4-CHLORO-*ortho*-TOLUIDINE

1. Exposure Data

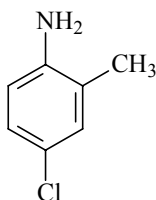## 1.1 Chemical and Physical Data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 95-69-2
CAS Name: 4-Chloro-2-methylbenzenamine
Synonyms: 2-Amino-5-chlorotoluene; 3-chloro-6-aminotoluene; 4-chloro-2-methylaniline; 4-chloro-6-methylaniline; (4-chloro-2-methylphenyl)amine; 4-chloro-2-toluidine; 4-chloro-*ortho*-toluidine; 5-chloro-2-aminotoluene; *para*-chloro-*ortho*-toluidine; 2-methyl-4-chloroaniline; 2-methyl-4-chlorobenzeneamine

1.1.2 *Structural formula, molecular formula, and relative molecular mass*

C_7H_8ClN Rel. mol. mass: 141.60

1.1.3 *Chemical and physical properties of the pure substance* (from Lide, 2008)

Description: Leaves (from alcohol); dark violet solid (Sigma-Aldrich, 2010)
Boiling-point: 244 °C
Melting-point: 30.3 °C
Solubility: Soluble in ethanol; slightly soluble in carbon tetrachloride

1.1.4   *Trade names*

Trade names for 4-chloro-*ortho*-toluidine include: Amarthol Fast Red TR Base; Azoene Fast Red TR Base; Azoic Diazo Componenet 11, Base; Brentamine Fast Red TR Base; Daito Red Base TR; Deval Red K; Deval Red TR; Diazo Fast Red TRA; Fast Red 5CT Base; Fast Red Base TR; Fast Red TR Base; Fast Red TR-T Base; Fast Red TRO Base; Kako Red TR Base; Kambamine Red TR; Mitsui Red TR Base; Red Base Ciba IX; Red Base IRGA IX; Red Base NTR; Red TR Base; Sanyo Fast Red TR Base; Tulabase Fast Red TR.

1.1.5   *Analysis*

4-Chloro-*ortho*-toluidine can be readily detected at the ppb (10^{-9}) level by means of GC/MS methods, with or without prior derivatization. Two recent studies have involved methods for detecting this amine in complex mixtures and as reduction products of azo dyes used as toy colourants. Table 1.1 presents a selection of recent studies on the analysis of 4-chloro-*ortho*-toluidine in various matrices.

1.2   **Production and use**

1.2.1   *Production*

4-Chloro-*ortho*-toluidine is produced by direct chlorination of acetyl-protected *ortho*-toluidine. After removal of the protecting group with base, 4-chloro-*ortho*-toluidine is separated by distillation from the 6-chloro-*ortho*-toluidine isomer (Bowers, 2000).

Commercial production of 4-chloro-*ortho*-toluidine began in Germany in 1924 and was first reported in the United States in 1939 (IARC, 1990, 2000). An IARC Working Group reported that commercial production of 4-chloro-*ortho*-toluidine in the USA stopped in 1979, and all importation and distribution of the compound were discontinued in 1986 (IARC, 1990).

The USEPA (2003, 2007) Inventory Update Rule regulation requires manufacturers and importers of certain chemical substances listed in the TSCA Chemical Substance Inventory to report manufacturing information (aggregate production volumes) for chemicals manufactured (including imported) in amounts of 10 000 pounds or greater (in 1986) or 25 000 pounds or greater (in 2003) at a single site. Table 1.2 presents the aggregate production volumes that were reported for 4-chloro-*ortho*-toluidine.

Available information indicates that 4-chloro-*ortho*-toluidine was produced and/or supplied in research quantities in the following countries: Belgium, China, Hong Kong Special Administrative Region, Japan, Switzerland, the United Kingdom, and the USA (Chemical Sources International, 2010).

Table 1.1. Selected methods of analysis of 4-chloro-*ortho*-toluidine in various matrices

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Water	Evaporate solution; derivatize using heptafluorobutyric acid and heat; add phosphate buffer; extract with methanol	GC/MS	0.4pg	Longo & Cavallaro (1996)
Toy products	Sodium dithionite reductive cleavage of azo dye and analysis of resultant amines	HPLC/UV	< 0.2μg/g	Garrigós *et al.* (2002)
Textiles	Solutions are prepared in dry tetrahydrofuran; add pentafluoropropionic anhydride and heat; cool; dilute with methaol	GC/MS	[2ng/mL]	Narvekar & Srivastava (2002)
Water	Dissolve amine in methanol; dilute aliquots with deionized water	SPE/HPLC	~1ng/mL	Chang *et al.* (2003)
Water	A mixture of 20 amines is dissolved in methanol, diluted to different concentrations for analysis. Other solvents are dichloromethane, ethyl acetate, and methanol/ dichloromethane (50:50).	GC/MS	5ng/mL	Doherty (2005)

GC, gas chromatography; HPLC, high performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; SPE, solid phase microextraction; UV, ultraviolet

1.2.2   *Use*

4-Chloro-*ortho*-toluidine and its hydrochloride salt have been used commercially to produce azo dyes for cotton, silk, acetate, and nylon, and as intermediates in the production of Pigment Red 7 and Pigment Yellow 49. As an azoic diazo component, 4-chloro-*ortho*-toluidine is used in the synthesis of some azoic dyes, which are prepared by a two-step process involving diazotization of a primary amine component and coupling of the diazotized amine with a naphthol-derived coupling component (IARC, 1990, 2000; National Cancer Institute, 1979). Since the 1960s, 4-chloro-*ortho*-toluidine has also been used in the manufacture chlordimeform, a pesticide and acaricide (IARC, 1990). As the hydrochloride salt, 4-chloro-*ortho*-toluidine occurs as a contaminant of chlordimeform. It is also a metabolite of this insecticide, which has recently been banned in several countries (IARC, 1983, 1990, 2000; Bowers, 2000). Chlordimeform is no longer used in the USA since 1989 (EPA, 2007).

