ortho-ANISIDINE

This substance was considered by previous working groups, in 1981 (IARC, 1982) and 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

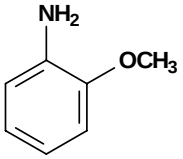
Chem. Abstr. Serv. Reg. No.: 90-04-0

Chem. Abstr. Name: 2-Methoxybenzenamine

IUPAC Systematic Name: *o*-Anisidine

Synonyms: *ortho*-Aminoanisole; 2-aminoanisole; *ortho*-aminomethoxybenzene; 2-aminomethoxybenzene; 1-amino-2-methoxybenzene; 2-methoxy-1-aminobenzene; *ortho*-methoxyaniline; 2-methoxyaniline; 2-methoxybenzenamine; *ortho*-methoxyphenylamine; 2-methoxyphenylamine

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_7H_9NO Relative molecular mass: 123.15

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Yellowish liquid; becomes brownish on exposure to air (Budavari, 1996)

(*b*) *Boiling-point*: 224°C (Lide, 1997)

(*c*) *Melting-point*: 6.2°C (Lide, 1997)

(*d*) *Density*: 1.096 g/cm^3 at 20°C (Lide, 1997)

(*e*) *Solubility*: Slightly soluble in water; soluble in ethanol, diethyl ether and acetone (Lide, 1997)

(*f*) *Stability*: Susceptible to oxidation in air (National Toxicology Program, 1991)

(*g*) *Octanol/water partition coefficient (P)*: log P, 0.95 (calculated), 1.18 (measured) (Verschueren, 1996).
(*h*) *Conversion factor*: mg/m^3 = 5.04 × ppm

1.2 Production and use

Information available in 1995 indicated that *ortho*-anisidine was produced in Armenia, China, France, Germany, India, Japan, the Ukraine and the United Kingdom (Chemical Information Services, 1995).

ortho-Anisidine is used as a chemical intermediate (e.g. in the manufacture of azo or triphenylmethane dyes and pharmaceuticals), as a corrosion inhibitor for steel storage and as an antioxidant for some polymercaptan resins (National Toxicology Program, 1991).

1.3 Occurrence

1.3.1 *Natural occurrence*

ortho-Anisidine is not known to occur naturally.

1.3.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 1998), approximately 700 workers in the United States were potentially exposed to *ortho*-anisidine. Occupational exposure to *ortho*-anisidine may occur during its production and during its use as a chemical intermediate, corrosion inhibitor or antioxidant.

1.3.3 *Environmental occurrence*

ortho-Anisidine has been identified in wastewater from chemical plants and from oil refineries, and in cigarette smoke (National Library of Medicine, 1998a).

According to the United States Environmental Protection Agency Toxic Chemical Release Inventory for 1987, 1600 kg of *ortho*-anisidine were released into the air, 280 kg were discharged into water, and 110 kg were released onto the land from manufacturing and processing facilities in the United States. By 1996, 690 kg were released into the air and 13 kg discharged into water (National Library of Medicine, 1998b).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (1997) has recommended an 8-h time-weighted average threshold limit value of 0.5 mg/m^3, with a notation for potential dermal absorption, for occupational exposure to *ortho*-anisidine in workplace air. Similar values have been used as standards or guidelines in many other countries (International Labour Office, 1991).

No international guidelines for *ortho*-anisidine in drinking-water have been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Previous evaluation

ortho-Anisidine hydrochloride was tested for carcinogenicity in one study in mice and one study in rats by oral administration in the diet. It produced transitional-cell carcinomas of the urinary bladder in animals of each species and sex (IARC, 1982).

New studies

In a model of urinary bladder carcinogenesis, groups of 15 male Fischer 344 rats, six weeks of age, were given 0.05% *N*-nitroso-*N*,4-hydroxybutylamine (NHBA) in the drinking-water for four weeks. They were then fed diets with or without a supplement of 1700 mg/kg diet (ppm) *ortho*-anisidine for the first two weeks and 425 ppm thereafter for an additional 30 weeks. Ten animals received *ortho*-anisidine without prior NHBA administration. The incidence of papillary or nodular hyperplasia in the urinary bladder, derived by assessing the number of lesions per unit length of mucosa, was significantly higher ($p < 0.01$) in the group receiving *ortho*-anisidine plus NHBA (13/16) than in the group given NHBA alone (2/13). No lesions were observed in animals receiving *ortho*-anisidine alone (Ono *et al*., 1992).

