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## Volatile Nitrosamines in Cured Meats Packaged in Elastic Rubber Nettings

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An investigation was carried out to determine the levels of volatile nitrosamines in elastic rubber nettings and cured meats packaged in such nettings. Most unused nettings analyzed contained only traces of nitrosamines (mainly *N*-nitrosodiethylamine and *N*-nitrosodi-*n*-butylamine) but the used nettings contained extremely high levels (up to 504 ppb) of the same nitrosamines. The homogenized meats from such packages also contained significant levels of *N*-nitrosodi-*n*-butylamine (up to 29 ppb) and traces of *N*-nitrosodiethylamine. There was also a definite concentration gradient of these two nitrosamines along the cross section of the meat. Cured meats packaged in plastic wrappings or cotton nettings were negative. The above two nitrosamines detected in the meats and used nettings most likely originated as a result of the interaction of the amine precursors (e.g., dithiocarbamates) in the rubber and nitrite additive commonly used for the preparation of cured meat products.

Man is exposed to *N*-nitrosamines through a variety of sources such as cigarette smoke, cosmetics, foods (e.g., cured meats, fried bacon, fish) and beverages (e.g., beer and ale, whiskies), industrial and agricultural chemicals (e.g., cutting fluids, pesticides), and rubber products (Fine et al., 1980). Since most *N*-nitrosamines are potent carcinogens in laboratory animals, their occurrence or formation in the above items has been a matter of concern (Preussmann and Stewart, 1984). Research during the past 10 years has shown that various rubber products used by man can contribute significantly toward his/her total body burden of nitrosamines (Fine et al., 1980; Spiegelhalter and Preussmann, 1983).

The first indication that rubber products may contain traces of nitrosamines originated from the studies by Fajen et al. (1979), who detected varying levels of *N*-nitrosodimethylamine (NDMA), *N*-nitrosomorpholine (NMOR), and *N*-nitrosodiphenylamine (NDPhA) in the air from

several rubber industry factories. Soon after, Fine et al. (1980) reported the occurrence of fairly high levels of nitrosamines (mainly NDMA and NMOR) in rubber tires and in the interior air of new automobiles. Working independently, Ireland et al. (1980) detected traces of volatile nitrosamines in a variety of household rubber products such as baby bottle rubber nipples, rubber gloves, and natural rubber condoms. This latter group of workers also suggested that most of the nitrosamines in rubber originated via nitrosation of various dialkylamino compounds (e.g., dialkyldithiocarbamates) used as vulcanization accelerators in the rubber industry. The nature of the amine accelerator used determined which nitrosamine would be found in the rubber.

The main nitrosating agents responsible for the formation of nitrosamines in rubber are believed to be NDPhA, a retarder used in the vulcanization of rubber and a good transnitrosating agent, and nitrogen oxide gases present in the factory air (Spiegelhalter and Preussmann, 1983). Several preventive measures can be taken to reduce nitrosamine levels in rubber. These include (a) the use of alternative accelerators that do not form nitrosamines or form only noncarcinogenic nitrosamines, (b) the replace-

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ment of NDPhA with retarders that are not nitrosating agents, and (c) control of indoor concentrations of nitrogen oxide gases in factories (Speigelhalder and Preussmann, 1983). The introduction of some of these measures has already resulted in a significant reduction in the levels of volatile nitrosamines in baby bottle rubber nipples and pacifiers (Havery and Fazio, 1985; Sen et al., 1985a).

The Canadian Health Protection Branch has been actively engaged in research on nitrosamines in baby bottle rubber nipples and pacifiers and their possible migration into babies' saliva or infant foods in contact with such products (Sen et al., 1984, 1985a). In continuation of this project we wanted to determine nitrosamine levels in other food contact rubber materials such as elastic rubber nettings used to package various cured pork products (e.g., ham, cottage rolls, pork picnic shoulders, sweet pickled pork cottage rolls). These nettings are mainly used for holding the meat pieces together during processing and cooking (consumers usually cook these products with netting on them). Because of their elastic nature, the nettings also help retain the specific shapes of various products during the entire course of processing and marketing.

