4-CHLORO-*ortho*-TOLUIDINE

This substance was considered by previous working groups in June 1977 (IARC, 1978), June 1982 (IARC, 1983), March 1987 (IARC, 1987a) and February 1989 (IARC, 1990). Since that time, new data have become available, and these have been incorporated in the monograph and taken into consideration in the evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

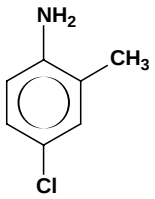
4-Chloro-*ortho*-toluidine

Chem. Abstr. Serv. Reg. No.: 95-69-2
Chem. Abstr. Name: 4-Chloro-2-methylbenzenamine
IUPAC Systematic Name: 4-Chloro-ortho-toluidine
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; *para*-chloro-*ortho*-toluidine; 2-methyl-4-chloroaniline; 2-methyl-4-chlorobenzeneamine

4-Chloro-*ortho*-toluidine hydrochloride

Chem. Abstr. Serv. Reg. No.: 3165-93-3
Chem. Abstr. Name: 4-Chloro-2-methylbenzenamine hydrochloride
IUPAC Systematic Name: 4-Chloro-*ortho*-toluidine hydrochloride
Synonyms: 4-Chloro-2-methylaniline hydrochloride; C.I. Azoic Diazo Component 11; 2-methyl-4-chloroaniline hydrochloride; *para*-chloro-*ortho*-toluidine hydrochloride

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_7H_8ClN Relative molecular mass: 141.6

Hydrochloride

$C_7H_8ClN.HCl$ Relative molecular mass: 178.07

1.1.3 *Chemical and physical properties of the pure substance*

4-Chloro-*ortho*-toluidine

(*a*) *Description*: Crystalline solid (Lewis, 1993)
(*b*) *Boiling-point*: 244 °C (Lide, 1999)
(*c*) *Melting-point*: 30.3 °C (Lide, 1999)
(*d*) *Spectroscopy data*: Infrared (grating [669], ultraviolet [5411], nuclear magnetic resonance (proton [558]) and mass [NIST 69505] spectral data have been reported (Lide & Milne, 1996)
(*e*) *Solubility*: Soluble in ethanol; slightly soluble in carbon tetrachloride (Lide, 1999)
(*f*) *Conversion factor*[1]: $mg/m^3 = 5.79 \times ppm$

4-Chloro-*ortho*-toluidine hydrochloride

(*a*) *Description*: Buff-coloured or light-pink powder (Department of Health and Human Services, 1999)
(*b*) *Conversion factor*[1]: $mg/m^3 = 7.28 \times ppm$

1.1.4 *Technical products and impurities*

Trade names for 4-chloro-*ortho*-toluidine include: Daito Red Base TR; Fast Red Base TR; Fast Red 5CT Base; Fast Red TR Base; Fast Red TR-T Base; Fast Red TRO Base; Kako Red TR Base; Mitsui Red TR Base; Red Base NTR; Red TR Base; Sanyo Fast Red TR Base.

[1] Calculated from: $mg/m^3 = (\text{relative molecular mass}/24.45) \times ppm$, assuming a temperature of 25 °C and a pressure of 101 kPa

1.1.5 *Analysis*

Gas chromatography (GC) with alkali flame ionization, thin-layer chromatography and GC with electron capture detection (ECD)/flame ionization detection have been used to determine 4-chloro-*ortho*-toluidine in food and plant materials (Kossman *et al.*, 1971; Fan & Ge, 1982). GC/ECD and reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet detection have been used to determine 4-chloro-*ortho*-toluidine in human urine samples (Geyer & Fattal, 1987). Liquid chromatography with electrochemical detection has been used to determine haemoglobin adducts of 4-chloro-*ortho*-toluidine (Riffelmann *et al.*, 1995).

1.2 Production

Commercial production of 4-chloro-*ortho*-toluidine began in Germany in 1924 (Uebelin & Pletscher, 1954) and was first reported in the United States in 1939 (Tariff Commission, 1940). It has been sold as both the free amine and the hydrochloride salt (Tariff Commission, 1940, 1945). Production of both forms has been discontinued in most countries (Stasik, 1988; Environmental Protection Agency, 1988).

