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Analysis of Nitrosamines Migration from Condoms in the Chinese Market Using a Proper Migration Experiment

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Abstract In 2008, a total of 37 condoms was sampled from the Chinese market. Released nitrosamines and nitrosatable substances from the samples were monitored according to EN12868 method. Furthermore, to simulate the process of nitrosamines migration from condoms, a new and proper migration experiment was proposed in this study. N-nitrosodimethylamine, N-nitrosodiethylamine and N-nitrodibutylamine were found in almost all samples. The release levels of nitrosamines varied from 15.62 to 792.89 μg/kg. The proposed method is feasible, sensitive and accurate.

Keywords Nitrosamines · Migration · Determination

Nitrosamines are potentially mutagenic and carcinogenic for animals and humans even at low concentration (IARC 1987). Nitrosatable substances are the precursors of nitrosamines and can be converted into nitrosamines. Nitrosamines have been found in various rubber products, such as baby teats and soothers (Glória 1991; Vieira et al. 2006), condoms (Biaudet et al. 1997; Pensabene et al. 1995), gloves (Fiddler et al. 1985; Feng et al. 2009), balloons (Inspectorate for Health Protection and Veterinary Public Health of Netherlands 2002; Altkofer et al. 2005), rubber netting (Bouma and Schothorst 2003). Commission Directive 93/11/EEC (1993) states that the release of nitrosamines from elastomer or rubber teats and soothers should not exceed 10 μg/kg material and nitrosatable substances should not exceed at 100 μg/kg material. The standard EN12868 (European Committee for Standardization 1999) is carried out and specified methods for the isolation, identification and determination of nitrosamine and nitrosatable substances released by artificial saliva for 24 h at 40°C from elastomer or rubber teats and soothers. Though short exposure about 10 min, it is very important to examine the release of nitrosamines from latex condoms because of their close contact with penis, vagina and cervix. Harington et al. (1973) detected N-nitrosodimethylamine (NDMA) in the human vaginal vault. Barrington et al. (1997) revealed it was theoretically possible for nitrosamines to be an important agent in the development of premalignant disease of the cervix. Some determinations for nitrosamines migration from condoms have been undertaken. Biaudet et al. (1997) detected the migration of nitrosamines from condoms into artificial saliva, diluted physiological secretions such as cow secretion, she-goat secretion and human cervical mucus at 40°C for 24 h. Altkofer et al. (2005) collected condoms from the German market and examined the migration of nitrosamines into artificial sweat at 37°C for 1 h. But there should be more proper and feasible detection method for nitrosamines migration from condoms. Presently, there are no standard detection method or prescribed limits for nitrosamines migration from latex condoms.

The aims of present study were to detect the migration of nitrosamines and nitrosatable substances from latex condoms commercially available in the Chinese market according to EN12868 (European Committee for Standardization 1999) method, and to propose proper and

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feasible migration experiment for determination of nitrosamines migration from condoms.

Materials and Methods

A total of 37 different brands of natural latex condoms was obtained from the Chinese market in 2008. A mixture solution of nitrosamines including of N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrodibutylamine (NDBA), N-nitromethylethylamine (NMEA), N-nitrosopiperdine (NPIP), N-nitropyrrobidine (NPYR), N-nitrosomorpholine (NMOR), N-nitrodipropylamine (NDPA), N-nitrosodiethanolamine (NDElA) was employed as reference standard. N-nitrodiisopropylamine (NDiPA) was employed as internal standard. The artificial saliva was prepared as described by the EN12868 (European Committee for Standardization 1999) method: 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 distilled water. The pH was adjusted to pH 9.0. The artificial sweat was prepared according to the German § 35 Method 82.10 (standard for the determination of colorfastness for toys with artificial sweat) (Altkofer et al. 2005): 4.5 g sodium chloride, 0.3 g potassium chloride, 0.3 g sodium sulfate, 0.4 g ammonium chloride, 3.0 g lactic acid and 0.2 g urea were dissolved in 1 L distilled water. In deviation of § 35 Method 82.10, the pH of artificial sweat was adjusted to 4.5 to simulate the pH of normal vagina condition of woman.

