Workplace Exposure Standard (WES) review

ANILINE AND HOMOLOGUES
(CAS NO: 62-53-3)

March 2020

New Zealand Government

WORKSAFE
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appendices

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1.0
Introduction

This WorkSafe New Zealand (WorkSafe) review considers whether the **WES** for aniline and homologues should be changed.

The WES review considers the potential for exposures to aniline and homologues in New Zealand, the health effects and risks, exposure standards from other jurisdictions around the world, and the practicability of measuring exposures.

The review includes a recommendation to retain the WorkSafe WES for aniline and homologues, which is currently set at a **WES-TWA** of 1**ppm** [4**mg/m³**] with ***skin*** and 6.7B notations, as published in the special guide *Workplace Exposure Standards and Biological Exposure Indices*, 11th Ed., November 2019 (WorkSafe, 2019), but to confine the standard to just aniline (not its homologues) and adopt a **WES-STEL** of 2ppm [8mg/m³].

Terms that are **bold** (first occurrence only) are further defined in the Glossary.
Synonyms: Benzenamine; Aminobenzene; Phenylbenzene; Phenylamine.

2.0 Chemical and physical properties

Aniline is a colourless, oily liquid at room temperature with a characteristic amine odour and burning taste (**SCOEL**, 2016; **NICNAS**, 2013; **ACGIH®**, 2001).

Aniline darkens to brown on exposure to light and air (NICNAS, 2013).

Aniline is reported to have an odour threshold at 1ppm (SCOEL, 2016; ACGIH®, 2001).

Chemical and physical properties aniline include:

Molecular weight	93.13g/mol
Formula	C_6H_7N
Specific gravity	1.0217 at 20°C
Melting point	-6°C
Boiling point	184.1°C
Vapour pressure	0.65hPa at 25°C
Relative vapour density [air = 1]	3.3
Flash point	Closed cup: 76°C
Auto-ignition	630°C
Log K_{ow}	0.9 at 20°C
Solubility	Water: 35g/L; soluble in benzene and most other organic solvents
Conversion factors	1mg/m³ = 0.258ppm 1ppm = 3.87mg/m³

SCOEL, 2016; **ECB**, 2004; ACGIH®, 2001

TABLE 1: Physicochemical properties of aniline

Health-related hazard classifications for aniline:

	HSNO CLASSIFICATION
Substance	Benzenamine [Aniline]
CAS No.	62-53-3
Classification	6.1C (All); 6.1C (O); 6.1C (D); 6.1C (I); 6.3A; 6.5B; 6.6A; 6.7B; 6.9A (All); 6.9A (O); 6.9A (D); 6.9A (I) 8.3A

TABLE 2:
HSNO health-related hazard classifications of benzenamine (**EPA**, 2019)

For a full listing of all HSNO health-related hazardous substances classification codes and their descriptions, see Appendix 2.

[All] Overall classification for that endpoint.

[O] Oral exposure route.

[D] Dermal exposure route.

[I] Inhalation exposure route.

3.0
Uses

Aniline is predominantly used as a chemical intermediate in various industries with some used in the manufacture of pharmaceuticals and plant protection products (SCOEL, 2016; ECB, 2004; ACGIH®, 2001).

The primary products from aniline are 4,4'-methylenedianiline [**MDA**], used to make polyurethane and similar products, and methylene diphenyl diisocyanate [**MDI**], while other products include: dyes; compounds used in the rubber industry; petroleum refining chemicals; phenolics; photographic chemicals; and, explosives (SCOEL, 2016; ECB, 2004; ACGIH®, 2001).

Occupational exposure to aniline can occur during production, storage, transportation and end-use.

Aniline can be released into the environment and contribute to occupational exposures as a result of degradation of plant protection products; degradation of rubber chemicals; thermal degradation of polyurethanes; coal carbonisation; and, from landfill leachate plumes (ECB, 2004).

Workers can be exposed to aniline aerosols and/or vapour via inhalation and eye or dermal contact (ACGIH®, 2001).

The number of workers exposed or potentially exposed to aniline in New Zealand workplaces is unknown.

Statistics New Zealand 2019 data indicate that 16,290 New Zealand workers were working in the areas of:

- other petroleum and coal product manufacturing
- basic organic chemical manufacturing
- basic polymer manufacturing
- pesticide manufacturing
- pharmaceuticals and medicinal product manufacturing
- polymer product and rubber product manufacturing (NZ.Stat, 2019).

4.0
Health effects

IN THIS SECTION:

4.1 Non-cancer

Humans

The SCOEL recommendation on aniline summarised the acute toxicity potential in humans:

> "Acute aniline poisoning in workers was frequent several decades ago. The effects are attributed to the formation of **MHb**. The victims suffered from cyanosis (which is why they were called "blue boys") and more severe symptoms at higher exposure (ECB 2004). Severe poisoning has been observed in combination with alcohol intake, which is reported to increase aniline toxicity (Henschler 1992).
>
> "According to earlier observations, 400–600mg/m^3 [103–155ppm] may be tolerated without much harm for up to one hour, whereas several hours of exposure to 100–250mg/m^3 [26–65ppm] produce slight symptoms. Concentrations of about 25000mg/m^3 [6,450ppm] or 0.35–1.43g/kg bodyweight are reported to be lethal. Workers may develop some tolerance to symptoms but the cyanosis may persist (Smyth 1931).
>
> "Generally, an increase in MHb above the normal background level in blood (about 1.1%) to 15% will be without signs and symptoms. However, as known from **CO**-induced oxygen deficiency for sensitive risk groups (persons with latent restricted coronary or arterial function), much lower MHb-levels may be tolerable (Bolt *et al* 1985). Clinical cyanosis but no hypoxic symptoms except for a possible slight euphoria will develop at about 15–20% MHb and more. Fatigue, anxiety, headache, weakness, dizziness, tachycardia, dyspnoea, and syncope will occur at 30–45% MHb. Higher concentrations will cause a decreased level of consciousness and finally coma, heart failure and death at more than 60–70% MHb (Henschler 1992; **HSE** 1997; **NRC** 2000)." (References cited in SCOEL, 2016).
>
> "The dose-response-relationship between oral aniline intake and MHb-formation was studied in 20 volunteers, which received a bolus dose of 5, 15 or 25mg/day on three consecutive days. Some volunteers were given higher doses on subsequent days. All volunteers were healthy with no evidence of glucose-6-phosphat [sic] dehydrogenase deficiency in screening tests (Jenkins *et al* 1972). The mean maximum increase in MHb-formation occurred in less than 4 hours. After intake of 5**mg** and 15mg the increase of MHb was not significant (1.2% or 1.8%, respectively). Significant increases were seen at 25mg (2.5% MHb) and more. Doses of 35, 45 and 55mg/person led to maximum MHb increases of 3.7% (n=5); 7.1% (n=5) and 5.2% (n=2). At the highest dose of 65mg, an MHb-level of 16% was reached in the only volunteer exposed to this dose. As these data are pivotal for the derivation of an **OEL**, they are also included as Figure 1 in the 'Recommendation' section. No Heinz bodies were detected at any dose. With respect to MHb-formation after aniline exposure, humans appear much more sensitive than rats which were orally exposed to aniline in the same study." (References cited in SCOEL, 2016).

The New Zealand EPA classifies aniline as a 6.1C – a substance that is acutely toxic. (EPA, 2019).

The **NIOSH** Skin Notation Profile for aniline noted:

> "Numerous instances of accidental and occupational exposures to aniline have been reported. Two cases of accidental spraying of the skin with aniline reported symptoms including discoloring of the skin, weakness, headache, sinus tachycardia, and methemoglobin (**MetHb**) levels of up to 70% [Phillips

> *et al*. 1990; Cummings *et al*. 1994]. Liao *et al*. [2002] reported dermal exposure to aniline in a worker. The worker was treated with methylene blue; however, methemoglobin recurred, followed by severe Heinz body hemolytic anemia. As is typical of accidental or occupational exposures, the doses of aniline that elicited these effects have not been quantified. Lee *et al*. [2013] reported a worker dermally exposed to 200cc of aniline that splattered on his face and upper body. The worker suffered from a burn around the exposed skin, and showed signs of cyanoderma on his entire body. The workers' MetHb level was 46.8%, and he was diagnosed with methemoglobinemia [Lee *et al*. 2013]. The National Research Council [NRC 2000] reported aniline is absorbed through the skin and is a methemoglobin-forming compound as seen in these case reports." (References cited in NIOSH, 2015).

