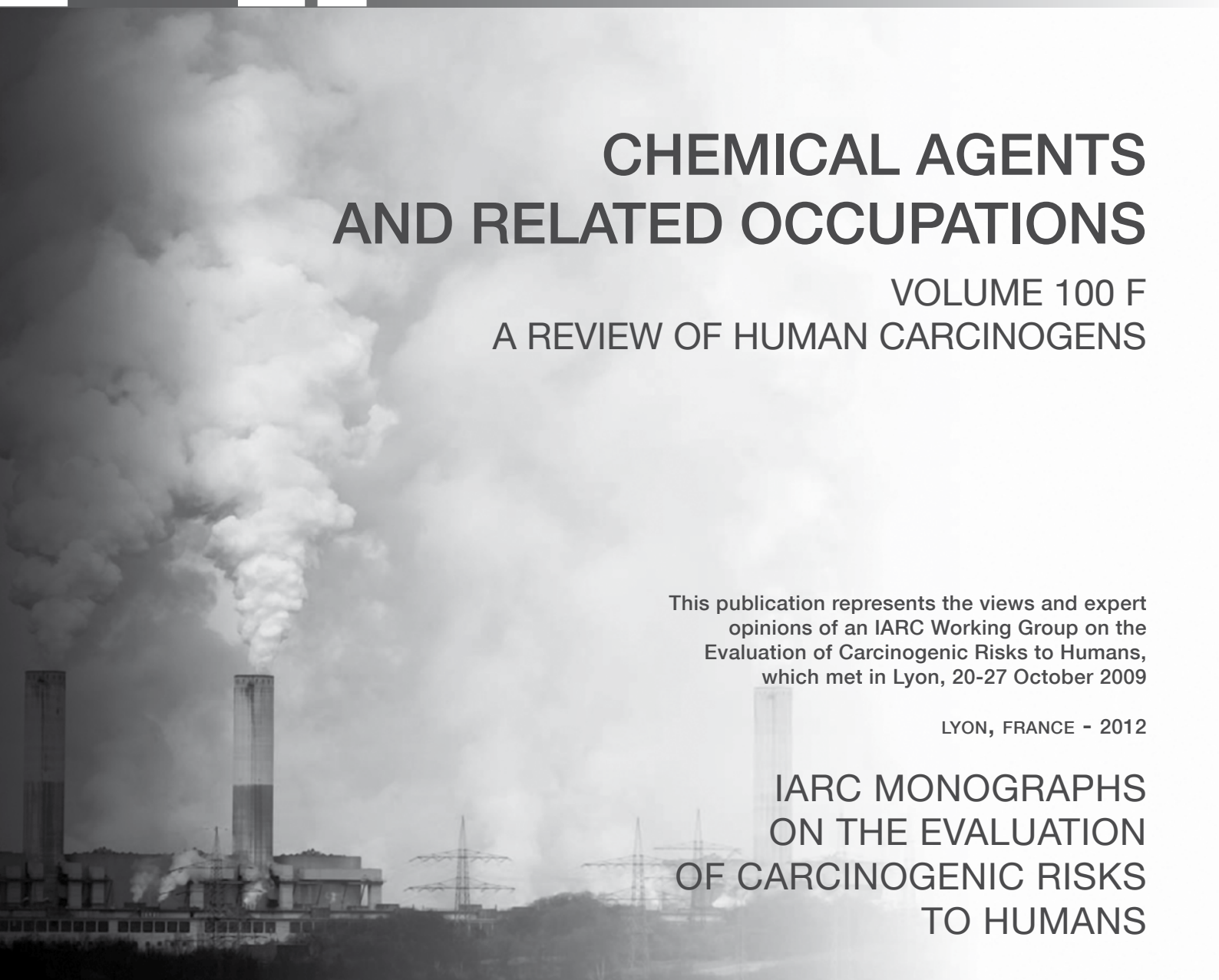
IARC MONOGRAPHS



CHEMICAL AGENTS AND RELATED OCCUPATIONS

VOLUME 100 F
A REVIEW OF HUMAN CARCINOGENS

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 20-27 October 2009

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer
World Health Organization

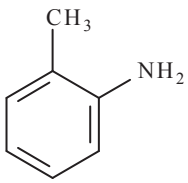
ortho-TOLUIDINE

ortho-Toluidine was considered by previous IARC Working Groups in 1977, 1981, 1987, 2000, and 2008 (IARC, 1978, 1982, 1987, 2000, 2010). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

