Australian Government
Department of Health and Aged Care
Australian Industrial Chemicals Introduction Scheme

Cyclohexanamine, 4,4'-methylenebis[2-methyl-

Evaluation statement

26 June 2023

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AICIS evaluation statement

Subject of the evaluation

Cyclohexanamine, 4,4'-methylenebis[2-methyl-

Chemical in this evaluation

Name	CAS registry number
Cyclohexanamine, 4,4'-methylenebis[2-methyl-	6864-37-5

Reason for the evaluation

Evaluation Selection Analysis indicated a potential human health risk.

Parameters of evaluation

The chemical is listed on the Australian Inventory of Industrial Chemicals (the Inventory). This evaluation statement includes a human health risk assessment for all identified industrial uses of the chemical in Australia.

Summary of evaluation

Summary of introduction, use and end use

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

Based on international use information, the chemical is mainly used as a hardener in the manufacture of epoxy resins and polyamides. The epoxy resins and polyamides have several commercial applications including paints and coatings. Although some of these commercial products may be used in domestic settings, based on available information this is not expected to be widespread. Chemicals manufactured from the chemical may also be used as food contact materials.

Human health

Summary of health hazards

The critical health effects of the chemical include the following:

- Acute toxicity
- Skin corrosion and eye damage
- Repeat dose toxicity
- Reproductive toxicity.

The chemical is corrosive, causing severe skin burns (full thickness necrosis after 3 minutes of exposure) and serious eye damage in studies in rabbits.

Case reports of skin abnormalities including scleroderma like symptoms in some workers was linked to chronic inhalation exposure to the chemical.

The chemical caused significant systemic toxicity following oral exposure in several guideline studies in rats. The main indicator of systemic toxicity was vacuolar degeneration in many organs and tissues, including the liver, kidney, adrenals, skeletal muscle and heart. Systemic vacuolation was consistently seen in most of the available guideline studies including a 90-day repeat dose toxicity study and an extended one generation reproductive toxicity study. Across several repeated dose oral toxicity studies, there was consistent evidence that the chemical caused effects at doses of 5 mg/kg bw (body weight)/day and above. Mild liver toxicity and disturbed haemoglobin metabolism were the main signs of systemic toxicity following repeated inhalation exposure in a guideline study in rats. The lowest no observed adverse effect concentration (NOAEC) for the chemical was 2 mg/m^3.

Based on the available data, the chemical may cause reproductive toxicity following repeated exposure. Reduced implantation sites along with reduced litter sizes was the main indicator of effects on fertility. Based on the findings of several studies, there is no clear evidence of effects on development. No treatment related effects were seen on the gestation parameters and no significant skeletal malformations were seen in the foetuses. In an extended one generation reproductive toxicity study the chemical caused no significant effects in pups from exposure during gestation and via lactation. Although decreased body weight changes in pups was observed it is unclear whether this was a result of reduced feed consumption in the dams. There were limited reported effects on developing immune systems and neurobehavioural changes, but in the absence of further information the significance of these findings is uncertain. In a pre-natal developmental toxicity study in rabbits, a lower number of implantation sites along with a decreased litter size was seen in the mid and high dose groups. The significance of the findings was uncertain. No effects were seen in a similar prenatal study in rats.

On acute exposure, the chemical is:

- fatal if inhaled (median lethal concentration (LC50) (aerosol) of 0.42 mg/L)
- toxic when in contact with skin (median lethal dose (LD50) of 200–400 mg/kg bw)
- harmful if swallowed (LD50 of 320–460 mg/kg bw).

Based on the available data the chemical is not considered:

- to be a skin sensitiser
- to have genotoxic potential
- to have carcinogenic potential
- to have endocrine disrupting properties.

Hazard classifications relevant for worker health and safety

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 worker health and safety as follows. This does not consider classification of physical and environmental hazards.

Health hazards	Hazard category	Hazard statement
Corrosion/skin irritation	Skin Corr. 1	H314: Causes severe skin burns and eye damage
Serious damage to eyes/eye irritation	Eye Damage 1	H318: Causes serious eye damage
Acute toxicity (inhalation)	Acute Tox. 2	H330: Fatal if inhaled
Acute toxicity (dermal)	Acute Tox. 3	H311: Toxic in contact with skin
Acute toxicity (ingestion)	Acute Tox. 4	H302: Harmful if swallowed
Specific target organ toxicity (repeated exposure)	STOT RE 1	H372: Causes damage to organs through prolonged or repeated exposure
Reproductive toxicity	Repr. 2	H361f: Suspected of damaging fertility

Summary of health risk

Public

There are no identified risks to the public that require management. Since Australian use data are not available for the chemical, use patterns in Australia are assumed to be similar to those overseas. Based on the available international use information, it is unlikely that the public will be exposed to the chemical directly. Although the public could come into contact with articles and/or coated surfaces, it is expected that the chemical will be bound within articles and coated surfaces. Based on available data, negligible exposure through identified use in food contact materials is expected.

