Australian Government
Department of Health
Australian Industrial Chemicals Introduction Scheme

Benzene, 1,4-dichloro-2-nitro- (2,5-dichloronitrobenzene)

Evaluation statement

30 June 2022

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AICIS evaluation statement

Subject of the evaluation

Benzene, 1,4-dichloro-2-nitro- (2,5-dichloronitrobenzene)

Chemical in this evaluation

Name	CAS registry number
Benzene, 1,4-dichloro-2-nitro-	89-61-2

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 is a human health risk assessment for all identified industrial uses of the chemical.

Summary of evaluation

Summary of introduction, use and end use

There is currently no specific information about the introduction, use or end use of the chemical in Australia. Based on international information, the chemical is predominantly used as an intermediate in the synthesis of other chemicals, including textile dyes, pigments, pesticides, and UV absorbents (IARC 2020; OECD 1996; REACH).

While the chemical was previously reported as not being contained in consumer products (OECD 1996), it has more recently been registered for consumer use in the European Union (EU) in washing and cleaning products (REACH). However, no consumer products containing the chemical as an ingredient have been identified.

Human health

Summary of health hazards

The critical health effects for risk characterisation include:

- systemic acute effects from oral exposure
- local effects from eye irritation
- systemic effects following repeated oral exposure
- carcinogenicity.

While the chemical may be genotoxic and have reprotoxic properties, there is insufficient evidence to meet the hazard classification criteria.

The chemical is a nitro-aromatic compound and is expected to be readily absorbed via the gastrointestinal tract and through the skin. It is rapidly metabolised, then excreted primarily in urine. Metabolism of the chemical forms 2,5-dichloronoaniline, through conversion of the nitro ($–NO_2$) substituent to amine ($–NH_2$).

Based on the available data, exposure to the chemical may result in moderate acute oral toxicity, and low dermal toxicity, with the lowest oral lethal median dose (LD50) reported being 800 mg/kg bw in guinea pigs, and the lowest dermal LD50 being 2000 mg/kg bw in rats. Therefore, the chemical is recommended for hazard classification for the oral route only. No data are available for acute inhalation exposure, so no conclusions could be made for this endpoint.

The chemical is not expected to be irritating to the skin according to a guideline in vitro study. However, it was reported to be irritating to the eye based on positive results from a guideline in vitro eye irritation/corrosion study and an in vivo Draize test, warranting hazard classification for this endpoint.

Results from a human patch test indicate that the chemical has skin sensitisation potential. However, there is insufficient information to conclude if the chemical meets the hazard classification criteria for this endpoint. Structural and mechanistic profiling of the chemical using the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure Activity Relationship (QSAR) Toolbox did identify potential skin sensitisation structural alerts (OECD QSAR Toolbox version 4.2). These alerts were also identified for the 2,4-isomer, 2,4-dichloronitrobenzene (CAS No. 611-06-3) which is considered to be sensitising to the skin (AICIS 2022a).

The chemical is expected to cause harmful systemic health effects following repeated oral exposure. A lowest observed adverse effect level (LOAEL) of 93 mg/kg bw/day in male rats was determined in a 13 week feeding study, based on hepatotoxic effects. The chemical also induced renal, haematological, and testicular toxicity. These effects were observed in a similar pattern in a 13 week feeding study in mice, supporting the conclusion that the chemical has target organ toxicity mostly in the liver, but also spleen, testes, and blood (and rat kidney). Methaemoglobinaemia was observed in subacute and chronic toxicity studies with the chemical, in both rats and mice. Several structurally similar chloronitrobenzene compounds, including its 3,4-isomer, benzene, 1,2-dichloro-4-nitro- (NICNAS 2013), 2-chloronitrobenzene (AICIS 2022b), 3- chloronitrobenzene (NICNAS 2016), and related dichloroaniline metabolites (NICNAS 2017), are also reported to be methaemoglobin inducers, typically leading to a regenerative anaemia and a variety of tissue changes secondary to oxidative erythrocyte injury, in addition to specific renal and hepato-toxicity.

According to rodent studies, the chemical has clear carcinogenic properties, inducing tumours in the liver of rats and mice and the kidney of rats following long term exposure, and warranting hazard classification in category 1B. The International Agency for Research on Cancer (IARC) has classified the chemical as ‘Possibly carcinogenic to humans’ (Group 2B) based on ‘sufficient evidence’ in animal studies (IARC 2020).

