Australian Government
Department of Health
Australian Industrial Chemicals Introduction Scheme

Benzene, 1-chloro-2-nitro- (2-chloronitrobenzene)

Evaluation statement

30 June 2022

Table of contents

Contents

AICIS evaluation statement

Subject of the evaluation

Benzene, 1-chloro-2-nitro- (2-chloronitrobenzene)

Chemical in this evaluation

Name	CAS registry number
Benzene, 1-chloro-2-nitro-	88-73-3

Reason for the evaluation

Evaluation Screening 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.

In this evaluation, the chemical is referred to by the common synonym of 2-chloronitrobenzene. However, relevant information on this chemical may also be found under the synonym of *ortho*-chloronitrobenzene.

Summary of evaluation

Summary of introduction, use and end use

There is no specific information about the introduction, use and end use of 2-chloronitrobenzene in Australia, but the available data report that the chemical is an important intermediate in the synthesis of other chemicals, including colourants and specialty chemicals overseas (IARC 2020; OECD 2001). 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
- systemic effects following repeated oral exposure
- carcinogenicity.

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

The chemical is a nitro-aromatic compound and is readily absorbed via the gastrointestinal tract and through the skin. It is rapidly metabolised, then excreted primarily in urine, with the liver and kidney reported to contain the highest detectable concentrations. Metabolism of the chemical forms 2-chloronoaniline (also commonly referred to as *o*-chloroaniline), through conversion of the nitro ($–NO_2$) substituent to amine ($–NH_2$).

Based on the available data, the chemical has high acute oral and dermal toxicity. The lowest reported median lethal dose (LD50) values are 144 mg/kg bw for oral toxicity in rats and 355 mg/kg bw for dermal toxicity in rabbits. For inhalation toxicity, a median lethal concentration (LC50) of 3200 mg/m^3 was reported in rats.

In regard to local effects, the chemical is not irritating to the skin and may be mildly irritating to the eyes. There is insufficient data to determine the skin and respiratory sensitisation potency of the chemical, with available data both limited and lacking detail. Structural and mechanistic profiling of the chemical using the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure Activity Relationship (QSAR) Toolbox did not reveal any skin sensitisation alerts (OECD QSAR Toolbox version 4.2). However, it is noted that the related 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 chemical is expected to cause serious systemic health effects following repeated oral and inhalation exposure. For repeat oral exposure, a lowest observed adverse effect level (LOAEL) of 4 mg/kg bw/day in rats is derived from a 13 week feeding study, based on haematological effects seen at this dose (Matsumoto et al. 2006a). No observed adverse effect level (NOAEL) values of 16 mg/kg bw/day for male mice and 24 mg/kg bw/day for female mice, were determined in a subchronic oral toxicity study. In repeat inhalation studies, a lowest observed adverse effect concentration (LOAEC) of 1.1 ppm was established in rats based on haematotoxicity, while in mice, a no observed adverse effect concentration (NOAEC) of 4.5 ppm was based on target organ toxicity (liver, kidney, and spleen).

Similar to other nitrobenzene compounds, including the metabolite 2-chloroaniline and the related para-isomer 4-chloronitrobenzene, the chemical is reported to be a methaemoglobin inducer, leading to a regenerative anaemia (methaemoglobinaemia) and a variety of tissue changes secondary to oxidative erythrocyte injury (NICNAS 2016; NICNAS 2017). Clearance of the increased levels of methaemoglobin by the spleen results in high concentrations of cytotoxic reactive metabolites in the spleen, causing local cell damage. Epigenetic changes and tumours are also reported to be formed due to this process (NICNAS 2019). Specific renal and hepato-toxicity is also identified.

The chemical is considered to induce methaemoglobin formation to a lesser extent than the para-isomer 4-chloronitrobenzene (Travlos et al. 1995). This difference in methaemoglobin-inducing potential across the isomers of chloronitrobenzene has also been identified in the chloroaniline metabolite isomers. Comparative studies have demonstrated that for the isomers of chloroaniline, the order of potency for methaemoglobin formation in rats and mice is para isomer (4-chloroaniline) > meta isomer (3-chloroaniline) > ortho isomer (2-chloroaniline) (Hejtmancik et al. 2002; NICNAS 2016). A similar order of potency was also seen in relation to changes in other haematological parameters, spleen weights, gross abnormalities, histopathological changes, and the severity of haemosiderin deposition. It is considered that this relative difference is likely due to a steric hindrance effect at the ortho

position for the chemical, with 2-chloronitrobenzene reported to be less readily metabolised than its para and meta isomers (REACH).

Results from rodent studies show that the chemical has clear carcinogenic properties, inducing tumours in the liver and kidneys of rats and mice in chronic toxicity studies. The International Agency for Research on Cancer (IARC) has classified 2-chloronitrobenzene as 'Possibly carcinogenic to humans' (Group 2B) based on experimental evidence from animal studies. The IARC considered there was moderate evidence of oxidative stress induced by the chemical in both rats and mice, as shown with increased methaemoglobin levels. There was moderate evidence of the chemical altering cell proliferation, death, and nutrient supply in multiple tissues, especially kidneys and spleen, in both rats and mice.