Information available in 1999 indicated that 4-chloro-*ortho*-toluidine was manufactured by two companies in China (Chemical Information Services, 1999).

1.3 Use

4-Chloro-*ortho*-toluidine and its hydrochloride salt have been used to produce azo dyes *in situ* on 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; Environmental Protection Agency, 1988). 4-Chloro-*ortho*-toluidine was also used from the 1960s until the 1980s in the manufacture of chlordimeform [*N*′-(4-chloro-2-methylphenyl)-*N*,*N*-dimethylformamide; see IARC, 1983], an acaricide and insecticide that is believed to be no longer produced or used worldwide (WHO, 1998).

1.4 Occurrence

1.4.1 *Natural occurrence*

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

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999), 250 chemists employed in health services in the United States were potentially exposed to 4-chloro-*ortho*-toluidine (see General Remarks). Only three laboratory workers were

identified as exposed to 4-chloro-*ortho*-toluidine or its salts by the Finnish Register of Employees Exposed to Carcinogens in 1997 (Savela *et al.*, 1999). No data on the extent of exposure in the manufacture of 4-chloro-*ortho*-toluidine, textile dyes, pigments or insecticide chlordimeform or in the use of 4-chloro-*ortho*-toluidine-based dyes were available to the Working Group.

4-Chloro-*ortho*-toluidine is a major metabolite of chlordimeform and it has been detected in the urine of workers exposed to chlordimeform in packaging and agriculture (Folland *et al.*, 1978; Geyer & Fattal, 1987). Exposure to 4-chloro-*ortho*-toluidine and chlordimeform were measured in the urine of chlordimeform production workers and was reported to be minimal after substantial improvement of working conditions in a chlordimeform-manufacturing plant in 1980 (Popp *et al.*, 1992). Stasik (1991) reported that 4-chloro-*ortho*-toluidine was detected in the urine of two workers at a chemical plant 12 and 24 h after exposure at concentrations of 1.7 and 2.1 mg/L, respectively.

1.4.3 *Environmental occurrence*

The principal source of 4-chloro-*ortho*-toluidine in the environment was as an impurity and as a decomposition (metabolic) product of chlorodimeform. This pesticide is no longer produced (WHO, 1998).

(*a*) *Water*

4-Chloro-*ortho*-toluidine occurred in water as a result of the hydrolysis of chlordimeform via hydrolysis of the intermediate *N*-formyl-4-chloro-*ortho*-toluidine (WHO, 1998).

(*b*) *Soil*

The microbial degradation of chlordimeform in soils by a number of bacterial and fungal species has led to formation of 4-chloro-*ortho*-toluidine (Johnson & Knowles, 1970). When ^{14}C-labelled 4-chloro-*ortho*-toluidine was incubated at a concentration of 5 ppm [mg/kg] in non-autoclaved soil, almost 20% of 4-chloro-*ortho*-toluidine was metabolized to carbon dioxide within 40 days (Bollag *et al.*, 1978).

(*c*) *Plants and foods*

4-Chloro-*ortho*-toluidine has been identified in field samples of plant materials treated with chlordimeform, e.g., in young bean leaves at concentrations of less than 0.1–0.2 ppm [mg/kg], in grape stems at 0.02–0.3 ppm [mg/kg], in a mixture of grape stems and berries at 0.02–0.5 ppm [mg/kg] and in prunes and apples at less than 0.04 ppm [mg/kg] (Kossmann *et al.*, 1971). In an experimental field application (one to three treatments with chlordimeform, harvesting 42 days after last treatment), 4-chloro-*ortho*-toluidine was found in rice grains at 3–61 ppb [µg/kg] and in straw parts at 80–7200 ppb [µg/kg] (Iizuka & Masuda, 1979). 4-Chloro-*ortho*-toluidine was detected as a metabolic product in cotton plants following treatment with chlordimeform (Bull, 1973).

