HAZARD REVIEW

OF

N-NITROSODIMETHYLAMINE (DMN)

PB87210787

July 1973

Prepared by

Office of Research and Standards Development

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
U.S. Public Health Service
National Institute for Occupational Safety and Health
Rockville, Maryland

REPRODUCED BY:
U.S. Department of Commerce
National Technical Information Service
Springfield, Virginia 22161
NTIS

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	PB87-210787

4. Title and Subtitle

Hazard Review of N-Nitrosodimethylamine (DMN)

5. Report Date
July 1973

6.

7. Author(s)

8. Performing Organization Rept. No.

9. Performing Organization Name and Address
NIOSH
4676 Columbia Parkway
Cincinnati, Ohio 45226

10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.
(C)
(G)

12. Sponsoring Organization Name and Address
NIOSH
4676 Columbia Parkway
Cincinnati, Ohio 45226

13. Type of Report & Period Covered

14.

15. Supplementary Notes

16. Abstract (Limit: 200 words)

The toxic hazards of exposure to N-nitrosodimethylamine (DMN) are discussed. The first extensive investigation of DMN toxicity is described in response to two reported cases of cirrhosis of the liver in exposed workers. The results from these studies are reviewed, including acute lethal doses in rats, rabbits, mice, guinea-pigs, and dogs, and chronic effects in rats. Findings are also summarized from numerous other acute and chronic toxicity tests, such as induction of liver damage and hepatic tumors in rats, rabbits, mice, and guinea-pigs. Dose relationships are discussed along with effects other than liver tumors, including kidney tumors, lung tumors, and ethmoid carcinoma. The carcinogenicity of N-nitrosodiethylamine in monkeys is described in lieu of available data on DMN carcinogenicity in nonhuman primates. Tumor induction times, total incidence, survival times, and histologic type of tumor are described. The author concludes that DMN has been shown to be carcinogenic in a variety of animal species by several routes of administration. DMN should be considered as a potential carcinogen for humans.

17. Document Analysis a. Descriptors

NIOSH-Publication NIOSH-Author Health-hazards Industrial-chemicals Employee-exposure Animal-studies Humans Case-studies Hepatotoxicity Dose-response N-Nitrosodimethylamine Carcinogens

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

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U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA 22161

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
AVAILABLE TO THE PUBLIC	UNCLASSIFIED	12
	20. Security Class (This Page) UNCLASSIFIED	22. Price

Hazard Review

of

N-Nitrosodimethylamine (DMN)

Barnes and Magee [1] undertook the first extensive study of the toxicity of N-nitrosodimethylamine, also called dimethylnitrosamine (DMN), in response to the request of the medical officer of a firm that had been using it in the laboratory as a solvent. Two of three men working in the laboratory had developed cirrhosis of the liver. In acute toxicity experiments with rats, rabbits, mice, guinea pigs, and dogs the lethal dose was found to be in the range of 10-50 mg/kg. In chronic exposure experiment with rats, 200 ppm DMN in the diet proved lethal in approximately one month, 100 ppm required two to three months and 50 ppm was tolerated for at least 110 days, at which time the study was terminated. The principal pathologic changes in the acute and in the higher dose chronic exposure tests were hemorrhage and necrosis of the liver. In those rats surviving the longest, fibrosis and bile duct proliferation were also observed. Little change was observed in other organs.

In 1956 Magee and Barnes [2] reported the results of a long-term feeding experiment with DMN in rats and a shorter term experiment in rabbits. In the rat experiment 10 males and 10 females were placed on a diet containing 50 ppm DMN. The first death occurred during the 27th week and the experiment was continued until the 42nd week, when the single remaining survivor was killed. All rats had nodular livers and 19/20 had developed primary hepatic tumors with metastases in seven. The tumors showed varying proportions of parenchymal and bile

duct proliferation with one fibrosarcoma observed. The sole surviving rat revealed only slight diffuse and nodular hyperplasia in the liver parenchyma.

In the rabbit experiment six males were initially fed a diet containing 20 ppm DMN. After 10 weeks the DMN level was increased to 30 ppm, and 4 weeks later the level was increased to 50 ppm for a further 8 weeks by the end of which time all rabbits had died. None of the rabbits demonstrated any evidence of neoplasia. Magee and Barnes concluded in their report that "Dimethylnitrosamine....may be of some value in the investigation of hepatic carcinogenesis." In the succeeding fifteen years the carcinogenicity of DMN for the rat has been confirmed in well over forty separate reports.