The SCOEL recommendation on aniline summarised the irritation/corrosion potential in humans:

> "Although aniline has been used in a variety of industrial applications for many years, no data on local irritant effects of aniline on eyes, skin, or mucous membranes are available. Also, no such effects were described in clinical human studies." (References cited in SCOEL, 2016).

The New Zealand EPA classifies aniline as a 6.3A and 8.3A substance – a substance that is irritating to the skin and corrosive to ocular tissue, respectively (EPA, 2019).

The SCOEL recommendation on aniline summarised the sensitisation potential in humans:

> "Skin sensitisation was observed in a maximisation test with 25 healthy volunteers. For the induction, undiluted aniline was applied five times to irritated skin (pre-treatment with sodium lauryl sulphate, **SLS**). The challenge was carried out with the highest non-irritating concentration of aniline (10%), again on SLS-pretreated skin. Seven of 25 volunteers showed a positive sensitisation reaction, which were judged by the author as a mild reaction rate (ECB 2004; HSE 1997).
>
> "Positive reactions in patch tests have also been reported in monitoring surveys and in studies with patients suffering from eczematous dermatitis. Here, the positive reactions are often associated with a group allergy to other aromatic amines, which are substituted at the para-position (para-group compound cross reactivity) (ECB 2004).
>
> "Data regarding the potential for aniline to produce respiratory sensitisation are not available." (References cited in SCOEL, 2016).

The New Zealand EPA classifies aniline as a 6.5B substance – a substance that is a contact sensitiser (EPA, 2019).

The SCOEL recommendation on aniline summarised the repeated dose toxicity in humans:

> "In an occupational study of workers in a plant producing diphenylamine in which aniline was used as the raw material, aniline and hydrogen chloride were reported to be the only harmful chemicals to which the workers were exposed. The concentration of aniline was in the range of 1.3–2.75mg/m^3.

> A "definite" increase in MHb content was claimed during the first year (no data presented) in the group of workers compared to the control; decreases in haemoglobin levels, erythrocyte count, and coagulation factors were also described. The validity of the effects claimed in this study cannot be assessed (Vasilenko *et al* 1972a, b)." (References cited in SCOEL, 2016).

The New Zealand EPA classifies aniline as a 6.9A substance – a substance that is toxic to human target organs or systems (EPA, 2019).

The SCOEL recommendation on aniline summarised the reproductive/ developmental toxicity in humans:

> "In an insufficiently documented study, menstrual disturbances, ovarian dysfunction and spontaneous abortion were mentioned to occur in women exposed to aniline and other chemicals. Due to incomplete documentation and missing exposure data, no conclusions can be drawn from this study (ECB 2004). Further data are not available." (References cited in SCOEL, 2016).

The SCOEL recommendation on aniline summarised the genotoxic potential in humans:

> "In cultures prepared from peripheral blood samples of workers exposed to aniline for 4–9 years, an increased incidence of chromosomal aberrations (fragments, gaps, breakages) were observed compared to healthy controls. No data were provided regarding exposure or co-exposure to other chemicals. The validity of these findings cannot be assessed (HSE 1997).
>
> "Further data are not available." (Reference cited in SCOEL, 2016).

The New Zealand EPA classifies aniline as a 6.6A substance – a substance that is a known or presumed mutagen (EPA, 2019).

Animals

The ECB review of aniline summarised the acute toxicity in experimental animals:

> "In experiments on rats and rabbits the acute toxicity of aniline is moderate, independent of the way of application: In rats oral **LD50** values of 780**mg/kg bw** in females and 930mg/kg bw in males were determined. Inhalation **LC50** values in rats are different depending on the kind of exposure: For head-only exposure 3.3mg/l/4 hours and for whole-body exposure 1.86mg/l/4 hours were detected. Acute dermal toxicity of aniline is characterised by LD50 values of 1,540mg/kg bw for rabbits and 1,290mg/kg bw for guinea pigs. Cats however, react much more sensitive (sic), with a dermal LD50 of 254mg/kg bw and death following oral application of as low as approximately 50–100**mg/kg**." (References cited in ECB 2004).
>
> "In dogs 24 hours after oral treatment with 15mg aniline/kg methaemoglobin levels are in the normal range of approximately 0.7% (being in the range of 19–29% after 3 hours). In an acute inhalation test with the same species peak methaemoglobin levels of 3–24% were determined within 3 hours after the start of the exposure and declined to normal levels (<1%) after approximately 20 hours. Methaemoglobin was restituted at a half time of 100 minutes. In rats an oral dose of 20mg aniline/kg resulted in a small increase of MetHb levels (3.3% versus 2.4% in controls)." (References cited in ECB 2004).

The SCOEL recommendation on aniline summarised the irritation/corrosion potential in experimental animals:

> "In standard tests on irritation, the application of undiluted aniline caused only slight erythema but no other effects on the skin of rabbits. However, in another test after a single application of 100–900mg/kg bw to the skin of rats and rabbits signs of dermatitis were observed within 3–5 days, which resolved after 2–3 weeks. In a test, which was to study the acute dermal toxicity of aniline, rabbits developed subdermal haemorrhages and severe erythema after dermal exposure to undiluted aniline (ECB 2004; HSE 1997).
>
> "After instillation of undiluted aniline into the eyes of rabbits, severe corneal opacity and severe conjunctival erythema and oedema were detected which were not reversible within 8 days. In other tests, undiluted aniline produced lacrimation, inflammation of the conjunctiva and damage to the cornea but the effects were reversible within 24–48 hours. Comparable effects were observed after application of a saturated aqueous solution of aniline (ECB 2004).
>
> "In an acute inhalation toxicity study with rats, mild to severe corneal damage with corneal clouding was observed after the 4-hour nose-only exposure to 681–896ppm aniline vapour and aerosol. The eye effects persisted for 2 weeks (ECB 2004)." (References cited in SCOEL, 2016).

The NIOSH Skin Notation Profile for aniline summarised the sensitisation potential in experimental animals:

> "In animals, tests of sensitizing potential have produced equivocal results. Goodwin *et al*. [1981] evaluated the skin sensitization potential of aniline using three guinea pig sensitization procedures and observed positive allergic reactions in 1 of 10 animals (10%) in the guinea pig maximization test (**GPMT**), 5 of 10 animals (50%) in the single injection adjuvant test (**SIAT**), but none in the modified Draize test. Subsequently, Godwin *et al*. [1981] classified the potential of aniline to cause sensitization in the guinea pigs as weak in the GMPT and moderate in the SIAT, and a mild sensitizer based on their assessment of clinical data in humans. Basketter and Scholes [1992] classified aniline as an extreme sensitizer based on the 90% positive reactions in the GPMT, although the **LLNA** test was borderline. In a later study, Basketter *et al*. [2003] concluded that aniline was a weak sensitizer in the LLNA procedure. The Haneke *et al*. [2001] database reported aniline as a skin sensitizer in the guinea pig maximization test/Buehler assay; however, reviewing the results of the murine local lymph node assays (LLNA), negative responses were reported. The structure activity relationship model, *DEREK* for Windows, also predicted aniline to be positive regarding skin sensitization potential.
>
> "The results from the **HMT** [Basketter *et al*. 1994; Haneke *et al*. 2001], GPMTs [Goodwin *et al*. 1981; Basketter and Scholes 1992], and the weight of evidence from the murine LLNAs [Basketter and Scholes 1992; Basketter *et al*. 2003], supported by the structure-activity relationship model prediction, demonstrate that aniline is a skin sensitizer. Therefore, on the basis of the data for this assessment, aniline is assigned the **SK: SEN** notation." (References cited in NIOSH, 2015).

The SCOEL recommendation on aniline summarised the repeated dose toxicity in experimental animals:

> "Studies with repeated administration have been carried out with rats and, to a very limited extent, with mice, guinea pigs and dogs.

"Irrespective of the route of administration, the toxicity of aniline in rats manifests in effects on the red blood cells and the haematopoietic system with corresponding effects on the spleen, bone marrow, liver and kidney. Cyanosis with increased MHb levels, erythrocyte lesions with formation of Heinz bodies and haemolytic anaemia are characteristic observations. Reticulocytosis and increased bone marrow and extramedullary erythropoiesis are compensatory reactions of red blood cell toxicity. The damaged erythrocytes are scavenged mostly in the spleen. Accumulation of haemosiderin in the spleen and sometimes also in liver and kidney, spleen congestion, dark colouration and increased spleen weight are observed after repeated administration. Splenitis, spleen hyperplasia and fibrosis are further signs after long-term exposure to aniline.

"Except for a black discolouration of the spleen, no histological evidence of haematotoxicity was found in mice. However, a more detailed clinical and haematological examination has not been performed in this species." (SCOEL, 2016).

