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

Aniline and its salts

Evaluation statement

26 June 2023

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AICIS evaluation statement

Subject of the evaluation

Aniline and its salts

Chemicals in this evaluation

Name	CAS registry number
Benzenamine	62-53-3
Benzenamine, hydrochloride	142-04-1
Benzenamine, sulfate (2:1)	542-16-5

Reason for the evaluation

Evaluation Selection Analysis indicated a potential human health risk.

Parameters of evaluation

The chemicals in this group are benzenamine (aniline) and its salts listed on the Australian Inventory of Industrial Chemicals (the Inventory). This evaluation is a human health risk assessment of all identified industrial uses of these chemicals.

Aniline was previously assessed under the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework, under the former National Industrial Chemicals Introduction and Assessment Scheme (NICNAS) and this evaluation will review new information on the weight of the evidence for its carcinogenicity. The previous assessment of aniline should be read in conjunction with this evaluation statement (NICNAS 2013).

These chemicals have been assessed as a group because they are structurally similar and have similar use patterns. The salts will reach a pH-dependent equilibrium with aniline in aqueous solutions such as body fluids.

The chemicals in this evaluation will be referred to as follows:

- CAS No 62-53-3 as aniline
- CAS No 142-04-1 as aniline hydrochloride
- CAS No 542-16-5 as aniline sulfate.

Summary of evaluation

Summary of introduction, use and end use

There is currently no specific information about the introduction, use and end use of these chemicals in Australia. Aniline may also be present in tattoo inks used in Australia.

Based on international information, these chemicals primarily have site-limited use as chemical intermediates including in the production of:

- polyurethane foams
- dyes and pigments
- rubber processing agents
- resins.

The chemicals may be present in low concentrations as a residual in consumer products such as marker pen inks.

Human health

Summary of health hazards

There is limited toxicological information for the hydrochloride salt and none for the sulfate salt of aniline. Available data for aniline were used to evaluate human health hazards for this group. The previous assessment of aniline should be read in conjunction with this evaluation statement (NICNAS 2013). This evaluation statement reviews new evidence on the carcinogenic potential of aniline and its mode of action based on toxicokinetics, repeated dose toxicity and genotoxicity data.

Based on read across and available data, critical health effects for risk characterisation include:

- systemic acute and chronic effects (acute and repeated dose toxicity from oral, dermal and inhalation exposure)
- carcinogenicity
- local effects (skin sensitisation).

The targets for toxicity are the spleen and red blood cells. Chemical-induced effects include:

- methaemoglobin formation (a dysfunctional form of haemoglobin that cannot transport oxygen) leading to cyanosis (blueish purple tissues due to reduced oxygen saturation in the blood), damaged erythrocytes (red blood cells) and haemolytic anaemia
- hyperplasia, fibrosis and tumours in the spleen.

There is sufficient evidence of carcinogenicity in experimental animals based on the increased incidence of tumours in two independent studies. Effects were largely seen in males, although an increased incidence of fibrosarcoma or sarcoma of the spleen or multiple organs of the body cavity (combined) was observed in female rats. Hyperplasia in the spleen (likely precursor lesions) has been observed in both males and females in these studies. Hyperplasia of the spleen has also been observed in a different strain of rat in sub-chronic repeated dose toxicity studies. The chemicals are structurally related to the known human carcinogen *o*-toluidine (CAS No. 95-53-4). The chemicals exhibit similar patterns of toxicokinetic and toxicological behaviour (NICNAS 2014).

While the mode of action for carcinogenicity is uncertain, there is strong evidence that aniline exhibits the key characteristics of carcinogens in experimental systems. A suggested mode of action relating to methaemoglobinaemia is considered relevant to humans. A genotoxic mode of action via the formation of reactive oxygen species (ROS), reactive nitrogen species (RNS) or nitrenium ions cannot be ruled out.

Positive results were obtained in several in vitro and in vivo genotoxicity studies including those investigating DNA damage, chromosomal aberrations, micronucleus formation and sister chromatid exchange (SCE). Although not directly demonstrated at every step, there is evidence for formation of aniline–DNA adducts. The International Agency for Research on Cancer (IARC) now considers aniline and aniline hydrochloride to both be probably carcinogenic in humans (Group 2A) based on:

- sufficient evidence of carcinogenicity in experimental animals
- strong mechanistic evidence that aniline belongs to a class of aromatic amines for which several members have been classified as carcinogenic to humans.

The weight of evidence supports an amendment of the carcinogenicity classification for aniline and its salts (see **Hazard classifications relevant for worker health and safety**).

Repeated exposure to the chemicals is expected to cause toxic effects on red blood cells and the haematopoietic system, with corresponding effects on the spleen, bone marrow, liver and kidney. A lowest observed adverse effect level (LOAEL) of 7 mg/kg bw/day, from a combined chronic/carcinogenicity study in rats was determined for systemic effects (non-neoplastic lesions). The LOAEL was based on haematological effects, haemosiderosis and splenic haematopoiesis at the lowest dose administered. Adverse health effects following acute and repeated exposure have been observed in humans. The most prominent symptoms of acute exposure to the chemical were:

- cyanosis
- lacrimation
- tremors
- tachypnoea
- weakness
- disorientation
- dizziness
- impaired gait
- lethargy
- drowsiness
- convulsions
- loss of consciousness
- coma.