The chemical may have some genotoxic potential, according to in vitro studies. Although positive results were observed in a few bacterial test systems and in mammalian chromosome aberration tests, negative results were also reported in most cases. Therefore, the overall information is not consistent with clear mutagenicity. According to a study author (Shimizu et al. 1983), positive results in bacterial mutagenicity assays may be correlated to

the chloro substituent in the ortho position of the nitrobenzene ring. There are no in vivo studies for the chemical. According to IARC, there is 'weak evidence' of genotoxic potential (IARC 2020).

The carcinogenic and genotoxic potential of the chemical, in addition to the methaemoglobin induced systemic toxicity, are consistent with findings from assessments of several structurally related nitro-aromatic compounds, including the metabolite 2,5-dichloroaniline (AICIS 2022a; AICIS 2022b; NICNAS 2013; NICNAS 2017). A former National Industrial Chemicals Notification and Assessment Scheme (NICNAS) assessment of the genotoxic and carcinogenic potential of monocyclic aromatic amine metabolites also supports a methaemoglobin induced systemic toxicity pathway for these chemicals (NICNAS 2019).

There is insufficient data to conclude on reproductive and developmental toxicity. The chemical was shown to induce toxic effects in dams and pups exposed to the chemical before and during pregnancy and through lactation at high dose. However, it is uncertain if the lower survival rate and growth rate observed in pups at high dose were directly due to the chemical or secondary to maternal toxicity. The chemical induced testicular toxicity in rats and mice in subchronic and chronic oral toxicity studies. In the reproductive toxicity study, the male reproductive system was impacted, but the extent of toxicity could not be determined. Several structurally related chloronitrobenzenes are reported to have potential reprotoxic properties with similar effects on the male reproductive system (AICIS 2022a; AICIS 2022b; NICNAS 2016).

Hazard classifications relevant for worker health and safety

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

Health hazards	Hazard category	Hazard statement
Acute Toxicity	Acute Tox. 4	H302: Harmful if swallowed
Eye irritation	Eye Irrit. 2	H319: Causes serious eye irritation
Specific Target Organ Toxicity (repeated exposure)	STOT RE 2	H373: May cause damage to organs through prolonged or repeated exposure
Carcinogenicity	Carc. 1B	H350: May cause cancer

Summary of health risk

Public

Based on the available use information it is unlikely that the public will be exposed to the chemical. While the chemical in the EU has been registered for potential consumer use in washing and cleaning products, no evidence of consumer products has been identified. Therefore, there are no identified risks to the public that require management. However, if information becomes available indicating the chemicals have more widespread consumer use, further risk management may be required.

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 health 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 **Proposed means for managing risks** section). Control measures implemented due to the carcinogenicity hazard classification are expected to be sufficient to protect workers from any potential genotoxic or reprotoxic health effects.

The data available indicate that a workplace exposure standard may be beneficial to mitigate the risk of adverse effects to workers. Methaemoglobin formation was observed in studies in animals. Exposure standards have been established for several nitro-aromatic related compounds to protect for methaemoglobinaemia in exposed workers (refer to **Supporting information – Existing Australian regulatory controls** section).

Guidance within the Interpretation of Workplace Exposure Standards for Airborne Contaminants (SWA 2019a) advises that 'exposure to carcinogens should be eliminated or minimised so far as is reasonably practicable'.

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.

It is recommended that SWA consider establishing a workplace exposure standard (WES). However, this may be more appropriate following finalisation of existing WES reviews currently underway for similar methaemoglobin inducers.

Information relating to safe introduction and use

The information in this statement including recommended hazard classifications, should be used by a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) to determine the appropriate controls under the relevant jurisdiction 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 manage risk arising from storing, handling and using this hazardous chemical depend on the physical form and how this 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.

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
- conducting air monitoring to ensure control measures in place are working effectively and continue to do so.

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.

Conclusions

The conclusions of this evaluation are based on the information described in the statement.

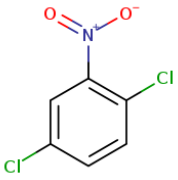
Considering the proposed means of managing risks, the Executive Director is satisfied that the identified human health risks can be managed within existing risk management frameworks. This is provided that all requirements are met under environmental, workplace health and safety and poisons legislation as adopted by the relevant state or territory and 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 one of 6 possible isomers of dichloronitrobenzene. It can be described as a benzene with a nitro group attached at the 1 position, and 2 chloro groups attached at the 2 and 5 positions, respectively. Note that the 2,4-isomer (CAS No. 611-06-3) has been assessed separately (AICIS 2022a), while the 3,4-isomer (CAS No. 99-54-7) has been previously assessed under the NICNAS IMAP (Inventory Multi-tiered Assessment and Prioritisation) framework (NICNAS 2013).

