Australian Government
Department of Health
Australian Industrial Chemicals Introduction Scheme

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

Evaluation statement

30 June 2022

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AICIS evaluation statement

Subject of the evaluation

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

Chemical in this evaluation

Name	CAS registry number
Benzene, 2,4-dichloro-1-nitro-	611-06-3

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 no specific information about the introduction, use, or end use of the chemical in Australia, but the available data report that it is an intermediate in the synthesis of other chemicals, including pigments, agrochemicals, and pharmaceutical products (IARC 2020; OECD 1996; REACH). It has mostly site limited use and is expected to be present in industrial settings only. No consumer uses have been reported for the chemical.

Human health

Summary of health hazards

The critical health effects for risk characterisation include:

- systemic acute effects from oral and dermal exposure
- local effects from skin sensitisation
- systemic effects following repeated oral exposure
- carcinogenicity.

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

The chemical is a nitro-aromatic compound and is readily absorbed via the gastrointestinal tract and through the skin. It is expected to be rapidly metabolised, then excreted primarily in urine, with metabolism of the chemical forming 2,4-dichloronoaniline, through conversion of the nitro ($–NO_2$) substituent to amine ($–NH_2$).

Based on the available data, the chemical has moderate acute oral and dermal toxicity, with an oral lethal median dose (LD50) of 387 mg/kg bw in male rats, and a dermal LD50 of 921 mg/kg bw in rats.

Regarding local effects, the chemical is not expected to be irritating to the skin or eyes, but it is considered to be a weak skin sensitiser. The chemical was considered to be a slight sensitiser in one guinea pig maximisation test (GPMT) (Basketter et al. 1996) and a strong skin sensitiser in the second test (REACH). The chemical also induced positive results in a local lymph node assay (LLNA) in which the concentration producing a three-fold increase in lymphocyte proliferation (EC3) was 20%, indicating weak sensitisation potential (Basketter et al. 1996). Structural and mechanistic profiling of the chemical using the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure Activity Relationship (QSAR) Toolbox also identified potential skin sensitisation structural alerts (OECD QSAR Toolbox version 4.2).

The chemical is expected to cause harmful systemic health effects following repeated oral exposure. In a combined repeat dose and reproductive toxicity screening test, a NOEL (no observed effect level) of <8 mg/kg bw/day was determined for systemic toxicity (NITE 1996; OECD 1996). In chronic feeding studies (Kano et al. 2012), the chemical induced renal toxicity and haematotoxicity in rats and mice and hepatotoxicity in mice. The chemical also induced pathological changes in the upper respiratory tract of mice following oral administration. Based on the effects seen in rats and mice at the lowest dose of 750 ppm, a lowest observed adverse effect level (LOAEL) of 36 mg/kg bw/day could be derived for rats. While methaemoglobinaemia is not specifically reported in studies of this chemical, structurally similar chloronitrobenzene compounds, including the 2,5- and 3,4-isomers of dichloronitrobenzene (AICIS 2022a; NICNAS 2013), 2-chloronitrobenzene (AICIS 2022b), 3-chloronitrobenzene (NICNAS 2016), and related dichloroaniline metabolites (NICNAS 2017), are reported to be methaemoglobin inducers, typically leading to a regenerative anaemia and a variety of tissue changes secondary to the oxidative erythrocyte injury, in addition to specific renal and hepato-toxicity.

Based on 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 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 the available data. Mixed results were reported in vitro. Sufficient data are not available to confirm whether the chemical is a specific genotoxin in vivo. According to the IARC, there is ‘weak evidence’ of genotoxic potential (IARC 2020).

The carcinogenic and genotoxic potential of the chemical, in addition to potential methaemoglobin induced systemic toxicity, are consistent with findings from assessments of several structurally related nitro-aromatic compounds, including the metabolite 2,4-dichloroaniline and related isomers (AICIS 2022a; AICIS 2022b; NICNAS 2013; NICNAS 2017). The former National Industrial Chemicals Notification and Assessment Scheme’s (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).

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. The chemical was reported to effect pup survival rate at the highest dose tested in a reproductive toxicity test. However, these effects were only observed at doses that were also toxic to parent animals. It is noted that several structurally related chloronitrobenzenes are reported to have potential reprotoxic properties (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 – oral	Acute Tox. 4	H302: Harmful if swallowed
Acute toxicity – dermal	Acute Tox. 4	H312: Harmful in contact with skin
Skin sensitisation	Skin Sens. 1B	H317: May cause an allergic skin reaction
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. Therefore, there are no identified risks to the public that require management.