Table 1.2. 4-Chloro-*ortho*-toluidine production volumes

Year	Volume (in thousands of pounds)
1986	10–500
1990	10–500
1994	NR
1998	NR
2002	<10
2006	NR

USEPA, 2003, 2007
NR, not reported

1.3   Occurrence and human exposure

1.3.1   *Natural occurrence*

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

1.3.2   *Occupational exposure*

Occupations with the greatest potential for exposure to 4-chloro-*ortho*-toluidine include those involved in the production and use of this chemical as an intermediate for the manufacture of dyestuffs, pigments, and in the production of chlordimeform. Since

4-chloro-*ortho*-toluidine is a major metabolite of this insecticide, exposure can also occur in field workers applying chlordimeform.

(a)   Exposure during the manufacture of 4-chloro-ortho-*toluidine, and 4-chloro*-ortho-*toluidine-based dyes*

Workers in production and processing of 4-chloro-*ortho*-toluidine in Germany were reported to have been exposed to this substance between 1929 and 1982 (Stasik, 1988). The same author reported that 4-chloro-*ortho*-toluidine was detected at concentrations of 1700 and 2100 μg/L, respectively, in the urine of two workers, at 12 and 48 hrs after exposure to [unknown concentrations of] chlordimeform (Stasik, 1991).

In a dye factory in Switzerland, 4-chloro-*ortho*-toluidine was manufactured from *ortho*-toluidine between 1924 and 1953 (Uebelin and Pletscher, 1954); in an organic-dye factory in the USA, 4-chloro-*ortho*-toluidine was used in the thioindigo production-area (Ott & Langner, 1983). No exposure measurements were reported for either study.

(b)   Exposure during the manufacture and use of chlordimeform

4-Chloro-*ortho*-toluidine has been detected in the urine of workers exposed to chlordimeform in the production, packaging and agricultural use of this insecticide.

After substantial improvement of working conditions in a chlordimeform-manufacturing plant in 1980, the concentrations of 4-chloro-*ortho*-toluidine and chlordimeform were measured in the urine of production workers and reported to be minimal (Popp *et al.*, 1992). Quantitative information on concentrations before and after the improvements was not given.

Among employees of a chemical plant, nine of 22 workers who packaged chlordimeform became suddenly ill; chlordimeform and 4-chloro-*ortho*-toluidine were present in their urine three days after exposure (Folland *et al.*, 1978).

Kurtz *et al.* (1987) monitored agricultural workers exposed to chlordimeform used as a pesticide on cotton in California, USA, during the 1982 application season. Chlordimeform and its metabolites were detected in the urine.

Geyer and Fattal (1987) found 4-chloro-*ortho*-toluidine at a concentration of 240 μg/L in the urine of an agricultural worker exposed to chlordimeform in a cotton-growing area.

Among 1000 urine samples from 130 cotton growers, two thirds had no detectable levels of 4-chloro-*ortho*-toluidine. For six mixer/loader/applicators, the concentration was higher than 1 ppm (Coye *et al.*, 1986).

(c)   Other uses

Workers can be exposed to 4-chloro-*ortho*-toluidine during its laboratory use as an immunochemical stain. Under the name Fast Red TR, 4-chloro-*ortho*-toluidine is also reported to be used in a colorimetric method to assess the authenticity of drugs (Green *et al.*, 2000). In a study from Finland (Kauppinen *et al.*, 2003), one of five molecular-

biology laboratories used 4-chloro-*ortho*-toluidine, albeit in very small amounts (< 1 g per year). According to the 1981–83 National Occupational Exposure Survey (NOES, 1983), 250 chemists employed in health services in the United States were potentially exposed to 4-chloro-*ortho*-toluidine.

1.3.3   *Environmental occurrence and exposure in the general population*

The general population can be exposed to 4-chloro-*ortho*-toluidine via the environment and in food products as a decomposition product of chlordimeform. Riffelmann *et al.* (1995) reported concentrations of 4-chloro-*ortho*-toluidine in the urine of occupationally non-exposed smokers ($n = 8$) and nonsmokers ($n = 8$) as 3.0 μg/L (0.0–8.0 μg/L) and 2.2 μg/L (0.0–6.3 μg/L).

(a)   *Water*

4-Chloro-*ortho*-toluidine can occur 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 several bacterial and fungal species has led to formation of 4-chloro-*ortho*-toluidine (Johnson & Knowles, 1970).

(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 < 0.1–0.2 ppm [mg/kg], in grape stems at 0.02–0.3 ppm, in a mixture of grape stems and berries at 0.02–0.5 ppm and in prunes and apples at < 0.04 ppm (Kossmann *et al.*, 1971). In an experimental field application (one, two or three treatments with chlordimeform, harvest 42 days after last treatment), 4-chloro-*ortho*-toluidine was detected in rice grains at 25, 53 and 61 ppb [μg/kg], respectively, and in straw parts at 1600, 7200 and 6900 ppb, respectively (Iizuka & Masuda, 1979). 4-Chloro-*ortho*-toluidine was detected as a metabolic product in cotton plants following treatment with chlordimeform (Bull, 1973) and similarly in cucumbers and apples (Grübner, 1977). 4-Chloro-*ortho*-toluidine could also potentially occur in honey, as chlordimeform is used in some countries against the mite *Varroa jacobsoni* Oud., which seriously affects honey beehives (Jiménez *et al.*, 2002). 4-Chloro-*ortho*-toluidine was not detected in cargo rice and husks sprayed or treated on the soil with chlordimeform (limit of detection, 0.028 ppm) (Fan & Ge, 1982).

(d)   *Finger paint*

Garrigós *et al.* (2000) detected 4-chloro-*ortho*-toluidine in five commercially available fingerpaints at concentrations between 0.3–0.6 ng/g dry paint [total number of

samples not indicated; values read from graph]. In an earlier study, 4-chloro-*ortho*-toluidine was detected in one of three tested fingerpaints (0.3 ng/g dry paint) (Garrigós *et al.*, 1998).

1.4 Regulations and guidelines

1.4.1 *Europe*

(a) *Directive 2002/61/EC*

Directive 2002/61/EC restricts the marketing and use of azo-colourants (European Commission, 2002). In this Directive, Annex I to Directive 76/769/EEC is amended. Azodyes that, by reductive cleavage of one or more azo groups, may release 4-chloro-*ortho*-toluidine in detectable concentrations, i.e. above 30 ppm in the finished articles or in the dyed parts thereof, according to the testing method established in accordance with Article 2a of this Directive, may not be used in textile and leather articles that may come into direct and prolonged contact with the human skin or oral cavity.

