IARC MONOGRAPHS

SOME AROMATIC AMINES AND RELATED COMPOUNDS

VOLUME 127

This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met remotely, 25 May–12 June 2020

LYON, FRANCE - 2021

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

ORTHO-NITROANISOLE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

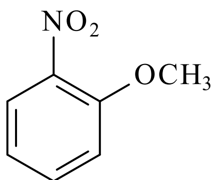
Chem. Abstr. Serv. Reg. No.: 91-23-6

EC No.: 202-052-1

IUPAC systematic name: 1-methoxy-2-nitrobenzene

Synonyms and abbreviations: 2-methoxy-nitrobenzene; 2-methoxy-l-nitrobenzene; *ortho*-nitroanisole; 2-nitroanisole; *ortho*-nitrobenzene methyl ether; 2-nitromethoxy-benzene; *ortho*-nitromethoxybenzene; 1-nitro-2-methoxybenzene; *ortho*-nitrophenyl methyl ether.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_7H_7NO_3$

Relative molecular mass: 153.14 (NCBI, 2020)

1.1.3 Chemical and physical properties of the pure substance

Description: *ortho*-nitroanisole is a colourless to yellow-red liquid (NCBI, 2020)

Boiling point: 277 °C (NCBI, 2020)

Melting point: 10.5 °C (NCBI, 2020)

Flash point: 142 °C, closed cup (NCBI, 2020)

Density: 1.254 g/cm^3 at 20 °C (NCBI, 2020)

Vapour density: 5.29 (air = 1) (NCBI, 2020)

Stability and reactivity: stable under normal temperatures and pressures; explosively reactive with sodium hydroxide and zinc (NTP, 2016)

Vapour pressure: 0.004 kPa at 30 °C (ILO, 2017); 3.6×10^{-3} mm Hg at 25 °C (NCBI, 2020)

Water solubility: none at 20 °C; 1.69 g/L at 30 °C. Miscible with ethanol, ethyl ether; soluble in carbon tetrachloride (NCBI, 2020)

*Octanol/water partition coefficient (*P*)*: log K_{ow}, 1.73 (NCBI, 2020)

Conversion factor: 1 ppm = 6.26 mg/m^3 (IARC, 1996) [calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming temperature (25 °C) and pressure (101 kPa)].

1.1.4 Technical grade and impurities

ortho-Nitroanisole is commercially available, with a purity ranging from 98% to 99% (IARC, 1996).

1.2 Production and use

1.2.1 Production process

ortho-Nitroanisole is prepared by slowly adding methanolic sodium hydroxide to a solution of 2-chloronitrobenzene in methanol at 70 °C and then gradually heating the mixture under pressure to 95 °C. After dilution with water, the product is separated as an oil, at a 90% yield; methanol can be recovered from the aqueous layer (Booth, 1991; Lewis, 1993). This process has been optimized by applying pressure earlier and keeping the temperature at 30–60 °C, before filtering and then washing with methanol. The final step is distillation (Xia et al., 2010).

1.2.2 Production volume

ortho-Nitroanisole is produced by several manufacturers in China and in India (HSDB, 2011; Aarti Industries, 2020; LookChem, 2020). In 2020, *ortho*-nitroanisole was available from 28 suppliers worldwide, including 4 in the USA and 18 in China (Chemical Register, 2020). USA imports of *ortho*-nitroanisole totalled more than 319 000 kg in 1976 and 246 000 kg in 1978 (HSDB, 2011). No more recent data on USA imports or exports of *ortho*-nitroanisole were available to the Working Group.

1.2.3 Uses

ortho-Nitroanisole is used primarily as a precursor for *ortho*-anisidine [see the monograph on *ortho*-anisidine in the present volume for a description of its uses].

1.3 Measurement and analysis

Several analytical methods are available. Gas chromatography-mass spectrometry for *ortho*-nitroanisole in cosmetics was reported with a detection limit of 25.4 ng/g (Huang et al., 2017). Sludge samples were analysed for *ortho*-nitroanisole content using both gas chromatography and liquid chromatography coupled with mass spectrometry (Liang et al., 2019). Several studies reported the use of high-performance liquid chromatography coupled to detection by ultraviolet light (HPLC-UV) for determination of *ortho*-nitroanisole in studies of metabolism in vitro conducted using human, rat, rabbit, and porcine hepatic microsomes and cytosol (Mikšanová et al., 2004a, b; Dracínska et al., 2006).

1.4 Occurrence and exposure

ortho-Nitroanisole is not known to occur naturally.

1.4.1 Environmental occurrence

ortho-Nitroanisole can be released into the environment by dye and pharmaceutical manufacturing facilities through various waste streams. Airborne *ortho*-nitroanisole will remain in the vapour phase and will be degraded by reactions with photochemically produced hydroxyl radicals, with an estimated half-life of 109 hours (NTP, 2016).

When released to water, *ortho*-nitroanisole may adsorb to sediments and suspended solids. Volatilization is very slow, with a half-life of 105 days in a model river and 772 days in a model pond (NTP, 2016). *ortho*-Nitroanisole has been detected in water samples in Japan (0.7 µg/L) and from the Rhine river in the Netherlands (0.3–1.0 µg/L) in 1978–1983. Water samples from the Rhine river in Germany were obtained in 1983–1984 and contained concentrations of all nitroanisole isomers combined ranging from 0.1 to 0.9 µg/L (BUA, 1987). *ortho*-Nitroanisole has been identified in drinking-water (NTP, 2016).

When released to soil, *ortho*-nitroanisole has moderate mobility. It is not expected to bioaccumulate in aquatic organisms. *ortho*-Nitroanisole has been detected in sediment samples taken in

Japan (0.01 μg/L) (BUA, 1987). *ortho*-Nitroanisole was among the 16 known, presumed, or suspected human carcinogens identified in several water and sediment samples in China (Greenpeace, 2017).

1.4.2 Occupational exposure

Occupational exposure to *ortho*-nitroanisole can occur via inhalation or skin contact during production and application. Information on number of workers exposed to *ortho*-nitroanisole was available only for Finland and Poland. No workers were registered with occupational exposure in Finland (Saalo et al., 2016). In Poland, 203 workers were exposed to *ortho*-nitroanisole in 2016 (Starek, 2019). No estimates of occupational exposure to *ortho*-nitroanisole in other countries were available (NTP, 2016; IFA, 2019).

1.4.3 Exposure of the general population

The Working Group found no measurements of exposure to *ortho*-nitroanisole within the general population. The general population can be exposed to *ortho*-nitroanisole via the environment either from drinking contaminated water or breathing contaminated air.

1.5 Regulations and guidelines

ortho-Nitroanisole is considered a hazardous material, and there are special requirements for marking, labelling, and transporting this material (NTP, 2016; ECHA, 2019a).

In the European Union, *ortho*-nitroanisole is regulated under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations, Annex III: criteria for 1–10 tonne registered substances (ECHA, 2019a). The European Chemicals Agency (ECHA) has classified *ortho*-nitroanisole as carcinogenic (Category 1B) and causing acute toxicity (Category 4) (ECHA, 2019b). It is banned from use in any cosmetic products marketed for sale or use in the European Union. Workers who are aged < 18 years, pregnant, or breastfeeding may not be exposed to *ortho*-nitroanisole. Employers are obliged to minimize other workers' exposure to *ortho*-nitroanisole as far as possible and must arrange for medical surveillance of exposed workers. *ortho*-Nitroanisole is also included in Classification, Labelling and Packaging (CLP) legislation, which lists classification and labelling data that have been notified to the ECHA by manufacturers or importers (ECHA, 2019a, b).

In the USA, *ortho*-nitroanisole is regulated under the Toxic Substances Control Act (TSCA), which requires the United States Environmental Protection Agency (US EPA) to compile, keep current, and publish a list of each chemical substance that is manufactured or processed, including imports, in the USA for uses under TSCA tracked in the Toxic Release Inventory (TRI). Three US EPA compliance-monitoring regulations for *ortho*-nitroanisole are: Emergency Planning and Community Right-To-Know Act (EPCRA 313), Standards of Performance for New Stationary Sources of Air Pollutants – Equipment Leaks Chemical List (CAA 111), and Organic Hazardous Air Pollutants National Emission Standards (CAA 112 (b) HON) (NCBI, 2020).

ortho-Nitroanisole is labelled according to the United Nations' Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as acute toxic category 4 for oral intake with hazard phrase H302: "Harmful if swallowed and a carcinogen, Category 1B with hazard phrase H350: May cause cancer 302." *ortho*-Nitroanisole is highly restricted in products destined for the general public but permitted for use by professionals (NCBI, 2020).

1.5.1 Exposure limits and guidelines

In the Russian Federation in 1993 the short-term exposure limit (STEL) for *ortho*-nitroanisole was set at 1 mg/m^3 (IARC, 1996). In Poland, a maximal admissible concentration of 1.6 mg/m^3, based on the no-observed-effect level of 8 mg/kg body weight (bw) per day and an uncertainty factor of 36, was adopted in 2018 (Starek, 2019). In other countries, occupational limit values or standards have not been set for this compound (ACGIH, 2018; DFG, 2019; IFA, 2019).

1.5.2 Reference values for biological monitoring of exposure

No reference values were available to the Working Group.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

ortho-Nitroanisole was previously evaluated by the *IARC Monographs* programme in 1995 (IARC, 1996). In its evaluation at that time, the Working Group concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of *ortho*-nitroanisole

See Table 3.1.

3.1 Mouse

Oral administration (feed)

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female B6C3F$_1$ mice (age, 40 days) were given feed containing *ortho*-nitroanisole (purity, > 99%) at a concentration of 0 (controls), 666, 2000, or 6000 mg/kg for 103 weeks (NTP, 1993; see also Irwin et al., 1996). Survival did not significantly differ between treated males and controls (controls, 35/50; 666 mg/kg, 43/50; 2000 mg/kg, 39/50; and 6000 mg/kg, 40/50), or between treated females and controls (38/50, 26/50, 33/50, and 45/50). The mean body weights of male and female mice at 2000 and 6000 mg/kg were significantly lower than those of controls throughout the study. Full histopathology was performed on grossly visible lesions and major organs and tissues.