From IARC (2010), unless indicated otherwise
Chem. Abstr. Serv. Reg. No.: 95-53-4
Chem. Abstr. Serv. Name:
2-Methylbenzenamine



C_7H_9N
Relative molecular mass: 107.15
Description: Light yellow liquid becoming reddish brown on exposure to air and light
Boiling-point: 200–202 °C (O'Neil, 2006)
Solubility: Slightly soluble in water; soluble in alcohol, ether, and dilute acids

1.2 Uses

ortho-Toluidine is used as an intermediate in the synthesis of the large-volume herbicides, metolachlor and acetochlor, in the manufacture of more than 90 dyes and pigments (e.g. acid-fast dyestuffs, azo pigment dyes, triarylmethane dyes, sulfur dyes, and indigo compounds), and as an intermediate for synthetic rubber and rubber-vulcanizing chemicals, pharmaceuticals, pesticides, and other chemicals. *ortho*-Toluidine is also used in the clinical laboratory as an ingredient in a reagent for glucose analysis, and for tissue staining (IARC, 2010; NTP, 2004).

1.3 Human exposure

1.3.1 Occupational exposure

Occupational exposure to *ortho*-toluidine can occur by inhalation or skin contact during its production, or during the production of dyes, pigments and rubber chemicals manufactured from this chemical. Laboratory and medical personnel may be exposed when using *ortho*-toluidine for staining tissues (IARC, 2010).

From the US National Occupational Exposure Survey (1981–83) it was estimated that 30000 workers, including approximately 15500 women, were potentially exposed to *ortho*-toluidine (NIOSH, 1990). No estimates of the number of exposed workers in the European Union have been reported.

At a chemical plant in the former Soviet Union where *ortho*-toluidine was produced via reduction of *ortho*-nitrotoluene, workers were exposed to concentrations of *ortho*-toluidine in the air that generally exceeded the maximum permissible concentration [of 3 mg/m^3, IARC (1982)] by 2–7-fold. In a total of 215 air samples, the highest exposure levels were observed during distillation and extraction processes (25–28.6 mg/m^3). Dermal exposures also were documented (Khlebnikova *et al.*, 1970). Measurements in the 1940s in a US dye-production plant indicated that the concentration of *ortho*-toluidine was < 0.5 ppm [2 mg/m^3] in the workroom air and in the breathing zone of the workers, and < 0.3–1.7 mg/L in the urine of workers engaged in the production of thioindigo (Ott & Langner, 1983). Exposure to *ortho*-toluidine was also reported to occur in plants involved in dye-production in Italy (Rubino *et al.*, 1982), Germany (Stasik, 1988), and the USA (New Jersey) (Delzell *et al.*, 1989), but no data on exposure levels were provided.

Concentrations of *ortho*-toluidine in indoor air in plants producing rubber antioxidants or vulcanising rubber articles ranged up to several hundred µg/m^3 and *ortho*-toluidine concentrations in post-shift urine samples were around 100 µg/L (Ward *et al.*, 1991; Teass *et al.*, 1993; Ward *et al.*, 1996; Korinth *et al.*, 2006).

Medical and laboratory personnel also are potentially exposed to *ortho*-toluidine, although air concentrations are reportedly low (EPA, 1984; Kauppinen *et al.*, 2003).

1.3.2 Non-occupational exposure

Significant non-occupational exposures to *ortho*-toluidine may result from the use of some hair dyes, the local anaesthetic prilocaine, or tobacco smoke. In a study from Turkey (Akyüz & Ata, 2008), *ortho*-toluidine was found in 34 of the 54 hair dyes tested, at levels up to 1547 µg/g. Prilocaine, a widely used anaesthetic, is metabolized to *ortho*-toluidine. In 25 patients who received local anaesthesia, the average amount of *ortho*-toluidine adducts to haemoglobin (Hb) increased 6–360-fold, from 0.54 ± 0.95 ng/g Hb before treatment to 22 ± 13.2 ng/g Hb at 24 hours after surgery (Gaber *et al.*, 2007). *ortho*-Toluidine has been measured in mainstream cigarette smoke at 9–144 ng per cigarette (Stabbert *et al.*, 2003), and concentrations in urine of smokers are higher than in non-smokers (Riffelmann *et al.*, 1995; Riedel *et al.*, 2006). *ortho*-Toluidine has also been detected in surface waters and industrial effluents (Shackelford & Keith, 1976; Neurath *et al.*, 1977; EPA, 1984; NTP, 2004), in vegetables such as kale, celery and carrots, in the volatile aroma of black tea (Vitzthum *et al.*, 1975; Neurath *et al.*, 1977), and in breast milk (DeBruin *et al.*, 1999), but levels are generally very low.

