5-Chloro-o-toluidine and its hydrochloride: Human health tier II assessment

12 December 2019

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Benzenamine, 5-chloro-2-methyl-	95-79-4
Benzenamine, 5-chloro-2-methyl-, hydrochloride	6259-42-3

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit:**www.nicnas.gov.au**

Disclaimer

ACRONYMS & ABBREVIATIONS

Grouping Rationale

The chemical, 5-chloro-o-toluidine hydrochloride (CAS No. 6259-42-3), is the hydrochloride salt of 5-chloro-o-toluidine (CAS No. 95-79-4; referred to as the parent base in this report). In solution, the hydrochloride salt is expected to dissociate into chloride ion and the parent base. Therefore, these two chemicals are considered to have similar toxicological profiles and are grouped together for purposes of this human health assessment. The speciation of these chemicals in biological fluids is pH dependent, but independent of the original form.

Import, Manufacture and Use

Australian

No specific Australian use, import, or manufacturing information has been identified.

International

The following international uses have been identified through the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers; Galleria Chemica; International Agency for Research on Cancer (IARC, 2000); National Toxicology Program (NTP, 1979a); Substances and Preparations in Nordic countries (SPIN) database; the United States (US) Environmental Protection Agency's Aggregated Computer Toxicology Resource (ACToR); and the US National Library of Medicine's Hazardous Substances Data Bank (HSDB).

The chemicals have reported site-limited uses as intermediates in the production of azo dyes and pigments for dying cotton, silk, nylon and cellulose.

Restrictions

Australian

These chemicals are not directly listed in the *Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP). However, the chemicals can fall under the scope of the following group entry in Schedule 5 (SUSMP, 2019).

Schedule 5:

'Amines for use as curing agents for epoxy resins (unless separately specified in the Schedules)'

Schedule 5 chemicals are described as 'Substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label.' Schedule 5 chemicals are labelled with 'Caution' (SUSMP, 2019).

International

These chemicals are listed under the entry 'Toluidines, their isomers, salts and halogenated and sulfonated derivatives' on the following (Galleria Chemica):

- EU Cosmetics Regulation 1223/2009 Annex II—List of substances prohibited in cosmetic products (EU Cosmetic Regulation);
- New Zealand (NZ) Cosmetic Products Group Standard—Schedule 4: Components cosmetic products must not contain (NZ EPA);
- Health Canada list of prohibited and restricted cosmetic ingredients (Health Canada); and
- 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 (ASEAN Cosmetic Directive).

Existing Worker Health and Safety Controls

Hazard Classification

These chemicals are not listed on the Hazardous Chemical Information System (HCIS) (Safe Work Australia).

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available.

Health Hazard Information

Due to the interconvertability of the parent base (CAS No. 95-79-4) and its hydrochloride salt (CAS No. 6259-42-3), data on both these chemicals are considered relevant for this human health hazard assessment. While the hydrochloride salt may have different properties with regards to local irritation effects, the systemic effects of these chemicals are expected to be similar.

Toxicokinetics

There is limited information on the toxicokinetics of these chemicals. Based on the available information, the chemicals can be absorbed via the oral, dermal and inhalation routes, and metabolised (Birner and Neumann, 1988; HSDB).

Similarly to other primary aromatic amines, the chemicals in this group can potentially be metabolised via N-hydroxylation to form N-hydroxylamine intermediates. These reactive intermediates can undergo co-oxidation reactions with oxy-haemoglobin to form methaemoglobin (Pathak et al., 2016). They can also subsequently form nitrosamines or pro-carcinogenic nitrenium ions which can covalently binding to haemoglobin and DNA, respectively (Sabbioni and Sepai, 1995; Pathal et al., 2016).