Nitrosamines and nitrosatable substances released from 37 condoms into artificial saliva for 24 h at 40°C were determined according to EN 12868 method. In deviation of EN12868 (European Committee for Standardization 1999), the condoms were not boiled for 10 min prior to the start of the migration experiment. A new proper migration experiment for condoms was proceeded. A total of 17 samples was choosed randomly from 37 condoms. Samples were treated with artificial sweat for 10 min at 37°C and shaken for these time periods. Nitrosamines and nitrosatable substances released from condoms into artificial sweat were detected according to EN 12868. Unlike Biaudet et al. (1997) and Altkofer et al. (2005), the lubricant was not removed and the migration time was reduced to 10 min.

A Varian 3900 GC coupled to a thermal energy analyzer (TEA, Thermedics Inc. Model 610) was used. A capillary column (50% methylphenyl polysiloxane, 30 m × 0.53 μm × 0.1 μm, Varian Inc.) was used. The carrier gas was He (99.999%) with a flow rate of 4.4 mL/min. The temperature of the injection port and the pyrolysis oven were 190 and 500°C. The initial temperature of the oven was 40°C. The temperature was then programmed at 10°C/min up to 60°C held for 5 min, then 15°C/min up to 75°C held for 10 min, and 20°C/min up to 230°C held for 4 min.

Recovery studies were performed for NDMA, NDEA and NDBA using three spike levels (10, 100 and 200 μg/kg sample) for each nitrosamine. In order to study the release rate of nitrosamines from condoms to artificial sweat, for one sample (condom C07) the migration of nitrosamines was determined at different migration time of 5, 10, 20, 30, 60, 120 min ($n = 3$ replicates). Statistical differences between the detection results of nitrosamines and nitrosatable substances migration from latex condoms with two treatments were determined with Paired-samples signed rank test (SAS 9.1.3 for Windows XP).

Results and Discussion

The total migration of nitrosamines and nitrosatable substances from 37 condoms in artificial saliva for 24 h ranged from 5.25 to 1289.76 μg/kg and 63.57 to 4705.53 μg/kg, respectively (Table 1). Following treatment with artificial sweat for 10 min, total nitrosamines and nitrosatable substances released from 17 condoms varied from 15.62 to 792.89 μg/kg and 26.29 to 1959.69 μg/kg, respectively (Table 2). NDMA, NDEA and NDBA were found in almost all condoms. The migration levels of nitrosamines from 17 condoms were all above the 93/11/EEC limit. Figures 1 and 2 show the chromatograms of the nitrosamines standard mixture and the migration of nitrosamines from one sample (C02) into artificial sweat.

Statistical analysis showed the release of nitrosamines from the samples was higher following treatment with artificial saliva for 24 h than with artificial sweat for 10 min (Paired-samples signed rank test, $S = 59.5$ and $P = 0.0032$) and the release of nitrosatable substances from the samples was higher following treatment with artificial saliva than with artificial sweat (Paired-samples signed rank test, $S = 62.5$ and $P = 0.0017$). The artificial saliva contained $NaNO_2$ (30 mg/L) which can react with aminated vulcanization accelerators through nitrosation in latex condoms, whereas $NaNO_2$ was not present in the artificial sweat used in the study. In addition, the migration time in artificial saliva was longer than that in artificial sweat. From Fig. 3, it concluded that a maximum migration of nitrosamines from condom C07 has taken place at short exposure about 10 min. Figure 4 shows the distribution of nitrosamines level within 17 analyzed samples. About 65% of 17 condoms released nitrosamines at 10–50 μg/kg. In a comparison, about 44% samples of 27 latex gloves in the Chinese market released nitrosamines into artificial sweat (pH 5.5) for 4 h at 50–100 μg per kg (Feng et al. 2009).

