para-CHLORO-*ortho*-TOLUIDINE AND ITS STRONG ACID SALTS

This monograph covers *para*-chloro-*ortho*-toluidine and its strong acid salts; data on chemistry, production and use and occurrence are presented only for *para*-chloro-*ortho*-toluidine and its hydrochloride salt, which are the major commercial products and the only forms for which biological data were available.

para-Chloro-*ortho*-toluidine (hydrochloride) was considered by a previous working group (IARC, 1978). Since that time, new data have become available and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms

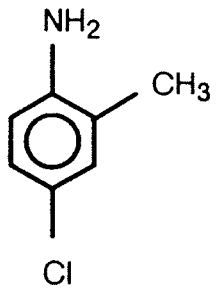
para-Chloro-_ortho_-toluidine

Chem. Abstr. Services Reg. No.: 95-69-2
Chem. Abstr. Name: Benzenamine, 4-chloro-2-methyl-
IUPAC Systematic Name: 4-Chloro-*ortho*-toluidine
Colour Index No.: 37085
Synonyms: 2-Amino-5-chlorotoluene; 3-chloro-6-aminotoluene; 5-chloro-2-aminotoluene; 4-chloro-2-methylaniline; 4-chloro-6-methylaniline; 4-chloro-2-toluidine; 2-methyl-4-chloroaniline

para-Chloro-_ortho_-toluidine hydrochloride

Chem. Abstr. Services Reg. No.: 3165-93-3
Chem. Abstr. Name: Benzenamine, 4-chloro-2-methyl-, hydrochloride
IUPAC Systematic Name: 4-Chloro-*ortho*-toluidine hydrochloride
Colour Index No.: 37085
Synonyms: 4-Chloro-2-methylaniline hydrochloride; C.I. Azoic Diazo Component 11; 2-methyl-4-chloroaniline hydrochloride

1.2 Structural and molecular formulae and molecular weight



C_7H_8ClN Mol. wt: 141.61

Hydrochloride: $C_7H_8ClN.HCl$ Mol. wt: 178.07

1.3 Chemical and physical properties of the pure substance

From Weast (1985), unless otherwise specified

para-Chloro-*ortho*-toluidine

(*a*) *Description*: Leaflets (from ethanol)

(*b*) *Boiling-point:* 241°C

(c) *Melting-point*: 29-30°C

(d) *Spectroscopy data*: Infrared (grating [669]; prism [730H] [17155]; prism-FT [1219B]), ultraviolet [5411] and nuclear magnetic resonance ([558]; [1027B]) spectral data have been reported (Sadtler Research Laboratories, 1980; Pouchert, 1981, 1983, 1985).

(*e*) *Solubility*: Soluble in ethanol

para-Chloro-*ortho*-toluidine hydrochloride

Description: Buff-coloured powder (Currie, 1933); light-pink powder (National Cancer Institute, 1979)

1.4 Technical products and impurities

para-Chloro-*ortho*-toluidine

Trade Names: Daito Red Base TR; Fast Red Base TR; Fast Red 5CT Base; Fast Red TR Base; Fast Red TRO Base; Fast Red TR-T Base; Kako Red TR Base; Mitsui Red TR Base; Red Base NTR; Red TR Base; Sanyo Fast Red TR Base

para-Chloro-*ortho*-toluidine hydrochloride

Trade Names: Azogene Fast Red TR; Devol Red K; Devol Red TR; Echtrot TR Base; Neutrosel Red TRVA

No other data were available to the Working Group.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

para-Chloro-*ortho*-toluidine was first synthesized in 1870 by Beilstein and Kuhlberg by the reduction of 2-nitrotoluene with tin and hydrochloric acid (Prager *et al.*, 1929). It has also been produced by the direct chlorination of *ortho*-toluidine (Schimelpfenig, 1975) and by the chlorination of 2-formylaminotoluene (Grieder, 1977) or 2-acetylaminotoluene followed by hydrolysis or alcoholysis (Society of Dyers and Colourists, 1971). *para*-Chloro-*ortho*-toluidine has been sold commercially both as the free amine and its hydrochloride salt (US Tariff Commission, 1940, 1945). Commercial production of *para*-chloro-*ortho*-toluidine began in Germany in 1924 (Uebelin & Pletscher, 1954) and was first reported in the USA in 1939 (US Tariff Commission, 1940). In Switzerland, *para*-chloro-*ortho*-toluidine and its salts were produced from 1956 through to 1976; production in 1976 was estimated at 100-200 thousand kg (IARC, 1978).

