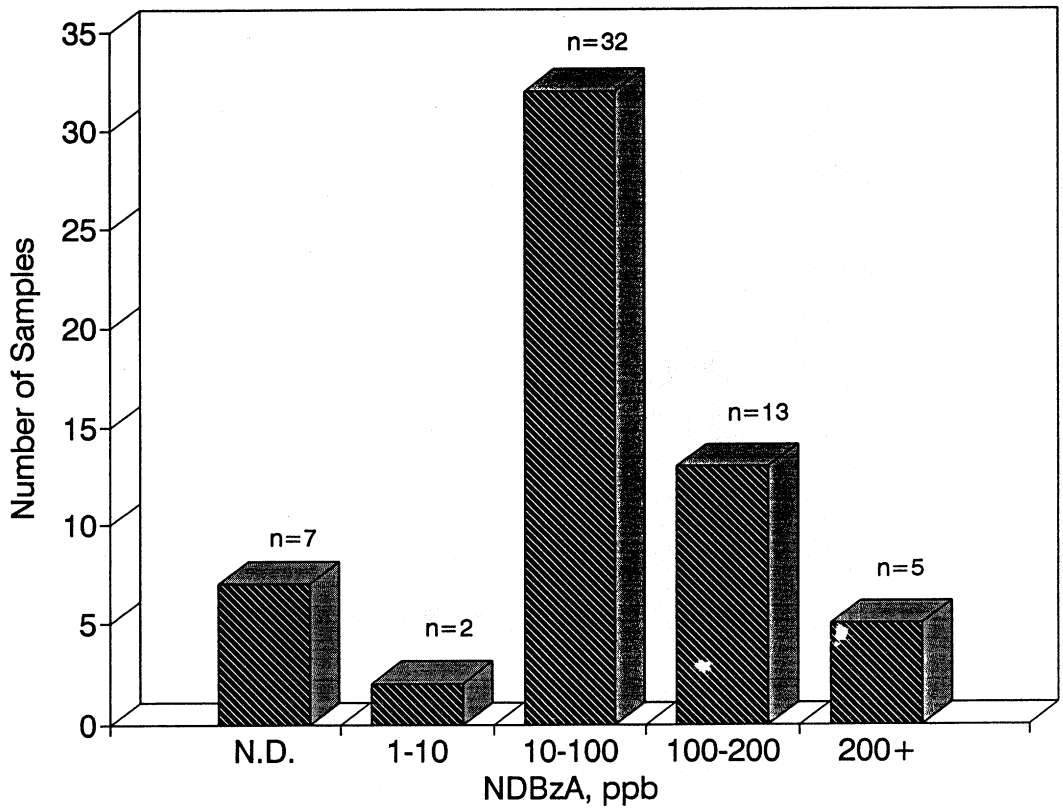

Figure 1. Incidence of NDBzA in the outermost layer of netted ham products.

ton thread burns, whereas the polyester thread melts to form a bead. In the third type of netting, designated R/C-P, the rubber is wrapped in cotton but the lengthwise attachments between the sections are made of polyester knitted thread. Ninety percent (28 of 31) of hams processed in all-cotton nettings, 94% (15 of 16) of those processed in all-polyester nettings, and 100% (5 of 5) of hams processed in the hybrid type were positive for NDBzA. Corresponding mean values from the exterior surface layers for each type of netting used were 79.3, 113.4, and 172.5 ppb NDBzA, respectively.

Although visual inspection of the data appears to reveal large differences among netting subgroups, no significant difference ($p < 0.05$) was found among their means because of the very high sample variability within each subgroup. For example, one sample in the R/C category was processed in a cotton elastic netting that, in turn, was covered with a polyester stocking. The 31.0 ppb NDBzA obtained for this sample was within the range of values for other hams processed in elastic nettings not covered with an outer stocking. Another ham was processed in a netting consisting of red-dyed cotton wrapped around the rubber filament, and undyed cotton was used for cross-linking. This sample contained 118.7 ppb NDBzA. Another ham was processed in a netting with 3 strands of rubber rather than the single rubber strand found in all the other elastic nettings. This sample contained 443.4 ppb NDBzA, one of the highest amounts found.