The chemical has some genotoxic properties, based on the available data. According to IARC, there is 'weak evidence' of genotoxic potential. Based on in vitro studies, the chemical is weakly mutagenic in bacterial test systems but not in mammalian cell test systems. In mammalian cells, the chemical increased sister-chromatid exchanges and chromosomal aberrations in some tests but not others. Only one in vivo somatic cell genotoxicity test was reported to give positive results, where DNA strand breaks were observed in the kidney, liver and brain of Swiss mice exposed to a single injection of the chemical. The chemical did not induce mutations in germ cells of *Drosophila melanogaster* according to two distinct studies. In one available study in humans, a non-statistically significant increase in chromosomal aberrations was reported in workers exposed to various chloronitrobenzenes including 2-chloronitrobenzene. However, the reported effects cannot be concluded as specifically caused by exposure to 2-chrolonitrobenzene alone.

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-chloroaniline (AICIS 2022a; AICIS 2022b; NICNAS 2016; 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).

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. Inhalation studies showed there was evidence of decreased spermatogenesis in male rats and decreased sperm motility in male mice. However, fertility was not affected in CD-1 mice orally exposed to the chemical at up to 160 mg/kg bw/day. While systemic toxicity was observed (significant changes in organ weights and methaemoglobin levels, decreased pup weights of the F1 generation), no significant effects on fertility were noted in F0 or F1 mice. Adverse effects on development were only observed at doses that were also toxic to parent animals. 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 – oral	Acute Tox. 3	H301: Toxic if swallowed
Acute Toxicity – dermal	Acute Tox. 3	H311: Toxic in contact with skin
Carcinogenicity	Carc. 1B	H350: May cause cancer
Specific Target Organ Toxicity (repeated exposure)	STOT RE 1	H372: Causes damage to organs through prolonged or repeated exposure

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 or reprotoxic health effects.

The data available indicate that a workplace exposure standard (WES) may be beneficial to mitigate the risk of adverse effects to workers. Methaemoglobinaemia was observed in inhalation studies in animals. Exposure standards have been established for several nitro-aromatic related compounds, including the para-isomer 4-chloronitrobenzene, 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 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 minimize 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 minimize 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.

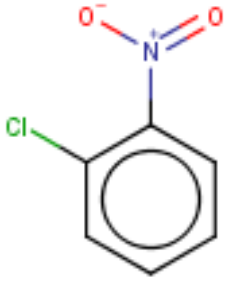
Considering the proposed means of managing risks, the Executive Director is satisfied that the identified human health risks can be managed within existing risk management frameworks. This is provided that all requirements are met under environmental, workplace health and safety and poisons legislation 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 the ortho (*o*-) isomer of chloronitrobenzene. It can also be described as chlorobenzene with a nitro group attached at the 2 position (ortho).

Chemical name	Benzene, 1-chloro-2-nitro-
CAS No.	88-73-3
Synonyms	*o*-chloronitrobenzene 2-CNB
Structural formula	
Molecular formula	C6H4ClNO2
Molecular weight (g/mol)	157.55
SMILES	[O-][N+](=O)c1ccccc1Cl

Relevant physical and chemical properties

Physical form	Solid
Melting point	32°C
Boiling point	246°C
Vapour pressure	0.018 mmHg at 25°C
Water solubility	441 mg/L at 25°C
log K_{ow}	2.52

Introduction and use

Australia

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

International

The chemical is an important intermediate in the manufacture of other chemicals, including 2-nitroaniline, dichlorobenzidine, 2-nitroanisole, and 2-chloroaniline. These chemicals are subsequently used to manufacture other chemicals with industrial end use in dyes and pigments and corrosion inhibitors. Reported non-industrial end uses include pesticide and pharmaceutical products (Chemwatch; IARC 2020; OECD 2001; SPIN).

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), although the total production has decreased in most countries. In 1995, the worldwide production of 2-chloronitrobenzene was 111,800 tpa, including 27,000 tpa in Western Europe, 39,000 tpa in China and 19,000 tpa in the USA. In 2015, manufacture of the chemical in the USA was reported to be less than 11 tpa (IARC 2020). The chemical is currently reported to be manufactured or imported in the European Union (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 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). In 2020, Safe Work Australia reviewed the WES for the para isomer, 4-chloronitrobenzene. A time weighted average (TWA) WES of 0.1 ppm (0.64 mg/m^3) was recommended to be retained to protect for methaemoglobinaemia in exposed workers (SWA 2020). The metabolite, 2-chloroaniline, 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. Exposure limits for the para isomer (4-chloronitrobenzene; CAS No. 100-00-5) have been established in several countries (NICNAS 2016; SWA 2020).

OECD

The chemical is listed on the OECD List of High Production Volume (HPV) chemicals (OECD 2007). However, this listing is based on world wide production volume data from 1995 (OECD 2001). More recent data indicate global production volumes of the chemical have significantly reduced (see **Introduction and use** section).

Other

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

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 (Chemwatch).

Human exposure

Workers

Dermal and inhalation exposure may occur at sites where the chemical is used. Accidental ingestion of the chemical may occur. Exposure data from China reported in 2006 showed that the mean 8 hour average exposure levels in factory workers exposed to the chemical was 0.4 mg/m^3 (IARC). Data from Bayer AG in 2001 reported 8 hour average exposure levels between 0.03–0.6 mg/m^3, the highest values corresponding with filling operations. In these cases, workers were wearing face masks to prevent inhalation of dust (OECD 2001).

