2-NAPHTHYLAMINE

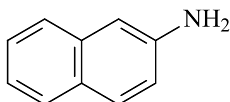
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 91–59–8
CAS Name: 2-Naphthalenamine
Synonyms: 2-Aminonaphthalene; β-aminonaphthalene; *ortho*-aminonaphthalene; C.I. 37270; β-naphthalenamine; naphthalen-2-ylamine; 2-naphthylamine; β-naphthylamine

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



$C_{10}H_9N$ Rel. mol. mass: 143.19

1.1.3 *Chemical and physical properties of the pure substance* (O'Neil, 2006)

Description: White to reddish crystals
Boiling-point: 306 °C
Melting-point: 111–113 °C
Solubility: Soluble in water, diethyl ether, and ethanol (Lide, 2008)
Octanol/water partition coefficient: log P, 2.25 (Verschueren, 2001)

1.1.4 *Technical products and impurities*

The commercial product contains several polycyclic hydrocarbons including dibenzo(*a,h*)phenazine, which is formed from 2-naphthylamine in the presence of air. 2-Amino-1,4-naphthoquinone-N^4,2-naphthylimine has also been reported as a contaminant of 2-naphthylamine (Radomski *et al.*, 1971).

Trade names for 2-naphthylamine include Fast Scarlet Base B.

1.1.5 *Analysis*

Analyses of 2-naphthylamine were first reported in the 1960s. Historically, water, urine, and textile samples have been analysed for 2-naphthylamine content with gas chromatography (GC) and liquid chromatography-mass spectrometry (LC-MS). These methods permit detection at concentrations down to the ng/L level. Recent studies involve the use of GC/MS to determine the concentrations of 2-naphthylamine (in derivative form) in cigarette smoke and in the urine of smokers. Table 1.1 presents selected recent studies of the analysis of 2-naphthylamine in various matrices.

1.2 Production and use

1.2.1 *Production*

2-Naphthylamine was commercially produced in the US from the early 1920s to the early 1970s. In 1955 (the latest year for which production data were found), 581 000 kg (1.3 million pounds) were produced (IARC, 1974). Since its commercial manufacture and use in the US were banned in the early 1970s, 2-naphthylamine has been available only in small quantities for laboratory research.

According to EU legislation, the production, manufacture and use of 2-naphthylamine has been prohibited since 1998 (European Commission, 1998).

Available information indicates that 2-naphthylamine was produced and/or supplied in research quantities in the following countries: Belgium, Germany, Hong Kong Special Administrative Region, the People's Republic of China, Switzerland, and the USA (Chemical Sources International, 2010)

1.2.2 *Use*

2-Naphthylamine is now used only in laboratory research. It formerly was used commercially as an intermediate in the manufacture of dyes, as an antioxidant in the rubber industry, and to produce 2-chloronaphthylamine (IARC, 1974; HSDB, 2003).

1.3 Occurrence

1.3.1 *Natural and environmental occurrence*

2-Naphthylamine is formed during the pyrolysis of nitrogen-containing organic matter and can occur as such in nature (Masuda *et al.*,1967; Talukder & Kates, 2000).

Table 1.1. Selected methods of analysis of 2-naphthylamine in various matrices

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Textiles	Solutions are prepared in dry tetrahydrofuran; add pentafluoropropionic anhydride and heat; cool; dilute with methanol	GC/MS	[2 ng/mL]	Narvekar & Srivastava (2002)
Urine	Extract twice with *n*-hexane; centrifuge; add dry pyridine; derivatize using pentafluoropropionic anhydride; extract with phosphate buffer; centrifuge; evaporate organic solvent under N_2 stream	GC/MS	75 ng/L	Weiss & Angerer (2002)
Water	Pass water samples at neutral pH through C18 cartridges; add 10 mM tributylamine–acetic acid and formic acid (pH 3); conduct ion-pair solid phase extraction	HPLC-ESI-MS	2 ng/L	Loos *et al.* (2003)
Water	A mixture of 20 amines is dissolved in methanol, diluted to different concentrations for analysis. Other solvents are dichloromethane, ethyl acetate, and methanol/dichloromethane (50:50)	GC/MS	5 ng/mL	Doherty (2005)
Dyes, cosmetics, inks and finger paints	Extract with methanol. Separate and detect on a phenyl ether-linked stationary phase	HPLC/MS	NR	Hauri *et al.* (2005)
Urine	Acid hydrolysis of arylamine conjugates in urine, extraction with n-hexane, derivatization with pentafluoropropionic anhydride, and analysis	GC/MS	1 ng/L	Riedel *et al.* (2006)
Cigarette smoke	Cigarettes are conditioned at 22 °C and 60% humidity, machine smoked; mainstream smoke constituents are collected in glass filters; constituents are extracted with hydrochloric acid and hexane, derivatized with pentafluoropropionic acid anhydride and trimethylamine, and analysed.	GC/MS	1 ng/cig. [0.5–0.8 g tobacco/cig.]	Hyodo *et al.* (2007)
Water	River water samples are filtered and then spiked with stock solutions of analyte.	HPLC/ECD	8.8 nmol/L	Zima *et al.* (2007)

