4-METHYLIMIDAZOLE

1. Exposure Data

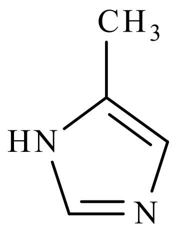
1.1 Chemical and physical data

From NTP (2007), GESTIS (2010) and HSDB (2010)

1.1.1 Nomenclature

Chem. Abstr. Services Reg. No.: 822-36-6
Chem. Abstr. Name: 4-Methylimidazole
Synonyms: 1*H*-Imidazole, 4-methyl; 1*H*-imidazole, 5-methyl-; imidazole, 4(or 5)-methyl; 4(5)-methylglyoxaline; 4(5),4(5)-methylimidazole
EINECS No.: 212-497-3

1.1.2 Structural and molecular formulae and relative molecular mass



$C_4H_6N_2$
Relative molecular mass: 82.11

1.1.3 Chemical and physical properties of the pure substance

Description: Light yellow crystalline solid
Boiling-point: 263 °C
Melting-point: 46–48 °C
Vapour pressure: 0.007 mm Hg at 25 °C
Solubility: Very soluble in water and alcohol
Flash-point: 157 °C
Octanol/water partition coefficient: log K_{ow}, 0.23
Henry's law constant: 4.14×10^{-6} atm.m^3/mol at 25 °C (estimated)

1.1.4 Technical products and impurities

No data were available to the Working Group.

1.1.5 Analysis

Ten alkylated imidazoles have been identified in cigarette smoke, of which 4-methylimidazole and imidazole were the most abundant, by high-performance liquid chromatography on LiChrosorb Si 60 after chemical derivatization with 4-chloro-7-nitro-benzo-2-oxa-1,3-diazole. This method is very selective because no clean-up procedure is necessary (Moree-Testa *et al.*, 1984).

1.2 Production and use

1.2.1 Production

Preparation of 4-methylimidazole involves cyclocondensation of an aldehyde and ammonia with methylglyoxal. Variations include the use of ammonium carbonate or ammonium oxalate as the source of ammonia and cyclocondensation of ammonia and formamide with hydroxyacetone. Another method to synthesize the compound is by catalytic dehydrogenation of imidazoline derivatives. 4-Methylimidazole may be synthesized from propanol and formamide, by catalytic cyclization of bisformamidipropane or by photolysis of alkenyltetrazole derived from alkenes by sequential epoxidation, ring opening and dehydration (NTP, 2007).

1.2.2 Use

4-Methylimidazole is used as a chemical intermediate, raw material or component in the manufacture of pharmaceuticals, photographic and photothermographic chemicals, dyes and pigments, agricultural chemicals and rubber. It has also been investigated for use as a raw material in the synthesis of cardiovascular stimulants, epoxy resin anticholesteraemics, neurotransmitter antagonists, disinfectants/antiprotozoal antiseptic agents and aromatase inhibitors (NTP, 2007).

The chemical is also used as a component in imidazolephenoxyalkanal oven cleaners, a cross-linking agent for epoxy resin hardeners, a corrosion inhibitor for cooling water in heat exchange apparatuses, a component of absorbents to remove acid gases from hydrocarbon or synthesis gas, and a raw material for inks and paper dyes (NTP, 2007).

1.3 Occurrence

1.3.1 Natural occurrence

4-Methylimidazole is not known to occur as a natural product.

1.3.2 Occupational exposure

Workers may be exposed to 4-methylimidazole by inhalation or dermal contact during its production, its use as a major pharmaceutical intermediate and from other uses (NTP, 2007).

1.3.3 Environmental occurrence

4-Methylimidazole may be released into ambient air, water and soil during its production and use.

4-Methylimidazole is expected to exist only in the vapour phase and to be degraded in the ambient atmosphere by a reaction with photochemically produced hydroxyl radicals; its estimated atmospheric half-life is 4.1 hours, and it is not expected to undergo photolysis by sunlight (HSDB, 2010).