Limited data are available for the salts of aniline. Oral median lethal dose concentration values (LD50) of 840 mg aniline hydrochloride/kg bw in rats and 841 mg aniline hydrochloride/kg bw in mice (no study details) have been identified (CCOHS 2022). Humans are reported to be more sensitive to acute effects of these chemicals compared to rats, with the formation of methaemoglobin occurring at much lower doses in humans than rats (NICNAS 2013). Based on acute toxicity information, local effects and reproductive toxicity previously assessed for aniline (NICNAS 2013), the chemicals are expected to:

- have high acute oral, dermal and inhalation toxicity based on observations in humans
- cause serious eye damage
- be skin sensitisers
- have no specific reproductive or developmental toxicity.

More information used to support conclusions for acute toxicity, local effects and reproductive toxicity is available in the IMAP report (NICNAS 2013).

Hazard classifications relevant for worker health and safety

These chemicals satisfy the criteria for classification according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UNECE 2017) for hazard classes relevant for worker health and safety as follows. This evaluation does not consider classification of physical hazards and environmental hazards. These recommended classifications are based on read across principles (see **Supporting Information – Grouping Rationale** section). If empirical data become available for a specific chemical, this data may be used to amend the default classification for that chemical.

Health hazards	Hazard category	Hazard statement
Carcinogenicity	Carc. 1B	H350: May cause cancer
Genotoxicity	Muta. 2	H341: Suspected of causing genetic defects
Acute toxicity	Acute Tox. 3	H331: Toxic if inhaled
Acute toxicity	Acute Tox. 3	H311: Toxic in contact with skin
Acute toxicity	Acute Tox. 3	H301: Toxic if swallowed
Specific Target Organ Toxicity (repeated exposure)	STOT Rep. Exp. 1	H372: Causes damage to organs through prolonged or repeated exposure
Serious eye damage	Eye Damage 1	H318: Causes serious eye damage

Summary of health risk

Public

Based on the available use information, significant public exposure is not expected.
The public could be exposed to the chemicals as an impurity in products such as marker pens. However, the limited data available indicate that impurity levels are low. Aniline is currently listed on Schedule 6 of the SUSMP except in preparations containing 1% or less. At concentrations greater than 1%, several warning statements, first aid instructions and safety directions relating to the chemical apply. The current controls are considered adequate to minimise the risk to public health posed by potential domestic products containing the chemical as an impurity.

The public could also be exposed to aniline as an impurity or azo dye breakdown product in tattoo inks. The risk to the public from this route of exposure will be considered in any subsequent evaluation of the relevant dye and pigment chemicals.

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 these chemicals at lower concentrations could also occur while using formulated products containing these chemicals. The level and route of exposure will vary depending on the method of application and work practices employed. Good hygiene practices to minimise incidental oral exposure are expected to be in place.

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

Inhalation exposure is expected to be greater for aniline (volatile liquid) than for the salts (solids). Therefore, a change in the exposure standard for aniline to include the salts is not considered necessary to manage the risk.

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.

The hydrochloride and sulfate salt should be included as individual chemicals. If the generic entry for salts of aniline is retained, the classification in the HCIS for this entry should also be updated.

Information relating to safe introduction and use

The information in this evaluation 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 exposure to these chemicals include, but are not limited to:

- using closed systems or isolating operations
- 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 these chemicals.

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

These control measures may need to be supplemented with:

- conducting health monitoring for any worker who is at significant risk of exposure to these chemicals if valid techniques are available to monitor the effect on the worker's health
- conducting air monitoring to ensure control measures in place continue to work effectively.

Personal protective equipment should not solely be relied upon to control risk. This equipment 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
- label containers of hazardous chemicals.

Your Work Health and Safety regulator should be contacted for information on Work Health and Safety laws and relevant Codes of Practice in your jurisdiction.

Conclusions

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

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

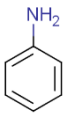
Note: Obligations to report additional information about hazards under *section 100* of the *Industrial Chemicals Act 2019* apply.

Supporting information

Grouping rationale

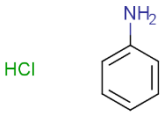
This group of chemicals consists of aniline and two of its salts. Although toxicokinetic data is limited to aniline, the toxicity data for aniline is relevant to the salts because they will reach a pH-dependent equilibrium with each other when dissolved in aqueous solutions such as bodily fluids. The chemicals are already classified for several endpoints based on data available for aniline.