Workers

During product formulation and packaging, 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 application and work practices employed. Good hygiene practices to minimise incidental oral exposure are expected to be in place.

Given the critical systemic long term, systemic acute and local health effects, the chemical could pose a risk to workers. Control measures to minimise dermal, ocular and inhalation exposure are needed to manage the risk to workers (see **Proposed means for managing risks** section).

Proposed means for managing risk

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 relating to safe introduction and use

The information in this statement should be used by a person conducting a business or undertaking at a workplace (such as an employer) to determine the appropriate control under the relevant jurisdictions and Work Health and Safety laws.

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

- using closed systems or isolating operations
- using local exhaust ventilation to prevent the chemical 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.

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

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.

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.

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 safety data sheets (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.

Conclusions

Considering the proposed means of managing risks, the Executive Director is satisfied that the identified human health risks can be managed within existing risk management frameworks. This is provided that:

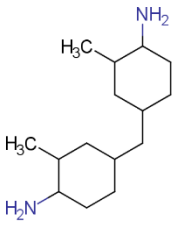
- all requirements are met under environmental, workplace health and safety and poisons legislation as adopted by the relevant state or territory
- the proposed means of managing the risks identified during this evaluation are implemented.

Note: Obligations to report additional information about hazards under *Section 100* of the *Industrial Chemicals Act 2019* apply.

Supporting information

Chemical identity

The chemical is a UVCB (unknown or variable composition, complex reaction products or of biological materials that comprises a large number of stereoisomers (ECHA 2022).

Chemical name	Cyclohexanamine, 4,4'-methylenebis[2-methyl-
CAS No.	6864-37-5
Synonyms	3,3'-Dimethyl-4,4'-diaminodicyclohexylmethane 2,2'-dimethyl-4,4'-methylenebis(cyclohexylamine) 4-[(4-amino-3-methylcyclohexyl)methyl]-2-methylcyclohexan-1-amine DMDC
Molecular formula	C15H30N2
Molecular weight (g/mol)	238.41
SMILES	CC1CC(CC2CCC(N)C(C)C2)CCC1N
Chemical description	UVCB
Structural formula	

Relevant physical and chemical properties

Physical form	Colourless to yellow liquid
Melting point	–7.1°C at 101.3 kPa
Boiling point	342°C at 101.3 kPa
Vapour pressure	0.08 Pa at 20°C
Water solubility	2.01 g/L at 20°C
pKa	10.3 at 25°C
log K_{ow}	2.3 at 23°C and pH 10

Introduction and use

Australia

No specific information about the introduction, use and end use of the chemical in Australia has been identified.

International

Most of the identified uses for the chemical are site limited applications (Chemwatch n.d.; ECHA 2022; OECD 2005; REACH n.d.; SPIN n.d.; US EPA 2012). The chemical is mainly used as a hardener in the manufacture of epoxy resins and polyamides. The chemical is also used as a monomer for specialty plastics and in the coatings industry.

Epoxy resins cross linked with the chemical are used mainly for coating concrete and other building materials, as raw material for varnishes, and in anti-corrosive paints. These resins can also be used in shipbuilding and for coating pipelines, as well as in the wet laminating of heavy duty fibre composite materials. Application of the substance without chemical conversion is not known (OECD 2005).

Some of these resins manufactured from the chemical may also have domestic applications. The US Consumer Product Information Database (CPID) listed one epoxy concrete floor coating product containing the chemical for use in home maintenance (DeLima Associates).

The chemical is registered for use in the manufacture of food contact materials in the EU and the US (see **International regulatory status**).

The chemical is:

- listed on the Organisation for Economic Co-operation and Development (OECD) List of High Production Volume (HPV) Chemicals
- listed on the US EPA High Production Volume Program Chemical List
- registered under REACH with a global range of 1000 to 10000 tonnes/year (Chemwatch n.d.; OECD 2005; REACH n.d.; US EPA 2012).

Existing Australian regulatory controls

AICIS

No specific controls are currently available for the chemical.

Public

The chemical is not specifically listed in the *Poisons Standard – The Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP). However, the chemical falls under the scope of the following Schedule 5 group entry for 'Amines used as curing agents for epoxy resins' (TGA 2022).

Schedule 5 chemicals are substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label (TGA 2022).

Workers

The chemical is listed on the HCIS (SWA n.d.) with the following hazard category and statements for human health:

Health hazards	Hazard category	Hazard statement
Skin corrosion	Skin Corr. 1A	H314: Causes severe skin burns and eye damage
Acute toxicity (inhalation)	Acute Tox. 3	H331: Toxic if inhaled
Acute toxicity (dermal)	Acute Tox. 3	H311: Toxic in contact with skin
Acute toxicity (ingestion)	Acute Tox. 4	H302: Harmful if swallowed

No exposure standards are available for the chemical.