4-Methylimidazole is not expected to adsorb to sediments and soils in the aquatic environment, but is expected to adsorb more strongly to soils that contain organic carbon and clay than to other types of soil in the terrestrial environment. Volatilization from water surfaces and moist soils is probable, but not from dry soil surfaces; the half-lives for volatilization were 194 hours in a model river and 62 days in a model lake. Its potential bioconcentration in the aquatic environment is low, and its estimated bioconcentration factor in fish is 3.2 based on an estimated octanol/water coefficient (log K_{ow}) of 0.23 (HSDB, 2010).

1.3.4 Occurrence in food

(a) Occurrence in milk through ammoniated forage

Exposure can occur from the consumption of foods contaminated with 4-methylimidazole, which is formed as a result of the interaction of ammonia with reducing sugars. Forage — typically hay and straw — is sometimes treated with anhydrous ammonia to improve its quality (e.g. increase the non-protein nitrogen content) and digestibility (Waagepetersen & Vestergaard, 1977). Imidazoles (such as 4-methylimidazole) and pyrazines appear to be the dominant groups of toxic by-products formed from the interaction of ammonia with reducing sugars. Experimental studies have shown that higher concentrations of sugar and ammonia, higher temperatures, higher water activity and longer reaction times increased the amount of 4-methylimidazole (formed at the pH achieved by the addition of ammonia) (Bergström, 1991). Perdok & Leng (1987) reported that 4-methylimidazole was not present in most types of untreated roughage, but was found at concentrations ranging from 8 to 43 mg/kg in thermo-ammoniated roughage. Average concentrations of 0.72 µg/mL 4-methylimidazole were detected in the plasma of sheep fed ammoniated tall fescue that contained an average concentration of 64.36 mg/kg of the chemical (Karangwa *et al.*, 1990a). 4-Methylimidazole has been identified in the plasma, urine and milk of cows and sheep fed ammoniated forage (Müller *et al.*, 1998a, b). Müller *et al.* (1998a) reported that the concentrations of 4-methylimidazole in an ammoniated forage-fed (90 µg/g dry matter) ewe were 0.07 µg/mL in plasma, 0.23–0.31 µg/mL in milk and 21 µg/mL in urine. The plasma concentration in one of the ewes' suckling lambs that developed toxicosis was 0.01 µg/mL. Similar concentrations were found in the plasma and milk of ewes fed ammoniated seed hay (with 4-methylimidazole concentrations greater than 100 µg/g dry matter) for 7 days (Sivertsen *et al.*, 1993). In a dairy cow fed ammoniated forage containing 4-methylimidazole (58 µg/g dry matter), concentrations of the chemical in plasma, milk and urine were 0.28, 2.7 and 5.8 µg/mL, respectively (Müller *et al.*, 1998a).

(b) Occurrence in food and drinks containing caramel colourings

4-Methylimidazole is found in ammonia and ammonia-sulfite process caramel colourings. Caramel colourings are produced by heating carbohydrates with specified reagents under defined temperatures and pressures, which results in a dark brown colouring with a characteristic odour of burnt sugar. Their use accounts for 95% by weight of the permitted colour additives used in food. They have been classified by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the European Union Scientific Committee for Food into four classes, two of which are prepared using compounds that contain ammonia (reviewed by Chappel & Howell, 1992; Houben & Penninks, 1994). Class III ammonia caramels are commonly used in various bakery products, soya-bean sauces, brown sauces, gravies, soup aromas, brown (dehydrated) soups, brown malt caramel blends for various applications, vinegars and beers, especially in certain dark-brown beers. Their use accounts for 20–25% of the total use of caramel colourings in the USA and for about 60% in Europe. Class IV ammonia-sulfite caramels are used in soft drinks, pet foods and soups (Houben & Penninks, 1994), and account for approximately 70% of the caramel colourings produced worldwide (Licht *et al.*, 1992a). Reported concentrations of 4-methylimidazole in caramel colourings are provided in Table 1.1. Licht *et al.* (1992b) reported 4-methylimidazole concentrations ranging from < 5 to 184 mg/kg in 40 commercial Class III caramel colourings that met JECFA guidelines for 2-acetyl-4-tetrahydroxybutylimidazole. Other studies have reported higher concentrations in some samples, ranging up to 463 mg/kg. In general, higher 4-methylimidazole levels have been found in Class IV caramel colourings; a study of 90 commercial products found levels ranging from 112 to 1276 mg/kg (see Table 1.1).