The American Conference of Governmental Industrial Hygienists (ACGIH) published a biological exposure index (BEI) for methaemoglobin inducers (ACGIH 2001), including 2-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 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

In an oral absorption study, a single dose of the radiolabelled (^{14}C) chemical was administered to Fisher 344 (F344) rats (8 males/dose) via oral gavage at 2, 20 or 200 mg/kg bw. Within 72 hours, minimum absorption of the chemical was reported to be 61–77% of the administered dose. At ≤20 mg/kg bw, the highest concentrations were detected in liver and kidney, with 4% of the radiolabel detected in liver at 24 hours, and 2% at 72 hours. At 200 mg/kg bw, the highest concentrations were detected in fat followed by liver and kidney, with 13% of the radiolabel detected in fat at 24 hours, and 1.6% in liver at 72 hours. At ≤20 mg/kg bw, 58–60% of the administered dose was excreted in urine, and 26–28% in faeces, primarily within the first 24 hours. At 200 mg/kg bw, 74% of the administered dose was excreted in urine and only 7% in faeces. There were up to 23 metabolites (unspecified) in urine (IARC 2020; REACH).

In a dermal absorption study, a single non-occlusive dose of the radiolabelled chemical was applied to the skin of F344 rats (3 males/dose) at 0.65, 6.5 or 65 mg/kg bw. Within 72 hours, 33–40% of the dose had been absorbed through the skin. Excretions were 21–28% in urine and 11–15% in faeces and was not significantly affected by dose over the range studied (OECD 2001; REACH).

In another toxicokinetics study, the chemical was orally administered to rats via gavage at a dose of 65 mg/kg bw/day for 11 days, with the radiolabelled chemical administered on days one, 5 and 9. Results showed that 71–74% of the administered dose was excreted in urine and 20–27% in faeces. On day 12, 5% of the dose was detected in tissues, with the highest concentrations in liver and kidney (REACH).

In a toxicokinetic study in rabbits, 100 mg/kg bw of the chemical was administered as a single oral dose. After 48 hours, nearly the entire administered dose (82% accounted for in total) was excreted in the urine as either 2-chloroaniline or derivatives of phenolic metabolites, with 0.3% of the administered dose excreted in faeces as 2-chloraniline. Metabolites identified in urine included 42% ether glucuronide, 24% ethereal sulfate, 7% mercapturic acid and 9% free chloroaniline (IARC 2020; REACH).

In an in vitro metabolism study, ^{14}C radiolabelled chemical was incubated with isolated male F344 rat hepatocytes. 2-Chloroaniline was reported to be the main metabolite detected (19.2% of total radiolabel), with 2-chloroaniline-N-glucuronide (14.2%) and S-(2-nitrophenyl)glutathione (13.3%) also detected (IARC 2020).

In an in vitro study, the comparative metabolism of chloronitrobenzenes was investigated in a purified milk xanthine oxidase-xanthine system. A steric hindrance effect at ortho position for the chemical was reported due to it being less readily metabolised by the enzyme than its para and meta isomers (REACH).

Acute toxicity

Oral

Based on the weight of evidence from available experimental data, the chemical is expected to have high acute oral toxicity, with reported oral LD50 values ranging from 144 to 457 mg/kg bw.

In an acute toxicity study similar to OECD Test Guideline (TG) 401 (no data on Good Laboratory Practice (GLP) compliance), Wistar rats (15 animals/sex/dose) were treated via gavage with the chemical in polyethylene glycol, at doses of 50, 100, 150, 200, 250, 300 and 500 mg/kg bw in males and 25, 50, 100, 250, 350, 500, 650 and 850 mg/kg bw in females. Animals were observed for 14 days following exposure, for mortality and clinical signs. The LD50 was 219 mg/kg bw for males and 457 mg/kg bw for females. Mortalities occurred at ≥150 mg/kg bw in males and at ≥250 mg/kg bw in females. Clinical signs of toxicity included reduced general condition and cyanotic appearance in all male animals given ≥100 mg/kg bw and in all female rats given ≥50 mg/kg bw. Signs of intoxication were not observed in either of the lowest dose groups for both males and females (REACH).

In another acute oral toxicity study similar to OECD TG 401 (no data on GLP compliance), Wistar rats (10 animals/sex/dose) were treated via gavage with the chemical in Lutrol, at doses of 100, 200, 250, 300 and 400 mg/kg bw in males and 100, 200, 300, 400 and 500 mg/kg bw in females. Animals were observed for 14 days following exposure for mortality and clinical signs. The LD50 was 251 mg/kg bw for males and 263 mg/kg bw for females. Mortalities and clinical signs of toxicity, including reduced general condition and cyanotic appearance, sedation, and narcosis, were reported in both sexes at ≥200 mg/kg bw. Treatment related effects were not observed at 100 mg/kg bw (OECD 2001; REACH).

In an acute toxicity study with limited detail, male Wistar rats (10 animals/dose) were administered 63, 100, 160 and 250 mg/kg bw of the chemical by oral gavage and observed for 14 days following exposure. The LD50 was 144 mg/kg bw. Reported clinical signs of toxicity included imbalance, rough fur, diarrhoea, and slight tremor. Mortalities occurred at doses ≥100 mg/kg bw (OECD 2001; REACH).