Production of *para*-chloro-*ortho*-toluidine in the USA ceased in 1979, and all importation and distribution of the substance was discontinued in 1986 (US Environmental Protection Agency, 1988); production and distribution in the Federal Republic of Germany were stopped in 1986 (Stasik, 1988). *para*-Chloro-*ortho*-toluidine and its hydrochloride were produced in the UK in 1930 (Currie, 1933); it is not known if they are still produced there. *para*-Chloro-*ortho*-toluidine has not been produced commercially in Japan (IARC, 1978).

(*b*) *Use*

para-Chloro-*ortho*-toluidine and its hydrochloride salt have been used to produce azo dyes for cotton, silk, acetate and nylon and as intermediates in the production of Pigment Red 7 and Pigment Yellow 49 (Society of Dyers and Colourists, 1971; US Environmental Protection Agency, 1988). As an azoic diazo component, *para*-chloro-*ortho*-toluidine is used with naphthol derivatives to form azo dyes *in situ* on fabric and yarns. Dyeing with naphthol dyes takes place in two phases: the textile is first immersed in a solution of azoic coupling component, naphthol, and then allowed to react with azoic diazonium component consisting of an aromatic amine first converted to a diazonium derivative (Priha *et al.*, 1988).

para-Chloro-*ortho*-toluidine has also been used since the 1960s in the manufacture of chlordimeform [N′-(4-chloro-2-methylphenyl)-*N*,*N*-dimethylformamidine; see IARC, 1983], an acaricide and insecticide (Kossmann *et al.*, 1971; Sittig, 1980).

(*c*) *Regulatory status and guidelines*

No regulatory standard or guideline has been established for *para*-chloro-*ortho*-toluidine.

2.2 Occurrence

(a) *Natural occurrence*

para-Chloro-*ortho*-toluidine and its hydrochloride salt are not known to occur as natural products.

(b) *Occupational exposure*

Exposures were reported to occur during the charging of mixing vats and the basification stage at a *para*-chloro-*ortho*-toluidine purification plant in the UK (Currie, 1933). Workers in a batch-operated chemical processing plant (at a bromindigo and thioindigo production area) in the USA were reported to be exposed by inhalation and dermal contact to *para*-chloro-*ortho*-toluidine (Ott & Langner, 1983). Workers were also reported to be exposed to this compound during its production and processing at a plant in the Federal Republic of Germany (Stasik, 1988). In none of these studies were data provided on exposure levels.

para-Chloro-*ortho*-toluidine has been detected in the urine of workers exposed to chlordimeform (Folland *et al.*, 1978; Geyer & Fattal, 1987). It is a major metabolite of chlordimeform in dogs, rats and goats (Knowles, 1970; Watanabe & Matsumura, 1987).

(c) *Food*

para-Chloro-*ortho*-toluidine has been isolated and identified in field samples of different plant materials treated with chlordimeform, at concentrations of less than 0.1 to 0.2 ppm (mg/kg) in young bean leaves, 0.02-0.3 ppm (mg/kg) in grape stems, 0.02-0.05 ppm (mg/kg) in a mixture of grape stems and berries, and less than 0.04 ppm (mg/kg) in prunes and apples (Kossmann *et al.*, 1971). *para*-Chloro-*ortho*-toluidine can also be formed from chlordimeform by enzymes present in the leaves of apple seedlings (Gupta & Knowles, 1969) and in cotton plants (Bull, 1973).

