveal how substrate structure modifies reactivity.

There have been numerous kinetic investigations of secondary amine acidic nitrosation, and the subject has been well reviewed from several perspectives [1-3]. The following slightly edited quotation provides a succinct summary [2].

"The most important factors determining the kinetics of amine nitrosation are represented by the acid-base equilibria of the amine and of nitrous acid. The nitrosating agent N_2O_3 reacts with the free electron pair of the unprotonated amine to the corresponding N-nitroso compound, displacing NO_2.

Since the nitrous acid/nitrite equilibrium has a pKa of 3.4, at a pH of 3 about half of the nitrite will be in the reactive, protonated form. Furthermore, since two molecules of HNO_2 form the nitrosating intermediate, N_2O_3, the rate of nitrosation follows second order kinetics with respect to nitrous acid [3]. Low pH favours N_2O_3 formation, but reduces concentration of the unprotonated amine, the reactive species available for nitrosation. The reverse is true for higher pH values. For most alkylamines therefore the pH-dependence of the reaction rate represents a bell-shaped curve, with optimal rates at pH 3-4 and mostly steep slopes above and below this optimal value[4]".

Moreover, it has been established that the intrinsic rate constants for the reaction of the free amine with N_2O_3 for a number of amines are remarkably similar[1, 3]. Consider, for example, the nitrosation rate constants (x 10^8 $M^{-1}sec^{-1}$) for morpholine and piperidine are 2.2 and 1.8, respectively. Yet, their pKa values (8.7 and 11.2, respectively) and experimentally observed nitrosation rates are vastly different. Morpholine, the less basic amine, reacts at least 316 times faster at pH 3.4 under comparable conditions. This comparison and numerous others point to the fact that basicity is the major factor that controls the nitrosation rate of amines.

Simple methods for estimating nitrosation rates based on the pKa of the ammonium ion of the secondary amine have been developed. [2, 3] The basicity of an amine is determined by its structure. Piperidine has a six-membered ring structure containing a single N-H in the ring. Morpholine has a similar structure, except that the CH_2 directly opposite to nitrogen, has been replaced by an oxygen atom. This structural change diminishes the basicity by 2.5 pKa units. The change results from the electron withdrawing capacity of the oxygen atom in comparison to the less electronegative carbon of piperidine. In general, compared to a normal alkyl chain, substitution of CH_2 by a more electronegative oxygen or, to a lesser extent, a nitrogen atom, will decrease the basicity and increase the nitrosation rate of the amine. Since the pKa values of many amines are available, nitrosation rates can be relatively easily predicted under specified conditions.

Replacement of one of the alkyl groups of a secondary amine by a benzene ring reduces the amines basicity by approximately 5 pKa units. Substituents in the benzene ring can have a powerful effect on the nitrosation rate of the secondary amine, because they affect both the amine basicity and the intrinsic rate constant for nitrosation[1]. So here, the simple relationship between basicity and nitrosation rate breaks down. Electron withdrawing substituents in the benzene ring decrease basicity, but they also decrease the intrinsic rate constant for nitrosation. Electron donating substituents in the benzene ring have the opposite effect and overall increase nitrosation rates.

Although not common, steric crowding adjacent to the nitrogen atom of the secondary amine reduces nitrosation rates. This effect was demonstrated for piperidines methylated at the 2- and 6-positions[5]. Stereo electronic effects are quite important in determining tertiary amine nitrosation rate, see Section below

Groupings

Primary amines

Primary alkyl amines react with nitrosating agents to give short-lived, highly reactive diazonium ions (the species also formed by metabolic activation of nitrosamines). These reactive intermediates decompose to give molecular nitrogen by substitution, elimination, and molecular rearrangement pathways. For example, the nitrosation of ethanolamine in acetic acid produces ethylene glycol, 2-hydroxyethyl acetate, and acetaldehyde[6]. The generated acetaldehyde can react with ethanolamine to form 5-methyloxazolidine which may be nitrosated to the corresponding NOC. Depending on the primary alkanolamine, other NOC may occasionally arise from such secondary processes (see section 4.4.5 below). Spermidine, a polyamine containing both primary and secondary amino groups, reacts with nitrosating agents to give N-nitrosopyrrolidine and other nitrosamines. It is likely formed from the reaction of a diazonium ion derived from one of primary amino groups with the secondary amine to close the ring (see section 4.8. [7, 8]).

Secondary amines, alkylamines and alkanolamines:

Any compound containing the secondary amine functional group is expected to react with nitrosating agents to produce a nitrosamine. The extent of nitrosamine formation will depend upon the concentrations of the amine and the nitrosating agent as well as structural features of the amine influencing reaction rates as discussed above.

Tertiary amines

Nitrosamines may also be formed from tertiary amines. The transformation requires the cleavage of the carbon-nitrogen bond of one of the alkyl groups attached to the nitrogen atom. The rates and the nature of these processes depend significantly on the structure of the tertiary amine[[1, 3, 9]. In the case of tri-alkyl amines, the scope, mechanism[10], and kinetics have been well studied[3, 10, 11]. A number of nitrosating agents are capable of affecting this transformation, but the reaction is slow compared to secondary amine nitrosation and often requires a significant molar excess of a nitrosating agent and temperatures above 60°C to occur at a reasonable rate. Because of these facts, tertiary alkanolamines and other tri-alkyl amines may be used in many commercial formulations of interest without concern for nitrosamine formation.

Aromatic di-alkyl amines are much more reactive toward nitrosation than are the tri-alkyl amines.[1, 12-14] The products of these reactions are arylalkyl nitrosamines. Only those compounds containing a powerful electron withdrawing group in the benzene ring do not react at an appreciable rate. Sun screen preparations containing esters of 4-dimethylaminobenzoic acid have been shown to contain N-nitroso compounds produced during formulation.[13] Significant advances have been made in the understanding of the chemical details of these transformations.[1, 12-15]

Some tertiary amines have unusually high reactivity towards nitrosation because of the presence of special structural features[9]. Some of these have been reviewed [1, 3, 9]. However, because the chemistry is hard to generalize, it is recommended that manufacturers of products containing aromatic di-alkyl amines use the Apparent Total N-nitroso group Content (ATNC) procedure defined and discussed in the COLIPA technical document[16] to demonstrate that their products are nitrosamine free.

Quaternary ammonium compounds:

Also quaternary ammonium compounds may be of relevance with respect to NOC formation, as may be deduced from reports on acute liver toxicity of combinations of nitrite and certain quaternary ammonium compounds (48). Moreover, amine nitrosation by nitrite has been reported to be catalyzed in presence of the cationic surfactant decyltrimethylammonium bromide and other micelle forming agents (49), suggesting amine impurities in such surfactants being prone to enhanced nitrosation risk.

Amides. The reactivity of amides and related carbonyl derivatives of amines and of ammonia toward nitrosating agents has been reviewed recently[1], but already a 1975

review [3] contains much pertinent information. Because of the electron withdrawing properties of the carbonyl group attached to the nitrogen, the reactivity of amides toward common nitrosating agents is low. Primary amides react with nitrous acid to give the parent acid of the amide and N_2, and can be used as nitrous acid traps. Powerful nitrosating agents such as fuming nitric acid, N_2O_4, and $NOBF_4$ are required to convert secondary amides into their N-nitroso derivatives. While these same reagents have little effect on tertiary amides, ionic nitrite can convert them into nitrosamines. Amides of dialkanolamines are especially subject to nitrosation by ionic nitrites [17, 18] See also section 4.4.3.
N-alkyl-carbamates are more readily nitrosated than amides [3]. In N- alkylureas, the presence of the second carbamoyl nitrogen brings about greatly enhanced nucleophilicity and such compounds are very easily nitrosated, forming nitrosoureas, most of which are strong carcinogens.

4.3.2. Cases where grouping with respect to their reactivity to nitrosating agents is not possible

I*dentify chemicals or groups/classes for which such grouping may not be possible and case-by-case assessments need to be made.*

This group may include dyes and other nitrogen-containing additives that are difficult to classify. It is recommended that manufacturers of products containing these substances use the Apparent Total N-nitroso group Content (ATNC) procedure defined and discussed in the COLIPA technical document[16] to demonstrate that their products are nitrosamine free.

Specific case of hair dyes ingredients:

Primary, secondary and tertiary amines are ingredients of both oxidative and non-oxidative hair dye formulations. These are used in concentrations of 0.1-3% (on head). In general, these amines invariably are attached to an aromatic, heteroaromatic or polycyclic ring system that may carry hydroxy-, amino-, alkylamino-, alkoxy- and other substituents.