Chemical name	benzene, 1,4-dichloro-2-nitro-
CAS No.	89-61-2
Synonyms	2,5-dichloronitrobenzene 1-nitro-2,5-dichlorobenzene 1,4-dichloro-2-nitrobenzene 2,5-DCNB nitro-*p*-dichlorobenzene
Structural formula	
Molecular formula	C6H3Cl2NO2
Molecular weight (g/mol)	192
SMILES	[O-][N+](=O)c1cc(Cl)ccc1Cl
Chemical description	solid, crystalline, pale yellow plates and prisms with a faint aromatic odour

Relevant physical and chemical properties

Physical form	Solid
Melting point	56 °C
Boiling point	267 °C
Vapour pressure	0.38 × 10^{-2} mm Hg [0.51 Pa] at 25 °C
Water solubility	95 mg/L at 25 °C; 83 mg/L at 20 °C
Henry's law constant	1.52 Pa m^3/mol
Density	1.67 g/cm^3

Flash point	135 °C
log K_{ow}	3.03

Introduction and use

Australia

No specific information is available for the introduction, use, or end use of this chemical in Australia.

International

Based on the available information, the chemical has mostly site limited use as an intermediate in the manufacture of diazo pigments, fabrics and textiles, textile dyes, pulp and paper products and ultraviolet absorbents (IARC 2020; OECD 1996; REACH).

The chemical is registered in the EU for consumer use in washing and cleaning products (REACH). However, no consumer uses are reported in the Substances and Preparations in Nordic countries (SPIN) database, or in a North American consumer product information database (DeLima Associates).

Reported non-industrial end-uses include agricultural chemicals and pharmaceutical products (IARC 2020; REACH).

The chemical is included in the OECD high production volume list of chemicals produced or imported at greater than 1000 tonnes per annum (tpa) (OECD 2007); this was based on annual production volumes reported between 1988 and 1992 of up to 1200 tpa in Japan and up to 2800 tpa in Germany (IARC 2020; OECD 1996). Increased production and import volumes (2100 tpa) were reported in Japan in 2000, while up to 227 tpa was reported in the USA between 1998 and 2002 (IARC 2020; OECD 1996). The chemical is currently reported to be manufactured or imported in the EU at volumes of less than 10 tpa (REACH).

Existing Australian regulatory controls

AICIS

No specific controls are currently available for the chemical.

Public

No specific controls are currently available for the chemical.

Workers

The chemical is currently not classified in the HCIS and no exposure standards are available for the chemical in Australia (SWA).

Workplace exposure standards have been established for several nitro-aromatic related compounds to protect for methaemoglobinaemia in exposed workers, with many of these under review by Safe Work Australia, including 4-chloronitrobenzene, the ortho, meta and para isomers of nitrotoluene, the para isomer of nitroaniline, nitrobenzene itself, and the meta and para isomers of toluidine (SWA 2020; SWA 2021). The metabolite, dichloroaniline, is considered to fall under the scope of the group entry for 'aniline and homologues' (NICNAS 2016). Safe Work Australia reviewed this WES in 2019 and recommended amending the TWA of 2 ppm (07.6 mg/m^3) to 0.5 ppm (1.94 mg/m^3) to protect for the risk of elevated blood methaemoglobin and associated effects in exposed workers (SWA 2019b). At the time of publication of this evaluation statement, these values were yet to be finalised.

International regulatory status

Exposure standards

No specific exposure standards have been identified for the chemical. An occupational exposure limits (OEL) for 'chloronitrobenzenes' of 1 mg/m^3 has been established in Estonia and Japan (Chemwatch).

OECD

The chemical is listed on the OECD List of High Production Volume (HPV) chemicals (OECD 2007).

European Union

The chemical has been classified as 'Possibly carcinogenic to humans' (Group 2B) by the IARC (IARC 2020).

United States of America

The chemical is included in the California Proposition 65 list of chemicals known to the state to cause cancer or reproductive toxicity, based on carcinogenic effects (OEHHA).