Long-term dietary exposure to caramels among children aged 1–10 years has been estimated based on analytical data in 11 European countries (EFSA, 2010). Median exposure ranged from 4.3 to 41 mg/kg body weight (bw) per day for ammonia-sulfite caramels (class IV) and from 32 to 105 mg/kg bw per day for ammonia caramels (class III).

Reported concentrations of 4-methylimidazole ranged from 1.58 to 28.03 mg/kg in dark beer (Klejdus *et al.*, 2006), from 0.3 to 1.45 mg/kg in coffee (Casal *et al.*, 2002; Lojková *et al.*, 2006) and from 0.30 to 0.36 µg/mL in

Table 1.1 Concentrations of 4-methylimidazole in caramel colourings and food and beverages

Product	Number of samples	Concentration (mg/kg)	Reference
Class III	40 commercial colourings[a]	< 5–184	Licht *et al.* (1992b)
Class III	6 commercial colourings[b]	ND–463	Allen *et al.* (1992)
Class III	5 colourings	85.6–187.8	Klejdus *et al.* (2006)
Class III	3 colourings[b]	34–463	Brusick *et al.* (1992)
Class III	3 commercial colourings		Ciolino (1998)
	Liquid	14–24	
	Powder	50	
Ammonia process caramel colourings	6 colourings	6.6–351 per 20 000 EBC units[c]	Thomsen & Willumsen (1981)
Ammonia caramel colourings	4 samples	7.5–210	Fernandes & Ferreira (1997)
Ammonia caramel colourings	3 samples	122–414	Kvasnička (1989)
Ammonia caramel colourings	5 samples	25–303	Fuschs & Sundell (1975)
Class IV	90 commercial colourings[d]	112–1276	Licht *et al.* (1992a)
Class IV	2 commercial colourings[b]	146–215	Allen *et al.* (1992)
Class IV	6 colourings[b]	ND–387	Brusick *et al.* (1992)
Class IV	3 colourings		Ciolino (1998)
	Liquid	130–300	
	Powder	480	
Ammonia caramel colourings (ammonia-sulfite process)	8 colourings	62–341 per 20 000 EBC units[c]	Thomsen & Willumsen (1981)
Malt extract	2 samples	ND	Fernandes & Ferreira (1997)
Coffee	10 real samples	0.39–2.05	Klejdus *et al.* (2006)
Coffee	5 types		Lojková *et al.* (2006)
	Solid	0.77–1.45	
	Liquid	0.35–0.77	
Coffee	7 samples		Casal *et al.* (2002)
	Roasted	0.307–1.241	
Dark beer	7 real samples	1.58–28.03	Klejdus *et al.* (2006)
Soda	5 brands	0.30–0.36	Moon & Shibamoto (2010)

Table 1.1 (continued)

Product	Number of samples	Concentration (mg/kg)	Reference
Soft drinks			Yoshikawa & Fujiwara (1981)
Cola type	7 samples	0.17–0.70	
Grape type	2 samples	0.15–0.16	
Alcoholic beverages			
Whisky	5 samples	ND–0.14	
Black beer	2 samples	ND	
Beer, wine brandy	1 sample each	ND	
Milk products	3 samples	Trace	
Seasoning sauces			
Worcestershire sauce	6 samples	1.6–3.4	
Soya sauce	4 samples	0.37–0.55	
Others	5 samples	0.11–1.5	
Foods cooked in soya sauce	5 samples	0.89–3.2	
Confectioneries	6 samples	ND–0.78	

[a] Represent full range of commercially available samples, limited to those that meet JECFA specification of 25 mg/kg or less of 2-acetyl-4(5)-tetrahydroxybutylimidazole when expressed per 0.1 colour intensity unit or varied between > 10 and 45 mg/kg on an ‘as is’ basis.
[b] Samples provided by the International Technical Caramel Association
[c] EBC units: caramel colour intensity unit of the European Brewery Convention
[d] Represent full range of commercially available samples from 11 manufacturers and seven countries.
ND, not detected

soda (Moon & Shibamoto, 2010). Yoshikawa & Fujiwara (1981) measured 4-methylimidazole in various foods and beverages (see Table 1.1). The highest levels were found in Worcestershire sauce (up to 3.4 mg/kg) or foods cooked in soya sauce (up to 3.2 mg/kg).