The SCOEL recommendation on aniline summarised the reproductive/ developmental toxicity in experimental animals:

"Fertility studies are not available.

"No treatment-related effects were observed on testes weight and histopathology at interim and terminal sacrifice in a chronic/carcinogenicity study in male rats at aniline doses up to 72mg/kg ·bw/d. In females, ovary weight was not reduced by aniline exposure at interim sacrifice at 26, 52, and 78 weeks but was lower at termination of the study in the high-dose group (CIIT 1982). No dose-dependent effects on histology of reproductive organs were observed in rats and mice in another chronic/carcinogenicity study (NCI 1978)."

"Aniline hydrochloride was evaluated for teratogenicity and postnatal effects in F344 rats. Pregnant dams were given the test substance by gavage at doses of 0, 10, 30 and 100**mg/kg bw/d** (0, 7.2, 21.6, 71.8mg aniline/kg bw/d) during gestational day 7–20 or from day 7 to parturition. Maternal toxicity manifested as a statistically significant decrease in absolute weight gain (high dose), a dose-dependent increase in spleen weight (significant at all doses) and in other signs characteristic of aniline toxicity (increased MHb formation, decreased erythrocyte count) at the highest dose. No embryo/ foetotoxic effects and no teratogenicity were observed at any dose. Foetal examination revealed a significant but marginal increase in liver weight and a marginally increased erythrocyte volume (**MCV**) in the high-dose group. A dose-dependent but non-significant increase in the number of litters in which one ore [sic] more postnatal deaths occurred was seen in all treated groups (total litters effected: 3/16, 4/15, 5/16) compared to control (2/15). After cessation of aniline exposure at parturition, maternal effects persisted at least for the period of nursing. Pups examined post-natally had increased MCV on day 0 (high dose). On the 25th post partum day, a decreased body weight (high dose), increased liver weight (low and mid, but not high dose) and a non-significant increase in spleen weight were observed. No signs of toxicity were seen in pups on day 60. No behavioural changes were detected in several tests (Price *et al* 1985).

"High doses of aniline hydrochloride (≥260mg/kg, single **i.p.** injection) may cause foetal cardiovascular malformations and cleft palate in rats. The effects are attributed to the MHb-induced maternal hypoxia (Matsumoto 2003).

> "In a screening study with mice treated by gavage with 560mg aniline/kg · d on gestation day 6–12, maternal toxicity including death of dams and a reduced birth weight and weight gain of offspring were observed but no effects on the number of live pups per litter or on the percentage survival during the first 2 weeks (Hardin *et al* 1987)." (References cited in SCOEL, 2016).

The SCOEL recommendation on aniline summarised the genotoxic potential in experimental animals and *in vitro* test systems:

> "In essence, mutagenicity/genotoxicity tests conducted with aniline mere mostly negative. Positive results were generally confined to high doses that were accompanied with signs of toxicity. However, it must be noted that distinct metabolites of aniline displayed genotoxicity, as explained in more detail [on page 30 of SCOEL, 2016]."
>
> "Aniline was not mutagenic in standard bacterial gene mutation test using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 in the absence or presence of metabolic activation by **S9** mix from Aroclor-induced rat or Syrian hamster livers. Also, no mutagenicity was observed in tests with *Escherichia coli* WP-2 uvrA and in yeasts. A mutagenic effect was observed in the strain TA98 in the presence of S9 mix and the co-mutagen **norharman**; a highly potent direct mutagen aminophenyl-norharman was identified as the reaction product of aniline and norharman (ECB 2004).
>
> "In assays using mammalian cells, aniline did not induce mutations at the **HPRT** locus in V79 cells in the absence of S9 mix. In the presence of S9 mix, a mutagenic response was observed at high concentrations, which exceeded the maximum concentration recommended by current test guidelines (Bomhard and Herbold 2005). Aniline showed mutagenic and clastogenic effects in several mouse lymphoma assays both in the absence and in the presence of exogenous metabolic activation system. Generally, the effects were weak or confined to high concentrations, which reached or were close to marked cytotoxicity (Bomhard and Herbold 2005).
>
> "Tests on chromosomal aberrations in Chinese hamster cells showed controversial results. A positive response was obtained, mostly at high concentrations, in five tests; in two of them, the positive response was restricted to the assay with S9 mix, while in two other tests the effect was seen in the absence of S9 mix. Aniline did not induce micronuclei in Syrian hamster embryonic cells; in Chinese hamster lung cells, an increase in the presence of S9 mix was observed but the effect was not dose-dependent (Bomhard and Herbold 2005).
>
> "Aniline induced a slight increase in sister chromatid exchanges (**SCE**) in cell lines from Chinese hamster, in rat liver epithelial cells and in human fibroblasts. SCE were also observed in concanavalin A induced human T-lymphocytes from whole blood cultures, but not in pure lymphocyte cultures. Therefore, it was concluded that erythrocytes contribute to the transformation of aniline into genotoxic intermediates. Two metabolites of aniline, o-aminophenol and N-phenylhydroxylamine, were much more effective in inducing SCE in human fibroblasts at 0.1mM and 0.05mM, respectively, than aniline at a far higher concentration (10mM) (ECB 2004; NRC 2000).
>
> "**DNA** strand breaks were found in mouse lymphoma cells in the presence of S9 mix, but only at an exceedingly high concentration of aniline (2g/l). Aniline did not induce unscheduled DNA synthesis (**UDS**) in primary cultures of rat, mouse, hamster and human hepatocytes (Bomhard and Herbold 2005; ECB 2004)." (References cited in SCOEL, 2016).

4.2 Cancer

The International Agency for Research on Cancer [**IARC**] evaluation of aniline concluded that:

> There is *inadequate evidence* in humans for the carcinogenicity of aniline.
>
> There is *limited evidence* in experimental animals for the carcinogenicity of aniline.

With an overall evaluation that:

> Aniline is *not classifiable as to its carcinogenicity to humans (Group 3)* (IARC, 1982; IARC, 1987).

The US National Toxicology Program [**NTP**] Report on Carcinogens [**RoC**], Fourteenth Edition has no evaluation on the carcinogenic potential of aniline (NTP RoC, 2019).

The New Zealand EPA classifies aniline as 6.7B – a substance suspected to be a human carcinogen (EPA, 2019).

Humans

The SCOEL recommendation on aniline summarised the carcinogenicity data in exposed humans:

> "In two cohort studies with 4622 and 1223 men, which were employed in the British dyestuff industry for at least 6 months, the incidence of bladder cancer was higher than expected in employees who had been exposed to several chemicals including aniline (but not the known carcinogens 2-naphthylamine and benzidine) (Case *et al* 1954; Case and Pearson 1954). In a further study in an industrial rubber plant, co-exposure to chemicals including aniline, *o*-toluidine and hydroquinone was associated with a significant increase of bladder cancer in the group of 708 workers who were definitively exposed and in that of 288 who were possibly exposed (NIOSH Alert 1990; Ruder *et al* 1992; Ward *et al* 1991). Overall, the workers in all of these studies were exposed to a number of different substances and the carcinogenic potential of aniline cannot be assessed from these findings." (References cited in SCOEL, 2016).

In a follow-up study of the industrial rubber plant cohort, Carreón *et al*. (2014) concluded that:

> "Bladder cancer incidence remains elevated in this cohort and significantly associated with estimated cumulative exposure. Results are consistent with earlier findings in this and other cohorts. Despite other concurrent chemical exposures, we consider *o*-toluidine most likely responsible for the bladder cancer incidence elevation and recommend a re-examination of occupational exposure limits." (Carreón *et al*., 2014).

The Health Canada follow-up assessment report on aniline noted:

> "Relevant human data were limited to the results of (limited) epidemiological studies, in which workers were exposed to aniline and other chemicals within the working environment; however, no clear relationship was established

between exposure to aniline and incidence of cancer (Sorahan and Pope, 1993; Mikoczy *et al*., 1996; Alguacil *et al*., 2000; Sathiakumar and Deizell, 2000). In an update of the Sorahan and Pope (1993) study, additional data analyses indicated no association between duration of employment in the aniline department and increased risk of bladder cancer in chemical product workers (Sorahan *et al* 2000)." (References cited in Health Canada, 2011).

Animals

The SCOEL recommendation on aniline summarised the carcinogenicity data in experimental animals:

"Carcinogenicity studies were carried out with oral administration of aniline hydrochloride in rodents. Most evidence is available from two feeding studies with rats.