ECD, electrochemical detection; GC, gas chromatography; HPLC-ESI, high performance liquid chromatography–electrospray ionization; MS, mass spectrometry; NR, not reported

2-Naphthylamine may occur in the waste streams from plants where it is produced and used; it has been reported to be present in the effluent from certain dyestuff factories in Japan (Takemura *et al.*, 1965).

1.3.2 *Occupational exposure*

Occupational exposure to 2-naphthylamine (2-NA) mainly occurs during its production and when used in the manufacture of azo dyes. Occupational exposure to 2-NA can also occur in laboratories where it is used for research purposes, and when workers are exposed to pyrolysis fumes containing 2-NA (e.g., foundry fumes, environmental tobacco smoke, heated cooking oils), or to 2-nitronaphthalene (e.g., foundry workers), a nitro-PAH that can be metabolized to 2-NA. Exposure can also occur in workers exposed to products containing 2-NA as a contaminant, such as certain rubber chemicals.

Subjects reported to have been exposed to 2-NA during its production include workers from Italy (Rubino *et al.*, 1982), Japan (Tsuchiya *et al.*, 1975; Morinaga *et al.*, 1982), the Russian Federation (Bulbulyan *et al.*, 1995), the United Kingdom (Case & Pearson, 1954) and the USA (Schulte *et al.*, 1986; Cassidy *et al.*, 2003).

Exposure measurement data were reported for a dye factory that manufactured 2-NA and benzidine in Moscow (Bulbulyan *et al.*, 1995). 2-NA levels in factory-air samples taken between 1939 and 1948 ranged from < 0.001 mg/L to > 0.003 mg/L. Factory-wall wipes contained 60.0–115 mg/m^2 wall surface. Dermal wipes taken after a shower at work showed amounts of 2-NA between 0.018–37.5 mg [data on the skin surface-area were not available].

In a study among a group of workers in Germany primarily exposed to aniline and 4-chloroaniline, urinary 2-NA concentrations ranged between 0–9.8 µg/L (mean 3.9±2.2) in 22 smokers and between 0.0–11.6 µg/L (mean 2.1±2.8) in 21 nonsmokers; levels measured in non-smoking non-exposed workers were 0.0–1.6 µg/L (mean 0.5±0.7). No difference was observed between slow and fast acetylators among smokers or non-smokers (Riffelmann *et al*., 1995).

In a study in two Danish iron foundries, 2-NA was proposed as a possible marker of 2-nitronaphthalene in the urine of PAH-exposed workers (Hansen *et al.*, 1994). The concentrations of 2-NA in urine were significantly higher in PAH-exposed workers compared with controls (matched for smoking habits), with hand moulders, finishing workers and truck drivers having the highest levels. This may be explained by the presence of 2-nitronaphthalene (which can be metabolized to 2-NA), of aromatic amines (e.g., in moulding sand) or of nitrogen oxides (e.g., in diesel exhaust). A study of 19 human volunteers showed that up to 0.03% of a single 10-mg dose of *N*-phenyl-2-naphthylamine is converted internally to 2-naphthylamine (IARC, 1978). More recently, it has been estimated that a maximum of 1% of total *N*-phenyl-2-naphthylamine uptake can be metabolized into 2-NA (Weiss *et al.*, 2007).