1.3.5 Other occurrence

Exposure to 4-methylimidazole from tobacco smoke may also occur. 4-Methylimidazole has been detected in the condensate of smoke from several brands of cigarettes, ranging from 2.3 (low tar) to 15 (non-filtered) μg/cigarette for dark, air-cured tobacco. Concentrations (μg/cigarette) in other types of tobacco were 2.3 for Virginia and 5.5 for American blend. 4-Methylimidazole is one of the most abundant imidazoles found in cigarette smoke (Moree-Testa *et al.*, 1984).

1.4 Regulations and guidelines

Specifications issued by the European Commission (2008) and JECFA (2006) stated that the maximum level of 4-methylimidazole in class III ammonia caramel and class IV ammonia-sulfite caramel should be restricted to ≤ 250 mg/kg on a colour intensity basis.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Oral administration

See Table 3.1

3.1.1 Mouse

In a 2-year carcinogenicity study, groups of 50 male and 50 female B6C3F$_1$ mice were fed diets containing 0, 312, 625 or 1250 ppm 4-methylimidazole (> 99% pure) for 106 weeks (equivalent to average daily doses of approximately 40, 80 and 170 mg/kg body weight (bw)) (NTP, 2007; Chan *et al.*, 2008). Food consumption of treated male and female mice was generally similar to that of controls. 4-Methylimidazole significantly increased the incidence of alveolar/bronchiolar adenoma in all treated groups of females, of alveolar/bronchiolar carcinoma in 1250-ppm males and of alveolar/bronchiolar adenoma or carcinoma (combined) in 1250-ppm males and 625- and 1250-ppm females. Although the incidence of alveolar/bronchiolar carcinoma in females was not statistically significant, that in the 1250-ppm exposure group exceeded the range (0–6%) in historical controls.

3.1.2 Rat

In a 2-year carcinogenicity study, groups of 50 male and 50 female F344/N rats were fed diets containing 0, 625, 1250 or 2500 ppm (males) and 0, 1250, 2500 or 5000 ppm (females) 4-methylimidazole (> 99% pure) for 106 weeks (equivalent to average daily doses of approximately 30, 55 or 115 and 60, 120 or 260 mg/kg bw in males and females, respectively) (NTP, 2007; Chan *et al.*, 2008). The food consumption of 5000-ppm females was lower than that of the controls. The incidence of mononuclear-cell leukaemia in 5000-ppm females was significantly higher than that in the controls, and exceeded the range (12–38%) in historical feed study controls. Mononuclear-cell leukaemia is a common finding with a highly variable incidence in F344/N rats and may have been exacerbated by exposure to 4-methylimidazole, as the onset in 5000-ppm females was earlier (day 368) than that in control females (day 624). [The Working Group also noted the significantly

Table 3.1 Carcinogenicity studies of oral administration of 4-methylimidazole in the diet to experimental animals

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, $B6C3F_1$ (M, F) 106 wk	0, 312, 625 or 1250 ppm 7 d/wk 50/group	Lung (alveolar/bronchiolar adenoma): M[a]–8/50, 11/50, 13/50, 15/50 F[b]–0/50, 8/50, 16/50, 8/50	*P* = 0.004 (low-dose F) *P* < 0.001 (mid-dose F) *P* = 0.003 (high-dose F) *P* = 0.017 (trend F)	> 99% pure
		Lung (alveolar/bronchiolar carcinoma): M[c]–2/50, 4/50, 4/50, 8/50 F[d]–3/50, 0/50, 2/50, 7/50	*P* = 0.042 (high-dose M) *P* = 0.024 (trend M)	
		Lung (alveolar/bronchiolar adenoma or carcinoma): M[e]–9/50, 13/50, 16/50, 22/50 F[f]–3/50, 8/50, 17/50, 14/50	*P* = 0.003 (high-dose M) *P* < 0.001 (trend M) *P* < 0.001 (mid-dose F) *P* = 0.002 (high-dose F) *P* = 0.002 (trend F)	
Rat, F344 (M, F) 106 wk	0, 625, 1250 or 2500 ppm (M) 0, 1250, 2500 or 5000 ppm (F) 7 d/wk 50/group	Haematopoietic system (mononuclear-cell leukaemia): M–15/50, 18/50, 22/50, 20/50 F[g]–9/50, 7/50, 16/50, 20/50	*P* = 0.013 (high-dose F) *P* < 0.001 (trend F)	> 99% pure