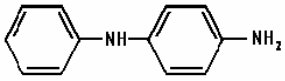
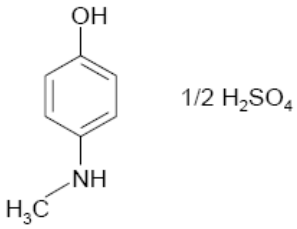
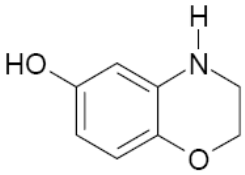
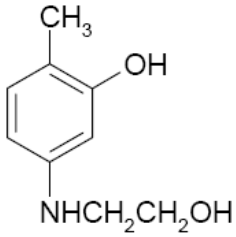
Monosubstituted nitrogens attached to aromatic rings are very easily nitrosated under nitrosating conditions. Examples for compounds carrying easily nitrosatable nitrogens are, for instance, COLIPA numbers A9, 22, 25, 31, 84, 98, 130, 138, B5, 70, 34, 36, 37 and similar compounds (table 1).
Disubstituted nitrogens attached to aromatic rings may generate NOC as well. In general the rate of NOC formation is expected to be lower for tertiary amines, as compared to secondary amines, but there are exceptions. When the N-nitrostable group is attached to an electron-rich aromatic ring, reactivity towards nitrosating agents to give nitrosamines by dealkylation is greatly increased. Furthermore, certain compounds may also react through a radical cation mechanism, as has been shown for compounds with similarity to A 50[1,2] A 50 is expected to produce a nitrosamine and a nitro compound in a radical cation mechanism, encompassing competition of N-dealkylation with ring nitration and with diazotization of the primary amino group. There are other tertiary amines on the list that may be conceptualized to react similarly, for example: A121, B31, B37, B73, B77 (see table 1 below)
It is difficult to predict the precise product ratios and rates. Of note, in presence of an aromatic nitro substituent in a hair dye constituent, it is important to ensure that no nitrosating potential is exerted.

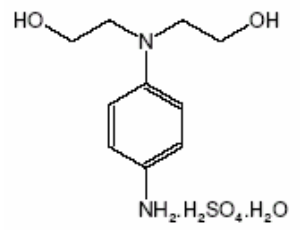
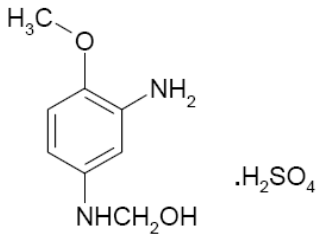
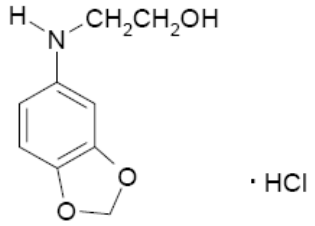
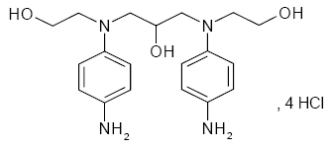
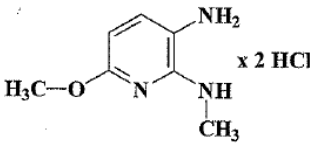
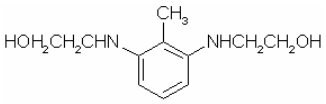
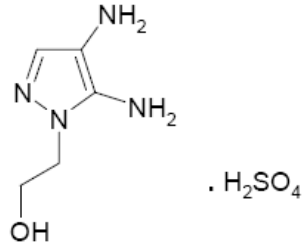
C117 is a compound that in addition to carrying a nitrosatable amino group as part of an aminoanthraquinone ring system also carries an N-Methyl-morpholinium substituent that under nitrosating conditions may give rise to N-Nitrosomorpholine.
B70, represents a urea structure, monosubstituted with a 2-(4 Nitrophenylamino) ethyl substituent. It has two easily nitrosatable nitrogens, one of which expected to form a directly acting NOC. Since during the oxidative hair dyeing process mostly basic conditions prevail, this NOC is expected to rapidly decompose.

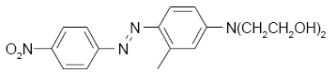
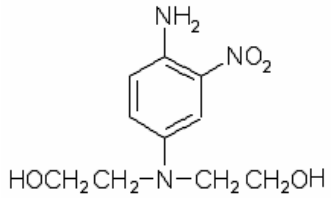
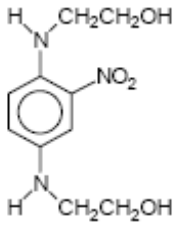
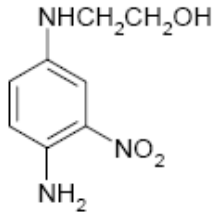
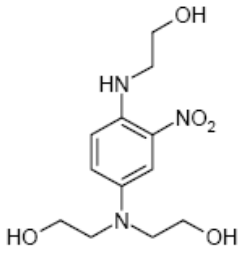
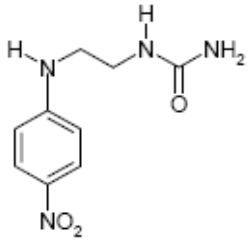
A154 represents an N-substituted diaminopyrazole. In the presence of nitrosating agents it is expected to form a diazonium intermediate that may generate the corresponding 1,2,3-triazole by ring closure reaction.

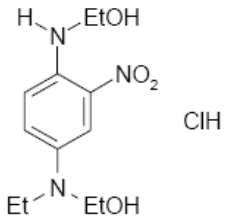
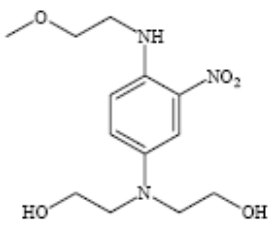
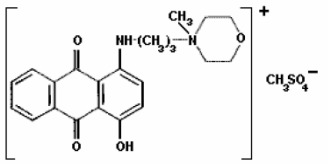
Summary
Since the nitrosation chemistry of hair dye constituents, product types, ratios and formation kinetics are difficult to predict, case by case investigations are necessary. In many cases, elucidation of structures and biological properties of the resulting NOC would represent an enormous task, since structures expected to result from nitrosation of such hair dye components are vastly diverse. Biological data on the corresponding array of generated N nitrosation products are not available. NDELA formation or contamination is only expected to result from nitrosation in very few cases. When information on a specific NOC structure is not available, the default assumption that all potentially generated NOC will be mutagenic/carcinogenic should be applied.
It is suggested to refer to the general purity specifications for amines in cosmetics, requiring that a given amine constituent contains not more than 50 ppb of an NOC. When compound specific analytical information is not available, generic determination of apparent total NOC (TNOC) should be used. It is suggested to follow the procedure as described in the COLIPA 2009 guidance document. In cases where aromatic NOC are expected to be generated, N-Nitroso-N-Methylaniline may serve as a reference instead of NDELA.

Table 1: Examples of hair dyes ingredients which may react with nitrosating agents

COLIPA n°	**INCI name - CAS number** *Common name in italic*	**Formula**
A9	N-Phenyl-p-phenylenediamine CAS: 101-54-2; 2198-59-6 (HCL); 4698-29-7, sulphate	
A22	p-Methylaminophenol CAS: 150-75-4	
A25	Hydroxybenzomorpholine CAS: 26021-57-8	
A31	2-Methyl-5-hydroxyethylaminophenol CAS: 55302-96-0	

COLIPA n°	INCI name - CAS number *Common name in italic*	Formula
A50	N,N-bis(2-Hydroxyethyl)-p-phenylenediamine sulfate CAS: 54381-16-7 (sulfate)	
A84	2-Amino-4-hydroxyethylaminoanisole CAS: 83763-47-7	
A98	Hydroxyethyl-3,4-methylenedioxyaniline HCl CAS: 94158-14-2	
A121	Hydroxypropyl bis (N-hydroxyethyl-p-phenylenediamine) HCl CAS: 128729-30-6 (free base); 128729-28-2 (hydrochloride)	
A130	6-Methoxy-2-methylamino-3-aminopyridine HCl CAS: 83732-72-3 (2HCl); 90817-34-8 (HCl)	
A138	2,6-Dihydroxyethylaminotoluene CAS: 149330-25-6	
A154	1-Hydroxyethyl-4,5-diamino pyrazole sulfate CAS: 155601-30-2	

COLIPA n°	INCI name - CAS number *Common name in italic*	Formula
B5	Disperse Red 17 (Ethanol, 2,2'-[[3-methyl-4-[(E)-(4-nitrophenyl)azo] phenyl]imino]bis-) CAS: 3179-89-3	
B31	HC Red n° 13 (2,2'-((4-Amino-3-nitrophenyl)imino)bisethanol) CAS: 94158-13-1	
B34	N,N'-bis(Hydroxyethyl)-2-nitro-p-phenylenediamine CAS: 84041-77-0	
B36	HC Red n° 7 (1-Amino-2-nitro-4-(β-hydroxyethyl)-aminobenzene) CAS: 24905-87-1	
B37	HC Blue n° 2 (2,2´-{[4-(2-hydroxyethyl)amino-3-nitrophenyl]imino} bisethanol) CAS: 33229-34-4	
B70	4-Nitrophenyl aminoethylurea CAS: 27080-42-8	

COLIPA n°	INCI name - CAS number *Common name in italic*	Formula
B73	HC Blue n° 12 (1-(beta-hydroxyethyl)amino-2-nitro-4-N-ethyl-N-(beta-hydroxyethyl)aminobenzene) CAS: 104516-93-0 (free base); 132885-85-9	
B77	HC Blue n° 11 (Ethanol, 2,2'-[[4-[(2-methoxyethyl)amino]-3-nitrophenyl]imino]bis-) CAS: 23920-15-2	
C117	Hydroxyanthroquinone aminopropyl methyl morpholinium methosulphate CAS: 38866-20-5	

4.4. Factors that influence the formation of nitrosamines

4.4.1. Nitrosation in the presence of nitrogen oxides

Is N-nitrosation expected to occur, for instance in the presence of nitrogen oxides (NOx), when pH is close to neutrality or even alkaline?