The importance of N-hydroxylation is supported by the formation of haemoglobin adduct in vivo. The parent base (~70 mg/kg bw) was orally administered to female Wistar rats and blood was collected after 24 hours. Hydrolysis of the heamoglobin yielded adducts that were confirmed to be the N-hydroxylated derivative. The heamoglobin binding index (HBI) of the parent base was reported as 28, the same as that for structurally related 4-chloro-o-toluidine (CAS No. 95-69-2). The HBI of the chemicals was 28-fold higher in female rats compared to female mice (Birner and Neumann, 1988).

Acute Toxicity

Oral

The available median lethal dose (LD50) values in rats (464–793 mg/kg bw) indicate that the chemicals in this group have moderate acute oral toxicity, warranting hazard classification (see **Recommendation** section).

The lowest LD50 reported for the parent base in rats was 464 mg/kg bw (RTECS) and the highest was 793 mg/kg bw (REACH). Sub-lethal effects were not reported for these studies.

Dermal

Only limited data are available for assessment. Dermal exposure to 1667 mg/kg bw or 1111 mg/kg bw of the parent base in female and male cats, respectively, resulted in 100 % mortality after 3–4 days. Sub-lethal effects were not reported (REACHa).

Inhalation

No data are available for the chemicals.

Observation in humans

No data are available.

A single exposure to related isomer, 4-chloro-o-toluidine (CAS No. 95-69-2) resulted in severe pain when passing urine and haemturia (bloody urine) (Lyons, 1947). Blue lips and fingernails (cyanosis) indicative of low oxygen levels in blood were also reported (Lyons, 1947).

Corrosion / Irritation

Skin Irritation

Based on the available data, the parent base may be slightly irritating to skin. However, the effects were not considered sufficient to warrant hazard classification. No data are available for the hydrochloride salt.

The parent base (in polyethylene glycol) produced mild skin irritation in New Zealand White rabbits (n=3) in a guideline study (Economic Co-operation and Development (OECD) Test Guideline (TG) 404) (REACH). All signs of irritation were completely reversed within 7 days. Scores were reported as mean values for the respective observation periods and not as individual scores for the treated animals. Signs of irritation included the following:

- redness (erythema)—highest mean score was 2, reported for 48 and 72 hour post-application observation periods;
- very slight swelling (oedema)—seen only at 24 hours post-application; and
- dry and brittle skin at the application sites seen 48 and 72 hours post-application.

Eye Irritation

Based on the available data for the parent base, the chemicals in this group are considered to be irritating to eyes, warranting hazard classification (see **Recommendation** section). Although individual occular irritation scores were not available, the effect on corneal opacity was considered sufficient for hazard classification. No data are available for the hydrochloride salt, and no classification is possible.

In a study reported to be conducted in accordance to EU guidelines (B.5) for eye irritation/corrosion, the parent base was found to be irritating to the eyes of Himalayan rabbits (REACH). Application of 100 µL of the parent base to the eyes of rabbits resulted in slight reddening and swelling. Notably, the cornea showed clouding for up to 7 days. All signs of irritation were reversed within 2 weeks. Ocular irritation scores were not reported.

Sensitisation

Skin Sensitisation

No human or animal data are available.

These chemicals contain structural alerts for skin sensitisation (aromatic primary amines). Predicted mechanism include formation of nitroso moieties and nitrenium ions that can react with skin proteins (DEREK Nexus, 6.0). The metabolism of the chemicals to intermediates that are able to bind to proteins is supported by in vivo studies (Sabbioni and Sepai, 1995).

Repeated Dose Toxicity

Oral

Based on the available data for the parent base, the chemicals are not considered harmful following repeated oral exposure. The effects observed in mice were reported as 'preneoplastic' indicators (NTP, 1979) (see **Carcinogenicity** section).

