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Migration of Nitrosamines from Condoms to Physiological Secretions

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Fajen et al. (1979) reported occurrence of volatile nitrosamines in the atmosphere of a rubber factory. These carcinogenic compounds were subsequently discovered in other rubber products including stoppers in blood collection tubes (Lakritz and Kimoto 1980) gloves, condoms and baby bottle nipples (Ireland et al. 1980), toys and balloons (Spiegelhalder and Preussmann 1982), and elastic rubber nettings used for processing cured meat products (Sen et al. 1987). Dialkylamines are additives used by rubber manufactures as accelerators and stabilizers during rubber vulcanization which have been shown to react with nitrosating agents during processing to form nitrosamines. The potential health hazard to infants using contaminated nipples and the demonstration that nitrosamines could migrate from the nipple towards an artificial saliva simulating physiological conditions focused attention on baby bottle nipples and pacifiers (Spiegelhalder and Preussmann 1982; Spiegelhalder 1983). Little research has been carried out into the other contaminated rubber products like condoms and gloves. Migration of nitrosamines from condoms or gloves via physiological liquids such as vaginal secretions or sweat could be significant.

The purpose of the present study was to investigate the occurrence of volatile nitrosamines and nitrosatable compounds in condoms and their eventual migration to physiological secretions.

MATERIAL AND METHODS

Volatile nitrosamines and nitrosatable compounds were extracted from condoms by three methods : artificial saliva in accordance with the European Union method used for nipples, bovin and she-goat vaginal secretions, and human cervical mucus. Condoms were purchased and the lubricant removed by washing with water. The designations « ref.

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condom A-G » refer to different manufacturers. Each model of condom was analyzed three times in the same extraction liquid. Cow and she-goat vaginal secretions were taken by National Institute of Agronomical Research, avenue de la Forêt de Haye, Vandoeuvre, France.

The secretions were stocked at 4°C until utilization within 48 hours. Woman's cervical mucus was collected by the Regional Maternity, rue du docteur Heydenreich, Nancy, France, and the secretions stored at 4°C until used within 48 hours. The artificial saliva was prepared as described by the European Committee for Standardization (CEN/TC252 WG5/TG Nitrosamines) to assess the conformity of teats and soothers to the European Commission Directive 93/11/CEE : 4.2 g sodium hydrogen carbonate, 0.5 g sodium chloride, 0.2 g potassium carbonate and 30 mg sodium nitrite were dissolved in 1 L water. The pH was adjusted to pH 9.0 if necessary, by adding 0.1 M hydrochloric acid solution or 0.1 M sodium hydroxide solution.

N-nitrosodiisopropylamine (100 µg/ml in ethanol) was used as an internal standard and a mixture of eight volatile nitrosamines were purchased from Greyhound-Chem Service, Birkenhead Merseyside, UK. The mixture consisted of N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA) , N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), and N-nitrosomorpholine (NMOR).

One condom was weighed and introduced into a flask containing 40 ml of artificial saliva. The flask was closed and stirred at 40°C for 24 hours. The solution was decanted from the flask into a 50 ml volumetric flask and the volume completed to 50 ml with artificial saliva. Internal standard and 1 ml of 1M sodium hydroxide were introduced in a 40 ml portion of this solution. Dichloromethane was added three times (30 ml at each addition) to extract nitrosamines from the aqueous artificial saliva. The combined dichloromethane extracts were concentrated at 52°C to a final volume of 1 ml in a three-stage Kuderna-Danish evaporator. Nitrosatable compounds were nitrosated by adding 1 ml of 1M hydrochloric acid in the remaining 10 ml portion of the solution and by allowing to stand for 30 minutes at a temperature of 20°C. Internal standard and 2 ml of 1M sodium hydroxide were

introduced. Nitrosatable substances as nitrosamines were extracted by liquid/liquid extraction with dichloromethane and the combined organic extracts were concentrated to 1 ml as for preformed nitrosamines.

