Volatile Nitrosamine Contamination of Laboratory Animal Diets

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ABSTRACT

Low levels of the carcinogen N-nitrosodimethylamine (1 to 4 ppb) were found in 11 of 12 samples of commercial pelleted diets for laboratory animals. Higher levels (5 to 50 ppb) of N-nitrosodimethylamine were found in three of seven samples of the NIH open-formula rat and mouse ration. The fish meal used in this diet contained N-nitrosodimethylamine in quantities sufficient to account for most of the contamination. In addition, several samples of the NIH diet contained low levels of N-nitrosopyrrolidine (0.3 to 2.1 ppb). The implications of these findings with respect to carcinogenicity testing are briefly discussed.

Recently, Kann et al. (7) reported that 37 of 46 laboratory animal diets from Germany contained NDMA at levels between 1 and 79 ppb (μg/kg). Twenty-seven samples also contained NPYR. They speculated that the major source of the contamination was probably fish meal, which was used as a protein source in the diets. Earlier reports by Ender et al. (4), Sen et al. (12), and others have described NDMA contamination of fish meal used in animal feeds. We now report the presence of N-nitroso contaminants in a small sampling of laboratory animal diets from the United States. The highest level was found in a sample of the NIH open formula rat and mouse ration (13). This diet, which contains 10% fish meal, has been recommended for use in carcinogenicity studies (13). By contrast, 8 samples of commercial pet food contained little or no volatile nitrosamines.

The diets analyzed for this study were obtained directly from the producer or a wholesaler and were stored in polyethylene bags until analyzed. Pelleted diets were powdered for 1 to 2 min in a Waring blender before analysis. All samples were analyzed in duplicate or quadruplicate.

The analysis of volatile nitrosamines was performed by methods similar to those described previously (5, 9). Briefly, these procedures involve distilling the volatile nitrosamines from the diet sample under reduced pressure, extracting the distillate with dichloromethane, and analyzing the concentrated extract with a gas chromatograph interfaced to a TEA nitrosamine detector (Thermo Electron Corp., Waltham, Mass.).

The sample was identified and quantitated by comparison with certified nitrosamine standards. Certain samples (as noted) were also analyzed by high-pressure liquid chromatography-TEA (9). Further details of the analytical techniques are available upon inquiry to the authors.

A number of precautions were taken to ensure the accuracy of the analytical procedures and to avoid artifactual formation of N-nitroso compounds. NDPA (5 ng/g), an NDMA analog which is not normally present in the diets, was added to each powdered sample prior to the vacuum distillation step as an internal (recovery) standard. All reported levels of NDMA and NPYR have been adjusted for the recovery of the NDPA (average, 80%; range, 68 to 108%). In addition, sodium ascorbate and α-tocopherol, 10 mg/g each, known inhibitors of nitrosation (2, 10), were added to each sample just prior to the NDPA. Other procedures which were used to avoid artifacts and to check on the accuracy of the results are described in a recent review of this subject (8).

We first analyzed 9 samples of commercially available pelleted laboratory rodent diets and one canine diet from 3 major suppliers in the United States. These feeds were labeled as containing fish meal (amount unspecified), and all contained between 0.9 and 3.7 ppb of NDMA. One of 2 tested samples of pelleted rabbit food contained NDMA (1.3 ppb) and NPYR (3.2 ppb). The other sample contained no detectable volatile nitrosamines (<0.1 ppb). Neither of the latter 2 samples included fish meal as a labeled ingredient.

We also analyzed several samples of dry pet food. Two samples of pelleted dog food and 6 samples of cat food, all marked as containing fish or "fish flavor," were purchased from local markets and analyzed for volatile nitrosamines. One sample (dog food) contained 0.1 ppb NDMA. Five other samples contained trace amounts of NDMA (<0.1 ppb), and 2 contained no detectable NDMA.