There were two reasons for concern that could arise from the use of such nettings: First, traces of nitrosamines present in these nettings could migrate to the meat. Second, there was also a possibility that some of the amine precursors (e.g., diethyldithiocarbamate, di-*n*-butyldithiocarbamate) present in the rubber could react with nitrite in the cured meat and form additional amounts of nitrosamines. The possibility of this happening has not been suggested or investigated before. The results of our investigations are presented here. Although elastic rubber nettings are often used to hold together pieces of various uncured meats (e.g., roast beef), this study does not cover such items because the chance of formation of nitrosamines in such cases is very remote (due to lack of nitrite).

#### EXPERIMENTAL SECTION

**(A) Samples.** They were either purchased locally from the retail outlets or procured directly from various meat packers in the two provinces of Ontario and Quebec. Matched pairs of unused elastic rubber nettings and cured meats packaged in such nettings (of the same lot as the unused netting) were picked up from the various meat plants by Agriculture Canada inspectors. However, unused nettings corresponding to the retail outlet samples were not available for analysis. In total, 10 smoked hams, 4 smoked cottage rolls, 5 smoked pork picnic shoulders (boneless), and 6 sweet pickled pork cottage rolls were analyzed.

**(B) Reagents and Solvents.** These were similar to those reported previously (Sen et al., 1979, 1987) except that glass-distilled dichloromethane was purchased from Burdick and Jackson, Muskegon, MI. *N*-Nitrosothiazolidine (NThZ) standard was obtained as a gift from W. Fiddler of the U.S. Department of Agriculture, Philadelphia, PA.

**(C) Gas-Liquid Chromatograph—Thermal Energy Analyzer (GLC-TEA).** GLC-TEA analysis was carried out as described previously (Sen et al., 1985a,b). It allowed simultaneous analysis of the following volatile nitrosamines: NDMA, *N*-nitrosodiethylamine (NDEA), *N*-nitrosodi-*n*-propylamine (NDPA), *N*-nitrosodi-*n*-butylamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR), NMOR, and NThZ.

**(D) GLC-High-Resolution Mass Spectrometry Confirmation (GLC-MS).** A VG 7070E mass spectrom-

eter, operating in the electron-impact ionization mode and connected to a Varian (Model Vista 6000) gas chromatograph, was used for the GLC-MS confirmation. GLC-MS confirmation was carried out as reported previously (Sen et al., 1985a,b).

**(E) Experimental Design.** Aliquots of the unused netting, the corresponding used netting, and meats from various cross sections (e.g., outermost 5-mm layer, second 5-mm outermost layer, and meat from the center) of a packaged meat sample were analyzed for volatile nitrosamines. This allowed us to determine whether a concentration gradient existed along the cross sections of the packaged product. This would be expected if the nitrosamines were migrating or forming from the rubber at the outer surface of the meat. An aliquot of the whole homogenized meat was also analyzed to determine the average concentration of various nitrosamines in the sample.

**(F) Preparation of Samples.** Most of the cooked meat products were warmed in an oven before analysis. Any plastic wrapping was first removed, and the sample was covered with aluminum foil and then heated in an oven (160–175 °C) for 1–1½ h depending on the size of the sample. Except in a few cases, the nettings were kept on the samples during heating. It should be emphasized that since most of the samples were fairly large (2–4 kg) and cold (~4 °C) to start with, the above cooking conditions were not excessive, just right to bring the temperature of the meat at the center to ~55 °C.

The sample was then allowed to cool to ~40 °C and the netting taken off and stored at 4 °C until analysis. The fat portions, if any, from the outer surface were carefully cut out and discarded. The meat sample was then cut into approximately four equal pieces, and two diagonally opposite portions were combined and homogenized thoroughly using a blender. Layers of meats were carefully carved out from the two remaining pieces to provide the following samples: (a) meat from the ~5-mm outermost layer; (b) meat from the ~5-mm second outermost layer; (c) meat from the center that was farthest away from the netting. Each of these aliquots was homogenized as above and stored at 4 °C until analysis, which was usually carried out within 1 week. If stored longer than 1 week, the samples were kept at -20 °C. For the ready-to-serve products (e.g., smoked ham), the samples were prepared as above but the preheating (in an oven) step was omitted.