1.3.3 *Occurrence as an impurity in other compounds*

Several dye intermediates have been shown to contain small amounts of 2-naphthylamine. 2-NA concentrations of up to 0.5% have been reported in commercially produced 1-naphthylamine (substance profile). A 4% contamination level has been reported in 1-NA produced in Japan, which after 1970 was reduced to less than 1% (Tsuchiya *et al.*, 1975). 2-NA has also been detected in 6-amino-2-naphthalene sulfonic acid (Bronner's acid) (Cassidy *et al.*, 2003), a pigment intermediate, as well as in auramine.

2-NA has been detected in hair dyes. A study from Turkey showed 2-NA in 20 out of 54 hair dyes and in 15 out of 25 hennas, in concentrations up to 5.47 and 4.15 μg/g respectively (Akyüz & Ata, 2008). [The Working Group noted that the hair dyes analysed in this study are not necessarily representative of hair dyes used in other countries].

2-NA has been detected in rubber antioxidants such as Nonox S and Agerite Resin at levels of 0.25% [2500 ppm] (Veys, 2004). *N*-phenyl-2-naphthylamine, also a rubber antioxidant, can be contaminated with 2-NA, and can also be metabolized (dephenylated) into 2-NA to some extent.

In a polyethylene pipe manufacturing plant in the USA, contamination of a resin additive with 2-NA was detected in 1996, which may have resulted in worker exposure to 2-NA from 1970 to 1996 (Felknor *et al.*, 2003).

1.3.4 *Exposure of the general population*

The general population can be exposed to 2-NA through environmental exposure, via tobacco smoke, via other fumes containing 2-NA, or when in contact with dyes and hair dyes contaminated with 2-NA. Exposure to the nitro-PAH 2-nitronaphthalene, which is formed by incomplete combustion of organic material and generally occurs in the environment as a mixture of other nitro-PAH and non-nitro-PAH compounds, can also represent an indirect source of exposure to 2-NA.

Mainstream cigarette-smoke from eight different US conventional market cigarettes contained 2-naphthylamine at concentrations of 1.47 to 14.06 ng per cigarette (Stabbert *et al.*, 2003). Amounts in mainstream cigarette-smoke range from 1–22 ng per cigarette (IARC, 2004), those in sidestream cigarette-smoke range from 113.5–171.6 ng per cigarette (IARC, 2004).

In a study from Germany (Riffelmann *et al.*, 1995), urinary concentrations were higher in smokers (3100 ng/L±2100; $n = 8$) compared with non-smokers (500 ng/L±700; $n = 8$). [The Working Group noted that the very high levels reported are likely due to the unspecific detection by the GC-EC method used (Riedel *et al.*, 2006)]. In a later German study (Grimmer *et al.*, 2000), freebase 2-NA was found at comparable levels in the urine of non-smokers (120.8 ng/24 hours), of smokers (84.5 ng/24 hours) and of individuals exposed to environmental tobacco smoke (94.9 ng/24 hours). In a study by Riedel *et al.* (2006), smokers ($n = 10$) excreted significantly higher amounts of 2-NA compared with nonsmokers ($n = 10$) (20.8 *vs* 10.7 ng/24 hours).

1.4 Regulations and guidelines

1.4.1 *Europe*

(a) Council Directive 89/677/EEC

According to Council Directive 89/677/EEC, 2-naphthylamine and its salts may not be used in concentrations equal to or greater than 0.1% by weight in substances and preparations placed on the market (European Economic Community, 1989).

(b) Council Directive 98/24/EC

According to EU regulations, the manufacture and use of 2-naphthylamine has been prohibited since 1998 (European Commission, 1998). The Council Directive 98/24/EC in Annex III prohibits the production, manufacture or use at work of 2-naphthylamine and its salts and activities involving 2-naphthylamine and its salts. The prohibition does not apply if 2-naphthylamine is present in another chemical agent, or as a constituent of waste, provided that its individual concentration therein is less than 0.1% w/w.

(c) Council Directive 2002/61/EC

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

(d) Directive 2004/37/EC

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

(e) Directive 2004/93/EC

The Commission Directive 2004/93/EC of 21 September 2004 amends the Council Directive 76/768/EEC for the purpose of adapting Annexes II and III thereto to technical progress (European Commission, 2004b). In this Directive, 2-naphthylamine and its salts are listed in *Annex II* as substances that must not form part of the composition of cosmetic products.

(f) Directive 2005/90/EC

In the Directive 2005/90/EC, the list in Directive 76/769/EEC of substances classified as carcinogenic, mutagenic or toxic to reproduction was amended to include 2-naphthylamine (European Commission, 2005).

