Australian Government
Department of Health
Australian Industrial Chemicals Introduction Scheme

Mercaptobenzimidazoles and their zinc salts

Evaluation statement

14 January 2022

Table of contents

Contents

AICIS evaluation statement

Subject of the evaluation

Mercaptobenzimidazoles and their zinc salts

Chemicals in this evaluation

Name	CAS registry number
2H-Benzimidazole-2-thione, 1,3-dihydro-	583-39-1
2H-Benzimidazole-2-thione, 1,3-dihydro-, zinc salt (2:1)	3030-80-6
[2H-Benzimidazole-2-thione, 1,3-dihydro-5-methyl-	27231-36-3
2H-Benzimidazole-2-thione, 1,3-dihydro-4(or 5)-methyl-zinc salt (2:1)	61617-00-3
2H-Benzimidazole-2-thione, 1,3-dihydro-4(or 5)-methyl-	53988-10-6

Reason for the evaluation

The Evaluation Selection Analysis indicated a potential risk to human health.

Parameters of evaluation

These chemicals are listed on the Australian Inventory of Industrial Chemicals (the Inventory). This evaluation is a human health risk assessment for all identified industrial uses of the chemicals.

These chemicals have been assessed as a group as they are structurally very similar and share the same use patterns.

Summary of evaluation

Summary of introduction, use and end use

There is currently no specific information about the introduction use and end use of these chemicals in Australia.

Based on international use information, these chemicals are used as intermediates in the production of tyres and rubber goods, as process regulators in polymerisation processes, in plastic products and in adhesive and sealants.

Although some possible domestic uses of these chemical in adhesives and sealants has been identified, based on available information this is not expected to be widespread.

Human health

Summary of health hazards

Toxicity studies for chemicals in this group are limited. Due to the similarity in structure and physico-chemical properties of the 5 chemicals in the group, their toxicological effects are expected to be similar. Although differences in toxicokinetics may impact the dose at which systemic effects occur. The critical health effects for risk characterisation include:

- systemic effects following repeated exposure, in particular, effects on the thyroid
- potential developmental neurotoxicity or developmental immunotoxicity
- local effects (skin sensitisation).

Zinc cations have low to moderate oral toxicity (NICNAS 2014), which is sufficiently lower compared with that of the mercaptoimidazoles. Therefore, the systemic toxicity is expected to be driven by the mercaptoimidazole moiety.

Based on the available information the chemicals are rapidly absorbed by the gastrointestinal tract and are detectable in all organs and tissues examined. Urine is the major route of excretion of this group of chemicals. The chemicals undergo metabolic desulfuration. Results from toxicokinetic studies suggest that metabolic elimination is faster for methylated derivatives compared to MBI and that metabolic desulfuration of MBI is reduced at high doses or following repeated exposure. Following exposure to similar doses, serum levels of the chemical were MBI >> 5-MeMBI>mixture of 4-MeMBI and 5-MeMBI, particularly following repeated exposure.

The chemicals have moderate to high oral toxicity with a median lethal dose (LD50) in the range of 50–400 mg/kg body weight (bw). They have low acute dermal toxicity. Limited data are available for acute inhalation toxicity.

These chemicals are not irritating to skin or eyes. Mixed results were obtained with skin sensitisations tests (LLNA and guinea pig maximisation test (GPMT)) and in human reactions to MBI. Based on weight of evidence and some positive results in human volunteers, chemicals in this group are considered to be sensitising to skin

Repeated dose oral and inhalation studies indicated that these chemicals can produce adverse health effects following repeated oral and inhalation exposure. The thyroid was the main target organ. Effects were also reported in the liver, kidneys, adrenals, spleen and thymus. Thyroid effects occurred at much lower doses for MBI compared with its methylated derivatives. This is likely due to differences in toxicokinetics which result in higher systemic exposures for MBI compared with its methylated derivatives.

For MBI, hypertrophy of follicular cells in the thyroid was observed at 1.2 mg/kg bw/ day (oral 28 day study in rats) and approximately 3 mg/m^3 (13 week inhalation studies in rats and mice). In an inhalation study with MBI that investigated thyroid hormones at 50 mg/m^3, T3 levels were temporarily depressed and T4 levels were below the limit of detection from 2 weeks until the end of the study.

In a 28 day oral study with a 1:1 mixture of 4-MeMBI and 5-MeMBI, exposure to 100 mg/kg bw/day caused histopathological changes in the thyroid but did not alter serum thyroid hormone levels. 4- or 5-MeMBI caused follicular cell hypertrophy in the thyroid at doses of 50 mg/kg bw/day in a combined repeated dose reproductive/developmental toxicity study and thyroid hypertrophy was observed in another reproductive toxicity study with ZnMeMBI at 15/40 mg/ kg bw/day.

Based on the weight of evidence from in vitro or in vivo mutagenic tests, the chemicals are not considered to be genotoxic. There is no information on the carcinogenic potential of these chemicals.

Based on the weight of evidence, the chemicals may cause specific adverse effects on fertility following exposure. Data for ZnMeMBI supports that methyl derivatives of MBI do not cause developmental neurotoxicity or developmental immunotoxicity. However, given the higher potency of thyroid toxicity for MBI these effects cannot be ruled out for MBI and ZnMBI.

No specific reproductive studies are available for MBI. Increases in oestrous cycle length were observed in female mice in subchronic inhalation studies. Some effects on sperm motility were noted in these studies but these were not consistent. In a modified extended one generation reproductive toxicity study with ZnMeMBI, prolonged parturition/dystocia occurred in females of both generations receiving 40 mg/kg/day. No adverse effects on sperm motility or morphology were apparent in males. There was no evidence of developmental neurotoxicity or developmental immunotoxicity in this study. Effects on fertility were also observed in another combined repeated dose reproductive/developmental toxicity study in rats with ZnMeMBI. At doses of 375 mg/kg bw/day, there was a marked reduction in the number of mating pairs with positive evidence of mating and pregnancies. Females with no evidence of mating generally showed a lack of oestrous cyclicity.

In developmental studies with MBI, ossification was observed in the presence of maternal toxicity. Foetal resorption rates were increased at doses of 60 mg/kg bw/day.

In developmental studies with ZnMBI there were no effects on implantations or resorptions. The incidence of major and minor foetal abnormalities and skeletal variants showed no dose response relationship to maternal treatment with ZnMeMBI.

Health hazard classification

The chemicals satisfy the criteria for classification according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UNECE, 2017) for hazard classes relevant for work health and safety as follows. This does not consider classification of physical hazards and environmental hazards.

The repeat dose toxicity classification applies to MBI (CAS No. 583-39-1) and ZnMBI (CAS 3030-80-6). The other chemicals in this group 5-MeMBI (CAS No. 27231-36-3), 4(or 5)-MeMBI (CAS No. 53988-10-6) and ZnMeMBI (CAS No. 61617-00-3) should be classified as STOT Repeated Exposure 2—H373: May cause damage to thyroid through prolonged or repeated exposure.

Health hazards	Hazard category	Hazard statement
Acute toxicity – oral	Acute Tox. 3	H301: Toxic if swallowed
Skin sensitisation	Skin Sens. 1B	H317: May cause allergic skin reaction
Specific target organ toxicity (repeat exposure)	STOT Rep. Exp 1	H372: Causes damage to thyroid through prolonged or repeated exposures
Reproductive toxicity	Repr. 2	H361f Suspected of damaging fertility

Summary of health risk

Public

Based on the available information, limited consumer use of the chemicals is expected in Australia. Therefore, there are no identified risks to the public that require management. However, if information becomes available indicating any of the chemicals have consumer uses, further risk management may be required.

