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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

last updated: 11/2000

2-Mercapto-benzimidazole

No. 218

CAS No. 583-39-1

BG Chemie
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2-Mercaptobenzimidazole

1 Summary and assessment

On oral administration, 2-mercaptobenzimidazole is rapidly and practically completely absorbed from the gastrointestinal tract. There are no studies available on absorption via the skin and/or respiratory tract. Following intravenous administration of [^{14}C]-2-mercaptobenzimidazole to male rats, radioactivity was detectable in all organs and tissues examined, with levels found in the liver, kidneys and lungs being higher than in the blood and plasma. Levels were lowest in the fatty tissue. Strikingly, the time course of radioactivity in the thyroid gland, the tissue with the highest levels shortly after intravenous injection, showed a subsequent short-lasting drop in radioactivity levels, which then rose again and several hours after administration clearly exceeded the levels seen in plasma. There are comparable results from another rat study with oral administration. Analysis also revealed relatively high concentrations in the gastrointestinal tract between 2 and 6 hours after intravenous administration, a finding which correlated with biliary excretion. In the thyroid gland, 2-mercaptobenzimidazole and benzimidazole accounted for most of the radioactivity recovered after oral administration and were accompanied by two polar metabolites, the identity of which was not established. In plasma, only 2-mercaptobenzimidazole, but no benzimidazole, was detected upon oral administration. Following intravenous injection, analysis of plasma, liver, kidneys, lungs and gastrointestinal tract revealed metabolites which were not further characterised as well as unchanged 2-mercaptobenzimidazole. In the brain, total radioactivity corresponded to that of the recovered unchanged [^{14}C]-2-mercaptobenzimidazole, from which the authors concluded that the blood-brain barrier is impermeable to metabolites of 2-mercaptobenzimidazole. With the aid of open multi-compartment models, elimination half-lives from blood and plasma were calculated to be 86 and 136 minutes, respectively, for initial elimination and 83 and 22 hours, respectively, for terminal elimination. The initial half-life for clearance of unchanged 2-mercaptobenzimidazole from plasma was determined as 125 minutes. After oral as well as after intravenous administration, most of the administered radioactivity was excreted in the urine (67 to 69% and 52%, respectively, in 72 hours), while a smaller amount appeared in the faeces (19 to 25% and 31%, respectively, in 72

hours). Another oral study in the rat gave comparable recovery rates, with 68 to 77% of the administered radioactivity being recovered from the excrements, which were presumably collected over a 24-hour period, and 66% of the recovered activity appearing in urine. Of an intravenously injected dose, 12% was excreted in the bile in 4 hours. No elimination as [^{14}C]-CO_2 was detected. In the rat, recovery rates from the examined organs and tissues, including the carcass but excluding blood and gastrointestinal contents, were still 2.22 and 0.902% of the orally administered radioactivity and 1.48% of the intravenously injected activity at 72 hours after dosing. By extrapolation of the fractions of radioactivity recovered from the blood, muscle, skin and fat samples, and taking into account their percentages of total body weight as well as the radioactivity recovered from the gastrointestinal tract, the residual radioactivity in the body was found to be approx. 6.3 and approx. 2.2% for the two oral dose groups and approx. 2.7% for the intravenously treated dose group 72 hours after dosing. The total recovery rates were between 85 and 96%. Two major metabolites and 6 quantitatively less important metabolites were excreted in urine. Only the structure of one of the major metabolites was elucidated, which was benzimidazole. Another compound, which was recovered from urine in smaller amounts, was identified as unchanged 2-mercaptobenzimidazole. In bile, neither benzimidazole nor 2-mercaptobenzimidazole was detectable.

On single oral administration, 2-mercaptobenzimidazole proves to be harmful (LD_{50} rat oral 1230 and 476 mg/kg body weight; LD_{50} mouse oral 1250 and 750 mg/kg body weight; depending on the source of information).

In two subacute 14-day inhalation studies conducted in rats which underwent a total of 12 6-hour exposures to 2-mercaptobenzimidazole at concentrations ranging from 5 to 400 mg/m³, all rats exposed to the highest concentration died. Concentration-dependent retardation of body weight development was observed. Increased liver weights were seen at and above 5 mg/m³ and organ weights of the brain, thymus, heart, lung, kidneys and testes were reduced at and above 15 mg/m³. Macroscopically, findings observed at and above 12.5 mg/m³ included enlarged thyroid glands, which corresponded with microscopically observed follicular cell hyperplasia of the thyroid gland, and from 25 mg/m³ pituitary atrophy was noted. Further histopathological alterations included changes in the liver, thymus, spleen and kidneys. In a 14-day inhalation study (6 hours/day, a total of 12 exposures), mice were exposed to 2-mercaptobenzimidazole concentrations

ranging from 5 to 400 mg/m³. Mortality at the high concentration level was 100%. From 5 mg/m³, the liver weights were increased, as compared with controls. Histopathological changes were observed in the liver, thyroid gland, pituitary gland, thymus, spleen and kidneys.

2-Mercaptobenzimidazole is not to moderately corrosive to the skin and eye of the rabbit.

In a modified maximisation test in guinea pigs, 2-mercaptobenzimidazole did not cause skin sensitisation. Similarly, the skin of guinea pigs showed no cross-reactions to chemically related products. In guinea pigs, the testing of technical-grade 2-mercaptobenzimidazole (no further details, particularly with respect to impurities) in a rarely used study design resulted in skin sensitisation and cross-reaction to 2-mercaptobenzothiazole (cf. Toxicological Evaluations, volumes 4 and 16 of the book series published by Springer).

Two subchronic inhalation studies (exposure to 2-mercaptobenzimidazole at levels of 0.1, 0.3, 1, 3 and 10 mg/m³ and at levels of 3.13, 6.25, 12.5, 25 and 50 mg/m³ for 6 hours daily, 5 days/week over a period of 13 weeks) show markedly increased mortality among male and female rats. From 3 mg/m³, the findings primarily include increased thyroid weights, which histopathologically corresponded to follicular cell hyperplasia, and reduced blood T_3 and T_4 levels starting at dose levels of 12.5 mg/m³. At and above a concentration level of 3.13 mg/m³ the adenohypophyseal cells are reported to have exhibited alterations identical to “thyroidectomy cells”. In addition, thymus weights were decreased at concentration levels of 3.13 mg/m³ and above, and histopathological examination revealed changes in terms of thymic atrophy. The following histopathological findings were observed at the higher concentration levels. There was hepatocyte hypertrophy in the liver, tubular atrophy and mineralisation in the kidneys, the adrenal glands exhibited necrosis and degeneration, there was hyperplasia of pancreatic islet cells, and in the nose cystic degeneration of the respiratory epithelium was noted. As changes in haematological and clinical parameters were observed even at the lowest test concentration of 0.1 mg/m³, a *no observable effect level* was not determined in the study. Following subchronic inhalation exposure (to 0.1, 0.3, 1, 3 and 10 mg/m³ and to 3.13, 6.25, 12.5, 25 and 50 mg/m³ for 6 hours daily, 5 days/week over a period of 90 days), mice are reported to have shown increased liver weights and thyroid

weights at and above dose levels of 3 mg/m³ and 10 mg/m³, respectively. In the females exposed to 0.1 mg/m³ and higher doses, kidney weights were found to be increased. Histopathologically, the animals showed a concentration-dependent increase in the incidence of thymic hyperplasia. Bronchial epithelial hyperplasia and centrilobular hypertrophy of liver cells were diagnosed in male and female mice exposed to 12.5 mg/m³ and higher levels, and again incidences increased in a dose-dependent manner. Only the female mice were found to have degenerative renal changes at and above a concentration level of 3.13 mg/m³. The *no observable effect level* for male mice is reported to have been 3.13 mg/m³. Seeing that changes in organ weights occurred even at the 0.1 mg/m³ exposure level in female mice, it was not possible to determine a *no effect level* for the females.

2-Mercaptobenzimidazole shows no mutagenic activity in the Salmonella/microsome test with or without metabolic activation. In a comparative test of pure and technical-grade 2-mercaptobenzimidazole in *Salmonella typhimurium* strains TA 98 and TA 100 which was carried out with and without metabolic activation, the technical product tested positive in strain TA 98 in the presence of metabolic activation; all other results were negative. A urine test with *Salmonella typhimurium* strain TA 98 as an indicator organism was carried out in workers who had been exposed to technical-grade 2-mercaptobenzimidazole and gave positive results in the presence of metabolic activation. This mutagenic effect is attributed to impurities in the technical product. On the basis of the results of a mouse lymphoma (L5178Y) assay, in which concentrations from 12.5 to 500 µg/ml were tested without metabolic activation and concentrations from 0.156 to 300 µg/ml were investigated in the presence of metabolic activation, it is not possible to deduce a definite gentoxic potential. From an HPRT test on V79 cells, there are no indications that 2-mercaptobenzimidazole (test concentration 100 µg/ml) induces gene mutations in the absence or presence of metabolic activation. In the absence of concentration dependency of the pathological changes and due to the lack of information on the methodology employed and any impurities which may have been present in the 2-mercaptobenzimidazole tested, the reported increase in numerical and structural chromosomal aberrations in human leukocyte cultures cannot be regarded as proof of chromosomal damage from this benzimidazole compound. For the latter reason and in view of the abnormal finding that no structural aberrations were present in the bone-marrow of the animals from

the two control groups, it is not possible to arrive at a final evaluation of the chromosomal changes in the bone-marrow of rats after intraperitoneal injection of 2-mercaptobenzimidazole at 30 mg/kg body weight. Two publications based on studies in which 2-mercaptobenzimidazole was tested for induction of recessive sex-linked lethal mutations in male *Drosophila melanogaster* report one negative and one positive result; however, the data are questionable and therefore can not be used for the purpose of evaluating the genotoxic potential of this benzimidazole compound. From a micronucleus test carried out on the blood taken from mice at the end of the 13-week inhalation study, there is no evidence that 2-mercaptobenzimidazole produces clastogenic effects. Based on the results of the various in vitro and in vivo studies, it appears unlikely that pure 2-mercaptobenzimidazole has a clear mutagenic potential.

In a modified BALB/c-3T3 cell transformation assay, 2-mercaptobenzimidazole is reported to have tested positive.

In a teratogenicity study in rats, oral administration of 2-mercaptobenzimidazole at dose levels of 3.3, 10 and 30 mg/kg body weight on days 7 to 17 of gestation and administration of the maternally highly toxic dose of 60 mg/kg body weight on gestational days 7 to 10, 11 to 14 and 15 to 17 produced adverse effects on embryonic and fetal development only at maternally toxic dose levels. The *no observed adverse effect levels* (NOAELs) of 2-mercaptobenzimidazole for the dams and the foetuses were therefore < 3.3 mg/kg body weight and 3.3 mg/kg body weight, respectively. Thus, at maternally nontoxic dose levels 2-mercaptobenzimidazole produces no embryotoxic or teratogenic effects in rats. When administered to rats on different gestational days as a single intraperitoneal injection of 50 mg/kg body weight, a dose suspected to cause maternal toxicity, 2-mercaptobenzimidazole proves to be embryolethal or fetotoxic, depending on the time of administration, but not teratogenic. Single oral administration of the high 2-mercaptobenzimidazole dose of 120 mg/kg body weight on days 12 or 13 of pregnancy also does not result in visceral or skeletal malformations in spontaneously born pups. In the placenta of rats, 2-mercaptobenzimidazole causes haemorrhages and lesions in the vascular walls. In the above-mentioned 13-week study conducted in rats and mice, the mice were found to have longer oestrous cycles while both the rats and mice showed reduced sperm motility. These changes were interpreted by the investigators as being indicative of effects on fertility.

In humans, 2-mercaptobenzimidazole appears not to cause primary irritation of the skin, but there is evidence that the chemical causes skin sensitisation. In Shanghai, occupational exposure to technical-grade 2-mercaptobenzimidazole is reported to have resulted in an increased cancer mortality rate in individuals with many years of exposure compared with the city's general population.