Human exposure

Workers

During the chemical synthesis and use as intermediate, inhalation is expected to be the main exposure route, followed by dermal exposure to a lesser extent (IARC 2020; OECD 1996). The level and route of exposure will vary depending on the method of application and work practices employed. The OECD report considered exposure in the workplace to be negligible due to workers wearing specific personal protective equipment such as a chemical cartridge respirator with an organic vapour cartridge, during filling processes (OECD 1996). However, accidental ingestion, inhalation and dermal exposure to the chemical may also occur.

The American Conference of Governmental Industrial Hygienists (ACGIH) published a biological exposure index (BEI) for methaemoglobin inducers (ACGIH 2001), including chloronitrobenzene, of 1.5% methaemoglobin in the blood. The BEI indicates the level of methaemoglobin most likely to be observed in samples collected from workers following inhalation exposure to the methaemoglobin inducing chemical at the threshold limit value

(TLV). The TLV refers to the airborne concentrations of a chemical that workers may be repeatedly exposed to without adverse health effects.

Public

Although the chemical has been registered for potential domestic use in washing and cleaning products in the EU, no evidence of consumer products has been identified.

As the public may come in contact with residues of the chemical from consumer products or coloured products, the potential for public exposure cannot be excluded. However, based on the available international use information it is unlikely that the public will be significantly exposed to the chemical.

Health hazard information

Toxicokinetics

The limited available data indicate that the chemical is expected to be absorbed through the gastrointestinal tract following ingestion of the chemical. It is also expected to be bioavailable following absorption through the skin, as supported by observations of effects following dermal exposure.

In a non-guideline metabolism study, rabbits (n=6–10) were administered a single oral dose of 400 mg/kg bw of the chemical. Urine samples were collected for 72 hours following exposure. About 92% of the administered dose was recovered in urine. Mercapturic acid (9–33%), glucuronide (8–56%), and sulfate (3–21%) metabolites were the main urinary metabolites. Out of the mercapturic acid metabolites, 13% was 2,5-dichloroaniline, 2% was N-acetyl-S-(4-chloro-2-nitrophenyl)-l-cysteine, and 1% was 4-amino-2,5-dichlorophenol (ECHA 2021; IARC 2020).

In a non-guideline metabolism study in rats, with limited details available, 3 males (F344/DuCrj species) were administered the chemical at 1% in the diet for 2 days, and urine samples were collected following exposure. The main metabolite identified in the urine was N-acetyl-S-(4-chloro-2-nitrophenyl)-l-cysteine (Ohnishi et al. 2004).

Acute toxicity

Oral

Based on the limited available data, the chemical is expected to have moderate acute oral toxicity. The following LD50 values were reported without further details on the studies:

- An LD50 of 1000 mg/kg bw in rats (ChemIDPlus; IFA 2018).
- An LD50 of 2503 mg/kg bw in male rats (OECD 1996).

In an acute toxicity study (no data on test guideline or Good Laboratory Practice (GLP) compliance), guinea pigs (14 animals/sex/dose) were treated via oral gavage with the chemical in 5% gum acacia solution, at doses of 500, 600, 800, 1000 or 1200 mg/kg bw. The LD50 was reported to be 800 mg/kg bw. Treatment related effects on the kidneys (noted as irritation) were reported. However, no details on mortality or clinical signs of toxicity were provided (REACH).

Dermal

Based on the available data, the chemical is expected to have low acute dermal toxicity.

An LD50 of >2000 mg/kg bw in rats was derived based on the absence of mortality in rats dermally exposed to a dose of 2000 mg/kg bw of the chemical. A ‘decrease in spontaneous activity’ was the only effect reported in exposed rats. No further details are available (REACH).

Inhalation

No data are available.

Corrosion/Irritation

Skin irritation

Based on the limited available data the chemical is not expected to be a skin irritant.

In an in vitro skin irritation study conducted in accordance with OECD Test Guideline (TG) 439 (noted as non-GLP compliant), the chemical was applied to reconstructed human epidermis (RhE) for an exposure period of 60 minutes, followed by a post exposure incubation period of 42 hours. A mean tissue viability value of 108.3% was reported for the chemical in this study, and it was determined to not be irritating to the skin (REACH).

In a skin irritation study, reported as following a standard Draize procedure (no data on test guideline and GLP compliance), 500 mg of the chemical was applied to the skin of rabbits (number or species not stated) for 24 hours. ‘Mild irritation’ was reported, but no further details were provided, such as irritation scores (REACH).

Eye irritation

Based on the weight of evidence from the available in vitro and in vivo studies, the chemical is expected to be irritating to the eyes.

