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International Agency for Research on Cancer
World Health Organization

PARA-NITROANISOLE

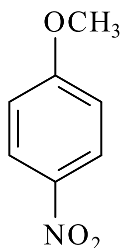
1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 100-17-4
Chem. Abstr. Serv. name: *para*-nitroanisole
IUPAC systematic name: 4-nitroanisole
Synonyms: 1-methoxy-4-nitrobenzene; *para*-methoxynitrobenzene.

1.1.2 Structural and molecular formula, and relative molecular mass



Molecular formula: $C_7H_7NO_3$
Relative molecular mass: 153.14

1.1.3 Chemical and physical properties of the pure substance

Description: solid, yellowish-grey substance (IFA, 2018)
Density (at 25 °C): 1.233 g/cm³ (IFA, 2018)
Octanol/water partition coefficient: log K_{ow} = 2.03 (IFA, 2018)
Melting point: 52 °C (IFA, 2018)
Boiling point: 258–260 °C (IFA, 2018)
Solubility: slightly soluble in water, 468 mg/L at 20 °C (IFA, 2018)
Flammable limits: lower explosion limit, 1.0 vol% (66 g/m³) (IFA, 2018)
Flash point: 130 °C (IFA, 2018)
Impurities: available with purity of greater than 99% (ThermoFisher Scientific, 2018).

1.2 Production and use

1.2.1 Production process

para-Nitroanisole can be prepared from *para*-nitrophenol by etherification with dimethyl sulfate. Alternatively, reaction of *para*-chloronitrobenzene with sodium methoxide can also be performed (Booth, 2005).

1.2.2 Production volume

para-Nitroanisole has not been listed as a chemical with a high production volume (OECD, 2009). Global production in 2002 was approximately 10 000 tonnes per year, with Japan importing approximately 400 tonnes (JBRC, 2005).

para-Nitroanisole is in Annex III of the European Union Registration, Evaluation,

Authorisation and Restriction of Chemicals regulations (ECHA, 2018), which lists substances with low production volumes (1–10 tonnes per year) or no known human exposure scenarios. Information on production volumes elsewhere in the world was not available to the Working Group.

1.2.3 Use

para-Nitroanisole is used as an intermediate in the manufacture of *para*-anisidine (4-methoxyaniline), a precursor to synthetic colorants (e.g. in cosmetics), and may therefore occur as a residue in coloured products. *para*-Nitroanisole can also be used as a chemical to detect oxidation products in fats and oils, particularly unsaturated aldehydes (Dijkstra, 2016).

1.3 Methods of measurement and analysis

1.3.1 Air

No specific methods have been described for the determination of *para*-nitroanisole in air samples.

1.3.2 Other environmental media

No specific methods have been described for the detection and measurement of *para*-nitroanisole in water and soil samples. A method based on gas chromatography with mass spectrometric confirmation was recently described for the quantitative determination of *para*-nitroanisole in cosmetics. Sample preparation was carried out by a combination of liquid-liquid and dispersive solid-phase extraction, depending on the matrix, and the limits of detection were reported as 8.6 μg/kg for aqueous cosmetics, and 13.9 μg/kg for cream and powdery cosmetics (Huang et al., 2017).

1.3.3 Biomonitoring

Early methods of measurement of *para*-nitroanisole in alkaline solutions applied semiquantitative polarography in pharmacological studies (Burgschat & Netter, 1977a). [The Working Group noted that, on the basis of the observed demethylation of *para*-nitroanisole by cytochrome P450 (CYP) 2A6 and 2E1 in human liver microsomes (Jones et al., 1997; Sai et al., 1999), *para*-nitrophenol may qualify as a biomarker in the urine of individuals and populations exposed to *para*-nitroanisole. There are multiple reliable analytical methods available to quantify *para*-nitrophenol in human urine samples (Hill et al., 1995; Sancho et al., 2002; Olsson et al., 2003; Hernández et al., 2004; Babina et al., 2012). However, measurement of *para*-nitrophenol in urine is not specific to the exposure to *para*-nitroanisole, as several other environmental and occupational pollutants can form this metabolite in humans; co-exposures to chemicals that are capable of forming *para*-nitrophenol (e.g. parathion, nitrobenzene) may therefore affect interpretation of the data (e.g. Ikeda & Kita, 1964; Padungtod et al., 2000).]