Chemical identity

Chemical name	Benzenamine
CAS No.	62-53-3
Synonyms	aniline aminobenzene phenylamine aminophen benzidam
Molecular formula	C6H7N
Molecular weight (g/mol)	93.13
SMILES	NC=1C=CC=CC1
Chemical description	-
Structural formula	

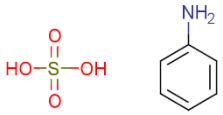
Chemical name	Benzenamine, hydrochloride
CAS No.	142-04-1
Synonyms	aniline hydrochloride
Molecular formula	C6H7N.ClH
Molecular weight (g/mol)	129.59
SMILES	Cl.NC=1C=CC=CC1
Chemical description	-

Structural formula:



Chemical name	Benzenamine, sulfate (2:1)
CAS No.	542-16-5
Synonyms	aniline sulfate dianilinium sulfate
Molecular formula	(C6H7N)2.H2O4S
Molecular weight (g/mol)	284.33
SMILES	O=S(=O)(O)O.NC=1C=CC=CC1
Chemical description	-

Structural formula:



Relevant physical and chemical properties

Aniline is a yellowish to brownish oily liquid with a musty, fishy odour detectable at 1 ppm. Aniline hydrochloride is a white to greenish coloured crystalline solid. Aniline sulfate is a white odourless powder. The respective melting points for aniline and aniline hydrochloride are -6ºC and 198ºC; the respective boiling points are 184.1ºC and 245ºC. The vapour pressure of aniline is 40 Pa at 20ºC. The water solubility for aniline and the hydrochloride are 36 g/L at 25ºC and 1070 g/L at 20ºC respectively. The pKa of the aniline conjugate acid is 4.6 at 25ºC. The log Kow is 0.9 for aniline and the same value is predicted for the hydrochloride salt (IARC 2021). No further information was available for aniline sulfate.

Introduction and use

Australia

Aniline may be present in tattoo inks to be used in Australia (NICNAS 2018).

International

Aniline primarily has site-limited use as an intermediate in the synthesis of many compounds (IARC 2021; NICNAS 2013), including:

- isocyanates (more than 90% of the volume is used in the production of 4,4-methylene diphenyl diisocyanate (MDI) which is used to produce polyurethane foam)
- dyes and pigments
- rubber processing agents
- resins.

There is limited available information on uses of the salts. The chemicals are designated as active on the Toxic Substances Control Act (TSCA) inventory, indicating that they are in commerce in the United States of America (US). Data available (Chemwatch) indicate that the chemicals are predominantly used as intermediates. Although application in dyeing and printing have been identified, this use is likely to refer to chemicals synthesised from the chemicals in this group. Aniline hydrochloride is used in the manufacture of textile dye, aniline black.

Aniline is reported to have potential domestic use in:

- paints
- lacquers and varnishes
- adhesives and binding agents
- colouring agents.

However, based on the weight of evidence from consumer product databases and international assessments (De Lima Associates; IARC 2021; NICNAS 2013), direct domestic use is not likely, with uses likely to be for chemicals synthesised from the chemicals.

Residual amounts of these chemicals may be present in consumer products. Aniline has been detected in certain marker pens in Denmark at concentrations of ≤0.02%. However, in a survey conducted by Health Canada, aniline was found at levels below the limit of quantification in inks in the pens and markers that were tested (IARC 2021). No consumer uses were identified for the chemicals in this group (REACH n.d.).

Existing Australian regulatory controls

AICIS

No specific controls are currently available for these chemicals.

Public

Aniline is listed in the *Poisons Standard – The Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP) under Schedule 6 (TGA 2022). The Schedule 6 entry states:

'ANILINE (excluding its salts and derivatives) except in preparations containing 1 per cent or less of aniline'.

Schedule 6 chemicals are labelled with 'Poison'. These are substances with a moderate potential for causing harm, the extent which can be reduced by using distinctive packaging with strong warnings and safety directions on the label.

Workers

The chemicals are currently listed on the HCIS (SWA) with the following hazard categories and statements for human health:

Health hazards	Hazard category	Hazard statement
Carcinogenicity	Carc. 2	H351: Suspected of causing cancer
Genotoxicity	Muta. 2	H341: Suspected of causing genetic defects
Acute toxicity	Acute Tox. 3	H331: Toxic if inhaled
Acute toxicity	Acute Tox. 3	H311: Toxic in contact with skin
Acute toxicity	Acute Tox. 3	H301: Toxic if swallowed
Repeated dose	STOT Rep. Exp. 1	H372: Causes damage to organs through prolonged or repeated exposure
Corrosion/irritation	Eye Damage 1	H318: Causes serious eye damage

The hydrochloride and sulfate salts are not listed by their CAS numbers (CAS No. 142-04-1 and CAS No. 542-16-5) but instead are covered by a generic entry for ‘salts of aniline’.

Aniline is listed on the HCIS (SWA) with the following exposure standard:

- Time Weighted Average (TWA): 2 ppm (7.6 mg/m^3)

In 2019 Safe Work Australia (SWA) reviewed and recommended the following new workplace exposure standards for aniline and homologues (SWA 2019):

- TWA: 0.5 ppm (1.94 mg/m^3)

At the time of publication of this evaluation statement, these values are under consideration.