Table 1 shows the results of analyses on several samples of the NIH open formula rat and mouse ration obtained from 2 suppliers during the summer and fall of 1978. All samples contained NDMA at levels ranging up to 52 ppb. Five of 7 samples also contained small amounts of NPYR. The possibility that these findings were the result of nitrosation during the analytical procedure was ruled out by confirming the Sample A data with experiments in which the nitrosation inhibitors (ascorbate and α-tocopherol) were omitted and/or excess dimethylamine was added. If such artifacts were a problem, one would expect higher values for NDMA in the absence of inhibitors and in the presence of excess dimethylamine. In all cases, the results were within the normal range of experimental variability (Table 1).

A major source of the contamination in these diets appears to be the fish meal, which constitutes 10% of the finished mixture. After our initial experiments revealed the presence of NDMA in Sample A, we obtained samples of fish meal from both producers and corn meal (24.5% of the diet) from Producer 1. The results of these analyses are also shown in Table 1.
The result on Sample A is the average of 4 replicate diet samples prepared as follows: (a) diet alone (NDMA = 57 ppb); (b) diet and 1 mg dimethyamine per g (45 ppb); (c) diet plus sodium ascorbate and α-tocopherol, 10 mg/g each (55 ppb). (d) diet plus dimethyamine, ascorbate, and α-tocopherol, as in a and b (52 ppb).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Form</th>
<th>NDMA (ppb)</th>
<th>NPYR (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIH-07 Sample A</td>
<td>Powder</td>
<td>52 (57–45)*</td>
<td>0.4</td>
</tr>
<tr>
<td>NIH-07 Sample B</td>
<td>Powder</td>
<td>8.0 (7.8–8.1)*</td>
<td>0.6</td>
</tr>
<tr>
<td>NIH-07 Sample C</td>
<td>Powder</td>
<td>5.1 (4.6–5.6)</td>
<td>0.5</td>
</tr>
<tr>
<td>NIH-07 Sample D</td>
<td>Pellets</td>
<td>1.8 (1.9–1.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>NIH-31 Sample E</td>
<td>Pellets</td>
<td>1.9 (2.0–1.8)</td>
<td>ND*</td>
</tr>
<tr>
<td>NIH-31 Sample F</td>
<td>Pellets</td>
<td>1.7 (1.8–1.5)</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Producer 2**

**NIH-07 Sample G** Powder 1.0 (1.0–1.0) 2.1

**Fish meal**

**Sample 1** 143 (156–128)* | 5.7
| **Sample 2** 324 (339–308)* | 3.9
| **Sample 3** 637 (647–631) | 8.8
| **Sample 4** 134 (136–131) | ND
| **Sample 5** 169 (183–150) | ND

**Producer 2**

**Sample 6** 2.2 (2.1–2.3) 0.7

**Corn meal**

**Producer 1**

**Sample 1** 4.2 (4.1–4.3) ND

We should note, for future reference, that some fish meal samples contained a small amount of an unidentified TEA-responsive material which cochromatographed with NDPA under our gas chromatography conditions. This material was resolved from NDPA by high-pressure liquid chromatography-TEA. The significance of these findings is difficult to assess, especially since only one diet sample in our rather limited survey contained over 10 ppb of NDMA. It is not likely that even the highest level of nitrosamines reported here (52 ppb) would cause a significantly increased incidence of cancer in a typical study involving a few hundred animals. However, it is possible that these dietary contaminants could act synergistically with other (test) carcinogens or cocarcinogens to increase tumor incidence or to alter the target organ. Such synergism has been reported for higher levels of NDMA (3, 6, 11) but we are unaware of any attempt to demonstrate it at the low levels found in these diets. It is interesting, however, to note that an even lower level of NDMA than that reported here (10 ppb in drinking water) was able to cause a significant increase (from 4.3 to 32%) in the incidence of lung tumors in male A/J mice dosed from 4 weeks preconception to 22 weeks of age (1). Thus, it is possible that such low NDMA levels may not be innocuous and that efforts should be made to minimize the nitrosamine content of animal diets.

REFERENCES


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