Residues of chlordimeform and its metabolites were measured in rice plants and paddy soil following experimental field application (one to three treatments, with harvesting 42 days after the last treatment). Residue concentrations of *para*-chloro-*ortho*-toluidine were 3-61 ppb (μg/kg) in rice grains, 80-7200 ppb (μg/kg) in straw parts, 2-68 ppb (μg/kg) in the upper layer of soil (0-5 cm) and from trace to 20 ppb (μg/kg) in the lower layer of soil (5-10 cm; Iizuka & Masuda, 1979). In another experimental field application of chlordimeform (one to three treatments, either sprayed or applied to the soil, with analysis 20-55 days after treatment), no residue of *para*-chloro-*ortho*-toluidine (< 0.02 ppm [mg/kg]) was found in rice grains or husks (Fan & Ge, 1982).

2.3 Analysis

Selected methods for the analysis of *para*-chloro-*ortho*-toluidine are given in Table 1.

Table 1. Methods for the analysis of *para*-chloro-*ortho*-toluidine

Sample matrix	Sample preparation[a]	Assay procedure[a]	Limit of detection	Reference
Air	Extract from filter with dichloromethane; evaporate	GC/MS	Not reported	Hunt & Hoyt (1982)
	Collect on membrane filter; desorb with water	HPLC/UV	3 µg/sample	Eller (1985)
Plants, soil	Extract with methanol/hydrochloric acid and methanol/dichloromethane; separate by TLC; treat eluate with acetic acid and sodium hydroxide; diazotize and couple with *N*-ethylnaphthylamine	Colorimetric	0.02-0.03 ppm (mg/kg)	Kossmann *et al.* (1971)
	Steam distill; extract into isooctane; diazotize and couple with *N*-ethyl-1-naphthylamine; clean up by column chromatography	Colorimetric	0.05 ppm (mg/kg)	Geissbühler *et al.* (1971)
Soil	Extract with ethanol; purify by TLC; extract with diethyl ether or dichloromethane	GC/FID and GC/MS	Not reported	Bollag *et al.* (1978)
Rice	Extract with ethanol; clean up on neutral alumina column; elute with ethanol	GC/FID	0.02 ppm (mg/kg)	Fan & Ge (1982)
Solid waste	Extract with dichloromethane; dry with anhydrous sodium sulfate and sonicate	GC/MS	1 ppm (mg/kg)	Wärner *et al.* (1983)
Urine, faeces	Add sodium bicarbonate and hexane; shake and centrifuge; add sulfuric acid to organic layer; shake, centrifuge and separate layers; inject aqueous phase	HPLC	5 ng/ml	Holdiness & Morgan (1983)
Urine	Buffer with ammonia/ammonium chloride; extract with toluene; analyse organic layer by high-performance TLC; visualize by coupling with *N*-ethyl-1-naphthylamine	TLC	6 ng/ml	Sistovaris & Bartsch (1984)
	Extract an alkaline hydrolysate with hexane; evaporate off the solvent; reconstitute residue with an aqueous acetonitrile	HPLC/UV	0.2 mg/l	Geyer & Fattal (1987)

[a]Abbreviations: GC/MS, gas chromatography/mass spectrometry; HPLC/UV, high-performance liquid chromatography/ultraviolet detection; TLC, thin-layer chromatography; GC/FID, gas chromatography/flame ionization detection; HPLC, high-performance liquid chromatography

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

Oral administration

Mouse: As part of a larger carcinogenicity study of several compounds, groups of 25 male and 25 female random-bred CD-1 albino mice (derived from HaM/ICR mice), six to eight weeks of age, were fed dietary levels of 0, 750 or 1500 (males) and 0, 2000 or 4000 (females) mg/kg of diet *para*-chloro-*ortho*-toluidine hydrochloride (97-99% pure) for 18 months and were observed for an additional three months. Haemangiosarcomas or haemangiomas were observed in 13/20 high-dose males, 12/20 low-dose males, 12/16 high-dose females and 18/19 low-dose females, mainly in the spleen and subcutaneous and retroperitoneal adipose tissues. Tumours of these types were not seen in the simultaneous controls but were found in 5/99 male and 9/102 female pooled controls from the larger study (Weisburger *et al.*, 1978).