In male mice, the incidence of hepatocellular adenoma at 0 (controls), 666, 2000, and 6000 mg/kg, respectively, was 14/50, 26/50, 41/50, and 29/50; the incidence of hepatocellular carcinoma was 7/50 (14%), 12/50 (24%), 11/50 (22%), and 7/50 (14%); the incidence of hepatoblastoma was 0/50, 3/50, 17/50, and 9/50; and the incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) was 21/50, 33/50, 46/50, and 34/50. There was a significant positive trend in the incidence of hepatocellular adenoma (P = 0.022), of hepatoblastoma (P = 0.015), and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (P = 0.049). The incidence of hepatocellular adenoma was significantly higher in males at 666 mg/kg (P = 0.012), 2000 mg/kg (P < 0.001), and 6000 mg/kg (P = 0.002) than in controls. The incidence of hepatoblastoma was significantly higher in males at 2000 mg/kg (P < 0.001) and 6000 mg/kg (P = 0.001) than in controls. The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) was significantly higher in males at 666 mg/kg (P = 0.013), 2000 mg/kg (P < 0.001), and 6000 mg/kg (P = 0.008) than in controls. The incidence of hepatocellular carcinoma in historical controls for male B6C3F$_1$ mice was 122/865 (mean, 14.1%; range, 3–27%).

In female mice, the incidence of hepatocellular adenoma in the control group and at 666, 2000, and 6000 mg/kg, respectively, was

Table 3.1 Studies of carcinogenicity with *ortho*-nitroanisole in mice and rats

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, $B6C3F_1$ (M) 40 days 103 wk NTP (1993)	Oral > 99% Feed 0, 666, 2000, 6000 mg/kg for 103 wk 50, 50, 50, 50 35, 43, 39, 40	*Liver* Hepatocellular adenoma 14/50, 26/50*, 41/50**, 29/50***	$P = 0.022$, Cochran–Armitage trend test; *$P = 0.012$, Fisher exact test; **$P < 0.001$, Fisher exact test; ***$P = 0.002$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; adequate number of animals; the duration of exposure and observation was adequate; use of males and females. Historical controls: hepatocellular carcinoma, 122/865 (14.1%, 3–27%).
		Hepatocellular carcinoma 7/50 (14%), 12/50 (24%), 11/50 (22%), 7/50 (14%)	NS	
		Hepatoblastoma 0/50, 3/50, 17/50*, 9/50**	$P = 0.015$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test; **$P = 0.001$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 21/50, 33/50*, 46/50**, 34/50***	$P = 0.049$, Cochran–Armitage trend test; *$P = 0.013$, Fisher exact test; **$P < 0.001$, Fisher exact test; ***$P = 0.008$, Fisher exact test	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (F) 40 days 103 wk NTP (1993)	Oral > 99% Feed 0, 666, 2000, 6000 mg/kg for 103 wk 50, 50, 50, 50 38, 26, 33, 45	*Liver* Hepatocellular adenoma 14/50, 20/50, 36/50*, 18/50	[$P < 0.001$, Cochran–Armitage trend test]; *$P < 0.001$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; adequate number of animals; the duration of exposure and observation was adequate; use of males and females. Historical controls: hepatocellular carcinoma, 28/863 (3.2%, 0–10%); hepatoblastoma, 1/863 (0.1%, 0–2%).
		Hepatocellular carcinoma 5/50 (10%), 2/50 (4%), 8/50 (16%), 3/50 (6%)	NS	
		Hepatoblastoma 1/50 (2%), 1/50 (2%), 2/50 (4%), 0/50	NS	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 17/50, 22/50, 37/50*, 20/50	[$P < 0.001$, Cochran–Armitage trend test]; *$P < 0.001$, Fisher exact test	
Rat, F344 (M) 40 days 103 wk NTP (1993)	Oral > 99% Feed 0, 222, 666, 2000 mg/kg for 103 wk 50, 50, 50, 50 32, 34, 24, 9	*Haematopoietic system*: mononuclear cell leukaemia 26/50, 25/50, 42/50*, 34/50 (68%)	$P = 0.041$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females; adequate number of animals; adequate duration of exposure and observation. Principal limitations: low survival rate (9/50) in the group at the highest dose (2000 mg/kg). Historical controls: mononuclear cell leukaemia, 385/800 (48.1%, 32–62%).
Rat, F344 (F) 40 days 103 wk NTP (1993)	Oral > 99% Feed 0, 222, 666, 2000 mg/kg for 103 wk 50, 50, 50, 50 33, 41, 26, 33	*Haematopoietic system*: mononuclear cell leukaemia 14/50, 11/50, 14/50, 26/50*	$P < 0.001$, Cochran–Armitage trend test; *$P = 0.012$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; the duration of exposure and observation was adequate; adequate number of animals; use of males and females. Historical controls: mononuclear cell leukaemia, 213/800 (26.6%, 14–52%).

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344 (M) 41 days 28 wk NTP (1993)	Oral > 99% Feed 0, 6000, 18 000 mg/kg for 27 wk (27 wk stop-exposure, with interim evaluation at 28 wk) 10, 10, 10 10, 10, 10	*Urinary bladder*: transitional cell carcinoma 0/10, 0/10, 10/10*	 *$P \le 0.01$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females.
Rat, F344 (F) 41 days 28 wk NTP (1993)	Oral > 99% Feed 0, 6000, 18 000 mg/kg for 27 wk (27 wk stop-exposure, with interim evaluation at 28 wk) 10, 10, 10 10, 10, 10	*Urinary bladder*: transitional cell carcinoma 0/10, 0/10, 10/10*	 *$P \le 0.01$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females.
Rat, F344 (M) 41 days 40 wk NTP (1993)	Oral > 99% Feed 0, 6000, 18 000 mg/kg for 27 wk (27 wk stop-exposure, with interim evaluation at 40 wk) 10, 10, 10 10, 10, 6	*Urinary bladder*: transitional cell carcinoma 0/10, 3/10, 6/6* *Large intestine*: adenoma (adenomatous polyp) 0/10, 2/10, 4/6*	 *$P \le 0.01$, Fisher exact test *$P \le 0.01$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females.

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344 (F) 41 days 40 wk NTP (1993)	Oral > 99% Feed 0, 6000, 18 000 mg/kg for 27 wk (27 wk stop-exposure, with interim evaluation at 40 wk) 10, 10, 10 10, 10, 6	*Urinary bladder*: transitional cell carcinoma 0/10, 1/9, 6/6*	*$P \leq 0.01$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females.
Rat, F344 (M) 41 days 65 wk NTP (1993)	Oral > 99% Feed 0, 6000 mg/kg for 27 wk (27 wk stop-exposure, with interim evaluation at 65 wk) 10, 10 9, 3	*Large intestine*: adenoma (adenomatous polyp) 0/9, 3/3*	*$P \leq 0.01$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females.
Rat, F344 (F) 41 days 65 wk NTP (1993)	Oral > 99% Feed 0, 6000 mg/kg for 27 wk (27 wk stop-exposure, with interim evaluation at 65 wk) 10, 10 8, 10	*Urinary bladder*: transitional cell carcinoma 0/8, 9/10*	*$P \leq 0.01$, Fisher exact test	Principal strengths: well-described and well-conducted GLP study; use of males and females.

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344 (M) 41 days 103 wk NTP (1993)	Oral > 99% Feed 0, 6000, 18 000 mg/kg for 27 wk (27 wk stop-exposure, with evaluation at 103 wk) 20, 20, 20 13, 1, 0	*Urinary bladder* Transitional cell carcinoma 0/21, 23/27*, 33/34* Sarcoma 0/21, 1/27, 7/34* *Kidney*: transitional cell carcinoma 0/21, 1/27, 6/34* *Large intestine*: adenoma (adenomatous polyp) 0/21, 21/27*, 24/34*	 *$P \leq 0.01$, Fisher exact test *$P < 0.05$, Fisher exact test *$P < 0.05$, Fisher exact test *$P \leq 0.01$, Fisher exact test	Principal strengths: GLP study; use of males and females. Principal limitations: low survival rate. At the end of week 103, 13/20 control rats predesignated for evaluation and 1/20 rats predesignated for evaluation from the group at 6000 mg/kg were alive, and all of 20 rats predesignated for evaluation from the group at 18 000 mg/kg had died before study termination. Rats surviving at least 28 wk but that died before the end of study, and rats alive at the end of the study were included in the histopathological examination of this 2-year stop-exposure study (total number of males: 21 control, 27 lower-dose, and 34 higher-dose animals).
Rat, F344 (F) 41 days 103 wk NTP (1993)	Oral > 99% Feed 0, 6000, 18 000 mg/kg for 27 wk (27 wk stop-exposure, with evaluation at 103 wk) 20, 20, 20 14, 4, 0	*Urinary bladder* Transitional cell carcinoma 0/20, 18/20*, 32/34* Sarcoma 0/20, 2/20, 12/34* *Large intestine*: adenoma (adenomatous polyp) 0/22, 5/20*, 17/34**	 *$P \leq 0.01$, Fisher exact test *$P \leq 0.01$, Fisher exact test *$P < 0.05$, Fisher exact test; **$P \leq 0.01$, Fisher exact test	Principal strengths: GLP study, use of males and females. Principal limitations: low survival rate. At the end of week 103, 14/20 control rats predesignated for evaluation and 4/20 rats predesignated for evaluation from the group at 6000 mg/kg were alive, and all of 20 rats predesignated for evaluation from the group at 18 000 mg/kg had died before study termination. Rats surviving at least 28 wk but that died before the end of study, and rats alive at the end of the study were included in the histopathological examination of this 2-year stop-exposure study (total number of females: 22 control, 20 lower-dose, and 34 higher-dose animals).

F, female; GLP, Good Laboratory Practice; M, male; NS, not significant; wk, week.