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

para-Nitroanisole is not known to occur in the environment naturally. If used as an intermediate in the production of dyes, *para*-nitroanisole can be released through various waste streams. Additionally, *para*-nitroanisole may be formed by microbial degradation in munition-contaminated soil (Torralba-Sanchez et al., 2017).

No data on the concentrations of *para*-nitroanisole have been reported in environmental media or food samples. If released to air, *para*-nitroanisole is expected to be susceptible to photodegradation by sunlight and to photochemically degrade in the atmosphere in the

presence of hydroxyl radicals (Wubbels et al., 2010). Photodegradation of *para*-nitroanisole to *para*-methoxyphenol and *para*-nitrophenol has also been observed in the presence of hydroxide ions in water samples (Wubbels et al., 2010). If released to soil, *para*-nitroanisole may be susceptible to microbial degradation, forming *para*-nitrophenol (Schäfer et al., 1996). Additionally, *para*-nitroanisole has been reported to be taken up by plants with steady state reached after 3 weeks; an experimental bioconcentration factor of 3.3 in plants has been determined from contaminated soil (Torralba-Sanchez et al., 2017).

1.4.2 Exposure in the general population

Exposure to *para*-nitroanisole in the general population has not been reported. *para*-Nitroanisole was not detected in 15 cosmetic samples collected from local markets in China. The limit of detection was 7 µg/kg for aqueous cosmetics, and 12 µg/kg for cream and powdery cosmetics (Huang et al., 2017).

para-Nitrophenol, a potential metabolite of *para*-nitroanisole in humans, is routinely assessed in urine samples of the general population in many countries including the USA. However, *para*-nitrophenol is not entirely specific for exposure to *para*-nitroanisole; *para*-nitrophenol is also a human metabolite of nitrobenzene, a common environmental pollutant, and a metabolite of selected crop protection agents (such as *O*-ethyl-*O*-(4-nitrophenyl)phenylthiophosphonate, ethyl-parathion, and methyl-parathion). The median concentration of *para*-nitrophenol in urine of the general population of the USA in 2009/2010 was reported to be 0.51 µg/L (CDC, 2009). Median concentrations of *para*-nitrophenol in urine samples of 2.9 µg/L in farmers in Thailand (Panuwet et al., 2009) and of 7.9 µg/L in preschool children in an area of southern Australia where methyl-parathion was used (Babina et al., 2012) were reported.

1.4.3 Occupational exposures

Occupational exposures to *para*-nitroanisole have not been reported in the literature. However, occupational exposures may occur through inhalation and dermal absorption where *para*-nitroanisole is produced or used as an intermediate in the manufacture of dyes. [The Working Group noted that exposure may also occur through inadvertent ingestion.]

1.5 Regulations and guidelines

Concerning human health, *para*-nitroanisole is suspected of causing mutagenic defects (H341, category 2) according to the Globally Harmonized System of Classification and Labelling of Chemicals (ECHA, 2018). On the basis of this classification, the use of personal protective equipment in occupational settings is required (IFA, 2018). Ireland, the Republic of Latvia, and Romania have reported occupational exposure limits of 1, 3, and 5 mg/m^3, respectively (IFA, 2018).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Oral administration

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female Crj:BDF$_1$ mice (age, 6 weeks) were fed diet containing *para*-nitroanisole (purity, 99.72%; containing 0.28% *meta*-chloronitrobenzene) at a

Table 3.1 Studies of carcinogenicity with *para*-nitroanisole in experimental animals