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 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 this statement.

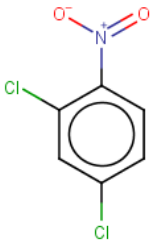
Considering the proposed means of managing risks, the Executive Director is satisfied that the identified human health risks can be managed within existing risk management frameworks. This is provided that all requirements are met under environmental, workplace health and safety and poisons legislation 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 4 positions, respectively. Note that the 2,5-isomer (CAS No. 89-61-2) has been assessed separately (AICIS 2022a), while the 3,4-isomer (CAS No. 99-54-7) has been previously assessed under the former NICNAS IMAP (Inventory Multi-tiered Assessment and Prioritisation) framework (NICNAS 2013).

Chemical name	Benzene, 2,4-dichloro-1-nitro-
CAS No.	611-06-3
Synonyms	2,4-dichloronitrobenzene 1,3-dichloro-4-nitrobenzene 2,4-DCNB
Structural formula	
Molecular formula	C6H3Cl2NO2
Molecular weight (g/mol)	192
SMILES	[O-][N+](=O)c1ccc(Cl)cc1Cl
Chemical description	solid, crystalline, pale yellow needles with a faint aromatic odour

Relevant physical and chemical properties

Physical form	solid
Melting point	34 °C
Boiling point	258 °C
Flash point	152 °C
Vapour pressure	1.43 x 10^{-2} mm Hg [190 Pa] at 25 °C
Water solubility	188 mg/L at 20 °C
Henry's law constant	3.22 x 10^{-5} atm m^3/mol [3.26 Pa m^3/mol] at 25 °C

Density	1.54 g/cm^3 at 15 °C
log K_{ow}	3.07

Introduction and use

Australia

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

International

Based on the available information, the chemical is an intermediate in the manufacture of other chemicals. These chemicals are subsequently used to manufacture other chemicals with industrial end-use in dyes and pigments (IARC 2020; OECD 1996). The chemical is currently reported to be manufactured or imported in the European Union (EU) for intermediate use only (REACH).

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). In 1990 an annual production volume of 1500 tpa was reported in Germany, while between 1998 and 1992, production volumes in Japan were reported to be less than 50 tpa (IARC 2020; OECD 1996). There is no information on current production volumes of the chemical.

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, 2,4-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 an 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 eyeglasses, chemical cartridge respirator and rubber gloves, during cleaning and maintaining equipment (the reaction vessel) (OECD 1996). This does not exclude accidental ingestion, inhalation, and dermal exposure to the chemical. While no measured data are available, estimated exposure levels calculated by an EU exposure model were reported to be 0–0.1 ppm by inhalation and 0–0.1 mg/cm^2/day for dermal contact (OECD 1996).

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

Given the site limited use of the chemical, the public is not expected to be 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 (GI) following ingestion of the chemical. It is also expected to be bioavailable following absorption through the skin.

In a dermal absorption study, mice were exposed to 13 µmol of the radiolabelled chemical in an acetone/olive oil (4:1 ratio) solution. Dermal absorption was estimated at 45% and 71% of the applied dose after 24 hours and 72 hours, respectively (IARC 2020).

The chemical is assumed to be readily absorbed through the GI based on urinary metabolites identified following ingestion of the chemical. Similar to other dichloronitrobenzenes (AICIS 2022a; NICNAS 2013), the major metabolic pathway of the chemical is mercapturic acid formation (Bray et al. 1957; IARC 2020; Ohnishi et al. 2009).

In a non-guideline toxicokinetic study, Fischer rats (F344) (n=3) were fed a diet containing 1% of the chemical for 2 days. Urine samples were collected for 24 hours following exposure. The main urinary metabolite was the N-acetylcysteine conjugate, N-acetyl-S-(5-chloro-2-nitrophenyl)-l-cysteine (Ohnishi et al. 2009).

In a non-guideline toxicokinetic study, rabbits (6 animals/dose) were given single oral doses of 200, 300 or 400 mg/kg bw of the chemical. Urine samples were collected for 72 hours following exposure. The main metabolites identified in urine were N-acetyl-S-(5-chloro-2-nitrophenyl)-l-cysteine (23% of dose) and 2,4-dichloroaniline as the acetyl conjugate (1%) (Bray et al. 1957).