In an in vitro eye corrosion study conducted according to OECD TG 492 (noted as non-GLP compliant), the chemical was topically applied to reconstructed human cornea like epithelium (RhCE) using the EpiOcular^{TM} model for an exposure period of 6 hours, followed by an observation period of 18 hours. Mean tissue viability was measured following exposure and a post treatment incubation period. The mean tissue viability was determined to be 22.4%, and the chemical was determined to be irritating to the eyes in this study (REACH). It should be noted however, that OECD TG 492 does not differentiate between UN GHS Category 1 (serious eye damage) and UN GHS Category 2 (eye irritation) (OECD 2019).

In an in vivo eye irritation study, reported as following a standard Draize procedure (no data on test guideline or GLP compliance), 100 mg of the chemical was applied to the eyes of rabbits (number or species not stated) for 24 hours. Moderate irritation was reported, but no further details were provided, such as irritation scores (REACH). Although the reported results are limited, they support the conclusion from in vitro results that the chemical is irritating to the eyes. There is sufficient evidence to classify the chemical in Eye irritation Category 2.

Sensitisation

Skin sensitisation

Only limited information is available. The chemical was reported to have a negative result for skin sensitisation in a GLP-compliant guinea pig maximisation test (guideline not stated), however no further details were provided (NITE 2009).

Observation in humans

In a human patch test, the chemical was applied to the skin under occlusive dressing (adhesive plaster) on the backs of 31 workers of a chemical factory (28 males aged 24–62 years, and 4 females aged 42–53 years) at concentrations of 0.1%, 0.5% and 1.0% in petrolatum, for an exposure period of 48 hours (REACH). A control group of 5 unexposed workers (4 males and one female) was also included in the study. Skin reactions were assessed 20 minutes after patch removal. The chemical was considered to be sensitising based on erythema observed in 3, 6 and 9 people at concentrations of 0.1%,0.5% and 1.0% of the chemical, respectively (NITE 2009).

In silico

Based on the mechanistic profiling function of the OECD QSAR Application Toolbox (OECD QSAR Toolbox version 4.2), the chemical has structural alerts for skin sensitisation potential by protein binding. According to the profiling results, arylation of skin protein may result from nucleophilic substitution at an appropriately activated aromatic centre, by protein amino or sulfhydryl groups. Also, it is noted that 'nucleophilic substitutions proceed slowly at aromatic carbons, but compounds of this type are susceptible to nucleophilic attack at the ring carbon attached to the halide atom, in the presence of electron withdrawing groups in the ortho and para ring positions. The presence of heterocyclic nitrogen atoms strongly activates toward nucleophilic attack good leaving groups such as halogens in ortho or para positions. The presence of two or more such activating groups allows reaction to occur under mild, e.g., physiological, conditions.'

It is noted that the 2,4-isomer, 2,4-dichloronitrobenzene (CAS No. 611-06-3), is considered to be sensitising to the skin based on sufficient evidence including mechanistic and structural alerts for protein binding identified by the OECD QSAR Toolbox (AICIS 2022a). The skin sensitisation structural alerts for the 2,4-isomer (CAS No. 611-06-3) are identified as being stronger than those identified for this chemical.

Repeat dose toxicity

Oral

In a GLP compliant 14 day oral toxicity study similar to OECD TG 407, F344 rats and BDF1 mice (10 animals/sex/dose) were fed with a diet containing 625, 1250, 2500, 5000 or 10000 ppm (w/w) nominal doses of the chemical for 14 days (Yamazaki et al. 2005a). No mortalities occurred in treated rats, while 2 male and 6 female mice fed 10000 ppm died before the end of the study. Marked retardation in growth rate and significantly decreased food consumption were noted at ≥5000 ppm in rats and at ≥10000 ppm in mice. The benchmark dose (lower confidence limit) values, at which a 10% increased risk in an adverse effect is expected ($BMDL_{10}$) were 12.9 mg/kg bw/day and 15.6 mg/kg bw/day for male and female rats, and 72 mg/kg bw/day and 83.2 mg/kg bw/day for male and female mice, respectively (actual

ingested doses). The most significant effects were lesions in the liver, testes and haematopoietic system for rats and mice, and kidney for rats only.

Effects on the liver included:

- increased liver weights in rats and mice
- centrilobular hypertrophy of hepatocytes in mice
- increased mitosis of hepatocytes in rats and mice
- increased levels of serum transaminases in rats and mice.