Similar preparation procedures were also used for the raw meat products (e.g., sweet pickled pork cottage roll) with the exception that the samples were cooked for 2–3 h at 175 °C or until the inside temperature (detected with a meat probe) was ~75 °C.

**(G) Analysis of Rubber Nettings for Volatile Nitrosamines.** Both the used and unused rubber nettings were cut up into small pieces, and a 4–5-g aliquot was analyzed for volatile nitrosamines by a method that we have developed for the analysis of the same compounds in baby bottle rubber nipples and pacifiers (Sen et al., 1987).

**(H) Analysis of Meats for Volatile Nitrosamines.** A 20-g aliquot of the homogenized meat was mixed with 200 mL of 3 N potassium hydroxide solution and 100 mg of NDPA (internal standard) and then analyzed by the low-temperature vacuum distillation method as reported previously (Sen et al., 1979, 1985b).

**(I) Test for Artifactual Formation of Nitrosamines.** Selected samples of meats containing very high levels of nitrosamines (e.g., meats taken from outer layer) were reanalyzed by a modified method that involved low-temperature vacuum distillation of the sample from 200 mL

**Table I. Levels of Volatile Nitrosamines Detected in Various Samples**

samples	no. analyzed	levels of nitrosamines detected, ppb					
		NDEA		NDBA		others	
		av	range	av	range		
unused rubber netting	14	1.3	N-4.7 <sup>a</sup>	4.6	N-27	NDMA, NPIP	
used rubber netting	25	2.7	N-36.7	116	N-504	NThZ	
whole homogenized meat (fat discarded)	16	tr <sup>b</sup>	N-2.4 <sup>c</sup>	10.3	N-29	NThZ	
outer layer (~5 mm) from meats packaged in rubber netting	21	1	N-6.9 <sup>d</sup>	26.2	N-64 <sup>d</sup>	NThZ, NMOR, NDMA	
meat from center	20	N	N-0.8	0.2	N-2.2	NThZ	

<sup>a</sup>N = negative (detection limit 1-2 ppb in the case of netting). <sup>b</sup>tr = trace (0.1-1 ppb). <sup>c</sup>N = negative (detection limit 0.1 ppb for NDMA and NDEA, 0.3 ppb for NDBA, and 1 ppb for NThZ in the case of meats). <sup>d</sup>Confirmed by GLC-high-resolution mass spectrometry in selected cases.

of 0.1 N H<sub>2</sub>SO<sub>4</sub> containing 1 g of dissolved sulfamic or ascorbic acids, both of which are known inhibitors of artifactual formation of nitrosamines (Hotchkiss et al., 1980; Mirvish et al., 1972). The rest of the procedure was same as described previously (Sen et al., 1979). The results obtained by the modified and the normal (distillation from 3 N KOH) methods were compared to determine whether any artifactual formation took place in the latter procedure. Also, two samples of cured meats were analyzed by the normal method (Sen et al., 1979) with the addition of 1000 µg of morpholine as a marker amine. Lack of formation of any detectable levels of NMOR in these cases could be taken as an evidence that the method was free of artifactual formation.

**(J) Test for Nitrosatable Amines in Unused Rubber Nettings.** The method was based on nitrosation of the product at pH 3-4 and analysis of the nitrosamines formed. A 2-g aliquot of unused rubber nettings (cut up in small pieces) was mixed with 100 mL of freshly prepared sodium nitrite solution (0.5 mg/mL), and the mixture was adjusted to pH 3-4 with 3 N hydrochloric acid. The flask containing the sample was stoppered, wrapped in aluminum foil, and shaken gently overnight at room temperature. Next morning, the sample was mixed with 100 mL of 6 N potassium hydroxide and the mixture analyzed for volatile nitrosamines by the low-temperature vacuum distillation method as described previously (Sen et al., 1979).