Acute toxicity

Oral

Based on the limited available data, the chemical may have moderate acute oral toxicity, with reported oral LD50 values ranging from 375 to 990 mg/kg bw in rats (OECD 1996; REACH). Clinical signs of toxicity included narcosis, face down and lateral body positioning, scrubby fur, heightened diuresis, and diminished general condition. No further details are available.

Dermal

Based on the limited available data, the chemical may have moderate acute dermal toxicity. A dermal LD50 of 921 mg/kg bw in rabbits was reported without further details (OECD 1996).

Inhalation

No data are available.

Corrosion/Irritation

Skin irritation

Based on the available data, the chemical is not expected to be irritating to the skin.

In a Good Laboratory Practice (GLP) compliant skin irritation study conducted in accordance with OECD TG 404, 3 New Zealand White (NZW) rabbits were treated with 500 mg of the chemical for 4 hours under semi-occlusive conditions. Observations were recorded at 24, 48 and 72 hours after patch removal. The overall mean irritation score was zero for all animals. Although slight erythema was observed in all animals within 30 to 60 minutes, no irritation was seen at any subsequent time point following exposure (REACH).

In a skin irritation study similar to OECD TG 404 (GLP compliance not specified), 0.5 g of the chemical was applied (using a small piece of cloth; type of coverage not specified) to the abraded skin of 3 NZW rabbits for 4 hours. Observations were recorded at 24, 48, 72 hours and 7 days after cloth removal. Mean erythema score was 0.55 and mean oedema score was 0.1 (both out of a maximum score of 4). All three animals showed slight erythema resolving within 72 hours. One animal had slight oedema. All effects were fully reversible (REACH).

Eye irritation

Based on the limited available data, the chemical may cause slight eye irritation.

In a GLP compliant skin irritation study conducted in accordance with OECD TG 405, 0.1 g of the chemical was instilled into one eye each of 3 NZW rabbits. The eyes were washed out with saline after 24 hours and observed at one, 24, 48, 72 hours and 7 days post exposure. Hyperaemia of the cornea was reported in 2 rabbits within 1 hour and resolved within 24 hours. No other sign of irritation was observed (REACH).

In an eye irritation study conducted in accordance with OECD TG 405 (GLP compliance not specified), 0.1 g of the chemical was instilled into one eye each of 3 NZW rabbits. The eyes were washed out with saline after 24 hours and observed at one, 24, 48, 72 hours and 7 days post exposure. All animals had slight conjunctivitis within 1 hour, that resolved before 24 hours. No other sign of irritation was observed, and the overall mean irritation score was zero (REACH).

Sensitisation

Skin sensitisation

Based on the available data, the chemical is considered to be a skin sensitiser. There is sufficient evidence to warrant hazard classification.

In an in vivo skin sensitisation study similar to OECD TG 406 (GPMT), female guinea pigs (n=20) were treated with an intradermal dose of 0.5% of the chemical, followed 7 days later by a dermal treatment with 1% of the chemical in acetone. The control group was composed of 10 guinea pigs receiving identical treatment with vehicle alone. The animals were then topically challenged 21 days later with the chemical at 0.1% in acetone. Skin reactions (grade one erythema) were observed in 2/20 exposed animals, compared to none in the 10 control animals (Basketter et al. 1996).

In a reportedly GLP compliant in vivo skin sensitisation study conducted in accordance with OECD TG 406 (GPMT), female Pirbright-White guinea pigs (n=20) were treated with an intradermal dose of 5% in paraffin and dermal dose of 25% of the chemical in Vaseline at induction phase. The control group was composed of 5 guinea pigs. The animals were then topically challenged with the chemical at 25% in Vaseline. No further details on methodology were provided. Slight to severe erythema and very slight to slight oedema could be observed after 24 to 48 hours in all treated animals (20/20), and the skin was reported to be dry and rough, whereas none of the control group animals showed any dermal responses (REACH).

In a modified LLNA, mice (BALB/c strain; 12 animals/group) were exposed to a dose of 20% of the chemical in acetone and olive oil vehicle (AOO) or to the vehicle alone, on one shaved flank under occlusive patch for 48 hours. After 5 days, mice were placed into small groups (3 animals/dose) and received daily topical applications of the chemical at doses of 0, 5, 10 or 20% in AOO on the dorsum of both ears, for 3 days. At the highest concentration of 20%, the reported stimulation indices (SI) were 3.43 for animals pre-exposed to the chemical and 3.05 for animals pre-exposed to the vehicle only. The EC3 value (concentration producing a three-fold increase in lymphocyte proliferation) is considered to be 20%, indicating weak sensitisation potential (Basketter et al. 1996).