International regulatory status

Exposure standards

The following exposure standards were identified (Chemwatch n.d.):

Protective action criteria (PAC) 1, 2, and 3 of 0.28, 3.1 and 19 mg/m^3, respectively, in the United States of America (US Department of Energy, US DOE n.d.).

European Union

The chemical is listed on the 'European Commission Regulation (EU) No. 10/2011 on plastic materials and articles intended to come into contact – Annex I'. The chemical is listed for use as a monomer with these restrictions: 'only to be used in polyamides' and a 'specific migration limit of 0.05 mg/kg food' (ECHA n.d.).

United States of America

The chemical is listed on the United States Food and Drug Administration (US FDA) Inventory of food contact substances – code of federal regulations (CFR), Title 21 (US FDA n.d.) as a component in the manufacture of Nylon resins.

Human exposure

Public

Direct public exposure to the chemical is not expected. Public exposure is most likely to be minimal, resulting from the use of products containing the chemical in a converted or bound state.

Even if unreacted starting material remains, emissions are considered unlikely due to the difficulty of diffusion in a cross linked system. The low vapour pressure would also reduce potential emissions from products (OECD 2005). It was reported that there is no evidence of migration of the chemical from different food contact materials. The detection limit was about 1.2 µg/dm^2. The investigations were reported to be carried out according to the guidance for food contact materials (OECD 2005).

Health hazard information

Toxicokinetics

No studies are available. Based on physio-chemical properties and toxicological experiments, oral, inhalation and dermal absorption and systemic distribution are expected (ECHA 2022; OECD 2005; REACH n.d.). Due to the corrosive nature of the chemical and the adverse systemic effects seen in acute toxicity studies (see **Acute Toxicity** section), a 100% absorption rate is expected for oral, dermal and inhalation routes (ECHA 2022).

Acute toxicity

Oral

The chemical is currently classified as hazardous in the Hazardous Chemical Information System (HCIS) (SWA n.d.) as ‘Acute Toxicity (Oral - Category 4)’. Data are consistent with this classification.

An oral median lethal dose (LD50) of 320–460 mg/kg bw was determined in a rat study conducted similarly to OECD Test Guideline (TG) 401 for acute oral toxicity (OECD 2005; REACH n.d.). Sprague Dawley (SD) rats (5/sex/dose) were administered the chemical at 0, 316, 464, 681 or 1000 mg/kg bw via oral gavage. Mortalities occurred in the 464 mg/kg bw dose group and above. Clinical observations in dosed animals included shortness of breath, apathy, diarrhoea, poor health state, abnormal salivation and blood in stool. Necropsy findings showed evidence of toxic effects in the gastro-intestinal tract (reddening in the stomach and intestines, scattered occurrence of gastric ulcers and diarrhoeic intestinal contents) and heart (acute dilatation of ventricles) (REACH n.d.).

Dermal

The chemical is currently classified as hazardous in the Hazardous Chemical Information System (HCIS) (SWA n.d.) as 'Acute Toxicity (Dermal - Category 3)'. Data are consistent with this classification.

A dermal LD50 of 200–400 mg/kg bw was determined in a rabbit study conducted similarly to OECD TG 402 for acute dermal toxicity (OECD 2005; REACH). Vienna White rabbits (5/sex/dose) were exposed to the neat chemical under occlusive conditions at doses equivalent to 200 or 400 mg/kg bw for 24 hours. Clinical observations in dosed animals included cyanosis, apathy, shortness of breath, accelerated breathing, abdominal position with flaccid extremities and tremors. Soft necrosis (tissue damage) was seen at the treatment site in all animals. Autopsy findings showed toxic effects in the heart (acute dilatation and acute congestion), lung (notable congestion) and liver (REACH n.d.).

Inhalation

The chemical is currently classified as hazardous in the Hazardous Chemical Information System (HCIS) (SWA n.d.) as 'Acute Toxicity (Inhalation - Category 3)'. The available data support an amendment to this classification. Based on available data, the chemical has very high acute inhalation toxicity with a calculated median lethal concentration (LC50) (inhalation, aerosol) of 0.42 mg/L.

An inhalation LC50 of 420 mg/m^3/4 hours was determined in a rat study conducted similarly to OECD TG 403 for acute inhalation toxicity (OECD, 2005; REACH n.d.). SD rats (10/sex/dose) were exposed via head/nose inhalation to the chemical as a liquid aerosol at 0.053, 0.31, 0.41, 0.62 mg/L air (analytical concentrations) for 4 hours. Clinical observations in dosed animals included apathy, staggering, squatting in abdominal position, ruffled fur and signs indicative of marked airway and eye irritation, such as eyelid closure, watery eyes, corneal opacity and nose discharge. Necropsy findings showed evidence of toxic effects in the heart (acute dilatation and acute congestive hyperaemia) and lung (moderate oedema and focal hyperaemia) (REACH n.d.).