The nitrosation of amines by NOx under neutral to alkaline conditions to produce nitrosamines has been well established, both in the laboratory and in practice [1, 19-27, Eisenbrand,Blankart Sommer, weber 1991)]. A review relevant to cosmetics and personal care items is provided in the COLIPA technical document (Section 4.1)[16]. Major sources of NOx include: air pollution; tobacco smoke, petroleum-or gas-powered machine exhaust, nitrite treated containers; and high temperature burners, such as used for heating buildings.

4.4.2. Role of nitrites as nitrosating agents

Ionic nitrite: It is important to realize that nitrite can be formed from NOx under neutral to basic conditions[1], as well as by the bacterial or chemical reduction of nitrate. Ionic nitrite can react with amides of alkanolamines to produce nitrosamines as discussed in section 4.4.3 below.

Ionic nitrite reacts with aldehydes (particularly formaldehyde) and amines to produce nitrosamines through the intermediacy of imines [1, 28]. Nitrous esters (alkyl nitrites) can also be formed by displacement reactions involving ionic nitrite from other esters [17, 18]. Nitrous esters are very effective nitrosating agents [1].

4.4.3. Risks of nitrosation during production/formulation

Amides of dialkanolamines react readily with ionic nitrite at temperatures which can be achieved from heats of mixing [17, 18]. Other amides in the presence of high-boiling alcohols, such as glycerol, also can react with nitrite to produce nitrosamines when heated. In the first case, the amide of the dialkanolamine, for example, a fatty acid amide, slowly rearranges both inter- and intramolecularly by acyl transfer from the nitrogen to the alcohol oxygen. Although this process involves an equilibrium that greatly favours the amide, the resulting ester rapidly reacts with ionic nitrite to form a nitrous ester which then reacts with the liberated secondary amine to form a nitrosamine. The lauryl amide of DEA forms NDELA in significant yields, when heated with nitrite. The quantities formed are increased when ethylene glycol is added.
Other tertiary amides have been shown to produce nitrosamines when heated with nitrite in a high-boiling hydroxylic solvent, such as glycerol or ethylene glycol [17, 18]. This process again involves acyl transfer from the amide to the alcohol to generate a nitrous ester which then rapidly reacts with the amine produced by the acyl transfer to give a nitrosamine.

4.4.4. Role of preservatives

Some preservatives such as Bronopol (INCI 2-Bromo-2-nitropropane-1,3-diol) and Bronidox (5-Bromo-5-nitro-1,3-dioxan), permitted to be used in cosmetics, have been found to be effective nitrosating agents. In aqueous alkaline solution diethanolamine and, to a lesser extent triethanolamine (20 mmol each) yielded NDELA in substantial amounts after prolonged storage in the presence of Bronopol (80 nmol). Bronidox was a less effective nitrosating agent under the same conditions. A similar ratio in nitrosating potency was observed in a near neutral (pH 6.8) non ionic model emulsion, containing 1% diethanolamine after 50 days of storage Bronopol decomposes into nitrosating agent(s) and formaldehyde and this may contribute to enhance nitrosamine formation by the catalytic effect of formaldehyde Thus, whenever these preservatives are coformulated with constituents having secondary amine structures, substantial nitrosamine formation is to be expected. Tertiary amines are considerably less reactive, yet can give rise to some nitrosamine formation after extended periods of storage. It is not known to what extent alkanolamides or substituted ureas might react but since their nitrosation by Bronopol/Bronidox cannot be ruled out, any co-formulation of these preservatives in cosmetics together with compounds having a nitrosatable nitrogen is to be avoided.

4.4.5. The role of catalysts like formaldehyde for nitrosation

Aldehydes (especially formaldehyde) may catalyze the formation of nitrosamines from secondary amines through the intermediacy of imines. These transformations occur readily at pH values ranging from 5-10. A similar reaction can occur with these ingredients in organic solvents that can serve as a source of formaldehyde, such as dichloromethane. The same applies also to formaldehyde donors like hydroxymethylurea and other formaldehyde releasing preservatives. Hydroxymethylsarcosine is a formaldehyde donor that may be expected to catalyse (its own) nitrosation. Thus, in addition to providing the nitrosation catalyst formaldehyde, so-called formaldehyde donor biocides can also be nitrosated as such to produce N–nitroso compounds. Practical methods for preventing such nitrosation chemistry are discussed in the Colipa document [16].

Halide ions, thiosulfate, thiols, and some phenolic compounds are all effective catalysts of nitrosation [1]. Nitrosation can also be catalyzed by a number of other substances (see reference 1 for a complete list).

Alkanolamines are known to react with aldehydes to form oxazolidines, which then may react with a nitrosating agent to give N-nitrosooxazolidines. Both 1,3-N-nitrosooxazolidine and 5-methyl-1,3-N-nitrosooxazolidine have been found in commercial formulations containing ethanolamine [31] and 1-amino-2-propanol [32], respectively. These transformations also involve formaldehyde. Such heterocyclic nitrosamines could form if their precursor ingredients are present or generated in formulations of the products being considered here.

4.4.6. Inhibitors of nitrosation

Cosmetic formulations require both hydrophobic and hydrophilic nitrosation inhibitors to be effective in both phases. Water soluble inhibitors may comprise ascorbic acid/ascorbates and other water soluble antioxidants, oil soluble inhibitors may include ascorbyl palmitate, tocopherols, butylated hydroxytoluene / hydroxyanisole (BHT/BHA), gallate esters, amongst others. There are several general reviews of methods for inhibiting the formation of nitrosamines under various circumstances [2, 3, 33-35]. Annex 1 of the Colipa document[16] addresses this subject, listing a number of inhibitors.

4.4.7. Mitigation of nitrosation in raw materials and during production

The use of raw materials complying with purity specifications is required, especially with respect to contents of secondary amine, and to avoiding any contact with adventitious nitrosating agents such as nitrite treated raw material containers, atmospheric NOX-sources at production, packaging and storage units and further mitigation measures as discussed before and in the COLIPA document (16).

4.4.8. Definition of nitrosating agent/system

Nitrosating agents have been covered under 4.4.1. to 4.4.4. Any situation where nitrosating agents are present simultaneously with N nitrosatable structures, irrespective of absence or presence of catalysts and/or inhibitors, may be viewed as a nitrosating system as mentioned in the present regulation. Nevertheless, the SCCS recommends using the term nitrosating agent instead of nitrosating system in the regulation.

4.5. Carcinogenic potency of nitrosamines in cosmetics/ranking/ dose descriptors

List the nitrosamines found in cosmetics and advise the Commission of approaches to rank nitrosamines that may occur in cosmetics with respect to their carcinogenic potency.

4.5.1. List of nitrosamines in cosmetics

Nitrosamines found in cosmetics are described below. Mainly NDELA and NBHPA have been found in cosmetics. The other nitrosamines of the list have been found only very rarely in cosmetics and/or raw materials. Recent analytical surveys, covering the time period from 2000 to present also confirm that these two nitrosamines were exclusively found (ref. 50 to 61).

N-nitrosated sunscreen agents, such as the NOC of 2-ethylhexyl-p-N,N-dimethylamino benzoate have been reported to occur in sunscreen formulations from the US market.

Table 2: List of Nitrosamines ever found in cosmetics and/or raw materials used for cosmetics with CAS Number and the most commonly used abbreviation when available

NDMA	N-Nitroso-dimethylamine,	CAS 62-75-9
NDEA	N-Nitroso--diethylamine,	CAS 55-18-5
	N-Nitroso-N-methyl-N-dodecylamine,	CAS 55090-44-3
NMOR	N-Nitroso- morpholine,	CAS 59-89-2
NPYR	N-Nitroso- pyrrolidine,	CAS 930-55-2
NBHPA	N-Nitroso-bis (2-hydroxypropylamine),	CAS 53609-64-6
NDELA	N-Nitroso- diethanolamine.	CAS 116-54-7
NPABA	N-nitroso-Para amino benzoic acid esters	

4.5.2. Ranking with respect to carcinogenic potential

Calculation of dose descriptors

The SCCS based the ranking of the NOCs listed in Table 2 with respect to their carcinogenic potential through dose descriptors commonly used in carcinogenic risk assessment, noting that other approaches to rank carcinogenic potency may be considered as well.

The T25

The T25 approach is defined as the chronic dose rate (usually expressed in units of mg per kg bodyweight per day) which will give tumours at a specific tissue site in 25% of the animals after correction for spontaneous incidence and within the standard life time of the species (Dybing et al., 1997). The T25 values are likely to be within the range of the experimental data. The use of data from the lowest dose giving a significant response should in most instances reduce the problem of intercurrent mortality to an acceptable degree. This often occurs in the two years cancer bioassay when e.g. calculating the TD50 value. The data profile needed for calculating a T25 value has to be less specific, e.g. time to tumour data are not needed. It is recognized that the potential loss of precision does not match the order of magnitude differences in carcinogenic potencies found between high and low potency substances in animals.

In a study of 110 substances an almost perfect (slope in log-log plot of 1.05) coincidence was found between potency estimates by the TD50 approach and the T25 method (Dybing et al., 1997). It was concluded that, given the very large variation in carcinogenic potency between individual carcinogens, any difference between the T25 value and a "true" potency value should be negligible.