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, the chemical could interact with proteins via nucleophilic substitution on an activated aryl carbon atom. The profiling results also note that for a chemical with an aromatic ring and single activating group (–NO_2 in this case) ortho or para to the leaving group, in combination with an additional activating group, such as a halogen (–Cl in this case) meta to the leaving group should be considered for classification as a Category 1B skin sensitiser.

It is noted that the OECD QSAR Toolbox also identified structural alerts for skin sensitisation potential by protein binding for the 2,5-isomer, 2,5-dichloronitrobenzene (CAS No. 89-61-2). However, the structural alerts for the 2,4-isomer are identified as being stronger than those identified for the 2,5-isomer (AICIS 2022a).

Repeat dose toxicity

Oral

In a GLP compliant combined repeat dose and reproductive toxicity screening test conducted in accordance with OECD TG 422, CRj:CD (SD) rats (12 animals/sex/group) were administered the chemical in corn oil by oral gavage at 0, 8, 40 or 200 mg/kg bw/day, once daily, for 7 days/week. Males were treated for a total of 45 days before mating; females were treated from 14 days before mating to day 3 of lactation, through premating, mating, gestation, and lactation periods. At the highest dose, one female died during delivery of pups, and body weight gain was lower compared to controls. Other clinical signs of toxicity included eye discharge in 2 males at 40 and 200 mg/kg bw/day and salivation in one male at 200 mg/kg bw/day. A no observed adverse effect level (NOAEL) could not be determined for this study; however, the LOAEL was 8 mg/kg bw/day, based on haematological changes and effects on the thymus at the lowest dose (NITE 1996a; OECD 1996).

The following effects were reported at ≥8 mg/kg bw/day:

- atrophy of the thymus in females at all dose levels
- decreased red blood cell count in males at all dose levels
- basophilic change of the renal tubules in all treated female groups.

The following additional effects were reported at ≥40 mg/kg bw/day:

- enlargement of the adrenals in females
- necrosis of the renal tubules and fibrosis of the renal tubular epithelium in females
- decreased haematocrit and haemoglobin, increased reticulocytes, and slight anaemia in males
- increased total protein, albumin and gamma-glutamyl transpeptidase (GTP), and decreased creatinine in males.

Atrophy of the testes and epididymis was also reported in one male from the control group and one male given 40 mg/kg bw/day.

The following effects were reported at the highest dose only (200 mg/kg bw/day):

- significantly increased absolute and relative liver and kidney weights in males
- increased absolute and relative (significant) liver weights in females
- enlargement of the liver (significant in females)
- enlargement of the kidneys in males
- swelling and single cell necrosis of liver cells in both males and females
- mitosis of liver cells in males
- slight increase in the incidence of hyaline droplets of the renal tubules in males
- a moderate degree of pigment deposits in the spleen of females
- ulceration in the stomach, duodenum, and large intestine in females
- increased total bilirubin and albumin/globulin (A/G) ratio in males.

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

The available information suggests that the chemical is a potential genotoxin. However, the data are not sufficient to meet hazard classification criteria.

Both positive and negative genotoxicity results were reported in bacterial in vitro assays as listed below:

- In a bacterial gene mutation assay (no guideline indicated), positive results were observed in *Salmonella (S.) typhimurium* TA100, at concentrations starting at 3.3 µg/plate with and without metabolic activation and in TA98 and TA1537 at 10 µg/plate with metabolic activation only; negative results were reported for strain TA1535 at

concentrations up to 215 µg/plate with and without metabolic activation (Health Council of the Netherlands 2018; IARC 2020; OECD 1996).
- In a non-guideline bacterial reverse mutation assay, positive results were reported for *S. typhimurium* TA100 at concentrations starting at 51 µg/plate without metabolic activation, with a dose related response; negative results were reported for the other strains tested, TA98, TA1535, TA1537, and TA1538 at concentrations up to 6554 µg/plate without metabolic activation (Health Council of the Netherlands 2018).