Groups of 50 male and 50 female B6C3F$_1$ mice, six weeks old, were fed *ad libitum* diets containing 3750 or 15 000 (males) and 1250 or 5000 (females) mg/kg of diet *para*-chloro-*ortho*-toluidine hydrochloride (purity, 99%) for 92 (high-dose females) and 99 weeks, when survivors were killed. A group of 20 male and 20 female untreated mice served as controls. The mean body weights of treated mice were lower than those of the corresponding controls. All males survived beyond 52 weeks; of the females, 49/50 high-dose, 48/50 low-dose and 19/20 controls were still alive at that time, but all high-dose females had died by 92 weeks. Haemangiosarcomas occurred in 3/50 low-dose males, 37/50 high-dose males, 40/49 low-dose females and 39/50 high-dose females, mainly in the fatty tissue adjacent to the genital organs. No such tumour occurred in the untreated controls (National Cancer Institute, 1979).

Rat: Groups of 25 male Charles River CD Sprague-Dawley-derived rats, six to eight weeks of age, were fed 2000 or 4000 mg/kg of diet *para*-chloro-*ortho*-toluidine hydrochloride (97-99% pure) for three months, after which time the doses were reduced to 500 and 1000 mg/kg of diet, respectively, for 15 months. A group of 25 untreated males served as controls. All animals were killed after 24 months. No statistically significant difference in the incidence of tumours was found between treated and control groups (Fisher exact test; Weisburger *et al.*, 1978). [The Working Group noted the small number of rats tested, the low doses used after three months and the relatively short duration of treatment.]

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were fed diets containing 1250 or 5000 mg/kg of diet *para*-chloro-*ortho*-toluidine hydrochloride (purity, 99%) for 107 weeks, at which time all surviving animals were killed. A group of 20 male and 20 female untreated rats served as controls. The mean body weights of the high-dose male and female rats were lower than those of the corresponding controls. Treated animals of each sex lived longer than controls. Chromophobe adenomas of the pituitary gland were observed in 1/19 control, 13/48 low-dose and 15/48 high-dose females [$p = 0.025$; one-sided Cochran-Ar-

mitage test for trend], and 2/19 control, 6/48 low-dose and 15/47 high-dose males ($p = 0.006$; Cochran-Armitage test for trend). In historical controls, pituitary adenomas were observed in 18% of male and 21% of female rats. Adrenal phaeochromocytomas were observed in 0/20 control, 0/49 low-dose and 4/49 high-dose males ($p = 0.014$; Cochran-Armitage test for trend) (National Cancer Institute, 1979). [The Working Group noted the small number and low survival of the controls.]

3.2 Other relevant data

(*a*) *Experimental systems*

(i) *Absorption, distribution, excretion and metabolism*

Following oral administration of [^{14}C-methyl]-*para*-chloro-*ortho*-toluidine to male and female white rats, 71% of the administered radioactivity was eliminated in the urine and 24.5% in the faeces within 72 h (Knowles & Gupta, 1970).

After intraperitoneal administration of 14 mg/kg bw [^{14}C-methyl]-*para*-chloro-*ortho*-toluidine hydrochloride to Osborne-Mendel rats, radiolabel was bound to DNA, RNA and protein of liver; in other tissues, these macromolecules contained little radioactivity. *In vitro*, a phenobarbital-inducible liver microsomal enzyme converted *para*-chloro-*ortho*-toluidine to a reactive metabolite, 5-chloro-2-hydroxyaminotoluene (Hill *et al.*, 1979).

After a single oral administration of 25 mg/kg bw [^{14}C-ring]-labelled *para*-chloro-*ortho*-toluidine hydrochloride to male mice and male Sprague-Dawley rats, the extent of binding to hepatic DNA in mice was about twice as high as that in rats at 6, 12 and 20 h. Two major DNA adducts were formed in both species, but one of these adducts was formed to a much greater extent (six to 30 fold) in mice than in rats. Binding to proteins was more pronounced in rats. Preliminary analysis of metabolite patterns in the urine indicated that *para*-chloro-*ortho*-toluidine was metabolized differently in the two species (Bentley *et al.*, 1986).

