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Izdelki za otroke - Metode za ugotavljanje sproščanja N-nitrozaminov in N-nitrozabilnih snovi iz dud za stekleničke, tolažilnih dud, nastavkov za dojenje, grizal/žvečil in podobnih predmetov, izdelanih iz elastomerov ali gume

Child use and care articles - Methods for determining the release of N-Nitrosamines and N-Nitrosatable substances from elastomer or rubber teats and soothers

Artikel für Säuglinge und Kleinkinder - Verfahren zur Bestimmung der Abgabe von N-Nitrosaminen und N-Nitrosierbaren Stoffen aus Flaschen- und Beruhigungssaugern aus Elastomeren oder Gummi

Articles de puériculture - Méthodes pour déterminer la libération de N-nitrosamines et de substances N-nitrosables par les tétines et les sucettes en élastomère ou en caoutchouc

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Child use and care articles - Method for determining the release of N-nitrosamines and N-nitrosatable substances from elastomer or rubber teats and soothers

Articles de puériculture - Méthode pour déterminer la libération de N-nitrosamines et de substances N-nitrosables par les tétines et les sucettes en élastomère ou en caoutchouc

Artikel für Säuglinge und Kleinkinder - Verfahren zur Bestimmung der Abgabe von N-Nitrosaminen und N-nitrosierbaren Stoffen aus Babysaugern aus Elastomeren und Gummi

This European Standard was approved by CEN on 30 October 2016.

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European foreword

This document (EN 12868:2017) has been prepared by Technical Committee CEN/TC 252 "Child use and care articles", the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by July 2017, and conflicting national standards shall be withdrawn at the latest by July 2017.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 12868:1999.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

Compared to EN 12868:1999, this document contains the following significant changes:

— The common practice to perform at least double determinations has been made a requirement, including the preparation of samples.

— Sample preparation has been defined and simplified, reducing this source of interlaboratory variation.

— The pre-boiling and migration steps have been separated for the determination of N-nitrosamines and of N-nitrosatable substances, allowing use of the same vessels and avoiding the possible loss of migrated substances. Amounts of sample have been adjusted, increasing the sample mass for the determination of N-nitrosatable substances.

— Extraction of N-nitrosamines from the aqueous migrates has been restricted to one method, reducing interlaboratory variability. A rinsing step has been introduced to avoid variability due to possible loss of analytes.

— The calculation of results has been revised including a repeatability requirement for multiple determinations and taking into account state of the art analytical procedures.

— The confirmation of N-nitrosamines and application of analytical tolerances have been clarified including a N-nitrosamine specific adjustment as suggested by the validation trial.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

It has been shown that feeding teats and soothers made of elastomer or rubber may release N-nitrosamines and substances capable of being converted into N-nitrosamines (N-nitrosatable substances). The Scientific Committee for Food of the European Union has given the opinion that N-nitrosamines and N-nitrosatable substances may endanger human health owing to their toxicity [5]. Hence in 1993, the European Commission issued Directive 93/11/EEC [1] controlling rubber and elastomeric teats and soothers releasing these substances. The Directive also provided basic rules for determining the release of these substances and criteria for the method of analysis to be adopted.

The purpose of this European Standard is to provide a detailed analytical method for the identification and determination of N-nitrosamines and N-nitrosatable substances released from teats and soothers in order that compliance with the requirements of Directive 93/11/EEC may be tested.

This method has been validated.

The testing laboratories should take special care to observe occupational health and safety standards. Persons using this European Standard should be familiar with normal laboratory practice. This European Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This European Standard specifies the method for determining N-nitrosamines and N-nitrosatable substances released from elastomer or rubber teats in contact with artificial saliva salt solution for testing compliance with Directive 93/11/EEC.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 3696, *Water for analytical laboratory use - Specification and test methods (ISO 3696)*

EN ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories (ISO/IEC 17025)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
teat
flexible elastomeric part designed to be placed in the mouth

3.2
soother
article which includes a teat and which is intended to satisfy the non-nutritive sucking need of children

Note 1 to entry: Soothers are also known as pacifiers or babies' dummies.

3.3
feeding teat
any teat that permits a child to obtain food or drink

3.4
elastomer
material which undergoes substantial, elastic (fully reversible) deformation when put under stress and consisting of three-dimensional networks of cross-linked flexible polymers

Note 1 to entry: The cross-links are chemical bonds generated by curing in rubbers (like natural rubber or synthetic rubber including silicones) or physical, thermo-reversible fixation points in thermoplastic elastomers (TPE) or the combination of both (TPEV).