Effects on the testes included:

- germ cell necrosis in rats and mice
- decreased spermatic count in the epididymis.

Methaemoglobin levels were increased in both rats and mice. However, it was noted that the methaemoglobin inducing effect of the chemical was considered to be comparatively lower than methaemoglobinaemia reported to be caused by other chloronitrobenzenes (Yamazaki et al 2005a).

In a GLP compliant 13 week oral toxicity study conducted in accordance with OECD TG 408, F344 rats and BDF1 mice (10 animals/sex/dose) were fed with a diet containing 1481, 2222, 3333, 5000 or 7500 ppm (w/w) nominal doses of the chemical (Yamazaki et al. 2005 b). A no observed adverse effect level (NOAEL) could not be established in this study based on significant liver and renal effects observed at the lowest dose tested.

In rats, no mortality occurred before the end of the study. Significantly decreased food intake and lower terminal body weights were reported at ≥2222 ppm. A LOAEL of 1481 ppm (equivalent to an actual ingested dose of 93 mg/kg bw/day and 106 mg/kg bw/day for males and females, respectively) is derived based on haematological changes and effects in the liver.

Macroscopic findings and organ weights observations included:

- Significantly increased relative liver weights in both males and females at all dose levels.
- Significantly increased relatively kidney weights at all dose levels.
- Significantly decreased testes weight in male rats at ≥2222 ppm.
- Significantly increased relative spleen weights at ≥2222 pm in males and at ≥5000 ppm in females (absolute weights in females were significantly increased at ≥2222 ppm).

Haematological observations included:

- Significantly decreased haemoglobin levels in males at ≥2222 ppm, and at ≥1481 ppm in females.
- Significantly decreased red blood cell (RBC) count at ≥3333 ppm in both males and females.
- Significantly decreased platelet count, haematocrit levels and increased mean corpuscular volume (MCV).
- Increased methaemoglobin levels in male rats fed 7500 ppm and female rats fed 5000 ppm.

Histopathological examinations showed the following:

- Significantly increased incidence of centrilobular hypertrophy of hepatocytes at all dose levels in the liver.
- Significantly increased incidence of haemosiderin deposits in the spleen at ≥2222 ppm in males and at ≥1481 ppm in females.
- Significantly increased incidence of extramedullar haematopoiesis in the spleen at ≥5000 ppm for both males and females.
- Significantly increased incidence of hyaline droplets in the cytoplasm of proximal tubular epithelial cells in the kidney of all treated male rats (however, this is considered to be possibly indicative of male- and species-specific alpha 2u-globulin nephropathy).
- Increased incidence of granular casts in the renal tubular lumen at corticomedullar junction in treated male rats, indicative of necrotic desquamation of the tubular epithelium.
- Significantly increased incidence of eosinophilic droplets in the cytoplasm of proximal tubular epithelial cells in the kidney of all treated female rats – the eosinophilic droplets were morphologically different from hyaline droplets in male rats, suggesting a different type of lesion.
- Germ cell necrosis and debris of spermatic elements in the epididymides in male rats fed 2222 ppm and above.

In mice, mortalities (4 males and 4 females) occurred at the highest dose of 7500 ppm before the end of the study. Significantly decreased food intake and lower terminal body weights were reported at this dose level. Macroscopic findings and organ weights observations included:

- significantly increased relative liver weight in all treated animals
- significantly increased relative kidney weight at ≥3333 ppm in males and ≥5000 ppm in females
- significantly decreased absolute and relative testis weight in male mice fed 7500 ppm
- significantly increased relative spleen weight at ≥2222 ppm
- atrophy of the thymus was observed in 3 male and 3 female mice fed 7500 ppm, and in one male mouse fed 3333 ppm. It is noted that all of these 7 animals were found dead or in a moribund state during the study period.

Haematological observations included:

- significantly increased methaemoglobin levels in mice fed 7500 ppm
- significantly increased MCV in females at all dose levels, and in males at ≥2222 ppm
- decrease in RBC count, platelet count, haemoglobin, and haematocrit levels.

Histopathological examinations showed the following:

- significantly increased incidence of centrilobular hypertrophy of hepatocytes in all treated groups in the liver
- increased incidence of focal liver necrosis in male mice fed 3333 and 5000 ppm
- significantly increased incidence of single cell necrosis in the liver at ≥3333 ppm in both males and females
- increased incidence of splenic haemosiderin deposit at all doses (significant at ≥2222 ppm in males and at all dose levels in females)
- increased incidence of splenic haematopoiesis (significant at ≥2222 ppm in males and at ≥3333 ppm in females)

- germ cell necrosis and debris of spermatic elements in the epididymides in male mice fed 7500 ppm and above.