The migration levels of nitrosamines from latex condoms treated with artificial sweat (pH 4.5) for 10 min

Table 1 Migration of nitrosamines and nitrosatable substances from condoms according to EN 12868 (treated with artificial saliva at 40°C for 24 h)

Condom no.	Nitrosamines (μg/kg condom)				Nitrosatable substances (μg/kg condom)			
	NDMA	NDEA	NDBA	Total	NDMA	NDEA	NDBA	Total
C01	2.81	32.12	12.56	47.49	–	105.38	52.99	158.37
C02	4.82	277.84	556.26	838.92	10.56	233.38	883.35	1127.29
C03	2.35	1.46	104.05	107.86	27.26	57.42	65.22	149.90
C04	2.29	14.56	11.08	27.93	19.81	236.10	98.82	354.73
C05	7.72	4.78	9.08	21.58	129.63	15.70	119.65	264.98
C06	2.37	53.95	22.14	78.46	57.75	311.75	88.24	457.74
C07	1.00	23.57	155.51	180.08	11.80	955.64	83.04	1050.48
C08	2.20	11.46	33.52	47.18	13.15	343.26	100.03	456.44
C09	2.64	32.84	343.60	379.08	12.42	838.36	5623.88	6114.66
C10	1.68	86.18	19.54	107.40	52.49	519.12	64.02	635.63
C11	2.41	42.07	36.86	81.34	9.66	168.27	147.44	352.37
C12	–	40.81	87.37	128.18	8.92	1294.22	30.51	1333.87
C13	1.34	3.57	16.45	21.36	–	294.89	326.00	620.89
C14	1.56	5.33	214.25	221.14	102.63	795.01	79.05	976.69
C15	10.74	3.61	16.66	31.01	–	341.22	98.43	439.65
C16	10.82	215.54	104.50	330.86	21.86	625.28	106.92	754.06
C17	6.34	1.49	240.90	248.73	3.89	29.98	115.82	149.69
C18	–	7.66	74.19	81.85	26.63	204.97	46.01	277.61
C19	–	32.45	172.23	204.68	–	20.14	57.23	77.37
C20	0.97	7.50	28.96	37.43	89.57	11.31	102.89	203.77
C21	1.03	4.78	26.18	31.99	58.79	146.52	90.98	296.29
C22	–	3.43	5.95	9.38	90.01	3.15	85.92	179.08
C23	8.45	21.72	1259.59	1289.76	52.37	123.70	61.42	237.49
C24	12.16	–	88.49	100.65	15.62	8.44	166.16	190.22
C25	0.82	5.54	4.50	10.86	7.96	2.09	4695.48	4705.53
C26	12.81	–	93.23	106.04	52.07	31.00	2197.41	2280.48
C27	36.51	5.01	111.79	153.31	102.50	0.95	2833.78	2937.23
C28	–	5.91	23.36	29.27	23.30	234.61	106.03	363.94
C29	2.05	27.04	30.01	59.10	52.21	56.47	80.97	189.65
C30	16.00	21.81	34.81	72.62	103.46	268.40	90.38	462.24
C31	1.65	22.11	253.25	277.01	2.61	88.42	1013.00	1104.03
C32	0.87	5.87	4.77	11.51	16.93	2.65	73.50	93.08
C33	–	2.86	4.38	7.24	–	15.60	47.97	63.57
C34	–	52.95	47.10	100.05	12.49	591.13	229.17	823.79
C35	1.76	–	3.49	5.25	26.48	14.80	50.92	92.20
C36	1.74	5.70	42.05	49.49	44.79	539.85	46.36	631.00
C37	14.13	4.18	9.57	27.88	32.95	46.79	54.03	133.77

"–": Not detected

ranged from 15.62 to 792.89 μg/kg. The finding showed that an individual could be exposed to approximately 23.43–1,189 μg/kg. The average weight of one condom was 1.5 g. If sample C02 is taken as an example (the release level of nitrosamines from condom C02 was 792.89 μg/kg), an inividual could be exposed to approximately 1.2 μg nitrosamines after one sexual intercourse about 10 min. In addition, estimating 1,500 contacts to condoms C02 during lifetime (50 condoms/year for 30 years) this may result in the adsorption of up to 1.8 mg nitrosamines in total. In a comparison, it was estimated that the release of nitrosamines was 1.4 μg per condom in 1 h (Altkofer et al. 2005), 0.2–0.5 mg per day from food, 1.5–6.0 mg per day from tobacco and <0.05 μg per day from cosmetics during normal use (Eisenbrand et al. 1996).