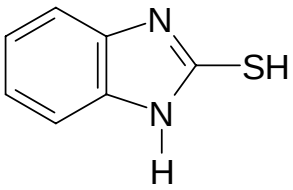
2 Name of substance

2.1	Usual name	2-Mercaptobenzimidazole
2.2	IUPAC name	1,3-Dihydro-2H-benzimidazole-2-thione
2.3	CAS No.	583-39-1
2.4	EINECS No.	209-502-6

3 Synonyms, common and trade names

Antiegene MB
Antioxidant MB
AOMB
ASM MB
2-Benzimidazolethiol
2H-Benzimidazole-2-thione, 1,3-dihydro-
2-Benzimidazolinthion
2-Benzimidazolthiol
1H-Benzimidazol-2-thiol
1,3-Dihydro-2H-benzimidazol-2-thion
MBI
2-Mercaptobenzimidazol
Mercaptobenzimidazole
Merkaptobenzimidazol
2-Merkaptobenzimidazol
Permanax 21
o-Phenylenethiourea
Vulkanox MB/MG

4 Structural and molecular formulae

4.1	Structural formula	
4.2	Molecular formula	$C_7H_6N_2S$

5 Physical and chemical properties

5.1	Molecular mass, g/mol	150.20
5.2	Melting point, °C	298 (Lide and Frederikse, 1996) 301–302 (Schmitt et al., 1983) 304 (Falbe and Regitz, 1991)
5.3	Boiling point, °C	–
5.4	Vapour pressure, hPa	No information available
5.5	Density, g/cm³	Ca. 1.42 (at 20 °C) (Bayer, 1988)
5.6	Solubility in water	Insoluble (Bayer, 1988) Ca. 350 mg/l (at 25 °C) (Bayer, 1994)
5.7	Solubility in organic solvents	Soluble in acetone, ethyl acetate and ethanol (Bayer, 1988) Soluble in ethanol and methanol (Falbe and Regitz, 1991) Dissolves very well in ethanol (Lide and Frederikse, 1996)
5.8	Solubility in fat	Partition coefficient n-octanol/water log P_{ow}: 1.62 (Bayer, 1994)
5.9	pH value	–
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 6.34 mg/m³ 1 mg/m³ ≙ 0.16 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

Used as an age-resister in the rubber industry. The sodium salt serves as a flotation aid in the concentration of sulfide ores (Schmitt et al., 1983).

7 Experimental results

7.1 Toxicokinetics and metabolism

The toxicokinetics and metabolism of 2-mercaptobenzimidazole were studied in male Fischer-344-rats (137 to 166 g) following oral and intravenous administration. [^{14}C]-2-Mercaptobenzimidazole (radiochemical purity > 96.7%) was administered by oral gavage as a mixture of Emulphor EL-620, ethanol and water at doses of 49 and 0.51 mg/kg body weight and by intravenous injection of approx. 0.5 mg/kg body weight. Urine, faeces and the exhaled CO_2 were collected at intervals over a total period of 72 hours. The animals receiving oral treatment were sacrificed after 72 hours, whereas those given intravenous doses were killed 0.5, 1, 2, 6, 24 or 72 hours after dosing. The residual radioactivity was determined in blood and plasma, the gastrointestinal tract, liver, kidneys, lungs, brain, skin and muscle as well as fatty tissue and, following intravenous administration, in the spleen and thyroid gland as well. In addition to total radioactivity, the plasma, liver, kidneys, lungs and brain of the intravenously treated animals were also analysed for unmetabolised [^{14}C]-2-mercaptobenzimidazole. In a biliary excretion study, older rats (259 to 281 g) were also given approx. 0.5 mg/kg body weight intravenously, bile samples being collected via previously implanted biliary-duct cannulas over a period of 4 hours. The urinary excretion data suggest rapid absorption of 2-mercaptobenzimidazole from the gastrointestinal tract. Following intravenous dosing, radioactivity was detectable in all organs and tissues examined, with higher levels being found in the liver, kidneys and lungs than in the blood and plasma. Levels were lowest in the fatty tissue. Strikingly, the time course of radioactivity in the thyroid gland, the organ which at first sampling (30 minutes after dosing) contained by far the highest levels, showed a marked drop in levels over the next 30 minutes, but then rose again and at 6 hours after administration exceeded the plasma levels by a factor of 11. Analysis also revealed relatively high concentrations in the gastrointestinal tract between 2 and 6 hours after administration, a finding which correlated with biliary excretion. At all times of assay,

total radioactivity found in the plasma, liver, kidneys, lungs and gastrointestinal tract was higher than the levels of radioactivity attributable to unchanged 2-mercaptobenzimidazole. In the brain, total radioactivity corresponded to that of the recovered unchanged [^{14}C]-2-mercaptobenzimidazole, which led the authors to conclude that the blood-brain barrier was impermeable to metabolites of 2-mercaptobenzimidazole. With the aid of the open multi-compartment models CSTRIP and NONLINE, the authors calculated elimination half-lives from blood and plasma as being 86 and 136 minutes, respectively, for initial elimination and 83 and 22 hours, respectively, for terminal elimination. The initial half-life calculated by the investigators for clearance of unchanged 2-mercaptobenzimidazole from plasma was 125 minutes. Upon oral as well as intravenous dosing, most of the administered radioactivity was excreted in the urine (67 to 69% and 52%, respectively, in 72 hours) and a smaller amount in the faeces (19 to 25% and 31%, respectively, in 72 hours). Of the intravenously injected dose, 12% was excreted in the bile in 4 hours. No elimination as [^{14}C]-CO_2 was detected. In the animals examined at 72 hours after dosing, the investigators still recovered 2.22 and 0.902% of the orally administered radioactivity and 1.48% of the intravenously injected activity from the examined organs and tissues, including the carcass but excluding blood and gastrointestinal contents. By extrapolation of the fractions of radioactivity recovered from the blood, muscle, skin and fat samples, taking into account their percentages of total body weight as well as the radioactivity recovered from the gastrointestinal tract, the residual radioactivity in the body was found to be approx. 6.3 and approx. 2.2% for the two oral dose groups and approx. 2.7% for the intravenously treated dose group at 72 hours after dosing. The total recovery rates were between 85 and 96%. Two major metabolites and 6 quantitatively less important metabolites were excreted in urine. The structure of only one of the major metabolites was elucidated, which was benzimidazole. Another compound, which was recovered from urine in smaller amounts, was identified as unchanged 2-mercaptobenzimidazole. Benzimidazole was not found among the metabolites excreted in bile, nor was unchanged 2-mercaptobenzimidazole detected in bile (El Dareer et al., 1984).

Janssen et al. (1981) also found that upon administration to rats (strain unspecified, 12 rats/sex, 3 rats/time point of investigation) of a single oral [^{14}C]-2-mercaptobenzimidazole dose of 8.3 mg/kg body weight in polyethy-

lene glycol 400, there was an accumulation of radioactivity in the thyroid gland as well as biotransformation to benzimidazole. The investigators detected maximum radioactivity levels of 4.8 µg/ml (expressed as 2-mercaptobenzimidazole equivalents) in plasma 2 and 4 hours following administration. Subsequently, the levels of radioactivity steadily dropped from 3.1 µg/ml at 8 hours, to 0.46 µg/ml at 16 hours and 0.10 µg/ml at 24 hours. In the thyroid gland, the values were 3.5 µg/g and 6.9 µg/g after 30 minutes and 60 minutes, respectively. As reported in the study by El Dareer et al. (1984; see above) the levels then dropped for a short period of time, but subsequently showed a marked increase again (3.8 µg/g at 2 hours, 5.1 µg/g at 4 hours and a maximum of 19.5 µg/g at 16 hours). After 24 hours, the value determined for the thyroid gland was 13.9 µg/g. The ratio of radioactivity in plasma (µg/ml) to radioactivity in the thyroid gland (µg/g) was in the range between 0.79 and 1.4 up to 4 hours following administration; at 8, 12, 16 and 24 hours after dosing, the ratio was 2.5, 9.7, 42 and 139, respectively. In the thyroid gland, 2-mercaptobenzimidazole and benzimidazole accounted for most of the recovered radioactivity and were accompanied by two polar metabolites, the identity of which was not established. In plasma, only 2-mercaptobenzimidazole but no benzimidazole was detected. Of the administered radioactivity, 68 to 77% was excreted in the faeces and urine, 66% thereof in urine (collection period not specified, but presumably 24 hours). In contrast to the findings of El Dareer et al. (1984), the authors of this study only found small amounts of benzimidazole in urine, most of the radioactivity representing polar metabolites which were not further identified; unchanged 2-mercaptobenzimidazole was excreted in urine only in small amounts (Janssen et al., 1981).

7.2 Acute and subacute toxicity

Acute toxicity

Groups of 5 male and 5 females Wistar rats (97 to 156 g and 94 to 116 g, respectively) were given single oral doses of a 20-percent (w/v) suspension of 2-mercaptobenzimidazole (Vulkanox MB) in propylene glycol at 1160, 1390, 1670, 2000 and 2400 mg/kg body weight. The LD_{50} value was 1230 (1010 to 1490) mg/kg body weight. The signs of intoxication observed in all dose groups within a few hours after dosing included sluggishness and de-

creased activity. Deaths occurred from 20 hours to 11 days after treatment. The survivors recovered rapidly and looked healthy again at the end of the 14-day observation period. At autopsy of the survivors, there were no indications of pathological alterations (no details of autopsy findings of the deceased animals; TNO, 1975).

Another oral LD_{50} of 2-mercaptobenzimidazole reported for rats was given as 476 mg/kg body weight (no further details; Marhold, 1986).

The oral LD_{50} value for white mice was 1250 mg/kg body weight. No signs of intoxication were mentioned in the report (Vorobieva et al., 1963; Vorobieva and Mezentseva, 1964).

In addition, LD_{50} values for 2-mercaptobenzimidazole were reported for mice following oral administration of 750 mg/kg body weight, intraperitoneal injection of 200 mg/kg body weight and intravenous injection of 180 mg/kg body weight (no further details; RTECS, 1996).

Following intraperitoneal injection, the LD_{50} for mice was 254 mg/kg body weight (no further details; Tarakhovskij et al., 1971).

Subacute toxicity

In a dose-finding study for a subchronic inhalation study (see Section 7.5; NTP, 1984 a), F344 rats were exposed to 2-mercaptobenzimidazole concentrations of 0 (controls), 5, 15, 45, 135 and 400 mg/m³ for 6 hours per day and a total of 12 exposures. All animals of the high concentration group died. In the control group and the 5 and 15 mg/m³ groups, the survival rate was 100%. Concentration-dependent retardation of body weight gain and decreased body weights were observed at the end of the study. As a clinical sign of toxicity, tremor occurred at the highest concentration level (3 animals). In the 15, 45 and 135 mg/m³ groups, the organ weights of the brain, thymus, heart, lungs, kidneys and testes were significantly lower than those of the controls. Liver weights were increased in the male rats of the 5 mg/m³ group and the female rats of the 5, 15 and 135 mg/m³ groups. Haematologically, there was impairment of haematopoiesis associated with mild anaemia at the higher concentration levels. The chemical suppressed thyroid function and possibly adenohypophyseal function. Gross necropsy findings were primarily enlarged thyroid glands. Histopathological changes

comprised diffuse follicular cell hyperplasia of the thyroid gland, pituitary hyperplasia, hypertrophy of centrilobular hepatocytes, renal calculi, depletion of thymic lymphocytes, bone marrow hypoplasia, splenic congestion and reduced prostatic secretory activity (no further details; NTP, 1984 a).