1.4.2 *Germany*

2-Naphthylamine is classified as a Category 1 carcinogen by the MAK Commission. The MAK Commission listed 2-naphthylamine as a substance for which percutaneous absorption may significantly contribute to systemic exposure. A MAK value was not set (MAK, 2007).

1.4.3 *Japan*

The Japan Society for Occupational Health (2007) follows the classification by IARC of 2-naphthylamine in Group 1.

1.4.4 *USA*

(a) ACGIH

2-Naphthylamine has been assigned an A1 notation, *Confirmed Human Carcinogen* (ACGIH, 2001). Accordingly, a numerical TLV (threshold limit-value) is not recommended for occupational exposure. As for all substances designated as A1 carcinogens without a TLV, workers should be properly equipped to eliminate all exposure to 2-naphthylamine to the fullest extent possible.

(b) NIOSH

The National Institute for Occupational Safety and Health (NIOSH, 2005) lists 2-naphthylamine as one of 13 OSHA-regulated carcinogens. Exposures of workers are to be controlled through the required use of engineering controls, work practices, and personal protective equipment, including respirators.

(c) NTP

2-Naphthylamine is listed in the NTP Report on Carcinogens as *known to be a human carcinogen* (NTP, 2005).

1.4.5 *Other*

(a) GESTIS

Table 1.2 presents some international limit values for 2-naphthylamine (GESTIS, 2007).

Table 1.2. International limit values (2007) for 2-naphthylamine

Country	Limit value – Eight hours		Limit value – Short-term	
	ppm	mg/m^3	ppm	mg/m^3
France	0.001	0.005		
Hungary				0.005
Italy				0.001

(b) Recent bans

The use and import of 2-naphthylamine was banned recently in the Republic of Korea in 2003, and in Switzerland in 2005 (UN/UNEP/FAO, 2007).

2. Studies of Cancer in Humans

Cancer risk related to exposure to 2-naphthylamine has been assessed in several cohort studies and case reports. In spite of the fact that, in general, production ceased several decades ago, follow-ups were continuously updated until very recent dates.

2.1 Case reports, case series

Several reports from the 1960s and 1970s described cases of bladder cancer among workers exposed to 2-naphthylamine in France (Billiard-Duchesne, 1960), Italy (Vigliani & Barsotti, 1962) and Japan (Tsuji, 1962, Tsuchiya *et al.* 1975)

Goldwater *et al.* (1965) found that the cumulative incidence of bladder cancer among British coal-tar dye workers exposed to 2-naphthylamine, and not to other aromatic amines, was 25% (12 cases among 48 exposed workers).

In another study from Japan, 112 cases of recognized occupational bladder cancer (10.3%) were found among 1085 workers employed in the synthesis and handling of 2-naphthylamine and/or benzidine (Nakamura *et al.*, 1980; Shinka *et al.,* 1991).

Hashmi *et al.,* (1995) found that among 250 consecutive bladder-cancer patients admitted to the Dow Medical College in Karachi, Pakistan, three had been exposed to 2-naphthylamine.

In a group of 438 persons engaged in the production of aromatic amines, including 2-naphthylamine, in Japan, 88 (20%) developed uroepithelial cancer (Hamasaki *et al.*, 1996).

[The studies listed above represent a mere sample of many case series spanning four decades.]

In addition to the case series presented above, three screening programmes detected cases of bladder cancer among workers exposed to 2-naphthylamine. These were the DuPont Chambers Works programme (Mason *et al.*, 1986, 1992; Mason & Vogler, 1990), the Drake programme (Marsh and Cassidy 2003) and the screening programme of a dyestuff plant in Tokyo, Japan (Miyakawa *et al.*, 2001). [The Working Group raised concerns about the validity of the results of these screening programmes as far as it concerns etiological research with a substantial potential for detection bias and because no measure of effect is available relating β-naphthylamine exposure to cancer.]

2.2 Cohort studies

One of the first studies to review cases of bladder cancer among workers exposed to 2-naphthylamine was that of Case *et al.* (1954), who reviewed 341 cases of bladder cancer of a total of 455 occurring in employees of participating firms in the British chemical industry who had been exposed to 2-naphthylamine only. There were 26 cases of bladder cancer mentioned on the death certificate, where only 0.3 would have been expected from the overall male population of England and Wales ($P < 0.001$). The mean induction period was 16 years.

