Migration of nitrosamines from rubber products – are balloons and condoms harmful to the human health?

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Studies performed in 2001 and 2003 surveyed the release of carcinogenic nitrosamines and nitrosatable substances from rubber toy balloons by extraction with artificial saliva and gas chromatography-thermal energy analysis (GC-TEA). 81% of the 16 in 2001 sampled balloons and 93% of the 14 in 2003 sampled balloons released nitrosamines above the recommended level in Germany of 10 µg per kg material. Furthermore, 32 rubber condom samples collected in 2004 from the German market were surveyed for nitrosamines by determining the amount migrating into an artificial sweat test solution. The levels released from condoms varied from <10 to 660 µg per kg material (*i.e.*, up to 1.4 µg nitrosamines per condom). In a model calculation, not considering the differences that may exist in the resorption rate, we have calculated that the exposure from condoms may exceed the exposure from food 1.5–3 fold. To our knowledge so far no legal binding legislation exists worldwide concerning nitrosamine migration from toy balloons or condoms.

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1 Introduction

1.1 General aspects

It has been known for many years that the exposure of humans to nitrosamines and/or their precursor compounds is associated with high risks. Exposure to nitrosamines in the general population occurs *via* different sources, *e.g.*, food, cosmetics, tobacco, and commodities [1] – here especially rubber products. Balloons and condoms are commonly made of natural rubber. In order to achieve the high elastic properties and necessary strength of the material, the rubber has to be vulcanized. However, additives, such as secondary amines, dithiocarbamates, and thiurams, used in this process, may have an adverse effect on the chemical safety. During vulcanization, dithiocarbamates, thiurams, and some sulfenamides (morpholine derivatives) can be converted to secondary amines. In the presence of nitrosating agents, secondary amines react to the corresponding *N*-nitrosamines, in short nitrosamines. It also has been shown that these "vulcanizing additives" can form nitrosamines directly in contact with nitrosating agents [2]. Many nitrosamines have demonstrated to exhibit carcinogenic activity in animals [3].

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The presence of nitrosamines in rubber products has been a concern since many years, but only few current studies have been published concerning the specific chemical safety of balloons [4–7] or condoms [3, 8]. Balloons are often taken into the mouth to inflate them. Children even tend to chew on them. Condoms are intended for close contact with the skin and mucous membranes. Due to this close contact with the human body and especially the mucous membranes, the chemical safety of these products is of great importance.

The aim of the present study was to investigate the release of nitrosamines from condoms using artificial sweat test solution, an issue that to our knowledge has not been investigated in other studies so far. The study furthermore aimed at describing the present situation concerning the release of nitrosamines and nitrosatable compounds from balloons into artificial saliva. Nitrosatable substances were analyzed in the migrates of the balloons, since they may be formed *in vivo* into nitrosamines. In the presence of nitrite (*e.g.*, in saliva from bacterially reduced nitrate from food), nitrosatable substances may react in the gastric juices to the corresponding nitrosamines [6]. The release of nitrosatable substances from condoms was not part of this study.

1.2 Legislation

To our knowledge, no legal binding limits are imposed on the migration of nitrosamines from condoms or toy balloons worldwide. Concerning balloons, Germany issued a guideline: Recommendation XXI of the BfR (Federal Institute for Risk Assessment) on commodities based on natural and synthetic rubber [9]. It states, that only up to 10 μg nitrosamines per kg material and 5 μg per dm^2 nitrosatable substances should migrate into artificial saliva in 1 h at 40°C. As to standards concerning other commodities coming into contact with the mucous membranes, soothers and teats for babies were regulated in the EU after high levels of nitrosamines were discovered in these products. Commission Directive 93/11/EEC [10] states that nitrosamines and nitrosatable substances (nitrosamine precursors) may not be released in detectable amounts from elastomer or rubber teats and soothers (detection limits: 10 μg nitrosamines per kg material and 100 μg nitrosatable substances per kg material). Other countries have also issued standards on nitrosamines in teats and soothers, *e. g.*, Canada has recently lowered the maximum permitted level from 60 to 10 μg per kg total volatile *N*-nitrosamines (dichloromethane extraction) [11], the USA have set a recommendation of 20 ppb total nitrosamines for pacifiers [12] and 10 ppb for baby bottle nipples [13].

2 Materials and methods

2.1 Sampling

In 2001 a total of 16 packages with rubber balloons were randomly sampled in Baden-Württemberg (Germany). 14 packages of balloons were sampled in 2003. 32 condom packages, sampled at random on the local market, were surveyed in 2004.

2.2 Experimental

For the balloons, the release of nitrosamines and nitrosatable substances was determined in a static migration using nitrite containing saliva simulant according to EN 12868 [14] (4.2 g $NaHCO_3$, 0.5 g NaCl, 0.2 g K_2CO_3, 30 mg $NaNO_2$ dissolved in 1 L distilled water; adjusted to pH 9). The balloons were cut into small pieces. If a package of balloons consisted of various colors, all colors were mixed and a homogeneous sample was prepared. 5–10 g of the homogeneous sample were then contacted with artificial saliva for 1 h at 40°C. Determination of the released nitrosamines was performed according to EN 12868 method A (standard for teats and soothers). The nitrosatable substances, were determined as their corresponding nitrosamines listed below after nitrosation as described in EN 12868.

