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

2-Propenamide, N,N'-methylenebis- (N,N'-methylenebisacrylamide)

Evaluation statement

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Draft

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AICIS evaluation statement

Subject of the evaluation

2-Propenamide, N,N'-methylenebis- (N,N'-methylenebisacrylamide)

Chemical in this evaluation

Name	CAS registry number
2-Propenamide, N,N'-methylenebis-	110-26-9

Reason for the evaluation

Evaluation Selection Analysis indicated a potential human health risk.

Parameters of evaluation

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

Summary of evaluation

Summary of introduction, use and end use

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

Based on international information, the chemical predominantly has site limited use as a crosslinking agent/monomer in polymer manufacture, or in scientific research and development.

Despite being included in cosmetic databases, based on available data the chemical is unlikely to be used in cosmetics. The chemical has reported commercial use in food contact materials.

Human health

Summary of health hazards

The identified health hazards are based on available data for the chemical, or where limited data were available, based on acrylamide, given structural and physico-chemical similarities.

Based on the available data the chemical:

- has low acute inhalation toxicity

- is not considered to cause skin irritation
- is not considered to cause eye irritation
- is not considered to be a potent skin sensitiser.

Based on the available data the chemical has high acute oral toxicity (median lethal dose (LD50)=50–300 mg/kg body weight (bw) in rats) and moderate acute dermal toxicity (LD50=1148 mg/kg bw in rats).

Limited data are available for the chemical to determine effects following repeated exposure. The structurally similar chemical, acrylamide, is a known neurotoxicant. Repeated exposure to acrylamide caused neurotoxicity in rats at lowest observed adverse effect levels (LOAELs) of 1 and 50 mg/kg bw/day, for oral and dermal exposure, respectively. Neurotoxic effects were not observed in available studies for the chemical. However, these studies may not have completely investigated the neurotoxicity potential of the chemical. Overall neurotoxic effects of the chemical cannot be ruled out.

Negative results were reported for the chemical and acrylamide in in vitro bacterial reverse mutation assays under most conditions. Acrylamide was reported to induce genotoxicity in an in vitro mammalian chromosome aberration assay, and in a mammalian gene mutation assay. Similar to acrylamide, the chemical produced positive results in several in vivo studies. This included inducing dominant lethal mutations when administered orally or by intraperitoneal injection. The available data provide clear evidence that the substance reaches the germ cells and interacts with germ cell DNA.

No carcinogenicity data are available for the chemical. In studies in rats with the structurally similar chemical, acrylamide, there was clear evidence of an increase in tumour incidence in several organs in both sexes. Observed lesions included, but were not limited to, carcinoma of the thyroid, and malignant tumours of the testes and uterus. The chemical contains the same structural alerts for carcinogenicity and has a similar genotoxicity profile.

In available studies of the chemical, observed fertility effects, including effects in male reproductive organs and a reduction in live births, are similar to those observed with acrylamide. Significant effects on development were not observed in a single study with the chemical.

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) (UNECE 2017) for hazard classes relevant for worker health and safety as follows. This evaluation does not consider classification of physical and environmental hazards.

Health hazards	Hazard category	Hazard statement
Acute toxicity	Acute Tox. 3	H301: Toxic if swallowed
Acute toxicity	Acute Tox. 4	H312: Harmful in contact with skin
Specific target organ toxicity (repeated exposure)	STOT Rep. Exp. 1	H372: Causes damage to organs through prolonged or repeated exposure
Germ cell mutagenicity	Muta. 1B	H340: May cause genetic defects

Health hazards	Hazard category	Hazard statement
Carcinogenicity	Carc. 1B	H350: May cause cancer
Reproductive toxicity	Repr. 2	H361f: Suspected of damaging fertility

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.

Although the public may come into contact with food contact articles and coated surfaces containing low levels of the chemical as a residual monomer, it is expected that the chemical will be bound within the article and hence, will not be bioavailable.