In a preliminary study for another 90-day study (see Section 7.5; NTP, 1988 a; Gaworski et al., 1991), groups of 5 male and 5 female F344/N rats (approx. 6 to 7 weeks old) underwent a total of 12 exposures to a dry aerosol of 2-mercaptobenzimidazole (> 98% pure) for 6 hours per day, 5 times per week. The concentrations were 0 (controls), 6.3, 12.5, 25, 50 and 100 mg/m³, and the particles had a mass median aerodynamic diameter of less than 3.0 µm. There were no deaths. Clinical signs of toxicity included transient lethargy and hunched posture. The animals of the 50 and 100 mg/m³ groups exhibited retarded body weight gain. Increased liver weights as well as decreased heart, lung and thymus weights were also observed (no indication of concentration levels at or above which these findings occurred). The rats exposed to the 4 highest concentrations exhibited enlarged thyroid glands, a finding which corresponded with microscopically observed thyroid follicular cell hyperplasia. Furthermore, the male rats and female rats which inhaled concentrations ≥ 12.5 mg/m³ and ≥ 50 mg/m³, respectively, were observed to have adrenal cortex fatty accumulation, and rats of both sexes exposed to levels ≥ 25 mg/m³ displayed pituitary atrophy. At the lowest test concentration, which was 6.3 mg/m³, no substance-related changes were noted (Gaworski et al., 1991).

In order to investigate the subacute inhalation toxicity of 2-mercaptobenzimidazole, white rats were exposed to the chemical as a powder at concentration levels of 300 to 400 mg/m³ for 2 hours/day for 15 days (no details of controls or analytical verification of the test substance levels in the atmosphere). Compared with the controls, the treated rats exhibited dishevelled fur, sluggishness, delayed reflexes, decreased body weight gain as well as retardation of the oxidising processes in the tissues. Changes in rheobase and chronaxy were interpreted as effects on the functional state of the nervous system. Furthermore, malfunction of the liver was observed (no further details). The kidney weights were increased, whereas liver and heart weights were unchanged. Examination of the lungs of the treated animals revealed mild emphysema, peribronchitis and haemorrhagic areas (Vorobieva et al., 1963; Vorobieva and Mezentseva, 1964). Insufficient documentation of the conduct and the results of the study render it unsuitable

for the assessment of the systemic toxicity of 2-mercaptobenzimidazole following repeated inhalation administration.

In a 14-day preliminary study for a subchronic inhalation study (see Section 7.5; NTP, 1984 b), male and female B6C3F1 mice (number of mice/group not specified) were exposed to 2-mercaptobenzimidazole concentrations of 0 (controls), 5, 15, 45, 135 and 400 mg/m³ for 6 hours per day and a total of 12 exposures. At the highest concentration level (400 mg/m³), all exposed animals died, while at the lowest concentration (5 mg/m³) and in the control group there were no deaths (no further mortality data given). Clinical signs of toxicity included loss of body weight and reduced activity. Exposure to concentration levels of 135 and 400 mg/m³ was observed to be associated with tremors. Final body weight changes noted in comparison with the controls were a depression in the 5 and 15 mg/m³ dose groups and an elevation in the 45 and 135 mg/m³ groups. Macroscopically, the animals surviving the exposure regimen predominantly had an enlarged liver. The absolute liver weights in all groups except the females of the 5 and 15 mg/m³ groups were significantly elevated relative to the controls. The relative liver weights also showed alterations. Histopathological observations included findings of cytoplasmic swelling of hepatocytes, thyroid and pituitary hyperplasia, lymphocytolysis and lymphocyte depletion within the thymus and to a lesser extent in the spleen. In one of the survivors from the 135 mg/m³ group, necropsy revealed renal calculi (no further details; NTP, 1984 b).

Thyrotoxicity studies

Ten male rats (strain unspecified) received daily intragastric doses of 21 mg 2-mercaptobenzimidazole/kg body weight for 12 days. In comparison with the 10 male rats used as controls receiving only the vehicle, polyethylene glycol 400, treatment resulted in a significant increase in relative thyroid weights and a marked reduction in plasma thyroxine levels (1.21 µg/100 ml compared with 7.11 µg/100 ml in the controls). As a mechanism of the chemical's anti-thyroid action, the authors proposed that the peroxidases present in the thyroid gland are inhibited, a process which interferes with iodide metabolism and finally leads to a decrease in thyroxine formation. As a result, increased amounts of thyroid-stimulating hormone (TSH), or thyrotropin, are secreted by the anterior lobe of the pituitary gland, a re-

sponse which ultimately results in hypertrophy of the thyroid gland (Janssen et al., 1981).

As early as 1950, Searle et al. showed that in rats the uptake of radioactive iodine (^{131}I) by the thyroid gland can be inhibited by approx. 95% by a single oral dose of 7.5 mg 2-mercaptobenzimidazole/kg body weight (radioisotope administration one hour after dosing with 2-mercaptobenzimidazole, thyroid gland preparation 4 hours after dosing with the test substance; Searle et al., 1950).

This specific thyrotoxicity correlates with the accumulation of the test compound and its main metabolite, benzimidazole, in the thyroid gland (cf. Section 7.1).

7.3 Skin and mucous membrane effects

In a standard Draize test in the rabbit, 2-mercaptobenzimidazole proved to be moderately irritating. A 500 mg dose was applied to the skin for 24 hours (no further details; Marhold, 1986).

When applied to the eyes of rabbits for 24 hours in a standard Draize test, 500 mg of 2-mercaptobenzimidazole were found to cause moderate irritation (no further details; Marhold, 1986).

In rats and mice, investigation of local skin and eye irritancy gave no indications of irritant effects (no further details; Vorobieva et al., 1963; Vorobieva and Mezentseva, 1964).

Equally, 2-mercaptobenzimidazole produced no irritation of the skin and eyes of rabbits (no further details; WTR, 1984; Bayer, 1988).

7.4 Sensitisation

In a modified maximisation test carried out in guinea pigs, 2-mercaptobenzimidazole, dissolved in olive oil as the vehicle, was tested for its sensitising potential at concentration levels of 0.5 and 5%. The first induction was carried out intradermally with 0.5 and 5% 2-mercaptobenzimidazole both with and without emulsified Freund’s adjuvant. In addition to a solvent control, the study included a positive control with 2,4-dinitrochlorobenzene (0.1%).

The second induction was performed dermally using a 20-percent formulation of 2-mercaptobenzimidazole. The challenge was carried out with 2-mercaptobenzimidazole at concentration levels of 0.05, 0.5 and 5%. Under these experimental conditions, 2-mercaptobenzimidazole and the fungicide methyl-2-benzimidazole carbamate, which was tested in parallel, did not cause sensitisation. By contrast, ethylene thiourea (cf. Toxicological Evaluations, volume 12 of the book series published by Springer), which was tested in parallel, proved to be a skin-sensitising agent. In addition, cross-reactivity was investigated. Animals which had undergone induction with ethylene thiourea showed no reaction following challenge with 2-mercaptobenzimidazole. Similarly, guinea pigs in which induction was carried out with 2-mercaptobenzimidazole or ethylene thiourea showed no skin reactions subsequent to treatment with products chemically related to 2-mercaptobenzimidazole (methyl-2-benzimidazole carbamate, 2-mercaptobenzothiazole, benzimidazole, benzene, imidazole, 2-mercapto-2-thiazoline, 2-mercapto-1-methylimidazole and thiourea). Ethylene thiourea (> 0.5%) elicited skin reactions in guinea pig which had received induction treatment with > 0.5% 2-mercaptobenzimidazole (Shimizu et al., 1994).

Technical-grade 2-mercaptobenzimidazole and technical 2-mercaptobenzothiazole (cf. Toxicological Evaluations, volumes 4 and 16 of the book series published by Springer; the publication contains no details of the types of impurities) which were used in a rubber production plant in Bratislava were tested in male albino guinea pigs with regard to their sensitising potential. Induction was carried out in different ways in 4 series of tests. In test series I, groups of 36 guinea pigs received daily epicutaneous applications of 0.03 ml of a 3-percent acetone solution of 2-mercaptobenzimidazole and 2-mercaptobenzothiazole for a period of 3 weeks. The total dose of 2-mercaptobenzimidazole and 2-mercaptobenzothiazole applied per animal was 18.9 mg. The animals in test series II (40 guinea pigs per group) were given intradermal injections of 0.1 ml heparin and subsequently, on 8 days within a period of 3 weeks, they each received epicutaneous applications of 0.03 ml of a 5-percent acetone solution of 2-mercaptobenzimidazole or 2-mercaptobenzothiazole in the area around the heparin injection site. The total dose of 2-mercaptobenzimidazole and 2-mercaptobenzothiazole administered per animal was 12 mg in both cases. The guinea pigs in test series III (groups of 40 animals) were given intradermal injections of 1000 µg 2-mercaptobenzimidazole or 2-mercaptobenzothiazole in 0.01 ml Freund's

adjuvant into the ear. In test series IV, 36 (2-mercaptobenzimidazole) and 42 (2-mercaptobenzothiazole) guinea pigs received daily epicutaneous 0.03 ml applications of a 3% acetone solution of 2-mercaptobenzimidazole and 2-mercaptobenzothiazole, respectively, plus intradermal injections of 100 µg 2-mercaptobenzimidazole and 2-mercaptobenzothiazole, respectively, as solutions in 0.01 ml Freund's adjuvant for 3 weeks. The total dose of 2-mercaptobenzimidazole and 2-mercaptobenzothiazole which was administered per animal was 18.3 mg. Each test series included a control group of 25 animals which was treated with the solvent in the appropriate manner. Eleven to 14 days after the end of the induction phase, the guinea pigs underwent epicutaneous challenge with 750 µg 2-mercaptobenzimidazole or 2-mercaptobenzothiazole which was applied to the untreated skin of the flank. In all 4 test series, the animals treated with 2-mercaptobenzimidazole or 2-mercaptobenzothiazole exhibited sensitisation reactions. The results of the studies are summarised in Table 1.

Table 1. The sensitising potential of 2-mercaptobenzimidazole and 2-mercaptobenzothiazole in guinea pigs as studied using a modified epicutaneous test with and without Freund's adjuvant

Test series	Substance	Number of animals with positive reactions relative to total number	
		Absolute figures	%
I	2-Mercaptobenzimidazole	4/36	11
	2-Mercaptobenzothiazole	6/36	17
II	2-Mercaptobenzimidazole	4/40	10
	2-Mercaptobenzothiazole	6/40	15
III	2-Mercaptobenzimidazole	2/40	5
	2-Mercaptobenzothiazole	4/40	10
IV	2-Mercaptobenzimidazole	7/36	19
	2-Mercaptobenzothiazole	18/42	43

With the exception of the guinea pigs from the 2-mercaptobenzothiazole group of test series IV, the skin changes were minor in all of the animals showing a positive reaction to 2-mercaptobenzimidazole or 2-mercaptobenzothiazole, and in general, technical-grade 2-mercaptobenzimidazole caused less skin sensitisation than did technical-grade 2-mercaptobenzothiazole. The authors evaluated the investigated technical-grade 2-mercaptobenzimidazole as a weak skin-sensitising agent according to the Magnusson

and Kligman classification. In cross-reactivity tests, challenge with 2-mercaptobenzothiazole induced skin reactions in 9 out of 36 animals having received pretreatment with 2-mercaptobenzimidazole (total dose/animal 18.3 mg), and conversely, following challenge with 2-mercaptobenzimidazole, 6 out of 42 guinea pigs pretreated with 2-mercaptobenzothiazole (total dose/animal 18.3 mg) developed skin reactions as a sign of group allergy. In a further series of tests, macrophage migration from spleens (samples of 50 µg tissue/ml each) of animals from test series IV were investigated in comparison with spleens taken from untreated guinea pigs. Following pretreatment with 2-mercaptobenzimidazole or 2-mercaptobenzothiazole, the migration of splenic macrophages was found to be inhibited, with cytotoxicity indices being 0.76 and 0.49, respectively, as compared with the control (0.9 to 1.16), a finding which according to the investigators' opinion suggested the development of allergy on a cellular basis, induced by 2-mercaptobenzimidazole and 2-mercaptobenzothiazole (Barlogova and Stolcova, 1984).

