Nitrosamine Formation in Processed Hams as Related to Reformulated Elastic Rubber Netting

ABSTRACT

A survey of boneless hams processed in elastic rubber nettings showed that 32 of 59 contained over 100 ppb N-nitrosodibenzylamine (NDBzA) on the surface. These findings demonstrated the need to reduce the nitrosamine content. Nettings made with rubber thread reformulated with zinc diisobutyldithiocarbamate vulcanizing agent were evaluated for their effect on reducing the nitrosamine content. The N-nitrosodiisobutylamine (NDiBA) values ranged from 4.6 to 33.5 ppb (mean 16.1 ppb) compared to 52.3 to 739.9 ppb NDBzA (mean 191.2 ppb) in the control hams. No detectable nitrosamines were found in the unused experimental nettings. The low amounts of NDiBA found on the outer surface of these hams suggested that the use of these new nettings would provide a beneficial alternative to nettings currently in use.

Key Words: cured meats, nitrite, nitrosamines, rubber

INTRODUCTION

NITROSAMINES HAVE BEEN REPORTED IN A WIDE VARIETY OF RUBber-containing products including tires, tubing, stoppers, gloves, toys, baby bottle nipples and pacifiers (Tricker et al., 1989). After the report of possible nitrosamine migration from rubber baby bottle nipples and pacifiers into drinks and formulas (Preussmann et al., 1982), such rubber items were the primary focus of concern and investigation, and became subject to regulatory restrictions regarding their nitrosamine content. In the U.S., the action level was originally set at 60 ppb for total volatile nitrosamines (Novitch, 1983) and later dropped to 10 ppb to ensure that manufacturers would develop means for their reduction. This led to the reformulation of the rubber with non-nitrosamine-producing agents or additives. In general, nitrosamines are formed from vulcanization accelerators that are used in the preparation of rubber to help impart desired physical properties (Ireland et al., 1980; Yeager et al., 1980). Metal dithiocarbamates, are among several classes of accelerators made from secondary amines which include thiuram mono- and poly-sulfides, sulfenamides and thioureas.

Another reported source of nitrosamine exposure from food-rubber contact has been hams processed in elastic rubber nettings (Sen et al., 1987). Nettings are typically made from cotton or polyester-wrapped rubber thread, which is used to help bind pieces of cured meat muscle together during the thermal processing of boneless hams in the smokehouse. While such nettings are also used for cooked and fresh meat products such as turkey breast, roast beef, and pork loin, boneless hams employ elastic nettings more extensively than any other product because of their widespread popularity. Sen et al. (1987) reported finding up to 29 ppb N-nitrosodibutylamine (NDBA) and 2.4 ppb N-nitrosodiethylamine in 16 cured meat products processed in elastic nettings containing rubber. There were also reports of NDBA on the outer surface of netted hams (Sen et al., 1988; FSIS, 1991; Pensabene et al., 1992; Peterson, 1993). All data indicated that the rubber in the nettings was responsible for the nitrosamines in hams, primarily on the product surface. Unlike the situation with rubber baby nipples and pacifiers, where the nitrosamines were leached out of the rubber, nitrosamines in hams are formed primarily from the reaction of precursors in the rubber and nitrite in the cured pork during smokehouse processing.

Sen et al. (1993) published additional data showing that the principal nitrosamine now found in ham was N-nitrosodibenzylamine (NDBzA), and not NDBA. In a limited survey, Pensabene and Fiddler (1994) also reported from 2.6 to 128.5 ppb NDBzA in the hams' outermost layer. These findings, and those by Sen et al. (1993), suggested that the rubber used for netting had been reformulated with a different accelerator, zinc dibenzyl- instead of dibutyl-dithiocarbamate, so as not to produce the carcinogenic NDBA.