Overall, 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 identified acute and long term systemic 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 (see **Proposed means for managing risks** section).

Proposed means for managing risk

Workers

Recommendation to Safe Work Australia

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

Information relating to safe introduction and use

The information in this statement including recommended hazard classifications, should be used by a person conducting a business or undertaking 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 oral, dermal, and inhalation 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 the chemical is used.

These control measures may need to be supplemented with:

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

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk.

Model codes of practice, available from the Safe Work Australia website, provide information on how to manage the risks of hazardous chemicals in the workplace, prepare 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 proposes to be satisfied that the identified human health risks can be managed within existing risk management frameworks.

This is provided that:

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

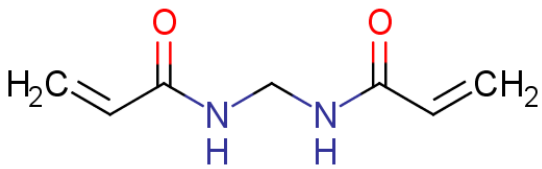
Note: Obligations to report additional information about hazards under *Section 100 of the Industrial Chemicals Act 2019* apply.

Supporting information

Chemical identity

Chemical name	2-Propenamide, N,N'-methylenebis-
CAS No.	110-26-9
Synonyms	N,N'-methylenebis[2-propenamide] N,N-methylenebis(acrylamide) N,N’-methylenebisacrylamide N,N'-methylenediacrylamide methylene bisacrylamide
Molecular formula	C7H10N2O2
Molecular weight (g/mol)	154.17
SMILES (canonical)	O=C(C=C)NCNC(=O)C=C
Chemical description	White crystalline powder

Structural formula:



Relevant physical and chemical properties

The following physico-chemical properties for the chemical were identified through the REACH dossiers (REACH n.d.).

Physical form	Solid
Melting point	173.7-185.9°C
Boiling point	333.8°C
Vapour pressure	3.0 x 10^{-6} Pa
Water solubility	34.1 g/L
log K_{ow}	-0.08

Introduction and use

Australia

No specific Australian information on introduction, use and end use have been identified for the chemical.

International

Based on international use information, the chemical predominantly has site limited use as a crosslinking agent/monomer in polymer manufacture, or in scientific research and development where it is used for the production of electrophoresis gels (ECHA 2021).

While there is information suggesting possible cosmetic use of the chemical (EC n.d.; Personal Care Products Council n.d.; SPIN n.d.), it is unlikely based on the weight of evidence.

The chemical is listed in the Personal Care Products Council database (n.d.) as being "*included in the International Nomenclature of Cosmetic Ingredients (INCI) database as a reference for the definition of other INCI names and might not be a marketed cosmetic ingredient*". The chemical has reported cosmetic use in the Substances and Preparations in Nordic countries (SPIN) database and is listed in the Cosmetic ingredient (CosIng) database (EC n.d; SPIN n.d.). However, it should be noted that SPIN does not distinguish between direct use of the chemical and use of the materials that are produced from chemical reactions involving the chemical (SPIN n.d.). In addition, the entry on SPIN was in 2001 and zero preparations were reported. The chemical is likely to be listed in CosIng based on it having an INCI name assigned (EC n.d.). No other supporting information was found that indicated direct use of the chemical in cosmetics (Bailey 2011; DeLima Associates n.d.; ECHA n.d.; NCBI n.d.).

The chemical has reported commercial use in papers, filters, or membranes intended to come into contact with food, and in adhesives intended for use in packaging, transporting, or holding food (Chemwatch n.d.).

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 not listed on the HCIS and no specific exposure standards are available in Australia (Safe Work Australia).

International regulatory status

Exposure standards

The United States of America's (USA) Department of Energy Subcommittee on Consequence Assessment and Protective Actions has derived temporary emergency exposure limits (TEELs) for the chemical of 0.64, 7.1, and 77 mg/m^3, for TEEL -1, -2, and -3, respectively (Chemwatch n.d.).