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, Crj:BDF$_1$ (M) 6 wk 104 wk JBRC (2000a,b, 2004a)	Oral 99.72% Diet 0, 5000, 10 000, 20 000 ppm 50, 50, 50, 50 36, 35, 27, 16	*Liver* Hepatocellular adenoma 12/50, 17/50, 18/50, 3/50	NS (for increase)	Principal strengths: well-conducted GLP study; multiple dose study; males and females used Incidence (range) in male historical controls: hepatocellular carcinoma, 20.1% (2–42%); hepatoblastoma, 0.6% (0–6%); hepatocellular adenoma, carcinoma, or hepatoblastoma, 35.5% (8–72%)
		Hepatocellular carcinoma 16/50, 11/50, 14/50, 39/50*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma 1/50, 12/50*, 18/50*, 38/50*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 22/50, 27/50, 33/50*, 43/50**	$P < 0.01$, Peto trend test, Cochran-Armitage trend test; *$P < 0.05$, **$P < 0.01$, Fisher exact test	
Mouse, Crj:BDF$_1$ (F) 6 wk 104 wk JBRC (2000a,b, 2004a)	Oral 99.72% Diet 0, 5000, 10 000, 20 000 ppm 50, 50, 50, 50 23, 27, 30, 13	*Liver* Hepatocellular adenoma 5/50, 18/50*, 13/50**, 4/50	*$P < 0.01$, **$P < 0.05$, Fisher exact test	Principal strengths: well-conducted GLP study; multiple dose study; males and females used Incidence (range) in female historical controls: hepatocellular adenoma, 5.3% (0–10%); hepatocellular carcinoma, 2.4% (0–12%); hepatoblastoma, 0.0%; hepatocellular adenoma, carcinoma, or hepatoblastoma, 7.5% (2–14%); liver histiocytic sarcoma, 1.3% (19/1448) (0–4%)
		Hepatocellular carcinoma 2/50, 12/50*, 41/50*, 46/50*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma 0/50, 0/50, 8/50*, 38/50*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, 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, Crj:BDF$_1$ (F) 6 wk 104 wk JBRC (2000a,b, 2004a) (cont.)		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined)		
		7/50, 24/50*, 45/50*, 48/50*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, Fisher exact test	
		Histiocytic sarcoma		
		1/50 (2%), 0/50, 0/50, 3/50 (6%)	$P < 0.05$, Peto trend test	
		All sites: histiocytic sarcoma		
		18/50, 17/50, 15/50, 15/50	NS	
Rat, F344/DuCrj (M) 6 wk 104 wk JBRC (2000c,d, 2004b)	Oral 99.72% Diet 0, 2000, 4000, 8000 ppm 50, 50, 50, 50 37, 39, 32, 2	*Liver*		Principal strengths: well-conducted GLP study; multiple dose study; males and females used Incidence (range) in male historical controls: hepatocellular adenoma, 1.6% (0–6%); testis, interstitial cell tumour, 85.6% (56–98%)
		Hepatocellular adenoma		
		0/50, 1/50, 13/50*, 11/50*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular carcinoma		
		0/50, 0/50, 0/50, 0/50	–	
		Testis: Interstitial cell tumour, benign		
		34/50 (68%), 45/50 (90%)*, 48/50 (96%)*, 48/50 (96%)*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.01$, 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
Rat, F344/DuCrj (F) 6 wk 104 wk JBRC (2000c,d, 2004b)	Oral 99.72% Diet 0, 2000, 4000, 8000 ppm 50, 50, 50, 49 45, 38, 35, 31	*Liver* Hepatocellular adenoma 0/50, 0/50, 0/50, 5/49* Hepatocellular carcinoma 0/50, 0/50, 0/50, 0/49 *Uterus:* adenocarcinoma 1/50, 4/50, 8/50*, 8/49*	$P < 0.01$, Peto trend test, Cochran–Armitage trend test; *$P < 0.05$, Fisher exact test – $P < 0.01$, Peto trend test; $P < 0.05$, Cochran–Armitage trend test; *$P < 0.05$, Fisher exact test	Principal strengths: well-conducted GLP study; multiple dose study; males and females used Incidence (range) in female historical controls: hepatocellular adenoma, 1.3% (0–6%); uterine adenocarcinoma, 0.4% (0–4%)

F, female; GLP, good laboratory practice; M, male; NS, not significant; ppm, parts per million; wk, week

concentration of 0, 5000, 10 000, or 20 000 ppm for 104 weeks (JBRC, 2000a,b, 2004a). Mice were housed alone. Mean daily intake of the test compound given at 5000, 10 000, and 20 000 ppm was estimated from food consumption and body weight to be 599, 1328, and 3314 mg/kg body weight (bw) per day in males and 745, 1663, and 3496 mg/kg bw per day in females, respectively. A decreased survival rate attributed to tumours of the liver was observed in males at 10 000 and 20 000 ppm and in females at 20 000 ppm; survival rates were 36/50, 35/50, 27/50, and 16/50 in males, and 23/50, 27/50, 30/50, and 13/50 in females. A significant reduction in final body weight was observed in males and females at 10 000 and 20 000 ppm. All mice, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy.