Observation in humans

Paleness, swelling of the lips, paralysis of neck muscles, and severe hypotension resulting from reduced cardiac output) with characteristic electrocardiographic anomalies were reported in a subject who unintentionally ingested a small amount of the chemical (OECD 2005). Lesions were not seen in the subject's mouth.

Corrosion/Irritation

Skin irritation

The chemical is classified as hazardous in the Hazardous Chemical Information System (HCIS) (SWA n.d.) as 'Skin corrosion/irritation - Category 1A'.

The two available in vivo studies in rabbits provide evidence that the undiluted chemical is corrosive or severely damaging to skin, with corrosive effects observed following 3 minute exposure in one study. However, there is insufficient in vivo data on the relevant time points at which effects were observed to determine the UN GHS skin corrosive sub-category (1-hour observations not reported). In vitro data show that the chemical is corrosive to skin with the results warranting a UN GHS skin corrosive Category 1B classification based on the

criteria of the prediction model. Overall, as data are not sufficient for sub-categorisation an amendment to the classification is warranted.

In a non-GLP compliant skin irritation study similar to OECD TG 404, Vienna White rabbits were treated with the undiluted chemical for three minutes (n=4) or one hour (n=2) under occluded conditions. Observations were recorded at 3 minutes, 1, 24, 48 hours and 8 days after the patch removal. In the 3 minute exposure group, the following mean scores were reported for observations at 3 minutes, 24 and 48 hours and 8 days: 0.5, 2.75, 2.5, 3.25 for erythema and 0, 2.25, 2, 0.5 for oedema, respectively. One animal had a reported score of 4 for erythema at the 24 hr observation. No scores were reported for the 1 hour observation period. Necrotic skin changes (irreversible full thickness necrosis) were reported in 3 out of 4 treated animals on day 8 of the study (OECD 2005; REACH n.d.). In the 1 hour exposure group, the following mean scores were reported for observations at 1, 24 and 48 hours and at 8 days: 2, 4, 4, 4 for erythema and 2, 3.5, 3.5, 2 for oedema, respectively.

In a non-GLP compliant skin irritation study similar to OECD TG 404, the chemical was identified as a strong irritant producing severe skin damage to rabbit skin (REACH n.d.). The chemical (undiluted or as a 30% preparation) was applied to rabbit skin (n=2/dose) under occlusive conditions sequentially for 1, 5 or 15 minutes in 3 separate patches. Following treatment, the skin was washed with 50% Lutrol. The animals were observed on the same day, at 24, 48 and 72 hours, 8 and 14 days following treatment. The chemical (undiluted and at 30%) produced inflammatory erythema (redness) followed by peeling of skin (desquamation) after 1, 5 or 15 minutes of exposure. After application for 15 minutes, slight swelling (oedema) and scaling were also observed. These effects were reversible within 14 days of application. In the animals treated with the undiluted chemical, the following mean scores were reported for erythema for observations on the same day, at 24, 48 and 72 hours: 1/0/0.5/1 (1 minute exposure), 2/0.5/1.5/1 (5-minute exposure), 2/2/2/2 (15 min exposure), respectively. In the animals treated with the chemical at 30%, the following mean scores were reported for erythema for observations on the same day, at 24, 48 and 72 hours: 0/0.5/1.5/0 (1 minute exposure), 0/0/1.5/0 (5-minute exposure), 0/0.5/2/1 (15 min exposure), respectively. Scaling was seen from day 8. No scores were reported for oedema.

The chemical was corrosive in a GLP compliant in vitro skin membrane barrier test (OECD TG 435). The mean breakthrough time was 6 min 41 seconds (REACH n.d.).

Eye irritation

Corrosive chemicals are also considered to cause irreversible effects in the eyes.

Severe eye damage was reported in an eye irritation study conducted similarly to OECD TG 405 (non-GLP compliant) (ECHA 2022; OECD 2005; REACH n.d.). The undiluted chemical (0.1 mL) was instilled into one eye each of 3 Vienna White rabbits. The eyes (unwashed) were observed at 24, 48 and 72 hours, and 8 days following treatment. The following mean scores were reported at 24, 48 and 72 hours: corneal opacity 3.4/4, iritis 0.75/2, conjunctival redness 2/3, chemosis 3.6/4. The study reported severe damage of ophthalmic tissue including corneal opacity. Due to severe oedema (swelling), the eyeball was not assessable at all readings. The study was terminated after 8 days as reversibility of the findings was not expected.

Sensitisation

Skin sensitisation

Based on the available data, the chemical is not considered to be a skin sensitiser.

The chemical showed no sensitising effect in a guinea pig maximisation test (GPMT) (non-GLP compliant) conducted similarly to OECD TG 406 with acceptable deviations (ECHA 2022; OECD 2005; REACH n.d.). Intradermal and topical induction was performed on 15 guinea pigs using the chemical at 0.5% in acetone. Following dermal challenge with the chemical at 2% in acetone, no skin reactions were observed (0/15 animals).