International regulatory status

Exposure standards

The following exposure standards were identified for aniline (Chemwatch n.d.):
TWA: 1.9–19 mg/m^3 (0.5–5 ppm) — Canada, Denmark, France, Ireland, Japan, Norway, Singapore, Spain, Sweden, Switzerland, United Kingdom, and the US.

Short term exposure limit (STEL): 3.8–20 mg/m^3 (1–5 ppm) — Canada, South Africa, Sweden, Switzerland, and the USA.

Canada

The chemicals are listed on the Canadian Cosmetic Ingredients Hotlist where use in cosmetics is not permitted (Government of Canada 2022).

The Government of Canada (2011) concluded that, based on estimates of exposure to the general population in Canada, the chemical aniline does not pose a danger to human health.

European Union

The chemicals are listed in Regulation (European Commission (EC)) 1223/2009 on cosmetic products, Annex II – List of substances prohibited in cosmetic products (EC).

Aniline is listed in the Regulation (EC) 2020/2081 on substances in tattoo inks or permanent make-up, Appendix 13 – List of substances with specific concentration limits. The maximum concentration of aniline allowed in tattoo inks and permanent make-up is 0.0005% (EC 2020).

New Zealand

The chemical is listed in the *New Zealand Cosmetic Products Group Standard* under *Schedule 4, Table 1 - Components Cosmetic Products 'Must Not Contain'* (EPA NZ 2019).

Asia

These chemicals are listed in the Association of Southeast Asian Nations (ASEAN) Cosmetic Directive Annex II Part 1: List of substances which must not form part of the composition of cosmetic products (HSA 2022).

Health hazard information

There is limited toxicological information for the hydrochloride salt and none for the sulfate salt. Available data for aniline were used to evaluate the health effects for this group. The previous assessment of aniline should be read in conjunction with this evaluation statement (NICNAS 2013). This evaluation reviews new data available for aniline and its hydrochloride salt relating to carcinogenicity and mode of action based on the outcomes from toxicokinetic, mechanistic, repeated dose toxicity and genotoxicity studies.

More information used to draw conclusions for acute toxicity, local effects and reproductive toxicity is available in the IMAP report (NICNAS 2013).

Toxicokinetics

Aniline is reported to be well absorbed following oral, dermal and inhalation exposure in animals. Following oral administration, the chemical was absorbed up to 89–96%, 72%, 80%, and 56% in rats, mice, sheep and pigs, respectively (NICNAS 2013). In humans, aniline is readily absorbed by the dermal, oral and inhalation routes (IARC 2021). A pulmonary retention of nearly 90% was reported in humans. A dermal absorption of up to 38% is estimated for human skin. Although details are not available, a biological half-life of about 3.5 hours has been reported for the chemical in humans. The rate of absorption of the chemical through human skin is reported to be approximately 1000 times lower in vapour form than in topically applied liquid form (NICNAS 2013).

Following a single oral administration of radiolabelled aniline in rats, peak plasma radioactivity was observed at 0.5, 1 and 2 hours at 10, 30, and 100 mg/kg bw, respectively. The radioactivity decreased to less than 2% of the peak concentration for all doses 24 hours after exposure. The distribution of the radioactivity was highest in the kidney, followed by

liver, plasma, lung, heart, spleen and brain for all doses. Less than 0.1% of the administered radioactivity remained in these tissues for all doses up to 48 hours after exposure. In another study in rats treated with the chemical at 100 mg/kg bw/day orally for one day, the highest radioactivity was present in erythrocytes followed by plasma, spleen, kidney, liver, lung, heart, brain, and adipose tissue. A greater accumulation of radioactivity was observed in the spleen following repeated administration of the chemical at the same dose for 10 days (NICNAS 2013).

The metabolic elimination pathway is qualitatively similar in humans and animals. Multiple studies of aniline metabolism in experimental animals, including rat, mouse, rabbit, guinea pig, gerbil, hamster, cat, dog, pig, and sheep, are consistent with the evidence in humans (IARC 2021). The chemical is mostly excreted as metabolites in urine. The chemical is metabolised primarily in the liver by three metabolic pathways: *N*-acetylation, aromatic ring hydroxylation and *N*-hydroxylation. While the *N*-acetylation of the chemical is catalysed by hepatic *N*-acetyltransferase, the cytochrome P-450 enzyme system (aniline hydroxylase) is responsible for the aromatic hydroxylation. It is believed that the *N*-acetylation pathway is an important route by which the chemical is detoxified, while *N*-hydroxylation is the principal route by which the chemical produces toxic effects through the formation of phenylhydroxylamine metabolite.

In humans, the predominant urinary metabolite is *N*-acetyl-*para*-aminophenol. Other metabolites include acetananilide and conjugates of *N*-acetyl-*para*-aminophenol. In exposed humans, aniline forms haemoglobin adducts. The formation of these adducts with haemoglobin is attributed to the formation of the metabolite phenylhydroxylamine via *N*-hydroxylation of aniline. *N*-hydroxylation of aniline to form phenylhydroxylamine has been demonstrated in many species (IARC 2021; NICNAS 2013).