14/50, 20/50, 36/50, and 18/50; the incidence of hepatocellular carcinoma was 5/50 (10%), 2/50 (4%), 8/50 (16%), and 3/50 (6%); the incidence of hepatoblastoma was 1/50 (2%), 1/50 (2%), 2/50 (4%), and 0/50; and the incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) was 17/50, 22/50, 37/50, and 20/50. There was a significant positive trend in the incidence of hepatocellular adenoma [$P < 0.001$] and in the incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) [$P < 0.001$]. The incidence of hepatocellular adenoma ($P < 0.001$) and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) ($P < 0.001$) was significantly higher in females at 2000 mg/kg than in controls. The incidence of tumours in historical controls for female B6C3F$_1$ mice was: hepatocellular carcinoma, 28/863 (mean, 3.2%; range, 0–10%); and hepatoblastoma, 1/863 (mean, 0.1%; range, 0–2%).

Regarding non-neoplastic lesions in the liver, there was a significant increase in the incidence of cytological alterations and eosinophilic foci in male and female mice, and of necrosis, haemorrhage, and Kuppfer cell pigmentation in males (NTP, 1993). [The Working Group noted that this was a well-conducted study that complied with GLP, the duration of exposure and observation was adequate, and males and females were used. In addition, *ortho*-nitroanisole caused tumours derived from tissues with different embryological differentiation pathways (epithelial and mesenchymal) in males and females.]

3.2 Rat

3.2.1 Oral administration (feed)

In a study that complied with GLP, groups of 50 male and 50 female Fischer 344 rats (age, 40 days) were given feed containing *ortho*-nitroanisole (purity, > 99%) at a concentration of 0 (controls), 222, 666, or 2000 mg/kg for 103 weeks (NTP, 1993; see also Irwin et al., 1996). Survival at the end of the study was 32/50, 34/50, 24/50, and 9/50 in males, and 33/50, 41/50, 26/50, and 33/50 in females, at 0, 222, 666, and 2000 mg/kg, respectively. Survival of males at 2000 mg/kg was significantly lower than that of controls. For female rats, there were no significant differences in survival rates between control and treatment groups. The final mean body weights of male and female rats at 2000 mg/kg were significantly lower than those of controls. Full histopathology was performed on grossly visible lesions and major organs and tissues.

The incidence of mononuclear cell leukaemia was 26/50, 25/50, 42/50, and 34/50 in male rats, and 14/50, 11/50, 14/50, and 26/50 in female rats, in the control group and at 222, 666, and 2000 mg/kg, respectively. There was a significant positive trend in the incidence of mononuclear cell leukaemia in males ($P = 0.041$) and females ($P < 0.001$). The incidence of mononuclear cell leukaemia was significantly higher in males at 666 mg/kg ($P < 0.001$) and in females at 2000 mg/kg ($P = 0.012$) than in controls. The incidence in males at 2000 mg/kg (68%) was slightly higher than in the control group (52%), but the difference was not significantly different by pairwise comparison [possibly due to the high mortality rate and corresponding early death of these animals]; however, this incidence was still above the upper bound of the historical control range for male Fischer 344 rats (32–62%) (NTP, 1993). [The Working Group noted this was a well-conducted study that complied with GLP, the duration of exposure and observation was adequate, and males and females were used.]

3.2.2 Oral administration (stop-exposure study)

In a study that complied with GLP, groups of 60 male and 60 female Fischer 344 rats (age, 41 days) were given feed containing *ortho*-nitroanisole (purity, > 99%) at a concentration

of 0 (controls), 6000, or 18 000 mg/kg for a predetermined duration of 27 weeks, and thereafter received feed only for up to an additional 76 weeks, for a total study duration of up to 103 weeks (NTP, 1993). Ten males and ten females per group were scheduled for interim evaluations at experimental weeks 13, 28, 40, and 65; the remaining rats were killed at experimental week 103. Full histopathology was performed on grossly visible lesions and major organs and tissues.

At experimental week 28 (1 week after the completion of *ortho*-nitroanisole administration), the incidence of transitional cell carcinoma of the urinary bladder was 0/10, 0/10, and 10/10 in males, and 0/10, 0/10, and 10/10 in females, in the control group and at 6000 and 18 000 mg/kg, respectively. The incidence of transitional cell carcinoma of the urinary bladder was significantly higher in males ($P \leq 0.01$) and females ($P \leq 0.01$) at 18 000 mg/kg than in controls.

At experimental week 40 (13 weeks after cessation of *ortho*-nitroanisole administration), 6 males and 6 females out of the 10 males and 10 females predesignated for interim evaluation were alive in the groups at 18 000 mg/kg. The incidence of transitional cell carcinoma of the urinary bladder was 0/10, 3/10, and 6/6 in males, and 0/10, 1/9, and 6/6 in females, in the control group and at 6000 and 18 000 mg/kg, respectively. The incidence of transitional cell carcinoma of the urinary bladder was significantly higher in males ($P \leq 0.01$) and females ($P \leq 0.01$) at 18 000 mg/kg than in controls. In addition, the incidence of adenoma of the large intestine was significantly higher ($P \leq 0.01$) in males at 18 000 mg/kg (4/6) than in controls (0/10).

At experimental week 65 (38 weeks after the completion of *ortho*-nitroanisole administration), all males (10/10) and females (10/10) predesignated for interim evaluation had died before evaluation in groups at 18 000 mg/kg. Three out of 10 males and 10 out of 10 females predesignated for interim evaluation were alive in the groups at 6000 mg/kg. Survival in the controls was 9/10 in males and 8/10 in females. The incidence of transitional cell carcinoma of the urinary bladder was: 0/9 and 1/3 in males, and 0/8 and 9/10 in females, in the control group and at 6000 mg/kg, respectively. The incidence of transitional cell carcinoma of the urinary bladder was significantly higher ($P \leq 0.01$) in females at 6000 mg/kg than in controls. In addition, the incidence of adenoma of the large intestine was significantly higher ($P \leq 0.01$) in males at 6000 mg/kg (3/3) than in controls (0/9).

At experimental week 103, 13/20 males and 14/20 females predesignated for evaluation in the control groups, and 1/20 males and 4/20 females predesignated for evaluation in the groups at 6000 mg/kg were alive. All 20 male and 20 female rats predesignated for evaluation in the groups at 18 000 mg/kg died before week 103. Rats surviving at least 28 weeks but dying before the end of study and rats alive at the end of the study were combined for histopathological examination (total number of males: controls, 21; lower dose, 27; and higher dose, 34; total number of females: controls, 22; lower dose, 20; and higher dose, 34). The incidence of transitional cell carcinoma of the urinary bladder was 0/21, 23/27, and 33/34 in males, and 0/20, 18/20, and 32/34 in females, in the control group and at 6000 and 18 000 mg/kg, respectively. The incidence of transitional cell carcinoma of the urinary bladder was significantly higher in males ($P \leq 0.01$) and females ($P \leq 0.01$) at 6000 and 18 000 mg/kg than in controls. The incidence of sarcoma of the urinary bladder was 0/21, 1/27, and 7/34 in males, and 0/20, 2/20, and 12/34 in females, in the control group and at 6000 and 18 000 mg/kg, respectively. The incidence of sarcoma of the urinary bladder was significantly higher in males ($P < 0.05$) and females ($P \leq 0.01$) at 18 000 mg/kg than in controls. The incidence of transitional cell carcinoma of the kidney was significantly higher ($P < 0.05$) in males at 18 000 mg/kg (6/34) than in controls (0/21). The

incidence of adenoma of the large intestine was 0/21, 21/27, and 24/34 in males, and 0/22, 5/20, and 17/34 in females, in the control group and at 6000 and 18 000 mg/kg, respectively. The incidence of adenoma of the large intestine was significantly higher in males ($P \leq 0.01$) at 6000 and 18 000 mg/kg, and in females at 6000 and 18 000 mg/kg ($P < 0.05$, $P \leq 0.01$, respectively) than in controls.

Regarding non-neoplastic lesions, the incidence of transitional cell hyperplasia of the urinary bladder and of transitional cell hyperplasia of the kidney was significantly higher in many of the treated groups of male and female rats than in controls [transitional cell hyperplasia of the urinary bladder and transitional cell hyperplasia of the kidney are considered to be pre-neoplastic lesions] (NTP, 1993).

[The Working Group noted this was a well-conducted study that complied with GLP, and males and females were used. The high incidence (100%) of transitional cell carcinoma of the urinary bladder after a short duration of exposure in treated males and females was also noted. In addition, *ortho*-nitroanisole caused tumours derived from tissues with different embryological differentiation pathways (epithelial and mesenchymal) in males and females.]

[The Working Group noted that the aromatic amines *ortho*-toluidine and 2-naphthylamine (both IARC Group 1, *carcinogenic to humans*) also caused malignant tumours of the urinary bladder when administered orally in rats, and that the aromatic amine 4-aminobiphenyl (IARC Group 1) caused malignant tumours of the urinary bladder in mice as well as in dogs when administered orally (IARC, 2012).]

3.3 Synthesis

In one well-conducted GLP study in male and female B6C3F$_1$ mice treated by oral administration (in feed), *ortho*-nitroanisole caused a significant increase, with a significant positive trend, in the incidence of hepatocellular adenoma, hepatoblastoma, and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) in males; and of hepatocellular adenoma, and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) in females (NTP, 1993).

In one well-conducted GLP study in male and female Fischer 344 rats treated by oral administration (in feed), *ortho*-nitroanisole caused a significant increase, with a significant positive trend, in the incidence of mononuclear cell leukaemia in males and females (NTP, 1993).

In a series of stop-exposure experiments in one well-conducted GLP study in male and female Fischer 344 rats treated by oral administration (in feed), *ortho*-nitroanisole caused a significant increase in the incidence of: transitional cell carcinoma of the urinary bladder in males and females in a first stop-exposure experiment; transitional cell carcinoma of the urinary bladder in males and females, and of adenoma of the large intestine in males in a second stop-exposure experiment; transitional cell carcinoma of the urinary bladder in females, and of adenoma of the large intestine in males in a third stop-exposure experiment; and transitional cell carcinoma of the urinary bladder, sarcoma of the urinary bladder, and adenoma of the large intestine in males and females, and of transitional cell carcinoma of the kidney in males in a fourth stop-exposure experiment (NTP, 1993).