Workers

During product formulation, dermal, ocular and inhalation exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemical at lower concentrations could also occur while using formulated products containing the chemical. The level and route of exposure will vary depending on the method of preparation and work practices employed. Good hygiene practices to minimise oral exposure are expected to be in place.

Given the critical systemic long-term effects, the chemical could pose a risk to workers. Control measures to minimise dermal and inhalation exposure are needed to manage the risk to workers (refer to **Recommendations** section).

Conclusions

The conclusions of this evaluation are based on the information described in this statement. Obligations to report additional information about hazards under section 100 of the Industrial Chemicals Act 2019 apply.

The Executive Director is satisfied that the identified human health risks can be managed within existing risk management frameworks provided all requirements are met under

environmental, workplace health and safety and poisons legislation as adopted by the relevant state or territory. The proposed means of managing the risks identified during this evaluation are set out in the **Recommendations** section.

Recommendations

Workers

Recommendation to Safe Work Australia

It is recommended that Safe Work Australia (SWA) update the Hazardous Chemical Information System (HCIS) to include classifications relevant to work health and safety.

Information on managing identified risks

The information in this report including recommended hazard classifications, should be used by persons conducting a business or undertaking (PCBU) at the workplace (such as an employer) to determine the appropriate controls under the Model Work Health and Safety Regulations.

Control measures that could be implemented to manage the risk arising from occupational exposure to the chemical include, but are not limited to:

- using closed systems or isolating operations
- using local exhaust ventilation to prevent the chemicals from entering the breathing zone of any worker
- minimising manual processes and work tasks through automating processes
- adopting work procedures that minimise splashes and spills
- cleaning equipment and work areas regularly
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

These control measures may need to be supplemented with:

- conducting health monitoring for any worker who is at significant risk of exposure to the chemical, if valid techniques are available to monitor the effect on the worker's health

Measures required to eliminate, or manage risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemical is used.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

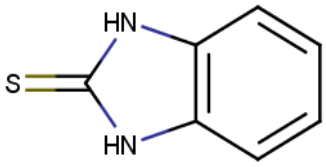
Model codes of practice, available from the Safe Work Australia website, provide information on how to manage the risks of hazardous chemicals in the workplace, prepare an SDS and label containers of hazardous chemicals. Your Work Health and Safety regulator should be contacted for information on Work Health and Safety laws and relevant Codes of Practice in your jurisdiction.

Supporting information

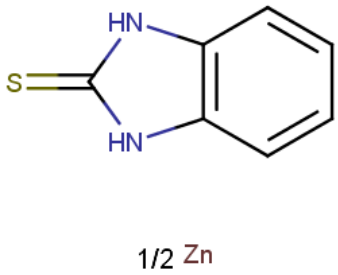
Grouping rationale

Chemicals in this group are mercaptobenzimidazole, methyl derivatives and their zinc salts. Zinc salts readily hydrolysed to Zn^{2+} ion and the corresponding weak acid. Earlier assessment of soluble zinc salts indicated that zinc cations have low to moderate oral toxicity (NICNAS 2014), which is sufficiently low compared with that of the mercaptoimidazoles. Systemic toxicity is expected to be driven by the mercaptoimidazole moiety. Given the close structural similarities of the chemicals in this group, they are expected to have similar systemic toxicological effects.

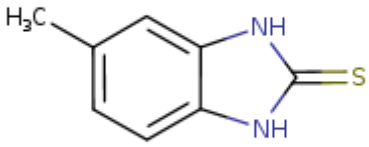
Chemical identity

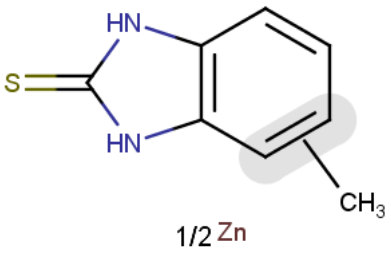
Chemical name	2H-Benzimidazole-2-thione, 1,3-dihydro-
CAS No.	583-39-1
Synonyms	mercaptobenzimazole 2-mercaptobenzimidazole (MBI) 2-benzimidazolinethione 2-thiol benzimidazole
Structural formula	
Molecular formula	C7H6N2S
Molecular weight (g/mol)	150.2
Smiles	S=C1NC2=CC=CC=C2N1

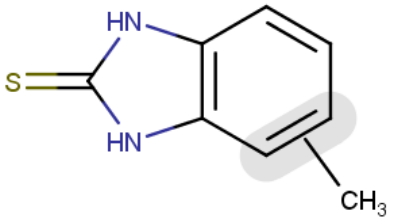
Chemical name	2H-Benzimidazole-2-thione, 1,3-dihydro-, zinc salt (2:1)

CAS No.	3030-80-6
Synonyms	2-mercaptobenzimidazole, zinc salt zinc(II) 1H-benzo[d]imidazole-2-thiolate 2-Benzimidazolethiol, zinc salt (ZnMBI)
Structural formula	
Molecular formula	C7H6N2S.1/2Zn
Molecular weight (g/mol)	363.8
Smiles	[Zn++].[S-]C1=NC2=CC=CC=C2N1.[S-]C1=NC2=CC=CC=C2N1

Chemical name	2H-Benzimidazole-2-thione, 1,3-dihydro-5-methyl-
CAS No.	27231-36-3
Synonyms	2-benzimidazolinethione, 5-methyl- 2-mercapto-5-methylbenzimidazole 5-methyl-1H-benzo[d]imidazole-2(3H)-thione 5-methyl-2-mercaptobenzimidazole(5-MeMBI)

Structural formula	
Molecular formula	C8H8N2S
Molecular weight (g/mol)	164.2
Smiles	CC1=CC2=C(NC(=S)N2)C=C1

Chemical name	2H-Benzimidazole-2-thione, 1,3-dihydro-4(or 5)-methyl-, zinc salt (2:1)
CAS No.	61617-00-3
Synonyms	4(5)-methyl-2-mercaptobenzimidazole, zinc salt (ZnMeMBI) methyl-2-mercaptobenzimidazole, zinc salt zinc methyl mercaptobenzimidazole
Structural formula	
Molecular formula	C16H14N4S2Zn
Molecular weight (g/mol)	391.8
Smiles	[Zn].[*]C.S=C1Nc2ccccc2N1

Chemical name	2H-Benzimidazole-2-thione, 1,3-dihydro-4(or 5)-methyl-
CAS No.	53988-10-6
Synonyms	2-mercapto-4(or 5)-methylbenzimidazole (4- or 5-MeMBI) 2-mercaptotoluimidazole 4(or 5)-methyl-2-mercaptobenzimidazole
Structural formula	
Molecular formula	C8H8N2S
Molecular weight (g/mol)	164.2
Smiles	Cc1cccc2NC(=S)Nc12

Relevant physical and chemical properties

These chemicals are white or off white powders. These chemicals are insoluble in water and have partition coefficients (Log Kow) reported in the range 0.4-0.9.

Introduction and use

Australia

No information is available on the introduction and industrial use of these chemicals in Australia.