Both positive and negative results were also reported in the available mammalian cell in vitro assays as detailed below:

- In a GLP compliant mammalian chromosome aberration test following Japanese guidelines, negative results were reported in Chinese hamster lung (CHL) cells exposed to the chemical at concentrations up to 140 µg/mL, with and without metabolic activation; although a statistically significant increase in cells with chromosomal aberrations (4%) was observed at the highest dose after 24 hours exposure, without metabolic activation. The overall result for the chemical was negative (Health Council of the Netherlands 2018; NITE 1996).
- In an in vitro mammalian chromosome aberration test conducted in accordance with OECD TG 473 (GLP compliance not specified), CHL cells were exposed to the chemical at concentrations of 0.025–0.3 mg/mL, with and without metabolic activation. Test concentrations were determined based on a cell growth inhibition test (no further details provided). Positive results were reported based on an observed increased frequency in cells with chromosomal aberrations (structural aberrations and polyploidy). The D20 value (concentration required to induce any aberration in 20% of metaphases) was reported to be 0.076 mg/mL (REACH).
- In a mammalian chromosome aberration test (guideline and GLP compliance not stated), negative results were reported in Chinese hamster ovary (CHO) cells exposed to the chemical at concentrations up to 3000 µg/mL with metabolic activation, and up to 500 µg/mL without metabolic activation (Health Council of the Netherlands 2018).
- In a sister chromatid exchange test, negative results were observed in CHO cells exposed to the chemical at concentrations up to 500 µg/mL without metabolic activation (Health Council of the Netherlands 2018).

In one in vivo sex linked recessive lethal mutations assay, negative results were reported in *Drosophila melanogaster* (fruit fly) injected with the chemical at doses of 0, 20 or 30 ppm (Health Council of the Netherlands 2018; REACH)

Carcinogenicity

In a GLP compliant 2 year carcinogenicity study in rats conducted in accordance with OECD TG 451 (Kano et al. 2012; REACH), F344 rats (50 animals/sex/dose) received the chemical in diet at nominal doses of 0, 750, 1500 or 3000 ppm (w/w) (equivalent mean ingested doses of 0, 36, 75 and 154 mg/kg bw/day in males, and 0, 43, 91 and183 mg/kg bw/day in females).

While there was no treatment related effect on survival, a dose related suppressed growth rate was reported across all treatment groups, with significantly decreased terminal body weight at ≥75 mg/kg bw/day in both males and females.

Macroscopic observations included:

- significantly increased absolute and relative liver weights in males at all dose levels (≥750 ppm) and in females at ≥1500 ppm
- significantly increased absolute kidney weights at all dose levels
- significantly increased relative kidney weights in males at all dose levels and in females at ≥1500 ppm
- greyish white nodules in the kidneys in both males and females at the highest dose (3000 ppm).

Haematological and blood chemistry observations included:

- increased plasma levels of total cholesterol and phospholipids in all dose levels
- increased gamma-GTP in males at all dose levels
- statistically significant increases in blood urea nitrogen (BUN) in all treated males and at ≥1500 ppm in females.

Histopathological observations (including neoplastic lesions) included:

- a significant dose related positive trend in the incidence of renal cell adenomas and carcinomas, with significantly increased incidences at the highest dose (3000 ppm)
- incidences of renal cell adenomas at 1500 ppm (6% of males and 6% of females) were reported to exceed the maximum incidences of historical controls (2%)
- atypical renal tubule hyperplasia at the proximal tubule in all treated groups, reported as a proliferative pre-neoplastic lesion
- increased eosinophilic droplets in the renal proximal tubule of all treated groups
- significantly increased incidences of marked and severe grades of chronic progressive nephropathy (CPN) in males at all dose levels and increased incidence in females at ≥1500 ppm
- increased incidences of urothelial hyperplasia in the pelvis and mineralization in the papilla in all treated males
- dose related occurrence of adenomas in the preputial glands of male rats, with a significantly increased incidence at 3000 ppm.

In a GLP compliant 2 year carcinogenicity study in mice (BCF1 strain; 50 animals/sex/dose) conducted in accordance with OECD TG 451, males received nominal doses of the chemical in diet at of 0, 750, 1500 or 3000 ppm (w/w) (equivalent mean ingested doses of 0, 82, 172 and 355 mg/kg bw/day), and female received 0, 1500, 3000 or 6000 ppm (w/w) nominal doses (equivalent to 0, 203, 416 and 942 mg/kg bw/day) (Kano et al. 2012).