Three hams were processed in what is more appropriately considered a stocking rather than a netting. The stocking was made of a tightly woven polyester mesh, and it did not leave rectangular-shaped marks on the ham surface after processing that are characteristic of nettings. Two of the hams were processed in stockings made only of polyester thread, designated S/P; they had no detectable nitrosamines. However, the one ham produced in a stocking in which rubber filaments were embedded, designated S/P-R, contained 94.0 ppb NDBzA on the surface, similar to that found in the other hams processed in rubber-containing elastic nettings.

There were 4 ham samples for which information on the type of netting used for processing was not provided. Three of these contained from 41.7–550.3 ppb NDBzA.

The data were analyzed to determine if the netting manufacturer was a significant factor. Data for samples where the netting manufacturer was unknown were removed. Analysis of variance showed highly significant ($p < 0.01$) differences among ham-processing plants, as would be expected, and also among netting manufacturers. Repeatability of analysis was 3.0 ppb (coefficient of variation, 3.1%). Results from Duncan's Multiple Range test for differences among NDBzA means for the most frequently represented netting producers, from highest to lowest, are shown in Table 1. For 6 netting manufacturers, only single samples were available. Data from these samples were omitted from the table, because they were not considered representative because of the high degree of variability found in hams produced from the same netting brand. Unfortunately, these omitted data included those of single samples from 2 manufacturers that gave no detectable nitrosamines

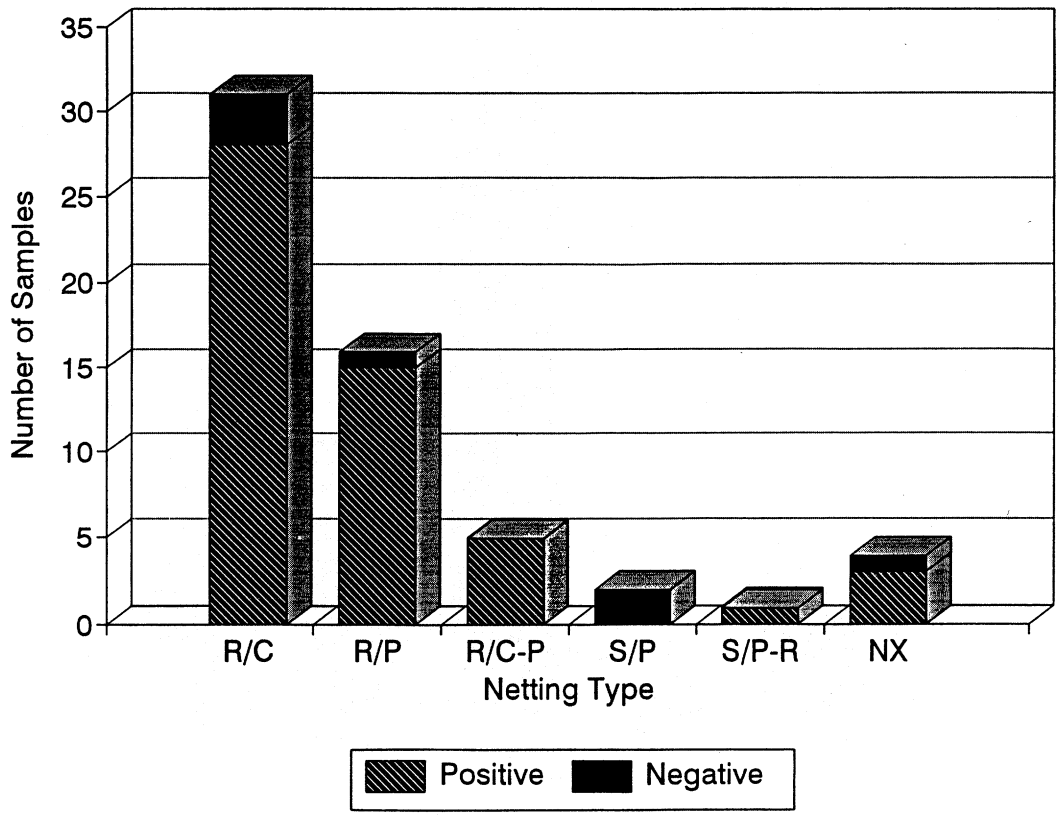

Figure 2. Effect of netting type on NDBzA in the netting: R/C = rubber/cotton; R/P = rubber/polyester; R/C-P = rubber/cotton-polyester; S/P = stocking/polyester; S/P-R = stocking/polyester-rubber; NX = netting type not known.