In males, the incidence of hepatocellular carcinoma (16/50, 11/50, 14/50, and 39/50) was significantly increased in mice at 20 000 ppm ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatoblastoma (1/50, 12/50, 18/50, and 38/50) was significantly increased in all treated groups ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (22/50, 27/50, 33/50, and 43/50) was significantly increased in mice at 10 000 ppm ($P < 0.05$, Fisher exact test) and at 20 000 ppm ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatocellular carcinoma, of hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) occurred with significant positive trends in males ($P < 0.01$, Peto trend test, Cochran–Armitage trend test).

In females, the incidence of hepatocellular adenoma (5/50, 18/50, 13/50, and 4/50) was significantly increased in mice at 5000 ppm ($P < 0.01$, Fisher exact test) and 10 000 ppm ($P < 0.05$, Fisher exact test) compared with controls. The incidence of hepatocellular carcinoma (2/50, 12/50, 41/50, and 46/50) was significantly increased in all treated groups ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatoblastoma (0/50, 0/50, 8/50, and 38/50) was significantly increased in mice at 10 000 and 20 000 ppm ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (7/50, 24/50, 45/50, and 48/50 ppm) was significantly increased in all treated groups ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatocellular carcinoma, of hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) occurred with significant positive trends ($P < 0.01$, Peto trend test, Cochran–Armitage trend test). The incidence of histiocytic sarcoma in the liver of female mice at 5000 ppm (0/50), 10 000 ppm (0/50), and 20 000 ppm (3/50, 6%) was not significantly increased compared with controls (1/50, 2%), but showed a significant positive trend ($P < 0.05$, Peto trend test) and a slightly higher incidence in the group at the highest dose compared with that in historical controls (19/1448 (1.3%); range, 0–4%). However, the incidence of histiocytic sarcoma (all sites) in the groups at 5000 ppm (17/50), 10 000 ppm (15/50), and 20 000 ppm (15/50) was not significantly increased compared with controls (18/50). [The Working Group concluded that the incidence of histiocytic sarcoma of the liver was not increased by the treatment.]

Regarding non-neoplastic lesions, a significant increase was observed in the incidence of hepatocellular hypertrophy in the centrilobular area in all groups of treated males and in females at 20 000 ppm, and in the incidence of hepatocytes with nuclear atypia in the centrilobular area in males at 10 000 and 20 000 ppm (JBRC, 2000a, b, 2004a). [The Working Group noted that this was a well-conducted GLP study that used multiple doses, a high number of mice per group, and males and females.]

3.2 Rat

Oral administration

In a study that complied with GLP, groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) were fed diet containing *para*-nitroanisole (purity, 99.72%; containing 0.28% *meta*-chloronitrobenzene) at a concentration of 0, 2000, 4000, or 8000 ppm for 104 weeks (JBRC, 2000c, d, 2004b). Rats were housed alone. Mean daily intake of the test compound given at 2000, 4000, and 8000 ppm was estimated from food consumption and body weight as 92, 191, and 413 mg/kg bw per day in males and 119, 229, and 475 mg/kg bw per day in females, respectively. A decreased survival rate was observed in males and females at 8000 ppm, attributed to chronic progressive nephropathy in males and females and to uterine tumours (adenocarcinomas) in females. Survival rates were 37/50, 39/50, 32/50, and 2/50 in males, and 45/50, 38/50, 35/50, and 31/49 in females. A significant reduction in final body weight was observed in males at 4000 ppm and in all treated females. All rats, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy.

In males, although no significantly increased incidence in malignant tumours was observed, the incidence of hepatocellular adenoma (0/50, 1/50, 13/50, and 11/50) was significantly increased in rats at 4000 and 8000 ppm ($P < 0.01$, Fisher exact test) compared with controls. The incidence of benign interstitial cell tumour of the testis (34/50 (68%), 45/50 (90%), 48/50 (96%), and 48/50 (96%)) was significantly increased in all treated groups ($P < 0.01$, Fisher exact test) compared with controls. The incidence of hepatocellular adenoma and of benign interstitial cell tumours of the testis occurred with significant positive trends ($P < 0.01$, Peto trend test, Cochran–Armitage trend test). [The Working Group noted that the incidence of interstitial cell tumour in this study was within the range for historical controls, 1368/1598 (85.6%) (range, 56–98%).]