Repeat dose toxicity

Oral

Based on the available data, the chemical is expected to cause significant systemic toxicity following chronic oral exposure. The main indicator of organ toxicity was vacuolar degeneration seen in many organs including the liver, kidney, adrenals, skeletal muscle and heart. Across a number of repeated dose oral toxicity studies, there was consistent evidence that the chemical caused effects at doses of 5 mg/kg bw/d and above.

In a 90 day oral toxicity study (OECD TG 408, GLP-compliant), Wistar rats (n=10/sex/dose) were administered the chemical via gavage at 0, 2.5, 12 or 60 mg/kg bw/day (5 days/week) for 3 months. No treatment related animal deaths were seen in any of the treatment groups. The following treatment related adverse effects were reported (ECHA 2022; OECD 2005; REACH n.d.):

- In the highest dose group, body weight gain and food consumption were clearly impaired in both sexes. The general state of health in these animals was poor. In the mid dose group, there was a slight reduction of feed consumption and a significant slowing of body weight gain in females.
- In the highest dose group, the relative weights of the liver, kidney, adrenals and testes were significantly increased in males. Absolute weight of adrenals was increased, and absolute weights of testes and liver were significantly decreased. The absolute kidney weights were unchanged. No significant organ weight changes were seen in females. In the mid dose group, significant increases in relative liver weights and absolute kidney weights were seen in males. Increased relative kidney weights were seen in both sexes.
- Histopathological changes corresponding to the changes in organ weights were seen in both sexes. In the highest dose group, signs of organ toxicity were seen in most animals in the liver (microvacuolar degeneration, more pronounced in females, and occasionally accompanied by single cell necrosis), kidney (vacuolar tubulopathy, more severe in males), heart (vacuolar myocardial degeneration), adrenals (hypertrophy of cortex), thymus (lymphocyte abnormalities) and mesenteric lymph nodes (slight depletion of lymphocytes). In the mid dose group, similar effects were in seen in the heart in most animals, and in the kidney in some animals. Vacuoles are membrane bound storage organelles within cells (Cooper 2000). They play an important role in cell storage and transportation (Cooper 2000). Cytoplasmic vacuolation (increase in size and number of vacuoles) is an adaptive cellular response to viral and bacterial pathogens or chemical inducers such as basic amine-containing lipophilic compounds (Shubin et al. 2016). Irreversible vacuolation resulting in cellular degeneration is indicative of organ toxicity (Shubin et al. 2016).

- Increased excretion of erythrocytes and bacteria and cellular debris sediments was seen in the urine of animals of both sexes in the highest and mid dose groups, indicative of kidney damage.
- Histopathological changes were seen in the testes (atrophy of seminiferous tubules, reduced contents of the seminal vesicles) in all high dose males. These changes accompanied by the significant decrease in absolute weight of testes were interpreted to be a consequence of marked impairment on body weight. As body weight was reduced more than the testes weight, the relative weights of the testes were increased.
- No changes were seen in the female reproductive organs (uterus and ovaries).

Based on the above effects, an NOAEL of 2.5 mg/kg bw/day was concluded for the study (OECD 2005). To account for a 7 days/week administration (the study only had 5 days/week exposure), the NOAEL was adjusted to be 1.8 mg/kg bw/day (ECHA 2022). The adjusted LOAEL is 8.5 mg/kg bw/day, warranting hazard classification.

In an extended one generation reproductive toxicity study (OECD TG 443), an NOAEL of 1.5 mg/kg bw/day was determined for systemic toxicity (ECHA 2022; REACH n.d.). Wistar rats (n=25/sex/dose) were administered the chemical via gavage at 0, 1.5, 5 or 15 mg/kg bw/day once daily for a total of at approximately 18 weeks – including 10 weeks prior to mating and during the mating gestation and weaning of their pups (F1 generation). At weaning, F1 pups were selected and assigned to 5 cohorts of animals for reproductive/developmental toxicity testing (cohort 1A and 1B), developmental neurotoxicity testing (cohort 2A and 2B) and developmental immunotoxicity testing (cohort 3). The F1 pups (except cohort 2B) received further treatment with the test substance from weaning to adulthood at the same doses as the parent (P) animals. Clinical observations and pathology examinations are performed on all animals (P and F1). The following treatment related adverse effects were seen in P animals – significant decrease in terminal body weights was seen in both sexes in the highest dose group. Cytoplasmic vacuolation was seen in the highest dose group in the:

- brain
- oesophagus
- eyes
- glandular stomach
- heart
- kidneys
- liver
- lungs
- axillary and mesenteric lymph nodes,
- pancreas
- pituitary gland
- skeletal muscle in both sexes
- adrenals and testes (left epididymis and seminal vesicles) in males.

Vacuolation was associated with signs of cytotoxicity (degeneration, inflammation, apoptosis or single cell necrosis) only in the kidneys, liver and skeletal muscle. Similar vacuolation was seen in some of the organs in the mid dose group in both sexes but there were no associated signs of cell toxicity.