1.5 Regulations and guidelines

4-Chloro-*ortho*-toluidine is listed as a carcinogen in Finland, Germany (Class 1 substances that cause cancer in man) and Switzerland. Switzerland has set an occupational exposure limit (time-weighted average) [8 h time-weighted average] of 12 mg/m^3 with a skin irritation notation for 4-chloro-*ortho*-toluidine (American Conference of Governmental Industrial Hygienists, 1999; Deutsche Forschungsgemeinschaft, 1999).

2. Studies of Cancer in Humans

2.1 Cohort studies

Two epidemiological studies (Ott & Langner, 1983; Stasik, 1988) concerning workers exposed to both 4-chloro-*ortho*-toluidine and *ortho*-toluidine are more fully described in the monograph on *ortho*-toluidine in this volume.

Ott and Langner (1983) studied the mortality of 342 employees assigned to three aromatic amine-based dye production areas from 1914 to 1958 in the United States. One of these areas, the bromoindigo and thioindigo production area, involved potential exposure to *ortho*-toluidine, 4-chloro-*ortho*-toluidine and 4-chloro-acetyl-*ortho*-toluidine. In a separate analysis of 275 individuals not exposed to arsenicals, vinyl chloride or asbestos, no deaths due to bladder cancer were observed, with 1.2 deaths expected from malignant neoplasms of the urinary organs. There were 23 deaths due to malignant neoplasms (17.5 expected; standardized mortality ratio (SMR), 1.3; [95% confidence interval (CI), 0.8–2.0]), 10 of which were coded to digestive organs (5.7 expected; SMR, 1.8; [95% CI, 0.8–3.2]). [The Working Group noted that the conclusions of this study were limited by the small size of the population exposed to 4-chloro-*ortho*-toluidine and the ascertainment of only deceased and not incident bladder cancer cases.]

In a historical mortality study of 335 male employees involved in the production and processing of 4-chloro-*ortho*-toluidine between 1929 and 1982 in Essen, Germany, no deaths from bladder cancer were identified. Four monocyclic amines had been used at the plant: *N*-acetyl-*ortho*-toluidine, 6-chloro-*ortho*-toluidine, *ortho*-toluidine and 4-chloro-*ortho*-toluidine; exposure to 4-chloro-*ortho*-toluidine was reported to be predominant. Urothelial carcinomas were subsequently recorded in eight of the employees between 1967 and 1985, two of whom had died as of December 1986. All eight had been employed in the 4-chloro-*ortho*-toluidine production plant before improvements in industrial hygiene were made in 1970. As a result of this discovery, an incidence study was initiated and the vital status ascertainment for 116 subjects with exposure before 1970 was extended through 1986. The expected number of incident bladder cancers in the cohort of 116 men was 0.11. The standardized incidence ratio (SIR) based on eight

observed cases from 1967 to 1985 was 72.7 (95% CI, 31.4–143) (Stasik, 1988). [The Working Group noted that the definition of the subcohort was made *a posteriori*, but this was justified by the authors'comment that improvements in industrial hygiene were introduced in 1970. It was also unclear in what year the follow-up started. The excess of bladder cancer could not be attributed with certainty specifically to 4-chloro-*ortho*-toluidine or to any one of the other compounds present.]