Dermal

Based on the weight of evidence from available experimental data, the chemical is expected to have moderate acute dermal toxicity, with reported dermal LD50 values ranging from 355 to 1796 mg/kg bw.

In a non-GLP compliant acute dermal toxicity study similar to OECD TG 402, Wistar rats (10 animals/sex/dose) were treated with a single dose of 250, 350, 500, 750, 1000 or 1500 mg/kg bw of the chemical in polyethylene glycol. The LD50 was 655 mg/kg bw in male rats and 1320 mg/kg bw in female rats. Reported sub-lethal signs of toxicity included reduced general condition, difficulties in breathing and cyanotic appearance (OECD 2001; REACH).

A dermal LD50 of 1796 mg/kg bw in female rats was estimated in a study where the chemical was dissolved in sesame oil at 40% before dermal application. No further details are available (REACH).

In a non-GLP compliant acute dermal toxicity study similar to OECD TG 402, rabbits (2 animals/sex/dose) were treated with a single dose of 251, 316, 398, 501 and 631 mg/kg of the undissolved chemical. The LD50 was 445 mg/kg bw in male rabbits and 355 mg/kg bw in

female rabbits. Reported sub-lethal signs of toxicity included lethargy for up to three days, increasing weakness and collapse. Gross autopsy revealed haemorrhagic areas in the lungs, discolouration in the liver, kidneys and spleen, gastrointestinal inflammation and enlarged gall bladder whereas in survivors the viscera appeared normal (OECD 2001; REACH).

A dermal LD50 of 450 mg/kg bw in rabbits is reported in an acute toxicity study with limited data provided (REACH).

Inhalation

In an acute inhalation toxicity study of limited reliability, male rats (CD strain; 10 animals per concentration) were exposed to the chemical as a mixture of vapour and aerosol, nose only for 4 hours, at concentrations of 1.56–3.33 mg/L. Reported mortalities were not dose dependent. A LC50 of 3200 mg/m^3 (495 ppm) was calculated in this study; however, this was reported to have no statistical significance. Clinical signs of toxicity included lethargy, slight to moderate cyanosis, slight to moderate corneal opacity, prostration, reddish brown nasal discharge and abnormally rapid breathing (OECD 2001; REACH).

Corrosion/Irritation

Skin irritation

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

In a non-guideline skin irritation study, 6 rabbits were treated with a solution of 10% of the chemical in sesame oil, applied to intact and abraded skin, for 24 hours under semi-occlusive conditions. Observations were recorded at 24, 48, 72 hours after patch removal. Only mild erythema (score of one) was reported for both intact and abraded skin after 24 hours, in 4 out of 6 rabbits. The chemical was considered as not irritating to the skin in this study (OECD 2001).

In a non-guideline study, the undiluted chemical was applied to the inner skin of the ear of 2 rabbits under an occlusive patch for 24 hours. No skin reactions were reported during the 7 day observation period following application (OECD 2001).

In a skin irritation study of low reliability, application of undiluted chemical to the skin of 6 rabbits resulted in no irritation up to 7 days after exposure. No further details were provided (OECD 2001).

Eye irritation

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

In a non-guideline eye irritation study, 100 mg of the undiluted chemical was instilled into one eye each of 6 Himalayan rabbits. The eyes were observed at 1, 7 and 24 hours post exposure. Conjunctival redness (maximum score of 2 out of 3) was observed in all treated rabbits after 1 hour and in 2 rabbits after 7 hours (score of one out of 3). Effects were reported to be reversed after 24 hours (OECD 2001).

In a non-guideline study, 0.1 mL of the chemical at 10% concentration was instilled into one eye each of 6 rabbits. The eyes were observed at one, 7 and 24 hours post exposure. Conjunctival redness (score of one out of 3) was reported in in 3 rabbits after one hour. No

irritation effects were observed after 7 hours post exposure. No further details were provided (OECD 2001).

In an eye irritation study with limited details available, the undiluted chemical was instilled into one eye each of 2 rabbits. Observations were conducted up to 7 days after exposure. Conjunctival redness (score of one out of 3) was observed in one rabbit and was reversed within 24 hours. No other signs of irritation were reported up to 7 days after exposure (OECD 2001).

Sensitisation

Skin sensitisation

In a non-guideline sensitisation test, guinea pigs (n=10) were treated with 1% of the chemical in acetone, on shaved skin for 5 consecutive days. The animals were challenged with the same concentration of the chemical in acetone after 7 days. As no positive responses were recorded, a subsequent test on day 22 was conducted; the animals were injected into the hind paw with a solution of 10% of the chemical with Freund's adjuvant. The animals were then challenged with a drop of a 10% solution on shaved untreated skin after 6 days. A total of 50% of the challenged animals had a positive response to the chemical. No other details were provided (OECD 2001; REACH). Due to the limited nature and low reliability of information available, the OECD concluded that the skin sensitisation potential of the chemical could not be determined (OECD 2001).

The chemical has no structural alerts for protein binding based on the mechanistic profiling functionality of the OECD QSAR Application Toolbox (OECD QSAR Toolbox version 4.2). However, it is noted that the structurally related 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).