Repeated Dose Toxicity

Oral

Repeated dose toxicity studies of aniline have been mainly carried out in rats. Systemic toxicity in these studies was seen at very low doses, mainly in the blood cells and the spleen. Based on these results, the chemicals in this group are expected to cause significant systemic toxicity following repeated oral exposure.

Repeated oral dose studies in rats show chemical-induced toxic effects on the red blood cells and the haematopoietic system. There are also corresponding effects on the spleen, bone marrow, liver and kidney. Clinical signs included cyanosis, reduced food consumption, reduced body weight gain, and premature deaths. Damaged red blood cells, haemolytic anaemia, methaemoglobinaemia, and higher levels of Heinz bodies were also observed. Since damaged red blood cells are scavenged predominantly in the red pulp of rat spleen, increased accumulation of haemosiderin was also noted, along with spleen congestion, dark colouration and increased spleen weight. Increased accumulation of haemosiderin was also occasionally noted in the liver and kidney. There was increased erythropoeitic activity in the bone marrow and spleen as a compensatory reaction to the haemolytic effect of the repeated administration of the chemical. Splenitis, spleen hyperplasia and fibrosis have also been reported (NICNAS 2013).

A lowest observed adverse effect level (LOAEL) of 7 mg/kg bw/day, from a combined chronic/carcinogenicity study in rats that complied mostly with the OECD Test Guideline (TG 407) (see **Carcinogenicity** section), was determined for systemic effects (formation of non-neoplastic lesions). The LOAEL was based on: haematological effects (reticulocytosis; decrease of red blood cell counts; haemoglobin and haematocrit; an increase in mean

corpuscular volume (MCV); haemosiderosis and splenic haematopoiesis at the lowest administered dose of 7 mg/kg bw/day. A no observed adverse effect level (NOAEL) could not be established. In this study, aniline hydrochloride was administered to Fischer 344 (F344) rats at dietary levels of 0, 200, 600, and 2000 ppm for two years. These doses were equivalent (as aniline hydrochloride) to 10, 30 or 100 mg/kg bw/day (equivalent to aniline doses of 7, 22 and 72 mg/kg bw/day). Although other repeated dose toxicity studies have been reported on the chemical, these were not considered here as these studies did not comply sufficiently with the current OECD test guidelines. In addition, an NOAEL could not be derived in these studies (NICNAS 2013).

Dermal

Although no data are available, aniline is reported to be well absorbed through all exposure routes. A dermal absorption of up to 38% has been estimated for human skin (see **Toxicokinetics** section). Relevant toxic effects following dermal exposure of aniline are expected to be similar to those observed following oral administration of the chemical (NICNAS 2013).

Inhalation

Due to lack of properly conducted studies in animals, there are no valid data on effects of repeated inhalation exposure to aniline. As reported above, the chemical is well absorbed through all exposure routes. A pulmonary retention of nearly 90% has been reported for humans (see **Toxicokinetics** section). Following repeated inhalation exposure in rats, reported effects on the haematopoietic system and spleen would be similar to those reported following repeated oral exposures. However, these effects are expected to occur to a lesser degree depending on the dose administered (NICNAS 2013).

Aniline was determined to have a lowest observed adverse effect concentration (LOAEC) of ≥17 ppm (approx. 66 mg/m³) based on a 14 day inhalation exposure study in rats (exposure on 5 days/week, 6 hours/day). The LOAEC was based on dose dependent effects seen in the spleen (reticuloendothelial system hypertrophy) and signs of dysregulated haemoglobin metabolism (haemosiderosis and increased haematopoiesis). In another inhalation study in rats, aniline was determined to have a LOAEC of 5 ppm (19 mg/m³) (26 weeks of exposure for 5 days/week, 6 hours/day). As no histopathology was performed in this study, the LOAEC was based on the development of cyanosis (NICNAS 2013).

In a more recent study, Wistar rats were exposed (nose-only) to aniline at 9.2, 32.4, 96.5, and 274.9 mg/m³ for five days/week, six hours/day, for two weeks followed by a two-week post exposure observation period. Methaemoglobin formation and associated adverse effects in red blood cells (sequestration of erythrocytes, iron accumulation, and lipid peroxidation) were the main signs of systemic toxicity. Cyanosis was also seen on initial exposure at concentrations ≥96.5 mg/m³ but did not persist through the 14 day exposure period. Anaemia, Heinz bodies, decreased haemoglobin and haematocrit, reticulocytosis and effects on the spleen (splenomegaly, hemosiderin accumulation, and haematopoiesis) were seen in the two highest doses. A borderline increase in splenic extramedullary haematopoiesis was observed in the 32.4 mg/m^3 dose group. But this effect was determined by the study authors to be a homeostatic response rather than an adverse effect. No significant effects were observed in the lowest dose group. Based on these observations, a no observed adverse effect concentration (NOAEC) of 32.4 mg/m³ was determined for the study (NICNAS 2013; REACH).