F1 animals: Decreased water consumption and decreased body weights were seen in both sexes in the mid and high dose groups. In the highest dose group, vacuolation was seen in various organs (including brain, liver, kidneys, pancreas, glandular stomach, lungs, pituitary, skeletal muscle) in F1 males and females, and the testes in F1 males. No degenerative

changes were specified. In the mid dose group, similar vacuolation was seen in some of the organs (including kidneys, pancreases, lymph nodes) in both sexes.

Based on the systemic occurrence of abnormal vacuolation in the high and mid dose groups in both generations, the study concluded an NOAEL of 1.5 mg/kg bw/day for general systemic repeat dose toxicity and LOAEL of 5 mg/kg bw/day.

In a combined repeat dose toxicity and reproductive toxicity/developmental toxicity screening test (OECD TG 422, GLP-compliant), an NOAEL of 5 mg/kg bw/day was determined for systemic toxicity (ECHA 2022; REACH n.d.). Wistar rats (n=10/sex/dose) were administered the chemical via oral gavage at 0, 1.5, 5 or 15 mg/kg bw/day once daily prior to mating (duration not specified) and during mating (at least for 2 weeks). In the females, dosing was continued until day 22 of lactation. Treatment related adverse effects in the parent animals and their pups were seen only in the highest dose group. These effects included:

- Significant decrease in food consumption and body weight in both sexes.
- Significant increase in total counts of white blood cells (WBCs), lymphocytes and platelets in males.
- Liver toxicity – significant increase in relative liver weight was noted in females. Slight liver vacuolation was seen in both sexes. There was a significant increase in aspartate-aminotransferase (AST) activity in males, and in inorganic phosphate levels in females.
- Vacuolisation was also seen in the brain (choroid plexus), axillary and mesenteric lymph nodes, and glandular stomach.
- No inflammatory or degenerative effects were seen in these organs.
- Staining of tissues showed vacuolation was phospholipidic in nature.

The main treatment related effect was phospholipidosis (abnormal accumulation of phospholipids in the vacuoles) in various organs. While phospholipidosis is in itself does not produce adverse effects, the abnormal vacuolation is treated as an indicator of chemical induced systemic toxicity since only a small number of chemicals are known to produce this effect (Graham 2011).

Vacuolar degeneration was also reported in three other non-guideline oral repeat dose toxicity studies in Fischer 344 rats with treatment time ranging from 10 days to 10 weeks with testing doses from 25 to 75 mg/kg bw (ECHA 2022). The electron microscopy findings form these studies identified degenerative, atrophic and fibroblastic lesions in skeletal muscles and vacuolar degeneration and swelling of choroid plexus cells in the brain (ECHA 2022).

Dermal

No data are available. Based on data from repeat dose oral and inhalation toxicity studies and the high rate of dermal absorption, the chemical is expected to cause some systemic toxicity following chronic dermal exposure.

Inhalation

Based on the available data, the chemical is expected to cause some systemic toxicity following chronic inhalation exposure. Mild liver toxicity and disturbed haemoglobin metabolism were the main signs of systemic toxicity. No direct adverse effects were seen in the male and female reproductive organs. The lowest NOAEC for the chemical was 2 mg/m^3.

In a 90 day inhalation toxicity study (OECD TG 413, GLP-compliant), Wistar rats (n=10/sex/dose) were exposed (nose/head) to aerosol concentrations of 0, 2, 12 or 48 mg/m^3 of the chemical for 3 months (6 hours/day and 5 days/week). No animal deaths were seen in any of the treatment groups. The following treatment related adverse effects were reported (ECHA 2022; OECD 2005; REACH n.d.):

- A clear and statistically significant depression of body weight gain was noted in animals of both sexes in the highest dose group.
- Local irritation: Signs of local irritation, typical of alkaline compounds such as amines, were seen in the highest dose group animals for skin (slight hyperkeratosis in 7/10 animals) and upper airways (nasal mucosa, slight vacuolisation of olfactory epithelium in 2/10 males, and in 1/10 females).
- Significant increases in relative organ weights of liver, lung and kidney weights were seen in both males and females in the highest dose group. Relative organ weights of adrenals and testes, and absolute lung weights were significantly increased only in the highest dose males. The changes in relative organ weights were largely influenced by reduced body weights and were not considered to be significant. No corresponding histopathological changes were seen.
- Effects on haemoglobin parameters: Significant reduction in haemoglobin, haemoglobin per red blood cell, and mean corpuscular haemoglobin concentration was seen in the highest dose males. Polychromatosis (abnormally high number of immature red blood cells) was also noted. Haemosiderin deposits seen in the spleen of all animals in the highest dose group, and extramedullary haematopoiesis seen in 9/10 high dose females were indicative of a mild anaemic effect.
- Effects on liver: Serum levels of transaminases (AST and alanine-aminotransferase, ALT) were significantly increased in males in highest dose group but not in the females. Absolute lung weights were significantly increased in the highest dose males. No corresponding histopathological changes were seen in any of the affected animals. There was a marginal but significant increase in ALT and alkaline phosphatase (AP) in males in the mid dose group. Since the increased AP levels were not seen in the highest dose group, they were not considered dose dependent.
- No direct adverse effects were seen in the male and female reproductive organs (testes, ovaries and uterus examined).