7.5 Subchronic and chronic toxicity

Groups of 10 male and 10 female F344 rats were exposed to 2-mercaptobenzimidazole concentration levels of 0 (controls), 0.1, 0.3, 1, 3 and 10 mg/m³ for 6 hours per day, 5 times per week for 13 weeks. At the highest concentration of 10 mg/m³, 5 of the exposed females died at weeks 12 and 13 of the study. Clinical signs of toxicity included emaciation and decreased activity. There was body weight depression in the 10 mg/m³ females. At the end of the study, body weights were lower in the 0.3 and 10 mg/m³ females and the 10 mg/m³ males than they were in the controls. Changes were observed with respect to various organ weights, particularly the thyroid gland weight (which presumably was increased) in both sexes of the 3 and 10 mg/m³ groups. The thyroids were found to be enlarged at necropsy as well. The relative weights of these organs also showed alterations. The haematology and clinical chemistry parameters exhibited changes even at the lowest test concentration, such as decreased erythrocyte parameters (no further details), the females showing increased lymphocyte counts, inhibition of the coagulation cascade, impairment of renal clearance, suppression of thyroid function, while at the high concentrations, an increase in plasma pseudocholinesterase activity and elevated serum protein levels (albumin and globulin) were seen. Histopathologically, the 10 mg/m³

exposure was found to be associated with follicular cell hyperplasia of the thyroid gland, while at concentration levels of 3 and 10 mg/m³ thyroids were hypertrophic. Cytological changes associated with the loss of and replacement of cells were observed in the adenohypophysis. In the two high dose groups, renal calculi were diagnosed together with tubular degeneration and biochemical evidence of nephrotoxicity (no further details). At the highest concentration, there was depletion of thymic lymphocytes and suppression of erythropoiesis. In 4 out of the 5 female rats of the highest dose group which died intercurrently, adrenocortical necrosis was observed (no further details; NTP, 1984 a).

In a further subchronic inhalation toxicity study, groups of 10 male and 10 female F344/N rats (approx. 6 to 7 weeks old) were exposed to a dry aerosol of 2-mercaptobenzimidazole (> 98% pure) for 6 hours per day, 5 times per week for a period of 13 weeks. The concentrations were 0 (controls), 3.13, 6.25, 12.5, 25 and 50 mg/m³ air, and the particles had a mass median aerodynamic diameter ranging between 2.0 and 2.3 µm. An additional 19 male rats/group were included in the control group as well as the 3.13, 12.5 and 50 mg/m³ dose groups for special studies regarding the pituitary and thyroid glands (see below; Norford et al., 1993). Prior to the beginning of exposure and at 2, 4 and 8 weeks, the levels of triiodothyronine (T_3) and thyroxine (T_4), inter alia, were determined in these animals. In the 50 mg/m³ group, 10 of the 29 male rats and all 10 females died in the course of the study or had to be sacrificed in a moribund condition. Moribund animals were often noted to be ataxic or comatose, and hypothermic. In the 50 and 25 mg/m³ dose groups, the animals had a hunched posture and displayed hypoactivity. Females had a greater overall incidence as well as an earlier time of onset of the clinical signs. From 25 mg/m³, both sexes exhibited a dose-related decrease in body weight gain. Concentration levels of and above 12.5 mg/m³ caused dose-dependent anaemia, which was statistically significant from 25 mg/m³. In the male rats of the 50 mg/m³ group and the female rats of the 25 mg/m³ group, prothrombin and activated partial thromboplastin times were increased. The leukocyte counts were depressed for the males of all concentration groups. The 25 mg/m³ males exhibited elevated levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and sorbitol dehydrogenase activity, while both sexes of the 25 mg/m³ group had increased levels of blood urea nitrogen and cholesterol as well as decreased blood levels of free fatty acids.

Males and females treated with doses ≥ 6.25 mg/m³ displayed statistically significant increases in absolute and relative thyroid weights. With regard to the thyroid hormones, the male rats of the 12.5 mg/m³ group showed a drop in T_3 levels at 2, 4 and 8 weeks, which, however, was reversible after 13 weeks. In the 50 mg/m³ group, T_3 levels were depressed at 2 weeks but found to have recovered to control levels by the end of the study. T_4 concentrations in the 50 mg/m³ group were below the limit of detection from 2 weeks until the end of the study. In the 12.5 mg/m³ group, decreased T_4 concentrations were also seen at 2, 4 and 8 weeks, but levels recovered by termination of the study. The 3.13 mg/m³ dose did not affect the levels of thyroid hormones in the male rats. Histopathologically, both sexes of the groups receiving 3.13 mg/m³ or higher doses exhibited thyroid follicular cell hyperplasia, the extent of which was concentration-dependent. In all exposure groups, there was a concentration-dependent decrease in absolute and relative thymus weights. Relative liver weights were increased in the males and the females at and above dose levels of 6.25 mg/m³ and 3.13 mg/m³, respectively. Histopathological examination revealed thymic atrophy from 12.5 mg/m³ and hepatocyte hypertrophy at exposure levels of 25 mg/m³ and above. Further histopathological lesions were noted in the kidneys (tubular atrophy and mineralisation from 25 mg/m³), the adrenal glands (necrosis and degeneration from 25 mg/m³), the pancreas (hyperplasia of islet cells from 25 mg/m³), the mesenteric lymph nodes (hyperplasia from 25 mg/m³), the pituitary gland (cytoplasmic vacuolisation from 12.5 mg/m³, proliferation of the thyrotropic hormone-producing basophilic cells of the anterior adenohypophysis), the bone marrow (hypocellularity from 25 mg/m³) and the nasal cavity (cystic degeneration of the respiratory epithelium from 12.5 mg/m³). Thyroid hyperplasia and decrease in thymus weight thus proved to be the most sensitive parameters. Both findings occurred even at the lowest test concentration of 3.13 mg/m³ so that it was not possible to determine a *no observable effect level* in this study (NTP, 1988 a; Gaworski et al., 1991).

The pituitary and thyroid glands of the 19 additional male rats of the 0, 3.13, 12.5 and 50 mg/m³ groups in the above-mentioned study were examined by electron microscopy and histochemical techniques. These investigations revealed that dose-dependent follicular hyperplasia and hypertrophy occurred in the thyroid gland and that all exposure groups were affected after only two weeks of exposure. The thyrotrophs of the pituitary gland

were hyperplastic in all three groups and they had varying numbers of hypertrophic cells with either eosinophilic stippled cytoplasm or with eosinophilic globules within one or more large vacuoles that displaced the nucleus. These cells were compared by immunohistochemistry and electron microscopy to “thyroidectomy cells” within the anterior pituitary glands of thyroid-parathyroidectomised rats and were determined to be identical to them. Immunohistochemical staining for the β-chain of thyroid-stimulating hormone (TSH) confirmed that the hyperplastic and hypertrophic cells were thyrotrophs. Electron microscopy demonstrated the presence of expanding cytoplasm containing endoplasmic reticulum with dilated cisternae, which displaced other cellular organelles. In this context, 2-mercaptobenzimidazole was considered to be comparable to other derivatives of thiourea which have been shown to produce low serum concentrations of triiodothyronine and thyroxine and to increase the synthesis and secretion of TSH, which results in thyroid hypertrophy and hyperplasia and ultimately leads to goitre (Norford et al., 1993).

Groups of 10 male and 10 female B6C3F1 mice were exposed to 2-mercaptobenzimidazole concentration levels of 0 (controls), 0.1, 0.3, 1, 3 and 10 mg/m³ for 6 hours per day, 5 days per week for 13 weeks. In the high concentration group one male died, and another 5 males and 9 females of the 3 mg/m³ group also died. Liver weights were increased in the male mice exposed to levels of 3 and 10 mg/m³ and in the female mice exposed to 10 mg/m³. Kidney weights were increased in the females of the 0.1, 0.3 and 10 mg/m³ groups, and the 10 mg/m³ males and females had higher thyroid weights compared with the controls. Decreased organ weights were observed for the brains of the females exposed to 1 mg/m³ and for the thymus glands of the males exposed to 3 mg/m³ or more. For those organs, relative organ weights were also altered. Macroscopically, no compound-related lesions were observed. Histopathological findings included thyroid hyperthrophy, hepatic centrilobular cytomegaly and lipoid degeneration of the zona reticularis of the adrenal glands (no further details; NTP, 1984 b).

In a further subchronic inhalation toxicity study in B6C3F1 mice, groups of 10 males and 10 females underwent whole-body exposure to a dry aerosol of 2-mercaptobenzimidazole for 6 hours per day, 5 days per week over a period of 90 days. The concentration levels were 0 (controls), 3.13, 6.25, 12.5, 25 and 50 mg/m³ air. The overall mean aerosol mass concentrations for the entire study period were within ± 2% of the target values in all expo-

sure chambers. The mean particle size was in the range of 2.0 to 2.3 µm mass median aerodynamic diameter. Results of monitoring for potential degradation products showed the test substance to be unaltered by the aerosol generation. No exposure-related deaths occurred in this study, and the animals exhibited no clinical signs of intoxication. The males which were exposed to 2-mercaptobenzimidazole concentrations of 12.5 mg/m³ or less, and the females which were exposed to 2-mercaptobenzimidazole concentrations of 25 mg/m³ or less gained slightly more body weight than did the controls in the study (weight increase in male mice was between 11 and 19%, in female mice between 15 and 28%). Body weight gain in the mice from the other dose groups was comparable to that of the mice serving as controls. At autopsy, the male and female mice of all exposure groups were found to have enlarged thyroid glands. Relative thyroid weights were significantly increased in the male mice of the 25 and 50 mg/m³ groups and in the females of the 50 mg/m³ group. In the lower exposure groups, the relative thyroid weights were also increased, but the changes were not statistically significant. The relative liver weights of the animals from the two highest exposure groups were significantly greater than controls. Light microscopy was used to diagnose the following exposure-related organ changes: thyroid follicular cell hyperplasia in the male mice exposed to a concentration of 6.25 mg/m³ and in the female mice at and above an exposure level of 3.13 mg/m³, the incidence increasing in a dose-dependent manner. At the two highest concentrations, all mice exhibited these ultrastructural tissue changes. In addition, 2 out of 10 males and 1 out of 10 females of the 50 mg/m³ group were found to have focal hyperplasia of the thyroid gland. Hyperplasia of the epithelium of the bronchioles and centrilobular hypertrophy of the liver with intranuclear inclusion bodies were diagnosed in both sexes at 2-mercaptobenzimidazole concentrations of 12.5 mg/m³ or above. The incidence of these alterations also increased in a concentration-dependent manner. Degeneration of the zona reticularis of the adrenal gland was seen only in the female mice, with 9 out of 10 females being afflicted in the 3.13 mg/m³ group and all 10 females in the groups exposed to 2-mercaptobenzimidazole concentrations of 6.25 mg/m³ or above. Based upon these histopathological changes, the *no observable effect level* (NOEL) of 2-mercaptobenzimidazole for male mice was 3.13 mg/m³. For female mice, a NOEL was not determined, as even the lowest exposure group (3.13 mg/m³) was found to have ultrastructural changes in the thyroid and adrenal glands (NTP, 1988 b).

Sperm motility and vaginal cytology investigations which were carried out in connection with the studies described above are discussed in Section 7.8.

Rabbits were given 2-mercaptobenzimidazole by oral gavage at 20 mg/kg body weight/day for 4 months. Treatment was administered every two days during the first two months and every day during the third and fourth month (no information given on controls). Haematological and clinical chemistry tests were carried out prior to study initiation and at study termination. 2-Mercaptobenzimidazole produced effects on serum proteins (altered albumin-globulin ratio) and the formation of prothrombin in association with haemorrhages. Whereas aldolase activity was elevated, there was no significant change in alkaline phosphatase or aminotransferase activities. In addition, anaemia developed and there was a leftward shift in white blood cell counts. Histopathological examination revealed focal fatty dystrophy of the liver, pulmonary emphysema as well as myocardial haemorrhages (no further details; Vorobieva et al., 1963; Vorobieva and Mezentseva, 1964). Insufficient documentation of the conduct and the results of the study render it unsuitable for the assessment of the systemic toxicity of 2-mercaptobenzimidazole following repeated oral administration.