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

(a) Exposed humans

In 1993, an accident (an explosion) in a chemical plant in Frankfurt, Germany, resulted in the emission of various chlorinated and azo compounds, as well as *ortho*-nitroanisole (Heudorf et al., 1994; Hengstler et al., 1995). The median levels of 2-nitrophenol [*ortho*-nitrophenol], a major metabolite of *ortho*-nitroanisole, in urine samples collected from inhabitants of the contaminated area a few days after the accident were three times higher (25.2 μg/L) than in the controls (8.2 μg/L) (Heudorf et al., 1994).

(b) Human hepatic microsomes and cytosols

An O-demethylated metabolite of *ortho*-nitroanisole, *ortho*-nitrophenol and two oxidation products of this metabolite, 2,5-dihydroxynitrobenzene and 2,6-dihydroxynitrobenzene, were obtained in vitro by human hepatic microsomes and cytochrome P450 (CYP) in the presence of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) (Mikšanová et al., 2004a; Dračínská et al., 2006). Two reductive metabolites of *ortho*-nitroanisole, *N*-(2-methoxyphenyl)hydroxylamine and *ortho*-anisidine, were obtained in vitro by incubation with human hepatic cytosolic samples and xanthine oxidase, particularly in the presence of hypoxanthine (Mikšanová et al., 2004b). The nitroreduction of *ortho*-nitroanisole to *N*-(2-methoxyphenyl)hydroxylamine and *ortho*-anisidine was not detected after incubation with human hepatic microsomes in the presence of NADPH (Mikšanová et al., 2004a). *N*-(2-Methoxyphenyl)hydroxylamine was metabolized by human hepatic microsomes predominantly to *ortho*-anisidine, whereas 2-aminophenol [*ortho*-aminophenol] and two metabolites were detected as minor products (Naiman et al., 2011).

In studies in vitro using human recombinant CYPs and purified rodent CYPs, 2E1, 1A1, and 2B6 were the most efficient isoforms oxidizing *ortho*-nitroanisole to *ortho*-nitrophenol (Mikšanová et al., 2004a), whereas 2E1 and 1A1 were the most effective in the formation of 2,5-dihydroxynitrobenzene and 2,6-dihydroxynitrobenzene (Dračínská et al., 2006). In a study in vitro using selective inhibitors of microsomal CYPs, 3A4, 2E1, and 2C were the most important in the metabolism of *N*-(2-methoxyphenyl)hydroxylamine to *ortho*-anisidine by human hepatic microsomes (Naiman et al., 2011).

4.1.2 Experimental systems

(a) In vivo

The absorption, distribution, and excretion of *ortho*-nitroanisole were studied in male Fischer 344 rats by Miller et al. (1985). Three dose levels of [^{14}C]-labelled *ortho*-nitroanisole (5, 50, or 500 mg/kg bw) were administered orally to rats, and daily excreta were analysed for radiolabel. *ortho*-Nitroanisole was readily absorbed from the stomach. Peak blood concentrations in vitro reflected the dose-dependence of absorption, with parent *ortho*-nitroanisole reaching maximal concentrations at 3 hours after a dose of 50 mg/kg bw and at 6 hours after a dose of 500 mg/kg bw. Within 7 days, 7% of the administered dose had been excreted in the faeces for all dose levels and about 70% of the administered dose had been eliminated in the urine. The predominant route of elimination was through metabolism to *ortho*-nitrophenol, subsequent sulfation to *ortho*-nitrophenyl sulfate, and glucuronidation to *ortho*-nitrophenyl glucuronide. Seven days after oral administration of *ortho*-nitroanisole, less than 0.5% of the administered dose remained in the carcass.

The distribution of *ortho*-nitroanisole–derived ^{14}C to tissues (muscle, 20%; skin, 10%; fat, 6.8%; blood, 6.5%; liver, 4.8%; plasma, 3.1%; kidney, 2.8%; and small intestine, 1.9%) occurred rapidly after intravenous administration of [^{14}C]-labelled *ortho*-nitroanisole at 25 mg/kg bw. Urinary and faecal elimination patterns were similar to those found after oral administration. The subsequent elimination of ^{14}C was rapid and biphasic. The initial elimination phase in all tissues had a half-life of 1–2 hours, and the terminal phase half-lives for all tissues ranged from 2.5 to 6.2 days. Elimination of parent *ortho*-nitroanisole from the blood was biphasic with initial and terminal half-lives of 30 minutes and 2.2 hours, respectively. Monophasic elimination of *ortho*-nitroanisole from the liver, kidneys, and small intestine occurred with half-lives of 0.35, 0.55, and 0.68 hour, respectively. Biliary excretion was similar to faecal elimination, indicating a lack of enterohepatic recirculation (Miller et al., 1985).

(b) In vitro

See Fig. 4.1.

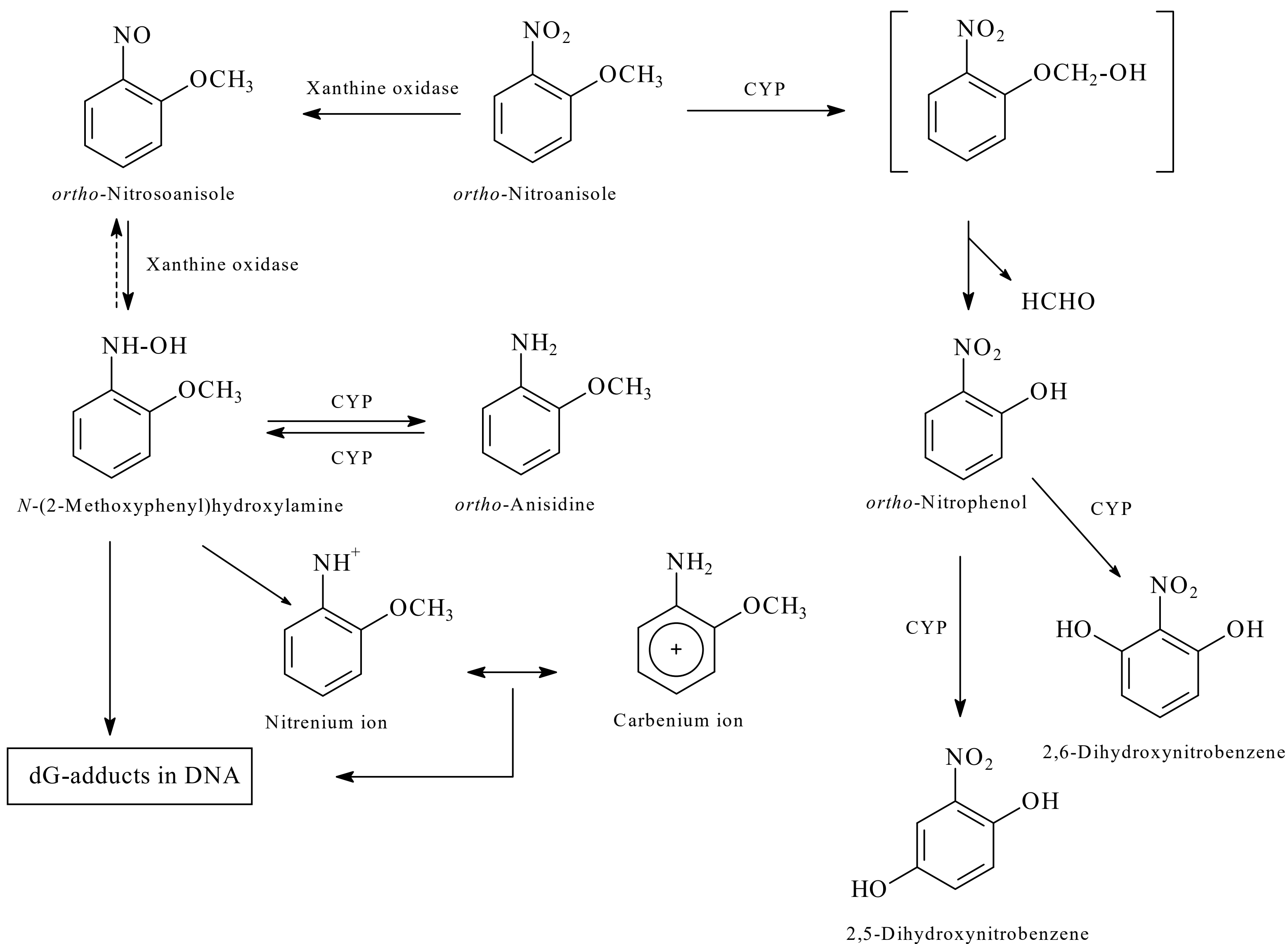
Three metabolites of *ortho*-nitroanisole obtained by incubation with human hepatic microsomes – *ortho*-nitrophenol, 2,5-dihydroxynitrobenzene and 2,6-dihydroxynitrobenzene – were produced in vitro by incubation with rat and rabbit hepatic microsomes in the presence of NADPH (Mikšanová et al., 2004a; Dračínská et al., 2006). 2,5-Dihydroxynitrobenzene was the predominant product of metabolism by human microsomes, whereas *ortho*-nitrophenol was the major metabolite generated by rat and rabbit microsomes (Dračínská et al., 2006). Oxidation of *ortho*-nitrophenol by rat hepatic microsomes to 2,5-dihydroxynitrobenzene was detected by HPLC, whereas reduction of *ortho*-nitrophenol to *ortho*-aminophenol was not observed (Svobodová et al., 2009). Two reductive metabolites of *ortho*-nitroanisole, *N*-(2-methoxyphenyl) hydroxylamine and *ortho*-anisidine, were obtained in vitro after incubation with rat, rabbit, and pig hepatic cytosolic samples and xanthine oxidase, particularly in the presence of hypoxanthine (Mikšanová et al., 2004b). The nitroreduction of *ortho*-nitroanisole to *N*-(2-methoxyphenyl)hydroxylamine and *ortho*-anisidine was not detected after incubation with rat and rabbit hepatic microsomes in the presence of NADPH (Mikšanová et al., 2004a). *N*-(2-Methoxyphenyl)hydroxylamine was metabolized by rat and rabbit hepatic microsomes mainly to *ortho*-aminophenol and *ortho*-anisidine, whereas *ortho*-nitrosoanisole was formed as a minor metabolite (Naiman et al., 2008). [Naiman et al. (2010) reported that *N*-(2-methoxyphenyl) hydroxylamine was metabolized by rat hepatic microsomes mainly to *ortho*-anisidine, whereas *ortho*-aminophenol and two other metabolites were minor products.]