Popp *et al*. (1992) reported the results of a bladder cancer incidence study conducted among a group of 49 male workers exposed to 4-chloro-*ortho*-toluidine in the synthesis of chlordimeform (evaluated as not classifiable as to its carcinogenicity to humans, Group 3 (IARC, 1987b)) from 1950 to 1986 in a German chemical plant. The period of follow-up was 1950–90. Seven cases of bladder cancer were identified between 1982 and 1990 in workers exposed to 4-chloro-*ortho*-toluidine while synthesizing chlordimeform before 1976, when working conditions were improved. Expected numbers of bladder cancers were calculated based on cancer registry data from the former German Democratic Republic, Denmark and Saarland. SIRs based on the three estimates of expected numbers of deaths were 89.7 (95% CI, 35.6–168.6) (German Democratic Republic), 35.0 (95% CI, 13.9–65.7) (Denmark) and 53.8 (95% CI, 21.3–101.1) (Saarland). No bladder tumours were noted among individuals exposed only to the final product, chlordimeform; however, the size of this group is unknown. The only other aromatic amine to which there was potential exposure was 4-chloroaniline (classified as possibly carcinogenic to humans, Group 2B (IARC, 1993)), which was used for appreciably shorter periods and in smaller quantities than 4-chloro-*ortho*-toluidine. [The Working Group noted that the nature of the facility and the methods of this study were not fully described. Concomitant exposure to chlordimeform and 4-chloroaniline could not be excluded as confounders. The Working Group considered that the excesses of bladder cancer reported were too large to have been due to smoking alone.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 25 male and 25 female Swiss CD-1 mice, four to six weeks of age, were fed 4-chloro-*ortho*-toluidine hydrochloride (97–99% pure) in the diet at dose levels of 0, 750 or 1500 mg/kg diet (ppm) (males) or 0, 2000 or 4000 ppm (females) for 18 months. Animals were kept without treatment for three further months and then killed. The doses were chosen on the basis of preliminary tests, the higher being the maximum tolerated dose. Additional control groups were prepared for the other compounds tested in the study, and tumour incidences of concurrent and pooled controls were compared statistically (both separately and together) with those of treated groups. Animals that

died during the first six months of the study were discarded without necropsy. In male mice, the incidence of vascular tumours (haemangiomas and haemangiosarcomas combined, observed mainly in the spleen and in the subcutaneous or subperitoneal adipose tissue) was 0/14, 5/99, 12/20 ($p < 0.025$, Fisher's exact test) and 13/20 ($p < 0.025$, Fisher's exact test) in concurrent controls, pooled controls, low-dose and high-dose groups, respectively. In female mice, the incidence of vascular tumours (haemangiomas and haemangiosarcomas combined) was 0/15, 9/102, 18/19 ($p < 0.025$, Fisher's exact test) and 12/16 ($p < 0.025$, Fisher's exact test) in concurrent controls, pooled controls, low-dose and high-dose groups, respectively [separate incidences for haemangiomas and haemangiosarcomas were not reported] (Weisburger *et al.*, 1978).

Groups of 50 male and 50 female B6C3F_1 mice, six weeks of age, were administered 4-chloro-*ortho*-toluidine (purity, > 99%) in the diet at concentrations of 3750 or 15 000 ppm (males) and 1250 or 5000 ppm (females) for 99 weeks, except high-dose females which had all died by 92 weeks. Concurrent controls consisted of 20 male and 20 female untreated mice. Mean body weights of all treated groups were lower than those of the corresponding controls and were dose-related. Mortality was dose-related for both males and females ($p < 0.001$, Tarone test for dose-related trend). In male mice, the incidence of haemangiosarcomas (originating in the adipose tissue adjacent to the genital organs) was 0/20, 3/50 and 37/50 ($p < 0.025$, Fisher's exact test) in control, low- and high-dose groups, respectively. In female mice, the incidence of haemangiosarcomas was 0/18, 40/49 ($p < 0.025$, Fisher's exact test) and 39/50 ($p < 0.025$, Fisher's exact test) in control, low- and high-dose groups, respectively (National Cancer Institute, 1979).

3.1.2 *Rat*

Groups of 25 male Sprague-Dawley CD rats, four to six weeks of age, were treated with 4-chloro-*ortho*-toluidine hydrochloride (97–99% pure) in the diet at dose levels of 2000 or 4000 ppm for three months and then at levels of 500 or 1000 ppm for a further 15 months. Animals were kept without treatment for an additional six months and then killed. The doses were chosen on the basis of preliminary tests, the higher being the maximum tolerated dose. A concurrent control group of 25 untreated male rats was used, plus additional controls used for the other compounds tested in the study, and tumour incidences of matched and pooled controls were compared with those of treated groups. Animals that died during the first six months of the study were discarded without necropsy. There was no treatment-related increase in the incidence of tumours at any site (Weisburger *et al.*, 1978). [The Working Group noted the non-standard protocol and that no information on survival was given.]