In a 78-week carcinogenicity study (see **Carcinogenicity** section), Fischer 344 rats (50/sex/dose) received 2500 ppm or 5000 ppm of the parent base in diet. This resulted in a calculated daily doses of approximately 210 or 420 mg/kg bw/day, respectively. The control group consisted of 20 male and 20 female untreated rats. Treatment was followed by a 25–26 week observation period. Mean body weights of female rats in both low and high dose treatment groups were reduced compared with controls. Mortality was comparable between treatment and control groups. An increased incidence of fatty metamorphosis of the liver was observed in all treatment groups (male and female rats, low and high dose treatment groups) compared with controls. There were increased incidences of chronic inflammation of the kidney in treated male rats at both doses. No other significant treatment-related effect was observed (NTP, 1979a; IARC 2000).

In a subchronic oral toxicity study, Fischer 344 rats (5/sex/dose) received the parent base in diet resulting in a daily dose of 31.5, 68, 146.5, 315.5 or 680 mg/kg bw/day for 4 weeks. No mortality was observed during the study period. Speckled liver and kidneys were reported even at the lowest dose tested (REACHa).

In a 78-week combined repeat dose and carcinogenicity study (see **Carcinogenicity** section), B6C3F mice (50/sex/dose) received 2000 ppm, or 4000 ppm of the parent base in diet. This resulted in a calculated daily dose of approximately 340 or 680 mg/kg bw/day. The control group consisted of 20 male and 20 female untreated mice. Treatment was followed by a 13-week observation period. Mean body weights of treated mice were reduced compared to controls. Mortality increased with dose for each sex ($p < 0.001$ and $p = 0.039$ for male and female groups, respectively). Observed lesions possibly related to compound administration included hepatocytic cytoplasmic changes in male and female mice in both treatment groups, myelosclerosis (fibrosis of the bones) in treated female mice at both high and low doses and mild glomerulonephritis (kidney inflammation) in both sexes in the high dose treatment group (NTP, 1979a; IARC 2000).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

No in vivo data are available for the chemical. The majority of in vitro studies gave negative results. However, due to limitations with the available data, genotoxicity cannot be ruled out.

In vitro the parent base was negative in:

- Bacterial reverse mutation assays in *Salmonella typhimurium* strains (TA 98, TA100, TA 1535, and TA 1537; using a preincubation procedure) with and without metabolic activation (rat and hamster liver S9) at concentrations up to 666 µg/plate (Haworth et al., 1983, REACHa);
- Microsuspension Ames assay in *S. typhimurium* strains TA97, TA98, TA100, TA102 and TA1535 (using a modified preincubation procedure) with and without metabolic activation at concentrations up to 500 µg/plate (Olaharski et al., 2009);

- Microscreen prophage-induction assay in *Escherichia coli* WP2(λ) with and without metabolic activation (S9) at concentrations up to 4.4 mM (DeMarini and Brooks, 1992);
- Yeast (*Saccharomyces cerevisiae*) RAD54-GFP GreenScreen genotoxicity assay (measures increased induction of DNA repair gene RAD54) at concentrations up to 3.28 mM (Knight et al., 2007);
- Gadd45α GreenScreen assay in human TK6 lymphoblastoid cells (measures increased activation of the growth arrest and DNA damage Gadd45α protein which is induced following DNA damage) without metabolic activation. Concentration information was not available for assessment (Olaharski et al., 2009);
- Mouse lymphoma assay (MLA) (similar to OECD TG 476) using L5178Y Tk+/- cells without metabolic activation at concentrations up to 375 µg/mL. The results with metabolic activation (S9) were inconclusive at concentrations up to 425 µg/mL (Mitchell et al., 1997);
- In vitro micronucleus assay in mouse lymphoma cell line L5178Y Tk+/- with and without metabolic activation. Concentration information was not available for assessment (Olaharski et al., 2009);
- In vitro sister chromatid exchange assay in Chinese hamster ovary (CHO) cells with and without metabolic activation (S9) at concentrations up to 16 µg/mL (Galloway et al. 1987);
- Chromosomal aberration assay in CHO cells with and without metabolic activation (S9) at concentrations up to 16 µg/mL (Galloway et al. 1987) and;
- DNA repair test in hepatocytes isolated from male rat liver at concentrations of up to 100 µM (Yoshimi et al., 1988).