International

The following international uses have been identified through the:

- European Union Registration, Evaluation and Authorisation of Chemicals (EU REACH)
- Chemwatch
- Substances in Preparations in Nordic countries (SPIN) database

- United States Environmental Protection Agency (US EPA) Chemical and Product Categories database (CPCat).

The predominant use of the 5 chemicals is site limited, including:

- as intermediates in the production of tyres and rubber goods
- as a vulcanisation accelerator for rubber.

The chemicals have reported commercial uses, including:

- in plastic products
- as process regulators in polymerisation processes
- in adhesive and sealants.

Although a REACH dossier indicated that adhesives and sealants containing MBI may be used by consumers there is no evidence from available databases that identify use of this chemical in consumer products, indicating that it is not likely to be widely available for domestic uses.

Existing Australian regulatory controls

AICIS

No specific controls are currently available for these chemicals.

Public

No specific controls are currently available for these chemicals. Zinc compounds for human internal use are listed in Schedule 4 of the Standard for the Uniform Scheduling of Medicines and Poisons, The Poisons Standard (TGA, 2021).

Workers

These chemicals are not listed on the HCIS and no specific exposure standards are available in Australia (SWA).

International regulatory status

Exposure standards

No specific exposure standards are available for these chemicals.

Health hazard information

Toxicokinetics

The toxicokinetics and metabolism of 2-mercaptobenzimidazole (MBI) were studied in male Fischer (F)344 rats (137 to 166 g) following oral and intravenous administration. [^{14}C]-MBI (purity >96.7 %) was administered by oral gavage (0.51 mg/kg bw) in a mixture of Emulphor EL-620, ethanol and water and by intravenous injection of approx. 0.5 mg/kg bw. The urinary excretion data suggested rapid absorption of MBI 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. Analysis also revealed relatively high concentrations in the gastrointestinal tract between 2 and 6 hours after administration that correlated with biliary excretion. The elimination half-lives from blood were calculated as 83 and 22 hours, following oral and intravenous administration, respectively. Most of the administered radioactivity was excreted in the urine (67 to 69 %) and a smaller amount in faeces (19 to 25 %) in 72 hours. Of the intravenously injected dose, 12 % was excreted in the bile in 4 hours. Two major metabolites and 6 quantitatively less important metabolites were excreted in urine. The structure of only one of the major metabolites was elucidated; this was benzimidazole (El Dareer et al., 1984).

Kinetics of MBI and its methyl derivative were compared after single oral administration in rats (Sakemi et al. 1999). Male Wistar rats received single oral doses of 2, 10, 50 and 250 mg/kg bw of MBI, or 4- or 5-MeMBI, by gavage. These chemicals were also administered by intraperitoneal (i.p.) injection at a dose of 50 mg/kg bw. Serum concentrations were monitored over time. The maximum concentration (C_{max}) levels were similar for both compounds with C_{max} levels higher after i.p administration compared with oral administration at the same dose. MBI degraded more slowly (longer $t_{1/2}$), and as a result its area under curve (AUC) in blood was 2−10 fold higher than MeMBI. The differences of AUC values were smaller at higher doses. When serum levels of 4-MeMBI and 5-MeMBI were determined separately after oral administration, the former disappeared more slowly than the latter, indicating an apparent effect of the position of methyl substituent on the toxicokinetics of MeMBI. Analyses of MBI, MeMBI and their desulfurated metabolites in urine suggested that metabolic elimination was faster for MeMBI compared to MBI but that metabolic saturation for desulfuration occurred for both chemicals at higher doses.

In a metabolic degradation study of MBI and its methylated derivatives (Sakemi et al. 2002), Wistar male rats were administered 0.3−0.6 mmol/kg bw of MBI, 4-MeMBI, 5-MeMBI and a 1:1 mixture of 4-MeMBi and 5-MeMBI by gavage for 2 weeks. Blood and urine samples were collected on day 1, 8, and 15. After repeated oral administration, the C_{max} and area under concentration time curve (AUC) of MBI were markedly increased, while the methylated derivatives essentially were cleared from the blood within 10 h. The C_{max} and AUC of 4-MMBI decreased markedly, suggesting metabolic enzyme induction. This was not observed with 5-MeMBI or the MeMBI mix. Consistent with these measured serum levels wereobservations in the urine analysis.

At lower doses, the amount of MBI excreted in the urine for 24 hours after administration was similar to that of its desulfurated metabolite, benzimidazole (BI); however, the amount of BI in urine was disproportionately low at higher doses, suggesting metabolic saturation of desulfuration of MBI (Sakemi et al. 1999). The 2 isomers of MeMBI (4-MeMBI and 5-MeMBI)

were desulfurised and excreted to different extents. Urinary excretions of the desulfurated metabolite (4-MeMBI) on day 14 increased significantly to about 2 fold of day 1 values. In contrast, the urinary excretion of 5-MeMBI did not change appreciably after repeated oral exposure.

Acute toxicity

Oral

Several guideline and non-guideline acute oral toxicity studies are available for MBI, MeMBI and their zinc salts. Based on the results of these studies the median lethal dose for acute oral toxicity for these chemicals was established to be between 50 mg/kg bw and 400 mg/kg bw. Similar acute toxicities for MBI and MeMBI are supported by similar C_{max} values at the same dose seen in toxicokinetic studies (Sakemi et al. 1999).

In a study conducted according to OECD Test Guideline (TG) 423 (Acute Oral toxicity - Acute Toxic Class Method), 3 female Sprague Dawley (SD) rats were first dosed with 300 mg/kg bw MBI. Two animals were deceased on day 1 after dosing. As mortality was observed at this dose, an additional 3 female animals were treated with the lower dose of 50 mg/kg bw MBI. Administration of the chemical at 50 mg/kg resulted in reduced locomotor activity and ataxic gait but no mortality occurred. The acute oral LD50 value for MBI was considered to be between 50−300 mg/kg bw (REACHa).

Male Wistar rats (3/sex/group) were treated with 140, 225, 370 or 600 mg/kg bw of the chemical. Rats treated with doses less than 225 mg/kg bw recovered within 24 hour but all rats treated with 370 and 600 mg/kg were deceased within 2 days. The LD50 value was considered to be 300 mg/kg bw (REACHa).

In a briefly described acute oral study, not conducted according to OECD Test Guidelines, 7 groups of young adult male Wistar rats (10/dose group) were administered 200, 300, 350, 380, 400, 450, or 500 mg/kg bw 4- or 5-MeMBI, in Lutrol. The animals were observed for mortality, body weights and clinical signs through to day 14. Mortality corresponding to dose 200, 300, 350, 380, 400, 450, or 500 mg/kg bw was 0, 20, 70, 70, 80, 80 and 100 % respectively. Deaths occurred between days 1 and 4. Sedation was the only clinical sign. The acute LD50 for male rats was determined to be 340 mg/kg bw (95 % confidence interval: 300 - 370 mg/kg bw) (REACHd).

An acute oral toxicity study was conducted with ZnMBI, according to OECD TG 423. A stepwise procedure was adopted, and all rats were deceased at doses of 2000 mg/kg bw and 300 mg/kg bw of the chemical. No animal died at 50 mg/kg bw dose level, and no significant pathological changes were noted in any of the treated groups. The LD50 was determined to be between 50 and 300 mg/kg bw (REACHb).