In females, the incidence of adenocarcinoma of the uterus (1/50 (2%), 4/50 (8%), 8/50 (16%), and 8/49 (16%)) was significantly increased in rats at 4000 and 8000 ppm ($P < 0.05$, Fisher exact test) compared with controls, with a significant positive trend ($P < 0.01$, Peto trend test; $P < 0.05$, Cochran–Armitage trend test). The incidence of hepatocellular adenoma (0/50, 0/50, 0/50, and 5/49) was significantly increased in rats at 8000 ppm ($P < 0.05$, Fisher exact test) compared with controls, with a significant positive trend ($P < 0.01$, Peto trend test, Cochran–Armitage trend test).

Regarding non-neoplastic lesions, a significant increase in incidence and/or grade was observed for spongiosis hepatis and basophilic cell focus in the liver of males at 4000 and 8000 ppm, and for chronic nephropathy in all treated males and in females at 4000 and 8000 ppm (JBRC, 2000c, d, 2004b). [The Working Group noted that this was a well-conducted GLP study conducted with multiple doses, a high number of rats per group, and in males and females.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Absorption, distribution, and excretion

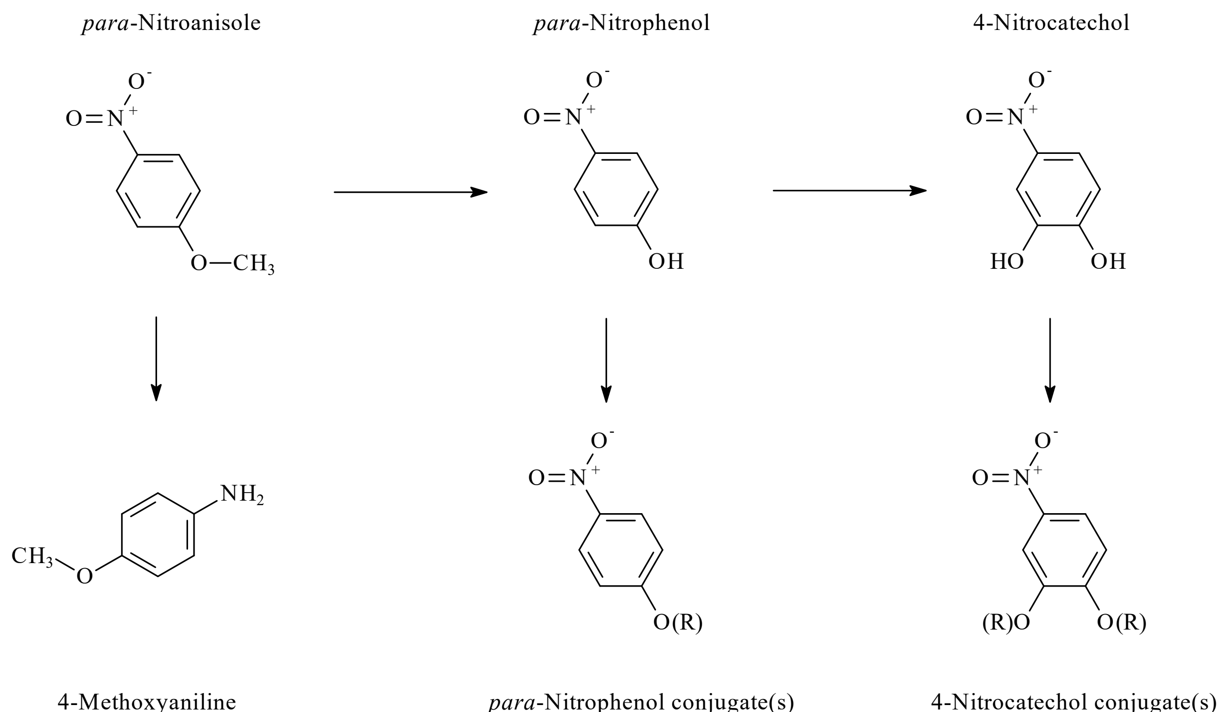
(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Only a few studies on the absorption, distribution, and excretion of *para*-nitroanisole have been published. One study in rabbits has indicated that *para*-nitroanisole can be transported

Fig. 4.1 Metabolic scheme for *para*-nitroanisole for humans and experimental animals



Compiled by the Working Group

across the skin membrane and rapidly metabolized to *para*-nitrophenol and *para*-nitrophenol conjugates (Henrikus et al., 1991).

A study in mice of a single intravenous dose or dermal application of *para*-nitrophenol provided detailed toxicokinetic information for the primary metabolite of *para*-nitroanisole, showing few differences in various toxicokinetic parameters between males and females (Eichenbaum et al., 2009).