Mancuso & el Attar (1967) followed a cohort of 639 male workers employed in a company manufacturing 2-naphthylamine and benzidine in Ohio (USA) for 27 years (1938–1965), with respect to cause-specific mortality. Based on 14 cases, the observed mortality rate for bladder cancer was elevated when compared with that expected for the population of Ohio (78/100 000 *vs* 4.4/100 000). Among white men, all cases occurred among the "exposed" group. There was an underreporting of cases of bladder cancer on the death certificates. The "attack rate", taking together those who had died and those who were living with bladder neoplasms, for those exposed to 2-naphthylamine was 952/100 000. Based on six cases, the mortality rate for pancreatic cancer was also elevated (39/100 000 *vs* 7.5/100 000).

Veys (1969, 2004) followed a cohort of male workers employed in the British rubber industry with respect to bladder-cancer morbidity and mortality. Among these workers, 2090 were considered likely to be exposed to 2-naphthylamine, as they were employed between 1945 and 1949, while 3038 workers who started their employment after 1950 were considered to be not exposed to 2-naphthylamine. For morbidity, 58 bladder cancers were identified in the first group, while 33.9 were expected according to national standardized incidence rates (SIR, 1.7; 95% CI, 1.3–2.2). In the second group, 39 cases were traced while 38.3 were expected (SIR, 1.0; 95% CI 0.7–1.4). Bladder-cancer mortality data from the same cohort did not show an increased risk (SMR, 1.0; 95% CI, 0.60–1.6); in only 16 of the 46 deceased was bladder cancer certified as the underlying cause of death.

Morinaga *et al.* (1982) ascertained the incidence of second primary cancers among 3322 workers exposed to 2-naphthylamine and benzidine during 1950–1978 in Japan. Among these workers, 244 had previously had genito-urinary cancer. Within this subgroup, 11 male workers developed subsequent cancers of the liver, gallbladder, bile duct, large intestine and lung. Compared with a control group of 177 male bladder-cancer patients, the excess occurrence of liver, gallbladder and bile-duct cancer was statistically significant ($P < 0.05$). One case of maxillary sinus cancer occurred in a worker exclusively exposed to 2-naphthylamine and one case of prostate cancer occurred in a worker exposed to both 2-naphthylamine and benzidine. The nine remaining cases appeared in workers exposed to benzidine.

Piolatto *et al.* (1991) carried out a cohort study among 906 dyestuff workers in Turin, Northern Italy, who were employed for ≥ 1 year between 1922 and 1970 and followed-up through 1989. Causes of death were ascertained through death certificates. Overall, 49 bladder-cancer cases were observed, while 1.6 were expected (SMR, 30.4; 95% CI, 23.0–40.2) Results were stratified by specific employment in the plant. Among those involved in manufacturing of naphthylamine or benzidine, the SMR for bladder cancer was 142.11 (27 deaths; 95% CI, 97.5–207.2) (Decarli *et al.*, 1985). Those who used naphthylamines or benzidine showed an SMR of 16.7 (three deaths; 95% CI, 5.4–51.7). An earlier study of the same cohort provided results specific to exposure during 2-naphthylamine manufacture; the SMR for bladder cancer was 150 (95% CI, 67.4–333.9) based on six cases (Rubino *et al.*, 1982).

A cohort study in the USA included 1384 workers employed between 1940 and 1972 in a chemical plant in Georgia manufacturing and using 2-naphthylamine and other aromatic amines. The cohort was followed-up until 1992. The initial cohort comprised approximately 70 female workers, but these were excluded from the mortality study because they were generally not employed in jobs involving exposure to 2-naphthylamine. The vital status at the end of follow-up was determined by use of the National Death Index and US postal service data. Incident cases of bladder cancer were identified by community neurologists and a bladder-cancer screening programme. The mortality experience of the cohort was compared with that of the US and Georgia populations. The SMR corresponding to bladder cancer as underlying cause of death was 2.4 (three deaths; 95% CI, 0.5–7.0) and that corresponding to bladder cancer as listed anywhere on the death certificate was 5.6 (eight deaths; 95% CI, 2.4–11.1). There was also an excess of deaths for esophageal cancer (seven deaths; SMR, 2.0; 95% CI, 0.8–4.1), lung cancer (41 deaths; SMR, 1.67; 95% CI, 1.20–2.3), and prostate cancer (11 deaths; SMR, 2.1; 95% CI, 1.1–3.8) (Schulte *et al.*, 1985, 1986; Stern *et al.*, 1985; Axtell *et al.*, 1998).