7.6 Genotoxicity

7.6.1 In vitro

2-Mercaptobenzimidazole was tested for its genotoxic potential in the Salmonella/microsome assay with preincubation using *Salmonella typhimurium* strains TA 97, TA 98, TA 100 and TA 1535 with and without metabolic activation (S9 mix from Aroclor 1254-induced rat and Syrian hamster livers). The substance was found not to cause gene mutations. The test concentrations were in the range from 3.3 to 1000 µg/plate (Zeiger et al., 1987).

From a further Salmonella/microsome assay (plate incorporation test) performed in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 it was also concluded that the substance was devoid of mutagenic potential both in the absence and presence of metabolic activation (S9 mix from Aroclor 1254-induced rat liver; Rannug et al., 1984).

Pure 2-mercaptobenzimidazole (melting point between 229 and 303 °C) and technical-grade 2-mercaptobenzimidazole, both produced at a chemi-

cal plant in Shanghai, were tested at concentrations of 25 to 500 µg/plate for their genotoxic potential in the Salmonella/microsome assay (unspecified whether plate incorporation or preincubation test) using strains TA 98 and TA 100 with and without metabolic activation (no details given regarding the preparation of the S9 mix), with DMSO serving as a solvent. Induction of positive control responses in the experiments without S9 mix and those with S9 mix was accomplished with 2 µg daunomycin and 5 µg 2-aminofluorene, respectively. With strains TA 98 and TA 100, pure 2-mercaptobenzimidazole gave negative results both in the presence and absence of metabolic activation. Technical-grade 2-mercaptobenzimidazole was also devoid of mutagenicity in strain TA 100 in the absence and presence of S9 mix and in strain TA 98 in the absence of metabolic activation. When tested in strain TA 98 in the presence of S9 mix, technical-grade 2-mercaptobenzimidazole produced a significant concentration-dependent increase in revertant counts compared with the solvent control (Feng et al., 1988).

Prompted by the mutagenic effect of technical-grade 2-mercaptobenzimidazole manufactured at a Shanghai chemical plant on *Salmonella typhimurium* strain TA 98 in the presence of metabolic activation, a test (in vivo/in vitro) employing the same Salmonella strain both with and without addition of S9 mix was carried out on urine samples obtained from exposed and unexposed individuals. For this purpose, urine samples were collected from 12 employees working at the 2-mercaptobenzimidazole production plant (7 men, 5 of them nonsmokers; 5 women, all of them nonsmokers) and 10 individuals who were not working at the plant (6 men, 4 of them nonsmokers; 4 women, all of them nonsmokers). After 4 consecutive working days, urine samples were collected from each subject over a period of 8 hours on the fifth day. The samples were then filtered, stored at –80 °C, thawed and filtered again prior to analysis. The substances contained in 100 ml of urine were concentrated and isolated, dissolved in DMSO (spectro grade) and diluted in a dilution series (from 1 : 2 to 1 : 64). The mutagenicity test was performed with aliquots of 100 µl, using DMSO as the solvent. Induction of positive control responses in the experiments without S9 mix and those with S9 mix was accomplished with 2 µg methyl methanesulfonate and 8 mg cyclophosphamide, respectively. An amount of 0.5 mg β-glucuronidase was added to each plate. All urine samples obtained from the 12 employees of the 2-mercaptobenzimidazole production plant gave positive results for one or several dilutions when tested in strain TA 98 with S9 mix.

The most pronounced increases in revertant counts were found in smokers. In 8 out of the 10 subjects who were not employed at the chemical plant, the urine samples were not found to produce a significant increase in revertant counts. These studies demonstrate that the risk of mutagenicity was increased in the employees of the Shanghai production plant, a finding which the investigators attributed to impurities in the technical-grade 2-mercaptobenzimidazole produced there (Feng et al., 1988).

2-Mercaptobenzimidazole was tested for mutagenic activity (forward mutation) in the mouse lymphoma assay (L5178Y) with and without metabolic activation (S9 mix from Aroclor 1254-induced rat liver). The concentration ranges investigated in the absence and presence of metabolic activation were 12.5 to 500 µg/ml (10 concentrations) and 0.156 to 300 µg/ml (24 concentrations), respectively. Without metabolic activation, 2-mercaptobenzimidazole proved not to be mutagenic at concentration levels of up to 75 µg/ml, while at 100 µg/ml there were three negative and two positive tests, one of the latter occurring in conjunction with high cytotoxicity. At 150 µg/ml, 2-mercaptobenzimidazole had no genotoxic effect in any of 4 test series which included that concentration. Levels of 200 and 300 µg/ml, which were included in two series, induced significantly increased mutation rates, which however were associated with high cytotoxicity. At concentration levels of 400 µg/ml and 500 µg/ml, 2-mercaptobenzimidazole produced complete inhibition of cellular growth. In the presence of metabolic activation, the results of the 7 test series varied greatly. On the one hand, positive effects were obtained with concentrations from 1.25 to 4.0 µg/ml. On the other hand, 2-mercaptobenzimidazole produced no mutagenicity even at concentrations of up to 100 µg/ml. Equally, the concentrations showing cytotoxic activity varied from 2 to 150 µg/ml in the different series. No evaluation was provided by the authors (NTP, 1995). Without metabolic activation, 2-mercaptobenzimidazole produced mutagenicity in the concentration range with marked to high cytotoxicity. In one of the 5 test series, a concentration dependency was observed which correlated with the cytotoxicity. As this finding was not reproducible in the other 4 test series, it is not possible to judge its relevance to the evaluation of the genotoxic potential of 2-mercaptobenzimidazole. The data from the studies carried out with metabolic activation are too heterogeneous to permit the assumption that 2-mercaptobenzimidazole has a genotoxic potential.

In the context of a study of 18 chemical compounds of different structure, 2-mercaptobenzimidazole used at a rubber production plant in Bratislava (cf. Section 7.4; Barlogova and Stolcova, 1984) was tested for mutagenicity in the HPRT test using the V79 cell line derived from lung tissue of a male Chinese hamster in the absence and presence of metabolic activation. The comparison included, inter alia, three directly alkylating carcinogens (methylmethane sulfonate, N-methyl-N-nitrosourea and N-methyl-N'-nitro-N-nitrosoguanidine) as well as two polycyclic aromatic hydrocarbons requiring metabolic activation to elicit carcinogenic activity (3,4-benzo(a)pyrene and 7,12-dimethylbenzo(a)anthracene). In contrast to methyl methanesulfonate, N-methyl-N-nitrosourea and N-methyl-N'-nitro-N-nitrosoguanidine, both of which induced gene mutations in the absence of S9 mix, and 3,4-benzo(a)pyrene as well as 7,12-dimethylbenzo(a)anthracene, which only did so upon addition of S9 mix, 2-mercaptobenzimidazole gave negative results at the test concentration of 100 µg/ml in the absence and presence of metabolic activation (Slamenová et al., 1986). Note: The duration of treatment was only 60 minutes in these experiments, which is considerably shorter than the 4-hour treatment that is generally used – and recommended by the international guidelines. However, a negative result – as reported in the study under discussion – should be evaluated with caution, not least because no cytotoxicity data are given on the 100 µg/ml concentration and the report contains only qualitative mutagenicity results but no absolute or relative figures.

Following a 72-hour incubation of human leukocyte cultures with the sodium salt of 2-mercaptobenzimidazole at concentration levels of 0.06 and 0.3 mg/ml, the metaphases were examined for numerical and structural chromosome aberrations. Leukocyte cultures which were treated with 0.1 ml of the solvent (distilled water) in a similar manner served as controls. The cytogenetic changes induced by 2-mercaptobenzimidazole were not concentration-dependent: at 0.06 and 0.3 mg/ml, pathological mitoses accounted for 42.8% and 33%, respectively. In the 0.3 mg/ml group, 21 out of 120 (17.5%) studied mitoses were aneuploid. In the control, the frequency of aneuploid cells was 5.6%. In the group treated with 0.3 mg/ml 2-mercaptobenzimidazole, the percentages of cells with hypodiploid and hyperdiploid chromosome complements were 12.5 and 5.0%, respectively, which was also higher than the controls. The frequencies of structural chromosomal aberrations were 25% and 21% in the 0.06 and the 0.3 mg/ml group,

respectively. Based on these findings, the investigator concluded that 2-mercaptobenzimidazole was clastogenic (Barilyak, 1974). Lack of information on the purity of the 2-mercaptobenzimidazole sodium salt used as the test substance and the absence of a detailed description of methods employed and data obtained limit the usefulness of the study results for the purposes of assessment.

7.6.2 In vivo

Eight male Wistar rats weighing from 150 to 180 g were sacrificed 48 hours after intraperitoneal treatment with a single 2-mercaptobenzimidazole dose of 30 mg/kg body weight, and 1220 bone-marrow metaphases obtained from the animals were examined cytogenetically. A control was included which consisted of 1500 metaphases from the bone-marrow of untreated rats. Compared with the control, which had an aneuploidy frequency of 13.8% and showed no structural aberrations, increases in aneuploidy (23.9%) and structural aberrations (4.5%) were observed in the rats which were sacrificed 48 hours after being injected with 2-mercaptobenzimidazole. Similarly, 4-week-old rats from dams treated intraperitoneally with a single 30 mg/kg body weight dose of 2-mercaptobenzimidazole on day 13 of gestation (12 rats, number of examined metaphases: 1000) were found to have a higher frequency of aneuploidy (50.9%) compared with controls (10 rats, number of examined metaphases: 1000; 13.6% aneuploid cells) and a higher frequency of structural aberrations (controls 0%; 2-mercaptobenzimidazole 5.3%) in the bone-marrow (Barilyak and Mel'nik, 1979). Note: The purity of the 2-mercaptobenzimidazole used in the tests was not specified. Moreover, the high frequencies of aneuploidy which were seen particularly in the two control groups and the complete absence of structural aberrations among 1500 and 1000 control cells, respectively, were noteworthy. There are no other reports of such a finding in the cytogenetic literature (Miltenburger, 1997). For the reasons given, it is not possible to evaluate the chromosomal changes described above.

In order to study the clastogenic effect of 2-mercaptobenzimidazole, two blood smears per mouse were obtained from B6C3F1 mice of the 90-day inhalation study (test concentrations of 2-mercaptobenzimidazole 0 (controls), 3.13, 6.25, 12.5, 25 and 50 mg/m³ air; see Section 7.5; NTP, 1988 b) at terminal sacrifice of the control group and of the mice exposed to 2-mer-

captobenzimidazole concentrations of 6.25, 12.5 and 50 mg/m³. At a contract cytogenetics laboratory, the blood smears were scored for micronuclei (10000 normochromatic erythrocytes and 2000 polychromatic erythrocytes/animal). It was not possible to incorporate a positive control because of the nature of the experimental design (90-day study). In none of the groups treated with 2-mercaptobenzimidazole were any of the male or female mice found to have increased numbers of micronucleated polychromatic or normochromatic erythrocytes as compared with the controls. Thus, 2-mercaptobenzimidazole was devoid of clastogenicity under these experimental conditions (NTP, 1995). The result of the micronucleus test carried out at the end of a 90-day inhalation study must be considered relevant in accordance with OECD guideline No. 474 (1995), as in this 90-day study systemic toxicity was observed in the female mice at all 5 test concentrations and in the male mice at concentration levels of and above 6.25 mg/m³, and also because the mice received 2-mercaptobenzimidazole treatment up to the time of sacrifice (when blood smears were obtained).