(b) *Directive 2004/37/EC*

4-Chloro-*ortho*-toluidine is regulated by Directive 2004/37/EC (European Commission, 2004), which applies to activities in which workers are exposed to carcinogens or mutagens of Category 1 and 2. Rules are fixed regarding the employers' obligations of reduction and replacement, prevention and reduction of exposure, unforeseen exposure, foreseeable exposure, access to risk areas, hygiene and individual protection, information for the competent authority, information and training of workers, consultation and participation of workers, health surveillance, record keeping and limit values.

(c) *Directive 2005/90/EC*

In the Directive 2005/90/EC, the list of substances classified as carcinogenic, mutagenic or toxic to reproduction of Directive 76/769/EEC was amended to include 4-chloro-*ortho*-toluidine (European Commission, 2005).

1.4.2 *Germany*

Deviating from the EU classification, 4-chloro-*ortho*-toluidine is classified as a Category-1 carcinogen by the MAK Commission (MAK, 2007). The MAK Commission listed 4-chloro-*ortho*-toluidine as a substance for which percutaneous absorption may significantly contribute to systemic exposure. A MAK value was not set. Furthermore, it was classified as germ-cell mutagen, Class 3A (Substances that have been shown to induce genetic damage in germ cells of humans or animals, or that produce mutagenic effects in somatic cells of mammals *in vivo* and have been shown to reach the germ cells in an active form).

1.4.3   *Japan*

The Japan Society for Occupational Health (2007) follows the classification by IARC of 4-chloro-*ortho*-toluidine in Group 2A.

1.4.4   *USA*

4-Chloro-*ortho*-toluidine and 4-chloro-*ortho*-toluidine hydrochloride are listed in the NTP Report on Carcinogens as *reasonably anticipated to be human carcinogens* (NTP, 2005).

2. Studies of Cancer in Humans

2.1   Cohort studies

Uebelin & Pletscher (1954) studied the occurrence of bladder tumours in workers at a factory in Switzerland producing dyestuff intermediates. The authors distinguished a group of 35 men who prepared 4-chloro-*ortho*-toluidine from *ortho*-toluidine during the years 1924–1953; no bladder tumours were found among these men [The Working Group noted that insufficient details concerning person-years at risk and period of follow-up were provided to evaluate the significance of this observation].

Ott & Langner (1983) studied the mortality of 342 male employees assigned to three aromatic amine-based dye production areas from 1914 to 1958 at a chemical plant in the United States. All study subjects had some period of employment at the plant after 1st January, 1940. Expected numbers of deaths for the period 1940–1975 were based on mortality rates for US white males. There were no deaths from cancer of the bladder and other urinary organs [SMR, 0.0; 95% CI, 0–2.46]. In one area of the plant, 117 men produced bromindigo and thioindigo with potential exposure to 4-chloro-*ortho*-toluidine and other raw materials and intermediates, including *ortho*-toluidine. No bladder cancer deaths occurred in this subcohort. [The expected figure was unspecified but estimated to be about 0.5.] [The Working Group noted that the interpretation of this study was limited by the small size of the population exposed to 4-chloro-*ortho*-toluidine, and the ascertainment of deaths only.] There was one death from lymphoma [SMR 0.83] and two deaths from leukaemia [SMR 2.22].

Stasik *et al.* (1985) reported findings from a mortality study of 335 male workers employed for at least twelve months in the period 1929–1982 in the production and processing of 4-chloro-*ortho*-toluidine at a dyestuffs-manufacturing plant in Saarland, Germany. Three other monocyclic amines had been used at the plant, *viz N*-acetyl-*ortho*-toluidine, 6-chloro-*ortho*-toluidine and *ortho*-toluidine. Exposure to 4-chloro-*ortho*-toluidine was reported to be predominant. No deaths from bladder cancer were identified in the period 1929–1982 [expected figure unspecified but estimated on the basis of

mortality rates for the Federal Republic of Germany to be about 0.2.] [The Working Group noted that the study had inadequate tracing of deaths and description of methods.] Two incident cases of urothelial carcinoma were subsequently identified in two workers employed in the 4-chloro-*ortho*-toluidine production-plant before improvements in industrial hygiene were made in 1970. As a result of this discovery, a cancer-incidence study was established (Stasik, 1988) for the 116 subjects employed at this plant before 1970. There was no cancer registry for the region in which the plant was located; expected numbers were based, therefore, on rates for Hessen, a neighbouring province of the Federal Republic of Germany. A marked excess of bladder-cancer incidence based on eight cases was reported (SIR, 72.7; 95% CI, 31.4–143.3). No quantitative measure of exposure to 4-chloro-*ortho*-toluidine was available, and exposure to other amines was also present. Cigarette smoking was not considered to be an important confounding variable as three of the eight cases were nonsmokers. [The Working Group noted that the definition of the subcohort for the cancer incidence survey was made *a posteriori,* the observational period was unspecified, and case ascertainment was inadequately described. Consequently, some bias in the estimate of excess risk may be present. The excess of bladder cancer could not be attributed with any certainty 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 among 49 male workers exposed on an irregular basis to 4-chloro-*ortho*-toluidine in the synthesis of chlordimeform from 1965 to 1986 in a German chemical plant. The period of follow-up was stated to be 1950–1990. Expected numbers were available from incidence rates based on cancer registry data from Saarland, Germany. There was a marked excess of bladder tumours based on seven observed cases (SIR, 53.8; 95% CI, 21.7 –110.9), all of which were found in workers exposed to 4-chloro-*ortho*-toluidine before 1976, when working conditions were improved. Some of these workers were also exposed to the aromatic amine 4-chloroaniline (classified as Group 2B, possibly carcinogenic to humans (IARC, 1993)), which was used for appreciably shorter periods and in smaller quantities than 4-chloro-*ortho*-toluidine. No bladder tumours were noted among a further group of 121 individuals exposed only to the final product, chlordimeform. [The Working Group noted that the study methods were not fully described, including allocation of person-years and case ascertainment. Concomitant exposure to chlordimeform and 4-chloroaniline could not be excluded as confounders. The Working Group considered that the excess of bladder cancer reported was too large to have been due to smoking alone.]