Since the extent of NDBzA occurrence in commercial boneless hams in the U.S. was not known, a survey was carried out in cooperation with FSIS (Fiddler et al., 1997). The results were obtained from a large number of producers where processing conditions varied considerably. Thirty-two of 59, or 1/3 of the hams tested, contained over 100 ppb NDBzA in the outermost layer. Ten percent contained nitrosamine levels higher than any previously encountered in a cured meat product; the highest level was 512 ppb. These survey findings demonstrated the need to reduce or eliminate the nitrosamine content in this type product. As a result, we conducted research in this area and published a report describing two possible approaches to reduce nitrosamines (Fiddler et al., 1996). Since nitrosamine formation is dependent on nitrite, one was the reduction of ingoing nitrite during processing. Another was the removal of nettings from the hams post-processing to reduce the contact time between any amine precursors in the netting and the nitrite in the product. However, a more effective alternative is needed to accomplish nitrosamine reduction. Our objective was to determine the effect of nettings made with reformulated rubber using a zinc dithiocarbamate containing a different secondary amine.

MATERIALS & METHODS

Rubber thread

Natural rubber thread samples were formulated with zinc diisobutyldithiocarbamate, sulfur, clay, potassium hydroxide, potassium oleate, a butylated reaction product of *p*-cresol and cyclopentadiene, zinc oxide, sodium salts of polymerized alkyl naphthalene sulfonate, casein, sodium polyacrylate, triethanolamine, sodium salts of sulfosuccinic acid and *o*-phenylphenate tetrahydrate.

Ham samples

Collaborating ham processors were given specific written instructions for this study. They were asked to provide two boneless hams from two different production runs, where experimental nettings were used. In addition, one ham, made with the nettings currently in use, was requested from one of the production runs. This sample served as a control for comparison. All hams were to be approximately identical in size, in the 2.3–3.6 kg range. The experimental hams were to be processed on the top rack, and not in direct contact with any other hams, in order to prevent cross-contamination by dripping or contact. The hams were to be wrapped or bagged with the nettings still on, and a sample of unused netting currently in use, was also requested. They

were to be kept chilled and shipped as soon after the second production run as possible. Overnight shipment was to be in an insulated container containing cold packs. After analyzing the hams from the first seven producers, the protocol was revised to include a control from each production run. Otherwise, all instructions were the same. When received at ERRC, the nettings were removed from the ham, the outer 0.6 cm surface removed from the ham, ground twice, vacuum-packaged, and stored in a −20°C freezer until analyzed.

Nitrosamine analysis

N-nitrosamines were extracted from the ham samples using a solid phase extraction procedure, and quantified by gas chromatography-Thermal Energy Analyzer (gc-TEA). The details of the procedure have been published (Pensabene and Fiddler, 1994). Samples were analyzed in duplicate, and the values corrected for recovery of the 10 ppb N-nitrosodipropylamine internal standard. The minimum detectable level (signal:noise > 2) for NDiBA was 0.5 ppb, and for NDBzA, 1.0 ppb. Recovery of NDiBA in blank ham samples (n=6) fortified with 10 ppb of this nitrosamine was 95.4±5.9%. N-nitrosamines were isolated from the netting after cutting the netting into small pieces. A 0.5-g sample was soaked for 18 h in 100 mL dichloromethane containing 250 mg propyl gallate, and the extract was analyzed by gc-TEA.

Nitrosation potential and data analysis

The complete details for determining nitrosation potential have been published (Fiddler et al., 1997). Data were analyzed by the General Linear Model and Means procedures of the Statistical Analysis System Institute, Inc. (1985). Results were interpreted according to the methods of Snedecor and Cochran (1979) and Youden and Steiner (1975). Significance was defined at p 0.05.