[a] Historical incidence (mean ± SD) for 2-year feed studies in male mice: 75/510 (15.8 ± 6.3%); range, 9–28%
[b] Historical incidence (mean ± SD) for 2-year feed studies in female mice: 19/509 (3.7 ± 3.8%); range, 0–10%
[c] Historical incidence (mean ± SD) for 2-year feed studies in male mice: 40/510 (7.8 ± 3.8%); range, 4–14%
[d] Historical incidence (mean ± SD) for 2-year feed studies in female mice: 16/509 (2.9 ± 2.5%); range, 0–6%
[e] Historical incidence (mean ± SD) for 2-year feed studies in male mice: 108/510 (22.2 ± 6.3%); range, 14–32%
[f] Historical incidence (mean ± SD) for 2-year feed studies in female mice: 35/509 (6.6 ± 4.2%); range, 0–12%
[g] Historical incidence (mean ± SD) for 2-year feed studies in female rats: 121/510 (23.8 ± 9.1%); range, 12–38%
d, day or days; F, female; M, male; SD, standard deviation; wk, week or weeks
From NTP (2007), Chan *et al.* (2008)

decreased incidence of pituitary (pars distalis) gland adenoma, of benign, complex or malignant pheochromocytoma (combined) of the adrenal gland in males, of pituitary (pars distalis) gland and clitoral gland adenoma, of mammary gland fibroadenoma and of uterine stromal polyps in females. These decreases in incidence could not be attributed to loss of body weight alone. The study in rats is also discussed in Murray (2011).]

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

(a) Absorption, distribution and excretion

Previous studies have shown species differences in the disposition of 4-methylimidazole.

In rats, 5 minutes after a single intraperitoneal injection of 216 mg/kg bw, the uptake of 4-methylimidazole was highest in the intestines, followed by the liver, blood, stomach and kidney (Hidaka, 1976). The compound was excreted unchanged in the urine, beginning approximately 30 minutes after injection, and reached approximately 90% within 8 hours (Hidaka, 1976).

In ewes, the absorption and elimination of a single oral dose of 4-methylimidazole followed first-order kinetics. One half of an oral dose (20 mg/kg bw) of 4-methylimidazole was absorbed within about 27 minutes, and the maximum plasma level was reached 5 hours after administration (Karangwa *et al.*, 1990b). Bioavailability calculated from plasma data from three ewes was 69%, and the biological half-life was 9.37 hours. Only 0.07 mg/kg of the dose was recovered in the urine as the unchanged parent compound. Metabolites of 4-methylimidazole were not detected by high-performance liquid chromatography. [The Working Group noted that the sensitivity of this assay was difficult to evaluate.]

In goats and heifers, the mean residence time of 4-methylimidazole administered orally or intravenously was about 5 hours, and the volume of distribution was 0.9 L/kg bw. 4-Methylimidazole and its metabolites were excreted mainly in the urine, but also in the milk and faeces, and the administered dose was distributed mainly in the liver, kidney and lung (Nielsen *et al.*, 1993). 4-Methylimidazole was found in the milk following its oral administration to pregnant and postpartum cows (Morgan & Edwards, 1986).

Following oral administration by gavage of 5, 50 or 150 mg/kg bw 4-methylimidazole (^{14}C-radiolabelled) to F344/N rats, peak plasma concentrations were reached at 0.5, 1.0 and 3.0 hours, respectively (Yuan & Burka, 1995). At 150 mg/kg, the plasma concentration of [^{14}C]4-methylimidazole was almost constant during the first 5 hours; at lower doses, the decline was more rapid. The estimated terminal half-life was dose-dependent. The authors suggested that the elimination of parent 4-methylimidazole was saturable. From the total urinary recovery of parent 4-methylimidazole, the estimated bioavailability was approximately 60–70%. Little or no metabolism of 4-methylimidazole was found. Only one minor hydrophilic metabolite was present in the urine and plasma. Faecal, biliary and respiratory elimination of radioactivity were negligible.

