



National Toxicology Program
Toxicity Report Series
Number 67

**NTP Technical Report
on the Toxicity Studies of**

2- and 4-Methylimidazole

(CAS No. 693-98-1 and 822-36-6)

**Administered in Feed
to F344/N Rats and B6C3F₁ Mice**

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FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Toxicity Study Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: <http://ntp-server.niehs.nih.gov>. Abstracts of all NTP Toxicity Study Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Perspectives (EHP) <http://ehp.niehs.nih.gov> (866-541-3841 or 919-653-2590). In addition, printed copies of these reports are available from EHP as supplies last. A listing of all the NTP Toxicity Study Reports printed since 1991 appears on the inside back cover.

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PEER REVIEW

The draft report on the toxicity studies of 2- and 4-methylimidazole was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the Toxicity Study Report presents the experimental results and conclusions fully and clearly.

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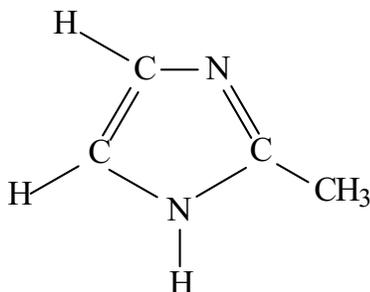
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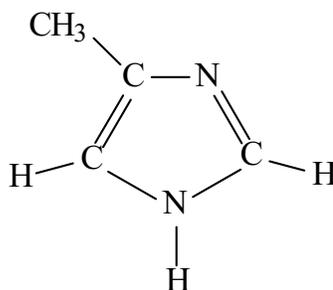
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ABSTRACT

2-Methylimidazole



4-Methylimidazole



CAS Number:	693-98-1	822-36-6
Molecular Weight:	82.11	82.11
Synonyms:	Imidazole, 2-methyl; 2-MeI; 2-methylglyoxaline; 2-MI; 2-MZ 2-methyl-1H-imidazole	Imidazole, 4-methyl; 4-MeI; 4(5)-methylglyoxaline; 5-methylimidazole; 4(5),4(5)-methylimidazole 4-methyl-1H-imidazole

2-Methylimidazole and 4-methylimidazole are intermediate/starting materials or components in the manufacture of pharmaceuticals, photographic and photothermographic chemicals, dyes and pigments, agricultural chemicals, and rubber; these chemicals have been identified as undesirable by-products in several foods and have been detected in mainstream and sidestream tobacco smoke. The National Cancer Institute nominated 2- and 4-methylimidazole as candidates for toxicity and carcinogenicity studies. Toxicity studies were carried out in male and female F344/N rats and B6C3F₁ mice. Animals were exposed to 2- or 4-methylimidazole in feed for 15 days or 14 weeks; clinical pathology studies were conducted in the 14-week studies on days 8, 29, and 86 and at week 14. Genetic toxicity studies were conducted in *Salmonella typhimurium*, rat and mouse bone marrow, and mouse peripheral blood.

Groups of five male and five female rats and mice were fed diets containing 0, 1,200, 3,300, or 10,000 ppm 2-methylimidazole (equivalent to average daily doses of approximately 115, 290, or 770 mg 2-methylimidazole/kg body weight to rats; 220, 640, or 2,100 mg/kg to male mice; 300, 800, or 2,400 to female mice) for 15 days. Groups of five male and five female rats and mice were fed diets containing 0, 300, 800, or 2,500 ppm 4-methylimidazole (equivalent to average daily doses of approximately 30, 80, or 220 mg/kg for rats and 65, 170, or 500 mg/kg for mice) for 15 days. In the 15-day 2-methylimidazole studies, all animals

survived to the end of the studies. The mean body weights of 10,000 ppm male rats and female mice were significantly less than those of the controls. Feed consumption by 10,000 ppm male and female rats was reduced. Enlarged thyroid glands were observed in 3,300 and 10,000 ppm male and female rats. The incidences of diffuse hyperplasia of follicular cells of the thyroid gland in 3,300 and 10,000 ppm male and female rats and pars distalis hypertrophy of the pituitary gland in 3,300 and 10,000 ppm males and 10,000 ppm females were increased compared to the controls. In all exposed groups of male and female mice, the incidences and severities of follicular cell hypertrophy of the thyroid gland and the severities of hematopoietic cell proliferation of the spleen generally increased with increasing exposure concentration. In the 4-methylimidazole studies, all animals survived to the end of the studies, and there were no significant differences in mean body weights, clinical findings, organ weights, or gross or microscopic lesions between exposed and control groups.