There are two publications from the literature reporting tests carried out with 2-mercaptobenzimidazole to investigate the induction of recessive sex-linked lethal mutations (Muller-5 test) in *Drosophila melanogaster*. In the context of a study of benzimidazole and 20 benzimidazole derivatives which were synthesised at the Institute of Physico-Organic Chemistry of the Byelorussian Academy of Sciences, 2-mercaptobenzimidazole was also tested at concentration levels of 2 to 5 mg/ml. Only one out of a total of 963 investigated chromosomes was found to carry a lethal mutation. The lethal mutation rate of 2-mercaptobenzimidazole was 0.17% and hence lower than that of the incorporated control, which was 0.21% (6 out of 2869 lethal mutations). Thus, 2-mercaptobenzimidazole tested negative in this study, as did benzimidazole itself and 18 of the other 20 benzimidazole derivatives (Goncarova and Levina, 1980). No further details were reported in terms of the chemical specifications of the compounds under investigation, including 2-mercaptobenzimidazole. According to an older study, a 2-mercaptobenzimidazole concentration of 0.5 mg/ml caused lethal mutations in 8 out of 267 chromosomes studied (3%). The lethal mutation rate in the control was 0.3% (Barilyak, 1974). Precise details of the methodology employed, the results obtained and the chemical specifications of the 2-mercaptobenzimidazole used in the study are lacking in Barilyak's work (1974). The results in both publications are questionable. Therefore, these publica-

tions can not be used for the purpose of evaluating the genotoxic potential of 2-mercaptobenzimidazole.

7.7 Carcinogenicity

Together with 23 other chemical compounds, 2-mercaptobenzimidazole was tested for its potential to induce transformation in a modified BALB/c-3T3 cell transformation assay which was more sensitive than previously published methods of testing for cell transformation. Each of the 24 chemicals investigated in the study was tested at 4 concentration levels in at least two independent trials. The 4 treatment levels were selected on the basis of the cytotoxic activity observed in the "co-culture cloning survival assay". Per transformation assay, 3 to 6 substances were tested together with 3,4-benzopyrene (0.200 and 0.0633 µg/ml) as the positive control substance. In addition, each trial included as a second positive control a substance which had tested positive in an earlier experiment. For 12 of the 24 chemicals tested, amongst them 2-mercaptobenzimidazole, the assay gave clear positive results at two or more consecutive concentration levels in the absence of a metabolic activation system. DMSO served as the solvent for 2-mercaptobenzimidazole. With an LC_{50} of 3.25 mM, equivalent to 0.488 µg/ml, 2-mercaptobenzimidazole was assessed by the investigators as being moderately cytotoxic (Matthews et al., 1993).

7.8 Reproductive toxicity

In vivo studies

In order to investigate the teratogenic potential of 2-mercaptobenzimidazole, female Wistar rats were given a single dose of 120 mg/kg body weight in corn oil by stomach intubation on day 12 or 13 of gestation (according to the investigators, higher doses caused maternal toxicity or death). On day 22 of gestation, the individual litter weight, litter size, number of deciduomas and corpora lutea were determined. Part of the foetuses were examined for skeletal anomalies, while the remainder were examined for visceral anomalies. At maternally tolerated dose levels, 2-mercaptobenzimidazole was not detected to possess embryocidal or teratogenic activity (Ruddick et al., 1976).

In a dose-finding study, groups of 5 or 6 3-month-old pregnant Wistar rats (SPF) received > 97% pure 2-mercaptobenzimidazole by oral gavage at dose levels of 0 (controls), 2.5, 5, 10, 20, 40 and 60 mg/kg body weight/day from day 7 to 17 after mating (day 0 after mating = day on which sperm was found in vaginal smears). Following laparotomy on day 20 after mating, the dams and foetuses were examined. Maternal body weight gain and food consumption were reduced following daily 2-mercaptobenzimidazole doses of ≥ 40 and ≥ 20 mg/kg body weight. Four out of 5 rats of the 60 mg/kg group and 1 out of 6 rats of the 40 mg/kg group died. The 8 foetuses from the surviving rat of the 60 mg/kg group had anasarca (extensive oedema of the subcutaneous tissue) and cleft palate. All foetuses from dams treated with daily doses of 40 mg/kg body weight were without appreciable findings. Based on the findings from the preliminary study, the main study was carried out with groups of 20 pregnant Wistar rats (SPF) which were treated by oral gavage with daily doses of 0 (controls), 3.3, 10 and 30 mg 2-mercaptobenzimidazole/kg body weight in olive oil from day 7 to 17 after mating. Following laparotomy on day 20 of gestation, the dams and foetuses were examined. Because 2-mercaptobenzimidazole was highly toxic at a daily dose level of 60 mg/kg body weight, additional groups of 17, 16 and 16 pregnant rats were treated by oral gavage with daily doses of 60 mg 2-mercaptobenzimidazole/kg body weight only on gestation days 7 to 10, 11 to 14 and 15 to 17, respectively. Even under these short-term treatment conditions, 2-mercaptobenzimidazole was severely toxic to dams at 60 mg/kg body weight per day, since all rats showed a substantial decrease in body weight and food consumption and 5 rats died out of the 16 treated from day 11 to 14 after mating. There was an increased foetal resorption rate in all three 60 mg/kg groups. Of the surviving foetuses from the dams treated with 60 mg 2-mercaptobenzimidazole/kg body weight from day 11 to 14 after mating, 40% were found to have a cleft palate. No other anomalies were observed. In the pregnant rats treated with 3.3, 10 or 30 mg/kg body weight from day 7 to 17 after mating, body weight gain was less in comparison with the controls. Furthermore, the thymus weights of the dams from all three 2-mercaptobenzimidazole-treated groups and the thyroid weights of the dams from the 10 and 30 mg/kg groups were significantly lower than those of the controls. Thus for the dams, the *no observed adverse effect level* (NOAEL) of 2-mercaptobenzimidazole was found to be < 3.3 mg/kg body weight/day. The foetal resorption rates were comparable to those seen in the control group. There were no visceral or skeletal mal-

formations. Only variations of organogenesis were diagnosed, which were seen in the form of kinked ureter and dilated renal pelvis in 20.5% of foetuses from the 10 mg/kg group and 47.6% of the foetuses from the 30 mg/kg group. Skeletal variations (unilateral or bilateral rudimentary lumbar ribs) were noted in 22% of foetuses. Ossification was retarded in the foetuses from dams treated with daily 2-mercaptobenzimidazole doses of ≥ 10 mg/kg body weight, in comparison with the controls in the study. The NOAEL for foetal toxicity of 2-mercaptobenzimidazole was 3.3 mg/kg body weight/day and hence was higher than the chemical's maternal NOAEL (< 3.3 mg/kg body weight/day). In these studies, maternal toxicity always preceded fetotoxicity. Malformations (such as cleft palate) were only seen upon oral administration of 60 mg/kg body weight, a dose which was fatal to many of the dams receiving such treatment. Therefore, the embryonic and foetal development of rats was affected by 2-mercaptobenzimidazole only when the chemical was given at maternally toxic doses. 2-Mercaptobenzimidazole thus did not cause teratogenicity in rats (Yamano et al., 1995).

Groups of 8 or 9 pregnant mongrel albino rats were treated intraperitoneally with a single dose of the sodium salt of 2-mercaptobenzimidazole at 50 mg/kg body weight, administered as an aqueous solution on day 1, 7, 9 or 13 of pregnancy (day 1 of pregnancy = the day on which sperm was detected in the vaginal smears). Twenty-six pregnant rats served as controls. Following laparotomy on day 20 of pregnancy, the dams and foetuses were examined. The number of dead embryos before implantation was increased to 36.4% only in the females treated on day 1 of pregnancy, compared with the controls (4.7%). Following injection of 2-mercaptobenzimidazole on day 7, 9 or 13 of pregnancy, there was no or no marked increase in preimplantational loss of embryos (11.8, 4.2 and 14.1%, respectively). In contrast, foetal resorption rates in the 4 groups treated with 2-mercaptobenzimidazole were all higher (36.4, 26.5, 72.1 and 22.2%) than the rate seen in the control group (4.9%). The highest number of resorbed foetuses was caused by 2-mercaptobenzimidazole when it was given intraperitoneally at a dose level of 50 mg/kg body weight on day 9 of pregnancy. On average, the foetuses in the 4 groups receiving 2-mercaptobenzimidazole treatment were smaller in size (26.1, 25.1, 24.8 and 26.3 mm) than those of the control group (28.5 mm). No visceral or skeletal malformations were noted in the foetuses from the pregnant rats which were treated with 2-mercapto-

benzimidazole. Thus, when administered to rats as a single intraperitoneal dose of 50 mg/kg body weight on different days of pregnancy, 2-mercaptobenzimidazole retarded foetal development but did not produce any teratogenic effects (Barilyak, 1974). The publication did not specify the purity of the sodium salt of 2-mercaptobenzimidazole which was used as the test substance, nor did it report any data on maternal toxicity. However, maternal toxicity is likely to occur after intraperitoneal injection of 50 mg 2-mercaptobenzimidazole/kg body weight seeing that at 60 mg/kg body weight oral administration of 2-mercaptobenzimidazole to pregnant rats on 3 consecutive days caused severe maternal toxicity in the studies conducted by Yamano et al., 1995 (see above).

Upon single administration to rats on day 13 of gestation, 2-mercaptobenzimidazole (dose and route of administration not specified) caused an increase in foetal resorptions (28.0% as compared with 4.7% in the controls) and, in the placental regions adjacent to the uterus, extensive haemorrhages and vascular wall lesions in the form of homogenisation and pyknotic endothelial cell nuclei (no further details; Barilyak, 1977 a).

Following treatment of 20 white rats with a 2-mercaptobenzimidazole dose of 30 mg/kg body weight (mode of administration not specified, but presumably intraperitoneal) on day 13 of gestation, an increase in foetal resorptions (26.4%) was also observed. The foetal resorption rate in the 40 controls was 7.0%. Malformations were observed neither in the 344 live foetuses from the control group nor in the 131 live foetuses from the group treated with 2-mercaptobenzimidazole (no further details; Barilyak, 1977 b).

Following injection of a solution of 2-mercaptobenzimidazole in acetone (5 µl) into the air chambers of eggs containing 3-day-old Leghorn chicken embryos and subsequent 11-day incubation, no embryotoxic effects were observed at concentrations up to 1.0 µmol/egg (highest concentration tested) as compared with the acetone control (20 eggs/concentration; Korhonen et al., 1982, 1983). It is hardly possible to extrapolate these findings to mammalian systems, however, because such comparison would fail to take into account the absorption barriers and detoxification mechanisms which are present in mammals. Furthermore, this test system is excessively sensitive and does not permit the distinction between teratogenic and embryolethal effects (Skofitsch, 1988; Neubert et al., 1992; Neubert, 1993; Heinrich-Hirsch, 1992).

The subchronic inhalation toxicity studies conducted in 1984 within the U.S. National Toxicology Program (NTP, 1984 a, b), which have been discussed in Section 7.5 above, included sperm motility and vaginal cytology studies in male and female rats which were exposed to 2-mercaptobenzimidazole concentrations of 0 (controls), 1, 3 and 10 mg/m³ as well as in male and female mice which were exposed to 0 (controls), 0.3, 1 and 10 mg/m³. Whereas the exposed male mice showed no change in body weight, testicular weight, epididymal weight or sperm motility in comparison with the controls, the male rats exhibited no change in body weight, testicular weight or epididymal weight, but did have significantly reduced sperm motility. No results on the female rats were reported. The female mice which inhaled 2-mercaptobenzimidazole were found to have an increased oestrous cycle length (no data given on concentration-dependency) relative to controls. The reduction in sperm motility and the increase in oestrus cycle length were interpreted by the authors as indicators of effects on fertility (NTP, 1984 a, b; Morrissey et al., 1988).