Table 2 Migration of nitrosamines and nitrosatable substances from condoms with artificial sweat (pH 4.5) and shaking at 37°C for 10 min

Condom No.	N-nitrosamines (μg/kg condom)				N-nitrosatable substances (μg/kg condom)			
	NDMA	NDEA	NDBA	Total	NDMA	NDEA	NDBA	Total
C01	–	26.17	13.15	39.32	11.83	41.66	–	53.49
C02	6.58	648.87	137.44	792.89	23.32	1187.99	758.28	1969.59
C03	–	16.36	5.24	21.60	–	26.29	–	26.29
C04	–	27.03	–	27.03	–	36.32	–	36.32
C05	5.54	20.69	–	26.23	7.36	31.20	–	38.56
C06	3.86	45.77	–	49.63	5.63	40.03	–	45.66
C07	–	18.21	20.63	38.84	4.91	44.08	–	48.99
C08	2.67	12.95	–	15.62	9.96	42.75	–	52.71
C09	4.81	48.21	52.95	105.96	8.86	42.17	25.27	76.31
C10	10.77	67.04	27.43	105.25	8.78	114.12	103.77	226.67
C11	5.49	68.81	27.68	101.99	11.87	95.58	89.84	197.29
C12	6.13	78.62	11.21	95.96	8.58	104.30	179.60	292.48
C13	1.07	10.48	7.04	18.60	9.06	37.64	110.40	157.10
C14	3.36	16.58	2.48	22.42	9.75	91.41	53.39	154.56
C15	6.85	7.33	6.25	20.43	21.21	53.63	63.35	138.19
C16	14.46	22.53	299.08	336.06	18.33	26.20	312.61	357.14
C17	3.36	10.80	11.95	26.11	6.92	22.67	51.93	81.53

"–": Not detected

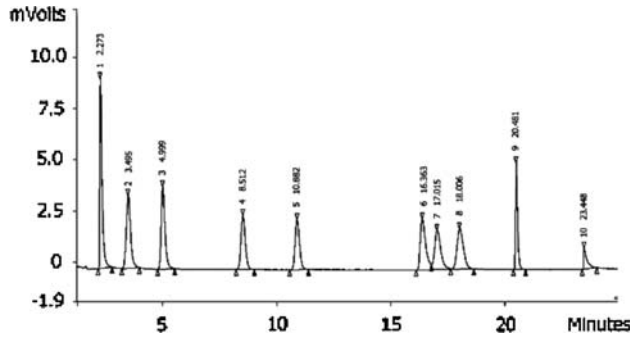

Fig. 1 Chromatogram of a nitrosamines standard mixture. *1* NDMA, *2* NMEA, *3* NDEA, *4* NDiPA (internal standard) *5* NDPA, *6* NPYR, *7* NMOR, *8* NPIP, *9* NDBA, *10* NDElA

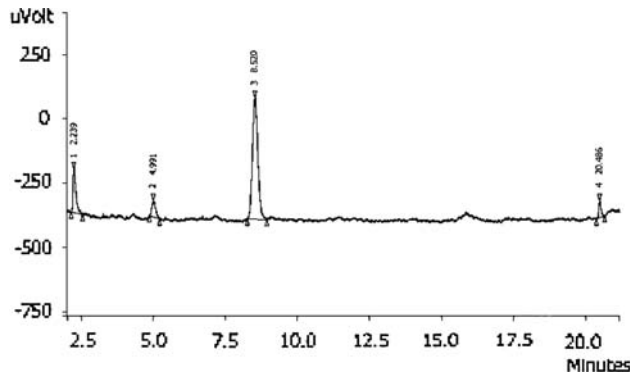

Fig. 2 Chromatogram of nitrosamines migration from condom C02 into artificial sweat for 10 min. *1* NDMA, *2* NDEA, *3* NDiPA (internal standard) *4* NDBA