Observation in humans

The chemicals are classified as hazardous with the hazard category ‘Specific target organ toxicity (repeated exposure) – Category 1’ and the hazard statement ‘Causes damage to organs through prolonged or repeated exposure (H372)' in the HCIS (SWA). The available data for aniline, particularly observations in humans, support this classification (NICNAS 2013).

Although limited information is available on repeated exposure of humans to aniline, it is expected that human health effects would be similar, in this case, to the human response following acute exposure to the chemical. These effects include:

- cyanosis
- lacrimation
- tremors
- tachypnoea
- weakness
- disorientation
- dizziness
- impaired gait
- lethargy
- drowsiness
- convulsions
- coma.

In a clinical study, the chemical was administered orally to 20 patients at doses of 5, 15, or 25 mg for three successive days. After initial administration at these doses, higher doses of the chemical were also given to some patients on successive days at the rate of 35 or 45 mg (n=5), 55 mg (n=2), and 65 mg (n=1). At doses of 25 mg or higher, a significantly higher production of methaemoglobin formation was observed, compared with the methaemoglobin level at the 5 mg dose. Following administration of 65 mg of the chemical, a maximum methaemoglobin level of 16.1% was reached 2 hours after administration. It was also concluded that, at dosages calculated to be from 0.4 mg/kg bw/day in this study, evidence of haemotoxicity (besides methaemoglobin formation) was present.

Methaemoglobinaemia (53%) was reported in one individual sitting in a car seat contaminated with the chemical, resulting in symptoms of cyanosis, dyspnoea, fatigue, and dizziness. Following contact with shoes that had been dyed with a preparation containing the chemical (10–25%), naphtha (50–100%) and ethyl alcohol (25–50%), 3 individuals were reported to experience grave methaemoglobinaemia as the main symptom, requiring urgent medical assistance (NICNAS 2013).

Genotoxicity

The chemicals are classified as hazardous with the hazard category ‘Germ cell mutagenicity’, Category 2 ’and hazard statement ‘Suspected of causing genetic defects (H341)’ in the HCIS (SWA). The positive findings seen in several in vitro and in vivo tests are sufficient to support this classification (IARC 2021; NICNAS 2013).

In vitro

Aniline and aniline hydrochloride were reported to be generally negative in bacterial mutation assays. Aniline induced intrachromosomal recombination in *Saccharomyces cerevisiae*

RS112 strain. The chemicals did not induce DNA repair (unscheduled DNA synthesis, UDS) in primary human or rat hepatocytes. However, positive results were obtained in the L5178+/- mouse lymphoma gene mutation assay, the Chinese hamster V79 *Hprt assay* and in comet assays investigating DNA strand breaks (non-human mammal and human cells). A significant induction of a biomarker for DNA damage was observed in the human urothelial cell line 1T1 when exposed at 7.5 mM. The response was reported to be related to chemical concentration.

The chemicals induced chromosomal aberrations (non-human mammalian cells), micronucleus formation (non-human mammalian cells) and sister chromatid exchange (SCE) (non-human mammalian and human cells).

Two metabolites of the chemical, 2-aminophenol and *N*-phenylhydroxylamine, also increased the frequency of SCE in human fibroblasts. Conflicting results were obtained in gene mutation assays.

In vivo

Following intraperitoneal (i.p.) administration of aniline, DNA binding was reported in the kidneys, liver and spleens of rats, but not mice. Binding index values were low (<15). Following i.p. administration of aniline, induction of DNA strand breakage was noticed in the various tissues of both mice and rats. DNA damage was also observed in a comet assay in mice. The tissues in which damage was observed changed with different sampling times. The spleen was typically not investigated in the majority of studies. In one study where the spleen was investigated DNA damage was not observed. DNA damage was not observed in the bladder tissues of F344 rats given feed containing 0.6% aniline hydrochloride. Gene mutation was not observed in the liver, spleen, bone marrow of Big Blue F344 rats following oral exposure to aniline for 28 days. Positive results were observed in a host-mediated mutation assay in *S. typhimurium* TA98 strain.

A negative result was obtained for the chemical in an in vivo chromosomal aberration assay with mouse bone marrow cells. A slight but significant increase in chromosomal aberrations was observed in male PVG rats at sampling time of 18 hours but not 30 hours.

Following exposure to aniline or aniline hydrochloride, an increase in the frequency of micronucleus formation was observed in several studies. Effects have been reported in both the peripheral blood and bone marrow in rats and mice. Although results were not consistent across studies and appear to be affected by the dosing regime, dosage and sampling time.

Aniline induced SCE in bone marrow cells in mice in 2 in vivo studies.

A dominant lethal assay for germ cell mutagenicity in rats was considered to be inconclusive, due to the weakness of the effects. The chemical also had no effect on the frequency of sperm head abnormalities in male mice. Because of relatively poor sensitivities in the last two test systems, the unclear results are of limited predictive value.