"In one study with F344 rats (50 animals/sex and treatment group; 25 animals/sex and control group), animals were fed diet with 0, 0.3% or 0.6% aniline hydrochloride (equivalent to aniline doses of 0, 174 or 360mg/kg bw/d) for 103 weeks followed by a post-treatment period of 5 weeks. Survival was not affected by treatment; body weight gain was slightly reduced in females and in high-dose males. There were no splenic tumours in control males and females. The incidences of several types of mesenchymal tumours, primarily of the spleen, were increased in male treated rats: haemangiosarcomas of the spleen were observed in 19/50 males at the low dose and in 20/46 at the high dose, fibrosarcomas in 3/50 at the low and 7/46 at the high dose. The combined incidence of fibrosarcomas and sarcomas of the spleen was also statistically significantly higher in male rats as was the combined incidence of fibrosarcomas and sarcomas of multiple body organs in male rats. In female rats, the number of animals with fibrosarcomas or sarcomas of either the spleen alone or multiple organs of the body cavity was significantly associated with treatment. Although this result was not supported by the Fischer exact tests, it was considered indicative of a compound-related carcinogenic effect because of the rarity of these tumours (observed incidences: 0/24 in the control group, 1/50 in the low-dose and 7/50 in the high-dose females). Non-neoplastic effects included splenic lesions (capsular fibrosis, fatty changes, hyperplasia, increased erythropoiesis, haemosiderosis) and increased haemosiderosis in the renal tubular epithelium in both sexes at both doses levels (NCI 1978). The splenic tumour incidence was confirmed in re-examination, which also showed that the occurrence of non-neoplastic splenic lesions was strongly correlated with tumour incidence. It was concluded that sarcomas arise from pre-existing fibrotic areas and splenic fibrosis and hyperplasia are likely to present pre-neoplastic lesions (Weinberger *et al* 1985).

"In another study, male and female F344 rats (130 animals/sex and group) received aniline hydrochloride for 104 weeks in food at body doses of 0, 10, 30 and 100mg/kg · d (equivalent to body doses of aniline of 0, 7, 22 and 72mg/kg · d). Survival was reduced in high-dose males, body weight was not affected by treatment. The incidence of splenic tumours in males of the high-dose group was much higher than in the control and the other two dose groups. Most of these tumours were stromal sarcomas (21), but there were also haemangiosarcomas (6) and several types of other splenic sarcomas (8). One stromal sarcoma was observed in the mid-dose group. No splenic tumours were seen in control animals of both sexes and in low- and mid-dose females, one splenic haemangiosarcoma was observed in a high-dose

female. The incidence of other tumours showed no significant increase. Non-neoplastic findings in the spleen of animals were fatty metamorphosis (high-dose males), stromal hyperplasia (high-dose, both sexes) and chronic capsulitis of the spleen, mostly at high-dose animals but less frequently also at the low- and mid-dose. Furthermore, haemosiderin deposition and extramedullary haematopoiesis were more severe in high-dose males, splenic atrophy occurred more frequently in high-dose males and females. In males and in high-dose females, the severity and frequency of splenic congestion were increased and several blood parameters (haematocrit, haemoglobin concentration and erythrocyte count) were decreased (CIIT 1982).

"In the same NCI-study as mentioned above, male and female B6C3F1 mice received 0, 0.6% or 1.2% of aniline hydrochloride in the diet for 103 weeks followed by a post-treatment period of 5 weeks. Survival was not reduced by treatment, body weight gain was reported to be slightly reduced in both groups of treated males. No treatment-related tumours were observed in any of the groups. The only non-neoplastic lesion related to treatment was a chronic inflammation of the bile duct in both groups of treated males (NCI 1978).

"The results of a further study (Hagiwara *et al* 1980) which was not reported in great detail were summarised by HSE (1997). In this study, groups of male Wistar rats received drinking water with 300 or 1200ppm aniline for 80 weeks with or without co-exposure to 0.05% norharman in the diet. No increase in tumour incidence was observed in any treatment group. Because of the lack of data and the short duration of exposure, the study was considered inadequate for the assessment of aniline carcinogenicity (HSE 1997)." (References cited in SCOEL, 2016).

4.3 Absorption, distribution, metabolism and excretion

The NICNAS review of benzenamine summarised the **ADME**:

"The chemical has been reported to be well absorbed following oral, dermal and inhalation exposure in animals. Following oral administration, the chemical was absorbed to the extent of 89–96, 72, 80, and 56% in rats, mice, sheep, and in pigs, respectively. A pulmonary retention of nearly 90% has been reported for humans and a dermal absorption of up to 38% has also been estimated for humans. Although details are not available, a biological half-life of about 3.5 hours has been reported for the chemical in humans (ECB, 2004; EC, 2010; **REACH**). The rate of absorption of the chemical though skin in humans has also been reported to be approximately 1000 times lower in vapour form than topically applied liquid form (REACH).

"Following a single oral administration of the chemical in rats, the peak plasma radioactivity was observed at 0.5, 1 and 2 hours at 10, 30, and 100mg/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 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 100mg/kg bw/day orally for one day, the highest radioactivity was present in erythrocytes followed by plasma, spleen, kidney, liver, lung, heart, brain, and fat. A greater accumulation of radioactivity was observed in spleen following repeated administration of the chemical at the same dose for 10 days.

> "The metabolism of the chemical is the main elimination pathway for the chemical and is qualitatively similar in humans and animals. Following a single dermal application of the chemical in rats and mice, the chemical is mostly excreted within 24 hours as metabolites in urine. The chemical is metabolised primarily in the liver by three metabolic pathways: N-acetylation, aromatic ring hydroxylation, 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. Small amounts of the chemical are hydroxylated to 2- and 4-aminophenols. The methaemoglobin forming ability of the chemical is strongly based on the formation of phenylhydroxylamine metabolite, but also to some extent on the formation of 2- and 4-aminophenol metabolites. The relative *in vitro* (rat erythrocyte suspensions) potencies for methaemoglobin formation for phenylhydroxylamine, 2-aminophenol, and 4-aminophenol metabolites were about 10:5:1. However, the relative potencies of aminophenols for methaemoglobin formation are lower in rats after intraperitoneal injections, the ratio being 100:4:1 (phenylhydroxylamine: 2-aminophenol: 4-aminophenol).
>
> "The toxic effects of the chemical are due to the formation of methaemoglobin leading to methaemoglobinaemia, cyanosis, tremors, lacrimation and respiratory problems. Methaemoglobin is produced as a result of oxidation of thiols within erythrocytes by phenylhydronitroxide radicals, which are produced as a reaction of phenylhydroxylamine with oxyhaemoglobin. After a single oral or inhalation exposure to dogs, the formation of methaemoglobin was 1–6 times higher following oral exposure than after inhalation exposure. The maximum methaemoglobin concentration was observed in less than one hour after cessation of inhalation exposure and three hours after oral administration.
>
> "The chemical has been reported to be able to pass the placental barrier in rats following a single subcutaneous dose. The total plasma concentrations of the chemical were slightly higher (10–15%) than the maternal plasma concentrations at 1, 2, and 4 hours after application. A similar plasma half-life of 1.5 hours was reported for foetal as well as for maternal plasma (ECB, 2004; REACH)." (References cited in NICNAS, 2013).

The SCOEL recommendation on aniline noted:

> "A comparative study on percutaneous penetration of a number of chemicals, including aniline, provided additional support for the assignment of a "skin" notation (Korinth *et al* 2012). The flux estimation for aniline was 752.2 ± 213.5**µg/cm²/h** (mean ± SEM)." (Reference cited in SCOEL, 2016).

The SCOEL recommendation on aniline summarised the mechanistic data for toxicity and carcinogenicity:

> "Aniline is categorised by SCOEL as a **Group C carcinogen** with a practical threshold, because carcinogenic doses caused early effects on haematological parameters, inflammatory reactions in the spleen and perturbations of iron metabolism as a result of haemolytic anaemia (Kan *et al* 1993, 1995, 1997). Recently, this has been further substantiated.

"Ma *et al* (2008) exposed male Sprague-Dawley rats subchronically to aniline (0.5mmol/kg/day *via* drinking water for 30 days). This aniline treatment led to a significant increase in splenic oxidative DNA damage, measured as 8-hydroxy-deoxyguanosine in spleen.