The T25 method (Dybing et al., 1997, Sanner et al., 2001) has been used within EU in setting specific concentration limits for carcinogens in preparations (EC 1999) and recently as a basis for calculation of lifetime cancer risk (LCR) and for quantitative hazard assessment of non-threshold carcinogens in several regulatory areas e.g. ECHA (2008), SCHER/SCCP/SCENIHR (2009) and SCCS (2010).

The Benchmark Dose (BMD)

The BMD approach has been increasingly used and recommended (EFSA, 2009, 2011; SCHER/SCCP/SCENIHR (2009); SCCS's Notes of Guidance (2010)). It is the dose level, derived from the estimated dose-response curve, associated with the specific change in the response defined through that BMR. It uses all available dose-response data of a study and fits a set of mathematical models. The lower one-sided confidence bound BMDL accounts for

the statistical uncertainty in the data (with the statistical certainty level of 95%) and is used as a point of departure (PoD), the reference point (RP) by EFSA. The BMDL is calculated for a specified Benchmark response level (BMR). A BMR=10% of extra risk over background has been set as default, (EFSA 2009) level when analyzing cancer bioassays.

The TD_{50}

The TD_{50} value has been introduced primarily for ranking of carcinogens in the Carcinogenic Potency Data Base (CPDB), not for the risk assessment and possible extrapolation to low doses. It is defined as dose in mg/kg bw/day which, if administered chronically for the standard lifespan of the species, will halve the probability of remaining tumourless throughout that period. Its definition requires linking with the statistical analysis method applied on individual time-to-tumour data. The ranking is based on a central tendency parameter, the median time to tumour (50% of the animals remaining tumour free), adjusted for the respective median in the control group. In principle the TD-50 is only applicable when individual time-to-tumour data are available and can be analysed by appropriate statistical methods, e.g. excluding competing risks and intercurrent mortality. In all other cases the determination of TD-50 values is complicated by intercurrent deaths due to causes other than tumorigenesis and the non-observability of the time of tumour onset. This requires assumptions on the nature of the data and the type of dose-response For instance, if an animal died bearing tumour(s), the time of death becomes the time of tumour onset. Consequently, the measure of tumour incidence is confounded with mortality and biased TD50 estimates can occur (Portier and Hoel, 1987). If, on the other hand, tumours do not significantly alter survival, then TD50 values become related to the rate-of-death-with-tumour, rather than the tumour incidence rate (Meier et al., 1993) . The TD-50 has been introduced as non-parametric statistical approach and thus not model dependent. However, it can also be used with parametric time-to-tumourmodels (e.g. the Weibull model).

Other Approaches

The Slope Factor (SF)

The slope factor has been used by US EPA as convenient descriptor of cancer potency ((http://www.epa.gov/iris/carcino.htm)) characterizing the slope of the dose-response curve at low doses (where the slope is still linear). It has dimensions of risk of cancer per unit dose and converts estimated daily intakes averaged over a lifetime of exposure directly to incremental risk of an individual developing cancer. The SF approach is performed in three steps:(1) selection of the appropriate data sets to use, (2) derivation of estimates at low doses from experimental data at high doses, using an extrapolation model and (3) choice of an equivalent human dose when animal data sets are used. Since the SF characterizes the slope of the dose-response curve at low doses by a linear approximation it can be considered as a specific modification of one model class (the linearized multistage model) also used in the BMD approach.

Quantitative structure toxicity relationship (QSTR) information may be used to qualitatively define carcinogenic potencies e.g., by establishing several categories of potency. TOPKAT(®) has been used to identify chemicals with a high probability of being chronically toxic and/or carcinogenic (46). Another approach to QSAR modelling is TOPological Substructural MOlecular DEsign (TOPS-MODE) for predictions of the carcinogenic potency of nitroso compounds (47).

Dose-response data in various *in vivo* genotoxicity studies. The *in vivo* genotoxicity potency (e.g. micronucleus assay (MN), the in vivo transgenic rodent mutation assay (TG), chromosome aberration and the comet assay) of different substances measured as the lowest effective dose giving response or using the BMD approach have been compared with the respective T25 or BMD10 from carcinogenicity studies in rats and mice. A good

correlation was found between the in vivo genotoxic potency and the carcinogenic potency. Thus the in vivo genotoxicity potency may be used as surrogate for carcinogenic potency, provided the in vivo genotoxicity dose-response data are of sufficient quality (Sanner et al., 2005, Hernandez et al., 2011)

The SCCS decided to use the three dose descriptors T25, BMD and TD50 for ranking carcinogenic potency. Comparative ranking is summarized in Table 3 (columns 2-4)

Table 3: Ranking of potencies of nitrosamines based on carcinogenicity studies on rats (the data are taken from the calculations presented in Tables 1 – 7, see annexes I and II)

Name	T25 (mg/kg bw/d) (SD)	BMDL-10 (mg/kg bw/d)	TD50 (mg/kg bw/d)
N-nitrosodimethylamine NDMA	0.058 (±0.028)	0.027	0.0959[m,v]
N-nitrosodiethylamine NDEA	0.085 (±0.065)	0.018 #	0.0265[m,v]
N-nitrosomorpholine MMOR	0.094 (±0.036)	NA	0.109[m]
N-nitroso-N-methyl-N-dodecylamine	0.46 (±0.08)	NA	0.537[m,P]
N-nitrosobis(2-hydroxypropyl)amine NBHPA	0.54	NA	0.846[m]
N-nitrosopyrrolidine NPYR	0.57 (±0.46)	0.16	0.799[m,P]
N-nitrosodiethanolamine NDELA	2.18 (±0.72)	1.74	3.17[m,v]
N-nitroso-Para amino benzoic acid esters NPABA	No data	No data	No data

NA: not available
[m] there is more than one positive experiment
[v] variation is greater than ten-fold among statistical significant (two-taled p<0.1) TD50 values from different positive experiments
[P] 100% of dosed animals had tumours at a target site in an experiment in this species)
[t]TD50 based on the same data as T25 was 0.161 mg/kg bw/d.
BMDL-10 value 0.034 of the study of Berger et al. (1987, 1990) was by factor of about 2 higher

In order to perform full BMD analysis, at least 3 dose groups should be available. This limits the number of substances/studies for the calculation of BMD. For the calculation of T25 only the lowest dose giving a significant increase in tumour frequency is used. It should be noted that in most cases, studies were the lowest tumour frequency was higher than 80% or the study period was less than 40 weeks were excluded.

T25 is calculated as described by Dybing et al (1997) and is based on experimental data on rats only as such data are available for all the nitrosamines considered. Suitable mice data are only available in the case of N-nitrosodimethylamine and N-nitrosopyrrolidine. In the case of N-nitrosodimethylamine the TD50 indicated less potency in mice than in rats, while in the case of N-nitrosopyrrolidine the potency appeared to be similar in both species. In most cases, the T25 has been calculated on the bases of malignant liver tumours. T25 values were determined for all studies available for seven of the above listed eight nitrosamines (no data were available for NPABA) and when more than one study was available a mean T25 with standard deviation (SD) was reported. Details on the data used and the derivation of the T25 are given in Annex I.

In contrast to the dose descriptor based on the T25 and TD50 which is an average of all data sets suitable where at least on control and one dose group was reported, the BMD approach reported the BMDL10 values of only those data sets where sufficient dose-response data were available to apply this method such that a set of models can be fitted to

the dose-response data. The absolute minimum number of experimental groups (e.g. one control and two dose groups) to apply the BMD technically is three. However, in this case major model with three parameters would not provide a reliable BMDL value. Therefore, in order to account for model uncertainty, only data sets with three dose groups and a control were subject to a BMD analysis (see Annex II) using BMDS 2.1.2 of http://epa.gov/NCEA/bmds/The smallest BMDL10 value among all acceptable models fitted was identified and used to characterize the BMDL10 of a study as long as the BMD and the BMDL did not differ by more than one order of magnitude which would indicate undue extrapolation. For a specific nitrosamine the smallest BMDL among all studies amenable to a BMD analysis was chosen when the study data were of comparable quality. Otherwise the BMDL of the study of highest quality was chosen. Details of all BMD analyses used to derive the dose descriptors in Table 8 are provided in an Annex.

Comments on the ranking
Based on T25 N-nitrosodimethylamine, N-nitrosodiethylamine, and N-nitrosomorpholine were the most potent nitrosamines with T25 values ranging from 0.058 to 0.094. N-nitroso-N-methyl-N-dodecylamine, N-nitroso(2-hydroxypropyl)amine, and N-nitrosopyrrolidine showed a similar potency with T25 ranging between 0.46 and 0,57 and were on the average 6 – 7 times less potent. Only N-nitrosodiethanolamine with a T25 =2.18 was about 30 times less potent than the most potent carcinogen amongst nitrosamines.

Based on the BMDL10 NDEA appeared as the most potent carcinogen (BMDL10 ranging between 0.018 and 0.034 mg/kg bw/day based on two independent studies. Slightly less potent was NDMA with a BMDL10=0.027 mg/kg bw/day. Less potent by a factor of about 10 compared to NDEA was NPYR with a BMDL10 = 0.16 mg/kg bw/day
NDELA was by a factor of about 100 less potent with BMDL10=1.74 mg/kg bw/day whereas for the other four nitrosamines no BMD/L value could be calculated. It must be noted that differences between the studies and the dose descriptors calculated using their data are not only due to different designs but also different types of carcinogenic endpoints. For example the BMDL10 of NPYR is based on total liver tumour incidence, whereas the BMDL10 of NDMA is based on fatal liver neoplasm. Such type of uncertainty should be taken into account when interpreting differences in ranking.