In the earliest report, Magee and Barnes [3] attempted to determine the critical dosage at which DMN is carcinogenic for the rat. Although the number of animals was limited to from 4 to 16 per treatment, an interesting pattern was evident nevertheless. The lowest dose, 5 ppm, produced no tumors in the 6 rats to which it was fed for 104 weeks. A combination of exposures to 10 and 20 ppm DMN for 102 weeks produced cancer of the liver in 7 of the 11 exposed rats. Both benign and malignant tumors of the kidney were induced by the higher dietary levels of 50 ppm for 12 weeks, 200 ppm for 1 week, or following a single dose of 30 mg/kg. With the appearance of kidney tumors, there was a corresponding decrease in the number of liver tumors.

In a more definitive study of dose-effect relationship, Terracini et al [4] exposed 201 rats for two years to DMN at dietary levels of 0, 2, 5, 10, 20, or 50 ppm. The incidence of liver tumors correlated well with the level of DMN in the diet. At 50 ppm DMN 10/12 animals developed liver cancer. As the dose decreased the incidence of liver tumors decreased steadily; at 2 ppm only one rat of 37 had a cancer in the liver. There were no kidney tumors in any of the rats.

The conditions favoring the production of kidney tumors by DMN have been the subject of several investigations. Most authors agree that feeding relatively high levels (100-500 ppm) for short periods of time (1-4 weeks), [5-7] or administering a large single dose (18-60 mg/kg), [8-10] will produce kidney tumors in the majority of rats. Unilateral nephrectomy performed two weeks prior to the administration of DMN increased the incidence of kidney tumors above that observed in non-nephrectomized rats exposed to DMN. [5] Partial hepatectomy 24-31 hours prior to an intraperitoneal dose of DMN, 6-9 mg/kg, increased the number of liver and kidney tumors over that in nonhepatectomized rats. [11]

Liver [12] and kidney [13-15] tumors produced by DMN have been subjected to light and electron microscopy. The kidney tumors have presented a particularly interesting pattern; Riopelle and Jasmin [16] suggested that the puzzling, mixed tumors may result from the "collision" of carcinomatous and sarcomatous elements which have arisen independently.

Various factors which affect the carcinogenic potency of DMN have been investigated and the diet was determined to be of great importance. Swann and McLean [10] found that a single dose of 60 mg/kg, which produced kidney tumors in only 35 percent of rats fed a commercial diet, induced tumors of the kidney in all rats when the DMN dosage was preceded by 8 days on a protein-free diet. Their data indicated that the protein-free diet results in less metabolism of DMN in the liver with the result that more reaches the kidney where it is metabolized into carcinogenic alkylating derivatives. The effects of age and sex on tumor induction were studied by Yang, [17] who concluded that the production of renal tumors by DMN was higher in male than in female rats and higher in young rats than in old ones.

Primary tumors of the lung have appeared in rats fed 125 ppm DMN in their diet, [18] or which were administered DMN 5 times per week by stomach tube in daily doses of either 0.4 mg for 35-48 weeks [19] or 0.2-0.5 mg for 20-24 weeks. [20] The tumors were usually adenocarcinomas but a few were squamous-cell carcinomas. The proportion of rats with lung tumors varied from experiment to experiment but averaged approximately one-third if animals dying in the early weeks were excluded. Liver tumors were generally observed less frequently than were lung tumors, while kidney tumors tended to be more numerous. Animals with tumors in multiple organs, in both the kidney and lung, for example, were more common than those with a single primary site.

There are three reports of tumors in the nasal area following inhalation of DMN vapors by the rat. Druckrey et al [21] exposed 6 rats for half an hour once a week to approximately 100 ppm DMN vapor. All animals died in 230-470 days with extensive cancer of the ethmoturbinal region. In a second experiment 12 rats were similarly exposed to half this concentration. All of the 9 surviving rats developed invasive cancer of the ethmoid cells in 260-450 days. In an earlier report Druckrey et al [22] noted ethmoidcarcinoma in one of the three rats surviving 300 days after a single inhalation dose of DMN stated by the authors to be 37 mg/kg. Thomas [23] exposed an unspecified number of rats to 100 ppm of DMN vapor for 30 minutes per week and 85 percent of the animals developed tumors of the nares with a median induction time of 336 days. The tumors induced by DMN were combined in the report with those induced by three other nitrosamines but were, apparently, principally neurogenic tumors and squamous carcinomas.