Carcinogenicity

The chemicals are classified as hazardous with the hazard category ‘Carcinogenicity – Category 2’ and hazard statement ‘H351 - Suspected of causing cancer’ in the HCIS (SWA). The weight of evidence from animal studies (neoplasms observed in two independent studies in one species of rat) and mechanistic investigations support amending this classification to the hazard category ‘Carcinogenicity – Category 1B’ and hazard statement ‘H350 – May cause cancer’.

Standard animal bioassays

Several carcinogenicity studies are available. A large number of them have limitations. The principal evidence of carcinogenicity is derived from two independent, well conducted studies in F344 rats.

In a carcinogenicity study, aniline hydrochloride was administered to F344 rats at dietary levels of 0, 200, 600 or 2000 ppm for 2 years. These doses were equivalent to 10, 30, 100 mg/kg bw/day (equivalent to aniline doses of 7, 22, 72 mg/kg bw/day). Male rats from the 2000 ppm group showed an increased incidence of primary splenic sarcomas. Males and to a lesser degree, females in this dose group also showed stromal hyperplasia and fibrosis of the splenic red pulp, which may represent precursor lesions of sarcoma. The majority of tumours seen in the spleen of high dose males were fibrosarcomas and/or haemangiosarcomas. Splenic fibrosarcomas were also described as invasive with widespread extension in multiple organs of the pleural and abdominal cavities in males and females in the highest dose groups. The incidence of mesothelioma of the tunica vaginalis of the testes was significantly higher in treated males than in controls, with a significant positive trend.

In another dietary carcinogenicity study, aniline hydrochloride was administered at 0, 3000 (0.3%) or 6000 ppm (0.6%) to F344 rats for 103 weeks. A statistically significant dose related trend in the incidence of haemangiosarcomas and sarcomas or fibrosarcomas in the spleen was noted in male rats. Male rats also had statistically significant increased incidences of sarcomas or fibrosarcomas and haemangiosarcoma in the spleen or in multiple organs in the body cavity (combined). In the females, the tumour incidence was markedly lower but an increased incidence of fibrosarcoma or sarcoma of the spleen or multiple organs of the body cavity (combined) was noted.

The effects in tumour incidence seen in the rat studies were not noted in a similar study in mice.

In a dietary carcinogenicity study in mice, the chemical (as aniline hydrochloride) was administered at 0, 6000 (0.6%) or 12000 ppm (1.2%) to B6C3F1 mice for 103 weeks. No statistically significant increase in any type of tumour in male or female mice relating to the feeding of the chemical was observed in this study (IARC 2021; NICNAS 2013).

Epidemiological evidence

Epidemiological data relating to cancers in humans following exposure to aniline was limited. Available data included:

- 4 cohort studies in aromatic amine dye and rubber chemical manufacturing plants
- 4 population-based case control studies
- several case reports and case series of bladder cancer following occupational exposure.

Overall, available data were not sufficient to determine causal association between aniline exposure and bladder cancer.

All cohort studies evaluated bladder cancer outcomes, but only two out of the available four studies were considered partially adequate for this evaluation. Both studies were of good quality but had their limitations. For one of these studies, it was not possible to separate any aniline-specific effect from the effect of other co-exposures to chemicals such as *ortho*-toluidine (*o*-toluidine, CAS No. 95-53-4). While the second cohort study evaluated the

association between aniline exposure and bladder cancer while controlling for concurrent exposures, the small sample size and strong correlations between the exposures resulted in statistically unstable estimates of the effect for aniline. Additionally, confounding effects from co-exposures to other potentially carcinogenic chemicals such as o-toluidine and 2-mercaptobenzothiazole could not be ruled out.

Data from the case control studies was inadequate. These studies were primarily based on work histories derived from interviews with study participants (IARC 2021). Information on duration, intensity or cumulative exposures to aniline was not reported.

Among the case reports and case series, confounding effects from co-exposures to occupational bladder carcinogens and/or tobacco smoking could not be ruled out (IARC 2021; NICNAS 2013).

Mechanistic studies

The mode of action for carcinogenicity of aniline is uncertain. Splenic tumours are commonly observed in studies with monoaromatic amines. The splenic tumours may be a secondary response resulting from red blood cell toxicity, although a genotoxic mode of action cannot be ruled out. The abnormal/dysfunctional red blood cells (i.e., cells with increased levels of methaemoglobin) are transported to the spleen for recycling and destruction. This process results in the release of high concentrations of cytotoxic reactive metabolites in the spleen leading to local cell damage, epigenetic changes and tumours. Methaemoglobinaemia and the resulting haematoxicity can also promote and sustain carcinogenic events (NICNAS 2019). Systemic toxicity induced by aniline and the formation of splenic tumours is similar to other aromatic amines which include o-toluidine (CAS No. 95-53-4), which have been classified as carcinogenic to humans (IARC 2021; NICNAS 2014). Carcinogenic potential of this class of aromatic amines is indicated by the characteristic formation of common DNA-reactive moieties, genotoxicity and methaemoglobin induced cytotoxicity. The spleen, bladder and testes are the common target organs of carcinogenicity in animal bioassays for chronic toxicity for these chemicals.