"A second study (Wang *et al* 2010) evaluated the potential contribution of haem oxygenase-1 (**HO-1**), which catalyses haem degradation and releases free iron. Male Sprague-Dawley rats were given 1mmol/kg bw/day aniline in water by gavage for 1, 4, or 7 days, and respective controls received water only. Aniline exposure led to significant increases in HO-1**mRNA** expression in the spleen (2- and 2.4-fold at days 4 and 7, respectively) with corresponding increases in protein expression, as confirmed by **ELISA** and Western blot analysis. Furthermore, immune-histochemical assessment of spleen showed stronger immune-staining for HO-1 in the spleens of rats treated for 7 days, confined mainly to the red pulp areas. The increase in HO-1 expression was associated with increases in total iron (2.4- and 2.7-fold), free iron (1.9- and 3.5-fold) and ferritin levels (1.9- and 2.1-fold) at 4 and 7 days of aniline exposure. The data suggested that HO-1 up-regulation in aniline-induced splenic toxicity could be a pro-oxidant mechanism, mediated through iron release.

"In a third study, Ma *et al* (2011) exposed male Sprague-Dawley rats to aniline (0.5mmol/kg bw/day) via drinking water for 30 days, while controls received drinking water only. The DNA base excision repair (**BER**) activity of the glycosylases **NEIL1** and 2 was assayed using a bubble structure substrate containing 5-hydroxyuridine (preferred substrates for the NEIL1 and **NEIL2**) and by quantitating the cleavage products. Aniline treatment led to a 1.25-fold increase in the NEIL1/2-associated base excision repair activity in the nuclear extracts of spleen compared to the controls. Real-time **PCR** analysis for NEIL1 and NEIL2mRNA expression in the spleen revealed 2.7- and 3.9-fold increases, respectively, in the aniline-treated rats compared to controls. Likewise, Western blot analysis showed that protein expression of NEIL1 and NEIL2 in the nuclear extract of spleens from aniline-treated rats was 2.0- and 3.8-fold higher than controls, respectively. The aniline treatment also led to stronger immuno-reactivity for NEIL1 and NEIL2 in the spleens, confined to the red pulp areas. The results were interpreted to show that aniline-induced oxidative stress is associated with an induction of NEIL1/2.

"A fourth study (Ma *et al* 2013) examined whether the repair enzymes **NTH1** and **APE1** contribute to the repair of oxidative DNA lesions in the spleen following aniline treatment. This was identical to that in the preceding studies. The treatment led to significant increase in NTH1- and APE1-mediated BER activity in the nuclear extracts of spleen of aniline-treated rats compared to the controls. NTH1 and APE1 mRNA expression in the spleen showed 2.9- and 3.2-fold increases, respectively. This was confirmed by Western blot analysis. The increased repair activity of NTH1 and APE1 was discussed as an important mechanism for the removal of oxidative DNA lesions.

"In essence, new data are fully in line with the view that the (experimental) carcinogenic effect of aniline is linked to a mode of action that is associated with a threshold: oxidative stress is involved in the spleen, and in response to oxidative stress DNA repair pathways are operative.

"Thereby, the categorisation in SCOEL group C of carcinogens is further supported." (References cited in SCOEL, 2016).

The Health Canada follow-up assessment report on aniline noted:

> "Elucidation of the mode of action of aniline has not become more defined since the release of the 1994 report. There is insufficient information to determine whether the tumourigenic response is mediated by direct interaction of aniline or its metabolites with splenic macromolecules (proteins, DNA or lipids) or if other possible cytotoxic responses of the spleen are involved. Possible involvement of a genotoxic or other multiple mode(s) of action needs further investigation." (Health Canada, 2011).

5.0 Exposure standards

IN THIS SECTION:

5.1 Other exposure standards

Table 3 below shows aniline exposure standards from around the world, as published by the Institute for Occupational Safety and Health of the German Social Accident Insurance (**IFA**, 2019).

JURISDICTION OR ADVISORY BODY	8-HOUR LIMIT VALUE		SHORT-TERM LIMIT VALUE	
	ppm	mg/m³	ppm	mg/m³
Australia	2	7.6		
Austria	2	8	10	40
Belgium[1]	2	7.7		
Canada – Ontario	2			
Canada – Québec	2	7.6		
Denmark	1	4	2	8
Finland	0.5	1.9	1.0[2]	3.9[2]
France	2	10		
Germany – **AGS**	2[3]	7.7[3]	4[2,3]	15.4[2,3]
Germany – **DFG**	2	7.7	4	15.4
Hungary		8		
Ireland	1	3.8		
Japan – **JSOH**	1	3.8		
Latvia		0.1		
New Zealand[4]	1	4		
People's Republic of China		3		
Poland		5		20
Romania	0.8	3	1.3[2]	5[2]
Singapore	2	7.6		
South Korea	2	10		
Spain[5]	2	7.7		
Sweden	1	4	2[2]	8[2]
Switzerland	2	8	4	16
USA – NIOSH	[6]			
USA – **OSHA**[4]	5	19		
UK	1	4		

TABLE 3: Exposure standards for aniline from around the world

[1] Additional indication "D" means that the absorption of the agent through the skin, mucous membranes or eyes is an important part of the total exposure. It can be the result of both direct contact and its presence in the air.

[2] 15 minutes average value.

[3] Inhalable aerosol and vapour.

[4] And homologues.

[5] skin.

[6] Lowest feasible concentration.

It is noted that the only organisations from whom we obtained information as to how and why they set occupational exposures standards on aniline were SCOEL, ACGIH® and Safe Work Australia. The DFG has recently updated its BAT Value Documentation on aniline (DFG, 2017).

5.2 SCOEL

The Scientific Committee on Occupational Exposure Limits [SCOEL] assessment of aniline recommended an 8-hour TWA of 2ppm with a **STEL** of 5ppm for occupational exposure to aniline (SCOEL, 2016).

The rationale for their recommendation was:

> "For the evaluation of the toxicity of aniline, the following effects must be taken into account:
> - carcinogenicity
> - methaemoglobin (MHb) formation, linked with
> - toxic effects on the haematopoietic system with erythrocyte toxicity and effects on the spleen.
>
> **Genotoxicity and carcinogenicity**
>
> "Aniline is not mutagenic in standard bacterial tests. In mammalian cells *in vitro*, the results are not uniform, but positive results were observed with respect to chromosomal effects, SCE and mutagenicity. *In vivo*, induction of micronuclei was observed in bone marrow cells of rats and mice. However, the doses in these studies were high and caused marked MHb-formation (Bomhard and Herbold, 2005). In general, the genotoxicity of aniline appears very low, if any. However, distinct metabolites of aniline are genotoxic, when individually tested. However, the balance of formation and detoxification of these metabolites has not been studied. Considering the distance between the high doses that produced genotoxicity in some tests and the proposed OEL, genotoxic effects of aniline appear negligible in practice.
>
> "Data on carcinogenicity of aniline in humans are inadequate for an evaluation. Experimentally, aniline is carcinogenic in rats, but not in mice. Tumours were mainly observed in the spleen of male rats, and the tumour incidence was clearly non-linear. It has been discussed whether the development of tumours is be [sic] connected to erythrocyte toxicity, which is indicated by the formation of MHb and Heinz bodies with the histopathological effects in the spleen being a consequence of this effect (ECB, 2004). If this is the case, it can be argued that repetitive toxic effects play a decisive role for the development of tumours, and that no increased tumour risk should be expected in the absence of an increased erythrocyte turnover. This view is experimentally be [sic] supported by Mellert *et al*. (2004), corroborating the contention that experimentally carcinogenic doses of aniline cause early effects on haematological parameters, inflammatory reaction in the spleen and perturbations in iron metabolism as a result of haemolytic anaemia. Recent studies into the mechanism of the experimental splenotoxicity and carcinogenicity in rats (section 7.7.2.) have provided further confirmation for a categorisation into SCOEL group C of carcinogens (as a compound with a practical threshold). Therefore, it is now well established that chronic splenotoxicity and subsequent carcinogenicity is a secondary process following an increased breakdown of erythrocytes because of aniline-induced methaemoglobenaemia. It follows that avoidance of excessive methaemoglobinaemia will protect against carcinogenesis in the spleen.

"Accordingly, the experimental carcinogenicity of aniline can reasonably be linked to a defined threshold-related process. *According to the delineations by SCOEL on the derivation of OELs for carcinogens and mutagens (Bolt and Huici-Montagud 2008), aniline is categorised into group C (carcinogens with a mode of action-based threshold)*.

Developmental toxicity

"In a developmental toxicity study with oral treatment of rats, maternal toxic effects were observed at 7.2mg aniline/kg · d (Price *et al*., 1985). There was no evidence of foetotoxicity or teratogenicity at maternally non-toxic doses. Accordingly, no developmental effects are expected at concentrations that protect from haematotoxic effects as derived above.