3. Studies of Cancer in Experimental Animals

Studies in experimental animals to assess the carcinogenicity of 4-chloro-*ortho*-toluidine, when given orally as the hydrochloride salt, were previously reviewed by IARC (1978, 1983, 1987, 1990, 2000). Those found to be adequate and/or reported more fully in later publications are included in this evaluation.

3.1   Oral administration

3.1.1   *Mouse*

Groups of 25 male and 25 female Swiss CD-1 mice, 6–8 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 mg/kg diet (ppm) (females) for 18 months. Animals were then 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. A parallel control group of 25 untreated mice of each sex was used, plus additional controls used for the other compounds tested in the study. Tumour incidences of matched 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 (5%), 12/20 (60%) ($P < 0.025$, Fisher exact test) and 13/20 (65%) ($P < 0.025$, Fisher 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 (9%), 18/19 (95%) ($P < 0.025$, Fisher exact test) and 12/16 (75%) ($P < 0.025$, Fisher exact test) in concurrent controls, pooled controls, low-dose and high-dose groups, respectively [the 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 given 4-chloro-*ortho*-toluidine hydrochloride (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). The incidence of haemangiosarcomas (originating in the adipose tissue adjacent to the genital organs) was 0/20, 3/50 (6%) and 37/50 (74%) ($P < 0.025$, Fisher exact test) in control, low- and high-dose group males, respectively, and 0/18, 40/49 (82%) ($P < 0.025$, Fisher exact test) and 39/50 (78%) ($P < 0.025$, Fisher exact test) in control, low- and high-dose group females, respectively (National Cancer Institute, 1979).

3.1.2   *Rat*

Groups of 25 male Sprague-Dawley CD rats, 6–8 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, due to toxicity, lowered to 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 dose 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. 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 the lack of information on survival.]

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were given 4-chloro-*ortho*-toluidine hydrochloride (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 treated groups of males was > 75% compared with > 55% in control males, and > 80% in treated females compared with > 75% in control females. No tumours occurred at incidences that could be related to the treatment (National Cancer Institute, 1979).

4. Mechanistic and Other Relevant Data

4.1   Absorption, distribution, metabolism, elimination

4.1.1   *Humans*

No data were available to the Working Group

4.1.2   *Experimental systems*

4-Chloro-*ortho*-[^{14}C]toluidine is a major metabolite of the insecticide chlordimeform, *N′*-(4-chloro-*ortho*-tolyl)-*N,N*-dimethyl-formamidine. Knowles & Gupta (1970) assessed the metabolism of both compounds in Sprague-Dawley rats. Chlordimeform ([^{14}C]-Galecron^R, tolyl methyl-[^{14}C], CIBA Agrochemical Co.) was orally administered to rats. Urinary excretion of [^{14}C]-Galecron after 72 hours represented 88% of a 3-μCi (specific activity, 1.43 mCi/mmol) dose, with 7.5% excreted in faeces. The chloroform-soluble radioactive material in urine (recovery not provided) was separated by means of silica-gel TLC and tentatively identified by co-chromatography with authentic standards.

Besides Galecron, the urine contained demethyl-Galecron, *N*-formyl-4-chloro-*ortho*-toluidine, and 4-chloro-*ortho*-toluidine. Three other metabolites were also observed, but not identified. The highest levels of radioactive equivalents were observed in liver > muscle > fat. In further experiments, [^{14}C]-4-Chloro-*ortho*-toluidine (specific activity, 1.43 mCi/mmol) was also orally administered to Sprague-Dawley rats. Urinary excretion accounted for 71% of the 3-μCi dose and faecal excretion was 25% after 72 hours. Approximately 50% of the radioactivity in urine was recovered in the ethyl-acetate extract and analysed by TLC as described for Galecron above. Besides 4-chloro-*ortho*-toluidine, urinary metabolites included 5-chloroanthranilic acid and 4-chloro-2-methylacetanilide. The latter is an *N*-acetylated metabolite, and its excretion never exceeded that of 5-chloroanthranilic acid. Five unknown metabolites were also indicated by TLC, but not further identified. The highest levels of 4-chloro-*ortho*-[^{14}C]toluidine equivalents were found in the fat > liver > kidney. The aqueous fraction from the urinary extracts was not further analysed. The major pathways proposed for chlordimeform metabolism in the rat are described as follows: *N*-demethylation forms demethyl-chlordimeform; both the latter and chlordimeform are cleaved at the carbon-nitrogen bond to form *N*-formyl-4-chloro-*ortho*-toluidine, which can undergo deformylation to 4-chloro-*ortho*-toluidine; oxidation of the methyl group on 4-chloro-*ortho*-toluidine produces 5-chloroanthranilic acid, while oxidation of *N*-formyl-4-chloro-*ortho*-toluidine produces *N*-formyl-5-chloroanthranilic acid; the latter can undergo deformylation to 5-chloroanthranilic acid.

4-Chloro-*ortho*-toluidine was one of thirteen different monocyclic aromatic amines given orally to female Wistar rats and female B6C3F1 mice (0.5 mmol [71 mg]/kg bw) (Birner & Neumann, 1988). The haemoglobin binding index (HBI) for 4-chloro-*ortho*-toluidine was 11-fold higher (HBI 28) in rats than in mice (HBI 2.5). Twenty-four hours after an oral dose of chlordimeform, 0.5 mmol/kg bw, to female Wistar rats, the HBI for 4-chloro-*ortho*-toluidine was 2.5 (Sabbioni and Neumann, 1990). The latter value is less than one tenth of that reported for 4-chloro-*ortho*-toluidine above in rats. This is because chlordimeform must be metabolized to 4-chloro-*ortho*-toluidine before it can be activated to bind Hb. In contrast to other aromatic amines, *N*-acetylation is not important in the metabolism and activation of 4-chloro-*ortho*-toluidine.