2 ml of either cow or she-goat vaginal secretions or human cervical mucus were diluted to 10 ml with distilled water to enable complete immersion of one weighed condom in the physiological secretion. The closed tube was maintained at 40°C for 24 hours. The solution was decanted from the tube into a 50 ml volumetric flask and the volume completed to 50 ml with distilled water. Nitrosamines and nitrosatable substances were extracted as previously described for artificial saliva.

The preformed nitrosamines and the converted nitrosatable substances were detected and identified by gas liquid chromatography coupled with a thermal energy analyzer (GLC-TEA). A Varian Model 3300 coupled with a TEA detector (Thermedics Inc. Model 610) was used for this work. A 10 µl sample aliquot of the concentrate was injected onto the GC-TEA using a stainless-steel column (5m x 1/8 in.) packed with 10% (w/w) Carbowax 20M on Chromosorb WAW (80-100 mesh). Argon was used as carrier gas. The injection port temperature was maintained at 220°C. The initial temperature of the oven was 168°C, held for 7 min., then programmed at 5°C/min. to a final temperature of 192°C held for 12 min.

RESULTS AND DISCUSSION

The human vaginal secretions would have been the adequate physiological liquid to perform the extraction of nitrosamines and nitrosatable substances from a condom. Because of the difficulty to obtain them in sufficient quantity, other extraction liquids had to be employed. Cow and she-goat vaginal secretions were used because their compositions are similar to humans. Artificial saliva was naturally inadequate for studying condom extraction, but has been extensively validated as a model of human saliva (Spiegelhalder 1983).
Nitrosatable compounds have been analyzed to take into account their possible endogenous nitrosation. This endogenous synthesis of nitrosamines has been demonstrated in the normal acidic stomach, the infected neutral achlorhydric stomach, the infected urinary bladder, and the infected vagina (Harington et al. 1973). Endogenous N-nitroso compounds are a potential hazard to health and could be a causative

factor in cancer of the stomach, urinary bladder, and vagina. Nitrosation can occur spontaneously in the normal acidic vagina (pH 5-5.5) through the reaction of nitrosating agents derived from the formation of nitrite and in neutral infected vagina through the action of nitrate-reducing bacteria.

Results show that regardless of the extraction liquid used, only NDMA, NDEA, and NDBA were detected from condoms.

The highest level was volatile nitrosamines and nitrosatable compounds after extraction with artificial saliva was NDBA (Table 1). This nitrosamine is the most common one in rubber products (Spiegelhalder and Preussmann, 1982). Previous studies showed that its occurrence was related to the use of zinc dibutyldithiocarbamate as a vulcanization accelerator during the rubber manufacturing process (Ireland et al. 1980). The nitrosatable compounds were particularly high and largely exceeded limits set by the European Union for teats and teeth. For example, condom of manufacturer E contained levels of 15,600 ng/g nitrosatable compounds whereas the limit set by European legislation for nipples and pacifiers is 100 ng/g. These contaminations are in agreement with the results obtained some years ago in teats and teeth (Ellen 1987), but the adoption of restrictive legislation in Europe led to modifications in the formulation and a noticeable decrease in contaminant levels in rubber products.

The extraction of nitrosamines and nitrosatable substances with animal vaginal secretions or women cervical mucus showed very different results than with artificial saliva (Table 1). Preformed nitrosamines were generally not extracted from condoms by vaginal secretions or cervical mucus, except for condoms of manufacturers B and C with vaginal secretions of a cow and for condom of manufacturer A with a woman’s cervical mucus. This phenomenon is probably linked to the difference of viscosity rather than chemical composition. With regard to nitrosatable compounds, the difference is remarkable where nitrosatable compounds were detected in minute quantities in physiological secretions. Less than 9 % and 2 % of nitrosatable compounds were detected in a woman’s cervical mucus for condom of manufacturer A and in cow vaginal secretions for condom of manufacturer C respectively. In all the other physiological secretions, nitrosatable compounds were not detected. These differences can be