In a non-guideline acute oral study, 5 groups of male Wistar rats (10/dose group) were dosed with 100, 300, 400, 500 or 600 mg/kg bw 4(5)-methyl-2-mercaptobenzimidazole, zinc salt (ZnMeMBI, CAS 61617-00-3) in Lutrol. The animals were observed for mortality, body weights and clinical signs through to day 14. Mortality corresponding to doses 100, 300, 400, 500, and 600 mg/kg bw was 0, 20, 50, 80 and 100 %, respectively. Sedation was seen in animals dosed at 300 mg/kg bw and above. The acute oral LD50 for male rats is 400 mg/kg bw (REACHc).

Dermal

Several dermal acute toxicity studies, conducted according to OECD TG 402 (Acute Dermal Toxicity), have been reported for MBI, MeMBI and their zinc salts. In all these studies the dermal LD 50 for all chemicals is above 2000 mg/kg bw (REACH a,c,d).

Inhalation

Limited information is available on the acute inhalation toxicity of the chemicals in this assessment. In the only available inhalation study, conducted according to US guidelines (EPA OPPTS 870.1300 Acute Inhalation Toxicity), male and female SD rats (5 per sex) were exposed to (nose only) a dust atmosphere containing 2.12 mg/L 5-MeMBI. Exposure duration was 4 hours. No deaths were reported. It was concluded that the acute inhalation LC50 for 5-MeMBI was greater than 2.12 mg/L (REACHd).

Corrosion/Irritation

Skin irritation

Several guideline skin irritation studies, conducted according to OECD TG 404, are available for the chemicals listed in this evaluation. No signs of skin reaction were observed at the site of application for any chemical (REACHa,b,c,d).

Eye irritation

In an eye irritation study conducted according to OECD TG 405, 100 mg MBI was applied in the conjunctival sac of New Zealand White (NZW) rabbits. The other eye which remained untreated, served as a control. The acute irritation to eye conjunctivae, cornea and iris was evaluated at 1, 24, 48 and 72 hours after the treatment and grades of ocular reaction (conjunctivae, cornea and iris) were recorded at each observation. To determine the reversibility of the effect, the animal was observed for 21 days. The study report indicated that the test chemical did not produce any eye irritation during the observation period. No other clinical signs were recorded after application of test compound (REACHa).

In another study, conducted according to OECD TG 492, 50 mg of the ZnMBI in 50 µL sterile deionized water was applied topically to a reconstructed human cornea-like epithelium model (EpiOcular™). Cell viability was determined by enzymatic conversion of vital dye MTT into a blue formazan salt and measurement of the formazan salt after extraction from tissues. The percentage reduction of cell viability in comparison to untreated negative controls was used to predict the eye irritation potential. Following treatment with the test chemical, the tissue viability was 102 %, compared to 42 % after treatment with a positive control (methyl acetate). The test substance was identified as non-irritant to the eye (REACHb).

A dose of 50 mg of ZnMeMBI was administered into one eye of one male and one female adult NZW rabbits. The treated eyes were rinsed with sterile water. The contralateral eye

remained untreated and served as a control. The eyes were examined and the grade of the ocular reaction was recorded until day 7. The test substance was reported to be non-irritating to the eye (REACHd).

Sensitisation

Skin sensitisation

The sensitisation potential of MBI, MeMBI and their salts was investigated by the Local Lymph Node Assay (LLNA) and GPMTs.

In a modified maximisation test carried out in guinea pigs, MBI, 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 % MBI. 2,4-Dinitrochlorobenzene (0.1 %) served as a positive control. The second induction was performed dermally using a 20 % MBI in olive oil. The challenge was carried out with MBI at concentrations of 0.05, 0.5 and 5 % in olive oil. Under these experimental conditions, MBI did not cause sensitisation (Shimizu et al. 1994).

In an LLNA test (OECD TG 429), BALB/c mice were exposed topically to increasing concentrations of MBI (5, 10 and 25 % v/v in 4:1 acetone/olive oil). The EC3 (concentration of the chemical induced an SI >3) was estimated using a benchmark approach. The EC3 value for the test chemical was 14.7 %. Based on this value the test chemical was considered to be moderate skin sensitiser (De Jong et al. 2002).

In the LLNA test with the ZnMBI, significantly increased lymphoproliferation (indicated by an SI ≥ 3) compared to the relevant control (DMSO) was noted at all applied test concentrations. The observed stimulation index values were 9.8, 10.1 and 4.8 for test item concentrations of 25 %, 10 % and 5 % (w/v), respectively (REACHb).

The chemical 4- or 5-MeMBI did not show any sensitising activity in the LLNA test. None of the parameters measured in the substance treated groups, i.e. cell counts and weights of the draining lymph nodes, ear weights and ear swelling, reached or exceeded the "positive levels" defined for this assay. These results showed that there was no indication for a skin sensitising effect after administration of 4- or 5-MeMBI, at a concentration of up to and including 50 % (REACHd).

In a guinea pig maximisation test (GPMT) conducted according to the OECD TG 406, female guinea pigs (10 animals/dose) were first injected with 0.1 mL of 5 % w/w 4- or 5-MeMBI in arachis oil intradermally. On day 7, the same area on the shoulder region was treated with a topical application of 50 % w/w 4- or 5-MeMBI. This occlusive dressing was kept in place for 48 hours. In the challenge phase, the animals were treated with 25 % w/w 4- or 5-MeMBI in arachis oil epicutaneously. After 24 hours, the dressing was carefully removed and discarded. The challenge sites were swabbed with cotton wool soaked in diethyl ether to remove residual material. Approximately 24 and 48 hours after dressingwas removal, the degree of erythema and oedema was quantified. A positive response was seen in 5 guinea pigs after 48 hours (REACHd).

In 1998, Isama and coworkers (Isama et al. 1998) evaluated skin sensitisation potential of MBI and its methyl derivatives by conducting aGPMT. Chemicals that had a mobile hydrogen at the thioamide/iminothiol moiety, such as MBI, 2-mercapto-4-methylbenzimidazole (4-MeMBI), 2-mercapto-5-methylbenzimidazole (5-MeMBI) and 2-mercapto-5,6-dimethylbenzimidazole (5,6-DMBI), were considered to be sensitisers, whereas those that lacked a mobile hydrogen at the corresponding moiety, such as 2-(methylmercapto) benzimidazole (2-MeMBI), 1-methyl-2-(methylmercapto)benzimidazole (1,2-DMBI) and 2-mercapto-1,3-dimethylbenzimidazole (1,3-DMBI), were considered to be non-sensitisers. The skin sensitisation potencies were of the following order: 5,6-DMBI > 4-MeMBI > 5-MeMBI > MBI. The study also reported a linear relationship between skin sensitisation potency and logarithm of the partition coefficient in n-octanol/water system (log P ow) among the MBI derivatives considered to be sensitisers.

Observation in humans

In 17 subjects allergic to 2-mercaptobenzothiazole, the reaction to MBI (1 % in petrolatum) was investigated in the epicutaneous test. The reactions were read after 48 hours, positive results were observed in 2 of the 17 subjects. All tests were reported as negative in 20 control subjects (Foussereau et al. 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 2 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 observed with 2,5-di-tert.-butylhydroquinone, the monobenzyl ether of hydroquinone, and MBI (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 MBI. 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 (Geier et al., 1994).

In a study of 12 patients (8 men, 4 women), who were sensitive to 2-mercaptobenzothiazole, patch-testing of 2 % MBI (in petrolatum) with 48 hour exposure were reported to produce negative results (reactions were read after an additional 24 hours). There was no cross-sensitisation reported (Fregert 1969).