A mixture of nitrosamines consisting of *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine (NDPA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosomorpholine (NMOR) was employed as reference standard. *N*-Nitrosodiisopropylamine (NdiPA) was used as internal standard. The nitrosamines and the internal standard were obtained from Promochem (Wesel, Germany). A Hewlett-Packard 5890 A GC coupled to a thermal energy analyzer (Thermedics, Woburn, USA) was used. A 5 μL aliquot of the extract was injected (splitless) onto a 30 m Stabilwax DB column (0.25 mm ID, 0.5 μm film). The injection port was maintained at 190°C. The initial temperature of the oven was 40°C, held for 1.5 min, then programmed at 45°C · min^{-1} to 130°C, 8°C · min^{-1} to 170°C and 2°C · min^{-1} to 185°C. The pyrolysis oven was set to 500°C. Evaluation of raw data was conducted using Chromeleon Version 6.60 software (Dionex, Idstein, Germany). Each analyzed nitrosamine was calculated separately. The individual nitrosamine values were summed up to give the total nitrosamine value. Simulating physiological conditions, the release of nitrosamines from condoms into artificial sweat test solution (4.5 g NaCl, 0.3 g KCl, 0.3 g Na_2SO_4, 0.4 g NH_4Cl, 3.0 g lactic acid, and 0.2 g urea dissolved in 1 L distilled water) according to the German § 35 Methode 82.10 [15] (standard for the determination of colorfastness of toys) was determined. Unlike Biaudet *et al.* [8], the lubricant was not removed, the extraction medium was altered, and migration time was reduced. The entire condom was fastened into a sealable tube and brought into contact by filling 40 mL sweat simulant inside the condom, plus 20 mL on the outside of the condom, and put in a shaking water bath for 1 h at 37°C (dynamic migration test). Determination of the released nitrosamines was performed analogously to the balloon samples. Recovery studies were performed for NDEA and NDBA using four spike levels: 37.5, 75, 150, and 375 μg · kg^{-1} material for each nitrosamine.

3 Results and discussion

3.1 Nitrosamines in balloons

The following nitrosamines were detected in the balloons and condoms: *N*-dimethylnitrosamine, *N*-diethylnitrosamines, and *N*-dibutylnitrosamine. Their structures are shown in Fig. 1. In balloons small amounts (<10 μg · kg^{-1}) of *N*-nitrosomorpholine were additionally detected. Often, a mixture of two or three of these nitrosamines was released from the samples. The detected nitrosamines are very potent carcinogens in animals [21], and thus classified as probably carcinogenic to humans.

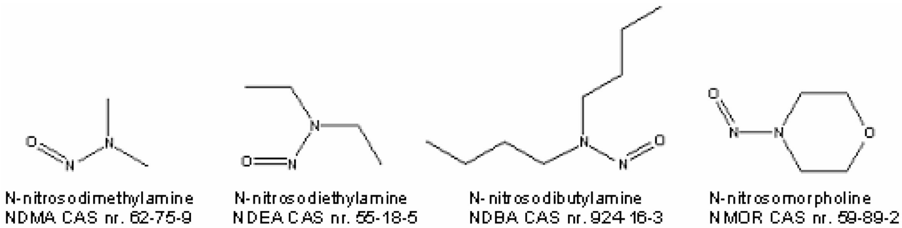

Figure 1. Structures of nitrosamines detected in the samples of this study.

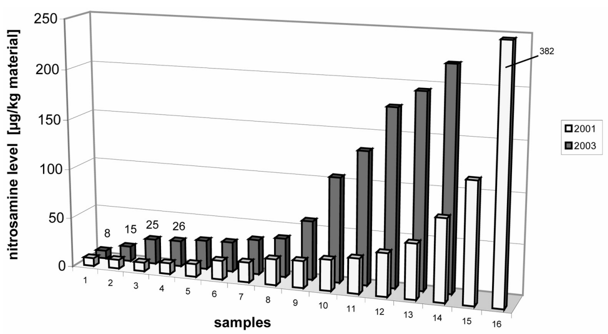

Figure 2. Levels of nitrosamines, expressed in µg per kg material, released from balloons into artificial saliva (contact conditions: 1 h at 40°C); maximum recommended level in Germany: 10 µg *N*-nitrosamines per kg material.