RESULTS & DISCUSSION

IN A NITROSAMINE SURVEY OF BONELESS HAMS PROCESSED IN elastic rubber nettings 30% were higher than 100 ppb NDBzA, with a maximum level of 512.2 ppb (Fiddler et al., 1997). Only 7 of the 59 exterior ham samples contained no detectable nitrosamine. Therefore, potential human exposure to nitrosamines from this cured meat product is still unknown. A report by Fiddler et al. (1996) indicated that nitrite reduction during processing, and netting removal post-processing, would lower the nitrosamine content in the product. However, the best approaches to eliminating this problem would be either removal of precursor amines from the rubber in the nettings or avoiding the use of rubber in the nettings. While all-polyester nettings are employed in ham production, their use is not widespread. Use of zinc dithiocarbamate vulcanization accelerators is essential in obtaining rubber with the proper elastic properties and hence, the use and presence of secondary amines cannot be avoided. Therefore, Wacker et al. (1987) proposed another approach, recommending accelerators containing "safe" amines that did not readily nitrosate, and on nitrosation would yield a non- or weakly carcinogenic nitrosamine. They reported 20 new secondary amine-containing dithiocarbamates and sulfenamides which may prevent formation of carcinogenic N-nitroso compounds. They included α-substituted tetramethyl-piperidine and piperazine, and tertiary-butyl-, amyl- and hexylamines that did not readily nitrosate due to steric hindrance of the amine nitrogen atom. The rate of nitrosation for such compounds is lower than for normal aliphatic or unsubstituted alicyclic secondary amines. Layer and Chaser (1996) described a means of curing rubbers with isoalkyl and cyclic dithiocarbamate accelerators where "the formation of nitrosamines is substantially eliminated." They included thiuram sulfides, sulfenamides and benzothiazoles and metal dithiocarbamates containing partially sterically hindered diisopropylamino and diisobutylamino groups. Prior to that report, we had hypothesized that the use of a zinc dithiocarbamate containing a β-methyl substituted dipropylamine, diisobutylamine, might enable the manufacture of elastic thread that would retain the general properties exhibited by this class of rubber accelerators (Fig. 1).

Initial experiments indicated that zinc diisobutyldithiocarbamate had the potential to produce low nitrosamine-containing rubber with the elasticity and retention of other properties required for use in nettings. As part of this assessment, the experimental unused nettings were checked for their nitrosamine content prior to use; none was found. An earlier survey (Fiddler et al., 1997) indicated the unused nettings accompanying 18 hams whose exterior NDBzA content ranged from 100.4 to 512.2 ppb (mean, 207.8 ppb) contained 30.8 to 943.7 ppb (mean, 226.5 ppb) NDBzA. Since the amount of formed nitrosamine is dependent on the contact of the nettings with the nitrite-treated pork muscle, the potential of such nettings to form nitrosamines on the surface of the hams was assessed. The "nitrosation potential" was evaluated by addition of an excess of nitrite using a described procedure (Fiddler et al., 1997). Here, a mean of 97.0 ppm NDBzA was found in unused nettings from 18 hams where the outer surface contained over 100 ppb NDBzA. With the experimental nettings we used, only 7.5 ppm NDiBA was found with no NDBzA.

Based on these findings, we obtained data on the surface nitrosamine content from commercial producers using these experimental nettings (Fig. 2). Statistical analysis indicated highly significant ($p < 0.01$) differences among companies and between the new netting and control ham samples. The means from different companies processed in the experimental nettings (n=26) ranged from 4.6 to 33.5 ppb NDiBA, with an overall mean of 16.1 ppb. No NDiBA was detected in the control hams (n=15). This compared to a range of 52.3 to 739.9 ppb NDBzA (mean 191.2) in the control hams processed with commercial nettings employed by the producers. This same nitrosamine, NDBzA, was found on the exterior surface of hams produced in experimental nettings from 5 of the 7 producers. In one case (F), this lack of cross- contamination could not be explained. In the case of the other producer (A), they did not follow directions as given. Here, the 4 hams processed in experimental nettings and the small number of hams made in control nettings were processed in a pilot plant smoke-

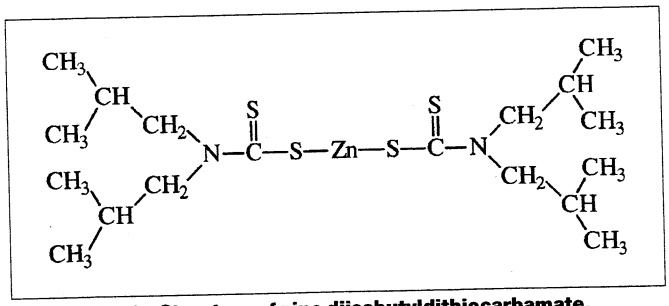

Fig. 1—Structure of zinc diisobutyldithiocarbamate.