and from 2 that had the highest levels of NDBzA, 512.2 and 443.4 ppb. Additional ham samples from the same processor were analyzed later in the study. Although more data were obtained from companies A and C compared with the single samples mentioned above, the number of data points was inadequate as a reliable predictor of the performance or NDBzA-forming potential of nettings from each of these companies. Identifying a particular manufacturer whose nettings may produce either more or less nitrosamine than the others may be difficult because they usually produce more than one type of netting for their product line. There is also the high probability that netting producers buy rubber thread from the same source.

Attempts were made to identify how the netting contributes to nitrosamine residues in hams. Unused nettings, accompanying 18 hams whose exterior NDBzA contents ranged from 100.4 to 512.2 ppb (mean, 207.8 ppb), were analyzed for nitrosamines. The results are shown in the second column of Table 2. The nettings contained 30.8–943.7 ppb (mean, 226.5 ppb) NDBzA. Regression analysis indicated no significant correlation ($p < 0.05$) between the NDBzA content on the ham surface and that found in the unused netting, probably because of the wide variation in processing conditions. The maximum amount of nitrosamine that could be formed from the nettings was obtained by nitrosating extracts of unused netting in the presence of excess nitrite; it is referred to as the nitrosation potential. These values are shown in the third column of Table 2. Here, too, no correlation was found between NDBzA values and nitrosation potentials (4.2–224.6 ppm; mean, 97.0 ppm). However, the results show that all the nettings contain sufficient amine precursors to form additional amounts of NDBzA and demonstrate the dependence of NDBzA formation on nitrite or other nitrosating agents.

The source of the variable amounts of preformed nitrosamine in the nettings was puzzling. One possible explanation is the use in rubber production of additives that contain the nitroso or nitro group that could act as transnitrosating agents. One of them, the vulcanization-retarding agent *N*-nitrosodiphenylamine, is commonly used in the manufacture of tires but is not typically used for the light-colored rubber stock required for the netting rubber (21). The "normal" amount of am-

Table 1. Concentration of NDBzA on exterior surface of ham

Netting manufacturer	*n*	Mean[a] NDBzA, ppb	Statistical group[b]
A	7	182.3	A
B	20	89.3	B
C	5	51.2	C
D	13	42.4	D

[a] Exterior portion.
[b] Netting means with different letters are significantly ($p < 0.05$) different from each other.

Table 2. Nitrite and NDBzA in unused elastic nettings from hams with >100 ppb NDBzA

NDBzA, ppb		NDBzA, ppm (nitrosated netting)	Netting type	$NaNO_2$, ppm (in netting fabric)
Ham	Unused netting			
107.7	594.3	93.0	R/C	6.0
115.5	51.8	162.1	R/C	51.8
139.6	107.9	183.7	R/C	4.3
157.3	76.8	159.4	R/P	6.6
138.5	303.0	161.8	R/C	28.3
125.3	56.6	15.1	R/C	4.0
139.9	669.5	176.9	R/C	8.0
443.4	150.8	84.2	R/C	26.8
244.2	41.4	24.5	R/P	0.9
512.2	301.7	91.3	R/P	ND[a]
370.6	193.6	136.4	R/P	2.0
486.8	943.7	224.6	R/C-P	31.2
118.7	187.9	4.2	R/C	4.9
124.9	103.8	82.3	R/P	3.5
100.4	33.3	22.2	R/C	72.6
174.1	85.3	16.1	R/C	96.3
125.5	30.8	37.8	R/C	0.7
115.2	144.4	71.0	R/C-P	1.9

[a] ND = none detected.

bient nitrogen oxides in the air was thought to be insufficient to account for the amounts of preformed NDBzA in the nettings. For this reason, other sources of nitrosating species were investigated.