Repeat dose toxicity

Oral

In a GLP compliant 5 week study conducted in accordance with OECD TG 407, B6C3F1 mice (12 animals/sex/dose) were administered the chemical in daily feed at nominal concentrations of 0, 50, 500 or 5000 ppm for 5 weeks; calculated intake (ingested amount) was reported to be 0, 16, 167 and 1120 mg/kg bw/day in male mice; and 0, 24, 220 and 1310 mg/kg bw/day in female mice, respectively. The NOAEL was reported to be 50 ppm, equivalent to 16 mg/kg bw/day and 24 mg/kg bw/day in males and females, respectively (OECD 2001; REACH). One mortality from the low dose male group was reported. Reduced body weight gain and reduced food intake were observed at 5000 ppm in males and at ≥500 ppm in females. Narrowed palpebral fissures and corneal opacity was also reported in males at the highest dose.

The following effects were reported at ≥500 ppm:

- increase in cholesterol content in the blood
- increased liver weights with hypertrophy of the centrilobular hepatocytes
- increase in liver enzyme activities.

The following effects were reported at the highest dose only (5000 ppm):

- morphology changes and reduced number of erythrocytes, increased values for bilirubin, methaemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC)
- increased spleen weights, dark red discoloration of the spleen and increased haemosiderin deposition
- gross changes in the liver including increased activity of ASAT, ALAT and alkaline phosphatase (males)
- decreased blood-urea in males
- decrease in gluconeogenesis and glycogen, activated pentose phosphate cycle, and increase of glycolysis
- decreased testes weight without reported histopathological changes.

In a GLP compliant, 13 week toxicity study conducted in accordance with OECD TG 408, F344 rats (10 animals/sex/dose) were fed 0, 63, 250, 1000, 2000 or 4000 ppm (w/w) nominal concentrations of the chemical, and BDF1 mice (10 animals/sex/dose) were fed 0, 78, 313, 1250, 2500 or 5000 ppm (w/w) nominal concentrations of the chemical (Matsumoto et al. 2006a).

In rats, a LOAEL of 63 ppm (equivalent to 4 mg/kg bw/day actual ingested dose) is derived based on the following effects in the blood:

- significantly decreased red blood cells (RBC) and haemoglobin at ≥63 ppm in females and at ≥1000 ppm in males
- significantly increased incidence of erythropoiesis in the bone marrow at ≥2000 ppm for both males and females.

Effects on the liver included:

- significantly increased incidence of haemosiderin deposit at ≥1000 ppm
- significantly increased incidence of centrilobular hypertrophy of hepatocytes at ≥2000 ppm in males and at ≥4000 ppm in females
- hydropic degeneration of the centrilobular hepatocytes at ≥1000 ppm in males and at ≥2000ppm in females
- significantly increased incidence of single cell necrosis at ≥1000 ppm for both males and females.

Effects on the spleen included:

- significantly increased incidence of congestion and haemosiderin deposit in at ≥250 ppm
- significantly increased incidence of extramedullar haematopoiesis at ≥1000 ppm.

In mice, a LOAEL of 313 ppm (equivalent to 43.6 mg/kg bw/day actual ingested dose) is derived based on haematological changes and the following effects in the spleen:

- significantly increased incidence of congestion and haemosiderin deposit at ≥313 ppm
- significantly increased incidence of extramedullar haematopoiesis at ≥1250 ppm.

Effects on the liver included:

- significantly increased incidence of haemosiderin deposition at ≥1250 ppm
- significantly increased incidence of centrilobular hypertrophy of hepatocytes and of nuclear enlargement with atypia of centrilobular hepatocytes at ≥313 ppm in males and ≥1250 ppm in females.

The main haematological effects included a significantly decreased RBC and haemoglobin at ≥250 ppm in both males and females.

Dermal

No data are available for the chemical.

Inhalation

In a 13 week study, conducted similar to OECD TG 413, F344 rats and B6C3F1 mice (10 animals/sex/dose) were exposed to the chemical vapour through whole body exposure at 0, 1.1, 2.3, 4.5, 9 or 18 ppm (approximately equivalent to 0, 7, 14.7, 28.8, 57.6 and 115.2 mg/m^3) for 6 hours/day and 5 days/week (NTP 1993; OECD 2001; Travlos et al. 1995).

In rats, significant increases in spleen weight (absolute and relative) were reported at the highest concentration (18 ppm) for males and at ≥4.5 ppm in females. All animals survived to the end of the study. Body weight gains were not impacted. Darkened spleen was observed at 18 ppm (one female and 2 males). Increased liver weights (absolute and relative) were reported at all treatment doses in males and at ≥2.3 ppm in females. Increased kidney weights were reported at ≥9 ppm in males and at ≥18 ppm in females. Hyperplasia of the nasal cavity respiratory epithelium was reported in all treated groups. Histopathologic observations included cytoplasmic basophilia in the liver at ≥9 ppm, renal tubule regeneration at all doses and tubule pigment at ≥4.5 ppm. Haematological effects included: methaemoglobinaemia; anaemia and haematopoiesis; methaemoglobin levels were significantly higher at all treatment dose levels in both males and females. Decreased levels of haematocrit, haemoglobin and red blood cell counts were reported along with increased leukocyte number at the highest concentration. Reticulocyte count was increased in all treated groups by the end of the study. A NOAEC could not be determined but the LOAEC was reported to be 1.1 ppm (7 mg/m^3), based on effects observed at the lowest concentration.