Although the human relevance of the observed mesothelioma of the tunica vaginalis of the testes in F344 rats is uncertain, this tumour was also observed in studies with the structurally related chemical o-toluidine. The mode of action for mesothelioma induction may involve DNA damage to the tunica vaginalis mesothelium in addition to the contribution of hormonal imbalance (Maronpot 2008).

There is strong evidence that aniline exhibits the following key characteristics of carcinogens in experimental systems.

- **Aniline is metabolically activated to electrophiles**. In exposed humans, aniline forms haemoglobin adducts, which are commonly used as biomarkers of aniline exposure. No data on DNA adducts in humans were available. In experimental systems, aniline was shown to bind DNA at low levels in the liver, spleen, and kidney of rats treated with aniline. Although not directly demonstrated at every step, there is a plausible pathway for formation of aniline-DNA adducts that parallels an established paradigm for aromatic amines (NICNAS 2019), including 4-aminobiphenyl (*para*-phenylaniline), 2-naphthylamine, and *o*-toluidine (*ortho*-methylaniline, CAS No. 95-53-4), which have been classified as carcinogenic to humans (IARC Group 1) by IARC. This proposed bioactivation pathway begins with CYP-catalysed *N*-hydroxylation to phenylhydroxylamine; followed by *O*-acetylation; and then spontaneous heterolysis of the *N*-acetoxy metabolite to give a DNA-reactive electrophilic nitrenium ion.

In humans the formation of phenylhydroxylamine is shown by the detection of haemoglobin adducts. There is evidence showing *N*-hydroxylation of aniline to phenylhydroxylamine in:

- dogs after intravenous administration
- rats after i.p. administration
- liver microsomal preparations from rat, mouse, and rabbit
- the isolated perfused rat liver, when the perfusion fluid contained human erythrocytes, to trap the oxidised metabolites by binding to haemoglobin. Synthetic *N*-acetoxyaniline was shown to react with DNA to give a guanine-C8 adduct as well as guanine-*N*7 and *N*2, and adenine-C2, C8, *N*7, and *N*6 adducts. As noted above, low but significant levels of DNA binding have been observed in the kidney, liver and spleen in F344 rats in studies with aniline (IARC 2021).

- **Aniline is potentially genotoxic.** Positive results were obtained in several in vitro and in vivo genotoxicity studies including those investigating DNA damage, chromosomal aberrations, micronucleus formation and sister chromatid exchange (SCE) (see **Genotoxicity** section).

- **Aniline induces oxidative stress.** Highly cytotoxic ROS are formed as a by-product of the metabolic activation reactions of aromatic amines (e.g. *N*-hydroxylation, redox cycling) (NICNAS 2019). Aniline was shown to increase 8-hydroxy-2′-deoxyguanosine concentrations (a biomarker of oxidative DNA damage) and upregulate DNA base-excision repair proteins in rats (IARC 2021). In rodent studies in vivo and in vitro, aniline exposure variously increased cytotoxic ROS and RNS, depleted glutathione (a cellular antioxidant), and increased malondialdehyde and protein carbonyl contents (indicators of toxic lipid peroxidation) (IARC 2021).

- **Aniline alters cell proliferation, cell death, or nutrient supply.** Hyperplasia (enlargement) of the spleen was seen in experimental animals following sub-chronic and chronic exposure (see **Repeat dose toxicity** and **Carcinogenicity** sections). Following short-term exposure to aniline, increased cell proliferation was reported in rats indicated by increased levels of proliferating cell nuclear antigen and nuclear Ki67 protein (IARC 2021).

Weight of evidence

There is sufficient evidence of carcinogenicity in experimental animals based on the increased incidence of tumours in two independent studies in rats. Effects were largely seen in males, although an increased incidence of fibrosarcoma or sarcoma of the spleen or multiple organs of the body cavity (combined) was observed in female rats. Hyperplasia in the spleen (likely precursor lesions) have been observed in both males and females in these studies. Hyperplasia of the spleen was also observed in rats in sub-chronic and chronic repeated dose toxicity studies. These chemicals are structurally and toxicologically related to a class of aromatic amines which include known human carcinogens such as *o*-toluidine. These chemicals have similar characteristic signs of systemic toxicity which can promote and sustain carcinogenic events. While the mode of action for carcinogenicity is uncertain, there is strong evidence that aniline exhibits the key characteristics of carcinogens in experimental systems. A suggested mode of action relating to methaemoglobinaemia is considered relevant to humans. A genotoxic mode of action via the formation of ROS, RNS and nitrenium ions cannot be ruled out. Although not directly demonstrated at every step, there is evidence for formation of aniline-DNA adducts.

The weight of evidence supports amending this classification to the hazard category ‘Carcinogenicity – Category 1B’ and hazard statement ‘H350 – May cause cancer’.

The IARC’s recent assessment concluded that the chemical is probably carcinogenic to humans (Group 2A) (IARC 2021). The US Environmental Protection Agency (US EPA) has also previously concluded that the chemical is a probable human carcinogen (B2) (US EPA 1988).

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