Binding of *para*-chloro-*ortho*-toluidine to haemoglobin was observed after oral administration of 85 mg/kg bw to female Wistar rats (Neumann, 1988).

para-Chloro-*ortho*-toluidine inhibited RNA synthesis in HeLa cells (Murakami & Fukami, 1974). It also inhibited thymidine incorporation in mouse testicular DNA *in vivo* (Seiler, 1977).

(ii) *Toxic effects*

The intraperitoneal LD_{50} of *para*-chloro-*ortho*-toluidine hydrochloride was 720 mg/kg bw in male and 680 mg/kg bw in female CD-1 albino mice and 560 mg/kg bw in male and 700 mg/kg bw in female Charles River CD (Sprague-Dawley derived) rats (Weisburger *et al.*, 1978).

Skin applications of 4 g *para*-chloro-*ortho*-toluidine in lard caused haematuria in cats (Lehmann, 1933). [The Working Group noted that the compound now known as *para*-chloro-*ortho*-toluidine was called 5-chloro-*ortho*-toluidine by Lehmann (1933), but that the chemical structures of the two are identical.] Application of 50 mg/kg bw to cats caused mild oedema and congestion of the bladder mucosa in cats; none of the cats, however, showed the

severe haemorrhagic cystitis reported by Lehman (1933) in workers exposed to *para*-chloro-*ortho*-toluidine (Kimbrough, 1980).

In male Sprague-Dawley rats, intraperitoneal injection of *para*-chloro-*ortho*-toluidine increased hepatic cytochrome P450, ethoxyresorufin-*O*-deethylase, ethoxycoumarin-*O*-deethylase, glutathione *S*-transferase and epoxide hydrolase activities. The activities of the 7α, 6β and 16β androstenedione hydroxylase pathways were also increased (Leslie *et al.*, 1988).

(iii) *Effects on reproduction and prenatal toxicity*

No data were available to the Working Group.

(iv) *Genetic and related effects* (see Appendix 1)

para-Chloro-*ortho*-toluidine was assayed in indirect tests for DNA repair using as an indicator the diameter of zones of growth inhibition in DNA repair-proficient and -deficient strains of bacteria. At doses at or above 1000 mg per disc, differential killing was observed in tests with *Salmonella typhimurium* TA1538 and TA1978 and with *Escherichia coli* WP2, WP2*uvr*A, WP67, CM611 and CM571, in the absence of an exogenous metabolic system (Rashid *et al.*, 1984). [The Working Group noted the extremely high doses required to elicit a positive response.]

Conflicting results have been reported with regard to the mutagenicity of *para*-chloro-*ortho*-toluidine to bacteria. It was mutagenic to *S. typhimurium* TA100 in the presence of an exogenous metabolic system from Aroclor 1254-induced rat liver and mouse liver. Liquid pre-incubation assays were less effective in demonstrating the mutagenicity of *para*-chloro-*ortho*-toluidine than standard pour-plate assays. In other studies, it was not mutagenic to strains TA1537 and TA98 in the presence or absence of exogenous metabolic systems (Zimmer *et al.*, 1980), nor to strains TA1535, TA1537, TA98 and TA100, in the presence or absence of an exogenous metabolic system from livers of rats or Syrian hamsters treated with Aroclor 1254 (Haworth *et al.*, 1983), nor to strains TA1537, TA1538, TA98 and TA100 in the absence of an exogenous metabolic system from Aroclor 1254-induced rat liver. The compound did not induce mutagenicity in *E. coli* WP2, WP2*uvr*A, WP67, CM611 or CM571, in the presence or absence of an exogenous metabolic system from Aroclor 1254-induced rat liver (Rashid *et al.*, 1984).

para-Chloro-*ortho*-toluidine caused DNA strand breaks in Chinese hamster V79 cells at 3 mM (Zimmer *et al.*, 1980). It induced sister chromatid exchange in the Chinese hamster CHO cell line *in vitro*, in the presence and absence of an exogenous metabolic system from Aroclor 1254-induced rat liver, but with a greater effect in the absence of activation. The compound induced chromosomal aberrations *in vitro* in CHO cells in the presence of metabolic activation, only at 400 μg/ml (Galloway *et al.*, 1987). It did not induce heritable translocations in SPF NMRI mice at toxic oral doses of 200 mg/kg bw (Lang & Adler, 1982). It was reported not to induce dominant lethal mutations or micronuclei in mice *in vivo* [details not given]. It induced mutations in female C57Bl/6J mice after oral administration to the dams of 100 mg/kg bw (Lang, 1984).