4.1.2 *Metabolism*

(a) Humans

See Fig. 4.1

O-Demethylation of *para*-nitroanisole into *para*-nitrophenol in human liver microsomes is mediated by both CYP2A6 and CYP2E1 (Jones et al. 1997; Gelboin & Krausz, 2006). CYP2A6 appears to be the major enzyme involved in humans, although the major enzyme in the rat is predominantly CYP2E1. Sai et al. (1999) found that human CYP2A6 further metabolized *para*-nitrophenol into *para*-nitrocatechol. A study that compared male and female hepatic metabolism of *para*-nitroanisole in humans and rats did not detect sex-specific differences (Kremers et al., 1981).

(b) Experimental systems

In rat primary hepatocytes, *para*-nitroanisole can be metabolized by CYP with subsequent glucuronide and sulfate conjugation (Eacho & Weiner, 1980; Eacho et al., 1981). In the rat liver, the metabolism of *para*-nitroanisole by CYP enzyme(s) and glucuronidation appears to be concentrated in the midzonal and periportal zones (James et al., 1981). Studies in vitro with rat hepatic microsomal fractions showed that *para*-nitroanisole is rapidly metabolized into *para*-nitrophenol, after which further hydroxylation at the *ortho* position can take place to produce

para-nitrocatechol (Burgschat & Netter, 1977b). No sex differences were observed in the rat for the sulfation and glucuronidation of *para*-nitrophenol, the primary metabolite of *para*-nitroanisole (Meerman et al., 1987). Metabolism of this compound to conjugates of *para*-nitrophenol was also observed after dermal application to the rabbit ear (Henrikus et al., 1991). Colonic mucosa of the rat also contains CYP enzymes that are able to efficiently metabolize *para*-nitroanisole (Strobel et al., 1980).

The metabolic rates of *para*-nitroanisole differ between rat tissues. Compared with the liver and lungs, the nasal epithelial membrane was found to have a higher metabolic capacity for this compound (Hadley & Dahl, 1982). These tissue-specific differences in the metabolism of *para*-nitroanisole were also observed in the rabbit. In pulmonary and hepatic microsomal fractions of this species, *para*-nitroanisole was found to be metabolized by the same CYP isoenzyme; however, from a quantitative point of view this metabolic rate differed between the types of tissues (Croft et al., 1986).

Based on total CYP content in rat primary hepatocytes, the metabolism of *para*-nitroanisole to *para*-nitrophenol was slower in females than males. After induction by phenobarbital, no sex-specific differences in metabolism were observed (Mazur & Petrenko, 1997). Moreover, it was shown that in pregnant and lactating female rats, the metabolism of *para*-nitroanisole increased during lactation (Borlakoglu et al., 1993). The metabolism of *para*-nitroanisole in rats was also found to be dependent on time during the postnatal period, with increasing O-demethylation activity until age 20 days followed by a decrease at age 30 days (Sonawane et al., 1981). Such a time dependency in the metabolism of *para*-nitroanisole was also found in the postnatal period of the beagle dog (Tanaka et al., 1998).

Several studies have addressed the specific role of certain isoforms of CYP in the O-demethylation of *para*-nitroanisole. Two studies in mice and rats, using specific antibodies against CYP3A3/4 and CYP2E1, indicated that *para*-nitroanisole is metabolized in these rodents by both CYP isoforms (Gelboin et al., 1995, 1996). In addition, CYP2B was also found to play a metabolic role in the rat (Sequeira et al., 1994). The role of CYP1A2 and CYP2E1 in the metabolism of *para*-nitroanisole in the rat was also identified using specific monoclonal antibodies against these isoforms of CYP. In the beagle dog, CYP1A1 was reported to be the major CYP isoenzyme responsible for the metabolism of *para*-nitroanisole (Tanaka et al., 1998).

It can therefore be concluded that several isoforms of CYP are involved in the primary metabolism of *para*-nitroanisole, in which CYP2A6 (humans) and CYP2E1 (humans and rats) play a dominant role.

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016), on whether *para*-nitroanisole is genotoxic; and alters cell proliferation, cell death, or nutrient supply.