After separation from the rubber filaments, the cotton was analyzed for NDBzA. Several samples indicated the presence of low levels of NDBzA that migrated from the rubber. Cotton used for the nettings does not undergo any thermal or chemical treatment before being spun into thread (22), thus precluding the role of these factors in nitrosamine formation. Nitrate is a normal constituent of cotton that could serve as a precursor for nitrite. Therefore, both cotton and polyester threads were separated from 18 samples of unused netting from processors whose hams contained >100 ppb NDBzA. The nitrite results are shown in the last column of Table 2. Levels of nitrite in the threads, calculated as $NaNO_2$, ranged from none detected (ND, <0.5 ppm) to 96.3 ppm (mean, 19.4 ppm). Only one of 18 samples had no detectable level of nitrite; 6 of 18 had over 25 ppm. Nitrite values for the cotton (n = 13) and polyester (n = 5) threads that had been in direct contact with the rubber were 25.9 and 2.6 ppm, respectively. The 2 samples designated R/C-P were considered R/C for this purpose, because the cotton was in direct contact with the rubber filament. These findings suggest that nitrite in cotton may have a role in formation of preformed nitrosamine in the netting. Later, the cotton from 6 of these same nettings, containing 51.8–943.7 ppb NDBzA and 26.8–96.3 ppm nitrite, was reanalyzed for nitrate. From 154.0 to 771.7 ppm $NaNO_3$ (mean, 443.4 ppm) was found in these samples, sufficient to form the amounts of nitrite detected. Nitrite could form from nitrate by endogenous and/or microbial nitrate reductase enzymes in or on the cotton. Analysis of cotton from an elastic netting accompanying a different ham, where both contained no detectable nitrosamine, gave 434.6 ppm of nitrate, which is within the range of values found for other samples, but a lower amount of nitrite, 16.6 ppm. These findings suggest that nitrite from cotton might have a role in NDBzA formation, but its contribution is small compared with that of nitrite in the cured pork muscle (average, 65 ppm). Analysis of polyester thread from 5 unused nettings gave from ND to 6.6 ppm nitrite (mean, 2.6 ppm), and from 26.4 to 105.7 ppm nitrate (mean, 58.1 ppm). These results were considerably lower than those obtained for the cotton, and may result from absorption of nitrogen oxides from the air. No relationship was found between nitrite and nitrate values in the polyester thread and NDBzA levels in unused nettings or in ham samples.

Additional ham and netting samples were obtained from producers whose hams contained >100 ppb NDBzA in the initial survey. These new hams were processed in an identical fashion to the original hams, and in most cases, the accompanying nettings were of the same type as those sent originally. Initial results are shown in the first column of Table 3. Contrary to instructions, the hams containing the highest NDBzA values (805.1 and 550.3 ppb) were sent without their nettings. There was no reason to suspect that the type of nettings used for these hams was different from that used originally, designated R/P and R/C-P, respectively. However, although it appeared that the type of netting was unchanged, the chemical composition of the netting itself may not have been the same.

Because rubber production is a batch process with technical-grade chemicals, a lack of batch uniformity could be a cause of nitrosamine formation. Hams from manufacturers A, E, and J were processed in different netting types. The values of NDBzA remained high for some of the repeats, but for oth-

Table 3. NDBzA in additional hams where the initial survey results were high

Ham manufacturer	NDBzA, ppb[a] (netting type)		
	Sample 1[b]	Sample 2	Sample 3
A	107.7[c] (R/C)	170.0[d] (R/C-P)	73.2 (R/C-P)
B	157.3 (R/P)	20.0 (R/P)	805.1 (NX[e])
C	138.5 (R/C)	ND[f] (R/C)	252.9 (R/C)
D	139.6 (R/C)	22.6 (R/C)	
E	244.2 (R/P)	ND (S/P)	
F	512.2 (R/P)	746.9 (R/P)	
G	370.6 (R/P)	291.0 (R/P)	
H	486.8 (R/C-P)	550.3 (NX)	
I	124.9 (R/P)	21.6 (R/P)	
J	115.2 (R/C-P)	72.1 (R/P)	
K	174.1 (R/C)	85.0 (R/C)	