(*b*) *Humans*

(i) *Absorption, distribution, excretion and metabolism*

No data were available to the Working Group.

(ii) *Toxic effects*

Chloroaniline derivatives have been reported to cause haematuria and to affect the bladder mucosa in humans (Currie, 1933; Lehmann, 1933; Folland *et al.*, 1978; Kimbrough, 1980); and haematuria and severe haemorrhagic cystitis have been reported in workers exposed to *para*-chloro-*ortho*-toluidine (Lehmann, 1933). [The Working Group noted that the compound now known as *para*-chloro-*ortho*-toluidine was called 5-chloro-*ortho*-toluidine by Currie (1933) and Lehmann (1933) but that the chemical structures of the two are identical.] Gross haematuria and strangury were observed among workers exposed to *para*-chloro-*ortho*-toluidine in a chemical plant in the UK; most of the 11 patients had suprapubic pain, and all developed symptoms within days after the first exposure. Follow-up examination of three patients within three years of their illness showed that one had had no subsequent bladder trouble, one had had slight cystitis and urethritis and one had a carcinoma of the bladder (Currie, 1933).

(iii) *Effects on reproduction and prenatal toxicity*

No data were available to the Working Group.

(iv) *Genetic and related effects*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

Investigations of the occurrence of bladder tumours among small groups of men exposed to *para*-chloro-*ortho*-toluidine were reported by Currie (1933) and Uebelin and Pletscher (1954); one case of bladder carcinoma was found (Currie, 1933).

Ott and Langner (1983) reported a cohort study of 342 men engaged in the manufacture of organic dyes in the USA between 1914 and 1958. In one area of the plant, involving 117 men in five processes in the production of brom- and thioindigos, there was potential exposure to *para*-chloro-*ortho*-toluidine and other raw materials and intermediates, including *ortho*-toluidine. During follow-up of this subcohort from 1940 to 1975, a nonsignificant excess of cancer deaths occurred (12 observed, 8.0 expected from age-specific US white male mortality rates), and no bladder cancer was observed [expected figure unspecified but estimated to be about 0.5]. [The Working Group noted that the study involved mixed exposures.]

Stasik (1988) re-examined a cohort of 335 male workers in *para*-chloro-*ortho*-toluidine production and processing plants in the Federal Republic of Germany who had been followed up for mortality from 1929 to 1982. No death from bladder cancer was found [expected figure unspecified but estimated to be less than 0.5] (Stasik *et al.*, 1985). The second study was limited to a subcohort of 116 men who had been exposed before 1970 (when improvements in industrial hygiene were introduced) to levels of *para*-chloro-*ortho*-toluidine that

were probably high, and was prompted by the occurrence of two urinary bladder carcinomas among the current work force. Excluding these two cases, six cases of bladder carcinoma were found between January 1983 and June 1986 through hospital and other institutions. These were compared with cancer registration rates for a different region of the Federal Republic of Germany from that in which the plant was located, as there was no cancer registry in the latter area. The expected number was 0.11 based on sex- and age-specific cancer registration rates. The latent periods of the eight tumours ranged from 17 to 38 years. Two of the patients had had haemorrhagic cystitis thought to be due to massive exposure to *para*-chloro-*ortho*-toluidine prior to diagnosis. Cigarette smoking was not thought to be a confounding variable on the basis of the smoking histories of the patients, three of whom were nonsmokers. No quantitative measure of exposure was available, but the predominant exposure was to *para*-chloro-*ortho*-toluidine; exposure to other amines was also possible. [The Working Group noted that the way in which the cases were ascertained and compared could have introduced bias.]