Rat recombinant enzymes CYP 2E1, 2D2, 2B2, 2C6, and 1A1 efficiently metabolized *ortho*-nitroanisole (Svobodová et al., 2008). In a study in vitro using selective CYP inhibitor and hepatic microsomes of rats pre-treated with specific CYP inducers, most oxidation of *ortho*-nitrophenol was produced by CYP 2E1 and 3A, followed by 2D and 2C (Svobodová et al., 2009). Rat hepatic CYP 2E1, 1A subfamily and, to a lesser extent, those of a 2B subfamily were capable of metabolizing *N*-(2-methoxyphenyl) hydroxylamine to *ortho*-aminophenol (Naiman et al., 2008). Rat CYP 2C, followed by 2E1, 2D, and 2A were the major enzymes metabolizing *N*-(2-methoxyphenyl)hydroxylamine to *ortho*-anisidine (Naiman et al., 2010).

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016) including whether *ortho*-nitroanisole is electrophilic or can be metabolically activated;

Fig. 4.1 Metabolic pathways for *ortho*-nitroanisole



CYP, cytochrome P450; dG, deoxyguanosine.
The compound shown in square brackets was not detected under experimental conditions.
Compiled by the Working Group using information from Stiborová et al. (2009).

is genotoxic; induces oxidative stress; or alters cell proliferation, cell death or nutrient supply. For the evaluation of other key characteristics of carcinogens, data were not available or considered insufficient.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

Table 4.1, Table 4.2, and Table 4.3 summarize the available studies on the genetic and related effects of *ortho*-nitroanisole.

(a) Humans

No data on exposed humans were available to the Working Group.

A study in vitro using [^{3}H]-labelled *ortho*-nitroanisole and the ^{32}P-post-labelling technique showed that the deoxyguanosine adducts formed from *N*-(2-methoxyphenyl)hydroxylamine were detected in calf thymus DNA incubated with *ortho*-nitroanisole and human hepatic cytosolic samples in the presence of hypoxanthine (Stiborová et al., 2004). In contrast, no DNA-adduct formation mediated by *ortho*-nitroanisole oxidation in human hepatic microsomes was detectable (Mikšanová et al., 2004a; see Table 4.3).

(b) Experimental systems

See Table 4.1 and Table 4.3.

In male Wistar rats treated intraperitoneally with *ortho*-nitroanisole (0.15 mg/kg bw per day) for 5 consecutive days, deoxyguanosine adducts formed from *N*-(2-methoxyphenol)hydroxylamine were detected in DNA from the urinary bladder and, to a lesser extent, the kidney, liver, and spleen by ^{32}P-post-labelling assay, whereas no adduct formation was observed in the lung, heart, or brain (Stiborová et al., 2004).

A study in vitro using [^{3}H]-labelled *ortho*-nitroanisole and the ^{32}P-post-labelling technique did not detect DNA-adduct formation mediated by *ortho*-nitroanisole oxidation in rabbit hepatic microsomes incubated with calf thymus DNA (Mikšanová et al., 2004a).

4.2.2 Is genotoxic

Table 4.1, Table 4.2, and Table 4.3 summarize the available studies of the genetic and related effects of *ortho*-nitroanisole.

(a) Humans

In 1993, an accident in a chemical plant in Frankfurt, Germany, resulted in the emission of various chlorinated and azo compounds, including *ortho*-nitroanisole (Heudorf et al., 1994; Hengstler et al., 1995). Levels of DNA single-strand breaks (by alkaline elution) in blood mononuclear cells were slightly but statistically significantly higher 19 days after the accident in 16 firefighters who participated in mechanically removing the precipitate in the contaminated area for about 8 hours than in two reference groups (19 unexposed firefighters living in the same town, and 28 people without any apparent occupational exposure to genotoxic substances) (Hengstler et al., 1995). [The Working Group noted that data on the firefighters' exposure to *ortho*-nitroanisole and co-exposures were lacking and that there were co-exposures to other genotoxicants.] After 3 months, the level of DNA single-strand breaks was no longer increased in comparison with the levels in the reference groups. [Limited data were reported on air concentrations in the community.]

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1.

In male rats treated by gavage, *ortho*-nitroanisole induced DNA strand breaks (as measured by the standard alkaline comet assay) in kidney cells in Sprague-Dawley rats (Nesslany et al., 2007), but not in liver and urinary bladder cells in male Sprague-Dawley and Fischer 344 rats (Wada et al., 2014, 2017). In male Fischer 344 rats

Table 4.1 Genetic and related effects of *ortho*-nitroanisole in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Reference
DNA adducts	Rat, Wistar (M)	Liver, kidney, spleen, and urinary bladder	+	0.15 mg/kg bw per day	Intraperitoneal, 5 consecutive days	Stiborová et al. (2004)
DNA adducts	Rat, Wistar (M)	Lung, heart and brain	–	0.15 mg/kg bw per day	Intraperitoneal, 5 consecutive days	Stiborová et al. (2004)
DNA strand breaks (comet assay)	Rat, Sprague-Dawley (M)	Kidney cells	+	250 mg/kg bw	Gavage, 2 doses, sampling 3–6 h and 22–26 h after dosing	Nesslany et al. (2007)
DNA strand breaks (comet assay)	Rat, Sprague-Dawley (M)	Urinary bladder and liver cells	–	700 mg/kg bw per day	Gavage, 2 doses on 2 consecutive days, sampling 3 h after final dosing	Wada et al. (2014)
DNA strand breaks (comet assay)	Rat, F344 (M)	Urinary bladder and liver cells	–	500 mg/kg bw per day	Gavage, 3 doses on 3 consecutive days, sampling 3 h after final dosing	Wada et al. (2017)
DNA strand breaks (modified comet assay, with hOGG1)	Rat, F344 (M)	Urinary bladder cells	+	500 mg/kg bw per day	Gavage, 3 doses on 3 consecutive days, sampling 3 h after final dosing	Wada et al. (2017)
DNA strand breaks (modified comet assay, with hOGG1)	Rat, F344 (M)	Liver cells	–	500 mg/kg bw per day	Gavage, 3 doses on 3 consecutive days, sampling 3 h after final dosing	Wada et al. (2017)
DNA strand breaks (phosphorylated histone γ-H2AX)	Rat, F344 (M)	Urinary bladder epithelial cell	+	1.8% or 1048 mg/kg bw per day	Feed, 4 wk with and without a 2-wk recovery period	Toyoda et al. (2015)
DNA strand breaks (phosphorylated histone γ-H2AX)	Mouse, $B6C3F_1$ (M)	Urinary bladder epithelial cell	–	0.6% or 638 mg/kg bw per day	Feed, 4 wk with and without a 2-wk recovery period	Sone et al. (2019)
Micronucleus formation	Rat, F344 (M)	Bone marrow	–	500 mg/kg bw per day	Gavage, 3 doses on 3 consecutive days, sampling 3 h after final dosing	Wada et al. (2017)

bw, body weight; h, hour; HID, highest ineffective dose; hOGG1, human 8-oxoguanine DNA-glycosylase 1; LED, lowest effective dose; M, male; wk, week.
[a] +, positive; –, negative.

Table 4.2 Genetic and related effects of *ortho*-nitroanisole in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Gene mutation, *Tk* locus	Mouse lymphoma L5178Y cells	+	NT	250 μg/mL	NTP (1993)
Chromosomal aberrations	Chinese hamster ovary cells	–	+	1060 μg/mL	Galloway et al. (1987)
Sister-chromatid exchange	Chinese hamster ovary cells	+	+	123 μg/mL	Galloway et al. (1987)

HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested; *Tk*, thymidine kinase.
[a] +, positive; –, negative.

given feed containing *ortho*-nitroanisole, there was a significant increase in levels of phosphorylated histone H2AX (γ-H2AX), a marker of DNA damage, in epithelial cells of the urinary bladder (Toyoda et al., 2015). In contrast, in male B6C3F$_1$ mice given feed containing *ortho*-nitroanisole, there was no increase in levels of γ-H2AX in bladder epithelial cells (Sone et al., 2019).

No increase in the frequency of micronucleus formation was seen in the bone marrow of male Fischer 344 rats given *ortho*-nitroanisole as three daily doses (up to 500 mg/kg bw per day) by gavage (Wada et al., 2017).

(ii) Non-human mammalian cells in vitro

See Table 4.2.

In single studies in cultured mammalian cells, *ortho*-nitroanisole induced mutation at the *Tk* locus of mouse lymphoma L5178Y cells (NTP, 1993), as well as chromosomal aberrations and sister-chromatid exchange in Chinese hamster ovary cells (Galloway et al., 1987). The clastogenic activity was modest and observed only in the presence of S9, whereas sister-chromatid exchange and *Tk* mutations were induced in the absence of S9.

