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Laboratory Animal Feed

The News and Comment briefing (13 Oct. 1978, p. 192), reporting the detection of more than 50 parts per billion (ppb) nitrosamines in a sample of National Institutes of Health (NIH) laboratory animal feed, generated considerable concern within the scientific community. We now provide information regarding the current status of this issue and report the preliminary results of more recent investigations regarding nitrosamine concentrations in laboratory animal feeds. Even though the issues regarding biological effects of low dietary nitrosamine concentrations have not been resolved, every effort should be made to identify and minimize contaminant levels in laboratory animal diets in order to facilitate the valid interpretation of results from various kinds of experiments.

The original report (*1*) indicated nitrosamines were present in concentrations of less than 0.1 ppb in nine commercial dog and cat feeds, between 1 and 3 ppb in eight of nine feeds used for small laboratory animals, and 52 ppb in the NIH feed. The authors suggested that the fish meal used in the formulation of the NIH diet was the primary source of nitrosamines, even though most of the other diets assayed also contained fish meal. Through conversations with an author of the report under consideration (*2*) and an inspection of records at the facility that manufactured the diet reported to contain 52 ppb nitrosamines, information has been obtained which appears critical to the evaluation of the original data and to its significance.

The NIH diet (*3*) from which the reported data were obtained was provided by the manufacturer in meal form. It was prepared by mixing appropriate amounts of ingredients in meal form and was packaged directly from the mixer into approximately 140 50-pound bags. The diet was not manufactured for delivery to NIH. All of the cat and dog foods assayed for the original report were either canned or extruded, and the nine laboratory animal feeds were pelleted and had to be ground before they were assayed (*2*). This suggests that the originally reported data (*1*) tested the effects of manufacturing procedures on nitrosamine stability rather than nitrosamine concentrations in specific diets. The single sample (NIH diet) that contained the highest nitrosamine concentration was also the only complete diet not subjected to a manufacturing process involving heat treatment. The potential effect of the heat associated with feed manufacturing processes on the concentration of volatile nitrosamines gives reason to reevaluate the significance of the reported assay results. A second point to reconsider is the amount of diet that was represented in the sample assayed. Apparently only a single bag of each diet was obtained for sampling. In the case of the NIH diet, the analytical results were reported as being representative of the "NIH diet." Data extrapolation of this magnitude does not seem reasonable because not enough information is available regarding the variability of contaminant concentrations in feeds.

In order to obtain more representative data regarding the distribution of volatile nitrosamines in laboratory animal diets, arrangements were made to assay samples collected from various production batches of open formula diets manufactured under NIH contracts. Results of analyses performed on the first three batches sampled indicate concentrations of 1.7, 1.8, and 1.9 ppb nitrosamines (*4*) in pelleted NIH diets formulated with 9 or 10 percent fish meal. Preliminary data regarding nitrosamine concentrations in NIH open formula diet have been provided by the National Institute of Environmental Health Sciences (*5*). Pelleted diets had concentrations of 1.3, 1.1, and 6.4 ppb nitrosamines (*6*), while the fish meal used in the diet formulation contained up to 172 ppb volatile nitrosamines. To confirm these preliminary data, we are collecting ingredient and diet samples for nitrosamine assay at five stages during the manufacture of an NIH diet. It is anticipated that at least four series of samples will be analyzed to provide the data required to make a rational judgment regarding the significance of volatile nitrosamine concentrations in laboratory animal diets.

Since preliminary information suggests that heat treatment of the complete diet substantially decreases volatile nitrosamine concentrations, it is tentatively recommended that all diets for laboratory animals be subjected to heat during their manufacture or to a steam sterilization process.

A high percentage of laboratory animal diets are marketed in extruded biscuits or pellets. To date, the potential problem with volatile nitrosamine concentrations appears to be only in diets purchased in meal form. A possible resolution is to require that diets purchased in meal form be manufactured by regrinding pellets.

An option being considered in the event that research and testing program objectives require decreased dietary volatile nitrosamine concentrations is to develop new diets using ingredients with a low probability of being contaminated with nitrosamines. The NIH open formula diets have the flexibility of reformulation to accommodate specific requirements of the biochemical research community. A series of experiments is now being designed to evaluate diets formulated to contain mineral nitrosamine concentrations in the event this type of diet is required.

The *Science* article also stated that the NIH diet was "Developed as the ideal diet for small rodents in carcinogen bioassays. . . ." The diet discussed (*3*) was in fact developed to maintain rodent production colonies and subsequently was recommended as a standard diet for small rodents in carcinogen bioassays.

The issue regarding a standard diet for carcinogen bioassays has received a considerable amount of attention and no doubt will remain unresolved for some time since the nutrient requirements of rodents beyond their reproductive life span have not been established (*7*). Laboratory animal nutrition is a dynamic discipline, and any specific diet for these species should not be presumed to be ideal. New information regarding dietary requirements of the various species and potential dietary contaminants are being reported with considerable frequency in the literature. Therefore, we must constantly seek to improve diets, a variable that can have a profound influence on experimental results.

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References and Notes

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