European Union

The chemical is listed in:

- Europe CEPE Code of Practice for Coated Articles where the Food Contact Layer is a Coating - Annexes II & III: Inventory List for Coatings Intended to Come Into Contact with Food - B. Temporary Appendix to List 1 of Monomers (Chemwatch n.d.).
- Germany Federal Institute for Risk Assessment (BfR) Database Recommendations on Food Contact Materials - Cooking Papers, Hot Filter Papers and Filter Layers (Chemwatch n.d.).
- Switzerland Annex 10 of the Ordinance of the FDHA on materials and articles intended to come into contact with foodstuffs – List of permitted substances for the production of packaging inks, and related requirements - Table 1: List of substances (Chemwatch n.d.)

United States of America

The chemical is listed in:

- USA FDA Food Ingredient & Packaging Inventories - Threshold of Regulation (TOR) Exemptions. N,N-methylenebis- (CAS No. 110-26-9) may be used as a hydrophilic crosslinker in the manufacture of PES filter membranes. The PES filter membranes may be used for filtration of aqueous, acidic, and alcoholic (50% alcohol or less) foods, except for infant formula and human milk, at room temperature or below (Chemwatch n.d.).
- USA FDA Indirect Food Additives: Adhesives and Components of Coatings - Substances for Use Only as Components of Adhesives - Adhesives (Chemwatch n.d.).

Health hazard information

Toxicology information for the chemical is limited for some endpoints. Available data from acrylamide (CAS No. 79-06-1) was used as a source of read across information, where necessary. The chemicals have similar structural and physico-chemical properties. Both are solid at room temperature, hydrophilic, water soluble and share the acrylamide moiety (ECHA 2021). For endpoints where data are available for both chemicals, a similar toxicological profile is observed.

Toxicokinetics

No studies on the experimental toxicokinetics of the chemical were available. Given the similar physical properties to acrylamide, the two chemicals are expected to behave similarly in the body.

Based on the chemical's molecular weight, log Kow and data for acrylamide, the chemical is expected to be absorbed following all routes of exposure. Acrylamide is extensively absorbed from the gastrointestinal tract and it, and its metabolites, are widely distributed to organs and tissues including the liver, kidney, lung, muscles, brain, testes, sciatic nerve, spinal cord, skin, fat and small intestines. Acrylamide is also transferred to the foetus and milk. Acrylamide is well metabolised, predominantly by conjugation with glutathione (GSH). Acrylamide is also metabolised by epoxidation to form glycidamide (GA) and is preferentially mediated by CYP2E1. Glutathione S-transferases (GSTs) promote the formation of GSH adducts of acrylamide and GA, which are processed to mercapturic acids and excreted via the urine. GA is also hydrolysed to 2,3-dihydroxypropionamide. Acrylamide metabolites are predominantly and rapidly excreted via the urine. There is no indication of tissue accumulation, except for residual protein adducts (ECHA 2021; EFSA 2015; NICNAS 2002).

Acute toxicity

Oral

Based on the available data, the chemical has high acute oral toxicity, warranting classification under GHS (see **Hazard classifications relevant for worker health and safety** section).

In a Good Laboratory Practice (GLP) compliant acute oral toxicity study conducted in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 423, female Sprague Dawley rats (3–6/dose) were treated with a single oral dose of the chemical by gavage. The median lethal dose was 50–300 mg/kg bw. Reported sublethal signs of toxicity included reduced motility, ataxia, tremor, and increased muscle tone (REACH n.d.-a).

The oral median lethal dose of acrylamide is reported to be 150–203 mg/kg bw in rats, 107 mg/kg bw in mice, 150–180 mg/kg bw in guinea-pigs, and 150–180 mg/kg bw in rabbits. Reported sublethal signs of toxicity included postural and motor incoordination, muscular dysfunction in the hindlimbs, hyperreflexia, tonic-clonic convulsions, tremors, dilatation of the pupils, and fatty liver (NICNAS 2002).