[a] All samples >100 ppb confirmed by GC/MS.
[b] Initial survey results.
[c] 3.8 ppb NDBA.
[d] 3.2 ppb NMOR.
[e] No netting supplied with sample.
[f] ND = none detected.

ers, they were lower than the samples analyzed initially. Overall, NDBzA results showed a great deal of variability. Because of this variability, it was not possible to pinpoint processing conditions that contributed to nitrosamine formation other than contact with the rubber netting. One of the most striking results was the failure to detect nitrosamine in manufacturer C's ham processed in a conventional R/C netting. This and other survey data suggest it may be possible to produce hams with no or low nitrosamine content. Perhaps tighter control of rubber formulation is the key to accomplishing this. The other repeat sample with nondetectable nitrosamine was processed in a non-rubber-containing polyester stocking, suggesting that use of such stocking may also be a means of producing hams with no or low nitrosamine content.

Additional ham samples were obtained from 2 manufacturers whose hams contained no detectable volatile nitrosamine or NDBzA (<1.0 ppb). Originally, no netting had been sent with one sample, and a conventional R/C netting had been enclosed with the other. The follow up duplicate of the first sample also contained no detectable nitrosamines. The absence of nitrosamines may be due to processing of this ham in a brown-colored polyethylene plastic netting, unique among other nettings in this study. The duplicate ham from the second manufacturer contained no detectable NDBzA but had 10.7 ppb NDBA. This suggested that this ham producer was still using some of the older R/C type nettings where the rubber was formulated with the zinc dibutyldithiocarbamate vulcanizing agent, not the one responsible for the occurrence of NDBzA.

Conclusions

This survey of nitrosamines in commercial hams processed in elastic rubber nettings covered a variety of nettings, producers, and processing conditions, and it was the largest done to date. It showed that 1/3 of the surveyed hams contain >100 ppb NDBzA in the outermost layer, with almost 10% containing nitrosamine levels higher than any previously encountered in a cured meat product (23). Except for consumption of end pieces of boneless hams, actual dietary exposure to NDBzA may not be as high as indicated by this survey because only a small portion of the surface is consumed in a cross-sectional slice. The nitrosamine content is highest on the outer surface, where there is direct contact with the netting, and is markedly less toward the interior of the ham (13). We reported (13) a highly significant ($p < 0.01$) correlation between concentrations of NDBzA in the outer surface and the cross-sectional slice (ratio = 7:1), when the slice area is removed as a statistical factor. The surface-to-slice ratio means that for hams with high levels of nitrosamine on the surface, exposure to nitrosamines in a cross-sectional slice could still be substantial. The study shows that the rubber used in nettings is the single most important factor in nitrosamine formation in netted hams. Action needs to be taken to reduce the nitrosamine content in hams. The best approach appears to be removal of precursor amines from the rubber in nettings or avoidance of rubber in nettings altogether.

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N-Nitrosodibenzylamine in Boneless Hams Processed in Elastic Rubber Nettings

Boneless hams processed in elastic rubber nettings contain high levels of nitrosamines in the outermost layer. The precursors of the nitrosamines are zinc dibutyl- or dibenzyldithiocarbamate used as a vulcanizing agent in the formulation of the rubber. The outermost layer from 59 commercial hams was analyzed for 11 volatile nitrosamines including *N*-nitrosodibutylamine (NDBA) and *N*-nitrosodibenzylamine (NDBzA). The principal nitrosamine, NDBzA, was detected in 32 (54%) ham samples at the 10–100 ppb range; it exceeded 100 ppb in 18 (30%) samples, with the highest at 512.2 ppb. No nitrosamine was detected in 7 of 59 ham samples. To determine the cause of the high NDBzA values, various types of unused nettings (from different manufacturers) accompanying the samples were analyzed for nitrosamines. No correlation was found between the NDBzA content of the hams and the nettings. The results suggest that the problem of nitrosamine formation in these products has not yet been resolved.