Methaemoglobin formation and other haematotoxic effects

"Following acute uptake of aniline, the critical toxic effect is the formation of methaemoglobin (MHb). Depending on the concentration of MHb, methaemoglobinaemia may have serious acute health effects. By analogy to tolerable levels of CO-Hb in carbon monoxide exposed persons (about 4% CO-Hb; see the SUM document on carbon monoxide), a MHb-level of about 5 % has been considered tolerable (Bolt *et al*., 1985). After repeated experimental exposure to aniline, the critical toxic effects are erythrocyte toxicity and toxic effects on the spleen. In a subacute inhalation study with rats, minimal splenic histopathological alterations developed at the lowest exposure concentration of 64.7mg/m^3 (17ppm) (du Pont de Nemours and Co, 1982). In another subacute inhalation study with rats, a borderline increase in splenic extra-medullary hematopoiesis was seen at 32.4mg/m^3 (8ppm), while 9.2mg/m^3 (2.4ppm) was not associated with any significant effect (Pauluhn, 2004). In a subchronic study, a "doubtful" slight cyanosis but not any other effects were reported in rats, dogs, mice and guinea pigs at the only exposure concentration of 5ppm, but the limited evaluation precludes definitive conclusions (Oberst *et al*., 1956). Inhalation exposure studies with rats have established a [sic] experimentally based NOAEC of 9.2mg/m^3 (2.4ppm) upon subacute exposure in rats. Both available subacute inhalation studies (du Pont de Nemours and Co, 1982; Pauluhn, 2004) did not include additional aniline exposure via the skin. In general, the experimental data in rats appear compatible with those observed in humans.

"In a subacute feeding study with rats, minimal erythrocyte toxicity with sporadic occurrence of Heinz bodies and minimal vascular congestion of the spleen were reported at the lowest dose of 4mg/kg · d (Mellert *et al*., 2004). As the effects were slight, the **NAEL** does not appear to be much lower. In a chronic/carcinogenicity study (CIIT, 1982), the lowest dose of 7mg/kg · d represented a **LOAEL** with respect to erythrocyte toxicity and effects on the spleen. No definite **NOAEL** is provided in either of these studies. With direct transformation into a corresponding air concentration (route-to-route extrapolation) a dose of 4mg/kg· d corresponds to 28mg/m^3 (7ppm) assuming a body weight of 70kg, a breathing volume of 10m^3 during an eight hour exposure and 100% absorption. Comparison of the effects in the subacute and chronic feeding study does not support a strong increase in severity of the effects with prolonged exposure. Again, both oral feeding studies (Mellert *et al*., 2004; CIIT, 1982) did not include additional exposure via the skin.

"Taking these data together, a health-based OEL may be derived, which protects against the relevant non-neoplastic effects, including methaemoglobinaemia. The experimental data in rats consistently point to beginning haematotoxic effects (MHb formation, associated with Heinz bodies) and spleen toxicity above repeated inhalation exposures of 5ppm aniline.

> "Inhalation studies are reported in dogs (Pauluhn, 2002, 2005) and rats (Pauluhn, 2004), but there are large species differences in the quantities of MHb production due to aniline exposure between experimental animal species and humans. Therefore, the derivation of an OEL must rely on available data from humans. A preference for human data is in line with the key methodology of SCOEL.
>
> "A new experimental human exposure study by Käfferlein *et al* (2014) provides a strong basis for standard setting. The study indicates that after a 6-hour exposure to 2ppm a level of MHb formation of 1.6% was reached, which is not expected to further increase after 8 hours. This level is more than 2-fold below the critical MHb level. A comparison of the methaemoglobin levels reached under the conditions (moderate exercise) of this study (Figure 1) and the critical methaemoglobin levels of 4–5% leads to the conclusion that an uncertainty factor to bridge the experimental conditions to those in industrial practice is not required. An OEL of 2ppm aniline is derived. No additional uncertainty factor is needed to compensate for possible additional human inter-individual variation that exceeds the variations recorded in the study of Käfferlein *et al* (2014). Carry-over effects to the next shift must not be taken into account, as the half-life of MHb after cessation of aniline exposure is about 3.5 hours.
>
> "A STEL is a preferred means to limit short-term exposures with possible methaemoglobin formation. In view of the short half-life of aniline and the rapid decrease of methaemoglobin, an excursion factor of 2 will provide adequate protection. Applying the preferred value approach of SCOEL, a *STEL of 5ppm is therefore recommended*." (References cited in SCOEL, 2016).

The SCOEL assessment of aniline also noted that:

> "Dermal absorption of aniline both in liquid and vapour form substantially contributes to total aniline uptake (Dutkiewicz, 1961; Korinth *et al*. 2008). *Therefore, a 'skin' notation was warranted.*
>
> "Aniline may cause contact allergy in humans, which is often associated with "para-group" cross-reactivity" (References cited in SCOEL, 2016).

5.3 ACGIH®

The American Conference of Governmental Industrial Hygienists [ACGIH®] review recommended a **TLV-TWA** of 2ppm [7.6mg/m^3] for occupational exposure to aniline, based on the increase in methaemoglobin in blood of animals exposed to 5ppm; skin absorption in humans; and, the TLV-TWA of nitrobenzene, a structurally similar chemical (ACGIH®, 2001).

ACGIH® noted that the TLV-TWA of 2ppm for aniline was only recommended if dermal absorption of aniline could be prevented (ACGIH®, 2001).

ACGIH® also noted that a Skin notation was warranted due to systemic toxicity after dermal absorption; an A3, Confirmed Animal Carcinogen with Unknown Relevance to Humans, notation was warranted, based on hemangiosarcomas, fibrosarcomas and sarcomas of the spleen reported in rodents fed aniline hydrochloride; but, there was insufficient data to recommend either a SEN notation or a **TLV-STEL** (ACGIH®, 2001).

5.4 DFG

The Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) review of aniline recommended a **MAK** value of 2ppm [7.7mg/m^3]; no Peak limitation; Skin notation "H", Carcinogenicity Category 4; no Pregnancy Risk or Germ cell mutation classifications (DFG, 2017).

The rationale for their conclusions included:

> "In a long-term study, aniline produced tumours of the spleen in rats (Greim 2007, translated), but it is not considered by the Commission to be primarily genotoxic, and is classified in carcinogen category 4. This allows the establishment of a MAK and a BAT value (biological tolerance value).
>
> "In the meantime, this assessment has been confirmed by mechanistic studies.
>
> "The methaemoglobinaemia caused by aniline leads to an increased degradation of erythrocytes in the spleen, combined with an increased accumulation of iron. The triggered oxidative stress results in secondary genotoxicity, which explains the tumour formation occurring at high doses (Ma *et al*. 2008, 2011, 2013; Wang *et al*. 2010). However, this also means that such a tumour formation is not to be expected when the methaemoglobin concentration is assessed as not adverse. With methaemoglobin levels of 4% to 5%, therefore, no tumour formation is to be expected in humans." (References cited in DFG, 2017).

5.5 Safe Work Australia

Safe Work Australia proposed an 8-hour TWA of 0.5ppm to protect for the risk of elevated blood methaemoglobin and associated effects in exposed workers.

In their review, they say, "The critical toxic effect of aniline exposure is formation of methaemoglobin (MHb) which results in methaemoglobinaemia and cyanosis. A NOAEL of 5ppm for increased MHb levels is reported in rats exposed for six months. A daily dose of 35mg, reported in humans, caused a concentration blood MHb of 3.7% which is considered tolerable and without adverse effects.

"The recommended TWA is based on the 35mg dose in human studies and corresponds to an airborne concentration of 0.5ppm (1.94mg/m^3). The TWA is expected to protect for the increase of MHb concentrations caused by exposure fluctuations around the TWA. Therefore, a STEL is not recommended". (Safe Work Australia, 2019).

6.0 Analytical methods for the assessment of airborne aniline

A common method to measure aniline exposure is using NIOSH Method 2017, Issue 1 (NIOSH, 1998).

Using this method an air sample of 5 to 50 litres is collected onto a sampling train consisting of a filter and solid sorbent (glass fibre filter, sulfuric acid treated and silica gel sorbent tube), with the sampling train set at a flow rate of 0.2 litres per minute. Following desorption of the analyte using ethanol, the sample is analysed using gas chromatography with flame ionisation detection.

This method can achieve a detection limit of 4µg per sample. This would allow quantitation of samples at an airborne concentration of 0.02 ppm for a 50L air sample.

It is acknowledged that determination of the airborne concentration of aniline for comparison with the WES-STEL cannot be achieved using this method.

7.0
Discussion

WorkSafe's WES for aniline and homologues has been unchanged since adoption in 2001.