## RESULTS AND DISCUSSION

An overall summary of the results obtained is presented in Table I. The data indicated that the unused rubber nettings contained only minute traces of nitrosamines, mainly NDEA and NDBA, whereas the used nettings contained much higher levels (up to 504 ppb) of the same nitrosamines, suggesting additional formation during processing or storage of the meat products. Both the frequency of occurrence and concentration of NDBA were much higher than those for NDEA. This was also true for the meat samples. NDBA was the predominant nitrosamine detected in both the outermost layers as well as in the homogenized meats. Traces of NThZ were also detected in most of the smoked meats, but it was never detected in unused nettings and only occasionally detected in used nettings that were in close contact with the smoked meat for a prolonged period. Previous studies from this (Sen et al., 1985b) and other laboratories (Mandagere et al., 1984; Pensabene and Fiddler, 1983) have clearly established that NThZ in smoked meats is formed as a result of the smoking process, and therefore, its occurrence in these products was not unexpected. Similarly, the occasional finding of traces of NDMA and NMOR in cured meats was consistent with the previously published data (Havery et al., 1978; Nitrite Safety Council, 1980; Sen et al., 1979). On the other hand, the consistent finding of the relatively high levels (up to 29 ppb in the homogenized

meat) of NDBA in cured meats was highly unexpected. In all previous surveys of cured meats, including fried bacon, carried out in the U.S.A. and Canada, NDBA was rarely detected (Havery et al., 1978; Nitrite Safety Council, 1980; Sen et al., 1979). It is possible that cured meats surveyed previously did not include samples that were packaged in elastic rubber nettings.

Detailed results of some 10 representative samples are presented in Table II so as to allow a direct comparison of nitrosamine levels in the unused netting, the used netting, and the corresponding meats in each individual case. The following observations could be made from these data:

(1) In most cases, the nitrosamine levels in the used rubber netting were markedly higher than those in the corresponding unused nettings.

(2) In general, the concentration of the nitrosamines was highest in the used netting, second highest in the meat taken from the ~5-mm outermost layer, followed by somewhat lower levels in the meat from the second outermost layer and practically none in the meat from the center of the packages.

(3) The whole homogenized meat (excluding fat and netting) in many cases contained significant levels of nitrosamines (mainly NDBA).

(4) All the meat samples (even the outer layers) packaged in cotton nettings (a total of four analyzed, only two shown in the table) were negative for both NDEA and NDBA.

In addition, one smoked ham packaged in plastic wrapping and two hams without any wrapping were analyzed. All three were negative for NDEA and NDBA, and one contained traces of NThZ and NMOR. All of these observations suggested that the nitrosamines (NDEA, NDBA) in cured meats packaged in rubber nettings most likely originated as a result of interaction of the amine additives in the rubber and nitrite in the meat. Since the concentration of the amine precursors would be expected to be highest in the rubber nettings and second highest in the outermost layers of various meats, the concentration of nitrosamines found in the various portions agreed extremely well with this hypothesis. Although we did not analyze the cured meats for nitrite, it is expected to be present in excess in all samples because most cured meats are processed with up to 200 ppm sodium or potassium nitrite (Canadian Food and Drug Act and Regulations, 1981).

It is well-known that diethyldithiocarbamate and *n*-butyldithiocarbamate are used as rubber vulcanization accelerators (International Agency for Research on Cancer, 1982; Ireland et al., 1980; Spiegelhalder and Preussmann, 1983) in fairly high concentrations. Nevertheless, we carried out the nitrosation experiments (see the Experimental Section) to reconfirm the presence of amine precursors in a few samples of unused rubber nettings. The

Table II. Volatile Nitrosamine Contents of Nettings and of Meats from Different Cross Sections of Packaged Products