In Osborne-Mendel rats that were given 4-chloro-*ortho*-[*methyl*-^{14}C]toluidine intraperitoneally at a dose of 14 mg/kg bw, the levels of adducts bound to protein (480 pCi), RNA (560 pCi), and DNA (170 pCi) were significantly higher in the liver than in 10 other tissues examined (Hill *et al.*, 1979). In in-vitro studies with liver microsomes, irreversible binding of radioactivity was detected which was dependent upon NADPH and linear with respect to incubation time and protein concentration. Binding to macromolecules *in vitro* was increased in 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. The major and minor microsomal metabolites were identified by mass spectrometry as 5-chloro-2-hydroxyaminotoluene and 4,4′-dichloro-2,2′-dimethylazobenzene, respectively. The former product suggested the presence of an *N*-OH intermediate, which is further oxidized to an *N*-nitroso derivative as indicated by

the latter product. Consistent with the studies described above (Birner & Neumann, 1988; Sabbioni & Neumann, 1990), these authors propose that the *N*-OH derivatives of these monocyclic aromatic amines are co-oxidized within the red blood cell to nitroso metabolites that bind to SH groups in haemoglobin.

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). Three times more radioactivity was shown to be covalently bound to rat liver protein (199 ± 18 pmol/mg) than to mouse-liver protein (62 ± 126 pmol/mg). The results with protein binding are consistent with the study mentioned above, which showed that the Hb-binding index for 4-chloro-*ortho*-toluidine was 11-fold higher in rats than in mice (Birner & Neumann, 1988). However, twice as much liver-DNA binding was detected in the mouse study than in the rat study. Two DNA adducts were observed by two-dimensional TLC on silica-gel plates for both mouse and rat. One adduct was present at six- to 30-fold higher levels in mice than in rats. Furthermore, more radioactivity was observed to bind to calf-thymus DNA when 4-chloro-*ortho*-toluidine was activated with mouse- *vs* rat-liver subcellular fractions. The higher levels of DNA-adduct formation of 4-chloro-*ortho*-toluidine in mice may mechanistically explain the greater susceptibility of the mouse compared with the rat to 4-chloro-*ortho*-toluidine-induced haemangiosarcoma.

The ability of chlordimeform and 4-chloro-*ortho*-toluidine to affect hepatic xenobiotic biotransformation in male Sprague-Dawley rats was assessed (Leslie *et al.*, 1988). Chlordimeform did not affect total CYP content, aniline *p*-hydroxylase or glutathione *S*-transferase activities, but induced ethoxyresorufin-*O*-deethylase, ethoxy-coumarin-*O*-deethylase and epoxide-hydrolase, while decreasing the activities of aldrin epoxidase and aminopyrine *N*-demethylase. These effects were observed after seven consecutive daily intraperitoneal injections of 50 or 100 mg/kg bw, but not with 1 or 10 mg/kg bw chlordimeform, except for a reduction in aminopyrine *N*-demethylase, which was also seen at 10 mg/kg bw chlordimeform. A similar experiment with 7 × 100 mg/kg bw 4-chloro-*ortho*-toluidine increased the CYP content and the activities of ethoxyresorufin-*O*-deethylase, ethoxycoumarin-*O*-deethylase, glutathione *S*-transferase and epoxide hydrolase. At 10 mg/kg bw, 4-chloro-*ortho*-toluidine also increased the activities of ethoxyresorufin-*O*-deethylase and ethoxycoumarin-*O*-deethylase. Both compounds caused an enhanced activity on certain hydroxylation sites of androstenedione and a small decrease in testosterone synthesis. Increased biotransformation by CYPs is likely to involve CYP1A1 and 1A2 (Leslie *et al.*, 1988).

The metabolism of chlordimeform ([^{14}C]-Galecron[R], tolyl methyl-[^{14}C], CIBA Agrochemical Co.) in animals and plants has been reviewed. The cumulative percentages of the dose excreted in 24-hour urine were 75% in mongrel dogs, 65% in a lactating goat, and 80% for a male goat. The percentage of the dose excreted in faeces after 72 hours was 0.6 and 1.8% in dog and goat, respectively. After 24 hours, 25% of radioactive material in rat urine partitioned into chloroform, but < 10% was extractable in organic solvents from dog and goat urine. The amounts of chlordimeform, referred to as chlorphenamidine,

expressed as a percentage of organic-soluble radioactivity at 24 and 72 hours were 9.9% and 2.1% for the rat, 1.3% and 0.2% for the dog, and 0.1% and < 0.1% for the goat. The organic-soluble metabolites in all three species included demethyl-Galecron, *N*-formyl-4-chloro-*ortho*-toluidine, 4-chloro-*ortho*-toluidine, *N*-formyl-5-chloroanthranilic acid, and 5-chloroanthranilic acid. The conversion of chlordimeform to *N*-formyl-5-chloroanthranilic acid, and 5-chloroanthranilic acid was goat > dog > rat. The water-soluble radioactivity was further analysed in dog and goat urine. Treatment with β-glucuronidase or β-glucuronidase-arylsulfatase yielded aglycones tentatively identified by means of TLC as glucuronide or sulfate conjugates. The biliary excretion was also examined in a mongrel dog treated orally with chlordimeform at 0.3 mg/kg bw. After 72 hours, 5% of the dose was recovered in bile, with peak concentrations of radioactivity occurring after eight hours. Metabolites in bile, present in free and conjugated form, included all those mentioned above and several unknown compounds. *N*-formyl-4-chloro-*ortho*-toluidine was the major metabolite in bile. Rat-liver microsomal metabolism of [^{14}C]-Galecron produced demethyl-Galecron, which was sensitive to SKF 525A, but not to diisopropyl-fluorophosphate. Microsomes from the abdomens of houseflies, *Musca domestica L.*, also produced demethyl-Galecron. In addition, [^{14}C]-*N*-formyl-4-chloro-*ortho*-toluidine was converted to 4-chloro-*ortho*-toluidine by a rat-liver soluble fraction, which was inhibited by diisopropylfluorophosphate, but not SKF 525A (Knowles, 1970; Knowles & Gupta, 1970).