Dermal

Insufficient data are available for the chemical. Based on the read across information from acrylamide, the chemical is expected to have moderate acute dermal toxicity, warranting classification under GHS (see **Hazard classifications relevant for worker health and safety** section).

In an acute dermal toxicity study similar to OECD TG 402, rabbits (strain not specified; 2/sex/dose) were treated with a single dose of an aqueous test solution containing 50% acrylamide in both sexes. The median lethal dose of acrylamide was 1148 mg/kg bw. Reported sublethal signs of toxicity included tremors and incoordination of the hindlimbs (NICNAS 2002; REACH n.d.-a).

Inhalation

No data are available for the chemical. Based on the read across information from acrylamide, the chemical is expected to have low acute inhalation toxicity.

In an acute inhalation toxicity study similar to OECD TG 433, male rats (strain not specified; 6/dose) were exposed to an aqueous test solution containing 50% acrylamide, as an aerosol (99% of particles under 6 microns), by nose only inhalation for 1 hour at 12.1 mg/L. No test substance related mortality or sub-lethal effects were reported (REACH n.d.-a).

In a GLP compliant acute inhalation toxicity study similar to OECD TG 433, male Fischer (F344) rats (10/dose) were exposed to acrylamide, as a vapour (mass median aerodynamic diameter not reported) by nose only inhalation for 6 hours at 16 mg/m^3. No test substance related mortality or sub-lethal effects were reported (REACH n.d.-a).

Corrosion/Irritation

Skin irritation

Based on an in vitro study, the chemical is not considered to be a skin irritant.

The chemical is considered to be not irritating to skin in a GLP compliant in vitro reconstructed human epidermis (RhE) (OECD TG 439) using the EpiDerm™ model. The mean tissue viability was 67.1% (REACH n.d.-a).

Eye irritation

Based on an in vitro study, the chemical is not considered to cause eye irritation.

The chemical does not warrant classification for eye irritation according to the UN GHS criteria, based on data from the in vitro bovine corneal opacity and permeability (BCOP) test (OECD TG 437). The chemical was tested as a 20% suspension in 0.9% sodium chloride solution w/v. The calculated in vitro irritancy score (IVIS) was -3.265. Based on the prediction model criteria, chemicals with IVIS values of >55 are considered to induce serious eye damage (REACH n.d.-a).

Sensitisation

Skin sensitisation

Based on the available data the chemical is not considered to be a potent skin sensitiser. A negative result was reported for the chemical when tested up to 50% in a local lymph node assay (LLNA). However, the chemical contains an alert for sensitisation and the structurally similar chemical, acrylamide, produced positive reaction in animal studies.

In vivo

In a GLP compliant LLNA conducted in accordance with OECD TG 442B, female CBA:JN mice (5/dose) received topical applications of the chemical (at 10, 25 or 50% w/w) in dimethylformamide vehicle. The chemical did not significantly increase any of the measured stimulation indices ((BrdU labelling, ear weight, and difference of ear thickness (TD 3 and TD

6)) at any concentration tested. The chemical was considered to have no skin sensitising properties in this assay (REACH n.d.-a).

The structurally similar chemical, acrylamide, is classified as hazardous as a Category 1 skin sensitiser with hazard statement “May cause an allergic skin reaction” in the HCIS (SWA n.d.). Positive results in up to 85% of test animals were reported following two maximisation tests carried out in guinea pigs (NICNAS 2002).

In silico

The chemical has structural alerts for skin sensitisation identified from the:

- OECD Quantitative Structure-Activity Relationship (QSAR) Application Toolbox (OECD 2023)—Bifunctional alpha, beta-carbonyl containing compounds)
- expert rule based system, DEREK (Deductive Estimation of Risk from Existing Knowledge) Nexus (Lhasa Limited n.d.)—alpha,beta-unsaturated amide.