(iii) Non-mammalian experimental systems

See Table 4.3.

ortho-Nitroanisole was tested in several laboratories for the induction of gene mutations in *Salmonella typhimurium*. Positive responses were obtained consistently with strains TA100 (Chiu et al., 1978; Haworth et al., 1983; Shimizu & Yano, 1986; Dellarco & Prival, 1989; NTP, 1993; JETOC, 1996; Wada et al., 2017) and YG3008 (Wada et al., 2017). Results were generally negative in strains used for detecting frameshift mutations (Chiu et al., 1978; Haworth et al., 1983; Shimizu & Yano, 1986; NTP, 1993; JETOC, 1996).

ortho-Nitroanisole gave positive results in the SOS/*umu* genotoxicity assay in *Salmonella typhimurium* strain TA1535/pSK1002 (Reifferscheid & Heil, 1996). *ortho*-Nitroanisole gave positive results in the rec assay in *Bacillus subtilis* strains H17 and M45 (Shimizu & Yano, 1986). *ortho*-Nitroanisole did not induce reverse mutation in the WP2*uvrA* strain of *Escherichia coli* in the presence or absence of exogenous metabolic activation (JETOC, 1996).

4.2.3 Induces oxidative stress

(a) Humans

No data were available to the Working Group.

Table 4.3 Genetic and related effects of *ortho*-nitroanisole in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA100	Reverse mutation	+	NT	1530 µg/mL		Chiu et al. (1978)
Salmonella typhimurium TA100	Reverse mutation	+	+	666 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA100, TA98 and TA1538	Reverse mutation	+	NT	1 µL/plate		Shimizu & Yano (1986)
Salmonella typhimurium TA100	Reverse mutation	NT	+	580 µg/mL		Dellarco & Prival (1989)
Salmonella typhimurium TA100	Reverse mutation	+	+	333 µg/plate		NTP (1993)
Salmonella typhimurium TA100	Reverse mutation	+	+	1000 µg/plate	Detailed methods were not available.	JETOC (1996)
Salmonella typhimurium TA100 and YG3008	Reverse mutation	+	+	313 µg/plate		Wada et al. (2017)
Salmonella typhimurium TA1535, TA1537 and TA98	Reverse mutation	–	–	1000 µg/plate		Haworth et al. (1983)
Salmonella typhimurium TA1535 and TA1537	Reverse mutation	–	NT	5 µL/plate		Shimizu & Yano (1986)
Salmonella typhimurium TA1535	Reverse mutation	+	–	1000 µg/plate		NTP (1993)
Salmonella typhimurium TA1535, TA1537, and TA98	Reverse mutation	–	–	1000 µg/plate	Detailed methods were not available.	JETOC (1996)
Salmonella typhimurium TA98	Reverse mutation	+	NT	765 µg/mL		Chiu et al. (1978)
Salmonella typhimurium TA98 and TA1537	Reverse mutation	–	–	1000 µg/plate		NTP (1993)
Salmonella typhimurium TA97	Reverse mutation	–	–	3333 µg/plate		NTP (1993)
Salmonella typhimurium TA1535/pSK1002	SOS/*umu* genotoxicity	+	NT	NR		Reifferscheid & Heil (1996)
Escherichia coli WP2*uvrA*	Reverse mutation	–	–	2000 µg/plate	Detailed methods were not available.	JETOC (1996)
Bacillus subtilis rec H17 and M45 strains	Differential toxicity	+	NT	625 µg/mL		Shimizu & Yano (1986)

Table 4.3 Genetic (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA isolated from calf thymus	DNA adducts	–	+	$[^3H]$-labelled *ortho*-nitroanisole, 20 µL/0.75 mL [0.5 mM]	Metabolic activation by human hepatic cytosolic protein in the presence of hypoxanthine.	Stiborová et al. (2004)
DNA isolated from calf thymus	DNA adducts	–	–	$[^3H]$-labelled *ortho*-nitroanisole, 20 µL/0.75 mL [0.5 mM]	Metabolic activation by human or rabbit hepatic microsomes and NADPH.	Mikšanová et al. (2004a)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NT, not tested; NR, not reported.
[a] +, positive; –, negative.

(b) Experimental systems

In male Fischer 344 rats treated with *ortho*-nitroanisole (500 mg/kg bw per day) by gavage for 3 consecutive days, oxidative damage to DNA in the urinary bladder (as measured by the modified comet assay) was significantly increased only in the presence of human 8-oxoguanine DNA-glycosylase 1 (hOGG1) which can recognize 8-oxoguanine and convert it into a DNA break. 8-Oxoguanine is not detectable by the standard comet assay. In contrast to the findings in the urinary bladder, no increase in the frequency of DNA damage was detected in liver cells regardless of the presence of hOGG1 (Wada et al., 2017).

In a test for reverse mutation in bacteria, strain YG3008 (which is sensitive to oxidative mutagens owing to a lack of the $mutM_{st}$ gene encoding an enzyme, formamidopyrimidine DNA-glycosylase, that recognizes and repairs oxidative DNA damage) was more sensitive to *ortho*-nitroanisole than the parent strain TA100 (Wada et al., 2017).

4.2.4 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In a dose-finding study for a carcinogenicity test during which Fischer 344 rats were given feed containing *ortho*-nitroanisole at concentrations of 200–18 000 mg/kg (10–720 mg/kg bw per day) for 13 weeks, the incidence of urothelial hyperplasia in the urinary bladder was significantly increased in male and female rats at 6000 and 18 000 mg/kg (NTP, 1993).

In Fischer 344 rats given feed containing *ortho*-nitroanisole at a concentration of 6000 or 18 000 mg/kg (340 or 1100 mg/kg bw per day) for 27 weeks followed by maintenance on feed only for additional 77 weeks (stop-exposure study), the incidence of urothelial hyperplasia in the urinary bladder was significantly increased in males and females from week 13. In addition, there was a significant increase in the incidence of hyperplasia of transitional epithelial cells in the renal pelvis in males at week 28, 40, and 103, and in females at week 103. Subacute inflammation and proliferation of connective tissue in the lamina propria of the urinary bladder were also observed. The lesions were characterized by scattered inflammatory cells, principally neutrophils and macrophages, and increased numbers of fibroblasts with immature collagen (NTP, 1993; Irwin et al., 1996).

In male Fischer 344 rats given feed containing *ortho*-nitroanisole at a concentration of 18 000 mg/kg (1048 mg/kg bw per day) for 4 weeks, there was a significant increase in the expression of Ki67 (a marker of cell proliferation activity) in the bladder urothelium (Toyoda et al., 2015). In contrast, in male $B6C3F_1$ mice given feed containing *ortho*-nitroanisole at a concentration of 6000 mg/kg (638 mg/kg bw per day) for 4 weeks, there was no increase in the frequency of Ki67-positive cells in the bladder urothelium (Sone et al., 2019).

In Fischer 344 rats given feed containing *ortho*-nitroanisole at a concentration of 222, 666, or 2000 mg/kg (10–90 mg/kg bw per day) for 2 years, the incidence of focal hyperplasia of epithelial cells in the forestomach was significantly increased in male rats of all dose groups and in female rats at the highest dose (NTP, 1993; Irwin et al., 1996).

4.2.5 Data on other key characteristics of carcinogens

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In a host-mediated in vivo/in vitro assay using male NMRI mice, the transformation of peritoneal macrophages was induced by a single dose of *ortho*-nitroanisole (1.3 mg/kg bw, or 0.1% of the median lethal dose, 1300 mg/kg bw) (Esmaeili et al., 2006).

4.3 Other relevant evidence

Methaemoglobin concentrations were significantly increased in male Fischer 344 rats fed *ortho*-nitroanisole (at concentrations at or above 1166 mg/kg in feed) for 14 days (NTP, 1993). In 13-week studies, increases in methaemoglobin concentrations were observed in male and female Fischer 344 rats (at 6000 and 18 000 mg/kg in feed) and in male B6C3F$_1$ mice (at 6000 mg/kg in feed).

Erythrocyte counts, haematocrit values, and haemoglobin concentrations were significantly lower in male Fischer 344 rats exposed to *ortho*-nitroanisole in the feed (583, 1166, 2332, 4665, or 9330 mg/kg) for 14 days. With the exception of depressed body-weight gain, no such treatment-related effect was observed in B6C3F$_1$ mice (NTP, 1993).

In 13-week feeding studies, observed haemoglobin and haematocrit values were lower in male and female Fischer 344 rats receiving *ortho*-nitroanisole at a concentration of 2000, 6000, or 18 000 mg/kg, and in male and female B6C3F$_1$ mice at 2000 or 6000 mg/kg than in controls (NTP, 1993).

5. Summary of Data Reported

5.1 Exposure characterization

ortho-Nitroanisole is an anisole compound with the formula $C_7H_7NO_3$, which is produced from methanolic sodium hydroxide, 2-chloronitrobenzene, and methanol.

Information on the production of *ortho*-nitroanisole was sparse, and indicated that manufacturing occurs in Asia. *ortho*-Nitroanisole is used primarily as a precursor for *ortho*-anisidine (see the monograph on *ortho*-anisidine in the present volume]. Waste containing *ortho*-nitroanisole can contaminate water and soil, but *ortho*-nitroanisole is not expected to bioaccumulate in aquatic organisms.

Scant information was found on occupational exposure and no information was identified on general population exposure, but *ortho*-nitroanisole has been detected in drinking-water. *ortho*-Nitroanisole is considered to be a hazardous material in the European Union and USA, and is labelled as such under the United Nations' Globally Harmonized System of Classification and Labelling of Chemicals. Only two countries (Poland and the Russian Federation) have established occupational exposure limits.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

ortho-Nitroanisole increased the incidence of malignant neoplasms in two species.

In B6C3F$_1$ mice, *ortho*-nitroanisole administered orally (in feed) in one study caused an increase in the incidence of hepatoblastoma in males. In addition, *ortho*-nitroanisole caused an increase in the incidence of a combination of benign and malignant liver tumours

(hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma) in male and female mice.

In Fischer 344 rats, *ortho*-nitroanisole administered orally (in feed) in one study caused an increase in the incidence of mononuclear cell leukaemia in male and female rats.

In Fischer 344 rats, *ortho*-nitroanisole administered orally (in feed) in four stop-exposure experiments caused an increase in the incidence of transitional cell carcinoma of the urinary bladder and sarcoma of the urinary bladder in male and female rats, and of transitional cell carcinoma of the kidney in male rats.