It was also reported that 1patient 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 MBI (1 % in petrolatum), for periods of 20 minutes as well as 2 days. No positive reaction to MBI was noted at the end of either period (Tuyp and Mitchell 1983).

Repeat dose toxicity

Oral

Several published oral repeat dose studies are available for MBI, methylated derivatives of MBI and their zinc salts. All studies indicated that they have adverse effects following repeated oral exposure. Their chemical structures include a thiourea unit, similar to potent thyrotoxic compounds, thiourea (TU) and ethylenethiourea (ETU).

In a sub-chronic oral dose study, Crj: CD (SD) rats 10/sex/ dose) were administered 1.2, 4, 8, 12 or 40 mg/kg bw/day MBI by gavage for 28 days (REACHa). Low food consumption and body weights were observed in males given 12 mg/kg bw/day and higher doses, and in females given 40 mg/kg bw/day MBI. Low platelet and reticulocyte counts and higher mean corpuscular haemoglobin concentration values were observed in males given 12 mg/kg bw/day or more and in females given 40 mg/kg bw/day. Haematocrit and prolonged prothrombin time were reported to be low in both sexes receiving 40 mg/kg bw/day.

Enlargement of thyroid was observed in both sexes receiving 4 mg/kg bw/day or more, and thyroid weights remained higher even after the recovery period. Higher values for absolute and relative thyroid weights were found in males receiving 4 mg/kg bw/day or more of the chemical and in females given 12 mg/ kg or more. On histopathological examination, both sexes receiving 1.2 mg/kg bw/day or more were reported to exhibit hyperplasia/hypertrophy of follicular cells in the thyroids.

Vacuolisation of cortical cells in the adrenals was reported to occur in animals receiving 40 mg/kg bw/day. The histopathological changes in the thyroids and adrenals were also reported as being present, although lower in degree after the recovery period. Based on the above results, it was concluded that MBI affects the thyroid, hematopoietic functions, and hepatic and renal functions.

The no observed adverse effect level (NOAEL) for MBI from the repeated dose oral exposure was considered to be less than 1.2 mg/kg bw/day for both sexes based on the effects on the thyroid at all doses tested.

In another 28 day oral repeat dose toxicity study, Wistar rats were administered 2, 10 and 50 mg/kg bw/day MBI by gavage. No mortality occurred during or after the treatment. Decreases in body weight gain and food consumption in the 50 mg/kg bw/day dose group were observed during the second half of the treatment period. Haematological examination and serum biochemical tests revealed decreased white blood cells and haemoglobin and increased serum urea nitrogen, cholesterol, phospholipid and gamma-glutamyl transpeptidase in this dose group. Marked thyroid enlargement (10 fold the control weight), associated with diffuse hyperplasia of follicles with decreased colloid and thickening of the fibrous capsule, was also observed. Even after a 2 week recovery period thyroid weights were 6 fold compared to the control weight. Reduction in thymus weight was observed in all dose groups in a dose dependent manner, without significant histopathological alteration. The NOAEL for MBI could not be established due to significant decrease in thymus weight at all doses (Kawasaki et al. 1998).

In a subacute oral toxicity study (Saitoh et al. 1999), male and female Wistar rats were treated with a 1:1 mixture of 4-MeMBI and 5-MeMBI by gavage at doses of 0 (corn oil), 4, 20

and 100 mg/kg bw/day for 28 consecutive days, followed by a 2 week recovery period. Relative weights of lung, liver and kidney, and serum cholesterol and phospholipid significantly increased in male rats treated with 20 and 100 mg/kg bw/day. The increase in weight was reversible and not accompanied by histopatholgical changes. Males in the100 mg/kg bw/day group were reported to exhibit a 1.8-fold increase in thyroid weight associated with histopathological changes but not altered serum thyroid hormone levels. Females administered 100 mg/kg bw/day were reported to exhibit significant increases in liver and kidney weights. Thyroid weight and serum cholesterol levels remained within normal range. The NOAEL for male and female rats was found to be 20 mg/kg bw/day.

In a combined, repeated dose reproductive/developmental toxicity study (details in the Reproductive and Developmental Toxicity Section), SD rats (10/sex/dose) were administered 4- or 5-MeMBI in the diet at 1000, 2750 or 7500 ppm (50, 138 or 375 mg/kg bw/day) (REACHd).

The study indicated that signs of systemic toxicity in adults included dose related decreases in body weight gain and food consumption in males and females from all treatment groups. Haematology of the high dose animals showed no significant trends despite the myeloid hypoplasia and splenic changes observed at histopathology. The clinical chemistry findings were indicative of alterations in metabolism including elevated cholesterol levels. Other blood chemistry changes including elevated plasma creatinine, phosphorus and chloride were not associated with renal changes at histopathology. Thyroid follicular cell hypertrophy was reported in rats in all treatment groups.

In a modified extended one generation reproductive toxicity study (REACHc) (described in detail in Reproductive and Developmental Toxicity Section), CD rats (25/sex/dose) were given 0, 5, 15, or 40 mg/kg bw/day ZnMeMBI by gavage from 2 weeks prior to mating (all animals) until weaning (females). There were no significant treatment related effects reported on mortality, clinical signs, body weight, body weight gain, haematology, clinical chemistry parameters or macroscopic findings. Mean T4 concentrations in F0 animals given 40 mg/kg bw/day ZnMeMBI were observed to be slightly higher than those observed in controls, with a dose response apparent in F0 males but not in F0 females. Mean serum TSH concentrations were statistically significantly higher than controls in all groups of F0 females, although there was no apparent dose response; a similar difference was not apparent in F0 males.

In the same study F0 animals, minimal to slight liver centrilobular hypertrophy was present in a number of rats (both sexes) given 15 or 40 mg/kg bw/day. Minimal follicular cell hypertrophy of thyroid was present in more than half of the males and females at 40 mg/kg/day and in some males at 15 mg/kg/day, and minimal to slight involution/atrophy of the thymus in some of the males and females at 40 mg/kg bw/day. These effects were considered treatment related. Based on the observations, the NOAEL for systemic toxicity of the F0 animals was 15 mg/kg bw/day based on increased incidences of liver hypertrophy, thyroid hypertrophy and involution/atrophy of the thymus.

In a 14 day study looking at toxicokinetics, a 2.7 fold increase in thyroid weight with moderate diffuse hyperplasia of the follicular cells was observed in rats exposed for 14 days to 5-MeMBI at 0.6 mmol/kg (Sakemi at al. 2002).

Inhalation

In a sub-chronic inhalation study, F344 rats (10/sex/dose) were exposed to 0 (controls), 0.1, 0.3, 1, 3 and 10 mg/m^3 MBI for 6 hours per day, 5 times per week for 13 weeks (BG RCI, 2000). It was reported that at the highest concentration (10 mg/m^3), 5 female rats died during week 12 of the study. Clinical signs of toxicity included emaciation and decreased activity. At the end of the study, body weights were lower in the 3 and 10 mg/m^3 (both sexes) compared to those of controls; changes were also observed with respect to various organ weights, particularly the thyroid, which were found to be enlarged at necropsy. The haematology and clinical chemistry parameters showed changes even at the lowest test concentration, such as decreased erythrocyte parameters, increased lymphocyte counts and inhibition of the coagulation cascade. At higher exposure levels, impairment of renal clearance, suppression of thyroid function, increase in plasma pseudocholinesterase activity and elevated serum protein levels (albumin and globulin) were observed. At concentration levels of 3 and 10 mg/m^3 thyroids were hypertrophic, while rats at the highest dose group had follicular cell hyperplasia of the thyroid. In these 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 and adrenocortical necrosis. A No Observed Adverse Effect Concentration (NOAEC) was not established in this study.