Repeat dose toxicity

The structurally similar chemical, acrylamide, is classified as hazardous as a Category 1 for specific organ toxicity with hazard statements “H372 (Causes damage to organs through prolonged or repeated exposure)” (SWA n.d.). The classification is based on neurotoxic effects observed at low doses (see **Neurotoxicity** section). Limited data are available for the chemical with no guideline repeated dose toxicity studies available. Neurotoxic effects were not observed in available studies that also investigated the reproductive toxicity of the chemical (see **Reproductive and developmental toxicity** and **Neurotoxicity** sections). However, these studies involved low doses or were of short duration. In addition, mixed results were observed for acrylamide in these studies. Based on the read across information from acrylamide, the chemical is expected to cause serious systemic health effects following repeated oral exposure, warranting classification under GHS (see **Hazard classifications relevant for worker health and safety** section).

Genotoxicity

Based on the available in vitro and in vivo studies, the chemical is considered to be genotoxic, warranting classification under GHS (see **Hazard classifications relevant for worker health and safety** section). Although the in vitro results were mostly negative, the classification is supported by consistently positive results from in vivo assays. The available data provide clear evidence that the substance reach the germ cells and interact with germ cell DNA.

In vitro

The following results were reported for the chemical in bacterial reverse mutation assays in *Salmonella typhimurium* (ECHA 2021):

- negative results with TA 98, 100, 1535, 1537, and 1538, with and without metabolic activation, at concentrations up to 5000 µg/plate
- negative results with TA 100 and 1535, without metabolic activation, at concentrations up to 10,000 µg/plate
- negative results with TA 97 and 98, with and without metabolic activation, at concentrations up to 10,000 µg/plate

- positive results with TA 100 and 1535, with metabolic activation, at concentrations up to 10,000 µg/plate.

Mixed results were reported for acrylamide in the following in vitro genotoxicity studies (REACH n.d.-b):

- negative results were reported in a bacterial reverse mutation assay (similar to OECD TG 471) in *S. typhimurium* TA 98, 100, 1535, and 1537, with and without metabolic activation, at concentrations up to 100 mg/plate
- positive results were reported in an in vitro mammalian chromosome aberration assay (similar to OECD TG 473) in Chinese hamster lung V79 cells without metabolic activation at concentrations up to 5 mM
- negative results were reported in a mammalian gene mutation assay (similar to OECD TG 476) in the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells, with and without metabolic activation, at concentrations up to 1500 µg/plate.

In vivo

In dominant lethal studies similar to OECD TG 478, male C3Hx101 mice (≥30/dose) were administered the chemical by intraperitoneal injection. Treatment groups included doses of 0 or 225 mg/kg bw, given once, before mating with untreated female C3Hx101 mice (between 1–50 days post-treatment) or untreated female SECxC57BL mice (between 1–9 days post treatment), and doses of 0 and 90 mg/kg bw/day, given for 5 days, before mating with untreated female C3Hx101 or SECxC57BL mice (between 1–5 days post treatment). Dominant lethal effects were observed in matings in all groups that were administered the chemical. An increased incidence of semi-sterile offspring, and heritable reciprocal translocations were also observed in this study (ECHA 2021).

In a mammalian erythrocyte micronucleus test similar to OECD TG 474, male B6C3F1 mice (5/dose) were treated with the chemical by intraperitoneal injection at 0, 25, 50, or 100 mg/kg bw/day for 2 days. The incidence of micronuclei in bone marrow polychromatic erythrocytes was increased in all of the treated groups, indicating clastogenicity (ECHA 2021).

In a comet assay, male CD-1 mice were treated with the chemical orally at doses of 0, 50, 100, or 190 mg/kg bw/day for 2 days. Increased DNA damage was observed in testicular cells from the animals administered 190 mg/kg bw/day (ECHA 2021).