After the first report of nitrosamines in baby bottle nipples (1) and the subsequent concern about exposure of infants to these potentially carcinogenic compounds, considerable research was performed to study the occurrence of nitrosamines in other rubber products and in foods in contact with rubber. Sen et al. (2) found up to 29 ppb *N*-nitrosodibutylamine (NDBA) and 2.4 ppb *N*-nitrosodiethylamine in 16 cured meat products processed in elastic nettings containing rubber. In the same study, Sen et al. (2) showed that netted hams had the highest concentration of NDBA in the outermost 5 mm of the surface, that this nitrosamine could also form in the product, and that it could migrate to deeper levels. These findings were later verified by a Danish report (3) that analyzed a single ham sample. In a study about a new method to extract volatile nitrosamines from hams processed in elastic nettings (4), from 10.8 to 49.9 ppb NDBA was detected in the outermost layer of 15 commercial samples. All these data indicate that the rubber in the nettings may be responsible for the nitrosamines in the product. Nettings are made from cotton- or polyester-wrapped rubber thread and are typically used to help bind pieces of cured meat muscle together during thermal processing of boneless hams in the smokehouse. Nettings also are used for cooked and fresh products such as roast beef, turkey breast, and pork loin. However, elastic nettings are used more extensively with boneless hams than any other product.

U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) found from 15 to 123 ppb NDBA in the outermost layer of 10 retail ham samples processed in nettings; only 2 samples had nondetectable levels of NDBA (5). None of the 5 hams processed without rubber netting contained detectable NDBA. In 1988 and 1993, Sen et al. reported more data showing that the principal nitrosamine in ham is *N*-nitrosodibenzylamine (NDBzA), not NDBA (6, 7). A limited survey found from 2.6 to 128.5 ppb NDBzA in the outermost layer of hams (8). This finding suggests that the rubber used for the netting had been reformulated with a different accelerator, most likely zinc dibenzyldithiocarbamate, so as not to produce the carcinogenic NDBA. A 1967 study of 65 different nitrosamines showed that NDBzA is noncarcinogenic in rats (9), but more recent investigations showed it to be genotoxic in mammalian assays (10–12).

Formation of nitrosamines in hams processed in elastic rubber nettings has been attributed to the nitrite used in curing meat and the zinc dialkyl- or dibenzyldithiocarbamate accelerator used in the formulation of rubber. Because the data on the extent of use of reformulated nettings and the amount of NDBzA in the outermost layer of boneless hams were limited, a survey was performed in cooperation with FSIS. Results are presented in this paper.

Experimental

Safety Note

Precaution should be exercised in the handling of nitrosamines, because they are potential carcinogens.

Ham Survey Study

Hams were obtained from FSIS. Initially, 59 hams weighing 4–8 lbs and processed in elastic rubber netting were obtained from FSIS inspectors nationwide in 1993 and 1994. The entire ham still in its netting and, with few exceptions, an unused netting from the same lot and an information sheet were shipped from the processing plant to the FSIS Athens, GA, laboratory. The information sheet contained establishment number, type of ham, netting producer and brand, source of heat for cooking, and other related processing data.

To prepare the sample, the FSIS laboratory separated the netting from the ham, sliced one-quarter inch of the ham exterior surface, and ground this portion. The used and unused netting and a portion of the ground outer ham sample were shipped to USDA's Eastern Regional Research Center, Wyndmoor, PA (ERRC), for nitrosamine analysis. Followup samples, requested after the initial survey was completed, were shipped by FSIS inspectors directly to ERRC. All samples, except unused netting, were stored in a –20°C freezer until analyzed.

Nitrosamine Analysis

N-Nitrosamines were extracted by solid-phase extraction and quantitated by gas chromatography–thermal energy analyzer (GC–TEA). Details of these procedures have been published elsewhere (13). Samples were analyzed for nitrosamines in duplicate, and the values obtained were corrected for recovery of a 10 ppb internal nitrosamine standard, *N*-nitrosodipropylamine. The minimum detectable levels (signal-to-noise ratio > 2) for NDBA and NDBzA were 1.0 ppb.