The toxicological database reviewed above indicates aniline is locally and systemically toxic to humans, causing eye irritation/corrosion, dermal sensitisation and methaemoglobinaemia; and locally and systemically toxic to laboratory species causing eye irritation/corrosion, dermal sensitisation, methaemoglobinaemia, and in rats splenotoxicity and carcinogenicity.

Based on the aforementioned documentation, informed by the conclusions of the SCOEL and ACGIH® reviews, and in particular the findings listed below, WorkSafe considers its current WES-TWA of 1ppm [4mg/m^3] of aniline to be adequate to manage health risks from possible workplace exposure:

- Aniline induced tumours in the spleens of male rats (SCOEL, 2016).
- Aniline is genotoxic: in mammalian cells *in vitro* aniline induces chromosomal aberrations, SCE and possibly gene mutations; in rats *in vivo* micronuclei; and, DNA strand breaks and DNA adducts were detected *in vivo* (ECB, 2004).
- The mechanism(s) by which aniline induces cancer in rats appear to be predominantly non-stochastic: oxidative stress, cytotoxicity and compromised DNA repair (SCOEL, 2016), although genotoxic involvement cannot be discounted (Health Canada, 2011).
- Epidemiological studies in workers revealed an increased risk of bladder cancers in industries involving exposure to aniline, but the latest analyses suggest *o*-toluidine was more likely the causative agent (SCOEL, 2016; Carreón *et al*., 2014).
- The SCOEL and ACGIH® reviews proposed OELs for workplace exposures to aniline, based on threshold effects.
- SCOEL proposed an OEL for aniline at 2ppm with a STEL at 5ppm, based on a report that a 6-hour exposure to 2ppm aniline induced a 1.6% level of methaemoglobin in volunteers undertaking moderate exercise. Critical levels of methaemoglobin were stated to be 4–5%, and no further uncertainty factor was applied (SCOEL, 2016).
- ACGIH® proposed an OEL for aniline at 2ppm [7.6mg/m^3], based on reports of an increase in methaemoglobin in animals exposed to 5ppm (ACGIH®, 2001).
- The current WES-TWA of 1ppm aniline is set at a point at which induction of methaemoglobin in exposed workers is expected not to be toxicologically significant, with some margin for potential dermal exposure.
- A WES-STEL of 2ppm aniline is proposed as the WES-TWA is set to minimise the potential for methaemoglobin formation, an acute toxic effect, and peak as well as cumulative exposures are significant for worker safety, noting that the half-life of methaemoglobin is 3.5 hours (SCOEL, 2016; DFG, 2017).

- Safe Work Australia holds the view that a dose of 35mg (0.5ppm) would result in a maximum blood MHb increase of 3.7% (as per the study cited in SCOEL, 2016). Based on this view, 1ppm would equate to a dose of 70mg. No data was available from the SCOEL report as to what MHb increase that could result in. The highest dose reported was 55mg which resulted in a MHb increase of 5.2%, and at 45mg the increase was 7.1%. The SCOEL report commented that "Generally, an increase in MHb above the normal background level in blood (about 1.1 %) to 15% will be without signs and symptoms. However, as known from **CO**-induced oxygen deficiency for sensitive risk groups (persons with latent restricted coronary or arterial function), much lower MHb-levels may be tolerable (Bolt *et al* 1985). Based on the limited data available it might be reasonable to assume at a concentration of 1ppm (dose around 70mg), the increase in MHb would be below 15%.
- The dermal absorption of aniline has been reported to potentially be significant (Korinth *et al*., 2013), more so with aqueous aniline solutions than vapour, and SCOEL has recommended a skin notation (SCOEL, 2016). ACGIH® also recommended a skin notation for aniline, based on systemic toxicity in experimental animals after dermal exposure (ACGIH®, 2001).
- A ***skin*** *notation* is justified for aniline, based on potential exposure contribution, reported systemic toxicity after dermal exposure, and potential for a simultaneous vapour phase.
- Available information indicates that while aniline is a dermal sensitiser, there is insufficient evidence about respiratory sensitisation (SCOEL, 2016). Therefore, a ***dsen*** *notation* is warranted and a ***rsen*** *notation* is not.
- Allergic sensitisation is considered an irreversible change (**OECD**, 2012), and while threshold levels exist for allergic sensitisation by allergenic substances (OECD, 2012), the data for aniline from human experience or animal studies was inadequate to quantitatively derive such a threshold.
- DFG have recommended BAT values for aniline in urine and blood to protect workers against unsafe levels of methaemoglobin, noting the demonstrable discrepancy in methaemoglobin formation between laboratory and field conditions due the contribution from dermal absorption (DFG, 2017). SCOEL has recommended a **BLV** for aniline in urine (SCOEL, 2016).

8.0
Recommendations

WorkSafe considers its current WES-TWA of 1ppm [4mg/m^3] of aniline with a *skin* notation to be adequate to protect workers exposed in the workplace, based on today's scientific understanding.

It is proposed that WorkSafe:

1. retain the WES-TWA for aniline of 1ppm [4mg/m^3]
2. retain the *skin notation* for aniline
3. adopt the *dsen notation* for aniline, and
4. adopt a WES-STEL for aniline of 2ppm [8mg/m^3].

Noting that the recommended WES-TWA of 1ppm and WES-STEL of 2ppm for aniline may not eliminate all risk, due to the genotoxic potential of aniline and its metabolites, the impact of dermal absorption, and the potential for dermal sensitisation, so exposures should be minimised.

Appendices

IN THIS SECTION:

Appendix 1: Glossary

TERM	MEANING
ADME	Absorption, Distribution, Metabolism and Excretion.
AGS	Ausschuss für Gefahrstoffe [Committee for Hazardous Substances] is a consultative body of the German Federal Ministry of Labour and Social Affairs on issues of the Ordinance on Hazardous Substances. Administered by the BAuA.
APE1	Apurinic/apyrimidinic (AP) endonuclease.
BAT	Biologische Arbeitsstoff-Toleranzwerte [Biological Tolerance Value], a DFG term.
BER	Base excision repair.
BLV	Biological Limit Value.
Carcinogen category 4	DFG MAK designation: Substances that cause cancer in humans or animals or that are considered to be carcinogenic for humans and for which a MAK value can be derived. A nongenotoxic mode of action is of prime importance and genotoxic effects play no or at most a minor part provided the MAK and BAT values are observed. Under these conditions no contribution to human cancer risk is expected. The classification is supported especially by evidence that, for example, increases in cellular proliferation, inhibition of apoptosis or disturbances in cellular differentiation are important in the mode of action. The classification and the MAK and BAT values take into consideration the manifold mechanisms contributing to carcinogenesis and their characteristic dose-time-response relationships.
CO	Carbon monoxide.
DFG	Deutsche Forschungsgemeinschaft (German Research Foundation), the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Federal Republic of Germany. The science-based MAK values are recommended to the German Minister of Labour and Social Affairs for possible adoption under the German Hazardous Substances Ordinance.
DNA	Deoxyribonucleic acid.
dsen	A substance that can 'sensitise' the skin, inducing a state of hypersensitivity to it, so that on subsequent exposures, an allergic reaction can occur (which would not develop in non-sensitised individuals). It is uncommon to become sensitised to a compound after just a single reaction to it. A WorkSafe term.
ECB	European Chemicals Bureau – an agency of the European Union and predecessor of the ECHA.
ECHA	The European Chemicals Agency (an agency of the European Union).
ELISA	Enzyme linked immunosorbent assay.
EPA	The New Zealand Environmental Protection Authority.
GPMT	Guinea pig maximization test.
Group C carcinogen	Genotoxic carcinogens for which a practical threshold is supported. SCOEL term.
"H"	DFG MAK designation: *danger of percutaneous absorption*. Equivalent to the 'skin' notation in the WorkSafe WES special guide.
HMT	Human maximisation test.
HO-1	Haem oxygenase-1.
hprt; HPRT; HGPRT	Hypoxanthine phosphoribosyltransferase or hypoxanthine-guanine phosphoribosyltransferase gene that codes for the enzyme.
HSE	Health and Safety Executive, UK.
HSNO	Hazardous Substances and New Organisms Act 1996, New Zealand.
IARC	International Agency for Research on Cancer, World Health Organization.
IFA	Institut für Arbeitsschutz der Deutschen Gestzlichen Unfallversicherung [Institute for Occupational Safety and Health of the German Social Accident Insurance].