In a 28 day oral toxicity study, Wistar rats (number not stated) were fed with the chemical at doses of 0, 10, 50 or 250 mg/kg bw/day. Clinical signs of toxicity included increased water consumption, salivation, crouching position, and impaired walk at the highest dose.

The following effects were observed at ≥50 mg/kg bw/day:

- decreased body weight gain
- increased hepatic weight and bilirubin value
- damage to the forestomach.

The following effects were reported only at the highest dose:

- hepatocellular hypertrophy
- testicle damage (necrosis of germ epithelium, azoospermia, depression of spermatogenesis).

A NOAEL of 10 mg/kg bw/day was derived based on this study (OECD 1996).

Dermal

In a 15 day toxicity study in rabbits of limited detail, the chemical was applied to the skin at doses of 100, 200 or 400 mg /kg bw/day. Mortalities were reported; however, no further details are available. Signs of systemic toxicity were decreased erythrocytes and haemoglobin level, hyperaemia, erythropoiesis, and iron pigmentation in spleen (ECHA 2021).

Inhalation

No data are available.

Genotoxicity

While only in vitro data are available for the chemical, the results gave some indication of genotoxic activity. Bacterial assays gave mostly positive results, while a well conducted chromosomal aberration study gave inconclusive results. The following results were reported (ECHA 2021; IARC 2020; OECD 1996; REACH):

- In a GLP compliant bacterial reverse mutation assay similar to OECD TG 471, positive results were observed in *Salmonella(S.) typhimurium* strain TA100 with and without metabolic activation at doses starting at 78 µg/plate; inconclusive results were observed in TA98 (first test negative, second test positive without metabolic activation); and negative results were reported for the remaining tested strains TA1535, TA1537 and *Escherichia coli* strain WP2uvrA with and without metabolic activation at doses up to 5000 µg/plate.
- In a non-guideline bacterial reverse mutation assay, positive results were observed in *S. typhimurium* strains TA98, TA100 and TA1538 without metabolic activation at doses starting at 102.4 µg/plate; and negative results were reported for strains TA1535 and TA1537 at doses up to 6553.6 µg/plate (Shimizu et al. 1983).

- In a non-guideline bacterial reverse mutation assay, positive results were observed in *S. typhimurium* strain TA100 with and without metabolic activation at doses starting at 250 µg/plate; and negative results were reported in TA198 with and without metabolic activation at doses up to 500 µg/plate.
- In a non-guideline Ames test, positive results were observed in *S. typhimurium* strain TA1535 containing plasmid pSK1002 without metabolic activation at doses starting at 1000 µg/plate.
- In a GLP compliant mammalian chromosome aberration study similar to OECD TG 473, a statistically significant increase in structural aberrations and number of polyploid cells was reported in Chinese hamster lung (CHL) cells exposed to the highest concentration tested 0.15 mg/mL for 48 h without metabolic activation; negative results were reported at other lower concentrations and/or shorter exposure. Cytotoxic effects were reported at the highest concentration of 0.15 mg/mL.
- In a non-guideline mammalian chromosome aberration study similar to OECD TG 473, a statistically significant increase in chromosome aberrations was observed in Chinese hamster V79 cells exposed to 200 µg/mL for 6 h with metabolic activation, but this concentration was cytotoxic; negative results were reported for lower concentrations and/shorter exposure with or without metabolic activation.
- In a mammalian cell HPRT gene mutation assay, negative results were reported in V79 cells exposed to 25–250 µg/mL with and without metabolic activation (no further details available).

Carcinogenicity

In a GLP compliant 2 year feeding study following OECD TG 451, F344 rats and BCF1 mice (50 animals/sex/dose) were fed with 0, 320, 800 or 2000 ppm (w/w) of the chemical (Yamazaki et al. 2005c). The chemical caused tumours in the male rat and mouse liver and in the male rat kidney. Signs of systemic toxicity included centrilobular hypertrophy of hepatocytes with nuclear atypia in mice, chronic progressive nephropathy (CPN) in rats, increased liver weight in rats, decreased haemoglobin levels and haematocrit, and increased bone marrow haematopoiesis in rats.