Similarly, the other subchronic inhalation studies which were conducted in rats and mice within the NTP (see Section 7.5; NTP, 1988 a, b; Gaworski et al., 1991) using higher concentrations (3.13, 6.25, 12.5 and 50 mg/m³) of 2-mercaptobenzimidazole also investigated sperm motility and vaginal cytology (no indication whether in all concentration groups). In the male mice, there were no changes in testicular and epididymal weights, sperm count per gram of tissue, total spermatid heads per testes and total spermatid heads per gram testes. Sperm motility was slightly but statistically significantly reduced in the 3.13, 12.5 and 50 mg/m³ groups. In the authors' judgement, however, the differences with respect to sperm motility were not biologically significant. In contrast, the male rats exhibited no changes in these study parameters, compared with the controls. The female mice treated with the highest concentration (50 mg/m³) were found to have a significant increase in oestrus cycle length (4.6 days, controls 4.1 days). The female rats exposed to 2-mercaptobenzimidazole at 6.25 mg/m³ exhibited a significant reduction in oestrus cycle length (no further details; NTP, 1988 c).

In vitro studies

Brain tissue cultures from 19-day-old Sprague-Dawley rat foetuses were cultured for 3 days and then exposed to 109 chemicals, including 2-mer-

captobenzimidazole, which were to be tested for cytotoxicity at concentration levels of 0.5, 1 and 2 mM (equivalent to 0.075, 0.150 and 0.300 µg/ml in the case of 2-mercaptobenzimidazole). The “neuroteratogenic” potential of a chemical was regarded as negligible if the cytocidal effect failed to occur in any cell type, neuron or non-neuron (glia-like cells), at a concentration ≥ 2 mM, or if the effect occurred in both cell types or only in non-neurons. Twenty-one structurally different chemicals which had a selective cytotoxic effect on neurons were assessed as being potential “neuroteratogens”. Ethylene thiourea and the derivative of thiourea, 2-mercaptobenzimidazole, were selectively cytotoxic to neurons at and above a concentration of 0.5 mM. Therefore, the investigators evaluated 2-mercaptobenzimidazole as a potential “neuroteratogen” (Khera and Whalen, 1988).

A study carried out to investigate the spermostatic effect of different amino alcohols showed that the sodium salt of 2-mercaptobenzimidazole possessed spermostatic activity and affected fructolysis in human spermatozoa. In contrast to other benzazoles, however, the sodium salt of 2-mercaptobenzimidazole did not affect hyaluronidase activity (no further details; Tarakhovskij et al., 1971).

7.9 Effects on the immune system

In order to study the effect of 2-mercaptobenzimidazole on the immune system, female Wistar rats were injected with a single dose of 30 mg/kg body weight from day 11 to 15 of gestation. At the age of one month, the pups were examined with regard to body weight, the weight of the thymus gland and spleen, and their humoral and cellular immune response. 2-Mercaptobenzimidazole caused a reduction in thymus weight in the progeny. In addition, significantly depressed humoral and cellular immunity was reported, as characterised by a decreased number of zones of haemolysis in the spleen cells as well as reduced haemolysin and haemagglutinin titres. Furthermore, peripheral leukocyte counts were found to be reduced (Barilyak et al., 1979; Mel’nik, 1979).

Following a single dose of 2-mercaptobenzimidazole (no details of the dose or the route of administration), rats exhibited considerable involution of the thymus gland 5 days after treatment. Mice, guinea pigs and rabbits did not exhibit this effect (Malmfors, 1976).

7.10 Neurotoxicity

In order to study the depressant or stimulating action of 2-mercaptobenzimidazole on the central nervous system, 3 male and 3 female Swiss-Webster mice (20 to 30 g) were injected intraperitoneally with a single dose of 500 mg/kg body weight as a suspension of the chemical in 5-percent gum acacia. The signs observed included sedation, loss of righting reflex, asphyxia and bulging eyeballs, which were interpreted as signs of CNS depression (Hussain and Lien, 1971). The intraperitoneal dose used in this study by far exceeded the published LD_{50} values for intraperitoneal administration in mice. Therefore, it seems unlikely that the signs of toxicity reported by Hussain and Lien reflect a specifically neurotoxic effect.

7.11 Other effects

In in-vitro studies with microsomal protein from rat liver microsomes, 2-mercaptobenzimidazole inhibited the deiodination of 3,3',5'-triiodothyronine to 3,3'-diiodothyronine (Visser et al., 1979).

Addition of 2-mercaptobenzimidazole to cell cultures of *Saccharomyces cerevisiae* at 4000 µg/ml caused 50-percent growth inhibition (determination by culture dry weight) following incubation, but it did not affect the processes of cell division (as ascertained by direct cell count; Loveless et al., 1954).

Following intraperitoneal treatment of rats with 30 mg 2-mercaptobenzimidazole/kg body weight on day 13 of gestation, the mitotic indices in the liver and kidney cells of foetuses removed by laparotomy on day 20 of gestation were lower than those of the controls included in the study (Barilyak, 1976).

After oral treatment of rats with 30 mg 2-mercaptobenzimidazole/kg body weight on day 13 of gestation, the hepatocytes from the foetuses removed by laparotomy on day 21 of gestation were studied with regard to the permeability of the lysosome membrane by establishing the concentration of neutral red capable of quenching the fluorescence of the granules within one minute at a given concentration of acridine orange. Based on data obtained under identical experimental conditions for teratogens, such as 6-mercaptopurine, and chemicals without adverse effects on embryonic foe-

tal development, for instance DMSO, it was observed that substances with teratogenic activity greatly stabilised the lysosome membrane whereas chemicals without adverse effects on embryonic and foetal development caused lability of the lysosome membrane. 2-Mercaptobenzimidazole, which in teratogenicity studies was embryolethal but not teratogenic at high dose levels (cf. Section 7.8), exhibited only a weak stabilising effect on the lysosomal membranes (Barilyak and Zabara, 1979).

8 Experience in humans

Percutaneous testing of 2-mercaptobenzimidazole was not observed to cause any irritant effects (no further details; Vorobieva and Mezentseva, 1964).

Three female patients with contact dermatitis (induced by spandex, a synthetic polyurethane elastomer), two of whom had a history of contact dermatitis due to rubber, were patch-tested with 2-mercaptobenzimidazole (2% in petrolatum). The result obtained after 48 hours was negative (van Dijk, 1968).

In 12 patients (8 men, 4 women), who were sensitive to 2-mercaptobenzothiazole, patch-testing of 2% 2-mercaptobenzimidazole (in petrolatum) with 48-hour exposure gave negative results when the reactions were read after an additional 24 hours. Thus, there was no cross-sensitisation (Fregert, 1969).

In a another study conducted in 17 subjects sensitive to 2-mercaptobenzothiazole, there was a mild positive reaction to 2-mercaptobenzimidazole, but this was seen only in two cases (no further details; Adams, 1975).

In 17 subjects allergic to 2-mercaptobenzothiazole, the reaction to 2-mercaptobenzimidazole (1% in petrolatum) was investigated in the epicutaneous test. The reactions were read after 48 hours, positive results being seen in 2 of the 17 subjects. All tests were negative in 20 control subjects (Foussereau et al., 1983).

One patient with recurrent facial dermatitis developed itching and redness of the face within 10 minutes of donning a diving mask. He underwent patch-testing with 10 rubber chemicals, including 2-mercaptobenzimidazole (1% in petrolatum), for periods of 20 minutes as well as 2 days. No positive

reaction to 2-mercaptobenzimidazole was noted at the end of either period (Tuyp and Mitchell, 1983).

A man aged 52 years, who had been employed in the production of synthetic rubber (polyurethane) for 10 years, had suffered from an itching infiltrating erythema of the face, neck, hands and lower arms for the last two years. Standard patch tests were carried out as well as tests with the polyurethane additives (10 and 1% in petrolatum). The reactions were read after 2, 3 and 6 days. Positive reactions were seen with 2,5-di-tert.-butylhydroquinone, the monobenzyl ether of hydroquinone, and 2-mercaptobenzimidazole (Higashi and Matsumura, 1987).

In another study, 198 patients (100 women, 98 men) with suspected contact allergy to rubber ingredients were patch-tested with 2-mercaptobenzimidazole, other rubber additives and a standard series. Five patients had positive reactions to 2-mercaptobenzimidazole. Among 6 patients with a positive reaction to 2-mercaptobenzothiazole, 3 also reacted to 2-mercaptobenzimidazole. Application to the skin was carried out with 1% formulations in petrolatum and 48-hour exposure, with readings being taken after 48 and 72 hours (IVDK, 1992; Geier et al., 1994).

The potential skin-protective action of 2-mercaptobenzimidazole was investigated in 4 healthy volunteers. Skin areas of the subjects' lumbar region were exposed to ultraviolet radiation from a mercury vapour lamp for 2½ to 3 minutes. Saturated solutions of 2-mercaptobenzimidazole in n-butanol which were applied locally either for 10 minutes prior to UV radiation exposure or after exposure were able to prevent the formation of UV-induced erythema, as indicated by the readings taken after 6 and 24 hours. It was hypothesised that this effect was due to inhibition of local prostaglandin synthesis (Ferguson et al., 1985).

Out of 65 workers (47 men and 18 women) registered in the period from 01.01.1972 to 31.12.1991 at a Shanghai chemical plant which had been producing technical-grade 2-mercaptobenzimidazole since 1963, one male worker died of carcinoma of the stomach and two female workers died of liver cancer at the age of 45 to 49 years. For the Shanghai men who died of stomach cancer at the age of 45 to 49 years between 1972 and 1979, the standard mortality ratio (SMR) was 39.6/10000, while for women of all age groups the SMR for liver cancer mortality was 14.3/10000. In the men (45

to 49 years of age) employed at the 2-mercaptobenzimidazole plant, the SMR for stomach cancer was 58.82, while in the female workers of all ages the SMR for liver cancer was 55.56. The workers who died of malignant tumours had worked at the factory for 12.3 years on average. Thus, the mortality rates for stomach cancer in the male workers and for liver cancer in the female workers were higher than the respective SMRs for the Shanghai population in general. This finding led the investigators to suspect that carcinogens occur during the production of 2-mercaptobenzimidazole. Therefore, Salmonella/microsome assays were carried out in order to assess the substances which occur during the production of 2-mercaptobenzimidazole (for the results of those studies, see Section 7.6.1; Feng et al., 1988).

9 Classifications and threshold limit values

No information available.

References

Adams, R.M.
Possible substitution for mercaptobenzothiazole in rubber
Contact Dermatitis, 1, 246 (1975)

Barilyak, I.R.
Embryotoxic and mutagenic effect of 2-mercaptobenzimidazol (English translation of the Russian)
Fiziol. Akt. Veshches., 6, 85–88 (1974)

Barilyak, I.R.
Relation between embryotoxic cell mitotic activity and the injurious effect of some agents on embryogenesis (in Russian with an English abstract)
Fiziol. Akt. Veshches., 8, 106–108 (1976)

Barilyak, I.R.
State of the placenta under the effect of some environmental factors (in Russian with an English abstract)
Pediatr. Akush. Ginekol., issue 6, 46–48 (1977 a)

Barilyak, I.R.
Influence of hydrolase enzymes of the lysosomes on rat embryos
Sov. J. Dev. Biol., 8, 451–455 (1977 b)

Barilyak, I.R., Mel'nik, E.K.
Dynamics of the cytogenetic effect of some chemical preparations (English translation of the Russian)
Dopov. Akad. Nauk Ukr. RSR, Ser. B: Geol., Khim. Biol. Nauki, issue 1, 68–71 (1979)

Barilyak, I.R., Zabara, A.M.
Influence of certain teratogens on the functional state of the lysosome-segregation apparatus of embryonic hepatocytes
Sov. J. Dev. Biol., 10, 540–546 (1979)

Barilyak, I.R., Rutskaya, G.V., Mel'nik, E.K.
Some immunological investigations in the presence of congenital chromosomal pathology (clinico-experimental data)
Cytol. Genet. (Engl. ed.), 13, 58–63 (1979)
English translation of: Tsitol. Genet., 13, 64–69 (1979)