An NOAEC of 2 mg/m^3 was determined for the study based on the slightly increased ALT levels seen in the mid dose male rats, which were considered as representing borderline toxicity (OECD 2005). For the same study, ECHA did not consider the marginal increase in ALT in the mid dose group to toxicologically relevant in the absence of an increase in AST levels or any other effects in the liver. An NOAEC of 12 mg/m^3 was concluded (ECHA, 2022; REACH). To account for daily (i.e., 7 days/week) exposure (exposure in the study was only 5 days/week), an adjusted NOAEC of 8.3 mg/m^3 was concluded for the study (ECHA 2022).

Observation in humans

Scleroderma like skin changes were reported in 6 of 233 workmen engaged in the polymerisation of epoxy resins (OECD 2005). A heavy or chronic exposure through inhalation was postulated and chemical was indicated as the most probable causative agent. Follow up investigation in 2 of the 6 affected subjects showed disappearance of the skin changes within 5 years (OECD, 2005; REACH n.d.).

In a cross sectional study 3 of 91 employees in the chemical's production showed nonspecific skin changes, but no scleroderma like symptoms (OECD 2005). Average employment duration was 11.8 years. Workplace conditions were not reported.

Genotoxicity

Based on in vitro data, the chemical is not considered to be genotoxic.

The chemical was not found to be genotoxic in any of the available in vitro studies (OECD 2005; REACH n.d.), as detailed below:

- Bacterial reverse mutation assay (Ames test) (OECD TG 471): the chemical did not induce mutations in bacteria (strains not reported) at concentrations up to 5000 µg/plate, with or without metabolic activation. Cytotoxicity was noted from concentrations of 2500 µg/plate.
- Mammalian cell gene mutation study (OECD TG 476): the chemical did not induce gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster V79 cells at concentration range of 0.03 to 1.2 µg/mL without activation and 0.1 to 2 µg/mL with activation. Higher concentrations could not be tested due to severe cytotoxic effects.
- Mammalian chromosomal aberration study (OECD TG 473, GLP compliant): the chemical did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cells when incubated with the chemical at 78 to 313 µg/mL without metabolic activation, and 156 to 625 µg/mL with metabolic activation. Cytotoxicity was noted at the highest doses with and without activation.

Carcinogenicity

No data are available. Based on the absence of genotoxicity in vitro, and lack of any signs of hyperplastic or pre-neoplastic lesions in repeat dose toxicity studies, the chemical is not expected to be carcinogenic.

Reproductive and development toxicity

Based on the available data, the chemical may cause reproductive toxicity following repeated oral exposure. Reduced implantation sites along with reduced litter sizes was the main indicator of effects on fertility. Based on the findings of several studies, there is no clear evidence of effects on development. No treatment related effects were seen on the gestation parameters and no significant skeletal malformations were seen in the foetuses. In an extended one generation reproductive toxicity study the chemical caused no significant effects in pups from exposure during gestation and via lactation. Although decreased body weight changes in pups was observed it is unclear whether this was a result of reduced feed consumption in dams. There were some reported effects on developing immune and neurobehavioural changes but in the absence of further information the significance of these findings is uncertain. In a prenatal developmental toxicity study in rabbits, a lower number of implantation sites along with a decreased number of pups was seen in the mid and high dose groups. The significance of the findings is uncertain. No effects were seen in a similar prenatal study in rats.

In an extended one generation reproductive toxicity study (OECD TG 443) in Wistar rats (see **Repeat dose toxicity – Oral** section), the following adverse effects were reported (test doses 0, 1.5, 5 or 15 mg/kg bw/day) (ECHA, 2022; REACH n.d.):

- Effects in P animals: The mean number of implantation sites was statistically significantly reduced (and below historical controls) in the high dose group. The mean litter size was significantly, and dose dependently decreased in the high and mid dose groups. There was no significant difference in post implantation loss between the treatment and control

group indicating an absence of intrauterine embryo or foetal toxicity. Signs of systemic toxicity included reduced food consumption and mean body weights in males and females in the high dose group. In the mid dose group reduced food consumption and mean body weights were observed in the females during gestation and lactation.