The ranking by TD50 exhibited NDEA as most potent carcinogen (TD50= 0.03 mg/kg/day) whereas NDMA and NMOR were less potent (TD50 = 0.10 and 0.11 mg/kg/day, respectively. N-nitroso-N-methyl-N-dodecylamine, NPYR and showed similar potencies (TD50 = 0.54 -0.84 mg/kg/day) whereas NDELA was less potent with a TD50 = 3.2 mg/kg/day. It should be noted that for NDMA, NDEA, and NDELA the variation was greater than ten-fold among studies used for calculation of the numbers given in Table 3.

As evident from Table 3, the most commonly found nitrosamine in cosmetics, NDELA, is much less potent as compared to NDMA or NPYR, nitrosamines that are commonly found in food.

4.6. Carcinogenic potential of nitrosamines in relation to their parent compounds

There is no obvious way to relate carcinogenic potential of nitrosamines formed with the parent chemical group. To predict the carcinogenicity potency of nitrosamines potentially generated, it has to be considered they have to undergo metabolic activation, in most cases by CYP-450 dependent hydroxylation in the alfa-position, to become (geno) toxic agents. The metabolic introduction of the hydroxyl group leads to a very unstable alfa-hydroxy-nitrosamine that decays by aldehyde elimination, liberating the ultimate electrophilic, genotoxic/mutagenic/ intermediate that subsequently binds to DNA, thereby causing mutations and cancer. Structural elements that inhibit this CYP dependent metabolic

activation/electrophil generation may decrease or even abrogate mutagenicity/carcinogenicity. As an example an alpha-t-butyl substituent adjacent to the N-Nitroso group, eliminates carcinogenicity, as demonstrated e.g. for ethyl-t-butyl nitrosamine. In addition, strongly basic or acidic centres present in a given NOC may also strongly reduce or even abrogate mutagenicity/carcinogenicity as shown e.g. for N-nitroso-N' methyl-piperazine or for most N-Nitroso-amino acids and their esters (Preussmann, R, 1990).
These limited structure activity observations indicate specific structural elements, when present in secondary amines may result in generation of non-carcinogenic nitrosamines. There might be other structures that can mitigate formation of carcinogenic NOC but this need to be demonstrated. It is recommended that case by case evaluations should be performed if this principle of using "safe amines" in cosmetics is to be applied (Janzowski, C et al, 2000).

SCCS recommends for situations where contact with nitrosating agents during production, formulation, storage and use cannot reliably be excluded, to use amines for cosmetics that are not easily nitrosated and /or give rise to noncarcinogenic nitrosamines.

4.7. Levels of nitrosamines in finished products and raw materials

Comment on the levels of 50 µg nitrosamine/ kg as set out currently in the Annexes of Directive 76/768/EEC. Should it apply to finished products or to raw materials? Should it be considered for all nitrosamines potentially formed? Should it be modified, following the ranking of carcinogenic potency of nitrosamines in question? Comment on the "maximum secondary amine content (5% in raw materials and 0.5% in finished products) ".

As currently regulated, the purity specification of 50 µg nitrosamine/ kg should apply to raw materials and to all nitrosamines potentially formed. The secondary amine content in raw materials should be as low as achievable, following GMP rules, but should not exceed 5% in raw material. In the finished cosmetic product, a maximum secondary amine content of 0.5% should be maintained. The SCCS supports the present regulation since the present regulation now provides high degree of consumer protection.

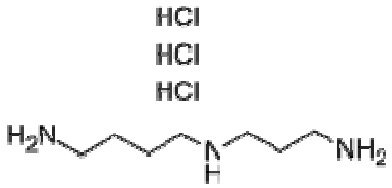
4.8. Specific cases

On the basis of the answers above SCCS to pronounce itself

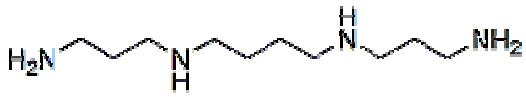
> *4.8.1- on the specific cases of spermidine (CAS 334-50-9), gerotine (CAS 71-44-3) and dipropylenetriamine (CAS 56-18-8);*
>
> *4.8.2- on the "Maximum secondary amine content: 5% (applies to raw materials)" and that "Maximum secondary amine content: 0.5%" in the finished cosmetic products" for the Fatty acid dialkylamides and dialkanolamines listed in entry 60 of Annex III, part I.*

4.8.1 Specific Cases

The SCCS is of the opinion that the specific cases of spermidine (CAS 334-50-9), gerotine (CAS 71-44-3) and dipropylenetriamine (CAS 56-18-8) fall under the entry 411 of annex II (secondary alkylamine) since each of these compounds contains at least one secondary amino group, with two alkyl substitutions.



spermidine trihydrochloride, (CAS 334-50-9)



Spermine, (CAS 71-44-3), Gerotine

dipropylenetriamine (CAS 56-18-8)

It has been shown that the acidic nitrosation of spermidine produces nitrosamines that are mutagenic [36-42]. The secondary amine is nitrosated in all of the nitrosamine products found. The primary amine groups are nitrosated and deaminated through the formation of short-lived reactive diazonium ions. It is reasonable to expect that other polyamines, will behave similarly. For example, some other nitrogen containing compounds, as exemplified by structures like chlorohexidine (1,1'-Hexamethylenebis[5-(4-chlorphenyl)biguanide, hexidine]) or hexetidine (1,3-Bis(2-ethylhexyl)-hexahydro-5-methylpyrimidine-5-amine,) can also be rapidly nitrosated to form nitrosamines. An excess of nitrous acid was used in that work (29, 30). Limited quantities of nitrosating agents may produce somewhat different results but it is highly likely that the secondary amine functionality will be nitrosated to give a nitrosamine. Subsequent research has verified the mutagenicity of nitrosospermidine [43] and demonstrated that these compounds may occur in the human environment. [44, 45].

4.8.2 Secondary amine content in raw material and in finished cosmetic products

As described above (see Section 4.7), the SCCS supports the present regulation on the *"Maximum secondary amine content: 5% (applies to raw materials)" and that "Maximum secondary amine content: 0.5%" in the finished cosmetic products" for the fatty acid dialkylamides and dialkanolamines listed in entry 60 of Annex III, part I.*

5. ABBREVIATIONS

ATNC	Apparent Total N-nitroso group Content
BMD	Benchmark dose
BMDL	Benchmark dose lower 95% confidence limit
CAS	Chemical Abstracts Service
Colipa	the European Cosmetics Association
CPDB	Carcinogenic Potency Database
DEA	Diethanolamine
NDELA	N-nitrosodiethanolamine
NO	nitrogen oxide
NOC	N-Nitroso compounds

QSAR	Quantitative structure Activity relationship
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers
SCCS	Scientific Committee on Consumer Safety
T25	The dose giving a 25% increase in the frequency of a specific tumour during the standard lifetime of the species studied.
TD50	Median Toxic Dose
WHO	World Health Organization

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SCCS's Notes of Guidance for the testing of Cosmetic Ingredients and their Safety evaluation, of 14 December 2010

Annex I

Tables of T25 for the different compounds:

The calculations below are based on experimental data on rats only as such data are available for all the nitrosamines considered. Suitable mice data are only available in the case of N-nitrosodimethylamine and N-nitrosopyrrolidine. In the case of N-nitrosodimethylamine the TD50 was less potent in mice than in rats, while in the case of N-nitrosopyrrolidine the potency appeared to be similar. In most cases, the T25 has been calculated on the bases of malignant liver tumours.

The T25 is calculated from the experimental data as described by Dybing et al. (1997). HT25 represent the human-equivalent dose estimated from the rat studies by using a scaling factor based on body weights to the power of ¾ as described by Sanner et al. (2001). Unless the specific weight is given, the following defaults are used: humans = 70 kg, male rats 500 g and female rats 350 g (ECHA, 2008).

N-nitrosodiethanolamine (1116-54-7) NDEA

Table 1: N-nitrosoethanolamine (CAS 1116-54-7). Calculation of T25 and HT25 from carcinogenicity studies on rats[1]

T25: HT25 (mg/kg bw/day)	Target	Species	Route	Expo-sure (week)	Observation (week)	Lowest tumour frequency %	Ref.
1.89 0.50	Liver carcinomas	F344 rats female	Drinking water	50	75	75	Lijinsky et al.,1984
2.19 0.64	Liver carcinomas	F344 rats male	Drinking water	100	104	20	Lijinsky and Kovatch, 1985
1.79 0.47	Liver carcinomas	F344 rats female	Drinking water	100	104	35	Lijinsky and Kovatch, 1985
2.22 0.65	Liver carcinomas	SD rats male	Drinking water	116	116	60	Preussman et al., 1982
2.93 0.86	Liver carcinomas	SD rats male	Drinking water	130	130	1.9	Berger et al., 1990
3.21 0.94	Liver carcinomas	SD rats male	Drinking water	104	104	33	Zerban et al., 1988
1.05 0.28	Liver unspecified	SD rats female	Drinking water	50	104	70	Hecht et al., 1989

[1] Studies with exposure time less than 50 weeks and studies where the frequency of hepatocellular tumours at the lowest dose tested were 80% or higher were excluded,

Mean T25 = 2.18 ± 0.72 mg/kg bw/d (range 1.05 - 3.21 mg/kg bw/d)

Mean HT25 = 0.62 ± 0.23 mg/kg bw/d (range 0.28 – 0.94 mg/kg bw/d)

TD50 = 3.17 (rat, there is more than one positive experiment, variation is greater than ten-fold among statistical significant (two-tailed $p<0.1$) TD50 values from different positive experiments).