5.4 Mechanistic evidence

No studies characterizing the absorption, distribution, metabolism or excretion of *ortho*-nitroanisole in humans were available. *ortho*-Nitrophenol, a major metabolite of *ortho*-nitroanisole, was detected in the urine of individuals exposed to contaminated air after an explosion that released *ortho*-nitroanisole, as well as various chlorinated and azo compounds. Human, rabbit, or rat hepatic microsomes catalysed cytochrome P450-dependent oxidation of *ortho*-nitroanisole to *ortho*-nitrophenol and further oxidation to 2,5-dihydroxynitrobenzene and 2,6-dihydroxynitrobenzene. Human, rabbit, rat, or pig hepatic cytosol catalysed nitroreduction of *ortho*-nitroanisole to *N*-(2-methoxyphenyl) hydroxylamine and *ortho*-anisidine. Human and rat hepatic microsomes reversibly reduced *N*-(2-methoxyphenyl)hydroxylamine to *ortho*-anisidine. In rats exposed orally, *ortho*-nitroanisole is readily absorbed, widely distributed to tissues, and excreted primarily via the urine as sulfate and glucuronide conjugates of *ortho*-nitrophenol.

There is consistent and coherent evidence that *ortho*-nitroanisole exhibits key characteristics of carcinogens in experimental systems. *ortho*-Nitroanisole is metabolically activated to electrophiles. No data on DNA adducts in exposed humans were available. In in vitro studies, *ortho*-nitroanisole is activated by human hepatic cytosol in the presence of hypoxanthine to form *N*-(2-methoxyphenyl)hydroxylamine-derived DNA adducts. In experimental systems, these DNA adducts were observed in several tissues of male Wistar rats exposed to *ortho*-nitroanisole, with the highest level found in the urinary bladder.

ortho-Nitroanisole is genotoxic, inducing mutations at the *Tk* locus in mouse lymphoma L5178Y cells in the absence of metabolic activation, and in multiple studies in base-pair substitution strains of bacteria, both in the presence and in the absence of metabolic activation. *ortho*-Nitroanisole also induces DNA damage, with positive findings in the comet assay in Sprague-Dawley rat kidney, but not urinary bladder; positive findings in Fischer 344 rat urinary bladder in the presence of human 8-oxoguanine DNA-glycosylase 1 in the comet assay; increased levels of γ-H2AX in urinary bladder epithelial cells in exposed Fischer 344 rats; and positive findings in two strains of *Bacillus subtilis* in the rec assay.

ortho-Nitroanisole also alters cell proliferation, cell death, or nutrient supply. *ortho*-Nitroanisole induced urothelial hyperplasia in the urinary bladder in male and female Fischer 344 rats, an effect that persisted after cessation of exposure. *ortho*-Nitroanisole also increased expression of Ki67 in bladder urothelial cells in male Fischer 344 rats.

Overall, the evidence is consistent and coherent that, in view of the metabolism of *ortho*-nitroanisole to the aromatic amine *ortho*-anisidine, *ortho*-nitroanisole belongs within a mechanistic class of aromatic amines. Members of this class, including 4-aminobiphenyl, 2-naphthylamine, and *ortho*-toluidine, have been classified previously by the *IARC Monographs* programme as *carcinogenic to humans* (IARC Group 1). *ortho*-Nitroanisole

exhibits concordance with aromatic amines with respect to the formation of common DNA-reactive moieties; genotoxicity; and target organs of carcinogenicity in chronic animal bioassays. The urinary bladder is a common target organ of carcinogenicity for these aromatic amines in experimental animals. For instance, *ortho*-nitroanisole causes malignant tumours of the urinary bladder when administered orally to rats, as do *ortho*-anisidine, *ortho*-toluidine, and 2-naphthylamine. 4-Aminobiphenyl causes malignant tumours of the urinary bladder when administered orally to dogs and mice. Therefore, these mechanistic considerations go beyond chemical structural similarity to encompass biological and biochemical similarities relevant to common key characteristics of carcinogens.

6. Evaluation and Rationale

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Mechanistic evidence

There is *strong evidence* that, in view of the metabolism of *ortho*-nitroanisole to the aromatic amine *ortho*-anisidine, *ortho*-nitroanisole belongs, based on mechanistic considerations, to a class of aromatic amines for which several members have been classified as carcinogenic to humans. There is also *strong evidence* that *ortho*-nitroanisole exhibits key characteristics of carcinogens in experimental systems.

6.4 Overall evaluation

ortho-Nitroanisole is *probably carcinogenic to humans (Group 2A).*

6.5 Rationale

The Group 2A evaluation is based on *strong* mechanistic evidence. In view of the metabolism of *ortho*-nitroanisole to the aromatic amine *ortho*-anisidine, there is *strong* evidence that *ortho*-nitroanisole, based on mechanistic considerations, belongs to a class of aromatic amines for which several members have been classified as carcinogenic to humans. *ortho*-Nitroanisole exhibits concordance with aromatic amines with respect to the formation of common DNA-reactive moieties, genotoxicity, and target organs of carcinogenicity in chronic animal bioassays.

There is also *sufficient evidence* of carcinogenicity in experimental animals, based on an increased incidence of malignant neoplasms in two species.

In addition, there is *strong evidence* that *ortho*-nitroanisole exhibits key characteristics of carcinogens in experimental systems. *ortho*-Nitroanisole is metabolically activated to electrophiles, it is genotoxic, and it also alters cell proliferation, cell death, or nutrient supply.

The evidence on cancer in humans was *inadequate* as no data were available.

References

Aarti Industries (2020). Ortho nitro anisole (ONA). Available from: https://www.aarti-industries.com/products/chemicals/detail/129/?name=Ortho-Nitro-Anisole-(ONA)(91-23-6), accessed 18 January 2021.

ACGIH (2018). American Conference of Governmental Industrial Hygienists TLVs and BEIs. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati (OH), USA: American Conference of Governmental Industrial Hygienists.

Booth G (1991). Nitro compounds, aromatic. In: Elvers B, Hawkins S, Schulz G, editors. Ullmann's encyclopedia of industrial chemistry. Volume A17. 5th rev. New York (NY), USA: VCH Publishers; pp. 411–55.

BUA (1987). GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance (BUA): *o*-Nitroanisole (1-Methoxy-2-nitrobenzene) (BUA Report 9) (June 1987). Weinheim, Germany: VCH Verlagsgesellschaft mbH.

Chemical Register (2020). 2-Nitroanisole (CAS No. 91-23-6) Suppliers. Chemical Register. Available from: https://www.chemicalregister.com/2-Nitro_Anisole/Suppliers/pid27982.htm, accessed 7 October 2020.

Chiu CW, Lee LH, Wang CY, Bryan GT (1978). Mutagenicity of some commercially available nitro compounds for *Salmonella typhimurium*. *Mutat Res*. 58(1):11–22. doi:10.1016/0165-1218(78)90090-3 PMID:362191

Dellarco VL, Prival MJ (1989). Mutagenicity of nitro compounds in *Salmonella typhimurium* in the presence of flavin mononucleotide in a preincubation assay. *Environ Mol Mutagen*. 13(2):116–27. doi:10.1002/em.2850130206 PMID:2645131

DFG (2019). List of MAK and BAT values 2019: Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. Report 55, Deutsche Forschungsgemeinschaft (German Research Foundation). Available from: https://onlinelibrary.wiley.com/doi/pdf/10.1002/9783527826155, accessed May 2020.

Dračínská H, Miksanová M, Svobodová M, Smrcek S, Frei E, Schmeiser HH, et al. (2006). Oxidative detoxication of carcinogenic 2-nitroanisole by human, rat and rabbit cytochrome P450. *Neuro Endocrinol Lett*. 27(Suppl 2):9–13. PMID:17159769

ECHA (2019a). Substance information. 2-nitroanisole. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.001.866.

ECHA (2019b). Summary of classification and labelling. 2-nitroanisole. Harmonised classification – Annex VI of Regulation (EC) No. 1272/2008 (CLP Regulation). Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/61842, accessed 12 January 2021.

Esmaeili A, Schlatterer K, Demirhan I, Schlatterer B, Nauck M, Chandra P, et al. (2006). Tumorigenic potential and the molecular mechanism of the carcinogenic effect exerted by 2-nitroanisole. *Anticancer Res*. 26(6B):4203–12. PMID:17201134

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, et al. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ Mol Mutagen*. 10(Suppl 10):1–175. doi:10.1002/em.2850100502 PMID:3319609

Greenpeace (2017). Hazardous chemical releases at large: an investigation at the Lianyungang Chemical Industrial Park, Jiangsu Province, China. Available from: https://www.greenpeace.org/static/planet4-eastasia-stateless/2019/11/f5588785-f5588785-big-bang-report_en-final.pdf.

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983). Salmonella mutagenicity test results for 250 chemicals. *Environ Mutagen*. 5(Suppl 1):1–142. doi:10.1002/em.2860050703 PMID:6365529

Hengstler JG, Fuchs J, Bolm-Audorff U, Meyer S, Oesch F (1995). Single-strand breaks in deoxyribonucleic acid in fire fighters accidentally exposed to *o*-nitroanisole and other chemicals. *Scand J Work Environ Health*. 21(1):36–42. doi:10.5271/sjweh.6 PMID:7784863

Heudorf U, Neumann HG, Peters M (1994). [Accident in the Hoechst AG company 22 February 1993. 2. Public health evaluation]. *Gesundheitswesen*. 56(7):405–10. [German] PMID:7919705

HSDB (2011). 2-Nitroanisole. Hazardous Substances Data Bank. PubChem. Bethesda (MD), USA: United States National Library of Medicine, National Center for Biotechnology Information. Available from: https://pubchem.ncbi.nlm.nih.gov/source/hsdb/5186, accessed 18 February 2020.