Delzell *et al.* (1989) followed a cohort of 2642 men employed at a dye- and resin-manufacturing plant in New Jersey (USA) from the opening of the plant in 1952 through 1988. Data on work history were obtained through several independent sets of records. A subcohort of 89 men who had previously worked in a chemical plant that produced benzidine and 2-naphthylamine showed an excess of total cancer deaths based on

17 cases (SMR, 1.99; 95% CI, 1.16–3.18). This excess was due essentially to bladder cancer (three cases observed, 0.25 expected; SMR, 12; 95% CI, 3.9–37.2), kidney cancer (two cases observed, 0.21 expected; SMR, 9.52; 95% CI, 2.4–38.1) and tumours of the central nervous system (two cases observed, 0.22 expected; SMR, 9.1; 95% CI, 2.3–36.3).

Morinaga *et al.* (1990) followed from 1970 to 1986 a cohort of 604 workers from two factories in Osaka (Japan) that produced 2-naphthylamine and benzidine in the period 1945–1971. The causes of death of these workers were compared with those expected based on the mortality of the Osaka population. Those who had been exposed exclusively to 2-naphthylamine showed a significant increase in urinary cancer (two cases observed; SMR, 11.76; 95% CI, 2.9–47.0). Those exposed to both 2-naphthylamine and benzidine had a higher risk with two cases observed (SMR, 25.00; 95% CI, 6.3–100.0). The increase in risk for other cancers (liver and lung) was not statistically significant.

Szeszenia-Dabrowska *et al.* (1991) conducted a cohort study among 6978 male workers employed in the rubber industry in Poland for at least three months during 1945 to 1973 and followed-up through 1985. These workers were predominantly engaged in rubber footwear manufacture. A total of 299 deaths from cancer were registered in the cohort (SMR, 112.7; 95% CI, 100.6–126.2). Bladder cancer was significantly increased for workers employed between 1945 and 1953, when 2-naphthylamine was used (six deaths; SMR, 2.76; 95% CI, 1.24–6.14).

Bulbulyan *et al.* (1995) evaluated cancer incidence and mortality in a cohort of 4581 aniline-dye production workers (2409 men and 2172 women) in Moscow, exposed to 2-naphthylamine or benzidine during ≥ 1 month, or employed during > 2 years, with a follow-up time of 15 years, from January 1975 to December 1989. Cases were ascertained through oncology registries that were available in the 33 Moscow regions. Job history for up to four jobs per person was ascertained through employment records. Incidence and mortality for several cancer sites were determined with regard to exposure to 2-naphthylamine and benzidine. Standardized incidence ratios were calculated by comparison with the Moscow general population. For workers ever exposed to 2-naphthylamine, the incidence of bladder cancer was significantly increased for those employed > 3 years (eight cases; SIR, 19.5; 95% CI, 8.4–38.5). There was a positive exposure-response relationship with years worked ($P = 0.07$); those who were first employed before the age of 20 showed a larger increase in risk (four cases; SIR, 49.4; 95% CI, 13.3–126.3) than those hired at later ages (p for trend = 0.04).

Naito *et al.* (1995) followed a cohort of 442 dyestuff workers in an urban area in Japan from date of entry (1935–1988) through 1992, on average for 39 years. The SMR for bladder cancer for those exposed to 2-naphthylamine was 48.4 (three deaths; 95% CI, 10.0–141.5). The SMR risk for all sites was 1.9 (nine deaths; 95% CI, 0.9–3.7) for 2-naphthylamine manufacture and 0.4 (2 deaths; 95% CI, 0.1–1.4) for 2-naphthylamine use. In addition to mortality, the authors reported on the incidence by periodic urologic screenings. The adjusted incidence rate of urothelial carcinoma increased with duration of exposure during 2-naphthylamine manufacture.