4.2   Genetic and related effects (see Table 4.1 for details and references)

Data on the genetic and related effects of 4-chloro-*ortho*-toluidine that were 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 several laboratories with a series of *Salmonella* strains, and the results have been generally negative. There were single positive results with strains TA100 and TA98 (with metabolic activation) 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 the induction of DNA damage.

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 exchange 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*.

4.2.1   *Macromolecular binding*

Bentley *et al.* (1986a) examined the covalent binding of 4-chloro-*ortho*-toluidine to macromolecules in rats and mice, and found that the extent of covalent binding of 4-chloro-*ortho*-toluidine to hepatic DNA in mice was about twice as high as that in rats. This was apparent at all time points, i.e. 6, 12, 28, and 68 hours after a single oral dose of 25 mg/kg bw of [^{14}C]-ring-labelled 4-chloro-*ortho*-toluidine. The extent of covalent binding to DNA decreased with time after the application, indicating that the DNA damage was being repaired, the extent and rate of repair being very similar in both species. Binding, assessed 20 hours after application, was proportional to the total dose given, following repeated daily application of 4-chloro-*ortho*-toluidine for up to five consecutive days in both rats and mice. Two major hydrophobic DNA-adducts were formed in both species, one of these adducts being 6–30-fold more prominent in the mouse than in the rat. The nature of the DNA adducts has not been clarified. Also, more radioactivity was bound to calf-thymus DNA *in vitro* when [^{14}C]-4-chloro-*ortho*-toluidine was activated by mouse-liver subcellular fractions than when rat-liver fractions were used. These findings suggest that mice were more efficient than rats at forming reactive metabolites from 4-chloro-*ortho*-toluidine. However, there was more covalent binding of this compound with hepatic proteins in rats than in mice. Twenty hours after a single application of [^{14}C]-4-chloro-*ortho*-toluidine, the amount bound to hepatic proteins of rats was 199 ± 18 pmol equivalents per mg, which was more than 3 times higher than in mice (62 ± 16 pmol equivalents per mg). The species differences in the metabolism of *4*-chloro-*ortho*-toluidine could account for the fact that mice were more susceptible to the carcinogenic effects of 4-chloro-*ortho*-toluidine.

A similar study conducted by the same group (Bentley *et al.,* 1986b) reported that at all time points after a single administration of [^{14}C]4-chloro-*ortho*-toluidine the extent of binding decreased in the order: protein > RNA > DNA in both species. In-vitro experiments showed that mouse-liver subcellular fractions catalysed the binding of *4*-chloro-*ortho*-toluidine to calf-thymus DNA more readily than did rat-liver fractions. Conversely, binding to protein and RNA was more marked in the rat than in the mouse. It seemed that different patterns of reactive metabolites are formed from *4*-chloro-*ortho*-toluidine in the two species, such that mice produced more DNA-reactive metabolites, while rats produced more metabolites with a high affinity for proteins and RNA.

The high incidence of haemangiosarcomas associated with chronic feeding of 4-chloro-*ortho*-toluidine suggests that blood-vessel endothelial cells are most susceptible to the effects of this compound. Binding of 4-chloro-*ortho*-toluidine to DNA isolated from non-parenchymal cells in mouse liver was investigated, but no preferential DNA-binding in these cells was found (Bentley *et al.*, 1986b). Metabolic activation by microsomal fractions and NADPH were essential for activation, but inducers of several CYP-dependent mono-oxygenases had no demonstrable effect on the rate of DNA binding. Cytosolic enzymes did not alter metabolic activation since the rate of binding to DNA was similar, whether microsomes or S9-supernatant were used for activation. When

Table 4.1. 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 TA100, 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)

Table 4.1 (contd)

Test system	Result[a] Without exogenous metabolic system	With exogenous metabolic system	Dose[b] (LED or HID)	Reference
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)
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.* (1986a,b)

[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
[c] Test performed with the hydrochloride salt of 4-chloro-*ortho*-toluidine
[d] Treatments on days 8, 9 and 10 of embryonic development

added, S9-supernatant cofactors for sulfate, acetate, or glutathione conjugation did not affect the DNA binding. This suggested that activation involving *N*-hydroxylation followed by sulfate conjugation did not happen in this system. Different reactive metabolites may be responsible for binding to DNA and proteins.

Capillary endothelial cells are most probably the target for 4-chloro-*ortho*-toluidine-induced carcinogenesis, but endothelial cells of the aorta (Juchau *et al.*, 1976) and the liver (Baron *et al.*, 1981) were reported to have very low CYP mono-oxygenase activity. This may also be the case for capillary endothelial cells, but endothelial cells are generally active producers of prostaglandins (Moncada *et al.*, 1977). The co-oxidation of several aromatic amines with arachidonic acid was catalysed by prostaglandin synthase (Zenser *et al.*, 1980). Reactive intermediates that bind to calf-thymus DNA may be formed from 4-chloro-*ortho*-toluidine by horse-radish peroxidase in the presence of H_2O_2. Therefore, the peroxidative activation of 4-chloro-*ortho*-toludine may be carried out by prostaglandin synthase in the capillary endothelial cells (Bentley *et al.*, 1986b).

Sabbioni & Neumann (1990) investigated haemoglobin adducts of 4-chloro-*ortho*-toluidine released from pesticides. Female Wistar rats were dosed orally with the pesticide chlordimeform—a precursor of 4-chloro-*ortho*-toluidine—up to 1 mmol/kg bw. Blood was obtained after 24 hours, and haemoglobin isolated and hydrolysed with 1N NaOH. The amines were extracted and quantified by gas chromatography with nitrogen-specific or mass-selective detection. The haemoglobin binding index (binding in nmol/mol Hb, per dose in nmol/kg) of 4-chloro-*ortho*-toluidine was 2.4. This indicated that 4-chloro-*ortho*-toluidine was produced from chlordimeform and bound to haemoglobin in rats.