3.5
rubber
types of elastomer

3.6
N-nitrosamine
substance characterised by the N-nitroso functional group, N-NO, usually formed by the reaction of an amine with a nitrosating agent, e.g. nitrite, at acidic pH

3.7
N-nitrosatable substance
substance which when released into the artificial saliva salt solution (5.5) and submitted to the conditions of step 8.3.3 undergoes nitrosation to form a N-nitrosamine

3.8
N-nitroamine
substance characterized by the N-nitro functional group bonded to an amine, N-NO_2, also called N-nitramine

3.9
ready to use product
product intended to be used without the need to clean before first use, but may be reusable

4 Principle

N-nitrosamines and N-nitrosatable substances are migrated into a nitrite-containing artificial saliva salt solution under specified conditions. Two migrations are carried out: migration A for the determination of N-nitrosatable substances (determined as N-nitrosamines from migrate A after nitrosation) and migration B for the determination of N-nitrosamines (from migrate B). After extraction from the migrate and concentration, the concentrates are examined for N-nitrosamines by gas chromatography employing a chemiluminescence detector (TEA). The N-nitrosamines and N-nitrosatable substances released are expressed as N-nitrosamines in milligram per kilogram of the sample.

5 Reagents

Unless otherwise specified, all chemicals shall be of analytical grade and free of N-nitrosamines and N-nitrosatable substances (see 8.8).

5.1 Distilled water, or water of equivalent purity conforming to at least grade 3 of EN ISO 3696.

5.2 Ammonia hydroxide aqueous solution, CAS 1336-21-6, c(NH_4OH) = 0,1 mol/l.

5.3 Hydrochloric acid (CAS 7647-01-0).

5.3.1 Aqueous solution, c(HCl) = 0,1 mol/l.

5.3.2 Aqueous solution, c(HCl) = 1,0 mol/l.

5.4 Sodium hydroxide (CAS 1310-73-2).

5.4.1 Aqueous solution, c(NaOH) = 0,1 mol/l.

5.4.2 Aqueous solution, c(NaOH) = 1,0 mol/l.

5.5 Artificial saliva salt solution.

Table 1 — Salts and their masses for 1 l of artificial saliva salt solution

Salts	CAS	Mass (g) [a]
Sodium hydrogen carbonate	144–55–8	4,2 ± 0,021
Sodium chloride	7647–14–5	0,5 ± 0,0025
Potassium carbonate	584–08–7	0,2 ± 0,001
Sodium nitrite	7632–00–0	0,03 ± 0,001
[a] Tolerances are ± 0,5 % of the mass, except for the sodium nitrite		

Prepare the artificial saliva salt solution by dissolving the salts given with the appropriate masses in Table 1 in (950 ± 5) ml of water (5.1).

The artificial saliva salt solution shall have a pH of (9,0 ± 0,1). If necessary adjust by adding 0,1 molar hydrochloric acid solution (5.3.1) or 0,1 molar sodium hydroxide solution (5.4.1) drop by drop. Transfer into a 1 l volumetric flask and dilute to the mark with water (5.1).

The artificial saliva salt solution has limited stability and shall not be used after more than 5 days.

5.6 Ethanol (CAS 64-17-5), **absolute**

5.7 Dichloromethane (CAS 75-09-2)

5.8 Glass wool, washed with the dichloromethane (5.7)

5.9 Diatomaceous earth

NOTE 1 Examples for suitable diatomaceous earth are Extrelute® or Toxelut® pH9,0 or Chromabond XTR®.

NOTE 2 To remove any N-nitrosamines the diatomaceous earth can be heated for 1 h to 200°C, cooled and washed with dichloromethane (5.7), or can be calcined, e.g. for 4 h at 550 °C.

5.10 Sea sand, acid washed and calcined

5.11 Purified nitrogen

5.12 Boiling chips

6 Apparatus

6.1 Normal laboratory apparatus.

Amber glassware and / or glassware protected from light by wrapping in aluminium foil shall be used to avoid degradation of N-nitrosamines.

To avoid loss of N-nitrosamines or N-nitrosatable substances, flasks shall be closed with ground glass stoppers.

The migration flasks shall be treated with ammonia solution (5.2), rinsed with water (5.1) and dried, prior to use in the tests.

NOTE This is to avoid uncontrolled nitrosation which could result from direct contact of the sample with acidic surfaces

6.2 Oven, maintained at a temperature of (40 ± 2) °C.

6.3 Columns for solid phase extraction (SPE columns).

Columns shall have capacities which when prepared in accordance with 8.5 allow the complete absorption of the entire amount of aqueous migrate (see 8.6).

NOTE 1 Suitable columns are normally glass columns with a length of approximately 450 mm and an internal diameter of (18 ± 2) mm or of length of approximately 300 mm and an internal diameter of (26 ± 2) mm. Columns may be equipped with a stoppered outlet (e.g. polytetrafluoroethylene stopper) to help adjusting the eluent flow.