In mice, 2 male mortalities occurred at the highest concentration. Body weight gains were not impacted. Liver and kidney weights were increased in most treated groups at ≥2.3 ppm. Histopathologic observations at ≥9 ppm included enlarged spleen and effects in the liver (hepatocellular necrosis, cytomegaly, mineralization and chronic inflammation). Increased haematopoietic activity of the spleen was observed in females at ≥9 ppm. At ≥18 ppm, discolouration of the liver was seen in 6 males and one female. The NOAEC was reported to be 4.5 ppm (28.8 mg/m^3).

Similar studies were conducted for the para isomer 4-chloronirobenzene (Travlos et al. 1995). Like for 2-chloronitrobenzene, toxic effects included methaemoglobin formation and oxidative damage to red blood cells, leading to a regenerative anaemia and a variety of tissue and biochemical changes secondary to erythrocyte injury. In rats, a LOAEC of 1.5 ppm was determined, while a NOAEC of 6 ppm was determined in mice. Both values are slightly higher than the values derived for 2-chloronitrobenzene. However, the para-isomer was considered to induced greater toxicity than 2-chloronitrobenzene, due to a higher degree of red blood cell and tissue injury with the para-isomer at similar exposure concentrations, and

a more pronounced methaemoglobinaemia and responsive anaemia and more extensive tissue deposition of haemosiderin. Also, the study authors found that the haematopoietic cell proliferation present in rats following exposure to 4-chloronitrobenzene was not observed following exposure to 2-nitrochlorobenzene. Additionally, pathological changes were reported to be seen across more tissue types in animals exposed to 4-chloronitrobenzene.

Observation in humans

Occupational exposure to the chemical and its para isomer (4-chloronitrobenzene) was described in 4 workers exposed via dermal contact and inhalation (EPA 2009). Only limited information was reported, with no indication on the total number of workers exposed or the air concentrations to which workers were exposed. Effects included methaemoglobinaemia, slate grey appearance, headache, dyspnoea on exertion, darkened blood serum, and large and occasionally deformed erythrocytes.

In a clinical study at a chemical manufacturing plant in China, the effects of the chemical and its para isomer (4-chloronitrobenzene) on workers were measured through clinical observations including physical examination, urinalysis, haematology, clinical chemistry, and analysis of blood for haemoglobin adducts with 2- and 4-chloronitrobenzene metabolites. A total of 39 exposed workers and 15 unexposed (control group) workers (from the same factory) were examined. Air concentrations were measured using personal air monitors in a subset of exposed workers (n=19). Median TWA (8 hour) air concentrations of the chemical and its para isomer were 0.37 and 0.87 mg/m^3, and mean TWA exposures were 0.49 and 1.17 mg/m^3, respectively. There was a higher, but not statistically significant, prevalence of reported symptoms (fatigue, headache, dizziness) and abnormalities (splenomegaly, hepatomegaly) among exposed workers compared with the control group. The median haemoglobin adduct levels were higher in workers with fatigue, eye irritation, splenomegaly, and cardiovascular effects. However, there was no significant difference between exposed and unexposed workers for urinalysis clinical chemistry or haematology including methaemoglobin concentrations, haemoglobin concentration, RBC counts, or leukocyte counts (EPA 2009; Jones et al. 2006).

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 (IARC 2020; REACH) as listed below, with mutagenic effects only reported in the presence of metabolic activation:

- Negative results were reported in a bacterial reverse mutation assay in *Salmonella typhimurium* TA1535 and TA1537 with and without metabolic activation at concentrations up to 128 µg/mL.
- Negative results were reported in bacterial reverse mutation assays in *S. typhimurium* TA98 and TA100 with and without metabolic activation only at concentrations up to 50 µg/mL.
- Positive results were reported in bacterial reverse mutation assays in *S. typhimurium* TA98 and TA100 and *Escherichia coli* WP2 uvrA with metabolic activation only (concentrations not stated).
- Positive results were reported in bacterial reverse mutation assays in *S. typhimurium* TA98 and TA100 with metabolic activation only, at concentrations from 38 µg/mL.

Both positive and negative results were also reported in the available mammalian cell in vitro assays (OECD 2001), as detailed below:

- Negative results were reported in a HPRT Test using Chinese hamster V79 lung cells, conducted according to OECD TG 476, at concentrations of 100–1200 µg/mL, both with and without metabolic activation. Cytotoxicity was noted at the highest concentration.
- Positive results were reported in two sister chromatid exchange (SEC) assays in Chinese hamster ovary (CHO) cells with metabolic activation at concentrations of 50–500 µg/mL.
- Positive results were reported in an in vitro mammalian chromosome aberration assay in CHO cells with metabolic activation at concentrations of 101–500 µg/plate.
- Positive results were reported in an in vitro mammalian chromosome aberration assay in Chinese hamster lung (CHL) cells without metabolic activation. However, this study is of limited reliability and concentrations were not stated.