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were administered 4-chloro-*ortho*-toluidine (purity, > 99%) in the diet at concentrations of 1250 or 5000 ppm for 107 weeks. Concurrent controls consisted of 20 male and 20 female untreated rats. Mean body weights of the high-dose males and females were

lower than those of the corresponding controls. Mortality was not significantly affected by treatment in rats of either sex. At the end of the study, survival in all groups was more than 50% in males and more than 70% in females. No tumours occurred at incidences that could be related to the treatment (National Cancer Institute, 1979).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

In Sprague-Dawley rats treated orally with 4-chloro-*ortho*-[^{14}C]toluidine, 71% of a 3 μCi (specific activity, 1.43 mCi/mmol) dose was excreted in the urine and 24.5% in the faeces after 72 h. In addition to 4-chloro-*ortho*-toluidine, the metabolites 5-chloro-anthranilic acid and 4-chloro-2-methylacetanilide were isolated from the urine. A number of other metabolites were isolated but their chemical structures were not determined. The highest levels of 4-chloro-*ortho*-[^{14}C]toluidine equivalents were found in the fat, liver and kidney (Knowles & Gupta, 1970). 4-Chloro-*ortho*-toluidine was given orally to female Wistar rats and female B6C3F$_1$ mice (0.5 mmol [71 mg]/kg bw) and found to bind to haemoglobin. It was concluded that binding of such monocyclic aromatic amines occurs via a reaction of the respective nitrosoarene metabolites with haemoglobin (Birner & Neumann (1988). In Osborne-Mendel rats administered 4-chloro-*ortho*-[*methyl*-^{14}C]toluidine intraperitoneally at a dose of 14 mg/kg bw, the level of adducts bound to protein, DNA and RNA was significantly higher in the liver than in 10 other tissues examined. In in-vitro studies, the major and minor microsomal metabolites were identified as 5-chloro-2-hydroxyaminotoluene and 4,4′-dichloro-2,2′-dimethylazobenzene, respectively. Binding to microsomal protein was increased in Osborne-Mendel rats pretreated with phenobarbital intraperitoneally at a dose of 100 mg/kg bw for two days, implying that cytochrome P450 is involved in the reaction (Hill *et al.*, 1979). Following oral administration of 25 mg/kg bw 4-chloro-*ortho*-toluidine to male Sprague-Dawley-derived [Tif:RAIf (SPF] rats and male mice [Tif:MAGf (SPF)] both from CIBA-GEIGY, covalent binding to both DNA and protein occurred (Bentley *et al.*, 1986a). Administration of 4-chloro-*ortho*-toluidine to male Sprague-Dawley rats induces a number of enzymes involved in xenobiotic metabolism: cytochrome P450, ethoxyresorufin-*O*-deethylase, ethoxycoumarin-*O*-deethylase, glutathione *S*-transferase and epoxide hydrolase (Leslie *et al.*, 1988).

4.2 Toxic effects

4.2.1 *Humans*

Toxic effects due to 4-chloro-*ortho*-toluidine result from inhalation or skin contact (Stasik, 1991). The first symptom of 4-chloro-*ortho*-toluidine toxicity is macroscopic or microscopic haematuria. Further symptoms include dysuria, reduced bladder capacity and generalized pain in the lower abdomen. Haemorrhagic cystitis is the leading symptom of acute toxicity.

Acute intoxications with 4-chloro-*ortho*-toluidine have occurred in workers in the chemical industry involved in the production and use of the compound. A single dermal exposure was sufficient to produce signs of toxicity. Endoscopic inspection of the urinary bladder revealed necrotic epithelial damage, bleeding and oedema. Methaemoglobinaemia was observed in about 50% of the intoxicated individuals (reviewed by Stasik, 1991). [The Working Group noted the incomplete reporting of these data.]