Groups of 10 male and 10 female rats and mice were fed diets containing 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm 2- or 4-methylimidazole (equivalent to average daily doses of approximately 40, 80, 160, 300, or 560 mg/kg 2- or 4-methylimidazole to rats; and 100, 165, 360, 780, or 1,740 mg/kg 2-methylimidazole or 100, 240, 440, 915, or 1,840 mg/kg 4-methylimidazole to male mice; and 90, 190, 400, 800, or 1,860 mg/kg 2-methylimidazole or 110, 240, 540, 1,130, or 3,180 mg/kg 4-methylimidazole to females) for 14 weeks. All animals survived to the end of the 14-week 2-methylimidazole studies. Compared to the controls, the mean body weights were significantly decreased in groups of male rats and mice exposed to 2,500 ppm or greater and in 5,000 and 10,000 ppm female rats and mice. In rats, 2-methylimidazole induced a transient erythrocytosis in females and a minimal, exposure concentration-related, microcytic, normochromic, nonresponsive anemia. 2-Methylimidazole increased thyroid-stimulating hormone concentrations and decreased thyroxine and triiodothyronine concentrations of male and female rats in an exposure concentration-related manner. 2-Methylimidazole induced a mild to moderate, exposure concentration-related, macrocytic, hyperchromic, responsive anemia in mice. Triiodothyronine concentrations were increased in exposed male and female mice, and thyroxine concentrations were decreased in exposed females. Relative to the control groups, clinical chemistry evaluations on day 29 and at week 14 identified decreases in alanine aminotransferase concentrations and total protein and albumin concentrations of rats.

In the 2-methylimidazole studies, absolute spleen weights were significantly increased in all exposed groups of male rats. The heart and liver weights were increased in all exposed groups of male mice, as were the spleen weights of female mice exposed to 2,500 ppm or greater. Spermatid heads per testis and mean spermatid count were significantly decreased in 10,000 ppm male rats. The estrous cycle of 10,000 ppm female rats was significantly increased. Gross pathology observations included enlarged thyroid glands, small uteri, and mottled

adrenal glands (females only) in 10,000 ppm rats and enlarged and/or darkened thyroid gland and enlarged spleen in 5,000 and 10,000 ppm mice. The incidences of diffuse follicular cell hyperplasia of the thyroid gland were significantly increased in male rats exposed to 1,250 ppm or greater and female rats exposed to 2,500 ppm or greater. The incidence of testicular degeneration was significantly increased in 10,000 ppm male rats, and two males in the 10,000 ppm group had follicular cell adenoma of the thyroid gland. In mice, there were generally significant increases in the incidences of follicular cell hypertrophy of the thyroid gland, hematopoietic cell proliferation of the spleen, and hemosiderin pigmentation of the renal tubule in males exposed to 1,250 ppm or greater and females exposed to 2,500 ppm or greater.