(b) Metabolism

Four metabolites were determined in the urine of goats and heifers given 4-methylimidazole (Nielsen *et al.*, 1993), three of which were identified as 5-methyl-hydantoin, 2-methyl-hydantoic acid and urea. The high polarity of the fourth product prevented further characterization.

(c) Toxicokinetic models

After a single oral administration by gavage of 4-methylimidazole (10, 50 or 100 mg/kg bw) to male and female F344/N rats, the plasma concentration versus time data could be described by a one-compartment model, with no lag phase, and first-order absorption and elimination for both males and females based on the findings of Yuan & Burka (1995) (NTP, 2007). The absorption half-life ranged from 5 to 23 minutes and decreased with dose. The elimination half-life ranged from 1 to 8 hours and increased with dose. The plasma concentration versus time data following intravenous administration of 10 mg/kg bw 4-methylimidazole was described as a one-compartment model with first-order elimination. From comparisons of the area under the concentration versus time curves for the two routes of administration, bioavailability was determined to be greater than 85%.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

The genetic effects of 4-methylimidazole have recently been reviewed (NTP, 2007).

(a) Mutations

4-Methylimidazole (up to 10 000 μg/plate) was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100 or TA1535 when tested in the presence or absence of 10% or 30% hamster or rat liver metabolic activation systems (detailed protocol presented by Zeiger *et al.*, 1988; NTP, 2007). Class III and IV caramel colourings contain various concentrations of 4-methylimidazole, and did not to induce mutagenic activity in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538. In these studies, concentrations of 4-methylimidazole in class III preparations were 9–463 mg/kg, and those in class IV were 146–215 mg/kg (Allen *et al.*, 1992). Class III and IV caramel colourings were also negative in the *S. typhimurium* Ames test and *Saccharomyces cerevisiae* gene conversion assays. In these studies, concentrations of 4-methylimidazole in class III preparations were 34–463 mg/kg, and those in class IV were 107–387 mg/kg (Brusick *et al.*, 1992).

(b) Chromosomal effects

No increases in the frequency of micronucleated erythrocytes were observed in the bone marrow of male rats or male mice (detailed protocol presented by Shelby *et al.*, 1993; NTP, 2007) administered three intraperitoneal injections of 4-methylimidazole at 24-hours intervals or in peripheral blood samples from male and female mice fed the compound in the diet for 14 weeks (detailed protocol presented by MacGregor *et al.*, 1990; NTP, 2007). No significant alterations in the percentage of polychromatic erythrocytes, an approximate indicator of bone marrow toxicity, were seen in the bone marrow or peripheral blood of mice; however, the percentage declined with increasing dose of 4-methylimidazole and was significantly depressed at the highest dose in the bone marrow of male rats (NTP, 2007).

Class III caramel colouring did not induce chromosomal damage in Chinese hamster ovary cells (Allen *et al.*, 1992). In the study of Brusick *et al.* (1992), class IV caramel colouring gave negative results in the chromosomal aberration assay while class III colouring was weakly clastogenic only in the absence of metabolic activation or in the presence of heat-inactivated metabolic activation. Class IV caramel colouring was not clastogenic in Chinese hamster ovary cells *in vitro* in either the presence or absence of metabolic activation, whereas the weak clastogenic effect of class III caramel colouring was abolished in the presence of metabolic activation. Moreover, *in vivo*, class III caramel colouring administered orally to mice did not induce micronuclei in the bone marrow.

4.3 Mechanistic data

4.3.1 Effects on cell physiology

In 15-day feed studies, 4-methylimidazole did not induce any histopathological changes in male or female F344 rats. In a 14-week feed study, some variation in serum thyroxine (males) or triiodothyronine and thyroid-stimulating hormone (females) was observed but the changes were sporadic and independent of dose. No histopathological alterations were observed in B6C3F$_1$ mice fed 4-methylimidazole for 15 days. However, transient decreases in serum thyroxine and increases in serum tri-iodo-thyronine levels were observed in males and females in a 14-week feed study; levels of thyroid-stimulating hormone were not determined (NTP, 2004; Chan *et al.*, 2006).