2. Cancer in Humans

Several cohort studies have been conducted among workers potentially exposed to *ortho*-toluidine (Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-06-Table2.1.pdf). Rubino *et al.* (1982) reported excess bladder-cancer risks in relation to *ortho*-toluidine exposure, however, other exposure to potential bladder carcinogens also occurred in this work environment. Ward *et al.* (1991) reported an excess in bladder cancer in 1749 US workers employed in the production of rubber additives from *ortho*-toluidine and aniline. Risks were greatest for workers with the strongest likelihood of exposure and for those with long-term exposure (> 10 years). Further cases of bladder cancer in this facility were reported by Markowitz & Levin (2004), but rates were not calculated. Exposure to low-level 4-aminobiphenyl was suspected, so a protein-adduct biomarker study was carried out (Ward *et al.*, 1996), which supported the conclusion that

ortho-toluidine was the most likely cause of the bladder-cancer excess, because 4-aminobiphenyl adducts to haemoglobin were unrelated to work in the facility. Using revised exposure categories, Carreón *et al.* (2010) conducted a re-analysis of the data and confirmed that workers in this plant have an increased risk for bladder cancer.

Sorahan *et al.* (2000) and Sorahan (2008) reported an excess in bladder-cancer risk in workers exposed to *ortho*-toluidine in the United Kingdom. Sorahan (2008) found increased risks with longer duration of employment in departments where *ortho*-toluidine was processed ($P < 0.05$), after adjusting for exposure to other bladder carcinogens in the factory.

Overall, the epidemiological studies show consistent associations between exposure to *ortho*-toluidine and bladder cancer. Although exposure to other bladder carcinogens occurred for several of the cohorts, the overall evidence is consistent with an association of exposure to *ortho*-toluidine and bladder cancer.

3. Cancer in Experimental Animals

Studies on the carcinogenicity of *ortho*-toluidine in the mouse, rat and hamster after oral administration or subcutaneous injection were reviewed in previous *IARC Monographs* (IARC, 2000, 2010). There have been no additional carcinogenicity studies in animals reported since the most recent evaluation (IARC, 2010).

ortho-Toluidine was tested for carcinogenicity as its hydrochloride salt by oral administration in the feed in two experiments in mice and in three experiments in rats, and as the free base in one limited subcutaneous-injection experiment in hamsters. Results of adequately conducted carcinogenicity studies are summarized in Table 3.1.

Oral administration of *ortho*-toluidine to male and female mice caused an increased incidence of haemangiomas and haemangiosarcomas (combined) in both sexes in one study (Weisburger *et al.*, 1978). The same result was found in male rats in another study, but the separate incidence for haemangiosarcomas was also increased (NTP, 1979). The incidences of hepatocellular carcinomas and of hepatocellular adenomas and carcinomas combined were increased in females in the latter study (NTP, 1979).

Oral administration of *ortho*-toluidine to male rats caused an increased incidence of subcutaneous fibromas and fibrosarcomas (combined) in one study (Weisburger *et al.*, 1978), and of skin and spleen fibromas, mammary gland fibroadenomas and peritoneal sarcomas in another (Hecht *et al.*, 1982). In a third study in male and female rats, *ortho*-toluidine increased the incidence of subcutaneous fibromas and of mesotheliomas of multiple organs or the *tunica vaginalis* in males, and of mammary gland fibroadenomas and urinary bladder transitional-cell carcinomas in females. An increased incidence of fibrosarcomas, angiosarcomas, osteosarcomas or sarcomas (not otherwise specified) (combined) of multiple organs (mainly subcutis and spleen or bone) was also observed in both sexes; and a significant increase in the incidence of fibrosarcomas and sarcomas of multiple organs in males, and of spleen angiosarcomas and osteosarcomas of multiple organs in females (NTP, 1979).