kind (producer)	type of netting (manufacturer)	levels of volatile nitrosamines detected, ppb						total formed during processing, <sup>b</sup> μg
		unused netting	used netting <sup>a</sup>	whole homog- enized meat	outermost layer	next layer	center	
(1) sweet pickled pork cottage roll (K)	rubber (A)	NDBA, 4.6	504	19.5	57 <sup>c,g</sup>	11.2	2.2	25.4
(2) smoked ham, type I (O)	rubber (C)	NDEA, tr <sup>d</sup>	tr	N <sup>e</sup>	N	N	N	neglig
(3) smoked ham, type II (O)	rubber (C)	NDBA, tr	tr	N	N	N	N	neglig
		NDEA, tr	4.9	2.4	6.9	4.9	0.8	3.1
		NDBA, tr	104	5.9	33.3	5.3	N <sup>e</sup>	8.5
(4) sweet pickled pork cottage roll (L)	rubber (B)	NThZ, N	N	1.7	3.8	2.7	tr	2.1
		NDEA, 3.8	16.0	0.7	5.1 <sup>c</sup>	1.0	N	1.4
(5) deboned picnic ham, smoked (M)	rubber (A)	NDBA, 9.9	51.5	7.2	28.2 <sup>c</sup>	14.7	N	4.8
		NThZ, N	N	5.1	7	5.8	4.8	3.1
(6) deboned ham (smoked) (N)	rubber (A)	NDBA, 13.7	281	29	f			22.5
		NThZ, N	N	1.4				0.37
(7) boneless smoked pork shoulder (K)	rubber (A)	NDBA, 4.6	168	5.3	33.1 <sup>c,g</sup>	9.3	tr	8.1
		NThZ, N	3.4	4.0	7.0 <sup>c</sup>	4.3	2.5	3.4
(8) boneless smoked pork picnic (P)	cotton (unknown)	NDBA, f	N	N	tr	N	N	N
		NThZ	N	4.1	2.9		2.7	1.5
(9) smoked cottage roll (K)	rubber (A)	NDBA, 4.6	400	17	60 <sup>c,g</sup>	45	N	24.1
		NThZ, N	N	2.9	5.6 <sup>c</sup>	4.7	1.2	2.7
(10) smoked cottage roll (S)	cotton (unknown)	NThZ	N	2.3	2.7		2.1	1.5

<sup>a</sup> After taking out of the package. <sup>b</sup> Including that in the used netting and assuming none originating from raw meat and other ingredients. <sup>c</sup> Confirmed by GLC-high-resolution mass spectrometry. <sup>d</sup> tr = trace (0.1–1 ppb). <sup>e</sup> N = negative (detection limit 0.1 ppb for NDEA, 0.3 ppb for NDBA, and 1 ppb for NThZ). <sup>f</sup> Not available or not analyzed. <sup>g</sup> Outermost layer in these cases also contained traces of NDMA (confirmed by GLC-MS).

Table III. Formation of Volatile Nitrosamines after Incubation of Elastic Rubber Nettings with Sodium Nitrite Solution under Acidic Conditions<sup>a</sup>

	levels of nitrosamines detected, <sup>b</sup> ppb	
	orig sample	after nitrosation <sup>a</sup>
brand 1, source A	NDBA, 4.6	NDBA, 945 NDMA, 58
brand 1, source B	NDBA, 13.7	(i) <sup>c</sup> NDBA, 5000 NDMA, 360
		(ii) <sup>c</sup> NDBA, 3000 NDMA, 86
		(iii) NDBA, 1100 NDMA, 55
brand 2	NDEA, tr <sup>d</sup>	NDEA, 2620 NDBA, 400
brand 3	NDBA, tr	NDEA, 336
	NPIP, 5.1	NPIP, 450
brand 4	NDBA, tr	NDMA, 49
	NDEA, tr	NDEA, 2620
blank (no netting used)	NDBA, tr	NDBA, 400
		NDMA, tr NMOR, tr

<sup>a</sup> Approximately 1–3 g of netting, cut into small pieces, incubated overnight with 100 mL of sodium nitrite solution (0.5 mg/mL) at room temperature at pH 3–4. <sup>b</sup> Based on the weight of the netting. <sup>c</sup> Incubation pH 3; in all other cases, incubation carried out at pH 4. <sup>d</sup> tr = trace (~1 ppb).

data (Table III) clearly indicate the presence of some amine precursors although the exact nature of the amines was not identified. It should be of interest that the predominant nitrosamines formed were NDBA and NDEA. One sample (brand 3) formed significant amounts of NPIP, but the corresponding matched pairs of meat packaged in such a netting was not available for analysis. It should be emphasized that these experiments were not designed to simulate conditions existing during processing of cured meats. Therefore, such high levels of nitrosamines formed in these experiments might not have any bearing on what could be found in cured meats packaged in such nettings.