TERM	MEANING
i.p.	Intraperitoneal.
IPCS	International Programme on Chemical Safety – a World Health Organisation Programme.
JSOH	Japan Society for Occupational Health.
LC_{50}	Lethal Concentration for 50% of the test population.
LD_{50}	Lethal Dose for 50% of the test population.
LLNA	Local lymph node assay.
LOAEL	Lowest Observed Adverse Effect Level.
MAK	Maximale Arbeitsplatz-Konzentration, (maximum workplace concentration) is defined as the maximum concentration of a chemical substance (as gas, vapour or particulate matter) in the workplace air which generally does not have known adverse effects on the health of the employee nor cause unreasonable annoyance (for example, by a nauseous odour) even when the person is repeatedly exposed during long periods, usually for 8 hours daily but assuming on average a 40-hour working week. A value set by the DFG.
MCV	Mean corpuscular volume.
MDA	4,4'-Methylenedianiline.
MDI	Methylene diphenyl diisocyanate.
µg/cm²/h	Micrograms of substance per square centimetre per hour. In the context, rate of dermal absorption per area of exposed skin.
mg	Milligram or one thousandth of a gram.
mg/kg	Milligrams per kilogram.
mg/kg b.w. mg/kg bw	Milligram of substance per kilogram body weight.
mg/kg b.w./day mg/kg bw/d	Milligram of substance per kilogram body weight per day.
mg/m³	Milligrams of substance per cubic metre of air.
MHb/MetHb	Methaemoglobin.
mRNA	Messenger ribonucleic acid.
NAEL	No adverse effect level.
NEIL1	Endonuclease VIII-like 1 – these glycosylases initiate the first step in base excision repair by cleaving bases damaged by reactive oxygen species (ROS) and introducing a DNA strand break via the associated lyase reaction.
NEIL2	Endonuclease VIII-like 2 – these glycosylases initiate the first step in base excision repair by cleaving bases damaged by reactive oxygen species (ROS) and introducing a DNA strand break via the associated lyase reaction.
NICNAS	National Industrial Chemicals Notification and Assessment Scheme is the Australian government's regulatory body for industrial chemicals.
NIOSH	The National Institute for Occupational Safety and Health is the United States federal agency responsible for conducting research and making recommendations for the prevention of work-related injury and illness.
NOAEL	No Observed Adverse Effect Level.
NRC	National Research Council: US body governed by the National Academies of Sciences, Engineering, and Medicine.
NTH1/NTHL1	Endonuclease III-like protein 1.

TERM	MEANING
NTP	National Toxicology Program, US Department of Health and Human Services.
OECD	Organisation for Economic Co-operation and Development.
OEL	Occupational Exposure Limit (equivalent to a WES).
OSHA	Occupational Safety and Health Administration, US Department of Labor.
PCR	Polymerase chain reaction.
ppm	Parts of vapour or gas per million parts of air.
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals. An EU program and regulation.
RoC/ROC	Report on Carcinogens.
rsen	A substance that can 'sensitise' the respiratory system, inducing a state of hypersensitivity to it, so that on subsequent exposures, an allergic reaction can occur (which would not develop in non-sensitised individuals). It is uncommon to become sensitised to a compound after just a single reaction to it. A WorkSafe term.
S9	Supernatant fraction obtained from an organ (usually liver) homogenate by centrifuging at 9000g for 20 minutes in a suitable medium; this fraction contains cytosol and microsomes. The microsomes component of the S9 fraction contain cytochrome P450 isoforms (phase I metabolism) and other enzyme activities. The cytosolic portion contains the major part of the activities of transferases (phase II metabolism). The S9 fraction is used in assays to observe the effect of metabolism of drugs and other xenobiotics on the assay endpoint(s).
SCE	Sister Chromatid Exchange.
SCOEL	The Scientific Committee on Occupational Exposure Limits is a committee of the European Commission, established in 1995 to advise on occupational health limits for chemicals in the workplace within the framework of Directive 98/24/EC, the chemical agents directive, and Directive 90/394/EEC, the carcinogens at work directive.
SEN	A notation indicating the substance is a sensitiser. DSEN and RSEN are used in place of SEN when specific evidence of sensitisation by the dermal or respiratory route, respectively, is confirmed by human or animal data. An ACGIH® term.
SIAT	Single injection adjuvant test.
skin	Skin absorption – applicable to a substance that is capable of being significantly absorbed into the body through contact with the skin. A WorkSafe term.
Skin	A notation indicating the potential for significant contribution to the overall exposure, by the cutaneous route, including mucous membranes and the eyes, by contact with vapours, liquids and solids. An ACGIH® term.
SK:SEN	Skin notation indicating the potential for immune-mediated reactions following exposure of the skin. A NIOSH term.
SLS	Sodium lauryl sulphate.
STEL	The 15-minute time-weighted average exposure standard. Applies to any 15-minute period in the working day and is designed to protect the worker against adverse effects of irritation, chronic or irreversible tissue change, or narcosis that may increase the likelihood of accidents. The WES-STEL is not an alternative to the WES-TWA; both the short-term and time-weighted average exposures apply. Exposures at concentrations between the WES-TWA and the WES-STEL should be less than 15 minutes, should occur no more than four times per day, and there should be at least 60 minutes between successive exposures in this range. A WorkSafe term.
TLV®	Threshold Limit Value (see TLV-STEL and TLV-TWA below). An ACGIH® term. Please see the Statement of Position Regarding the TLVs® and BEIs® and Policy Statement on the Uses of TLVs® and BEIs®
TLV-STEL	TLV-Short-Term Exposure Limit; a 15 minute TWA exposure that should not be exceeded at any time during a work day, even if the 8-hour TWA is within the TLV-TWA. An ACGIH® term.

TERM	MEANING
TLV-TWA	TLV – Time-Weighted Average; the TWA concentration for a conventional 8-hour workday and a 40-hour workweek, to which it is believed that nearly all workers may be repeatedly exposed to, day after day, for a working lifetime without adverse effect. An ACGIH® term.
UDS	Unscheduled DNA Synthesis.
WES	Workplace Exposure Standard – WESs are values that refer to the airborne concentration of substances, at which it is believed that nearly all workers can be repeatedly exposed to, day after day, without coming to harm. The values are normally calculated on work schedules of five shifts of eight hours duration over a 40 hour week. A WorkSafe term.
WES-STEL	The 15-minute time-weighted average exposure standard. Applies to any 15-minute period in the working day and is designed to protect the worker against adverse effects of irritation, chronic or irreversible tissue change, or narcosis that may increase the likelihood of accidents. The WES-STEL is not an alternative to the WES-TWA; both the short-term and time-weighted average exposures apply. Exposures at concentrations between the WES-TWA and the WES-STEL should be less than 15 minutes, should occur no more than four times per day, and there should be at least 60 minutes between successive exposures in this range. A WorkSafe term.
WES-TWA	The average airborne concentration of a substance calculated over an eight-hour working day. A WorkSafe term.

Appendix 2: HSNO health-related hazardous substance classifications

This is the full list of all health-related hazardous substances classifications that are listed by the NZ EPA, including those that apply to this substance.

CLASSIFICATION CODE	MEANING
Acutely toxic	
6.1A	Substances that are acutely toxic – Fatal
6.1B	Substances that are acutely toxic – Fatal
6.1C	Substances that are acutely toxic – Toxic
6.1D	Substances that are acutely toxic – Harmful
6.1E	Substances that are acutely toxic – May be harmful, aspiration hazard
Skin irritant	
6.3A	Substances that are irritating to the skin
6.3B	Substances that are mildly irritating to the skin
Eye irritant	
6.4A	Substances that are irritating to the eye
Sensitisation	
6.5A	Substances that are respiratory sensitisers
6.5B	Substances that are contact sensitisers
Mutagens	
6.6A	Substances that are known or presumed human mutagens
6.6B	Substances that are suspected human mutagens
Carcinogens	
6.7A	Substances that are known or presumed human carcinogens
6.7B	Substances that are suspected human carcinogens
Reproductive/developmental toxicants	
6.8A	Substances that are known or presumed human reproductive or developmental toxicants
6.8B	Substances that are suspected human reproductive or developmental toxicants
6.8C	Substances that produce toxic human reproductive or developmental effects on or via lactation
Target organ toxicants	
6.9A	Substances that are toxic to human target organs or systems
6.9B	Substances that are harmful to human target organs or systems
Skin corrosive	
8.2A	Substances that are corrosive to dermal tissue (UN PGI)
8.2B	Substances that are corrosive to dermal tissue (UN PGII)
8.2C	Substances that are corrosive to dermal tissue (UN PGIII)
Eye corrosive	
8.3A	Substances that are corrosive to ocular tissue

Source: www.epa.govt.nz/industry-areas/hazardous-substances/rules-for-hazardous-substances/hazardous-substances-classification-codes

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