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*

Horseradish peroxidase oxidized *ortho*-anisidine via a nitrogen-centred cation radical to the diimine, quinone imine and an azo dimer *in vitro*. The metabolism led to covalent binding to calf thymus DNA. Metabolites of *ortho*-anisidine were consistently more reactive with protein and glutathione than metabolites of *para*-anisidine (Thompson & Eling, 1991). [The Working Group noted that studies with mammalian enzymes have not been carried out.]

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

The oral LD_{50} of *ortho*-anisidine has been reported to be 2000 mg/kg bw in rats, 870 mg/kg bw in rabbits and 1400 mg/kg bw in mice. Its subacute effects include haematological changes, anaemia and nephrotoxicity (Prosolenko, 1975).

ortho-Anisidine induced methaemoglobinaemia in CBA mice and Alpk:APfSD rats after oral administration (Ashby *et al*., 1991). The authors suggested that their results indicate that *ortho*-anisidine is distributed and *N*-oxidized in rodents.

Male and female $B6C3F_1$ mice were fed diets containing up to 30 000 mg/kg of diet (ppm) *ortho*-anisidine hydrochloride for seven weeks. A dose-dependent depression in mean body-weight gain of up to 40% was observed. The spleens of mice given doses > 10 000 mg/kg of diet were black and enlarged. Female mice that received doses of 2500 or 5000 mg/kg of diet for up to 103 weeks developed more cystic hyperplasia of the uterus and endometrium than did control animals. Mice of each sex at 30 000 mg/kg of diet had an increased incidence of hyperplasia of the bladder (National Cancer Institute, 1978).

Feeding of diets containing up to 30 000 mg/kg (ppm) *ortho*-anisidine hydrochloride to Fischer 344 rats for seven weeks led to reductions in weight gain of up to 52% in males and 27% in females. Feeding of diets containing 1000 or 3000 mg/kg resulted in granular spleens in males but not in females; the spleens of rats of each sex given diets containing *ortho*-anisidine at > 10 000 mg/kg were dark and granular. A dose of 10 000 mg/kg of diet for up to 103 weeks resulted in depressions of body-weight gain of 21% in males and 11% in females. Male and female rats fed diets containing 5000 or 10 000 mg/kg *ortho*-anisidine hydrochloride developed non-neoplastic lesions of the thyroid gland and kidney more frequently than did control animals (National Cancer Institute, 1978).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

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)

ortho-Anisidine did not induce reverse mutation in *Escherichia coli* or in *Salmonella typhimurium* strains TA100, TA1535, TA1537, TA1538, TA98 or YG1012. In the presence of exogenous metabolic activation, it induced mutations in strain YG1029, both YG strains having elevated levels of *N*-acetyltransferase. *ortho*-Anisidine did not induce sex-linked recessive lethal mutations in *Drosophila*. It gave rise to gene mutations in