4. Summary of Data Reported and Evaluation

4.1 Exposure data

para-Chloro-*ortho*-toluidine and its hydrochloride have been produced since the 1920s and have been used as chemical intermediates in the manufacture of azo dyes for textiles and pigments and, since the 1960s, in the manufacture of chlordimeform, an insecticide. Occupational exposure can occur during production and use of *para*-chloro-*ortho*-toluidine; however, no data on levels were available. *para*-Chloro-*ortho*-toluidine has been detected as a metabolite of chlordimeform in plants and in humans.

4.2 Experimental carcinogenicity data

para-Chloro-*ortho*-toluidine hydrochloride was tested for carcinogenicity by administration in the diet in two strains of mice and in two strains of rats. It produced haemangiomas and haemangiosarcomas in one strain of mice and haemangiosarcomas in the other. In one study in rats, an increase in the incidence of adrenal phaeochromocytomas was seen in male animals given the high dose.

4.3 Human carcinogenicity data

A mortality study of workers in the manufacture of organic dyes with mixed exposures, including potential exposure to *para*-chloro-*ortho*-toluidine, showed a small, nonsignificant excess of cancers at all sites. Following two reported cases of bladder cancer among workers exposed before 1970 in the production and processing of *para*-chloro-*ortho*-toluidine, who were probably exposed to higher levels than in the previous study, a large excess of bladder carcinoma was found on further follow-up.

4.4 Other relevant data

para-Chloro-*ortho*-toluidine caused bladder irritation and haematuria in men exposed occupationally. It formed DNA adducts in rats and mice and bound to haemoglobin in rats treated *in vivo*.

In a single study, *para*-chloro-*ortho*-toluidine did not induce heritable translocations in mice *in vivo*; in another study, it induced somatic specific locus mutations in mice *in vivo*. In single studies in rodent cells in culture, it caused DNA strand breaks, sister chromatid exchange and chromosomal aberrations. It was mutagenic to bacteria in one study in the presence of an exogenous metabolic system.

4.5 Evaluation[1]

There is *sufficient evidence* for the carcinogenicity of *para*-chloro-*ortho*-toluidine hydrochloride in experimental animals.

There is *limited evidence* for the carcinogenicity of *para*-chloro-*ortho*-toluidine in humans.

In formulating the overall evaluation, the Working Group took note of the fact that any salt of *para*-chloro-*ortho*-toluidine with a strong acid can be expected to behave chemically in a manner similar to the hydrochloride salt in solution and *in vivo*.

Overall evaluation

para-Chloro-*ortho*-toluidine and its strong acid salts *are probably carcinogenic to humans (Group 2A)*.

5. References

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[1]For description of the italicized terms and criteria for making the evaluation, see Preamble, pp. 25-29.

Summary table of genetic and related effects of *para*-chloro-*ortho*-toluidine

Nonmammalian systems													**Mammalian systems**																											
Prokaryotes		Lower eukaryotes				Plants			Insects				*In vitro*														*In vivo*													
													Animal cells								Human cells								Animals							Humans				
D	G	D	R	G	A	D	G	C	R	G	C	A	D	G	S	M	C	A	T	I	D	G	S	M	C	A	T	I	D	G	S	M	C	DL	A	D	S	M	C	A
	+												+[1]		+[1]		+[1]												+[1]	?										

A, aneuploidy; C, chromosomal aberrations; D, DNA damage; DL, dominant lethal mutation; G, gene mutation; I, inhibition of intercellular communication; M, micronuclei; R, mitotic recombination and gene conversion; S, sister chromatid exchange; T, cell transformation

In completing the tables, the following symbols indicate the consensus of the Working Group with regard to the results for each endpoint:

\+ considered to be positive for the specific endpoint and level of biological complexity

+[1] considered to be positive, but only one valid study was available to the Working Group

? considered to be equivocal or inconclusive (e.g., there were contradictory results from different laboratories; there were confounding exposures; the results were equivocal)

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