In the samples collected in 2001, nitrosamines released from the balloons ranged from <10 to 380 µg · kg^{-1}. 81% of the 16 analyzed balloons were above the German recommendation of 10 µg · kg^{-1}. 380 µg · kg^{-1} equal theoretically 0.46 µg nitrosamines per balloon. Obviously no efforts on behalf the manufacturers to minimize the release of nitrosamines were carried out, since the levels did not decrease significantly in the past years. In 2003, only one of the 14 sampled balloons did not exceed the recommended migration level of 10 µg nitrosamines per kg material. The levels ranged from <10 to 220 µg · kg^{-1}. Figure 2 shows the levels of nitroamines released in 2003 compared to 2001. As far back as 1991, Majerus and Otteneder [6] criticized high levels of nitrosamines ranging from 10 to 300 µg · kg^{-1} (10 balloons) and appealed to the manufacturers to alter their recipes regarding the vulcanization accelerators.

In the samples surveyed in 2001 the concentrations of nitrosatable substances released ranged from <10 to 4300 µg · kg^{-1}, while in samples from 2003 levels ranged from <10 to 2500 µg · kg^{-1}. This equals a maximum level of nitrosatable substances of 12,4 µg per dm^2 balloon in 2001 and 5.2 µg per dm^2 in 2003, respectively. In 2001 five samples were found to contain nitrosatable compounds above the German recommended level of 5 µg per dm^2, while in 2003 only one sample did not meet this requirement.

In a total of 30 samples, the nitrosamines most often detected were *N*-dimethylnitrosamine (in 97% of the balloons) and *N*-dibutylnitrosamine (93%). In 34% of the samples *N*-diethylnitrosamine and in 9% of the samples *N*-nitrosomorpholine was detected. Regarding the vulcanization accelerators, curiously, only dibutlydithiocarbamate was determined in the rubber, while dimethyldithiocarbamate and diethyldithiocarbamate were not detected in any of the samples (experimental data not shown).

3.2 Nitrosamines in condoms

Up to 660 µg nitrosamines per kg material were released into the artificial sweat. Using the highest level found as a calculation basis, this equals approximately 1.4 µg nitrosamines per condom. In comparison, it is estimated, that the consumer is exposed to 0.2 – 0.5 µg per day from food, 1.5 – 6.0 µg per day from tobacco, and <0.05 µg per day from cosmetics [1]. Thus, in the worst case, if highly contaminated condoms are used daily, the exposure from condoms might exceed the exposure from food by a factor of 1.5 – 3 per person (considering exposition to 50% of the migrate per person). Encouragingly though, in three samples nitrosamines were not detected. This shows that it is possible to produce condoms without nitrosamine contamination.

Figure 3 shows the distribution of nitrosamine levels within the 32 analyzed samples. The limit of quantification (by means of S/N) was on average 10 µg · kg^{-1} for the analyzed nitrosamines. Recoveries determined for four spike levels

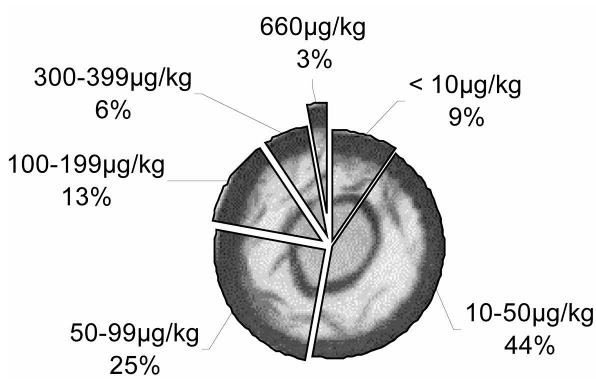

Figure 3. Distribution of nitrosamine levels, expressed in µg per kg material, released from condoms into artificial sweat (*n* = 32 samples; contact conditions: one whole condom for 1 h at 37°C in a shaking water bath).

ranged from 89 to 102% for *N*-diethylnitrosamine and 76 to 103% for *N*-dibutylnitrosamine with an average relative standard deviation of 7%. Although the contact time of 1 h is not common practice in the use of a condom, the levels obtained do provide an informative basis. A comparison between short exposure (10 min) and 1 h were performed. While this was a single experiment, the results suggest, that the bulk of nitrosamines are released within the first few minutes, since no significant difference between amounts released within 10 min (260 $\mu g \cdot kg^{-1}$) or 60 min (263 $\mu g \cdot kg^{-1}$) were found.

As expected, *N*-dibutylnitrosamine was most commonly found (in 91% of the sample migrates), since the vulcanization accelerator dibutyldithiocarbamate was determined in 88% of eight samples (experimental data not shown). In 34% of these samples *N*-diethylnitrosamine and in 9% of these samples *N*-dimethylnitrosamine was released.

The example of teats and soothers showed that adoption of restrictive legislation led to modifications in the recipes and to a significant decrease in nitrosamine levels [6, 16–19]. Due to the fact that balloons have not met the BfR Recommendation XXI in recent conducted surveys in Germany and these results were also confirmed by a recently conducted survey of The Netherlands [7], Germany is discussing to impose a legally binding limit for nitrosamines and nitrosatable substances in balloons [19]. A limit for nitrosamines and their precursors might follow for condoms. Nonetheless, due to the high carcinogenic potency of the detected nitrosamines, the aim should be the prevention of exposure to these substances – or if that does not succeed, to keep the exposure as low as reasonably achievable (ALARA principle).

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