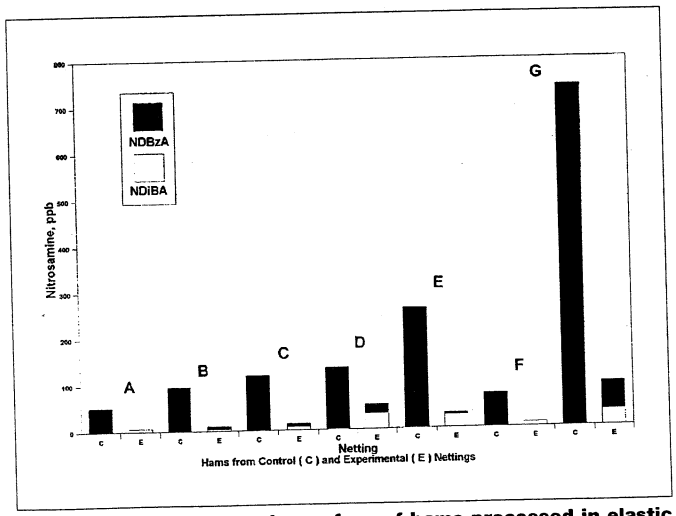

Fig. 2—Nitrosamines on the surface of hams processed in elastic rubber nettings from commercial producers (A-G).

house, not as part of a normal production run. As a result, the hams were subjected to a cook time shorter than usual because of the lower mass of product in the smokehouse. This shorter processing time also provided much less opportunity for cross-contamination noted in other ham processing. Where there was cross-contamination in the hams processed with experimental nettings, the NDBzA varied considerably (6.8, 6.3, 20.3, 3.8 and 60.0 ppb). The amount of NDBzA cross-contamination was unrelated to the amount of NDBzA in the controls. This can be seen in a comparison between companies D and E, where there was almost two times more NDBzA in the company E controls, but only 18% as much NDBzA for the experimental nettings.

Following the early suggestion that liver carcinogenesis in rats by NDMA might be related to methylation of liver nucleic acids (Magee et al., 1962), other dialkylnitrosamines were considered alkylating carcinogens. According to the accepted mechanism, enzymatic oxidative dealkylation of the nitrosamine occurs after metabolic hydroxylation at the α-carbon by cytochrome P450, although hydroxylation can occur in the β, γ and ω positions. With increasing chain length (Bellec et al., 1996), and presumably branched chains, the rate of alkyl formation becomes less efficient. Therefore, the rate of alkylating agent formation for NDiBA would also be reduced when compared to straight chain dialkylnitrosamines or NDBzA.

Mutagenicity (Andrews and Lijinski, 1980; Rao et al., 1979) and carcinogenicity studies (Althoff et al., 1975; Althoff and Kruger, 1975; Lijinski and Taylor, 1979; Lijinski and Reuber, 1981; Lijinsky, 1987), suggest that NDiBA is a weak carcinogen. This is especially true when compared to NDBA that was present in hams prior to reformulation of the rubber with zincdibenzyldithiocarbamate used in nettings. As for NDBzA, the carcinogenicity studies have been limited; NDBzA was reported to be noncarcinogenic in rats (Druckrey et al., 1967). However, other investigations showed this nitrosamine was mutagenic by the Ames *Salmonella* assay (Pool et al., 1986) and genotoxic in mammalian assays (Boyes et al., 1990; Schmerzer et al., 1990; Brendler et al., 1992) suggesting NDBzA may be a weak carcinogen. Schmerzer et al. (1990) postulated a mechanism for the formation of a benzylating species, consistent with the carcinogenicity exhibited by N-nitrosobenzylurea (Ivankovic, 1978) and other nitrosamines containing the benzyl group.