Barlogova, S., Stolcova, E.
The observation of allergogenic effect of 2-mercaptobenzothiazole and 2-mercaptobenzoimidazole (Czech publication with an English abstract and an abridged translation)
Prac. Lék, 36, 13–16 (1984)

Bayer AG, Geschäftsbereich Kautschuk
DIN safety data sheet Vulkanox MB/MG (1988)

Bayer AG
Safety data sheet Vulkanox MB/MG (1994)

Dijk, van, E.
Contact dermatitis due to Spandex
Acta Derm. Venereol., 48, 589–591 (1968)

El Dareer, S.M., Kalin, J.R., Tillery, K.F., Hill, D.L.
Disposition of 2-mercaptobenzimidazole in rats dosed orally or intravenously
J. Toxicol. Environ. Health, 14, 595–604 (1984)

Falbe, J., Regitz, M. (eds.)
Römpp Chemie Lexikon
9th ed., vol. 4, p. 2694
Georg Thieme Verlag, Stuttgart (1991)

Feng, B., Zhang, Y., Zhu, J., Zhang, M.
Überprüfung der Mutagenität des Alterungsschutzmittels 2-Mercaptobenzimidazol aufgrund von Urinproben von Arbeitern eines Herstellungswerkes (German translation of the Chinese)
Zhonghua Loadong Weisheng Zhiyebing Zazhi, 6 (5), 261–264 (1988)

Ferguson, M.M., MacFadyen, E.E., Moseley, H., Simpson, N.B.
Anti-inflammatory action of topical thiocarbamide drugs: possible use in protecting skin during radiotherapy
Lancet, 11, 325 (1985)

Foussereau, J., Menezes-Brandao, F., Cavelier, C., Herve-Bazin, B.
Allergy to MBT and its derivatives
Contact Dermatitis, 9, 514–516 (1983)

Fregert, S.
Cross-sensitivity pattern of 2-mercaptobenzothiazole (MBT)
Acta Derm. Venereol., 49, 45–48 (1969)

Gaworski, C.L., Aranyi, C., Vana, S., Rajendran, N., Abdo, K., Levine, B.S., Hall III, A.
Prechronic inhalation toxicity studies of 2-mercaptobenzimidazole (2-MBI) in F344/N rats
Fundam. Appl. Toxicol., 16, 161–171 (1991)

Geier, J., Pilz, B., Frosch, P.J., Schnuch, A.
Contact allergy due to 2-mercaptobenzimidazole
Dermatosen, 42 (5), 190–193 (1994)

Goncarova, R.I., Levina, A.B.
Untersuchung der genetischen Aktivität von Benzimidazolen an Drosophila (German translation of the Russian)
Vetsi Akad. Navuk BRRS, Ser. Biyal. Navuk, issue 3, 44–48 (1980)

Heinrich-Hirsch, B.
Possible contribution of in vitro methods to risk assessment; discussion of the presentation
In: Neubert et al., p. 471 (1992)

Higashi, N., Matsumura, T.
A case of occupational contact dermatitis from peroxide curing agents for rubber
Skin Res., 29, 563–567 (1987)

Hussain, M.H., Lien, E.J.
Centrally acting cyclic urea, thiourea, and their N,N'-dialkyl derivatives. Structure activity correlations
J. Med. Chem., 14, 138–144 (1971)

IVDK (Informationsverbund Dermatologischer Kliniken, Göttingen)
Written communication to BG Chemie of 21.12.1992

Janssen, F.W., Young, E.M., Kirkman, S.K., Sharma, R.N., Ruelius, H.W.
Biotransformation of the immunomodulator, 3-(p-chlorophenyl)-2,3-dihydro-3-hydroxy-thiazolo[3,2a]benzimidazole-2-acetic acid, and its relationship to thyroid toxicity
Toxicol. Appl. Pharmacol., 59, 355–363 (1981)

Khera, K.S., Whalen, C.
Detection of neuroteratogens with an in vitro cytotoxicity assay using primary mono-layers cultured from dissociated foetal rat brains
Toxicol. in Vitro, 2 (4), 257–273 (1988)

Korhonen, A., Hemminki, K., Vainio, H.
Embryotoxicity of benzothiazoles, benzenesulfohydrazide, and dithiodimorpholine to the chicken embryo
Arch. Environ. Contam. Toxicol., 11, 753–759 (1982)

Korhonen, A., Hemminki, K., Vainio, H.
Toxicity of rubber chemicals towards three-day chicken embryos
Scand. J. Work Environ. Health, 9, 115–119 (1983)

Lide, D.R., Frederikse, H.P.R. (eds.)
CRC handbook of chemistry and physics
77th ed., p. 3-66
CRC Press, Boca Raton, New York, London, Tokyo (1996)

Loveless, L.E., Spoerl, E., Weisman, T.H.
A survey of effects of chemicals on division and growth of yeast and Escherichia coli
J. Bacteriol., 68, 637–644 (1954)

Malmfors, T.
Thymus, the forgotten organ in toxicological testing
Toxicol. Appl. Pharmacol., 37, 185 (1976)

Marhold, J.
Prehled Prumyslove Toxikologie, Organicke Latky, p. 990
Avicenum, Praha (1986)

Matthews, E.J., Spalding, J.W., Tennant, R.W.
Transformation of BALB/c-3T3 cells: IV. Rank-ordered potency of 24 chemical responses detected in a sensitive new assay procedure
Environ. Health Perspect. Suppl., 101, Suppl. 2, 319–345 (1993)

Mel'nik, E.K.
Condition of the immune system of rats exposed to 6-mercaptopurine and 2-mercapto-benzimidazol in the antenatal period of active organogenesis (English translation of the Russian)
Pediatr. Akush. Ginekol., 2, 46–47 (1979)

Miltenburger, H.
Personal communication (1997)

Morrissey, R.E., Schwetz, B.A., Lamb IV, J.C., Ross, M.D., Teague, J.L., Morris, R.W.
Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program 13-week studies
Fundam. Appl. Toxicol., 11, 343–358 (1988)

Neubert, D., Kavlock, R.J., Merker, H.J., Klein, J. (eds.)
Risk assessment of prenatally-induced adverse health effects
Springer-Verlag (1992)

Neubert, D.
Personal communication (1993)

Norford, D.C., Meuten, D.J., Cullen, J.M., Collins, J.J.
Pituitary and thyroid gland lesions induced by 2-mercaptobenzimidazole (2-MBI) inhalation in male Fischer-344 rats
Toxicol. Pathol., 21 (5), 456–464 (1993)

NTP (U.S. National Toxicology Program)
Thirteen-week subchronic study in F344 rats – 2-mercaptobenzimidazole
Unpublished report, Abstract and Summary Tables (1984 a)

NTP (U.S. National Toxicology Program)
Thirteen-week subchronic study in B6C3F1 mice – 2-mercaptobenzimidazole
Unpublished report, Abstract and Summary Tables (1984 b)

NTP (U.S. National Toxicology Program)
Prechronic toxicity studies of 2-mercaptobenzimidazole: 90-day subchronic study report on inhalation exposure of F344/N rats
Unpublished report, Abstract and Summary Tables (1988 a)

NTP (U.S. National Toxicology Program)
Prechronic toxicity studies of 2-mercaptobenzimidazole: 90-day subchronic study report on inhalation exposure of B6C3F1 mice
Unpublished report, Abstract and Summary Tables (1988 b)

NTP (U.S. National Toxicology Program)
Sperm motility vaginal cytology evaluation in rodents – 2-mercaptobenzimidazole
Unpublished report, Results and Discussion (1988 c)

NTP (U.S. National Toxicology Program)
Unpublished studies
Written communication to of 05.12.1995

OECD (Organisation for Economic Co-operation and Development)
OECD guideline for the testing of chemicals. Proposal for updating guideline 474. Mammalian erythrocyte micronucleus test
Revised Draft Document (1995)

Rannug, A., Rannug, U., Ramel, C.
Genotoxic effects of additives in synthetic elastomers with special consideration to the mechanism of action of thiurames and dithiocarbamates
Prog. Clin. Biol. Res., 141, Iss. Ind. Hazards Plast. Synth. Elastomers, 407–419 (1984)

RTECS (Registry of Toxic Effects of Chemical Substances)
2-Benzimidazolethiol, RTECS Number DE1050000
produced by NIOSH (National Institute for Occupational Safety and Health) (1996)

Ruddick, J.A., Newsome, W.H., Nash, L.
Correlation of teratogenicity and molecular structure: ethylenethiourea and related compounds
Teratology, 13, 263–266 (1976)

Schmitt, H.G., Rippel, R., Schubart, R., Habersang, S.
Thiole, Sulfide, Polysulfide und Thioglykolsäure
In: Ullmanns Encyklopädie der technischen Chemie
4th ed., vol. 23, p. 191–208
Verlag Chemie, Weinheim (1983)

Searle, C.E., Lawson, A., Hemmings, A.W.
Antithyroid substances. 1. The mercaptoglyoxalines
Biochem. J., 47, 77–81 (1950)

Shimizu, M., Noda, T., Yamano, T., Morita, S.
Contact allergenicity and cross-reactivity among 2-mercaptobenzimidazole, methyl 2-benzimidazolecarbamate and ethylene thiourea for use in house products (English abstract of a Japanese publication)
Jpn. J. Toxicol. Environ. Health, 40, 558–566 (1994)

Skofitsch, G.
Vom Hund zum Ei? Große und kleine Laboratoriumstiere
In: Lembeck, F. (ed.)
Alternativen zum Tierversuch, p. 71–79
Georg Thieme Verlag Stuttgart, New York (1988)

Slamenová, D., Budayová, E., Gábelová, A., Dusinská, M.
Pre-screening of carcinogens by studying of DNA synthesis inhibition and gene mutations in mammalian cells
Neoplasma, 33, 699–706 (1986)

Smiley, R.A.
Phenylene- and toluenediamines
In: Ullmann's encyclopedia of industrial chemistry
5th ed., vol. A19, p. 405–410
VCH Verlagsgesellschaft mbh, Weinheim (1991)

Tarakhovskij, M.L., Nexynov, E.P., Pel'kis, P.S., Najgertsik, I.E., Rybakova, V.V., Lozinskij, M.O., Dubenko, R.G., Grabenko, A.D.
Spermostatische Wirkung einiger Aminoalkohole (translated in parts from Russian into German)
Fiziol. Akt. Veshches., 3, 119–130 (1971)

TNO (Central Institute for Nutrition and Food Research, Zeist, The Netherlands)
Determination of the acute oral toxicity of Vulkanox MB in rats
Unpublished report, CIVO/TNO Report 22/8/75 (1975)
On behalf of the Working Group on Toxicology of Rubber Auxiliaries (WTR)

Tuyp, E., Mitchell, J.C.
Scuba diver facial dermatitis
Contact Dermatitis, 9, 334–335 (1983)

Visser, T.J., van Overmeeren, E., Fekkes, D., Docter, R., Hennemann, G.
Inhibition of iodothyronine 5'-deiodinase by thioureylenes: structure-activity relationship
FEBS Lett., 103, 314–318 (1979)

Vorobieva, R.S., Zhilova, N.A., Kasparov, A.A., Mezentseva, N.V.
Comparative toxicity of mercaptobenzimidazole and N-phenyl-N-isopropylparaphenyle-neamine
Sov. Rubber Technol., 22, 11–12 (1963)

Vorobieva, R.S., Mezentseva, N.V.
Toxicological characteristics of new rubber mixture ingredients (English translation of the Russian)
Gig. Tr. Prof. Zabol., issue 8, 39–43 (1964)

WTR (International Working Group on the Toxicology of Rubber Additives)
Safety data sheet 2-mercaptobenzimidazole (1984)

Yamano, T., Noda, T., Shimizu, M., Morita, S.
The adverse effects of oral 2-mercaptobenzimidazole on pregnant rats and their fetuses
Fundam. Appl. Toxicol., 25, 218–223 (1995)

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., Speck, W.
Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals
Environ. Mutagen., 9, Suppl. 9, 1–110 (1987)