4.2.2   *Cytogenetic effects*

Galloway *et al.* (1985) developed a screening protocol for detecting chemically-induced cytogenetic changes *in vitro*. This protocol was used to test several chemicals including 4-chloro-*ortho*-toluidine for their ability to induce chromosomal aberrations and sister chromatid exchange (SCE) in Chinese hamster ovary cells, with and without a rat-liver metabolic activation system (S9 mix). The SCE test of 4-chloro-*ortho*-toluidine was positive under conditions of cell-cycle delay; the response was stronger without S9 mix (Galloway *et al.*, 1987). Chromosomal aberrations were not seen without S9 when cells when fixed at 19.5 hours, but this may be due to insufficient allowance for cell-cycle delay, since aberrations were observed on SCE slides in the first metaphase (MI) cells at later fixation times (26–33 hours). With S9, there was a high frequency of aberrations in cells fixed at 19.5 hours (37% aberrant cells; 1.4 aberrations per cell) at the high dose of 400 μg/ml. A high frequency of endoreduplicated mitoses (over 20% of the mitotic cells) was noticed in all of the treated cultures (Galloway *et al.*, 1987).

4.2.3   *Mutagenicity*

Zimmer *et al.* (1980) tested the mutagenicity and DNA-damage induction by several substituted anilines including 4-chloro-*ortho*-toluidine, and found that this amine caused mutations in a dose-dependent manner with the *Salmonella*/microsome mutagenicity assay with tester strain TA100 (but not TA98), with metabolic activation by S9 mix. When the DNA damage in Chinese hamster lung fibroblast (V79) cells was measured by alkaline elution as described by Swenberg *et al.* (1976), 4-chloro-*ortho*-toluidine caused a slight increase in the elution of DNA from the filters following a 2-hour exposure and a significant increase when the incubation time was 4 hours.

Lang and Adler (1982) studied the mutagenic potential of 4-chloro-*ortho*-toluidine in the mouse heritable translocation assay. The maximal tolerated dose of the compound was given daily to male mice, by gavage, for seven consecutive weeks. After mating with untreated females, about 1000 F1 male offspring per group including a vehicle-control group and a positive control (tretamine, TEM) group were tested for their reproductive performance by use of a sequential decision procedure on litter sizes to select males with translocation heterozygosity. Partially sterile, sterile, and non-classifiable F1 males were examined cytogenetically by scoring chromosomes for translocation multivalents or analysing mitotic divisions for marker chromosomes. 4-Chloro-*o*-toluidine tested at doses that showed toxic effects did not induce translocation heterozygosity.

The mutagenic potential of 4-chloro-*ortho*-toluidine was further tested with the mammalian spot test. Female C57BL/6J mice were mated to T-stock males that had been treated by gavage with a maximal tolerated dose of 4-chloro-*ortho*-toluidine. Mutation induction was monitored in the off-spring by checking the fur of the young mice for coloured spots that resulted from expression of a recessive gene involved in coat-colour determination. It was found that 4-chloro-*ortho*-toluidine was mutagenic in the spot test, which is in line with in-vitro experiments and with its carcinogenic potential in the mouse. However, its precursor chlordimeform and another metabolite of chlordimeform, *N*-formyl-4-chloro-*ortho*-toluidine, were negative in this assay (Lang, 1984).

4-Chloro-*ortho*-toluidine is a metabolite of chlordimeform, a pesticide. The bacterial mutagenicity of chlordimeform, its metabolite 4-chloro-*ortho*-toluidine, and another metabolite, 4-chloro-*N*-formyl-*ortho*-toluidine, was evaluated (Rashid *et al.*, 1984). It was found that 4-chloro-*ortho*-toluidine did not cause mutation in strains TA98, TA1537, TA1538 and TA100, with or without S9 activation. However, 4-chloro-*ortho*-toluidine caused a dose-dependent response in tester strain TA1535. At a concentration of 325 μg/plate the number of revertant colonies was almost threefold increased over the control. At > 400 μg/plate, the number of revertant colonies declined. The same authors reported that 4-chloro-*ortho*-toluidine did not cause mutation in tryptophan-dependent *E. coli* WP2, with or without activation by rat-liver microsomal enzymes. However, 4-chloro-*ortho*-toluidine was active in inducing DNA damage in the *S. typhimurium* TA1538/TA1978 and *E. coli* multirepair-deficient systems (Rashid *et al.*, 1984).

Hamzah & Eltorkey (1995) studied the mutagenicity of aromatic amines including 4-chloro-*ortho*-toluidine, *ortho*-toluidine, 4,4'-methylene dianiline (MDA), 4,4'-methylene-bis(2-chloroaniline) (MOCA) and its three possible substitutes, ethacure 300, cyanacure, and polacure 740M in *Salmonella typhimurium* tester strains TA98 and TA100. *ortho*-Toluidine, 4-chloro-*ortho*-toluidine, MDA, MOCA and its substitutes ethacure 300 and cyanacure showed mutagenic activity, while polacure 740M showed no mutagenic activity. All of the mutagens caused an increase in ethoxyresorufin *O*-deethylase (EROD) activity, while polacure 740M showed no appreciable increase in EROD activity. Thus, there was excellent correlation between mutagenicity and EROD induction. [The ability of a chemical to induce EROD activity has been suggested to bear some relationship to its carcinogenic potential.]

Göggelmann *et al.* (1996) studied the genotoxicity of 4-chloro-*ortho*-toluidine in *S. typhimurium*, human lymphocytes, and V79 Chinese hamster cells. In the absence of a metabolizing system (S9 mix), 4-chloro-*ortho*-toluidine did not induce mutations in *S. typhimurium* or chromosome aberrations and sister chromatid exchange in human lymphocytes. It also did not induce spindle disturbances in V79 Chinese hamster cells. In the presence of S9 mix, 4-chloro-*ortho*-toluidine induced revertants in *S. typhimurium* strains TA98 and TA100, but it was inactive in producing structural or numerical chromosomal changes in mammalian cells.

Sekihashi *et al.* (2002) conducted a comparative investigation of some carcinogenic chemicals including 4-chloro-*ortho*-toluidine in multiple organs from mice and rats, using the single-cell gel electrophoresis (Comet) assay. The species difference in genotoxic sensitivity was analysed at an equitoxic level but not at an equidose. Groups of four mice or rats were treated intraperitoneally or orally with a test chemical at the highest dose that did not cause death or distinct toxic effects. Mice were treated with 600 mg/kg bw, rats with 500 mg/kg bw. The stomach, colon, liver, kidney, bladder, lung, brain, and bone marrow were sampled at 3, 8, and 24 hours after treatment. Significantly increased DNA migration was observed in the liver (both species), kidney (rats only) and bladder, lung and brain (mouse only).

