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Levels of Volatile *N*-Nitrosamines in Baby Bottle Rubber Nipples Commercialized in Belo Horizonte, Mina Gerais, Brazil

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Several reports have been published describing the occurrence of volatile N-nitrosamines in rubber products (Fajen et al. 1979, Mcglothlin et al. 1981). The first evidence of the presence of these compounds in baby bottle nipples was published by Ireland et al. (1980). Their study showed the presence of N-nitrosamines in elastomers compounded with dialkylamine accelerators and stabilizers.

N-nitrosamines are formed by the interaction between amines and nitrosating agents. The amines originate from chemicals added during processing. These additives function as accelerators, retarders or stabilizers of the vulcanization process and as antioxidant or texture modifier (El Assaf et al. 1984, Tricker and Preussmann 1988). The nitrosating agents could be nitrates or nitrites, used as additives (El Assaf et al. 1984) and nitrogen oxides or nitrous anhydride found in the industry environment (Havery and Fazio 1982). N-nitrosamines are also present due to the use of N-nitrosamines themselves as additives (El Assaf et al. 1984, Sen et al. 1985) or to the use of additives containing up to 3.5 ppm of N-nitrosamines as contaminants (Spiegelhalder and Preussmann 1982, Sen et al. 1987).

The types of N-nitrosamines found in baby nipples and pacifiers depend on the amine moiety of the additives used during their manufacture (Havery and Fazio 1982, Sen et al. 1985). In general, the volatile N-nitrosamines found are N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosomorpholine (NMOR) and N-nitrosopyrrolidine (NPYR) (Havery and Fazio 1982, Osterdahl 1983, El Assaf et al. 1984, Sen et al. 1985, 1987, Tricker and Preussmann 1988).

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Most of these N-nitrosamines are potent carcinogens in laboratory animals (Magee 1976), infants and children being more vulnerable to the malignant effect of these compounds (Sen et al. 1985, Westin et al. 1987).

Because of the potential health hazard to infants, several analytical methods have been developed for monitoring the levels of N-nitrosamines in rubber nipples. Volatile N-nitrosamines can be extracted from cut-up rubber nipples with methylene chloride by Soxhlet extraction (Gray and Stachiw 1987, Tricker and Preussmann 1988), or by room temperature column extraction (Sen et al. 1987) followed by distillation from aqueous alkali, reextraction into methylene chloride, concentration using a Kuderna-Danish concentrator, and determination by gas chromatography with thermal energy analysis-TEA (Gray and Stachiw 1987, Sen et al. 1987, Westin et al. 1987, Tricker and Preussmann 1988).

In the method of Spiegelhalder and Preussmann (1982), the cut pieces of the rubber nipples are extracted with artificial saliva, and the extract is analyzed for both N-nitrosamines and nitrosatable amines. The procedure is appropriate as it is carried out under conditions that resemble saliva contact in an infant's mouth. Furthermore, it is a valuable information of the potential for 'in vivo' formation of N-nitrosamines.

Several countries, through active cooperation with industry, have made considerable progress in eliminating or significantly reducing the levels of N-nitrosamines in baby bottle nipples (Havery and Fazio 1985). Canada, USA, Germany and Holland have established limits for volatile N-nitrosamines in baby bottle nipples of 30, 10, 10 and 1 ppb, respectively (Havery and Fazio 1985, Gray and Stachiw 1987, Tricker and Preussmann 1988). Germany and Holland have also set limits for nitrosatable compounds (Tricker and Preussmann 1988).

The purpose of the present study was to investigate the occurrence of volatile N-nitrosamines in baby bottle rubber nipples in the market of Belo Horizonte, MG, Brazil.

MATERIALS AND METHODS

Six types of baby bottle nipples were purchased in Belo Horizonte, MG, Brazil. Four aleatory samples of each type were cut into pieces of approximately 5mm each and mixed thoroughly. Three subsamples of each type were analyzed. Methylene chloride, from Burdick & Jackson, was distilled in glass and tested for its nitrosation

potential (Sen et al. 1987). Water was distilled twice. N-nitrosamines were purchased from Sigma (St. Louis) and a working standard solution containing 0,1µg of each N-nitrosamine/ml of methylene chloride was prepared (Gray and Stachiw 1987). N-nitrosodipropylamine (NDPA) was used as an internal standard at a concentration of 0.1µg/ml.