The following in vivo study results were reported for the chemical (Cesarone et al. 1980; IARC 2020):

- Negative results were reported for DNA adduct formation in female Wistar rats orally given the chemical, although haemoglobin adducts were formed.
- Negative results were reported for induction of sex linked recessive lethal mutations in adult males of *Drosophila melanogaster* (fruit fly) fed with 125 mg/kg(feed) of the chemical.
- Positive results were seen in two studies using Swiss CD-1 mice treated with intraperitoneal injections of 60 mg/kg bw of the chemical. Nuclei from target tissues were isolated 4 hours after administration and DNA elution rate was measured to identify genotoxic damage. DNA single-strand breaks were observed in the liver, kidney, and brain.

The 2-chloroaniline metabolite has also been tested for genotoxicity in a variety of assays. While generally negative results are reported for reverse mutations in *S. typhimurium*, positive results were observed in several clastogenicity assays in vitro. In vivo micronucleus tests were mainly negative although a few positive results were reported at higher doses (NICNAS 2017).

Observation in humans

In a study in humans, the lymphocytes of workers exposed to various chloronitrobenzenes, including 2-chloronitrobenzene, were assessed for the formation of chromosomal aberrations (IARC 2020; OECD 2001). Samples from 24 exposed workers and 13 unexposed workers (controls) were analysed. While a positive trend (non-statistically significant) is reported between the frequency of chromosomal aberrations and levels of 2-chloroaniline–haemoglobin adducts in exposed workers, there was no reported increase when comparing the group exposed to chloronitrobenzenes with the unexposed group of workers. It should also be noted that the workers were also exposed other chloronitrobenzenes in this study, therefore reported effects cannot be specifically contributed to exposure to 2-chloronitrobenzene.

Carcinogenicity

In a GLP compliant 2 year carcinogenicity study conducted in accordance with OECD TG 451 (Matsumoto et al. 2006b), F344 rats (50 animals/sex/dose) received the chemical in diet at 0, 80, 400 or 2000 ppm (equivalent to 0, 4, 19, and 99 mg/kg bw/day in males and 0, 4, 22, and 117 mg/kg bw/day in females, respectively). At the highest dose, all male rats were deceased before the end of the study due to chronic progressive nephropathy. A significant difference in food consumption, and markedly suppressed body weights after week 20 of the study, was observed in males at this dose. There was no significant difference in the survival rate between the remaining treated and control groups for either sex. A significant decrease in body weight at the end of the study was reported in female rats given 2000 ppm (18% decrease) and male rats given 400 ppm (10% decrease), compared with controls. Noting that 2000 ppm males had been excluded from some analysis due to the high mortality rate, relative liver weight was significantly higher in male rats fed ≥80 ppm and in female rats fed ≥400 ppm. Relative spleen weight was significantly higher in female rats fed 2000 ppm. Relative kidney weight was significantly higher in male and female rats fed ≥400 ppm. Haematological changes in female rats given 2000 ppm included a significant decrease in RBC count and a significant increase in reticulocyte count in. Haematological changes in both males and females at ≥400 ppm included significantly decreased haemoglobin concentration and haematocrit values, significantly lower MCV and MCH levels, and significantly higher platelet counts. Methaemoglobin levels were also significantly higher in male and female rats fed ≥400 ppm.

Neoplastic lesions included a number of adenomas and carcinomas in liver and kidneys. There was a dose related increase in hepatocellular adenomas and carcinomas in treated groups compared with controls. However, only female rats given 2000 ppm had statistically significant increase in hepatocellular adenomas (20/50 vs 0/50 in controls). Although not statistically significant, the incidence of hepatocellular adenomas exceeded the maximum incidence of historical control data for male rats given 400 ppm (14% compared with 6% in historical control data). Similarly, the incidence of hepatocellular carcinomas for male rats given 400 ppm and female rats given 2000 ppm (6% and 8% respectively) was not significantly higher than controls but exceeded the maximum incidence of historical controls.

Non-neoplastic lesions were observed in the liver, spleen, and kidneys. In the liver, there was significant increases of clear cell foci in female rats fed 2000 ppm, acidophilic cell foci in male rats fed 400 ppm and female rats fed ≥400 ppm, and basophilic cell foci and spongiosis hepatis in male rats fed 400 ppm. Single cell necrosis, centrilobular hydropic degeneration and deposit of brown pigment, were observed only at the highest dose in both sexes, with statistical significance in female rats only. In the spleen, non-neoplastic lesions included capsule hyperplasia, angiectasis (abnormal dilation of blood vessels), engorgement of erythrocytes, increased extramedullary haematopoiesis and deposit of haemosiderin. These lesions were statistically significant only in female rats fed 2000 ppm. In the kidneys, chronic progressive nephropathy (CPN) was the most prevalent non-neoplastic lesion affecting both treated and control rats. The incidence of CPN was significantly higher in all treated female groups compared with controls, and in males fed 400 ppm. There was a dose related trend in the severity of the lesion, more pronounced in males than females, and 47/50 male rats fed 2000 ppm and 3/50 male rats fed 400 ppm died of CPN. Other non-neoplastic lesions in the kidneys included tubular hyperplasia, cortex mineralisation, urothelial hyperplasia, and deposit of brown pigment in the proximal tubule.