4.2.1 Genetic and related effects

See Table 4.1

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In rat hepatocytes exposed to *para*-nitroanisole, unscheduled DNA synthesis was not increased (Probst et al., 1981). Mutagenicity was observed in *Salmonella typhimurium* strain TA100, without metabolic activation, in a study by Probst et al. (1981); two other studies in strains TA98, TA100, TA1535, TA1537 or TA1538

Table 4.1 Genetic and related effects of *para*-nitroanisole and its major metabolite *para*-nitrophenol in experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
para-*Nitroanisole*						
Rat, primary hepatocytes	Unscheduled DNA synthesis	–	NT	0.050 µmol/mL [7.5 µg/mL]		Probst et al. (1981)
Salmonella typhimurium TA100	Reverse mutation	+	NT	6.5 nmol/mL [1 µg/mL]		Probst et al. (1981)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	100 µg/plate		Suzuki et al. (1983)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	NT	5000 µg/plate		Shimizu & Yano (1986)
para-*Nitrophenol*						
Mouse, Crl:CD-1, bone marrow, polychromatic erythrocytes, in vivo (M, F)	Micronucleus formation	–	NA	30 mg/kg bw, gavage, 1×		Eichenbaum et al. (2009)
Mouse, L5178Y, lymphoma cells	*Tk* gene mutation	NT	–	440 µg/mL		Amacher & Turner (1982)
Rat hepatocytes	Unscheduled DNA synthesis	–	NT	0.1 µmol/mL [14 µg/mL]		Probst et al. (1981)
Chinese hamster lung V79 cells	DNA strand breaks, comet assay	–	–	100 µM [14 µg/mL]	Cytotoxicity was specifically taken into account, but negative results across the whole concentration range were found	Hartmann & Speit (1997)
Chinese hamster ovary, CHO-K1 cell line	*Hprt* gene mutation	–	–	800 µg/mL		Oberly et al. (1990)
Chinese hamster ovary cells (CHO)	Sister-chromatid exchange	–	–	1500 µg/mL		NTP (1993)
Chinese hamster lung cells	Chromosomal aberrations	(+)	+	800 µg/mL (–S9), 600 µg/mL (+S9)	Induced chromosomal aberrations at non-toxic concentrations (+S9); cytotoxic at 800 µg/mL (–S9)	Noda (1995)
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	NA	7500 ppm by feeding 1500 ppm by injection		NTP (1993)

Table 4.1 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium G46	Reverse mutation; host-mediated assay (mouse)	–	NA	75 mg/kg bw		Buselmaier (1972)
Serratia marcescens	Reverse mutation; host-mediated assay (mouse)	–	NA	75 mg/kg bw		Buselmaier (1972)
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	500 µg/plate		McCann et al. (1975)
Salmonella typhimurium TA1538	Reverse mutation	–	NT	1 µg/plate		Commoner (1976)
Salmonella typhimurium TA98, TA100, TA1537, TA1538, D3052, G46, C3076	Reverse mutation	–	–	0.1 µmol/mL [14 µg/mL]		Probst et al. (1981)
Salmonella typhimurium TA1535, TA1537, TA98, TA100	Reverse mutation	–	–	1000 µg/plate		Haworth et al. (1983); NTP (1993)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	100 µg/plate		Suzuki et al. (1983)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	1000 µg/plate		Shimizu & Yano (1986)

bw, body weight; F, female; HIC, highest ineffective concentration; *Hprt*, hypoxanthine (guanine) phosphoribosyltransferase; LEC, lowest effective concentration; M, male; NA, not applicable; NT, not tested; S9, 9000 × *g* supernatant; *Tk*, thymidine kinase

[a] +, positive; –, negative; (+), positive in a study of limited quality

reported negative results (Suzuki et al., 1983; Shimizu & Yano, 1986).

The genotoxicity of *para*-nitrophenol, the major metabolite of *para*-nitroanisole, has also been studied. *para*-Nitrophenol gave negative results in an in vivo mouse micronucleus assay (Eichenbaum et al., 2009). In studies of mouse, rat and Chinese hamster cells in vitro, *para*-nitrophenol gave negative results for various end-points including unscheduled DNA synthesis, DNA strand breaks, gene mutation, (Probst et al., 1981; Amacher & Turner, 1982; Oberly et al., 1990; NTP, 1993; Noda, 1995; Hartmann & Speit, 1997). *para*-Nitrophenol was not mutagenic in *Drosophila melanogaster* (NTP, 1993), in the host-mediated assay in mice (Buselmaier, 1972) or in various *Salmonella typhimurium* strains including TA98, TA100, TA1535, and TA1537, with or without metabolic activation (McCann et al., 1975; Commoner, 1976; Probst et al., 1981; Haworth et al., 1983; Suzuki et al., 1983; Shimizu & Yano, 1986; NTP, 1993).