In a similar study in B6CF1 mice, with identical concentrations of MBI and exposure duration as in the rat study, 5 male and 9female mice from the 3 mg/m^3 group died. Liver weights were higher in the male mice exposed to 3 and 10 mg/m^3 MBI and in the females exposed to10 mg/m^3, when compared to controls. Female mice at 0.1, 0.3 and 10 mg/m^3 had higher kidney weights, and males and females at the highest dose had significantly higher thyroid weights compared with the controls. Macroscopically, no compound related lesions were observed. Histopathological findings included thyroid hypertrophy, hepatic centrilobular cytomegaly and lipoid degeneration of the zona reticularis of the adrenals. No further details were available (BG RCI, 2000).

In a subchronic inhalation toxicity study (Gaworski et al. 1991) F344/N rats (10/sex/dose) were exposed to a dry aerosol of MBI (>98 % pure) for 6 hours per day, 5 times/week for a period of 13 weeks. The concentrations were 0, 3.13, 6.25, 12.5, 25 and 50 mg/m^3 air. An additional 19 male rats/group were included in the 3.13, 12.5 and 50 mg/m^3 dose groups for special studies regarding the pituitary and thyroid (See Endocrine Effects)Almost all animals in the highest group died during the study, or had to be sacrificed in a moribund condition. Concentrations of MBI at and above 12.5 mg/m^3 caused body weight loss and dose dependent anaemia. Males and females exposed to 6.25 mg/m^3 displayed statistically significant increases in absolute and relative thyroid weights. Histopathologically, rats exposed to 3.13 mg/m^3 or higher doses exhibited dose dependent thyroid follicular cell hyperplasia. In all exposure groups, there was a concentration dependent decrease in absolute and relative thymus weights and increase in liver weights. Histopathological examination revealed thymic atrophy from 12.5 mg/m^3 and hepatocyte hypertrophy at exposure levels of 25 mg/m^3 and above. Lesions were also observed in the kidneys (tubular atrophy and mineralisation), the adrenals (necrosis and degeneration), the pancreas (hyperplasia of islet cells) and bone marrow (hypocellularity), and proliferation of the thyrotropic hormone-producing basophilic cells of the anterior adenohypophysis.

Thyroid hyperplasia and increase in thymus weight thus proved to be the most sensitive parameters. Both findings were reported to occureven at the lowest test concentration of

3.13 mg/m^3. Therefore, it was not possible to determine a no observable adverse effect concentration (NOAEC) in this study.

A similar study was conducted in B6CF1 mice with identical concentrations of MBI and exposure duration as described in the above study. The study reported that no exposure related mortalities occurred, and the animals exhibited no clinical signs of intoxication. At autopsy, male and female mice of all exposure groups had enlarged thyroids. Relative thyroid weights were significantly increased. Relative liver weights from the 2 highest exposure groups were significantly greater than controls. Dose dependent increase in thyroid follicular cell hyperplasia in males exposed to 6.25 mg/m^3, and females exposed to 3.13 mg/m^3 was noted. In addition, 2 out of 10 males and 1 out of 10 females of the 50 mg/m^3 group were found to have focal hyperplasia of the thyroid. Degeneration of the zona reticularis of the adrenals was seen only in the females, at all dose levels. Based upon these histopathological changes, the NOAEC of 3.13 mg/m^3 was established in males. For females, a NOAEC was not determined, as even the lowest exposure group (3.13 mg/m^3) was found to have ultrastructural changes in the thyroid and adrenals (Gaworski et al., 1991).

Genotoxicity

Based on reported negative results in the in vitro and in vivo mutagenicity tests, the chemicals in this group are not considered to have genotoxic potential.

In vitro

MBI was tested for its genotoxic potential in a bacterial reverse mutation assay in *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 test concentrations were in the range from 3.3 to 1000 µg/plate. Negative results for the chemical were reported (Zeiger et al. 1987).

MBI was tested for mutagenic activity in the mouse lymphoma assay (L5178Y) with and without metabolic activation (S9 mix from Aroclor 1254-induced rat liver) (BG RCI, 2000). 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. Generally negative results were obtained at lower concentrations. Levels of 200 and 300 ug/mL, which were included in 2 series, induced significantly increased mutation rates, but were associated with high cytotoxicity. At concentration levels of 400 ug/ml and 500 µg/ml, MBI produced complete inhibition of cellular growth.

The zinc salt of MBI (ZnMBI) tested negative in the reverse mutation assay (OECD TG 471) with *S. typhimurium* TA98, TA1537, TA1535 and TA100 strains and *Escherichia coli* WP2 uvrA. The concentrations tested were of 5, 16, 50, 160, 500, 1600 and 5000 µg/plate (REACHb).

Negative results were reported for 4- or 5-MeMBI in a reverse mutation test using the *S. typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and TA 102. The concentrations tested were 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate (REACHd).

Negative results were reported for 4- or 5-MeMBI in a mammalian cell gene mutation assay following OECD TG 476 at concentrations up 1000 ug/mL. No relevant and reproducible increases in mutations were observed in the main experiments up to the maximum concentration. Cytotoxic effects were reported at 900 µg/mL and above in 1 of the experiments without metabolic activation following 24 hours of exposure (REACHd).

The chemical ZnMeMBI tested negative in the mammalian chromosomal aberration test (OECD TG 473). It did not induce any statistically significant increases in the frequency of cells with chromosome aberrations in either the absence or presence of a liver enzyme metabolizing system. ZnMeMBI was therefore considered to be non-mutagenic in vitro (REACHc).

In vivo

In order to study the clastogenic effect of MBI, 2 blood smears per mouse were obtained from B6C3F1 mice at the end of the 90 day inhalation study described above. Test concentrations of MBI were 3.13, 6.25, 12.5, 25 and 50 mg/m^3 air (NTP, 1988 b). The blood smears were scored for micronuclei (10000 normochromatic erythrocytes and 2000 polychromatic erythrocytes/animal). None of the groups treated with MBI were found to have increased numbers of micronucleated polychromatic or normochromatic erythrocytes as compared with the controls. Thus, MBI was not clastogenic under these experimental conditions (BG RCI 2000).

The result of the micronucleus test carried in this 90 day inhalation study are considered relevant in accordance with OECD TG 474, as in this study the mice received MBI treatment up to the time of sacrifice, when blood smears were obtained. It was not possible to incorporate a positive control because of the nature of the experimental design (90 day study).

Reproductive and development toxicity

In subchronic inhalation toxicity studies discussed above, in which in rats and mice were exposed to 0.1, 0.3, 1, 3 and 10 mg/m^3 MBI for 6 hours per day for 13 weeks, testicular weight and epididymal weight were not altered in mice or rats, but sperm motility was significantly reduced in rats. Female mice showed an increased oestrous cycle length relative to controls (no data provided on dose dependency). The reduction in sperm motility and the increase in oestrus cycle length were interpreted by the authors as indicators of effects on fertility (Morrissey et al. 1988).