There were significantly decreased survival rates (higher mortality) in both males and females at ≥3000 ppm, attributed to malignant liver tumours. There was dose related suppressed growth rates and significantly decreased terminal body weights both males and females at ≥1500 ppm (noting that this was the lowest dose tested in females).

Macroscopic observations included:

- increased absolute and relative liver weights at ≥1500 ppm for both males and females
- a dose dependent increase in the incidence and size of liver nodules in all treated groups of both sexes
- a dose dependent increase in the incidence of peritoneal nodules in all treated groups of both sexes – reported predominantly in the peritoneum around the pelvic viscera.

Haematological and blood chemistry observations included:

- decreased red blood cell (RBC) count and haemoglobin, and increased mean corpuscular volume (MCV) in the 3000 ppm fed males
- increased total cholesterol levels in both sexes at all treatment doses
- increased phospholipid levels at ≥1500 ppm for both males and females
- increased alkaline phosphatase (ALP) both sexes at all treatment doses
- increased aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in males at dose levels and in females at ≥3000 ppm
- increased gamma-GTP at ≥3000 ppm in both males and females
- increased lactate dehydrogenase (LDH) and creatine kinase at ≥1500 ppm for both males and females
- increased BUN in females at ≥3000 ppm
- increased total bilirubin was increased in males at ≥1500 ppm and in the 6000 ppm fed females.

Histopathological observations (including neoplastic lesions) included:

- a dose dependent increase in the incidence of hepatocellular adenomas and carcinomas, and hepatoblastomas in both sexes, with significantly increased combined incidences in all the treated groups of both sexes
- significantly increased incidence of hepatocellular adenomas in both males and females at all dose levels
- significantly increased incidence of hepatocellular carcinomas at ≥3000 ppm in both males and females
- significantly increased incidence of hepatoblastomas at ≥1500ppm in males and at ≥3000 ppm in females, with incidence at all doses exceeding historical control data
- a dose dependent increase in the incidence of acidophilic cell foci in females at ≥3000 ppm
- increased incidence of centrilobular hypertrophy of hepatocytes in males at all doses and in the 6000 ppm fed females
- a dose dependent occurrence of peritoneal haemangiosarcomas in both males and females (significant at ≥3000 ppm in females) – predominantly found around the pelvic viscera (e.g., urinary bladder, uterus, and male accessory sex gland) – with incidences, apart from the 750 ppm group males, exceeding historical control data
- increased incidence of eosinophilic globules in the nasopharynx in the 3000 ppm fed males and all treated females
- increased incidences of brown pigment deposition and respiratory metaplasia in the olfactory epithelium and submucosal gland in the nasal cavity in both males and females.

It is reported that the hepatocellular carcinomas and hepatoblastomas metastasized predominantly to the lung, followed by the peritoneum, lymph nodes, stomach (whole layer infiltration), ovary and pancreas.

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 GLP compliant combined repeat dose and reproductive toxicity screening test conducted in accordance with OECD TG 422, CRj:CD (SD) rats (12 animals/sex/group) were

administered the chemical in corn oil by oral gavage at 0, 8, 40 or 200 mg/kg bw/day, once daily, for 7 days/week. Males were treated for a total of 45 days before mating; females were treated from 14 days before mating to day 3 of lactation, through premating, mating, gestation, and lactation periods. At the highest dose, one female died during delivery of pups, and body weight gain was lower compared to controls. Details from this study are also presented in the **Repeat dose toxicity** section.

No effect on mating, fertility, or the oestrous cycle was reported. However, 5 of the 12 females from the highest dose group (200 mg/kg bw/day) were reported not to give birth to live pups. Necropsy in these female rats showed moderate degree of pigment deposit in the spleen, atrophy of the thymus, swelling of the liver cells, ulcer action in the stomach, duodenum and large intestine, single liver cell necrosis and fibrosis of the renal tubular epithelium. At the highest dose, in comparison with the control group, there was also a significantly lower number of live pups born (80 versus 159), live birth index (65% versus 99%) and viability index on day 4 of lactation (42% versus 89%). Atrophy of the testes and epididymis was reported in one male from the control group and one male from the 40 mg/kg bw/day group; however, it was noted that this animal was not part of the mating phase. The NOAEL for reproductive toxicity was considered to be 40 mg/kg bw/day in this study (NITE 1996a; OECD 1996).

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