In vitro the parent base was positive in a cell transformation assay in BALB/c-3T3 cells (mouse cell line) at concentrations of up to 2.26 mM (Matthews et al., 1993).

Data on the related isomer 4-chloro-o-toluidine (CAS No. 95-69-2) indicate that it was generally found negative in bacterial reverse mutation assays (NICNAS), including when using pre-incubation procedures, but found positive when using plate incorporation methods (OEHHA, 1997). This isomer is classified for germ cell mutagenicity category 2 (HCIS). Similar behaviour for the chemicals in this assessment is; therefore, not excluded.

In silico these chemicals and their metabolites (in vivo rat liver metabolism and rat liver S9 metabolism) have structural alerts (primary aromatic amine, nitrosoamine, N-hydroxylamines) for DNA binding via nucleophilic substitution and following radical formation (OECD Toolbox v.4.2).

Carcinogenicity

Based on the weight of evidence from available carcinogenicity studies, the chemicals in this group are expected to have carcinogenic properties and warrant hazard classification (see **Recommendation** section).

In an NTP study, the parent base was carcinogenic in B6C3F1 mice, inducing hemangiosarcomas (mostly of adipose tissue) and hepatocellular carcinomas in both sexes. No carcinogenicity was observed in Fischer 344 rats. This data was used by the International Agency for Research on Cancer (IARC) to classify the parent base the chemical, 5-chloro-o-toluidine, as Group 3—not classifiable as carcinogenic in humans. The results from the 78-week NTP bioassays in the two species (see **Repeat Dose Toxicity: Oral** section) are detailed as follows:

- Fischer 344 rats (50/sex/dose) received the parent base in their diet. Treatment was followed by a 25–26 week observation period. In male rats a positive association between dose and the incidence of phaeochromocytomas of the adrenals (control 0/20, low dose 2/49, high dose 7/48, p = 0.019) was identified. However, there was no significant difference in the incidence between treatment groups and the control (NTP, 1979a; IARC, 2000). A similar trend was also reported for the 4-chloro-o-toluidine isomer (p = 0.014; NTP, 1979b). Notably, the statistical power of this study was reduced due to the low number of animals in the control group.
- B6C3F mice (50/sex/dose) received the parent base in their diet. Treatment was followed by a 13-week observation period. In male mice a positive association between dose and incidences of haemangiosarcomas (control 1/20, low dose 11/50, high dose 37/47; p <0.001, Cochran-Armitage trend test) was identified. A significant difference in the incidence between treatment groups and the control was also reported (p <0.001, Fisher's exact test). The incidences of haemangiosarcomas were also increased in female mice (control 0/20, low dose 6/50, high dose 22/43; p <0.001, Fisher's exact test; p <0.001 trend test). Notably, haemangiosarcomas in both male and female treatment groups predominantly originated in the adipose tissue adjacent to genital organs. The incidences of hepatocellular carcinomas were also increased in both male (control 4/20, low dose 19/50, high dose 25/47, p = 0.011, Fisher's exact test; p = 0.007, trend test) and female mice (control 0/20, low dose 19/50, high dose 26/43; p <0.001, Fisher's exact test; p <0.001, trend test) (NTP, 1979a; IARC, 2000).

While a genotoxic mode of action has not been identified for the chemicals in this group, the parent base has been shown to induce cell proliferation which may in turn increase the rate of mutagenesis (Miyagawa et al., 1995). In an in vivo—in vitro replicative DNA synthesis (RDS) assay in hepatocytes from male B6C3F1 mice, a single oral dose of the parent base (50 or 100 mg/kg bw) resulted in increased cell proliferation (increased incidence of [methyl-^{3}H]thymidine-incorporating cells) relative to controls (Miyagawa et al., 1995).