Huang J, Zhou L, Xun Z, Wang Q, Lin S, Guo X, et al. (2017). Simultaneous determination of seven nitrogen-containing phenyl ethers in cosmetics by gas chromatography with mass spectrometry and dispersive solid-phase extraction. *J Sep Sci*. 40(8):1718–23. doi:10.1002/jssc.201601192 PMID:28230323

IARC (1996). 2-Nitroanisole. In: Printing processes and printing inks, carbon black and some nitro compounds. *IARC Monogr Eval Carcinog Risks Hum*, 65:369–80. Available from: https://publications.iarc.fr/83 PMID:9097112

IARC (2012). Chemical agents and related occupations. *IARC Monogr Eval Carcinog Risks Hum*, 100F:1–599. Available from: https://publications.iarc.fr/123 PMID:23189753

IFA (2019). 2-Nitroanisole. GESTIS substance database. Germany: Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (Institute for Occupational Safety and Health of the German Social Accident Insurance). Available from: https://www.dguv.de/ifa/gestis/gestis-stoffdatenbank/index-2.jsp, accessed 2 March 2020.

ILO (2017). 2-Nitroanisole. Physical and chemical information. International Labour Organization. Available from: https://www.ilo.org/dyn/icsc/showcard.display?p_card_id=1520&p_edit=&p_version=2&p_lang=en.

Irwin RD, Chhabra R, Eustis S, Pinter A, Prejean JD (1996). Tumors of the bladder, kidney, and intestine of F344 rats and liver of B6C3F$_1$ mice administered *o*-nitroanisole in feed. *Fundam Appl Toxicol*. 30(1):1–12. doi:10.1006/faat.1996.0037 PMID:8812201

JETOC (1996). *o*-Nitroanisole. Mutagenicity test data of existing chemical substances based on the Toxicity Investigation System of the Industrial Safety and Health Law. Judgement for chromosomal aberration in CHL; positive. Tokyo, Japan: Chemical Industry Ecology-Toxicology and Information Center. Available from: https://anzeninfo.mhlw.go.jp/user/anzen/kag/pdf/C/C91-23-6.pdf.

Lewis RS (1993). Hawley's condensed chemical dictionary. 12th ed. New York (NY), USA: Van Nostrand Reinhold Co.; pp. 824–5.

Liang J, Ning XA, Song J, Lu X, Sun J, Zhang Y (2019). Treatment of 3,3-dimethoxybenzidine in sludge by advance oxidation process: Degradation products and toxicity evaluation. *J Environ Manage*. 238:102–9. doi:10.1016/j.jenvman.2018.11.135 PMID:30849594

LookChem (2020). 2-Nitroanisole. Hangzhou, China: LookChem Network Technology Co., Ltd. Available from: https://www.lookchem.com/newsell/search.aspx?p=2&key=91-23-6&businessType=Manufacturers.

Mikšanová M, Novák P, Frei E, Stiborová M (2004a). Metabolism of carcinogenic 2-nitroanisole in rat, rabbit, porcine and human hepatic cytosol. *Collect Czech Chem Commun*. 69(3):589–602. doi:10.1021/tx0499721

Mikšanová M, Šulc M, Rýdlová H, Schmeiser HH, Frei E, Stiborová M (2004b). Enzymes involved in the metabolism of the carcinogen 2-nitroanisole: evidence for its oxidative detoxication by human cytochromes P450. *Chem Res Toxicol*. 17(5):663–71. doi:10.1021/tx0499721 PMID:15144223

Miller MJ, Sipes IG, Perry DF, Carter DE (1985). Pharmacokinetics of *o*-nitroanisole in Fischer 344 rats. *Drug Metab Dispos*. 13(5):527–31. PMID:2865097

Naiman K, Dračínská H, Dračínský M, Martínková M, Martínek V, Hodek P, et al. (2008). Cytochrome P450-mediated metabolism of *N*-(2-methoxyphenyl)-hydroxylamine, a human metabolite of the environmental pollutants and carcinogens *o*-anisidine and *o*-nitroanisole. *Interdiscip Toxicol*. 1(3–4):218–24. doi:10.2478/v10102-010-0045-8 PMID:21218119

Naiman K, Frei E, Stiborová M (2010). Identification of rat cytochromes P450 metabolizing *N*-(2-methoxyphenyl) hydroxylamine, a human metabolite of the environmental pollutants and carcinogens *o*-anisidine and *o*-nitroanisole. *Neuro Endocrinol Lett*. 31(Suppl 2):36–45. PMID:21187827

Naiman K, Martínková M, Schmeiser HH, Frei E, Stiborová M (2011). Human cytochrome-P450 enzymes metabolize *N*-(2-methoxyphenyl)hydroxylamine, a metabolite of the carcinogens *o*-anisidine and *o*-nitroanisole, thereby dictating its genotoxicity. *Mutat Res*. 726(2):160–8. doi:10.1016/j.mrgentox.2011.09.010 PMID:21946300

NCBI (2020). 2-Nitroanisole. PubChem Compound Summary for CID 7048. Bethesda (MD), USA: National Center for Biotechnology Information, United States National Library of Medicine. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/7048, accessed 1 October 2020.

Nesslany F, Zennouche N, Simar-Meintières S, Talahari I, Nkili-Mboui EN, Marzin D (2007). In vivo comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds. *Mutat Res*. 630(1–2):28–41. doi:10.1016/j.mrgentox.2007.02.010 PMID:17507283

NTP (1993). NTP toxicology and carcinogenesis studies of *o*-nitroanisole (CAS No. 91-23-6) in F344 rats and B6C3F$_1$ mice (feed studies). *Natl Toxicol Program Tech Rep Ser*. 416:1–482. PMID:12616295

NTP (2016). *o*-Nitroanisole. Report on carcinogens, 14th Ed. Research Triangle Park (NC), USA: U.S. Department of Health and Human Services, Public Health Service. Available from: https://ntp.niehs.nih.gov/ntp/roc/content/profiles/nitroanisole.pdf, accessed February 2020.

Reifferscheid G, Heil J (1996). Validation of the SOS/umu test using test results of 486 chemicals and comparison with the Ames test and carcinogenicity data. *Mutat Res*. 369 3–4:129–45. doi:10.1016/S0165-1218(96)90021-X PMID:8792833

Saalo A, Soosaar A, Mikkola J (2016). ASA 2014. Syöpäsairauden vaaraa aiheuttaville aineille ja menetelmille ammatissaan altistuneiksi ilmoitetut Suomessa. Helsinki, Finland: Työterveyslaitos, Available from: http://www.julkari.fi/bitstream/handle/10024/131073/ASA_2014.pdf?sequence=1&isAllowed=y, accessed 16 November 2020. [Finnish]

Shimizu M, Yano E (1986). Mutagenicity of mono-nitrobenzene derivatives in the Ames test and rec assay. *Mutat Res*. 170(1–2):11–22. doi:10.1016/0165-1218(86)90077-7 PMID:3083245

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of

carcinogenesis. *Environ Health Perspect*. 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

Sone M, Toyoda T, Cho YM, Akagi JI, Matsushita K, Mizuta Y, et al. (2019). Immunohistochemistry of γ-H2AX as a method of early detection of urinary bladder carcinogenicity in mice. *J Appl Toxicol*. 39(6):868–76. doi:10.1002/jat.3775 PMID:30701581

Starek A (2019). 2-Nitroanisole. Documentation of occupational exposure limits (OELs). *Podstawy i Metody Oceny Środowiska Pracy*. 2(100):83–99. doi:10.5604/01.3001.0013.2534

Stiborová M, Miksanová M, Smrček S, Bieler CA, Breuer A, Klokow KA, et al. (2004). Identification of a genotoxic mechanism for 2-nitroanisole carcinogenicity and of its carcinogenic potential for humans. *Carcinogenesis*. 25(5):833–40. doi:10.1093/carcin/bgh061 PMID:14729594

Stiborová M, Naiman K, Martínková M, Martínek V, Svobodová M, Schmeiser HH, et al. (2009). Genotoxic mechanisms for the carcinogenicity of the environmental pollutants and carcinogens *o*-anisidine and 2-nitroanisole follow from adducts generated by their metabolite *N*-(2-methoxyphenyl)-hydroxylamine with deoxyguanosine in DNA. *Interdiscip Toxicol*. 2(1):24–7. doi:10.2478/v10102-009-0004-4 PMID:21217841

Svobodová M, Dračínská H, Martínková M, Hudeček J, Hodek P, Frei E, et al. (2008). Oxidation of carcinogenic 2-nitroanisole by rat cytochromes P450 - similarity between human and rat enzymes. *Interdiscip Toxicol*. 1(2):182–5. doi:10.2478/v10102-010-0035-x PMID:21218109

Svobodová M, Martínková M, Dračínská H, Frei E, Stiborová M (2009). Rat cytochromes P450 oxidize 2-nitrophenol, a human metabolite of carcinogenic 2-nitroanisole. *Neuro Endocrinol Lett*. 30(Suppl 1):46–51. PMID:20027144

Toyoda T, Cho YM, Akagi J, Mizuta Y, Hirata T, Nishikawa A, et al. (2015). Early detection of genotoxic urinary bladder carcinogens by immunohistochemistry for γ-H2AX. *Toxicol Sci*. 148(2):400–8. doi:10.1093/toxsci/kfv192 PMID:26338220

Wada K, Katoh Y, Ohnuma-Koyama A, Takahashi N, Yamada M, Matsumoto K (2017). 2-Nitroanisole-induced oxidative DNA damage in *Salmonella typhimurium* and in rat urinary bladder cells. *Mutat Res Genet Toxicol Environ Mutagen*. 816–817:18–23. doi:10.1016/j.mrgentox.2017.03.002 PMID:28464992

Wada K, Yoshida T, Takahashi N, Matsumoto K (2014). Effects of seven chemicals on DNA damage in the rat urinary bladder: a comet assay study. *Mutat Res Genet Toxicol Environ Mutagen*. 769:1–6. doi:10.1016/j.mrgentox.2014.04.015 PMID:25344106

Xia E, Xia E, Yue Y, Xia N (2010). Method for preparing *o*-nitroanisole. Publication of CN101643419A. Google patents. Available from: https://patents.google.com/patent/CN101643419A/en.