4.2.2 *Experimental systems*

The following LD_{50} values have been reported for intraperitoneally administered 4-chloro-*ortho*-toluidine: 720 mg/kg bw in male mice, 680 mg/kg bw in female mice, 560 mg/kg bw in male rats and 700 mg/kg bw in female rats (Weisburger *et al*., 1978). Administration of 4-chloro-*ortho*-toluidine in the diet for 99 weeks to B6C3F_1 mice (3750 and 15 000 ppm [mg/kg diet] for male mice; 1250 and 5000 ppm for female mice) led to a high incidence of haemosiderin deposit in the renal tubular epithelium, particularly in mice with haemangiosarcoma (National Cancer Institute, 1979).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

[The Working Group noted that many aromatic amines, including 4-chloro-*ortho*-toluidine, induce methaemoglobinaemia (Watanabe *et al*., 1976; Sachsse *et al*., 1980, cited in WHO 1998; Stasik, 1991; Coleman & Coleman, 1996). The effect of methaemoglobinaemia on fetal development has not been well studied, but may be associated with suboptimal fetal outcome (Fan & Steinberg, 1996; Kilpatrick & Laros, 1999).]

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

The data available up to 1990 and 1993 have been reviewed by IARC (1990) and Jackson *et al.* (1993), respectively.

4-Chloro-*ortho*-toluidine has been tested in a number of laboratories with a range of *Salmonella* strains, and in general the results have been negative. There were single positive results with TA100 and TA98 (with metabolic acivation) and with TA1535 (without metabolic activation). Mutation tests (with and without activation) and DNA repair assays (without activation only) in *Escherichia coli* were negative, while a positive response was observed for differential toxicity in *S. typhimurium*, indicating DNA damage.

However, positive results have been obtained in several tests in mammalian systems, including induction of DNA strand breaks *in vitro*, unscheduled DNA synthesis in rat primary hepatocytes, sister chromatid exchanges and chromosomal aberrations (only in the presence of an external metabolizing system) in Chinese hamster cells *in vitro*, and transformation of BALB/c 3T3 mouse cells. The mouse spot test was also positive. 4-Chloro-*ortho*-toluidine bound to hepatic DNA and RNA in mice and rats *in vivo*, with a greater extent of binding in mice than in rats. In contrast, it gave negative results in both the sister chromatid exchange and chromosomal aberration assays in human lymphocytes *in vitro* and in the mouse heritable translocation assay *in vivo*.

4.5 Mechanistic considerations

Like other aromatic amines 4-chloro-*ortho*-toluidine has been shown to undergo metabolic activation resulting in covalent binding to tissue proteins, DNA and RNA both *in vivo* and *in vitro* (Hill *et al*., 1979; Bentley *et al*., 1986a,b; Birner & Neumann, 1988).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

4-Chloro-*ortho*-toluidine and its hydrochloride salt were produced commercially in substantial amounts as intermediates in the manufacture of azo dyes and chlordimeform, an insecticide. Since the 1980s, production and use of 4-chloro-*ortho*-toluidine have been discontinued in most countries.

5.2 Human carcinogenicity data

Three small cohort studies of workers exposed to 4-chloro-*ortho*-toluidine, one each among dye, 4-chloro-*ortho*-toluidine and chlordimeform production workers, were