In the other subchronic inhalation study which was conducted in rats and mice using higher concentrations (3.13, 6.25, 12.5 and 50 mg/m^3) of MBI (Gaworskiet al. 1991), no changes were seen in testicular and epididymal weights, sperm count per gram of tissue, or total spermatid heads per testes in male mice. Sperm motility was slightly but statistically significantly reduced in the 3.13, 12.5 and 50 mg/m^3 groups. The male rats exhibited no changes in these study parameters compared with the controls. The female mice treated with the highest concentration (50 mg/m^3) were found to have a significant increase in oestrus cycle length (4.6 days, controls 4.1 days). The female rats exposed to MBI at 6.25 mg/m^3 exhibited a significant reduction in oestrus cycle length.

In a modified extended one generation reproductive toxicity study (EOGRTS) (OECD TG 443) (REACHc), CD male and female rats (25/sex/dose) were given 0, 5, 15, or 40 mg/kg bw/day ZnMeMBI (99.5 % pure) by gavage for 2 weeks prior to mating, during the 14 day mating period, throughout gestation, and through lactation until weaning. Study design and cohort assignment are summarised below:

F0 animals: Two weeks before pairing until termination after litters are weaned.

F1 Generation: Each set of F1 offspring was maintained on the test diet from the time of weaning until termination. Although direct exposure starts at weaning on Day 21 of age, all offspring have potential indirect exposure in utero and through the milk during lactation. F1 offspring were divided into 5 groups (Cohorts 1A, 1B, 2A, 2B, and 3) at weaning on PND21, and evaluated for potential effects on the nervous system, reproductive and endocrine systems, thyroid function, and other systemic toxicity parameters, as follows:

> **Cohort 1a** (22/sex/group): Assessment of reproductive and systemic toxicity – treated from weaning to 13 weeks of age.
>
> **Cohort 1b** (20/sex/group): Reproductive /developmental toxicity testing - treated from weaning to 21/22 weeks of age following breeding.
>
> **Cohort 2a** (10/sex/group): Developmental neurotoxicity testing - treated from weaning up to day 75 of age.
>
> **Cohort 2b** (12/sex/group): Developmental neurotoxicity testing - assigned to neuro-histopathology assessment at weaning.
>
> **Cohort 3** (12/sex/group): Developmental immunotoxicity testing - treated from weaning up to Day 60 of age.

Unselected F1 offspring: Retention of brain, spleen, thymus and mammary tissue and organ weights - no direct treatment, sacrificed after weaning.

Parental toxicity (F0 and F1 adult animals): There were no significant treatment related effects observed for F0 or F1 parental mortality, clinical signs, body weight, body weight gain, haematology, clinical chemistry parameters, urinalysis or macroscopic findings. On day 22-24 of gestation, F0 and F1 females in the 40 mg/kg bw/day group were observed to have prolonged parturition/dystocia, with deteriorating clinical condition, and therefore were sacrificed. These premature deaths were considered to be ZnMeMBI-related. Mean T4 concentrations in F0 animals given ZnMeMBI were slightly higher than those observed in controls, with a dose response apparent in F0 males but not in F0 females. Mean serum TSH concentrations were statistically significantly higher than controls in all groups of F0 females, although there was no apparent dose response; a similar difference was not apparent in F0 males. There was no effect of ZnMeMBI administration on mean serum TSH concentration in F1 offspring on day 22 of age.

In F0 and Cohort 1A (F1) animals, minimal to slight liver centrilobular hypertrophy was present in a number of rats (both sexes) at 15 or 40 mg/kg bw/day. Minimal follicular cell hypertrophy of thyroid was present in more than half of the males and females given 40 mg/kg/day and in some males at 15 mg/kg/day, and minimal to slight involution/atrophy of

thymus in some of the males and females at 40 mg/kg bw/day. These effects were considered treatment related.

No adverse effects on sperm motility, concentration, motion or morphology in F0 males were apparent following treatment with ZnMeMBI at doses up to and including 40 mg/kg bw/day. Oestrous cycle regularity of the F0 females during the 2 week pre-pairing treatment period and at termination were unaffected by treatment. There were no effects on reproductive performance.

The ano-genital distance of F1 male offspring in the 15 mg/kg/day group and of both sexes of F1 offspring in the 40 mg/kg bw/day group on Day 1 of age was slightly, but statistically significantly increased when compared to controls.

Histopathological observations in F1 animals included minimal liver hypertrophy in a small number of animals (both sexes) at 15 or 40 mg/kg bw/day, minimal thyroid hypertrophy in some of the males and females at 40 mg/kg bw/day and some males at 15 mg/kg bw/day. There was no effect of ZnMeMBI on the mean number of implantation sites, litter size or post implantation survival in the Cohort 1B females. Live birth index (F2 generation); however, was statistically significantly lower than control in the 40 mg/kg/day group, with offspring mortalities occurring between birth and formal assignment to day 1 of lactation.

Based on the observations, the NOAEL for systemic toxicity and reproductive performance of the F0 and F1 animals was 15 mg/kg bw/day based on increased incidences of liver hypertrophy, thyroid hypertrophy and involution/atrophy of the thymus, and incidences of prolonged parturition/dystocia in females of both generations receiving 40 mg/kg/day.

The NOAEL for the F1 and F2 offspring up to weaning was concluded to be 15 mg/kg/day due to reduced early post-partum survival at 40 mg/kg/day in both generations. There was no evidence of developmental neurotoxicity or developmental immunotoxicity in this study, therefore the NOAEL for these endpoints was concluded to be 40 mg/kg/day.

In a combined, repeated dose reproductive/developmental toxicity study (REACHc), SD male and female rats (10/sex/dose) were administered ZnMeMBI orally in the diet at 1000, 2750 or 7500 ppm (ca. 50, 138 or 375 mg/kg bw/day). Treatment began 2 weeks prior to mating and continued throughout maturation, mating, gestation and up to day 5 of lactation (ca. 47 days). The dose levels were reduced to 900, 2500 or 6750 ppm (ca. 45, 125 or 338 mg/kg bw/d) on day 29. On study day 33, the high dose group was further reduced to 5500 ppm (ca. 275 mg/kg bw/d) due to observed toxicity. A control group of similar size was given untreated diet only. Following 2 weeks of dosing, male and female rats were paired within their dose groups to produce litters. At day 5 post-partum, necropsies were performed on all surviving females and offspring and all adult male animals.

One female at the high dose and eight females on intermediate dose were sacrificed during the gestation phase of the study due to possible dystocia. Six of the eight female mortalities were found to have offspring in utero at post mortum examination indicating possible impairment of parturition. There were no mortalities at low dose or in the controls. No major clinical signs were observed in any of the treated animals.

At the high dose there was a marked reduction in the number of mating pairs with positive evidence of mating and pregnancies. The females with no evidence of mating generally

showed a lack of oestrous cyclicity. Of the mating pairs, where positive evidence of mating was observed, pregnancy was established in 50 % of these cases. Two of the mating pairs with positive evidence of mating also showed increased pre-coital interval. These findings were considered to be treatment related and of toxicological importance. At the intermediate and low doses there were no treatment related effects on fertility. All mating pairs at these doses showed positive evidence of mating and pregnancy and offspring bodyweight were not affected.

At the high dose, the low number of pregnant females resulted in too few animals. Statistically significant reductions in absolute weights of kidneys, epididymides, heart, thymus, spleen ($p<0.001$) and adrenals ($p<0.01$) compared to controls were observed. Relative liver, testes and brain weights were significantly higher ($p<0.001$) than those of controls. Centrilobular hepatocyte enlargement and follicular cell hypertrophy of the thyroid were observed in rats of either sex at all treatment levels.