When administered as the free base by subcutaneous injection to male and female Syrian golden hamsters, *ortho*-toluidine produced no increase in tumour incidence compared with controls (Hecht *et al.*, 1983).

Table 3.1 Carcinogenicity studies in experimental animals fed *ortho*-toluidine

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Swiss CD-1 (M, F) 21 mo Weisburger *et al.* (1978)	Groups of 25 M and 25 F mice were fed a diet containing 0, 16000 or 32000 ppm *ortho*-toluidine hydrochloride. After 3 mo, due to toxicity, doses were lowered to 8000 or 16000 ppm for a further 15 mo. Treated animals were then kept without treatment for an additional 3 mo.	Haemangiomas and Haemangiosarcomas (combined): M–0/14 (concurrent control), 5/99 (pooled control), 5/14*, 9/11* F–0/15, 9/102, 5/18**, 9/21*	*$P < 0.025$ (vs all controls **$P < 0.05$ (vs all controls)	Purity, 97–99% Pooled controls: additional controls used for the other compounds tested in the study. Tumour incidence of concurrent and pooled controls were compared statistically (both separately and together) with those of treated groups. Separate incidence for haemangiomas and haemangiosarcomas NR.
Mouse, $B6C3F_1$ (M, F) 103 wk NTP (1979)	Groups of 50 M and 50 F mice, were fed a diet containing 1000 or 3000-ppm *ortho*-toluidine hydrochloride for 103 wk. A group of 20 M and 20 F mice served as untreated controls.	*Males* Haemangiomas and Haemangiosarcomas (combined): 1/19, 2/50, 12/50* Haemangiosarcomas: 1/19, 1/50, 10/50* *Females* Hepatocellular adenomas and carcinomas (combined): 0/20, 4/49, 13/50** Hepatocellular carcinomas: 0/20, 2/49, 7/50***	*$P < 0.005$ (trend test) **$P < 0.007$ (Fisher's exact test), $P < 0.001$ (trend test) ***$P = 0.015$ (trend test)	Purity > 99%
Rat, Sprague-Dawley CD (M) 24 mo Weisburger *et al.* (1978)	Groups of 25 M rats were fed a diet containing 0, 8000 or 16000-ppm *ortho*-toluidine hydrochloride. After 3 mo, due to toxicity, doses were lowered to 4000 or 8000 ppm for a further 15 mo. Treated animals were then kept without treatment for an additional 6 mo.	Subcutaneous fibromas and fibrosarcomas (combined): M–0/16 (concurrent control), 18/111 (pooled control), 18/23*, 21/24* Urinary bladder transitional-cell carcinomas: M–0/16, 5/111, 3/23, 4/24	*$P < 0.025$ (vs all controls)	Purity, 97–99% Pooled controls: additional controls used for the other compounds tested in the study. Tumour incidences of concurrent and pooled controls were compared statistically (both separately and together) with those of treated groups
Rat, Fischer F344 (M) 93 wk Hecht *et al.* (1982)	Groups of 30 M rats were fed a diet containing 0 or 4000 ppm *ortho*-toluidine hydrochloride for 72 wk. Total dose of *ortho*-toluidine hydrochloride ingested was 31.3 g/rat.	Skin fibromas: 1/27, 25/30* Spleen fibromas: 0/27, 10/30* Mammary gland fibroadenomas: 0/27, 11/30* Peritoneal sarcomas: 0/27, 9/30**	*$P < 0.001$ (Fisher's exact test) **$P < 0.01$ (Fisher's exact test)	Purity NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Fischer F344 (M, F) 104 wk NTP (1979)	Groups of 50 M and 50 F rats, were fed a diet containing 3000 or 6000-ppm *ortho*-toluidine hydrochloride for 101–104 wk. A group of 20 M and 20 F rats served as untreated controls.	*Males* Sarcomas NOS, fibrosarcomas, angiosarcomas or osteosarcomas (combined) of multiple organs (mainly subcutis and spleen or bone): 0/20, 15/50**, 37/49* Sarcomas NOS of multiple organs: 0/20, 3/50, 11/49*** Fibrosarcomas of multiple organs: 0/20, 8/50, 20/49* Subcutaneous integumentary fibromas: 0/20, 28/50*, 27/49* Mesotheliomas of multiple organs or *tunica vaginalis*: 0/20, 17/50*, 9/49*** *Females* Sarcomas NOS, fibrosarcomas, osteosarcomas or angiosarcomas (combined) of multiple organs (mainly subcutis and spleen or bone): 0/20, 3/50, 21/49* Osteosarcomas of multiple organs: 0/20, 0/50, 18/49**** Spleen angiosarcomas: 0/20, 7/49, 9/49*** Urinary bladder transitional-cell carcinomas: 0/20, 9/45***, 22/47* Mammary gland fibroadenomas: 6/20, 20/50, 35/49*****	*$P < 0.001$ **$P = 0.003$ ***$P < 0.05$ ****$P = 0.001$ *****$P = 0.002$	Purity > 99% Mortality of male and female rats was significantly increased by treatment ($P < 0.001$).