Although we did not investigate in detail the extent of formation of nitrosamines at various stages of processing

Table IV. Comparison of Nitrosamine Levels in Fatty Layers with That in Lean Meat Portions from Samples Packaged in Rubber Nettings

samples	levels of nitrosamines detected, <sup>a</sup> ppb	meat	
		meat	fat
pork cottage roll, smoked	NDBA	15.1	27.8
	NMOR	3.4	2.8
smoked ham	NDEA	2	0.8
	NDBA	7.1	7.3
smoked cottage roll	NDBA	23.8	63.6

<sup>a</sup> In outermost layer.

and cooking, we have some data that suggest that the nitrosamines in the cured meat samples were already formed before they reached the consumers. For example, all six of the ready-to-serve products, which were analyzed without warming or heating, contained appreciable levels of NDBA (7–36 ppb in the outermost layers). Also, in the case of a cottage roll, heating did not seem to affect the nitrosamine levels. The outermost layers in the unheated and heated halves of the sample contained 15.1 and 14.1 ppb NDBA, respectively.

Furthermore, three pork picnic shoulders were warmed after removing all elastic rubber nettings. Even then, the homogenized meats in all cases contained fairly high levels (11, 15, 20 ppb) of NDBA. At least in these cases heating the samples with netting on was not responsible for NDBA formation. Since most consumers warm or cook (for raw products) such items with nettings on them, the sample preparation procedure used in this study was clearly justified.

As mentioned above, most of the fat was removed from the meat before sampling and analysis. Therefore, the data presented above only represent the nitrosamines detected in the edible portions. However, analyses of three fat samples suggested that they might contain higher levels of NDBA than the corresponding edible meats (Table IV). This might be attributed to either higher solubility of NDBA in the fat or to the presence of organic nitrosating

agents formed due to the interaction of nitrite and components of fat. The presence of such an organic nitrosating agent has been demonstrated in cooked-out bacon fat (Hotchkiss et al., 1985).

Although we have previously shown (Sen and Seaman, 1981) that our low-temperature vacuum distillation method used for the determination of volatile nitrosamines in meats is free of artifactual formation, additional experiments were carried out to reconfirm it. When selected samples of cured meats were analyzed with added morpholine (see the Experimental Section), no evidence for the formation of NMOR was observed. Also, when two other samples were reanalyzed by the modified acidic vacuum distillation with added sulfamic or ascorbic acids, the results obtained for NDEA or NDBA were practically the same as obtained with the alkaline distillation method. Since both sulfamic and ascorbic acids are proven inhibitors of N-nitrosation and since there was no significant change in the levels of nitrosamines detected by the modified method, it is highly unlikely that any artifactual formation of nitrosamines took place during analyses of the samples.

However, when used rubber nettings were analyzed with added morpholine, traces of NMOR were formed as an artifact. Formation of this artifact could be minimized by adding propyl gallate, an N-nitrosation inhibitor (Sen et al., 1987), but it could not be prevented completely. Therefore, it appeared that a small portion of the nitrosamines detected in the used nettings might have formed as a result of artifact formation. In the absence of a better method we were unable to determine exactly the extent of this formation. Since the used netting is not an edible item and since it was taken off before analysis of the meats, the above observation may not have much significance in the present context. Further work is in progress to improve the methodology for determining volatile nitrosamines in used nettings.

In summary, the data presented above suggest that the use of elastic rubber nettings in packaged cured meats can lead to the formation of nitrosamines (mainly NDEA and NDBA) in the finished meat products. Since both NDEA and NDBA are potent carcinogens in a wide range of animal species (Preussmann and Stewart, 1984), it would be advisable to reduce the concentration of these chemicals in such meat products as low as technically feasible. Perhaps, the use of nettings made from improved rubber or alternative materials might help resolve the problem. Further research along these lines is urgently needed.

#### SAFETY NOTE

Since most nitrosamines are potent carcinogens, adequate precautions should be taken while working with these chemicals.

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**Registry No.** NMOR, 59-89-2; NDMA, 62-75-9; NThZ, 73870-33-4; Et<sub>2</sub>N(NO), 55-18-5; Bu<sub>2</sub>N(NO), 924-16-3; NO<sub>2</sub><sup>-</sup>, 14797-65-0.

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