At lower dose levels similar signs of toxicity were observed but at a lower incidence. There were no effects upon mating performance or fertility when compared to the highest dose level, and offspring bodyweight was not affected.

Histopathology of the reproductive organs showed no specific effects. The live litter size at birth was low but offspring bodyweight appeared unaffected by treatment. These effects were seen at a dose level that was toxic to the adult and, therefore reproductive failure was a consequence of the toxicity seen. A NOAEL was not established in this study.

In a teratology study with MBI, pregnant Wistar (SPF) rats were administered daily doses of 0, 3.3, 10 or 30 mg MBI/kg bw by oral gavage (in olive oil) on gestation days (GD) 7-17 (period of organogenesis). In the pregnant rats from the 10 and 30 mg/kg bw/day dose groups, thymus and thyroid weights of the dams were significantly lower than those of the controls. The NOAEL for the dams was found to be <3.3 mg/kg bw/day based on reduction in thyroid and thymus weights.

Foetal resorption rates were comparable to those seen in the control group. There were no visceral or skeletal malformations. Only some variations of organogenesis were diagnosed, which were seen in the form of kinked ureter and dilated renal pelvis in 20 % of foetuses from the 10 mg/kg group and 48 % of the foetuses from the 30 mg/kg bw/day 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 10 mg/kg bw/day and above MBI, in comparison with the controls. The NOAEL for foetal toxicity of MBI was 3.3 mg/kg bw/day and hence, was higher than the chemical's maternal NOAEL (<3.3 mg/kg bw/day). In these studies, maternal toxicity was reported to always precede foetotoxicity. Malformations (such as cleft palate) were only observed upon oral administration of 60 mg/kg bw/day, a dose which was fatal to many of the dams receiving such treatment (Yamano et al. 1995).

Three groups of pregnant rats were treated with 60 mg/kg bw/day MBI (highly toxic dose) on GD 7 to 10, GD 11 to 14 and GD 15 to 17, (specific periods of organogenesis). Even under these short term treatment conditions, MBI was severely toxic to dams at 60 mg/kg bw/day, since all rats showed a substantial decrease in body weight and food consumption and 5 out of the 16 rats, treated from day 11 to 14 after mating, died. There was increased foetal resorption in all 3 60 mg/kg bw/day groups. Of the surviving foetuses from the dams treated

with 60 mg /kg bw/day MBI from day GD11 to GD14, 40 % were found to have a cleft palate. No other anomalies were observed.

In a developmental toxicity study (REACHc), female Crl:CD(SD) rats (20/group) received ZnMeMBI at doses of 8, 25 or 70 mg/kg bw/day (in 5 mL/kg bw corn oil) by oral gavage, from GD 6 to 19. The control group received just the vehicle (corn oil at the same volume). Animals were sacrificed on day 20 after mating for reproductive assessment and foetal examination. The report indicated that there were no treatment related macroscopic abnormalities detected among the parent females or foetuses at scheduled termination.

There was no effect of ZnMeMBI treatment on litter data, as assessed by the mean number of implantations, resorptions, live young, sex ratio and pre- and post-implantation losses. Placental and litter weights were essentially similar in all groups, but foetal weights were slightly low at 70 mg/kg bw/day.

The reported incidence of major and minor foetal abnormalities and skeletal variants indicated no dose response relationship to maternal treatment with ZnMeMBI. Across all treated groups, there was a reported increase in the incidence of incompletely ossified cranial bones when compared to concurrent control, the foetal and litter incidences of which exceeded the historical control data range. The assessment of ossification is; however, an evaluation at a snapshot in time, occurring at a transitory stage in foetal development, and ossification would continue as the animals matured. Therefore this minor skeletal abnormality is considered to have no long term consequence and is not adverse. Based on the results of this study, the NOAEL for maternal toxicity and embryo-foetal survival and development was concluded to be 70 mg/kg bw/day.

Immunotoxicity

In order to study the effect of MBI on the immune system, female Wistar rats were injected with 30 mg/kg bw every day from day 11 to 15 of gestation. At the age of 1 month, the pups were examined for body weight, the weight of the thymus and spleen, and their humoral and cellular immune response. MBI caused a reduction in thymus weight in the progeny. 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).

Following a single dose of MBI (no details of the dose or the route of administration), the study indicated that rats showed considerable involution of the thymus 5 days after treatment. Mice, guinea pigs and rabbits did not show this effect (Malmfors 1976).

Endocrine effects

Enzyme induction

In an in vitro assays with rat liver microsomes, cultured in the presence of MBI and its methyl derivatives (4-MeMBI, 5-MeMBI, and (4(5)-MeMBI), it was shown that these chemicals have potential for metabolic drug-drug interactions. At low concentrations, 4-MeMBI and 5-MeMBI inhibited CYP3A2 activity, but at higher concentrations (≥100 µM) they induced CYP1A1/2 activity (Miyajima et al. 2017; 2020).

Thyrotoxicity studies

Ten male rats (strain unspecified) received daily intragastric doses of 21 mg MBI/kg bw for 12 days. Treatment resulted in a significant increase in relative thyroid weights and a marked reduction in plasma thyroxine levels (1.21Ug/100 ml compared with 7.11 ~g/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 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, a response which ultimately results in hypertrophy of the thyroid (Janssen et al., 1981). This specific thyrotoxicity correlates with the accumulation of the test compound and its main metabolite, benzimidazole, in the thyroid.

In the subchronic inhalation toxicity study described in the report above (Inhalation Section) (Gaworski et al. 1991), additional 19 F344/N male rats/group were included in the 3.13, 12.5 and 50 mg/m^3 concentration groups for special studies regarding the pituitary and thyroid (Norford et al., 1993). Prior to the beginning of exposure and at 2, 4 and 8 weeks, the levels of triiodothyronine (T3) and thyroxine (T4), were determined.

The 3.13 mg/m^3 concentration did not affect the levels of thyroid hormones in the male rats. In the 12.5 mg/m^3 group, rats showed a drop in T3 and T4 levels at 2, 4 and 8 weeks, which was reversible after13 weeks. In the 50 mg/m^3 group, T3 levels were depressed at 2 weeks but found to have recovered to control levels by the end of the study. T4 levels in this group were below the limit of detection from 2 weeks until the end of the study.

The pituitary and thyroid of the 19 additional male rats were examined by electron microscopy and histochemical techniques. These investigations revealed that dosedependent follicular hyperplasia and hypertrophy occurred in the thyroid, and that all exposure groups were affected after only 2 weeks of exposure. The thyrotrophs of the pituitary were hyperplastic in all 3 groups and had varying numbers of hypertrophic cells with either eosinophilic stippled cytoplasm or with eosinophilic globules within 1 or more large vacuoles that displaced the nucleus. These cells were compared by immunohistochemistry and electron microscopy to "thyroidectomy cells" within the anterior pituitary of thyroid-parathyroidectomised rats and were determined to be identical to them. Immuno-histochemical staining for the B-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, MBI 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).

In a toxicokinetic study (Sakemi, 2002) repeated treatment with MBI resulted in significant decrease of serum T3 and T4 levels and increase of TSH levels. Slight changes were observed for 4-MeMBI but no changes of these hormone levels were found on treatment with the MMBI mix or 5-MEMBI.

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