- Effects in in F1 animals: In the highest dose group, the mean body weight changes were significantly below the concurrent control values during the entire lactation period. On post-natal day (PND) 13, there was a significant increase in the incidence of F1 male pups showing a retention of areolas/nipples in the highest dose group. However, no nipples/areolae were detected on PND 20 in any male pups at any dose.
- Effects relating to developmental neurotoxicity (seen in Cohorts 2A and 2B): Significant findings in cohort 2A animals included increased rearing in both sexes, and an increase in the amplitude and latency of the auditory startle response at PND 24 and a 3% decrease in brain weight at PND 22 in the high dose males. At PND 69, no adverse clinical signs nor clear dose-response findings in the locomotor-activity test were seen. There were no treatment related effects on neuropathology. While the study concluded no specific treatment related effects on neurodevelopment, for findings from the neuro-behavioural testing at PND 24 a developmental aetiology could not be excluded. The histopathological findings of cohort 2A animals were comparable to those seen in the P animals indicating systemic toxicity resulting from continuous treatment with the test substance. No adverse effects on neuropathology, motor activity and behaviour were seen in cohort 2B animals (weanlings, PND 22) indicating that there were no effects through lactation.
- Effects relating to developmental immunotoxicity (seen in Cohort 3): a significantly lower anti-SRBC (anti-sheep red blood cells) IgM (immunoglobulin M) antibody titre was detected in a T-cell dependent antibody response assay in females in all dose groups.

Based on the above effects, the study concluded a NOAEL of 1.5 mg/kg bw/day for fertility effects and 5 mg/kg bw/day for developmental toxicity. However, based on observations in cohort 3 females, a LOAEL of 1.5 mg/kg bw/day was suggested for developmental toxicity (ECHA 2022; REACH n.d.). Any potential developmental changes seen in cohort 2 would also be covered by the LOAEL of 1.5 mg/kg bw/day (ECHA 2022).

In a combined repeat dose toxicity and reproductive toxicity/developmental toxicity screening test (OECD TG 422) in Wistar rats (see **Repeat dose toxicity – Oral** section), an NOAEL of 15 mg/kg bw/day was determined for reproductive and developmental toxicity (ECHA 2022; REACH n.d.). No treatment related effects were seen in the oestrus cycle, corpora lutea, spermatogenesis or reproductive performance at any of the doses. The only adverse effect seen was a significantly higher number of areolas/nipples per pup on PND 13 in the mid dose males although the incidence of males displaying areolas/nipples was not affected.

In a prenatal developmental toxicity study in rats (OECD TG 414, GLP-compliant), an NOAEL of 5 mg/kg bw/day was determined for the chemical (ECHA 2022; OECD 2005). Pregnant SD rats (n=25/dose) were administered the chemical via gavage at doses 0, 5, 15 or 45 mg/kg bw/day from day 6 to day 19 of gestation. The dams were sacrificed on day 20 and assessed. The only treatment related effect was a reduced corrected body weight gain seen in the mid and high dose groups. No treatment related effects were seen on the gestation parameters. There was a slight retardation of ossification of skull bones in the foetuses of the females in the highest dose group. Based on these effects, the study concluded an NOAEL of 5 mg/kg bw/day for maternal toxicity, 15 mg/kg bw/day for fetotoxicity and 45 mg/kg bw/day for teratogenicity.

In a prenatal developmental toxicity study in rabbits (OECD TG 414, GLP-compliant), an NOAEL of 1 mg/kg bw/day was concluded for reproductive toxicity (ECHA 2022). Pregnant New Zealand White rabbits (n=25/dose) were administered the chemical via gavage at doses

0, 1, 3 or 9 mg/kg bw/day from day 6 to day 28 of gestation. The does were sacrificed on day 29 and assessed. Reduced food consumption and reduced body weight/body weight gain were seen in the mid and high dose groups. No treatment related effects were seen on the gestation parameters. No significant skeletal malformations were seen in the foetuses. A lower number of implantation sites along with a decreased number of pups was seen in the mid and high dose groups. The significance of the findings is uncertain given that the animals were dosed around the time of implantation and the number of implantations were within historical controls.). However, since the reduced implantation sites and reduced litter sizes were dose related this may be a treatment related effect (ECHA 2022).

Endocrine effects

Based on available data, the chemical is not considered to have effects on the endocrine system.

- In vivo data: Although the testes atrophy in the repeat dose toxicity studies (see **Repeat Dose Toxicity** section) may indicate a potential endocrine modulating effect, the observed effects are considered likely a consequence of high general toxicity. Although the increased retention of areolas/nipples seen in the male pups in the reproductive/developmental studies (see **Reproductive and Developmental Toxicity** section) indicates potential anti-androgenic activity, the increase was weak and showed full recovery upon re-examination after weaning. In the repeat dose toxicity studies, no changes were seen in the thyroid and changes in the thyroid hormone levels had no apparent dose response (ECHA 2022).
- Structure-activity considerations indicate that the chemical has a low oestrogen receptor binding potential (ECHA 2022).
- It is reported that in vitro data do not show oestrogen-androgen steroidogenesis or thyroid mediated endocrine activity for the chemical (ECHA 2022).

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