Other than the rat, the mouse has been the animal most extensively used in the study of the carcinogenic potential of DMN. In an early study the effect of a single subcutaneous dose of 1.5-75 μg in newborn mice was compared to that of 0.001 percent in the drinking water of adult mice. [24] Lung adenomas, hemangiomas, and hemangiosarcomas appeared in both groups, but only those treated when newborn developed a significant number of benign and malignant liver cell tumors. Frei [25] compared the tumorigenic effect of a single intra-

peritoneal dose of DMN in adult and newborn mice. There was an increased incidence of pulmonary adenomas in both groups but again only those treated when newborn developed hepatomas (14/30), all of which were locally invasive. In a study concerned primarily with the methylation of DNA by DMN, Den Engelse et al [26] used two strains of mice which differed markedly in their tumor response to DMN fed in the drinking water at a level of 10 ppm for 1 month, followed by 1 ppm for 2 months. The GR strain males were strikingly more sensitive than C3Hf strain males with regard to the formation of lung tumors following DMN treatment but just the opposite occurred with respect to liver tumor formation.

The characteristic response of the hamster to DMN is the development of malignant liver tumors. With doses of 0.5 to 1 mg weekly for 1-1/2 to 5 months, such tumors are principally hemangioendothelial sarcomas and cholangiocarcinomas. [27] Olfactory neuroepithelial tumors were also produced by this dosage schedule. Administration of 0.0025 percent of DMN in drinking water for eleven weeks (interrupted) caused mainly liver-cell carcinomas and cholangiocarcinomas. [28] One to 3 doses (1.6-3.6 mg total) by stomach tube resulted in liver cell carcinomas and a few cholangiomatous lesions. [29]

LePage and Christie [30] fed male guinea pigs diets containing 0, 12.5, 25 and 50 ppm DMN. Tumors of the liver cell type developed in 5/9 animals receiving 25 and 50 ppm and surviving for about a year.

LePage and Christie [31] observed liver-cell tumors in 3 of 10 rabbits receiving 25 ppm DMN in their diet for 17-60 weeks and in 1 of 5 receiving 50 ppm for 17-23 weeks. In three of the cases, there were metastases in the lungs. Presumably, the failure of Magee and Barnes [2] to obtain tumors in their rabbit experiment was due to the limited number of animals used; only 5 were still alive by the twelfth week. It may be noted, however, that Magee and Barnes used only male rabbits while 3 of the 4 animals with liver tumors in LePage and Christie's experiment were females.

In an experiment with adult mastomys injected subcutaneously with DMN at a dose of 0.1 mg twice a week for 10 to 44 weeks, Fujii and Sato [32] found an increase in liver tumors and a decrease in the spontaneously occurring tumors of the glandular stomach.

DMN was included by Halver [33] among the chemicals tested in the diet of rainbow trout because of demonstrated high carcinogenic activity in mammals. At a percentage of 0.48 of the total ration, DMN induced hepatomas in 33 of 81 trout examined at 15 months and in all 65 trout fed a ration containing 1.92 percent of DMN. In a second experiment at lower dosages, 0.03 percent DMN in the ration resulted in hepatomas in 42/100 trout and 0.12 percent resulted in hepatomas in 59/101 trout after 1 year when both groups were examined. A detailed description of the histopathology of the liver tumors produced in these experiments is given by Ashley and Halver. [34] The lesion is essentially a hepatic cell carcinoma. Metastases were rare, but

invasion of adjoining visceral fascia and pancreatic adipose tissue sometimes occurred.

Codegone et al [35] fed rainbow trout 0.08 percent DMN in a diet of fresh beef liver for 18-22 months and failed to produce hepatomas. The liver diet, therefore, may have had a protective action.

Khudolei [36] added DMN to the aquarium water of guppies (Lebistes reticulatus) at a concentration of 100 ppm. After 8 weeks, tumors were present in 6/50 fish. The tumors were characterized as adenomas, cholangiomas, and hepatocellular cancers.

Ingram [37] studied the effect of DMN in newts (Triturus helveticus). Six or 7 injections of 16 mg/g given over a period 3-4 weeks resulted in liver tumors in three of the four newts that survived for more than 2 months. One was an anaplastic liver tumor while the other two were moderately-to-well-differentiated hepatic cell tumors.

The literature reveals no tests of the carcinogenic potential of DMN in nonhuman primates. The next member of the homologous series, N-nitrosodiethylamine, has been so studied, however, and the results are worth mentioning. [38] Fourteen compounds which readily induced tumors in rodents were administered to monkeys. The only compound stated to produce cancer was N-nitrosodiethylamine, which caused hepatocellular carcinoma in 42 of 64 monkeys which had received a total dose of 1.35-57.8 g by either oral or intraperitoneal route. Induction time ranged between 13 to 40 months.

DMN has produced cancer in a variety of sites and by several methods of administration in the rat, mouse, hamster, guinea pig, rabbit, mastomys, trout, guppy, and newt. In view of this broad spectrum of carcinogenic activity in experimental animals, DMN must be regarded as potentially carcinogenic for man.

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