Nitrosamines were isolated from elastic nettings after the netting was cut into small pieces. A 0.5 g portion was soaked for 18 h in 100 mL dichloromethane (DCM) containing 250 mg propyl gallate and then analyzed as described previously (13).

Nitrosation Potential of Netting

One-half gram unused netting was extracted for 18 h in 50 mL 0.2N HCl. The netting was removed from the acid, and 10 mL aqueous solution containing 1000 ppm sodium nitrite was added to the acid, and the mixture was stirred for 1 h at room temperature. The reaction was quenched with 2 g sulfamic acid, the acidic solution was extracted 3 times with 50 mL DCM, and the DCM extract was dried over anhydrous sodium sulfate and concentrated to 1.0 mL. Nitrosamines were determined by GC–TEA (13).

Sodium Nitrite Analysis

Residual sodium nitrite was determined in 10 g comminuted ham sample by the Griess–Saltzman procedure as modified by Fiddler (14). Nitrite (measured as sodium nitrite) also was determined in unused nettings as follows: the string was separated from the rubber, 1–5 g string was soaked in water for 24 h, an aliquot was taken, and nitrite was measured spectrophotometrically by the Griess–Saltzman procedure.

Data Analysis

Data were analyzed by the General Linear Model and Means procedures of the Statistical Analysis System PC software (15). Results were interpreted according to the methods of Snedecor and Cochran (16) and Youden and Steiner (17).

Results and Discussion

The exterior portion from 59 commercial hams was analyzed for 10 volatile nitrosamines and the semivolatile nitrosamine NDBzA. Figure 1 shows the incidence of NDBzA in the samples. The values for NDBA, the other nitrosamine associated with the netting, are not shown because NDBA was detected in only 3 samples. One sample had 33.8 ppb NDBA and 4.0 ppb NDBzA; the other 2 had low levels of NDBA (3.8 and 4.2 ppb) with much higher quantities of NDBzA. The predominance of NDBzA indicates the widespread use of nettings containing rubber that has been reformulated with the zinc dibenzyldithiocarbamate vulcanizing agent. Only 7 of 59 samples contained no detectable level of either rubber-derived nitrosamine. Four hams contained 0.9–3.4 ppb *N*-nitrosomorpholine (NMOR), whose source is most likely morpholine, an anticorrosion agent used in steam boilers (18). From 0.5 to 1.6 ppb (mean, 1.1 ppb) NDMA was detected in 13 samples, representing what appears to be the normal incurred level, because it falls within the range of values previously reported for most cured meat products (19). Most samples, 32 (54%), fell in the 10–100 ppb range for NDBzA; the mean value for these samples was 49.4 ppb. Thirty percent of the samples contained >100 ppb NDBzA, all of which were confirmed by GC/mass spectrometry (MS). The maximum level was 512.2 ppb NDBzA. Thus, a wide range of NDBzA was detected in the exterior layer of the commercial hams tested. Statistical analysis of the surface NDBzA values versus curing ingredients and processing conditions showed no significant associations, because of confounding by the wide variation in processing conditions used by ham producers.

Because the presence of nitrosamine depends on contact of the nettings with nitrite-treated pork muscle, attention was placed on analysis of nettings. To understand the role of netting in NDBzA formation, the data were broken down according to the type of netting used (Figure 2). Elastic nettings that contain rubber can be classified into 3 types that are physically and visually distinguishable. The first and most common—made by a number of manufacturers—contains a single rubber strand wrapped with 2 layers of cotton thread around the circumference of the netting. This is the type of netting permitted by USDA's FSIS Chemistry Laboratory Guidebook (20). This cotton-covered rubber filament netting is designated R/C and is distinguished from the second type of netting—a similarly wrapped polyester thread designated R/P—by its off-white or creamy white color versus the true white color of the polyester. The 2 types of threads were identified by a flame test: the cot-