In the 14-week 4-methylimidazole studies, one 10,000 ppm male mouse was found dead during week 4, and seven 10,000 ppm female mice were found dead during weeks 1 and 2. Mean body weights were significantly less than those of the controls for male rats exposed to 2,500 ppm or greater, 5,000 and 10,000 ppm female rats, male mice exposed to 1,250 ppm or greater, and all exposed groups of female mice. Reduced feed consumption was observed in 5,000 and 10,000 ppm male and female rats. Clinical findings included nasal/eye discharge, ruffled fur, thinness, ataxia, and abnormal breathing in rats, and ruffled fur and dull coats in female mice. On days 29 and 82, functional observations in 5,000 and 10,000 ppm rats included labored or increased respiration, mild tremors, walking on tiptoes, hunched posture, piloerection, crouching over, impaired coordination of movement, ataxia, and pupillary constriction. 4-Methylimidazole induced a transient erythrocytosis and a minimal, exposure concentration-related, microcytic, normochromic, nonresponsive anemia in male and female rats. Clinical chemistry evaluations generally showed a cholestatic effect in exposed male and female rats. At week 14, there was a significant decrease in total protein and albumin concentrations of female rats exposed to 5,000 or 10,000 ppm. In mice, 4-methylimidazole induced a macrocytic, hyperchromic, responsive anemia and, particularly in males, increases in triiodothyronine concentrations and transient decreases in thyroxine concentrations.

In the 4-methylimidazole studies, the liver weights of male rats exposed to 2,500 ppm or greater were significantly increased; spleen weights of female rats exposed to 2,500 ppm or greater were decreased. The absolute liver weight was decreased in 10,000 ppm male mice, and relative weights were significantly increased in all exposed groups of mice. In female mice, there was a significant decrease in the absolute weights and increase in the relative weights of the heart, right kidney, and liver in groups exposed to 2,500 ppm or greater. The epididymal spermatozoal concentration was significantly increased in 5,000 ppm male rats. Gross pathology observations included pale livers in male rats exposed to 2,500 ppm or greater and small testes and uteri in 10,000 ppm male and female rats. Microscopic analysis identified significantly increased incidences of cytoplasmic hepatocyte vacuolization of the liver of male rats exposed to 2,500 ppm or greater and

10,000 ppm female rats, hypospermia of the epididymis in 10,000 ppm male rats, atrophy and inflammation of the prostate gland in 10,000 ppm male rats, and degeneration of the testes in 5,000 and 10,000 ppm male rats.

2-Methylimidazole and 4-methylimidazole were negative in the *S. typhimurium* mutation assay when tested in strains TA97, TA98, TA100, and TA1535, with and without S9 activation enzymes. Testing of 2-methylimidazole *in vivo* for induction of chromosomal damage, as measured by micronucleated erythrocyte frequency, produced mixed results. When administered by intraperitoneal injection three times at 24-hour intervals, 2-methylimidazole produced negative results in bone marrow micronucleus tests in rats and mice. However, in the 14-week study of 2-methylimidazole, a significant exposure-related increase in the frequency of micronucleated normochromatic erythrocytes was noted in peripheral blood of male and female mice. *In vivo*, 4-methylimidazole produced uniformly negative results in three-injection bone marrow micronucleus tests in rats and mice and in 14-week peripheral blood micronucleus tests in male and female mice.

TABLE G5
Results of Analyses of Dose Formulations Administered to Rats and Mice
in the 14-Week Feed Studies of 4-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)	
Mice					
January 24, 1994	January 24, 1994	625	642	+3	
		1,250	1,280	+2	
		2,500	2,450	-2	
		5,000	5,250	+5	
		10,000	9,980	0	
	February 14, 1994 ^b	625	640	+2	
		1,250	1,230	-2	
		2,500	2,480	-1	
		5,000	4,920	-2	
		10,000	8,990	-10	
	March 7, 1994	March 7, 1994	625	655	+5
			1,250	1,330	+6
			2,500	2,700	+8
			5,000	5,260	+5
			10,000	10,400	+4
April 10, 1994 ^b		625	550	-12	
		1,250	1,140	-9	
		2,500	1,950	-22	
		5,000	4,180	-16	
		10,000	9,040	-10	
April 18, 1994	April 18, 1994	625	680	+9	
		1,250	1,310	+5	
		2,500	2,760	+10	
		5,000	5,260	+5	
		10,000	10,500	+5	
	May 13, 1994 ^b	625	645	+3	
		1,250	1,280	+2	
		2,500	2,510	0	
		5,000	4,750	-5	
		10,000	10,100	+1	

^a Results of duplicate analyses

^b Animal room samples