N-nitrosobis(2-hydroxypropyl)amine (53609-64-6), NBHPA

Table 2. N-nitrosobis(2-hydroxypropyl)amine (Cas no. 53609-64-6). Calculation of T25 and HT25 from carcinogenicity studies on rats

T25: HT25 (mg/kg bw/day)	Target	Species	Route	Exposure (week)	Observation (week)	Lowest tumour frequency %	References
0.56 0.15	Esophagus carcinomas	F344 rats female	Drinking water	42	42	65	Lijinsky et al.,1984
0.52 0.15	Lung adenomas	Wistar rats male	Drinking water	52	52	60	Konishi et al., 1978

T25 = 0.54 ± 0.03 mg/kg bw/d (range 0.52 – 0.56 mg/kg bw/d

HT25 = 0.15 mg/kg bw/d.

TD50 = 0.846

N-nitrosodimethylamine (62-75-9), NDMA

Table 3: N-nitrosodimethylamine (CAS 62-75-9). Calculation of T25 and HT25 from carcinogenicity studies on rats

T25: HT25 (mg/kg bw/day)	Target	Species	Route	Exposure (week)	Observation (week)	Lowest tumour frequency %	References
0.11 0.032	Lung scc	F344 rats male	Gavage	30	45	32	Lijinsky et al., 1987
0.041 0.012	Liver carcinomas	F344 rats male	Drinking water	30	80	50	Lijinsky and Reuber, 1984
0.032 0.0085	Liver carcinomas	F344 rats female	Drinking water	30	90	45	Lijinsky and Reuber, 1984
0.73 0.22	Leydig cell	Wistar rats male	Feed	54	69	47	Terao et al., (1978)
0.059 0.017	Liver malignant	Wistar rats male	Drinking water	104	104	22	Peto et al., 1991
0.044 0.012	Liver malignant	Wistar rats female	Drinking water	104	104	45	Peto et al., 1991
0.061 0.016	Liver carcinomas	Wistar rats female	Feed	93	93	18	Arai et al., 1979

Terao et al., (1978) not included in the risk assessment as it is based on Leydig cell tumours)
Peto, 1991 (cited)
"The linear relationship observed at low dose rates (below 1 ppm) suggests that under these experimental conditions, among rats allowed to live their natural life span, a dose of 1 ppm of NDEA or NDMA in the drinking water will cause about 25% to develop a liver neoplasm."
Male rat, 25 ml per day, 500 mg; 1 ppm represent 0.001 mg/ml, 0.025 mg/0.500 = 0.050 mg/kg bw/d; female rat 20 ml per day, 350 g; 0.001 mg/ml, 0.020 mg/0.350 = 0.057

mg/kg bw/d. The above citation implies a T25 of 0.050 – 0.057 mg/kg bw/d both for NDMA and NDEA.

Mean T25 = 0.058 ± 0.028 mg/kg bw/d (range 0.032 – 0.11 mg/kg bw/d)

Mean HT25 = 0.016 ± 0.008 mg/kg bw/d (range 0.0085 – 0.032 mg/kg bw/d)

TD50 = 0.096 (rat, there is more than one positive experiment, variation is greater than ten-fold among statistical significant (two-tailed $p<0.1$) TD50 values from different positive experiments)

N-nitrosopyrrolidine (930-55-2), NPYR

Table 4: N-nitrosopyrrolidine (CAS 930-55-2). Calculation of T25 and HT25 from carcinogenicity studies on rats[1]

T25: HT25 (mg/kg bw/day)	Target	Species	Route	Exposure (week)	Obser-vation (week)	Lowest tumour frequency %	References
0.14 0.039	Liver carcinomas	Wistar rats male + female	Drinking water	104	104	42	Gray et al., 1991
0.62 0.17	Liver carcinomas	SD rats male	Drinking water	132	132	15	Berger et al., 1987
0.31 0.090	Liver carcinomas	SD rats male	Drinking water	86	104	66	Hoos et al., 1985
1.19 0.33	Liver carcinomas	SD rats male+ female	Drinking water	104	104	21	Preussman et al., 1977
0.43 0.12	Liver carcinomas	F344 rats male	Drinking water	80	80 weeks	87	Chung et al., 1986
0.27 0.070	Liver carcinomas	F344 rats female	Drinking water	30	104	100	Michejda et al., 1986
0.44 0.12	Liver carcinomas	F344 rats female	Drinking water	50	100	100	Lijinsky, Reuber 1981
0.61 0.18	Liver tumours	F344 rats male	Drinking water	50	80	86	Lijinsky, Taylor 1976
0.81 0.22	Liver tumours	F344 rats female	Drinking water	50	75	87	Lijinsky, Taylor 1976

[1] Studies with exposure time less than 50 weeks and studies where the frequency of hepatocellular tumours at the lowest dose tested were 80% or higher were excluded,

Mean T25 = 0.57 ± 0.46 mg/kg bw/d (range 0.14 – 1.19 mg/kg bw/d)

Mean HT25 = 0.16 ± 0.13 mg/kg bw/d (range 0.039 – 0.33 mg/kg bw/d

(Note: Including all results will not change the results.
Mean T25 = 0.54 ± 0.32 mg/kg bw/d (range 0.14 – 1.19 mg/kg bw/d)
Mean HT25 = 0.15 ± 0.09 mg/kg bw/d (range 0.039 – 0.33 mg/kg bw/d)

TD50 = 0.799 (rat, there is more than one positive experiment, 100% of dosed animals had tumours at a target site in an experiment in this species)

N-nitrosomorpholine (CAS 59-89-2), NMOR

Table 5: N-nitrosomorpholine (CAS 59-89-2). Calculation of T25 and HT25 from carcinogenicity studies on rats

T25: HT25 (mg/kg bw/day)	Target	Species	Route	Exposure (week)	Observation (week)	Lowest tumour frequency %	References
0.123 0.033	Liver carcinomas	F344 rats female	Drinking water	50	100	10	Lijinsky et al., 1988
0.047 0.014	Liver carcinomas	F344 rats male	Drinking water	50	80	90	Lijinsky, Reuber 1982
0.122 0.032	Liver carcinomas	F344 rats female	Drinking water	50	55	85	Lijinsky, Reuber, 1982
0.082 0.022	Liver carcinomas	F344 rats female	Drinking water	50	65	95	Hecht et al., 1989

Mean T25 = 0.094 ± 0.036 mg/kg bw/d (range 0.047 – 0.123 mg/kg bw/d)

Mean HT25 = 0.025 ± 0.009 mg/kg bw/d (range 0.014 – 0.033 mg/kg bw/d)

TD50 = 0.109 (rat, there is more than one positive experiment)

N-nitroso-N-methyl-N-dodecylamine (55090-44-3),

Table 6: N-nitroso-N-methyl-N-dodecylamine (CAS 55090-44-3). Calculation of T25 and HT25 from carcinogenicity studies on rats

T25: HT25 (mg/kg bw/day)	Target	Species	Route	Exposure (week)	Obser-vation (week)	Lowest tumour frequency %	References
0.40 0.11	Bladder carcinomas	F344 rats female	Gavage	30	50	84	Lijinsky et al., 1983
0.52 0.15	Bladder carcinoma	F344 rats male	Gavage	30	104	95	Lijinsky et al., 1981

Mean T25 = 0.46 ± 0.08 mg/kg bw/d (range 0.40 – 0.52 mg/kg bw/d)

Mean HT25 = 0.13 ± 0.03 mg/kg bw/d (range 0.11 – 0.15 mg/kg bw/d)

Note: Very high tumour frequency at the lowest doses.

TD50 = 0.537 (rat, there is more than one positive experiment, 100% of dosed animals had tumours at a target site in an experiment in this species)

N-nitrosodiethylamine (55-18-5), NDEA

Table 7: N-nitrosodiethylamine (CAS 55-18-5). Calculation of T25 and HT25 from carcinogenicity studies on rats

T25: HT25 (mg/kg bw/day)	Target	Species	Route	Exposure (week)	Obser-vation (week)	Lowest tumour frequency %	References
0.11 0.032	Liver carcinomas	F344 rats male	Gavage	30	45	32	Lijinsky et al., 1987
0.11 0.031	Liver Malignant	Wistar rats male	Drinking water	104	104	8	Peto et al., 1991
0.042 0.011	Liver Malignant	Wistar rats female	Drinking water	104	104	32	Peto et al., 1991
0.0088 0.0023	Liver carcinomas	F344 rats female	Drinking water	60	104	30	Lijinsky et al., 1981
0.19 0.054	Liver carcinomas	SD rats Male	Drinking water	122	122	26	Berger et al., 1987
0.030 0.0081	Esophagus unspecified	F344 rats female	Drinking water	30	45	89	Lijinsky et al., 1983
0.049 0.014	Liver unspecified	SD rats male	Drinking water	116	116	45	Habs, Schmähl, 1980

Mean T25 = 0.085 ± 0.065 mg/kg bw/d (range 0.0088 – 0.19 mg/kg bw/d)

Mean HT25 = 0.024 ± 0.019 mg/kg bw/d (range 0.0023 – 0.054 mg/kg bw/d)

TD50 = 0. 0265 (rat, there is more than one positive experiment, variation is greater than ten-fold among statistical significant (two-tailed $p<0.1$) TD50 values from different positive experiments) (TD50 based on the same data as T25 gives TD50 = 0.161 mg/kg bw/d).