In a continuous breeding reproductive assessment study, Swiss CD-1 mice were administered the chemical in drinking water at doses of 1.6, 4.7, or 9.3 mg/kg bw/day. Mice received the chemical for 7 days, were combined into mating pairs and received the chemical for a subsequent 98 days, and then separated and received the chemical for a further 6 weeks. Offspring delivered during the last dosing period were designated the F1 generation, and received the chemical at doses of 2.6, 9.6 and 16.3 mg/kg bw/day, until 74 days post-partum. F1 males were cohabited with untreated females, and embryo implantation and dominant lethal effects were evaluated on gestation day 16. Incidences of early resorption and post-implantation loss were seen in pregnant females after mating with males treated with 16.3 mg/kg bw/day of the chemical, indicating dominant lethal effects (Chapin et al., 1995; ECHA 2021).

In a study on sperm count and morphology, and testicular histopathology, male ddY mice (>3/dose) were treated with the chemical once, orally, at doses of 0, 50, 100, or 200 mg/kg bw. Testicular weight loss was observed 25 and 30 days post treatment in animals

administered 200 mg/kg bw. Sperm count was decreased, and abnormal sperm morphology was increased, in a dose dependent manner in response to the chemical (ECHA 2021).

In a sex linked recessive lethal (SLRL) test in *Drosophilia melanogaster* similar to OECD TG 477, the chemical was administered a dose of 0 or 600 ppm in water for 72 h. Positive evidence of sex linked lethal mutations was observed (ECHA 2021).

Similar in vivo effects are observed with acrylamide. Acrylamide has been shown to induce heritable genetic damage in in vivo germ cell studies, such as the dominant lethal assay, specific locus mutation and heritable translocation assays. Positive results have also been reported in in vivo mammalian tests, such as the comet assay and the micronucleus test (ECHA 2021; NICNAS 2002).

Carcinogenicity

No data are available for the chemical. In rat studies with the structurally similar chemical, acrylamide, there was clear evidence of an increase in tumour incidence in several organs in both sexes. The chemical contains the same structural alerts for carcinogenicity and has a similar genotoxicity profile. Therefore, based on the read across information from acrylamide, the chemical is considered to be carcinogenic, warranting classification under GHS (see **Hazard classifications relevant for worker health and safety** section).

The structurally similar chemical, acrylamide, is classified as hazardous as a Category 1B carcinogen with hazard statements “May cause cancer (H350)” in the HCIS (SWA n.d.). In a GLP compliant ~2 year carcinogenicity study conducted similar to EPA OPP 83-2, F344 rats (≥75 males/dose, ≥50 females/dose) received acrylamide via drinking water at doses of 0.1, 0.5, or 2 mg/kg bw/day (males), and 1 or 3 mg/kg bw/day (females) for ~2 years. Increased mortality and decreased body weight gain were observed in females receiving 1 and 3 mg/kg bw/day, and in males in the 2 mg/kg bw/day group. Sciatic nerve degeneration was observed in females receiving 3 mg/kg bw/day and in males receiving 2 mg/kg bw/day. Increased incidences of thyroid gland adenoma, with and without carcinoma, and tunica vaginalis mesothelioma, were observed in males receiving 2 mg/kg bw/day. Increased incidences of mammary gland fibroadenomas, with and without carcinoma, were observed in females receiving 1 and 3 mg/kg bw/day. Increased incidence of thyroid gland follicular cell adenomas, with and without carcinoma, were observed in females receiving 3 mg/kg bw/day (NICNAS 2002; REACH n.d.-a).

In a GLP compliant 2 year study similar to OECD TG 453, F 433 rats (60/sex/dose) were administered acrylamide in drinking water at 0, 0.01, 0.1, 0.5 or 2 mg/kg bw/day. Neoplastic effects included increased incidences of benign follicular cell adenoma of the thyroid (males, 2 mg/kg bw/day), malignant adenocarcinomas in the uterus (2 mg/kg bw/day), and malignant testicular mesothelioma (0.5 and 2 mg/kg bw/day). Benign papillomas in the oral cavity (females, 2 mg/kg bw/day) and focal hyperplasia of the hard palate (males, 2 mg/kg bw/day), were also observed (NICNAS 2002; REACH n.d.-a).