Table 1. Genetic and related effects of 4-chloro-*ortho*-toluidine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium, repair-deficient strains, TA1538, TA1978, differential toxicity	+	NT	250 mg/disc	Rashid *et al*. (1984)
Escherichia coli rec strains, differential toxicity	+	NT	1000 mg/disc	Rashid *et al*. (1984)
Salmonella typhimurium TA100, reverse mutation[c]	–	+	17.8 µg/plate	Zimmer *et al*. (1980)
Salmonella typhimurium TA100, reverse mutation[c]	–	–	333 µg/plate	Haworth *et al*. (1983)
Salmonella typhimurium TA1535, TA1537, TA98, reverse mutation[c]	–	–	1000 µg/plate	Haworth *et al*. (1983)
Salmonella typhimurium TA100, TA1537, TA1538, TA98, reverse mutation[c]	–	NT	325 µg/plate	Rashid *et al*. (1984)
Salmonella typhimurium TA100, reverse mutation	–	+	100 µg/plate	Göggelmann *et al*. (1996)
Salmonella typhimurium TA1535, reverse mutation[c]	+	NT	200 µg/plate	Rashid *et al*. (1984)
Salmonella typhimurium TA1535, TA1537, reverse mutation	–	–	1500 µg/plate	Göggelmann *et al*. (1996)
Salmonella typhimurium TA1537, TA98, reverse mutation[c]	–	–	NR	Zimmer *et al*. (1980)
Salmonella typhimurium TA98, reverse mutation	–	+	375 µg/plate	Göggelmann *et al*. (1996)
Escherichia coli WP2 *uvrA*, WP2, other strains, reverse mutation[c]	–	–	2000 µg/plate	Rashid *et al*. (1984)
DNA strand breaks, cross-links or related damage, Chinese hamster V79 lung cells *in vitro*[c]	(+)	NT	534	Zimmer *et al*. (1980)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	14.2	Williams *et al*. (1989)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*[c]	+	+	50	Galloway *et al*. (1987)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*[c]	–	+	400	Galloway *et al*. (1987)
Cell transformation, BALB/c 3T3 mouse cells[c]	+	NT	75	Matthews *et al*. (1993)
Sister chromatid exchange, human lymphocytes *in vitro*	–	–	283	Göggelmann *et al*. (1996)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, human lymphocytes *in vitro*	(+)	(+)	283	Göggelmann *et al.* (1996)
Mouse spot test, C57BL6J×T mice[c]	+		100 po × 3[d]	Lang (1984)
Mouse heritable translocation test, NMRI/SPF mice[c]	–		200 po; 7 d/w, 7 w	Lang & Adler (1982)
Binding (covalent) to DNA, rat and mouse liver *in vivo*[c]	+		25 po × 1	Bentley *et al.* (1986a)
Binding (covalent) to RNA or protein, rat and mouse liver *in vivo*[c]	+		25 po × 1	Bentley *et al.* (1986b)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; NR, not reported
[c] Test performed with the hydrochloride salt of 4-chloro-*ortho*-toluidine
[d] Treatments on days 8, 9 and 10 of embryonic development

available. Two of them showed high relative risks of bladder cancer. Despite problems in the cohort definitions in these two studies, the high relative risks observed for bladder cancer most likely represent a true excess. However, confounding cannot be excluded due to the presence of other exposures including potential bladder carcinogens. The third study had limited power to detect a risk due to use of mortality data only in a small cohort.

5.3 Animal carcinogenicity data

4-Chloro-*ortho*-toluidine or its hydrochloride was tested for carcinogenicity by oral administration in two experiments in mice and in two experiments in rats. The compounds increased the incidence of haemangiosarcomas in the spleen and adipose tissue in both male and female mice, but no increase in the incidence of tumours was observed in rats.

5.4 Other relevant data

4-Chloro-*ortho*-toluidine undergoes extensive metabolism in rodents *in vivo*. Like other aromatic amines, it undergoes metabolic activation via initial formation of the *N*-hydroxy derivative. The further metabolic processing of this metabolite has not been investigated.

In humans, 4-chloro-*ortho*-toluidine induces acute toxicity in the urinary bladder and causes methaemoglobinaemia. In rodents, 4-chloro-*ortho*-toluidine and/or its metabolites bind to macromolecules in liver cells.

4-Chloro-*ortho*-toluidine gave variable results in the majority of bacterial tests for mutagenicity. While most of the mammalian tests were positive, chromosomal aberration assays gave conflicting results. These data overall indicate that 4-chloro-*ortho*-toluidine causes DNA damage in mammalian cells.

5.5 Evaluation

There is *limited evidence* in humans for the carcinogenicity of 4-chloro-*ortho*-toluidine.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-chloro-*ortho*-toluidine.

Overall evaluation

4-Chloro-*ortho*-toluidine is *probably carcinogenic to humans (Group 2A)*.

6. References

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