Table 1. Genetic and related effects of *ortho*-anisidine

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA98, TA1535, TA1537, reverse mutation	–	–	10 800 µg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	–	–	10 000 µg/plate	Dunkel *et al.* (1985)
Salmonella typhimurium YG1012 (TA1538 with *N*-acetyltransferase gene), reverse mutation	–	–	Not reported	Thompson *et al*. (1992)
Salmonella typhimurium YG1029 (TA100 with *N*-acetyltransferase gene), reverse mutation	–	+	62 µg/plate	Thompson *et al*. (1992)
Escherichia coli WP2*uvrA*, reverse mutation	–	–	10 000 µg/plate	Dunkel *et al.* (1985)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		2000 ppm inj	Yoon *et al.* (1985)
DNA strand breaks/cross-links, mouse lymphoma L5178Y cells *in vitro*	–	+	150	Garberg *et al.* (1988)
Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	123	Wangenheim & Bolcsfoldi (1988)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	+	38	Galloway *et al.* (1987)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	+	1200	Galloway *et al.* (1987)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	500	Kerkaert *et al.* (1998)
Host-mediated assay, *Escherichia coli* in blood of mouse, DNA repair *in vivo*	–		1300 po × 1	Hellmer & Bolcsfoldi (1992)
Host-mediated assay, *Escherichia coli* in blood of mouse, DNA repair *in vivo*	+		310 ip × 1	Hellmer & Bolcsfoldi (1992)
DNA single-strand break, liver, thymus and testis of Sprague-Dawley rats *in vivo*	–		700 po × 1	Ashby *et al*. (1991)
DNA single-strand break, liver, kidney, spleen and bladder of Wistar rats *in vivo*	–		500 po × 1	Ashby *et al*. (1991)
DNA single-strand break, liver and bladder of Wistar rats *in vivo*	–		750 ip × 1	Ashby *et al*. (1991)
DNA breaks, bladder and colon of CD-1 mice *in vivo*	+		690 po × 1	Sasaki *et al.* (1998)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		1104 po × 1	Ashby *et al*. (1991)
Unscheduled DNA synthesis, rat kidney cells *in vivo*	–		500 ip × 1	Tyson & Mirsalis (1985)
Gene mutation, mouse bladder cells *in vivo*, *lacI* transgenic model	(+)		750 po × 3	Ashby *et al*. (1994)
Gene mutation, mouse liver cells *in vivo*, *lacI* transgenic model	-		750 po × 10	Ashby *et al*. (1994)
Micronucleus formation, CBA mouse bone-marrow cells *in vivo*	–		690 po × 1–3	Ashby *et al*. (1991)
Micronucleus formation, $B6C3F_1$ mouse bone-marrow cells *in vivo*	–		500 ip × 3	Ashby *et al*. (1991)
Micronucleus formation, mouse bone-marrow cells *in vivo*	?		800 ip × 1	Morita *et al*. (1997)
Micronucleus formation, AP and Fischer 344 rat bone-marrow cells *in vivo*	–		1380 po × 1	Ashby *et al*. (1991)
Micronucleus formation, rats liver cells *in vivo*	–		1104 po × 1	Ashby *et al*. (1991)
Binding (covalent) to DNA, $B6C3F_1$ mouse bladder and liver *in vivo*	–		750 po × 1	Ashby *et al*. (1994)
Inhibition of gap-junctional intercellular communication, 3PC mouse keratinocytes *in vitro*	+	NT	1232	Jansen & Jongen (1996)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; unless otherwise stated, in-vitro test, μg/mL; in-vivo test, mg/kg bw per day; inj, injection; po, oral; ip, intraperitoneal

mouse lymphoma L5178Y cells *in vitro* both in the presence and absence of exogenous metabolic activation, while DNA breaks and cross-links were observed only in the presence of an exogenous metabolic system. Sister chromatid exchange and chromosomal aberrations were induced in Chinese hamster ovary cells *in vitro*, both in the presence and absence of exogenous metabolic activation. In the absence of exogenous metabolic activation, *ortho*-anisidine induced cell transformation in Syrian hamster embryo cells *in vitro*. *ortho*-Anisidine did not bind covalently to DNA in mouse bladder or liver cells *in vivo*; it did not induce single-strand breaks in liver, thymus, testis, kidney, spleen or bladder DNA of rats treated *in vivo*, nor did it induce unscheduled DNA synthesis in rat hepatocytes or kidney cells *in vivo*. In mice treated *in vivo*, *ortho*-anisidine gave rise to breaks in bladder and colon DNA but not in DNA of stomach, kidney, liver, lung, brain or bone marrow. *ortho*-Anisidine induced DNA repair in *E. coli* in a host-mediated assay in male mice when the animals were treated intraperitoneally but not when they were treated by oral gavage. *ortho*-Anisidine weakly induced gene mutation in the *lacI* transgene in mouse bladder but not in liver. It did not induce micronuclei in bone marrow of mice or in bone-marrow or liver cells of rats treated *in vivo*.

ortho-Anisidine inhibited gap-junctional intercellular communication in mouse hepatocytes in the absence of an exogenous metabolic system *in vitro*.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to *ortho*-anisidine may occur during its production and its use as a chemical intermediate, a corrosion inhibitor and an industrial antioxidant.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

ortho-Anisidine hydrochloride was tested for carcinogenicity in one study in mice and one study in rats by oral administration in the diet. It produced transitional-cell carcinomas of the urinary bladder in animals of each species and sex.

5.4 Other relevant data

Limited information was available to the Working Group on the metabolism of *ortho*-anisidine. It was shown to be *O*-dealkylated in rat liver microsomes.

ortho-Anisidine at a high dose increased the incidence of hyperplasia of the bladder in male and female mice.

No data were available on the developmental and reproductive effects of *ortho*-anisidine.

No data were available on the genetic and related effects of *ortho*-anisidine in humans. No conclusion can be drawn about its genotoxicity in experimental animals *in vivo*; however, *ortho*-anisidine induced gene mutation in bladder cells in an assay in transgenic mice. There is no evidence that it has genotoxic effects in mammalian cells *in vitro*. *ortho*-Anisidine was not mutagenic to bacteria.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of *ortho*-anisidine.

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

Overall evaluation

ortho-Anisidine is *possibly carcinogenic to humans (Group 2B).*

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