In a GLP compliant 2 year carcinogenicity study conducted in accordance with OECD TG 451 (Matsumoto et al. 2006b), Crj:BDF1 mice (50 animals/sex/dose) received the chemical in diet at 0, 100, 500 or 2500 ppm; calculated intake (ingested amount) was reported to be 0, 11, 54, and 329 mg/kg bw/day in males and 0, 14, 69, and 396 mg/kg bw/day in females,

respectively. There was a significant decrease in survival rate (mortality) in males fed ≥500 ppm and females fed ≥2500 ppm, attributed to malignant liver tumours. Significantly decreased body weights were reported for male and female mice fed ≥500 ppm. There was no significant difference in food consumption in any of the treated groups compared with controls. Relative weights of the liver and kidneys were all significantly higher in both male and female mice fed ≥500 ppm, and relative spleen weight was increased in male mice fed 2500 ppm, when compared with control group animals. The only significant haematological change was an increase in reticulocyte count in both male and female mice fed ≥500 ppm.

There was a dose related increase in the incidence of liver tumours, including a significant increase in hepatocellular adenomas, in both male and female mice. A statistically significant increase in the incidence of hepatocellular carcinomas and hepatoblastomas was also reported in male and female mice fed ≥500 ppm.

Non-neoplastic lesions were observed in the liver, spleen, and kidneys. In the liver, there was a significant increased incidence of centrilobular hypertrophy in both sexes at the two highest doses, and in male mice fed 100 ppm. Centrilobular nuclear enlargement of hepatocytes was observed in male mice fed ≥500 ppm. In the kidney and the spleen, deposit of haemosiderin was reported with statistical significance in both sexes at ≥500 ppm. Increased extramedullary haematopoiesis was also observed in the spleen at these dose levels.

Reproductive and development toxicity

In a study following the US National Toxicology Program (NTP) continuous breeding protocol, groups of CD-1 mice (20 animals/sex/dose) were fed with doses of 40, 80 or 160 mg/kg bw/day of the chemical in corn oil for 7 days before cohousing them for 98 days of continuous breeding (OECD 2001). An additional control group of 40 pairs of mice received only corn oil. At the end of the breeding period, the last litters born (F1) from the control group and the high dose group were reared and exposed to the same doses as the parental (F0) animals. After weaning, 20 non-sibling F1 mice of each sex were cohabited for 7 days and then housed singly through delivery of pups.

Apart from cyanotic appearance at the highest dose, no other clinical signs of toxicity in F0 animals were reported. Necropsy showed an increase of 50–100% in spleen weight and increased levels of methaemoglobin at the highest dose. Reproductive functions in F0 animals were not impacted by treatment, as the number of litters, pup weight and viability showed no difference with controls. However, in the final litter of the holding period following the continuous breeding phase, pup weight gain during suckling was lower in the treated groups. At weaning, pups of the high dose group weighed 12% less than control. No clinical signs of toxicity were seen in the pups. Reproductive functions in F1 animals appeared unchanged, as proportion of mated pairs, number of litters per group, number of live pups per litter and pup weight or viability were not different between control and treated groups. Adult F1 mice dosed with 160 mg/kg bw/day of the chemical had higher methaemoglobin levels, were heavier than controls, and liver and spleen weights were increased by 40–60%. F1 male mice had an increase in right epididymis and kidney and/or adrenals weights, and a decrease in seminal vesicle-to-body weight, compared with controls. Epididymal sperm motility, sperm count, and percentage of abnormal sperm were unchanged. Oestrus cycle was unaffected in F1 females. The NOAEL for fertility was determined to be 160 mg/kg bw/day.

At the end of the 13 week inhalation studies (see **Repeat-dose Toxicity** section), vaginal cytology and sperm morphology evaluations were performed on rats and mice (10 animals/sex/group) from the 0, 4.5, 9, and 18 ppm groups. In male rats exposed to 18 ppm, there was a decrease in cauda epididymis weights and in the spermatid count and

spermatid heads/testes. Female rats were not affected by treatment. All treated male mice had a decrease in sperm motility, while female mice were not affected by treatment.

In a developmental toxicity study, 25 pregnant female Sprague Dawley (SD) rats were given 0, 25, 75 or 150 mg/kg bw/day of the chemical in corn oil by gavage, during gestation days (GD) 6–15. All females from the highest dose group were euthanised before the end of the study due to severe toxicity. Clinical signs of toxicity included slightly decreased body weight gain, urinary staining, and alopecia at mid dose. The mean number of early resorptions and post implantation loss were significantly higher at 75 mg/kg bw/day compared with controls, but no other effects on reproductive parameters were reported. There was no difference in the total number of litters exhibiting external and skeletal malformations in the treated groups compared with controls. An increase in the number of litters exhibiting some known variations occurred in the treated groups, but it was statistically significant only at 75 mg/kg/day. A NOAEL of 25 mg/kg bw/day for maternal toxicity and a LOAEL of 75 mg/kg bw/day for developmental toxicity were reported based on this study (EPA 2009; NTP 1983).

In a developmental toxicity study, 25 pregnant female SD rats were given 100 mg/kg bw/day of the chemical in corn oil by gavage, during GD 6–15. One female died on GD 20, and food consumption and body weights were lower in the treated group at the end of the study compared with an untreated control group of animals. No other signs of toxicity were reported. No effects on development were reported. A NOAEL of 100 mg/kg bw/day for developmental toxicity was reported for this study (EPA 2009; NTP 1983).

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