CONCLUSIONS

HAMS WERE PRODUCED IN NETTINGS WITH RUBBER FROM ZINC diisobutyldithiocarbamate that was lower in nitrosamine than nettings currently in use. This was due to the rate of nitrosation of the partially sterically hindered secondary amine precursor and the reduced amount of precursor, as indicated by nitrosation potential results. The absence of the NDBzA contaminant suggests that the overall nitrosamine content, i.e., NDiBA and NDBzA, could be reduced, since the NDBzA component would be eliminated if hams were processed in the experimental nettings. The low amount of NDiBA formed in these boneless hams suggests that use of this reformulated rubber for manufacture of the elastic nettings should be recommended.

REFERENCES

Althoff, J., Eagan, M., and Grandjean, C. 1975. Carcinogenic effect of 2,2'-dimethyldipropylnitrosamine in Syrian hamsters. J. Natl Cancer Inst. 55: 1209.

Althoff, J. and Kruger, F.W. 1975. Sprague Dawley rats. Cancer Lett. 1: 15.

Andrews, A.W. and Lijinsky, W. 1980. The mutagenicity of 45 nitrosamines in *Salmonella typhimurium*. Teratogenesis, Carcinogenesis and Mutagenesis 1: 295.

Bellec, G., Goasduff, T., Dreano, Y., Menez, J.F., and Berthou, F. 1996. Effect of chain length on the cytochrome P450 dependent metabolism of N-nitrosodialkylamines. Carcinogenesis 17: 2029.

Boyes, B.G., Rogers, C.G., Matula, T.I., Stapley, R., and Sen, N.P. 1990. Evaluation of genotoxicity of N-nitrosodi-benzylamine. Mutation Res. 241: 379.

Brendler, S.Y., Tompa, A., Hutter, K.F., Preussmann, R., and Pool-Zobel, B.L. 1992. *In vivo* and *in-vitro* genotoxicity of several N-nitrosamines in extrahepatic tissues of the rat. Carcinogenesis 13: 2435.

Druckrey, H., Preussmann, R., Ivankovic, S., and Schmahl, D. 1967. Organotropic carcinogenic activity of 65 different nitrosamines to BD-rats. Z. Krebsforsch 69: 103.

Fiddler, W., Pensabene, J.W., Gates, R.A., and Foster, J.M. 1996. Investigations on nitrosamine reduction in boneless hams processed in elastic rubber nettings. J. Muscle Foods 7: 389.

Fiddler, W., Pensabene, J.W., Gates, R.A., Custer, C., Yoffe, A., and Phillipo, T. 1997. Survey of boneless hams processed in elastic rubber nettings for N-nitrosodibenzylamine. J. AOAC Int'l. (in press).

FSIS. 1991. Studies show nitrosamines in elastic netted hams. FSIS Food Safety Review 1: 19.

Ireland, C.S., Hytrek, F.P., and Lasoski, B.A. 1980. Aqueous extraction of N-nitrosamines from elastomers. Am. Ind. Hyg. Assoc. J. 41: 895.

Ivankovic, S. 1978. Carcinogenic activity of N-benzyl-N-nitroso-urea to BD-rats. Z. Krebsforsch. 91: 63-86.

Layer, R.W. and Chaser, D.W. 1996. Method of curing rubbers without forming nitrosamines. U.S. Patent 5,554,699.

Lijinsky, W. 1987. Structure-activity relations in carcinogenesis by N-nitroso compounds. Cancer Metastasis Rev. 6: 301.

Lijinsky, W. and Taylor, H.W. 1979. Carcinogenicity of methylated derivatives of N-nitrosodiethylamine and related compounds in Sprague-Dawley rats. J. Natl Cancer Inst. 62: 407.

Lijinsky, W. and Reuber, M.D. 1981. Comparative carcinogenesis by some aliphatic nitrosamines in Fischer rats. Cancer Lett. 14: 297.

Magee, P.N. and Faber, E. 1962. Toxic liver injury and carcinogenesis. Methylation of rat-liver nucleic acids by dimethylnitrosamine *in vivo*. Biochem. J. 83: 114.

Novitch, M. 1983. Action levels for total volatile N-nitrosamines in rubber baby bottle nipples: availability of compliance policy guide. Fed. Reg. 48: 57014.

Pensabene, J.W., Fiddler, W., and Gates, R.A. 1992. Solid-phase extraction method for volatile N-nitrosamines in hams processed with elastic rubber netting. J. AOAC Int'l. 75: 438.

