

### Acute Toxicity of Mononitrobenzene in Mice.

MICHAEL B. SHIMKIN. (Introduced by H. L. Stewart.)

*From the National Cancer Institute, United States Public Health Service, Bethesda, Maryland.*

In a comprehensive review of nitrobenzene intoxication, cover. 265 papers, Schneider<sup>1</sup> states that "the minimal toxic dose in animal experiments is nowhere noted." The investigations on dogs, cats, rabbits, guinea pigs, rats<sup>1</sup> and frogs<sup>2</sup> are concerned primarily with the manifestations and effects of mononitrobenzene poisoning, and with the study of methemoglobinemia or, as in guinea pigs<sup>3</sup> and rabbits<sup>4</sup> some other non-oxygen-carrying derivative of hemoglobin formed with the agent.

The minimal lethal dose of mononitrobenzene (oil of mirbane) was determined in mice in the course of experiments on its effect upon tumor growth. The report was stimulated because mice have not been generally used in the studies of this important industrial poison, and because the toxic manifestations in mice were practically identical with those described in man.<sup>1, 2, 5</sup>

*Experimental:* Eighteen female mice of the C<sub>3</sub>H strain, weighing 35-40 g, were shaved over the abdomen and were painted lightly with a brush soaked in mononitrobenzene over an area less than one-tenth of the body surface. In one hour, 15 mice were in partial collapse, but all recovered within 24 hours. The animals were painted again; next day, 3 were dead. The rest were painted a third time; within 48 hours, 9 died. Thus, after 3 applications, 24 hours apart, the mortality was 12 out of 18 animals.

Ten male mice of strain A, about 30 g in weight, were painted vigorously over the unshaved abdomen for about 20 seconds. In 30 minutes, all were in partial collapse, and in one hour, 6 were motionless, cold and breathing forcibly; 2 were dead within 3 hours, and 4 within 20 hours. Two more mice, which apparently had recovered

<sup>1</sup> Schneider, W., *Veröffentl. a. d. Gebiete. Medizinalverwaltung*, 1933, **39**, 585.

<sup>2</sup> Mancini, M. A., and Guidi, G., *Arch. Internat. Pharmacodynamie et Therapie*, 1922, **26**, 247.

<sup>3</sup> Levin, S. J., *J. Am. Med. Assn.*, 1927, **89**, 2178.

<sup>4</sup> Stimson, B. B., *J. Biol. Chem.*, 1927, **75**, 741.

<sup>5</sup> (a) Adams, S. S., *Tr. Assn. Am. Physicians*, 1912, **27**, 503; (b) Stifel, R. E., *J. Am. Med. Assn.*, 1919, **72**, 395; (c) Hamilton, A., *Industrial Poisons in the United States*, Macmillan, N. Y., 1925, p. 497.

TABLE I.  
Toxicity of Mononitrobenzene Subcutaneously.  
(Sesame oil dilutions 1:5 and 1:10.)

Mononitrobenzene in sesame oil cc	No. mice used	Died	
		No.	Time in hr
.1	3	3	3-12
.05	10	10	6-18
.025	10	10	24-48
.010	15	2	96-120
.005	10	0	—
.001	9	0	—

from collapse, died within 3 days; the total mortality, therefore, was 8 out of 10 animals.

To determine the amount of mononitrobenzene absorbed, graded doses were dissolved in sesame oil in 1:5 and 1:10 dilutions, and were injected subcutaneously into mice. As summarized in Table I, the minimal lethal dose was about 0.01 cc, or 0.0004 cc per gram of body weight.

*Symptoms and Signs:* About 30 minutes after receiving the compound percutaneously or subcutaneously, the mice were prostrated and lay motionless or with occasional twitching movements. They were cold and the respirations were slow and labored. Some of the animals recovered from this collapse in one to 3 hours and remained well; others remained completely motionless and cold, with extremely slow respiration, for over 24 hours before they died. Death was due to respiratory failure; the heart continued to beat many minutes after the cessation of breathing.

One to 3 hours after exposure to mononitrobenzene the skin developed a dark grey-blue hue, and the blood became chocolate-colored and more viscous than normally. Spectroanalysis of the blood revealed a strong absorption band at 6350 Å (methemoglobin).<sup>6</sup> The urine was orange in color and had a strong odor of mononitrobenzene.

Three hours after the application of the compound the white blood cell count dropped from the normal 11,000 to 14,000 per cmm to 5,000 per cmm, with a normal differential count. The red blood cell count was not affected, remaining over 9 million per cmm. Twenty-one hours later, when the animals were moribund, the white blood cell count was 1000 to 1500 per cmm or lower, with a normal differential count (PMN, 30%, with 6% nonfilaments and 3% metamyelocytes;

<sup>6</sup> (a) Heubner, W., *Zentralbl. f. Gewerbehygiene*, 1914, 2, 409; (b) Hamilton, A., *Industrial Poisons in the United States*, p. 493.

lymphocytes, 60% ; monocytes, 4% ).<sup>7</sup> The red blood cell count was 7 to 8 million, and the smears showed hypochromia and hemolysis. The methemoglobinemia precluded routine hemoglobin determinations.

*Necropsy Findings:* All animals that died or were killed when moribund had the same pathological findings: dark grey-blue skin, dark chocolate colored blood, orange urine with a strong mononitrobenzene odor ; the livers were white and soft, some having only small areas of normal red tissue, and others having latticed white areas throughout the organ. Other organs were grossly normal.

Histologically, the most marked lesion was in the liver.<sup>8</sup> There was diffused necrosis of the outer portions of the liver lobules, sometimes involving two-thirds of the lobules. The cell outlines were still visible but the cytoplasm was pale and granular, and many of the nuclei had completely disappeared. There was a large amount of dark, brownish pigment in the Kupffer cells ; the pigment was more prominent in the necrotic portions of the lobules. No notable changes were seen in the bile ducts. The vessels and the sinusoids contained numbers of red blood cells which were pale, and very few white blood cells. The kidneys showed slight swelling of the glomeruli and of the tubular epithelium. No morphologic changes were observed in the spleen, lungs, or testes.<sup>9</sup>

*Effect on Tumors:* Sublethal doses of mononitrobenzene, administered every other day either by painting or by subcutaneous injections diluted in sesame oil, failed to influence the growth of cutaneously transplanted sarcoma 37 in 15 strain A back-cross mice, or in 8 strain C<sub>3</sub>H females bearing spontaneous mammary carcinomas. The absorption of mononitrobenzene is apparently more effective over highly vascularized areas. Ten C<sub>3</sub>H females with spontaneous breast tumors were divided into 2 groups : in 5, the compound was painted over the shaved abdomen and in 5 over an approximately equal area of the skin covering the tumor. In 24 hours, all of the former had recovered, while all of the latter group were moribund.

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<sup>7</sup> Carter, F. W., *Med. J. Australia*, 1936, **2**, 558.

<sup>8</sup> Leader, S. D., *Arch. Pediat.*, 1932, **49**, 245.

<sup>9</sup> Dresbach, M., and Chandler, W. L., *Proc. Soc. Exp. Biol. and Med.*, 1918, **15**, 136.