Carcinogenicity
of Nitrosothiomorpholine and 1-Nitrosopiperazine in Rats*

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Summary. Two cyclic nitrosamines, nitrosothiomorpholine and 1-nitrosopiperazine, have been tested by long term feeding to rats in drinking water, at 50 mg/l and 200 mg/l. Nitrosothiomorpholine produced tumors, both benign and malignant, of the esophagus and tongue. Nitrosopiperazine appeared to be definitely tumorigenic, although it showed no selectivity of site for tumor induction and induced tumors in a wide range of organs and tissues. Nitrosopiperazine was very much less acutely toxic than dinitrosopiperazine.

The two cyclic N-nitrosamines, nitrosothiomorpholine (I) and 1-nitrosopiperazine (II), were considered for biological testing because of their close structural similarity to nitrosomorpholine (III), which is a potent hepatotoxin and liver carcinogen in rats (Banasch and Müller; Druckrey et al).

Materials and Methods

Nitrosothiomorpholine. The amine to be nitrated was prepared by the slow addition of 50 g of 3-thiomorpholinone (m.p. 89—92°, Aldrich Chemical Co.) as the dry powder to a magnetically stirring slurry of 20 g of powdered lithium aluminium hydride in a convenient

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volume of anhydrous diethyl ether (Sommers and Horrom). The reaction mixture was refluxed for 4 hours, whereupon stirring and heating were interrupted until the next day. After refluxing for an additional 8 hours, the mixture was again allowed to stand overnight. The excess metal hydride was decomposed by the cautious addition, with vigorous stirring, of several hundred ml of ether saturated with water, followed by several drops of water. The resulting slurry was filtered, and the filter cake was washed thoroughly with ether. The filtrate was concentrated under reduced pressure, then distilled in vacuo. The yield was 27 g of thiomorpholine, b. p. 51–53° at 0.2 mm.

To prepare the nitroso compound, 62 g of thiomorpholine was dissolved in an excess of aqueous sodium nitrite solution. The reaction mixture was cooled to −5° in ice/salt, and 100 ml of concentrated hydrochloric acid was added slowly with stirring. After warming to room temperature, the mixture was saturated with sodium carbonate and extracted several times with chloroform. The combined extracts were dried over calcium chloride, filtered and concentrated using a rotary evaporator. The remaining yellow oil was twice distilled in vacuo, giving 55 g, b. p. 87–90° at 0.2 mm. The distillate eventually crystallized to a pale yellow solid, m. p. 50–51°.

Analysis: C 36.36, H 6.16, N 20.92, S 24.02; calc. for C₆H₆N₂O₂S − 36.34% C, 6.10% H, 21.19% N, 24.27% S.

The mass spectrum and nuclear magnetic resonance spectrum of the product were consistent with the structure N-nitrosothiomorpholine.

1-Nitrosopiperazine was prepared according to Berg. Anhydrous piperazine (52 g) was dissolved in 200 ml of 4 N HCl and cooled to −5° in ice/salt. A solution of 44 g NaNO₂ in 84 ml water was added drop by drop with stirring; the stirring was continued for 15 minutes after addition of the nitrite. The solution was adjusted to pH 6 with 50% NaOH and the dinitrosopiperazine removed by filtration. The filtrate was made strongly alkaline and extracted with 3 × 75 ml of chloroform. The chloroform was removed from the combined extracts using a rotary evaporator and the residual oil distilled in vacuo; the major portion distilling at 80–85° (0.1 mm) was collected as 1-nitrosopiperazine. The mass spectrum was consistent with this structure and the NMR spectrum indicated high purity of the product, with virtual absence of dinitrosopiperazine, as well as piperazine and other impurities.

Animal Treatment. The animals were MRC rats, bred in our colony, both male and female, and were 9 to 10 weeks old at the beginning of the treatment.

The acute toxicity of both nitrosamines was determined by administration by gavage to groups of 4 male rats. Nitrosopiperazine was dissolved in water and nitrosothiomorpholine was dissolved in olive oil; the doses given ranged from 50 to 3200 mg/kg body weight. The animals were observed for 48 hours following the treatment and the number of animals dying in each group was recorded.

For chronic administration the animals were divided into groups of 5 per cage and fed Rockland diet in pellets ad libitum. The nitrosamines were administered at two concentrations in drinking water, 50 mg/l and 200 mg/l. The treatment consisted of 100 ml of solution given to each cage (5 animals) each night, 5 nights per week; during daylight and at weekends the animals were given tap water. This method of administration has been found very satisfactory for nitrosamines (Goodall, Lijinsky, and Tomatis). The nitrosamine treatments were continued for a measured time and the animals observed until death. Each animal was subjected to complete autopsy and all lesions observed were examined histologically.

Results

The acute toxicities were calculated according to Weil and the LD₅₀'s were 800 mg/kg for nitrosothiomorpholine and 2260 mg/kg for 1-nitrosopiperazine. Necropsy showed with nitrosopiperazine brain hemorrhage, lung congestion with petechiae on the surface and alveolar edema, cloudy degeneration in liver cells, and cloudy degeneration of tubular cells in the kidney, with vacuolar degeneration of the epithelia of collector tubules. With nitrosothiomorpholine, the pathology observed was brain hemorrhage and intraalveolar hemorrhage in the lungs.