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Annex II

Dose descriptors derived form a BMD analysis and ranking information:

The SCCS applied the Benchmark Dose (BMD) Approach to derive for the eight NOCs identified above dose descriptors form available dose response studies where applicable. The BMD approach has been increasingly used and recommended (EFSA, 2009, 2011) to calculate a point-of departure for risk assessment which is a dose level, derived from the estimated dose-response curve, associated with a specific change in the response defined through a Benchmark Response level, denoted BMR, which for cancer incidences has been set by default equal to 10% extra risk over background. Ideally the BMD uses all available dose-response data of a study and fits a set of mathematical models. The lower one-sided confidence bound BMDL accounts for the statistical uncertainty in the data (with the statistical certainty level of 95%) and is used as a point of departure.

Since the BMD approach requires however a sufficient quality of the dose response data in terms of the number of dose groups it cannot be applied to studies with only two groups (a control and one dose group) and should not be applied to studies with only three groups (a control and two dose groups) since in that case not all models available for the BMD approach could be fitted such that a statistically sound BMDL can be calculated. Therefore the BMD analysis of the eight NOCs under consideration was restricted to those data sets where this criterion applied.

This Annex reports details of the BMD Analysis and those dose descriptors which represent the BMD approach in Table 8.

Table NDMA.1: Results of dose-response modelling of the NDMA data from Peto et al. (1991) on Colworth/Wistar male rats exposed by drinking water with fatal liver neoplasm incidence as endpoint (liver cells, bile ducts, mesenchyme, Kupffer cells, unknown) for the low dose range (control and 6 lowest dose groups), defined by Peto et al. (1991) as of lower than 1.1 ppm, for all models available in US EPA Software BMDS 2.1.1 and larger dose ranges for selected models (see lines with No. of Parameters equal to 9 or 16).

Model	Number of Parameters/ -loglikelihood[a)]		Model Acceptance p-value	BMD10 ppm	BMDL10 ppm
Full	7	76.4			
Reduced	1	84.3			
Probit	2	80.6	0.15	1.2	0.84
	16		<10-8	1.50	1.37
	9				
LogProbit	**3**	**76.9**	**0.91**	**3.5**	**0.64**
	16		0.054	1.46	1.24
Logistic	2	80.6	0.14	1.2	0.90
	16		<10-6	1.59	1.46
LogLogistic	3	76.9	0.90	3.1	0.65
	16		0.074	1.44	1.22
	9		0.65	1.94	1.63
Weibull	3	76.9	0.90	3.0	0.65
	16		0.004	1.00	0.79

Gamma	3	76.9	0.90	2.9	0.66
	16		0.01	1.22	0.96
MS CA	2	79.4	0.30	1.1	0.63
Slope factor =	0.16				
	16		no fit possible		
Quantal Linear	2	79.4	same as MS CA		
	16		<0.05	0.50	0.45

bw: body weight; BMD: benchmark dose; BMDL10: 95 % lower confidence limit of the benchmark dose with 10 % extra risk, MS CA: Multistage Cancer model
[a)] the negative loglikelihood value is reported only for the data set of the control group and the 6 lowest dose groups used for ranking

The BMDL value of 0.64 ppm was chosen as the BMDL10 of this study. Based on the conversion of the dosing in ppm to mg/kg bw/d calculated by OBrien et al. (2006) this value was converted by the factor of 24 into the **BMDL10 of 0.027 mg/kg bw/d.**

Table NDMA.2: Results of dose-response modelling of the NDMA data from Peto et al. (1991) on Colworth/Wistar female rats exposed by drinking water with fatal liver neoplasm incidence as endpoint (liver cells, bile ducts, mesenchyme, Kupffer cells, unknown) for the low dose range (control and 6 lowest dose groups) defined by Peto et al. (1991) as of lower than 1.1 ppm, for all models available in US EPA Software BMDS 2.1.1 and larger dose ranges for selected models (see lines with No. of Parameters equal to 9 or 16).

Model	Number of Parameters	-loglikelihood[a)]	Model Acceptance p-value	BMD10 ppm	BMDL10 ppm
Full	7	66.7			
Reduced	1	77.3			
Probit	2	71.0	0.14	1.03	0.79
	16				
	9				
LogProbit	**3**	**68.1**	**0.59**	**1.13**	**0.56**
	16		0.004	0.57	0.45
Logistic	2	77.1	0.11	1.05	0.83
	16				
LogLogistic	3	68.1	0.56	1.10	0.58
	16		0.03	0.60	0.48
	9				
Weibull	3	68.2	0.56	1.10	0.59
	16		<10-5	0.35	0.27
Gamma	ND				
MS CA	2	68.6	0.57	0.92	0.59

Slope factor =	**0.17**				
	16		<10-5	0.31	0.28
Slope factor =	**0.36**				
Quantal Linear	2	68.6	same as MS CA		

bw: body weight; BMD: benchmark dose; $BMDL_{10}$: 95 % lower confidence limit of the benchmark dose with 10 % extra risk; MS CA: Multistage Cancer model,
ND: model could not be fitted
[a)] the negative loglikelihood value is reported only for the data set of the control group and the 6 lowest dose groups used for ranking

The BMDL value of 0.56 ppm was chosen as the BMDL10 of this study. Based on the conversion of the dosing in ppm to mg/kg bw/d calculated by OBrien et al. (2006) this value was converted by the factor of 13.8 into the **BMDL10 of 0.041 mg/kg bw/d.**

Table NDEA.1: Results of dose-response modelling of the NDEA data from Peto et al. (1991) on Colworth/Wistar male rats exposed by drinking water with fatal liver neoplasm incidence as endpoint (liver cells, bile ducts, mesenchyme, Kupffer cells, unknown) for the low dose range (control and 6 lowest dose groups), defined by Peto et al. (1991) as of lower than 1.1 ppm, for all models available in US EPA Software BMDS 2.1.1 and larger dose ranges for selected models (see lines with No. of Parameters equal to 9 or 16).

Model	Number of Parameters	-loglikelihood[a)]	Model Acceptance p-value	BMD10 ppm	BMDL10 ppm
Full	7	75.8			
Reduced	1	91.0			
Probit	2	81.6	0.045	0.89	0.71
	16				
	9				
LogProbit	**3**	**79.1**	**0.16**	**0.79**	**0.42**
	16		0.07	0.74	0.50
Logistic	2	81.9	0.03	0.92	0.75
	16				
LogLogistic	3	79.1	0.16	0.79	0.44
	16		0.13	0.74	0.52
	9			0.84	0.50
Weibull	3	79.1	0.16	0.79	0.45
	16		0.16	0.69	0.48
Gamma	ND				
MSCA	2	79.7	0.16	0.72	0.48
Slope factor =	0.21				
	16		0.03	1.11	0.98
Slope factor = 0.10					
Quantal Linear 2	same as MS CA				

bw: body weight; BMD: benchmark dose; $BMDL_{10}$: 95% lower confidence limit of the benchmark dose with 10 % extra risk; MS CA: Multistage Cancer model
ND: model could not be fitted
[a)] the negative loglikelihood value is reported only for the data set of the control group and the 6 lowest dose groups used for ranking

The BMDL value of 0.42 ppm was chosen as the BMDL10 of this study. Based on the conversion of the dosing in ppm to mg/kg bw/d calculated by O'Brien et al. (2006) this value was converted by the factor of 24 into the **BMDL10 of 0.018 mg/kg b.w./d.**

Table NDEA.2: Results of dose-response modelling of the NDEA data from Peto et al. (1991) on Colworth/Wistar female rats exposed by drinking water with fatal liver neoplasm incidence as endpoint (liver cells, bile ducts, mesenchyme, Kupffer cells, unknown) for low dose range (control and 6 lowest dose groups) defined by Peto et al. (1991) as of lower than 1.1 ppm, for all models available in US EPA Software BMDS 2.1.1 and larger dose ranges for selected models (see lines with No. of Parameters equal to 9 or 16).