The available data are insufficient to support a consensus view on a clear biological mechanism of action for acrylamide-induced tumours and it may be that different mechanisms are acting concurrently or in different organs/tissues (NICNAS 2002).

In silico

The chemical has structural alerts for carcinogenicity identified from the:

- OECD Quantitative Structure-Activity Relationship (QSAR) Application Toolbox (OECD 2023)— Acrylamide Reactive Functional Groups (Oncologic) and alpha, beta-unsaturated carbonyls
- Expert rule based system, DEREK (Deductive Estimation of Risk from Existing Knowledge) Nexus (Lhasa Limited n.d.)—alpha, beta-unsaturated amide, nitro compound

The same alerts were identified for the structurally similar chemical, acrylamide.

Reproductive and development toxicity

Limited data are available for the chemical. In available studies, observed fertility effects, including effects in male reproductive organs and a reduction in live births, are similar to those observed with the structurally similar chemical, acrylamide. Overall data is sufficient to warrant classification for fertility. Significant effects on development were not observed in a single study.

In a study investigating effects on testes and neurotoxicity, male ddY mice were administered the chemical (or vehicle; 0.9% saline), orally, twice per week, for 8 weeks, at a concentration of 1.3 mmol/kg bw (200 mg/kg bw)(n=5). Observations included a decrease in testes weight (without a change in body weight) and lesions of the seminiferous tubules which included degeneration of spermatids and spermatocytes, reductions of spermatozoa, and the presence of multinucleate giant cells (Hashimoto et al. 1981).

In a continuous breeding reproductive assessment, Swiss CD-1 mice were administered the chemical in drinking water at doses of 1.6, 4.7 or 9.3 mg/kg bw/day, for approximately 21 weeks, as described in **Genotoxicity** section (Chapin et al. 1995; ECHA 2021)**.** At the conclusion of the administration period, these animals (designated as the F0 generation) were examined for indices of reproductive toxicity. In the 4.7 and 9.3 mg/kg bw/day dose groups, there was a reduction in live pups per litter, and in the 9.3 mg/kg bw/day dose group, a decrease in raw and adjusted live pup weights. As part of this study, animals from the F1 generation, once weaned, received the chemical in drinking water at doses of 2.6, 9.6 or 16.3 mg/kg bw/day, until 74 days post-partum, when they were mated with partners from the same treatment group, and examined for indices of reproductive toxicity. In the 16.3 mg/kg bw/day group, there was a decrease in live pups per litter, and in the 9.6 and 16.3 mg/kg bw/day dose groups, a decrease in dam weight, and raw and adjusted live pup weights. On necropsy, F1 male animals in the 9.6 and 16.3 mg/kg bw/day group had decreased corpus/caput epididymis weight, testis weights, and spermatid count. F1 male animals in the 16.3 mg/kg bw/day group also had decreased cauda epididymis weight. F1 female animals in the 16.3 mg/kg bw/day group had decreased raw and relative ovary weights. A crossover mating study was also performed in F0 animals. There was no impact on the number of live births in this study. F0 animals were also assessed for dominant lethal potential (see **Genotoxicity** section). A slight but significant increase in early resorption was noted in the high dose group.