4.2.2 Altered cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Esmaeili et al. (2006) did not report activity with *para*-nitroanisole in a host-mediated assay conducted in mice. The role of β-catenin, an essential contributor to Wnt signalling, was studied in the formation of hepatocellular neoplasms that occurred during the 104-week study in Crj:BDF$_1$ mice exposed to *para*-nitroanisole. No functional abnormalities in β-catenin could be detected in this particular mouse strain (Kushida et al., 2006).

4.3 Other adverse effects

Male and female Fischer 344/DuCrj rats exposed to *para*-nitroanisole at 8000 ppm demonstrated an increased incidence of chronic progressive nephropathy. Decreased erythrocyte counts and haemoglobin concentrations were observed in male rats (JBRC, 2004b).

In Crj:BDF$_1$ mice, an increased incidence of centrilobular hepatocyte hypertrophy was observed. Dose-related increases in the incidence of non-neoplastic lesions in the nasal cavity, nasopharynx, and lung were also noted, as well as haemosiderin deposition in the spleen and kidneys in males and females (JBRC, 2004a).

4.4 Data relevant to comparisons across agents and end-points

See the monograph on 2-chloronitrobenzene in the present volume.

5. Summary of Data Reported

5.1 Exposure data

para-Nitroanisole is a nitrobenzene. Global production quantities are unknown, although production volumes in and import into Europe are known to be low. It is used as an intermediate in the manufacture of synthetic dyes used for cosmetics and other consumer products.

para-Nitroanisole is not known to occur naturally. It may be released to the air or water during its production and downstream use, or be found as a degradation product in munition-contaminated soil. Quantitative information on levels in the environment was not available.

Occupational exposure to *para*-nitroanisole has not been reported, although this would be expected to occur through inhalation and dermal absorption in workplaces where *para*-nitroanisole

is produced or used. Quantitative information on exposure to *para*-nitroanisole in occupational settings or in the general population was not available.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

para-Nitroanisole was tested for carcinogenicity in the same laboratory in one well-conducted good laboratory practice (GLP) study of oral exposure by diet in male and female mice, and one well-conducted GLP study of oral exposure by diet in male and female rats.

In male mice, *para*-nitroanisole induced a significant positive trend in the incidence and a significant increase in the incidence of hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined).

In female mice, *para*-nitroanisole induced a significant positive trend in the incidence of hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined), and a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined).

In male rats, *para*-nitroanisole induced a significant positive trend in the incidence and a significant increase in the incidence of hepatocellular adenoma. In addition, there was a significant positive trend in the incidence and a significant increase in the incidence of interstitial tumours of the testis.

In female rats, *para*-nitroanisole induced a significant positive trend in the incidence and a significant increase in the incidence of adenocarcinoma of the uterus. In addition, *para*-nitroanisole induced a significant positive trend in the incidence and a significant increase in the incidence of hepatocellular adenoma.

5.4 Mechanistic and other relevant data

Information about the absorption, distribution, and excretion of *para*-nitroanisole in humans and experimental animals was sparse, but the occurrence of dermal uptake has been reported. In humans and rodents, *para*-nitroanisole is rapidly metabolized, predominantly by cytochrome P450 2A6 and 2E1, to *para*-nitrophenol, followed by catechol formation, conjugation, and excretion. In rodents, tissue-specific differences in metabolism were observed in the liver, lungs, and nasal epithelium. No differences in metabolic rate were detected between sexes in rodents.

Concerning the key characteristics of carcinogens, there is *weak* evidence that *para*-nitroanisole and its primary metabolite *para*-nitrophenol are genotoxic. No data in humans or in experimental animals in vivo were available. Results were largely negative in multiple studies in vitro in rodent cells and in bacteria for end-points including DNA strand breaks, mutation, and unscheduled DNA synthesis.

In chronic studies with *para*-nitroanisole, an increased incidence of chronic progressive nephropathy was observed in male and female rats, and hepatotoxicity was observed in male and female mice.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

para-Nitroanisole is *possibly carcinogenic to humans (Group 2B).*

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