Model	Number of Parameters	-loglikelihood[a)]	Model Acceptance p-value	BMD10 ppm	BMDL10 ppm
Full	7	69.4			
Reduced	1	116.1			
Probit	2	69.4	0.87	0.64	0.57
	16				
	9				
LogProbit	**3**	**70.0**	**0.57**	**0.63**	**0.49**
	16		<10-5	0.33	0.25
Logistic	2	69.4	0.88	0.70	0.62
	16				
LogLogistic	3	69.7	0.66	0.64	0.49
	16		<10-5	0.33	0.25
	9			0.56	0.11
Weibull	3	69.6	0.69	0.64	0.49
	16		<10-11	0.18	0.13
Gamma	ND				
MultiStage Ca	2	70.6	0.52	0.54	0.46
Slope factor = 0.22					
	16		<10-15	0.37	0.41
Slope factor = 0.29					
Quantal Linear	2	77.7	<0.05	0.42	0.31

bw: body weight; BMD: benchmark dose; $BMDL_{10}$: 95% lower confidence limit of the benchmark dose with 10 % extra risk¸ MS CA: Multistage Cancer model
ND: model could not be fitted
[a)] the negative loglikelihood value is reported only for the data set of the control group and the 6 lowest dose groups used for ranking

The BMDL value of 0.49 ppm was chosen as the BMDL10 of this study. Based on the conversion of the dosing in ppm to mg/kg bw/d calculated by OBrien et al. (2006) this value was converted by the factor of 13.8 into the **BMDL10 of 0.036 mg/kg b.w./d.**

Table NDEA.3: Results of dose-response modelling of the NDEA data from Berger et al. (1987, 1990) on SD male rats exposed by drinking water with liver tumour incidence as endpoint for all models available in US EPA Software BMDS 2.1.1. for all 4 groups (Incidence: control: 3/500, 0.01:2/80;0.0.032:2/80;0.1:36/80)

Model	Number of Parameters	-loglikelihood	Model Acceptance p-value	BMD10 mg/kg bw/d	BMDL10 mg/kg bw/d
Full	4	95.5			
Reduced	1	166.9			
Probit	2	96.0	0.66	0.052	0.046
LogProbit	**3**	**96.6**	**0.15**	**0.047**	**0.034**
Logistic	2	96.1	0.59	0.058	0.052
LogLogistic	**3**	**96.4**	**0.19**	**0.048**	**0.034**
Weibull	3	96.3	0.21	0.048	0.034
Gamma	ND				
MS CA	2	96.5	0.17	0.043	0.033
Slope factor =	**3.03**				
Quantal Linear	2	101.9	0.002	0.025	0.019

bw: body weight; BMD: benchmark dose; $BMDL_{10}$: 95% lower confidence limit of the benchmark dose with 10 % extra risk, MS CA: Multistage Cancer model

Table NDELA.1: Results of dose-response modelling of the NDELA data from Berger et al. (1987, 1990) on SD male rats exposed by drinking water with liver tumour incidence as endpoint for all models available in US EPA Software BMDS 2.1.1 for all 4 groups (Incidence: control: 3/500, 0.2:2/80;0.63:1/80;2.0:6/80)

Model	Number of Parameters	-loglikelihood	Model Acceptance p-value	BMD10 mg/kg bw/d	BMDL10 mg/kg bw/d
Full	4	61.4			
Reduced	1	54.4			

Probit	2	55.2	0.45	2.40	1.81
LogProbit	**3**	**55.2**	**0.19**	**4.11**	**1.74**
Logistic	2	55.2	0.44	2.33	1.83
LogLogistic	**3**	**55.2**	**0.22**	**3.44**	**1.74**
Weibull	**3**	**55.2**	**0.22**	**3.37**	**1.74**
Gamma	**3**	**55.2**	**0.22**	**3.39**	**1.74**
MS CA **Slope factor =**	2 **0.33**	55.2	0.46	3.20	1.76

Quantal Linear = MS CA

bw: body weight; BMD: benchmark dose; $BMDL_{10}$: 95% lower confidence limit of the benchmark dose with 10 % extra risk, MS CA: Multistage Cancer model

Table NDELA.2: Results of dose-response modelling of the NDELA data from Preussmann et al. (1982) on SD male rats exposed by drinking water with liver tumour incidence as endpoint for all models available in US EPA Software BMDS 2.1.1 for 4 groups (Incidence: control: 0/88, 1.5:7/72;6:43/72;25:33/36) Without the two largest dose groups

Model	Number of Parameters	-loglikelihood	Model Acceptance p-value	BMD10 mg/kg bw/d	BMDL10 mg/kg bw/d
Full	4	81.8			
Reduced	1	165.9			
Probit	2	105.6	<10-10	2.81	2.34
LogProbit	3	82.3	0.64	1.41	0.98
Logistic	2	103.0	<10-9	2.45	2.04
LogLogistic	**3**	**82.1**	**0.74**	**1.41**	**0.95**
Weibull	3	84.5	0.07	0.95	0.55
Gamma	ND				
MS CA **Slope factor =**	2 **0.14**	84.5	0.14	0.89	0.73

Quantal Linear = MS CA

bw: body weight; BMD: benchmark dose; $BMDL_{10}$: 95% lower confidence limit of the benchmark dose with 10 % extra risk, MS CA: Multistage Cancer model

Table NPYR.1: Results of dose-response modelling of the NPYR data from Berger et al. (1987, 1990) on SD male rats exposed by drinking water with liver tumour incidence as endpoint for all models available in US EPA Software BMDS 2.1.1 for all 4 groups (Incidence: control: 3/500, 0.04:1/80;0.133:4/80;0.4:17/80)

Model	Number of Parameters	-loglikelihood	Model Acceptance p-value	BMD10 mg/kg bw/d	BMDL10 mg/kg bw/d
Full	4	81.0			
Reduced	1	109.3			
Probit	2	81.5	0.56	0.29	0.25
LogProbit	3	81.0	0.84	0.22	0.15
Logistic	2	81.9	0.41	0.30	0.27
LogLogistic	**3**	**81.0**	**0.98**	**0.23**	**0.16**
Weibull	3	81.0	0.93	0.24	0.17
Gamma	ND				
MS CA	2	81.0	0.83	0.25	0.17
Slope factor =	**0.60**				
Quantal Linear 2	81.7		0.46	0.21	0.15

bw: body weight; BMD: benchmark dose; $BMDL_{10}$: 95% lower confidence limit of the benchmark dose with 10 % extra risk, MS CA: Multistage Cancer model

For N-nitrosodimethylamine **NDMA** data sets from a total of 5 studies were available. Only two studies fulfilled the quality criteria required for applying the BMD approach. One of the studies (Arai et al., 1979) reported the incidence of hepatocellular carcinoma separately for males and female Wistar rats in a dose range up to 10 ppm. A BMDL10 was calculated to 2.0 ppm in standard diet for hepatocellular carcinoma in males and 0.5 ppm in females, respectively. The study with highest quality available for NDMA was that of Peto et al. (1991) where ample dose-response data were available both for male and female Wistar rats. Restricting the BMD analysis on the lower dose range (up to 1.056 ppm) the BMDL for males and females was determined as 0.64 and 0.56 ppm, respectively. Those values were transformed using the calculations of O'Brien at al. (2006) into 0.027 and 0.041 mg(kg b.w./d, respectively. Therefore, a BMDL10 = 0.027 mg(kg b.w./d was established as dose descriptor for NDMA.

For N-nitrosodiethylamine **(NDEA)** data sets from total of 6 studies were available. Only two studies (Peto et al. (1991), Berger et al. (1987, 1990)) fulfilled the quality criteria required for applying the BMD approach. When restricting the study of Peto et al. (1991) where ample dose-response data were available both for male and female Wistar rats on the lower dose range (up tp 1.056 ppm) the BMDL10 for males and females was determined as 0.42 ppm both for males and females. Those values were transformed using the calculations of O'Brien at al. (2006) into 0.018 and 0.030 mg(kg bw/d respectively. The study of Berger et al. (1987, 1990) gave a BMDL = 0.034 mg/ kg bw/d as dose descriptor

for NDEA. The lowest BMDL10 of 0.018 mg/kg bw/d was established as dose descriptor for NDEA.

Only sparse data were available for N-nitrosomorpholine **(NMOR)** (4 studies), for N-nitroso-N-methyl-N-dodecylamine (1 study) and for N-nitroso-bis(2-hydroxypropyl)amine **(NBHPA,** 2 studies. None of those studies allowed to application of the BMD approach.

For N-nitrosopyrrolidine **(NPYR)** data from a total of 8 studies were available. However only the study Berger at al. (1987, 1990) was amenable to a BMD analysis which resulted in a BMDL10 = 0.16 mg/kg/d.

For N-nitrosodiethanolamine (**NDELA)** data sets from six studies of different quality were available. There were three studies for which the BMD approach could be applied: Preussman et al. (1982), Berger et al. (1987, 1990) and Zerban et al. (1988). The study of Zerban et al. (1988) investigated a control and five dose groups (0.2, 0.63, 1.5, 6, and 25 mg/kg bw/d) for hepatocellular carcinomas and hepatic nodules and their incidence over a time period of 24 months with interim sacrifice every 3 months after 12 months such that only a small portion of animals was available at end of study for calculating a total incidence. Therefore, the BMDL10 = 1.6 mg/kg/d derived from that study was not considered as reliable as the values obtained from the other two studies which had much higher numbers of evaluable animals. Not all dose-response data of the Preussmann et al. (1982) study could be used for the BMD analysis since the incidence of liver tumours reached at the dose of 25 mg/kg bw/d almost the level of 100%. Then restricting the BMD analysis to doses up to 25 mg/kg bw/d a BMDL=0.95 mg/kg bw/d was found. In the case of the higher quality study of the Berger at al. (1987, 1990) a BMDL10 = 1.74 mg/kg/d was calculated which was supported by the data of Zerban et al. (1988) and was used for the ranking.