Structurally related chemicals o-toluidine (CAS No. 95-53-4) and 4-chloro-o-toluidine (CAS No. 95-69-2) are classified for carcinogenicity in the HCIS (Category 1A and 1B, respectively). Exposure to these chemicals also resulted in an increased incidence of haemangiosarcomas and hepatocellular carcinomas in animal models (IARC, 2010; IARC, 2012). This suggests that these chemicals may share a common mode of action and in turn similar carcinogenic properties.

Reproductive and Developmental Toxicity

No reproductive or developmental studies are available for the chemicals in this group.

A single oral dose of 200 mg/kg bw of the parent base was reported to inhibit DNA synthesis in mouse testicular tissue by 52 % (Seiler, 1977).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include:

- systemic long-term effects (potential carcinogenicity);
- local effects (eye irritation); and
- systemic acute effects (acute toxicity from oral exposure).

Public Risk Characterisation

Based on the current information available, the intentional inclusion of the chemical in consumer products is not expected. Hence, the public risk from this chemical is not considered to be unreasonable.

However, the public could be exposed to the chemical as an impurity, or following release from dyes (reductive cleavage of azo dyes used in textiles) and pigments manufactured using the chemicals. The risk to the public from the exposure from dyes and pigments may be considered in a further evaluation of these dyes.

Occupational Risk Characterisation

During product formulation, oral, ocular and dermal exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemicals at lower concentrations could also occur while using formulated products containing the chemicals. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical health effects (carcinogenicity, eye irritation and acute toxicity), the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise exposure are implemented. The chemicals should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine the appropriate controls.

The data available support an amendment to the hazard classification in the HCIS (Safe Work Australia) (refer to **Recommendation** section).

NICNAS Recommendation

Assessment of these chemicals is considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Recommendations for additional regulatory controls might be required to limit exposure to the chemical due to its presence as an impurity in, or release due to breakdown from, dyes and pigments. This will be considered in subsequent IMAP assessments of the relevant dye and pigment chemicals.

Regulatory Control

Work Health and Safety

The chemicals are recommended for classification and labelling aligned with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as below. The hazard classifications for Acute Toxicity – Category 4 (H302; Harmful if swallowed), and Carcinogenicity – Category 2 (H351; Suspected of causing cancer) apply to both the parent base (CAS No. 95-79-4) and the hydrochloride salt (CAS No. 6259-42-3). The classification for Eye Irritation – Category 2B (H320; Causes eye irritation) is applicable to the parent base only. This does not consider classification of physical hazards and environmental hazards.

From 1 January 2017, under the model Work Health and Safety Regulations, chemicals are no longer to be classified under the Approved Criteria for Classifying Hazardous Substances system.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Not Applicable	Harmful if swallowed - Cat. 4 (H302)
Irritation / Corrosivity	Not Applicable	Causes eye irritation - Cat. 2B (H320)
Carcinogenicity	Not Applicable	Suspected of causing cancer - Cat. 2 (H351)

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

[*] Existing Hazard Classification. No change recommended to this classification

Advice for industry

Control measures

Control measures to minimise the risk from oral, ocular and dermal exposure to the chemicals should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemicals are used. Examples of control measures that could minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- health monitoring for any worker who is at risk of exposure to the chemicals, if valid techniques are available to monitor the effect on the worker's health;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemicals.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemicals are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of these chemicals has not been undertaken as part of this assessment.