A Hewlett-Packard 5710A gas chromatograph (GC) interfaced with thermal energy analyzer (TEA) model 502L (Thermedics, Inc.) was used. GC-TEA conditions included a column of 2.7m x 4mm internal diameter glass column packed with 10% Carbowax 1540/5% KOH on 100-120 mesh Chromosorb W(HP) (Supelco); nitrogen carrier gas flow at 40ml/min; column temperature programmed from 90°C to 180°C at 4°C/min.; injection port at 200°C; TEA furnace at 450°C; and liquid nitrogen as TEA trap.

The quantitative determination of N-nitrosamines in the baby bottle nipples was performed according to Gray and Stachiw (1987) with a few modifications. The method was based on extraction of the volatile N-nitrosamines from the cut up nipples with methylene chloride by Soxhlet extraction; alkalinization of the solution; distillation of the methylene chloride and then of the water; partition of the N-nitrosamines in the aqueous phase back into the methylene chloride; and concentration of the extract in a Kuderna-Danish apparatus to 1ml for determination by gas chromatography with thermal energy analysis. NDPA was added as internal standard before distillation in order to check on the performance of the method. To ensure the absence of interfering peaks, blank tests were run on methylene chloride and water. One µl of the standards or samples were injected in the GC. The identification of the peaks was performed by comparing their retention time with that of standards. The quantification was done by comparing the area of individual peak with that of standard at the same retention time.

RESULTS AND DISCUSSION

Blank tests indicated that there was no artifactual formation of N-nitrosamines during the determination. The concentration of the N-nitrosamines in the standards and in the internal standard was observed to be adequate as it permitted standards and sample extracts to be run at the same instrument attenuation, eliminating concerns over the linearity of detector response.

Seven N-nitrosamines - N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine

(NDPA), N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR) and N-nitrosomorpholine (NMOR) - were present in the standard mixture. These N-nitrosamines, with the exception of NDPA, are the types generally found in nipples (Havery and Fazio 1982,1983, Osterdahl 1983, El Assaf et al. 1984, Sen et al. 1985, 1987, Tricker and Preussmann 1988). Because NDPA has not been found in rubber products, it was chosen as internal standard. During separation of the standards at the GC-TEA conditions established, N-nitrosamines eluted at 2.65; 3.37; 4.68; 7.04; 7.58; 7.92; and 8.74 minutes corresponding to NDMA; NDEA; NDPA; NDBA; NPIP; NPYR and NMOR respectively, showing good resolution.

Percent recoveries varied from 75 to 104% with the exception of one sample that recovered only 67% of the internal standard. Since recoveries below 75% reflect a bad performance of the method (Gray and Stachiw 1987), this sample was rejected.

Table 1. Levels of N-nitrosamines (ppb) in six types of baby bottle nipples commercialized in Belo Horizonte, MG, Brazil

SAMPLE	N-NITROSAMINE	CONCENTRATION (ppb)
1	NPIP	19.51 ± 2.62
2	NDMA	6.90 ± 0.23
3	NDMA	2.43 ± 0.06
4	NDMA	11.86 ± 3.40
5	NPIP	16.75 ± 2.37
6	NDMA	9.93 ± 0.20

Average of triplicate ± SD; NPIP: N-piperidine; NDMA: N-nitrosodimethylamine

Table 1 indicates the types and levels of N-nitrosamines in the baby bottle nipples. These results are not corrected for internal standard recovery because of differences in the volatility of N-nitrosamines (Gray and Stachiw 1987). Reproducibility was observed in all triplicates with the exception of type 4 that had a higher standard deviation. However, this can be due to sampling. According to Havery and Fazio (1982) N-nitrosamines levels varied by as much as 140% from nipple to nipple within the same manufacturer. NDMA or NPIP were found in all samples at levels varying from 2.43 to 19.51 ppb. NDMA, considered to be a stronger carcinogen (Shank 1975) was present in 66% of the samples.

In Brazil no regulatory limit has been set for N-nitrosamines. However, according to American and German and Dutch legislations, 50 and 100%, respectively, of these samples would not be allowed in the market.

Pacifiers and other types of baby bottle rubber nipples commercialized in Brazil should be evaluated for their N-nitrosamine content. The levels of nitrosatable amines extracted from the saliva should also be known, as it is well known that nitrosatable amines from the nipple can be extracted by the saliva (Tricker and Preussmann 1988) and be nitrosated 'in vivo', specially at the pH conditions of the stomach (Sen et al. 1985, Hill 1988, Craddock 1990). The effect of sterilization on N-nitrosamines and nitrosatable amines as well as the migration of these compounds into water, milk, infant formulas, juices and tea should also be investigated so that recommendations can be given in order to minimize health hazard.

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