F, female; M, male; mo, month or months; NOS, not otherwise specified; NR, not reported; vs, versus; wk, week or weeks

4. Other Relevant Data

A general Section on "Aromatic amines: metabolism, genotoxicity, and cancer susceptibility" appears as Section 4.1 in the *Monograph* on 4-aminobiphenyl in this volume.

ortho-Toluidine is a constituent of tobacco smoke and it is excreted in larger amounts in the urine of smokers than of non-smokers (Riedel *et al.*, 2006). *ortho*-Toluidine induced urinary bladder and mammary gland tumours in rats and liver tumours and haemangiosarcomas in mice. The risk for cancer of the urinary bladder was elevated in workers exposed to *ortho*-toluidine. This substance has been evaluated in a large number of genetic toxicology studies (IARC, 2010); however, there has been much inconsistency in the results reported.

The metabolism of *ortho*-toluidine has not yet been fully characterized, but the available data indicate a preferential ring-oxidation or *N*-acetylation rather than *N*-oxidation (Son *et al.*, 1980). Similarly, cancers of the urinary bladder associated with occupational exposure to *ortho*-toluidine may result from peroxidative activation of the chemical, catalysed by prostaglandin H synthase in the epithelium of the urinary bladder (Zenser *et al.*, 2002). *ortho*-Toluidine-haemoglobin adduct levels were increased in patients treated with the anaesthetic prilocaine (Gaber *et al.*, 2007) and in workers employed in the rubber chemicals manufacturing area of a chemical plant (Ward *et al.*, 1996). Metabolites are excreted primarily as sulfate or glucuronide conjugates, since *ortho*-toluidine is not a substrate for human NAT1-mediated acetylation (Zhang *et al.*, 2006).

ortho-Toluidine induces tumours in rodents and DNA lesions in multiple organs. Most studies reported that *ortho*-toluidine was not mutagenic in *S. typhimurium*, other studies showed positive responses in the same strains. The *N*-oxidized metabolite of *ortho*-toluidine, *N*-hydroxy-*ortho*-toluidine, was mutagenic in *S. typhimurium* strain TA100 (Gupta *et al.*, 1987). *ortho*-Toluidine induced intrachromosomal recombination in *Saccharomyces cerevisiae* in an assay that is responsive to the induction of DNA deletions (Carls & Schiestl, 1994); this response was reduced in the presence of an antioxidant. Other reported effects of *ortho*-toluidine (Danford, 1991) include the induction of sister chromatid exchange, aneuploidy, unscheduled DNA synthesis, DNA strand breaks, and cell transformation *in vitro*, and the induction of micronuclei in peripheral blood of rats treated *in vivo* (Suzuki *et al.*, 2005). The formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine in calf thymus DNA incubated *in vitro* with 4-amino-3-methylphenol, a metabolite of *ortho*-toluidine, suggests a potential role of reactive oxygen species in the DNA-damaging effects of this aromatic amine (Ohkuma *et al.*, 1999). *ortho*-Toluidine induced DNA lesions – measured by means of the comet assay – in multiple organs of exposed rats and mice (Sekihashi *et al.*, 2002): increased DNA migration was observed in the liver, bladder, lung, and brain of mice, and in the liver, bladder, and stomach of rats.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of *ortho*-toluidine. *ortho*-Toluidine causes cancer of the urinary bladder.

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

There is moderate mechanistic evidence indicating that the carcinogenicity of *ortho*-toluidine involves metabolic activation, formation of DNA adducts, and induction of DNA-damaging effects.

ortho-Toluidine is *carcinogenic to humans (Group 1)*.

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