In rats, survival, body weight gain and food consumption did not appear affected by treatment, but terminal body weights had decreased in the high dose groups by 15% and 20% in males and females, respectively. There was a significant increase in relative liver weight for all treated groups, a significant increase in relative kidney weight in males at all dose levels and in females at ≥800 ppm, and a significant increase in relative testes weight in male rats fed 2000 ppm.

Neoplastic lesions included:

- significantly increased incidence of hepatocellular adenomas and combined hepatocellular adenomas/carcinomas at 2000 ppm in males, with the incidence of hepatocellular carcinomas exceeding the historical control data range
- significant dose dependent increase in the incidence of basophilic foci at ≥800 ppm in males
- significant dose related increased incidence of renal adenomas/carcinomas in males, with the incidence of renal adenomas in 2000 ppm fed males exceeding the historical control data range
- significant dose related increased incidence of Zymbal gland adenomas in males, with the incidence in 2000 ppm fed male rats exceeding the historical control data range
- high incidences of interstitial cell tumours in both control and treated groups.

Non-neoplastic lesions included:

- dose dependent increase in CPN and liver nodules in males
- significant decrease in haematocrit at ≥2000 ppm and haemoglobin levels at ≥800 ppm in females
- increased incidence of urothelial hyperplasia in the pelvis of all treated males
- increased bone marrow haematopoiesis in the females fed 2000 ppms.

In mice, survival, body weight gain and food consumption showed no difference when compared with controls, although survival rate in the high dosed group was slightly lower by week 65. The decrease in survival in the high dosed group was correlated with the higher incidence of liver tumours causing death. Terminal body weights had decreased in the high dose groups by 34% and 17% in males and females, respectively. There was a significant increase in relative and absolute liver weights at ≥800ppm for both males and females, a significant increase in relative kidney weight at ≥800 ppm in males and at 2000 ppm in females, and a significant increase in relative testes weight in male mice fed 2000 ppm.

Neoplastic lesions included:

- significantly increased incidence of hepatocellular adenomas at ≥800 ppm in females
- significantly increased incidence of hepatocellular carcinomas at ≥800 ppm in females and at ≥2000 ppm in males
- significantly increased incidence of hepatoblastomas at 2000 ppm in males
- significant dose dependent increase in the incidence of acidophilic foci at ≥800 ppm in males
- 18/69 and 4/47 hepatocellular carcinomas in males and females, respectively
- 17/47 hepatoblastomas in males, with metastasis reported in the lung.

Non-neoplastic lesions included:

- dose dependent increase in liver nodules in all treated groups
- significantly increased incidence of centrilobular hypertrophy of hepatocytes with nuclear atypia in all treated groups
- significantly increased incidence of haemosiderosin deposit in the kidney of the females fed 2000 ppm
- significantly increased bone marrow erythropoiesis in the males fed 2000 ppm.

Reproductive and development toxicity

The chemical has some potential reprotoxic properties. However, based on the available data, it is unclear if adverse effects on fertility or development occur only secondary to parental toxicity.

In a preliminary reproductive toxicity study (guideline not stated), Sprague Dawley (SD) rats were orally administered the chemical at doses of 0, 6, 20, 60 or 200 mg/kg bw/day. Male rats were exposed for 49 days before mating, and female rats were exposed for 14 days before mating to day 3 of lactation. Clinical signs of toxicity included yellow urine, salivation, decreased activity and perigenital soiling. Lower food consumption and suppressed body weight gain were reported at the highest dose. At the highest dose, 6 dams died before the end of the study, with necropsy showing atrophy and/or haemorrhage in the thymus, lymphoid atrophy and decreased cellularity of the marginal zone in the spleen, congestion in the lungs and liver, and glandular stomach ulcers. In surviving female rats, atrophy of the thymus and decreased cellularity of white pulps in the spleen were reported. Lack of care

behaviour was reported at the highest dose in 7 dams. Growth rate and survival of the pups were lower at 200 mg/kg bw/day. One dam given 60 mg/kg bw/day only delivered dead pups. In male treated rats, degeneration of seminiferous epithelium in the testes and debris in the ducts of the epididymides were reported at the highest dose. Copulation and fertility indexes were not affected by treatment. Based on the effects seen in the study, the following NOAELs could be derived: 200 mg/kg bw/day and 20 mg/kg bw/day for male and female reproductive performance, respectively, and 60 mg/kg bw/day for developmental toxicity (NITE 1996; OECD 1996).

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