No thyroid lesions were observed following 15 days or 14 weeks of exposure to 4-methylimidazole. In contrast, the structural analogue, 2-methylimidazole, induced thyroid lesions in rats and mice in both 15-day and 14-week feed studies (NTP, 2004; Chan *et al.*, 2006).

Class IV caramel colouring was evaluated for toxicity in male and female F344 rats at doses up to 30 g/kg bw for 13 weeks (MacKenzie *et al.*, 1992). Although food and water consumption, body weight and urine volume were decreased, these were considered to be adaptive non-specific changes.

4.3.2 Effects on cell function

4-Methylimidazole forms complexes with haeme-containing enzymes such as cytochrome P450 (CYP) and results in the inhibition of mixed-function oxidase activity (Wilkinson *et al.*, 1983; Karangwa *et al.*, 1990b). It was reported that 4-methylimidazole significantly inhibited CYP2E1 activity in rat liver (Hargreaves *et al.*, 1994) and tolbutamide hydroxylase (CYP2C9) activity in human and rat microsomes (Back & Tjia, 1985, Back *et al.*, 1988). Moreover, it stimulated the phosphorylation of rabbit kidney (Na^+ and K^+)-adenosine triphosphatase (Schuurmans Stekhoven *et al.*, 1988), and exhibited significant antioxidant activity in a lipid peroxyl radical activity trapping assay (Kohen *et al.*, 1988).

4.4 Mechanisms of carcinogenesis

The incidence of hyperplasia of the lung alveolar epithelium was significantly increased in female mice fed 1250 ppm [60 mg/kg bw] 4-methylimidazole for 2 years (NTP, 2007). Hyperplasia of the alveolar epithelium is thought to be a precursor of neoplastic development. [In this study, hyperplasia was analysed only at the end of the 2-year study, which does not ensure that hyperplasia appeared before adenoma.] Interestingly, 4-methylimidazole had no effect on the respiratory epithelium in a 14-week toxicity study at concentrations as high as 10 000 ppm [3180 mg/kg bw] (NTP, 2004). 4-Methylimidazole induced neither mutations nor chromosomal aberrations *in vitro* or *in vivo*. The mechanism of action of 4-methylimidazole in mouse lung tumorigenesis is not clear.

5. Summary of Data Reported

5.1 Exposure Data

4-Methylimidazole is used as a raw material, chemical intermediate or component in the manufacture of pharmaceuticals, dyes, pigments or agricultural chemicals. Occupational exposure may occur by inhalation or dermal contact. 4-Methylimidazole is formed as a result of the interaction of ammonia with reducing sugars.

The general population is exposed to 4-methylimidazole in food through its presence in class III and IV caramels, which are widely used food colourings, especially in beverages. It has been detected in ammoniated forage and ammoniated molasses that were fed to animals, and in the milk from these animals.

4-Methylimidazole has been detected in tobacco smoke.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

4-Methylimidazole was tested for carcinogenicity by oral administration in the diet to mice and rats. It increased the incidence of alveolar/bronchiolar adenoma in female mice, alveolar/bronchiolar carcinoma in male mice and alveolar/bronchiolar adenoma and carcinoma combined in male and female mice. Oral administration of 4-methylimidazole increased the incidence of mononuclear-cell leukaemia in female rats.

5.4 Other relevant data

No data were available on the toxicokinetics of 4-methylimidazole in humans. After oral administration to mammals, 4-methylimidazole was rapidly absorbed and widely distributed. In rats, ewes, goats and heifers, 4-methylimidazole and its metabolites were mainly excreted in the urine. Three urinary metabolites were identified in goats and heifers (5-methyl-hydantoin, 2-methyl-hydantois acid and urea) but none was characterized in rats.

4-Methylimidazole induces neither mutations nor chromosomal aberrations in experimental test systems. It caused no observable histological lesions in rodents following 15 days or 14 weeks of exposure in the diet. The mechanism of action of 4-methylimidazole that leads to lung tumours in mice is unknown.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-methylimidazole.

6.3 Overall evaluation

4-Methylimidazole is *possibly carcinogenic to humans (Group 2B)*.

References

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