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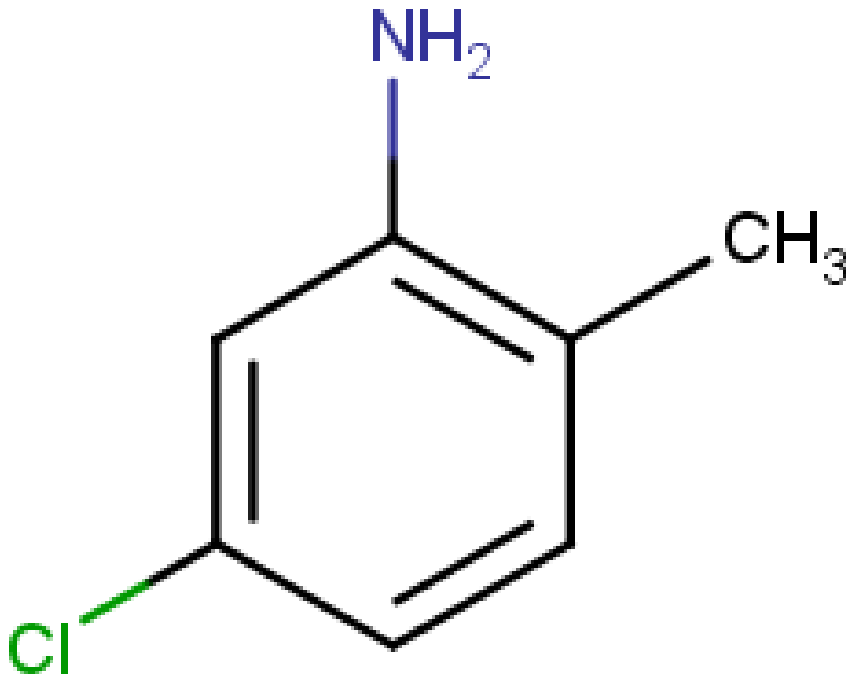
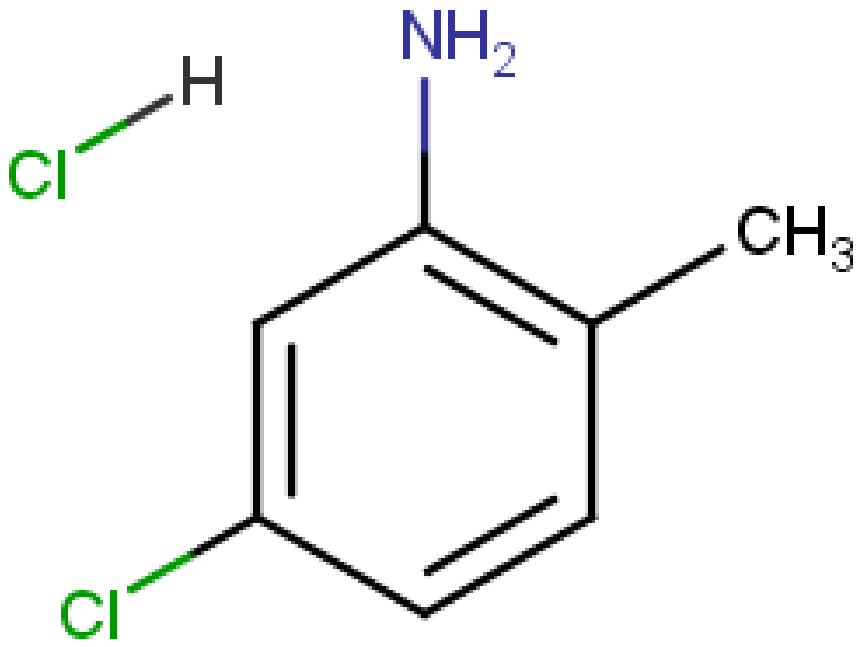
Last Update 12 December 2019

Chemical Identities

Chemical Name in the Inventory and Synonyms	**Benzenamine, 5-chloro-2-methyl-** 5-chloro-o-toluidine 5-chloro-2-methylaniline 2-amino-4-chlorotoluene

CAS Number	95-79-4
Structural Formula	
Molecular Formula	C7H8ClN
Molecular Weight	141.60

Chemical Name in the Inventory and Synonyms	**Benzenamine, 5-chloro-2-methyl-, hydrochloride** 5-chloro-o-toluidinium chloride 2-amino-4-chlorotoluene hydrochloride Fast Red KB salt
CAS Number	6259-42-3
Structural Formula	

	
Molecular Formula	C7H8ClN.ClH
Molecular Weight	178.06

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