In a developmental toxicity study, mated female Swiss CD-1 mice (≥25/dose) were administered the chemical, in water, by oral gavage from gestational day (GD) 6 to 17 at doses of 3, 10 or 30 mg/kg bw/day. The maternal and developmental no observed adverse effect levels (NOAELs) were reported to be 10 and 3 mg/kg bw/day, respectively. Exposure to the chemical did not result in any dose related clinical signs, including neurotoxicity. Observations of maternal toxicity included decreased maternal body weight (at GD 17; 10 and 30 mg/kg bw/day), decreased maternal weight gain (30 mg/kg bw/day), and decreased gravid uterine weight (30 mg/kg bw/day). Observations of maternal toxicity included increased percentage of live foetuses with anatomical variations per litter (extra ribs on first

lumbar vertebra; 10 and 30 mg/kg bw/day) and decreased foetal body weights per litter (30 mg/kg bw/day). The chemical was reported to not increase incidence of foetal malformations. There were no impacts on post-implantation loss or number of live foetuses per litter (George et al. 1998).

The structurally similar chemical, acrylamide, is classified as hazardous: Category 2 reproductive toxicant with hazard statements “Suspected of damaging fertility (H361f)" (SWA n.d.). The potential reproductive and developmental toxicity of acrylamide has been investigated in multiple studies in rats and mice. Toxic effects of acrylamide to reproduction included reduced sperm count, reduced incidence of fertilised ova, increased incidence of pre- and post-implantation loss, and reduced proportion of live pups per litter. These effects were concluded to not be secondary to general or other specific toxic effects (NICNAS 2002). Toxic effects of acrylamide to development included retardation in pup development, reduced proportion of live pups per litter, increased embryotoxicity, and increased in malformations. These effects were not able to be clearly excluded as consequences of maternal toxicity and the decreased numbers of live births may be related to dominant lethality effects (NICNAS 2002).

Neurotoxicity

Forelimb and hindlimb grip strength were assessed as part of a continuous breeding reproductive assessment, in which Swiss CD-1 mice were administered the chemical in drinking water at doses of 1.6, 4.7 or 9.3 mg/kg bw/day, for approximately 21 weeks, as described in the **Genotoxicity** section (Chapin et al. 1995; ECHA 2021). Grip strength was assessed in half the mice in each dose group at various time points. Grip strength was reported as being not decreased in F0 male or female animals, at any time point. Grip strength in the F1 generation (doses of 2.6, 9.6 and 16.3 mg/kg bw/day) was reported to be variably decreased, by up to 28%, without consistent dose or time dependency. No structural alterations were found in the nerves of the high dose animals. However, minimal effects on grip strength and no detectable neuropathology were observed for acrylamide in this study.

In a study investigating effects on the testes and neurotoxicity, as described in the **Reproductive and developmental toxicity** section, there was no evidence of neurotoxicity reported for the chemical. Reported signs of neurotoxicity for acrylamide in this study included weakness and ataxia of hindlimbs and a decrease in grip strength (Hashimoto et al. 1981).

The NOAEL and LOAEL for repeat dose oral administration of the structurally similar chemical acrylamide in rats was reported to be 0.2–5 and 1–20 mg/kg bw/day, respectively. Exposure duration varied between studies, ranging between 90 days and 2 years. The NOAEL and LOAEL in monkeys was reported to be 1 and 3–10 mg/kg bw/day, respectively. Exposure duration varied between studies, ranging from 6 weeks to 1 year. The LOAEL in mice, cats, and dogs, was reported to be 26–36, 1–15, and 6–7 mg/kg bw/day, respectively. In all studies, clinical signs of neurotoxicity (including, but not limited to, weakness, disturbed gait, inactivity, reduced motor performance, discoordination) were reported to be the critical effects. Histopathological observations included nerve cell degeneration, and peripheral nerve neuropathy. In some studies, degenerative effects extended to the white matter of the cervical and lumbar spine, and the trigeminal and dorsal root ganglia. Neurotoxic effects have also been observed in rabbits and mice following dermal exposure. The NOAEL and LOAEL for repeat dose dermal administration of acrylamide in rabbits was reported to be 5 and 50 mg/kg bw/day, respectively. No inhalation data are available but it is considered that inhalation exposure contributed to human poisonings (NICNAS 2002).

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