NOTE 2 Alternatively to self-prepared columns, suitable pre-filled SPE columns for single use are commercially available. They can be used provided that they are free from N-nitrosamines and of sufficient capacity.

6.4 Sintered glass frits for columns (6.3).

6.5 Evaporator flask, glassware for concentration step (8.7) or any suitable automated evaporator capable to reduce the volume of Extract A or B (60 ml or above, see 8.6) to (0,9 ± 0,1) ml.

6.6 Water bath, capable of maintaining temperatures in the range 40 °C to 60 °C.

6.7 GC glass vials with septa free from N-nitrosamines.

6.8 UV lamp suitable for confirmation of N-nitrosamines according to 10.1 a). Illumination of the highest concentration of the calibration solutions in a UV transparent vial within a reasonable time (1–3 h) shall degrade N-nitrosamines and significantly decrease the intensity of their peaks.

NOTE Longwave (365 nm) UV lamps have been shown to significantly decrease the intensity of peaks from the highest concentrated calibration standard solution, but midrange and shortwave UV lamps are generally also suitable.

6.9 UV transparent glass vials to be used for confirmation of N-nitrosamines according to 10.1 a).

6.10 pH-meter with minimum (±0,2) pH-relative accuracy.

6.11 Chemiluminescence detector (Thermal Energy Analyser, TEA, see A.7).

6.12 Gas Chromatography (GC).

The GC system shall separate the N-nitrosamines named in this standard, such that their peak areas can be compared with that due to the internal standard (see 7.4). It shall also separate N-nitroamines (3.8) from the named N-nitrosamines.

Should other than the N-nitrosamines named in this standard (Table 2) be found, the GC system shall be adaptable for their separation and identification.

N-nitrosamines which cannot be identified shall be confirmed according to 10.1 a) and be quantified and reported as given in 9.1.

NOTE Annex B provides an example for gas chromatography settings suitable to obtain the required separations.

7 Standard Solutions of N-nitrosamines

7.1 General

WARNING — Owing to their toxicity, some N-nitrosamines can be detrimental to human health. After use, the apparatus which has come into contact with N-nitrosamines should be carefully treated to destroy remains of N-nitrosamines, for example by rinsing with 15 % HBr (CAS 10035-10-6) / glacial acetic acid (CAS 64-19-7), UV light exposure or other suitable methods.

The concentration of N-nitrosamine standard solutions may change during storage due to UV-light, evaporation and/or adsorption. Therefore, amber glassware and/or glassware protected from light by wrapping in aluminium foil as well as ground glass stoppers shall be used. Any solutions shall be homogenized by rigorously shaking the containers before any liquid is transferred (some N-nitrosamines and N-nitrosatable substances adsorb at the internal surfaces of the vessels).

Certified N-nitrosamine standards and their mixtures can be purchased from several suppliers. Certification shall include storage and stability information as well as purity which shall be taken into account when calculating the quantities for the standard solutions.

Stock solutions (approximately 1 mg/ml) of N-nitrosamines shall be in ethanol (5.6) and stored in a freezer.

Internal standard solution and calibration standard solutions (7.4 and 7.3) shall be stored at temperature below 5°C in the dark and can be used for a maximum of 2 weeks.

7.2 N-nitrosamines identified in teats

The N-nitrosamines listed in Table 2 and / or the respective N-nitrosatable substances have been identified in teats and are relevant for testing and for calibration standards.

Table 2 — Names, abbreviated names and CAS numbers of N-nitrosamines relevant for this standard, and the necessary limits of quantification

Name	Abbreviated Name	CAS	LOQ (mg/kg)
N-nitrosodimethylamine	NDMA	62–75–9	0,001
N-nitrosodiethylamine	NDEA	55–18–5	0,001
N-nitrosodipropylamine	NDPA	621–64–7	0,001
N-nitrosodiisobutylamine	NDiBA	997–95–5	0,001
N-nitrosodibutylamine	NDBA	924–16–3	0,001
N-nitrosopiperidine	NPIP	100–75–4	0,001
N-nitrosopyrrolidine	NPYR	930–55–2	0,001
N-nitrosomorpholine	NMOR	59–89–2 or 67587–56–8	0,001
N-nitroso N-ethyl N-phenylamine	NEPhA	612–64–6	0,005
N-nitroso N-methyl N-phenylamine	NMPhA	614–00–6	0,005
N-nitroso-N,N-di(3,5,5-trimethylhexyl)amine also known as N-nitrosodiisononylamine	NDiNA	1207995–62–7	0,005
N-nitrosodibenzylamine	NDBzA	5336–53–8	0,005
NOTE N-nitrosamines are listed in order of elution as shown in Figure B.1			