Pensabene, J.W. and Fiddler, W. 1994. Gas chromatographic- Thermal Energy Analyzer method for N-nitrosodibenzylamine in hams processed in elastic rubber netting. J. AOAC Int'l. 77: 981.

Peterson, A. 1993. N-nitrosodibutylamine and other volatile nitrosamines in cured meat packaged in rubber nettings. J. Food Sci. 58: 47.

Pool, B. L., Eisenbrand, G., Preussmann, R., Schlehofer, J. R., Schmezer, P., Weber, H., and Wiessler, M. 1986. Detection of mutagens in bacteria and DNA damage and amplified DNA sequences in mammalian cells as a systematic test strategy for elucidating biological activities of chemical carcinogens. Food Chem. Toxicol. 24: 685-691.

Preussmann, R., Spiegelhalder, B., and Eisenbrand, G. 1982. Reduction of human exposure to environmental N-nitroso compounds. In *N-Nitroso Compounds*, R.A. Scanlon and S. Tannenbaum (Ed.), Vol. 174, p. 217. Am. Chem. Soc. Symp. Ser., Washington, DC.

Rao, T.K., Young, J.A., Lijinsky, W., and Epler, J.L. 1979. Mutagenicity of aliphatic nitrosamines in *Salmonella typhimurium*. Mutation Res. 66: 1.

Schmerzer, P., Pool, B.L., Lefevre, P.A., Callender, R.D., Ratpan, F., Tinwell, H., and Ashby, J. 1990. Assay-specific genotoxicity of N-nitrosodibenzylamine to rat liver in vivo. Environ. Molec. Mutat. 15: 190.

Sen, N.P., Baddoo, P.H., and Seaman, S.W. 1987. Volatile nitrosamines in cured meats packaged in elastic rubber nettings. J. Agric. Food Chem. 35: 346.

Sen, N.P., Seaman, S.W., Baddoo, P.A., and Weber, D. 1988. Further studies on the formation of nitrosamines in cured pork products packaged in elastic rubber nettings. J. Food Sci. 53: 731.

Sen, N.P., Baddoo, P.A., and Seaman, S.W. 1993. Nitrosamines in cured pork products packaged in elastic rubber nettings: an update. Food Chem. 47: 387.

Senedecor, G.W. and Cochran, W.G. 1979. Statistical Analysis, 6th Ed., Iowa State University Press, Ames.

Statistical Analysis System Institute, Inc. 1985. *SAS User's Guide: Statistics*, Cary, NC.

Tricker, A.R., Spiegelhalder, B., and Preussmann, R. 1989. Environmental exposure to preformed nitroso compounds. Cancer Surveys 8: 251.

Yeager, F.W., Van Gulick, N.N., and Lasoki, B.A. 1980. Dialkylnitrosamines in elastomers. Am Ind. Hyg. Assoc. J. 41: 148.

Youden, W.J. and Steiner, E.H. 1975. *Statistical Manual of the Association of Official Analytical Chemists*, AOAC, Arlington, VA.

Wacker, C.D., Spiegelhalder, B., Borzsony, M., Brune, G., and Preussmann, R. 1987. Prevention of exposure to N-nitros-amines in the rubber industry: new vulcanization accelerators based on 'safe' amines. In *The Relevance of N-Nitroso Compounds to Human Cancer: Exposure and Mechanisms*, H. Barsch, I. O'Neil and R. Schulte-Hermann (Ed.), p. 370-374. IARC Sci. Publ. Vol. 84, Oxford University Press, Lyon, France.

Ms received 6/9/97; revised 10/16/97; accepted 10/27/97.

We thank Judith M. Foster for her technical assistance, and Marcus Mintz, Jif-Pak Manufacturing Inc., for cooperation in this study. This work was done under a cooperative agreement between USDA's Agricultural Research Service and Globe Rubber Manufacturing Company (CRADA No. 58-3K95-M-476). Mention of brand or firm names does not constitute an endorsement by the U.S. Dept. Of Agriculture over others of a similar nature not mentioned.