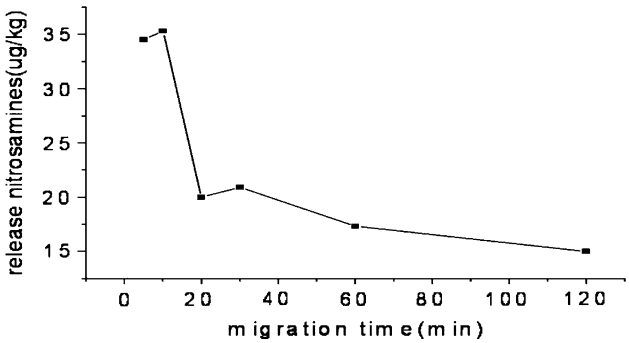

Fig. 3 Migration of nitrosamines from sample C07 in artificial sweat (pH 4.5) at 37°C as a function time

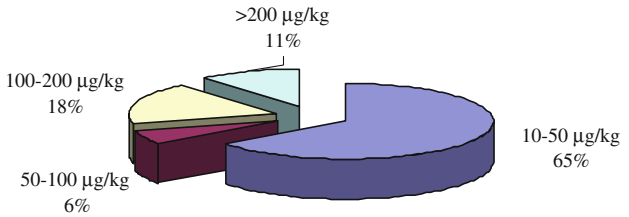

Fig. 4 Distribution of nitrosamine levels within the 17 analyzed samples, expressed in μg/kg sample, migration from condoms into artificial sweat at 37°C for 10 min ($n = 17$ samples)

The detection limit (3 times the noise) of NDMA, NDEA and NDBA was 1.47, 0.83 and 1.65 μg/L, respectively. Average recovery determined for three spike levels was 78% for NDMA with an average relative standard

deviation (RSD) of 6%. Average recovery for NDEA was 92% with an average RSD of 4% and for NDBA was 85% with an average RSD of 6%.

Biaudet et al. (1997) determined the migration of nitrosamines from condoms into diluted physiological secretions such as cow secretion, she-goat secretion and human cervical mucus. But the pH of physiological secretions may change after they are diluted. In addition, physiological secretions are difficult to obtain and may be instable in view of different individuals and different times for sampling. The exact components of the vaginal fluid of woman are still not certain, but the list of ingredients is longing: inorganic salts, urea, lactic acid, amino acids and other proteins. The ingredients of artificial sweat are similar to them of vaginal fluid, furthermore, artificial sweat is stable and convenient to obtain. So the artificial sweat was used in this study.

Lactobacillus can produce lactic acid in normal vagina of woman, so the pH of normal vaginal fluid is 3.8–4.5. If vagina is infected, the pH of vaginal fluid will be up to 5.0–6.0. In this study, the pH of artificial sweat was adjusted to 4.5 to simulate the pH of normal vaginal fluid of woman. The pH of normal semen of man is 7.2–8.0. But nitrosamines migration from condoms into semen was not taken into account in this study because nitrosamines may stay in vagina and cervix of woman and make more influence on woman than man.

In the study of Altkofer et al. (2005), the migration time was shortented from 24 to 1 h. In fact, the average time of one sexual intercourse is about 10 min. So in this migration experiment, to simulate the migration of nitrosamines from condoms, the migration time of 10 min was applied. From this study, it can be seen that the maximum nitrosamines migration from the condom C02 had taken place already after 10 min of exposure.

Until now, no standard detection method are imposed regarding the migration of nitrosamines from latex condoms. We recommend this migration experiment because it is proper and feasible for the determination of nitrosamines released from latex condoms. The overall method is sensitive and accurate. In view of the potential toxicity of nitrosamines, it is suggested that both standard detection method and prescribed limits should be imposed.

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