4-Chloro-*ortho*-toluidine induced chromosomal aberrations and sister chromatid exchange in Chinese hamster ovary cells (Galloway *et al.*, 1987) and gave conflicting results for mutagenicity in *S. typhimurium* strain TA1535 (negative, Haworth *et al.*, 1983; positive, Rashid *et al.*, 1984) (see Jackson *et al.*, 1993).

4.3   Mechanistic considerations

As a general rule, *ortho*-alkylsubstitution increases the biological activity of aniline, as does blocking the *para*-position relative the amino-group. *ortho*-Toluidine has a higher biological activity than aniline, and 4-chloroaniline is also more active than aniline. It could therefore be expected that 4-chloro-*ortho*-toluidine is exceptionally potent among the monocyclic aromatic amines. This is not really the case as far as carcinogenicity in rats is concerned. Two cancer bioassays were considered negative (IARC, 2000). In mice,

however, the typical hemangiosarcoma and hemangioma in spleen developed at doses of 2000 and 4000 mg/kg diet (ppm), i.e. considerably lower doses than with *ortho*-toluidine. All three of these amines are acutely toxic and produce methaemoglobin and Heinz bodies as well as anaemia upon chronic administration. Toxic effects are seen in the spleen, liver and kidney, the LD50s in rats being in a range comparable with *ortho*-toluidine and 4-chloro-*ortho*-toluidine (600–1300 mg/kg bw, oral dose), but lower than 4-chloro-aniline (300–400 mg/kg bw). Excessive binding to haemoglobin reflects the greater toxicity of 4-chloro-aniline. The haemoglobin binding index is over 300, as compared to 4 with *ortho*-toluidine and 28 with 4-chloro-*ortho*-toluidine. However, it is not clear how acute toxicity participates in the carcinogenic process and how to explain the difference between the formation of rodent sarcoma and the human bladder tumours.

Evaluating the available information, the Working Group determined that 4-chloro-*ortho*-toluidine has no properties that would elevate it from the group of monocyclic aromatic amines. The toxicological profile rather supports the idea of a common mode of action for the aromatic amines in general and the monocyclic amines in particular.

5. Summary of Data Reported

5.1   Exposure data

4-Chloro-*ortho*-toluidine and its hydrochloride derivative are not known to occur naturally. 4-Chloro-*ortho*-toluidine is used primarily in the manufacture of organic colourants. Traditionally, it has been used in the manufacture of the acaricide and insecticide chlordimeform.

Occupational exposures to 4-chloro-*ortho*-toluidine occur predominantly among workers involved in its production or use in the manufacture of organic colorants (dyes and pigments) and of chlordimeform. 4-Chloro-*ortho*-toluidine was detected at levels of 1700–2100 µg/L in the urine of workers involved in the production of this amine. Other occupational exposures to 4-chloro-*ortho*-toluidine occur among workers in research laboratories who use this amine as a biological stain or in colorimetry.

The general population can be exposed to 4-chloro-*ortho*-toluidine as a contaminant in certain consumer products. 4-Chloro-*ortho*-toluidine has been detected at ppm levels in field samples of plant-based foods (e.g. bean leaves, grape stems, and berries) and cotton plants treated with chlordimeform. Similar levels have been found in finger paints.

5.2   Human carcinogenicity data

Three small cohort studies of workers exposed to 4-chloro-*ortho*-toluidine were available: one among dye production workers in the USA, and two studies of 4-chloro-*ortho*-toluidine production workers in Germany. The US study did not show an excess of bladder cancer, but the study was small and had limited power to detect any excess. The

two German studies showed high relative risks of bladder cancer. Co-exposure to *ortho*-toluidine could not be excluded as the cause of the excess risk in the 4-chloro-*ortho*-toluidine production workers.

5.3   Animal carcinogenicity data

4-Chloro-*ortho*-toluidine was tested for carcinogenicity by oral administration of its hydrochloride salt in two experiments in mice and in two experiments in rats. A significant increase of hemangiosarcomas or hemangiomas was observed in both sexes of two strains of mice that received chronic dietary administration of 4-chloro-*ortho*-toluidine hydrochloride. 4-Chloro-*ortho*-toluidine hydrochloride, however, was not found to be a carcinogen when administered chronically in the diet to two strains of rats.

5.4   Other relevant data

The toxicokinetics of 4-chloro-*ortho*-toluidine were studied in experimental animals by administering the compound itself or the insecticide chlordimeform, which is metabolized to 4-chloro-*ortho*-toluidine.

When 4-chloro-*ortho*-toluidine was given orally to rats and mice the haemoglobin binding index was 11-fold higher in the rat than in the mouse. After intraperitoneal administration of 4-chloro-*ortho*-toluidine to rats, the level of adducts bound to protein and DNA was higher in the liver than in ten other tissues.

In studies with liver microsomes isolated from rats treated with radiolabelled 4-chloro-*ortho*-toluidine, irreversible binding of radioactive material to macromolecules was detected, which was increased in rats pretreated with phenobarbital. The major and minor microsomal metabolites were identified as 5-chloro-2-hydroxy-aminotoluene and 4,4'-dichloro-2,2'-dimethylazobenzene, respectively. The former product suggests the presence of an *N*-OH intermediate, which is further oxidized to an *N*-nitroso derivative. *N*-Oxidized metabolites of 4-chloro-*ortho*-toluidine bind to protein, RNA and DNA. Bacterial mutagenicity tests with 4-chloro-*ortho*-toluidine gave predominantly negative results, but tests with cultured mammalian cells gave positive results. *In vivo*, 4-chloro-*ortho*-toluidine binds to hepatic DNA in mice and rats; the damage seems to be repaired rapidly and the structure of the adducts has not been elucidated. The only positive in-vivo mutagenicity test reported is a mouse spot test.

There are no data on toxicokinetics or genetic effects of 4-chloro-*ortho*-toluidine in humans.

6. Evaluation

6.1   Cancer in humans

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

6.2   Cancer in experimental animals

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

6.3   Overall evaluation

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

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