Table 1. Nitrosamines and nitrosatable compounds extracted from condoms by artificial saliva, cow and she-goat vaginal secretions, and woman cervical mucus. The designations « Ref. condom A-G » refer to different manufacturers. Each value is the mean of three condoms (± SE) :

Ref. condom	Extraction solution	Nitrosamines (ng/g)			Nitrosatable compounds (ng/g)		
		NDMA	NDEA	NDBA	NDMA	NDEA	NDBA
A	artificial saliva	ND	ND	7.2 ± 0.6	58 ± 11	19 ± 3	1050 ± 80
	cow vaginal secretion	ND	ND	ND	ND	ND	ND
	she-goat vaginal secretion	ND	ND	ND	ND	ND	ND
	woman cervical mucus	ND	ND	9.3 ± 1.0	ND	ND	ND
	woman cervical mucus	ND	ND	ND	ND	ND	88 ± 6
B	artificial saliva	3.8 ± 0.4	ND	ND	220 ± 34	820 ± 65	310 ± 30
	cow vaginal secretion	4.2 ± 0.6	ND	ND	ND	ND	ND
	she-goat vaginal secretion	ND	ND	ND	ND	ND	ND
	woman cervical mucus	ND	ND	ND	ND	ND	ND
C	artificial saliva	ND	3.5 ± 0.5	31 ± 6	60 ± 9	340 ± 28	1680 ± 250
	cow vaginal secretion	ND	3.7 ± 0.7	30 ± 8	ND	ND	28 ± 6
	she-goat vaginal secretion	ND	ND	ND	ND	ND	ND
	woman cervical mucus	ND	ND	ND	ND	ND	ND
D	artificial saliva	ND	ND	23 ± 3	232 ± 28	440 ± 21	350 ± 25
E	artificial saliva	ND	ND	13 ± 2	245 ± 31	2560 ± 240	12850 ± 1025
F	artificial saliva	ND	3.4 ± 0.4	ND	26 ± 2	4440 ± 320	6440 ± 350
G	artificial saliva	ND	ND	ND	30 ± 8	3730 ± 130	1370 ± 60

NDMA: N-nitrosodimethylamine, NDEA: N-nitrosodiethylamine, NDBA: N-nitrosodibutylamine, ND: not detected

explained by two factors : the ability to extract amines from condoms and the ability to nitrosate extracted amines are probably more important for artificial saliva than for physiological secretions. Moreover, the lubricant of condoms was removed prior to experiments. Natural use of lubricant would probably reduce migration of chemical compounds from the condom to physiological secretions. However, human vaginal secretions have not been tested and their nitrite concentration could depend also on bacterial contamination.

Vaginal secretions are probably nitrate-rich, but nitrate-reducing bacteria and nitrite (Hill 1988), amines (Chen et al. 1979) and nitrosamines (Harington et al. 1973) are generally absent in the normal vagina. On the contrary, infection of the cervix by organisms such as *Trichomonas vaginalis* or *Neisseria gonorrhoea* can be associated with a neutral vaginal pH and consequently the colonization of the vagina by nitrate-reducing bacteria and by putrefactive bacteria. NDMA has been detected in vaginal exudates of women with *T. vaginalis* infection living in the Transkei region of South Africa (Harington et al. 1973) and a strong association between *T. vaginalis* infection and cervical cancer has been shown (Robertson et al. 1971). Therefore, nitrosamine formation in the vagina has been suggested as a cause of induction of cervical cancer. Considering the extremely high levels of nitrosatable compounds in tested condoms, an unusually high nitrite concentration in vaginal secretions could result in a significant formation of nitrosamines.

Because of the quantity of nitrosatable compounds in condoms, the variable conditions of extraction, pH, and nitrite concentrations of vaginal secretions, it should be wise to limit the content of nitrosamines and nitrosatable compounds in condoms. Nevertheless, this problem is not comparable with teats and teeth and so we do not recommend restricting the use of condoms as a prevention factor against AIDS.

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