The Drake Health Registry Study provides for bladder-cancer screening of all former employees of the Drake Chemical Co. (Lock Haven, Pennsylvania, USA). The company and its predecessors produced 2-naphthylamine as a main product from 1940 until 1962 and possibly as a residual contaminant through 1981, when the plant was closed. Based on the Drake Health Registry, a cohort study was conducted among 400 workers (374 men, 26 women) with sufficient data for analysis. Causes of death were determined through the National Death Index and death certificates. The mortality experience of the cohort from 1960 to 1998 was examined, and expected numbers of deaths were computed with the US population and the local area population as a standard. For all cancers, based on 28 deaths, the SMR was 3.1 (95% CI, 2.1–4.5). For bladder cancer, the SMR was 16.8 (95% CI, 4.6–43.1) based on four deaths. For cancer of the respiratory system, based on 12 deaths, the SMR was 3.9 (95% CI, 2.0–6.8) (Cassidy *et al.*, 2003). Two decades earlier in an ecological study, Budnick *et al.* (1984) had analysed age-, sex-, and race-adjusted cancer-mortality rates for the county where the plant was located, and reported a statistically significant increase in bladder cancer among white men.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

A group of 13 male and 12 female IF mice, weighing 25 g, were given 5 mg of 2-naphthylamine (BDH; purified by distillation and crystallization) in arachis oil via stomach tube twice weekly at a dose of 400 mg/kg bw per week for life (90 weeks). A group of six males and five females served as arachis-oil controls. Liver cholangiomas were observed in 5/13 (38%) male and 5/12 female (41%) mice. No tumours were observed in control animals (Bonser *et al.*, 1952).

Groups of nine male and 14 female CBA mice, weighing 30 to 35 g (age not specified) were given five mg 2-naphthylamine (BHD; purified by distillation and crystallization) in arachis oil by gavage, twice weekly for a weekly dose of 240 mg/kg bw for life (90 weeks). A group of seven male and seven female mice served as arachis-oil controls. Hepatomas were observed in 6/9 males (67%) and 7/14 females (50%). No tumours were observed in the control animals. The authors reported the incidence of hepatomas in the control breeding mice of the laboratory to be 8% (Bonser *et al.*, 1952).

Groups of 14–15 male and 12–15 female CBA mice (weight and age not given) were fed four different synthetic diets containing 2-naphthylamine (concentration not specified) for a dose of 160 mg/kg bw per week for 90 weeks. No information on use of controls was provided. Hepatomas occurred at similar incidences in all four diet groups, with a total of 24/57 (42%) hepatomas observed in males and 25/54 (46%) in females. The

authors reported that malignant hepatomas were found in 16 mice (sex not specified) (Bonser *et al.*, 1952). [The Working Group noted the lack of controls.]

Groups of 20 female BALB/c mice, eight weeks of age, were fed a diet containing 2000 ppm 2-naphthylamine [purity not specified] for 40 weeks, followed by basal diet for 15 weeks. At 55 weeks, hyperplasia of the bladder epithelium was seen in 6 of 16 (37%) surviving mice. In the livers of these mice, hyperplastic nodules were seen in 14 animals (87%), adenomas in 10 (62%) and hepatomas in three (19%) (Yoshida *et al.*, 1979).

A group of 16 female and 16 male A/J mice, 6–8 weeks of age, received 2-naphthylamine in tricaprylin by gavage three times per week for eight weeks, resulting in a total dose per animal of 600 mg/kg bw. At 24 weeks, 8 of 14 (57%) male and 4 of 13 (31%) female survivors had lung tumours. This was not significantly different from the lung tumour rate in the vehicle controls. Tumour multiplicity was significantly increased in male mice (0.93 ± 1.00 *vs* 0.27 ± 0.59; $P < 0.05$) (Stoner *et al.*, 1986).

3.1.2 *Rat*

A group of 18 male, 29 female and three unknown albino rats were fed 0.01% 2-naphthylamine [purity unspecified] in the diet for life; this resulted in four bladder papillomas among 50 rats that survived for up to 102 weeks. No carcinomas were seen in the treated group. There were no tumours observed in the 43 controls fed an untreated diet (Bonser *et al.*, 1952).

Oral administration by gavage of 0.5–150 mg 2-naphthylamine [purity unspecified] per week to rats for 52 weeks, followed by an observation period until up to 80 weeks did not result in a significant increase in tumours compared with controls (Hadidian *et al.*, 1968).

A group of 25 female Wistar rats, 6–8 weeks of age, received 2-naphthylamine [purity unspecified] suspended in arachis oil by stomach tube at a dose rate of 300 mg/kg bw per week for one year, and were then kept until symptoms of bladder disease were seen. A group of 55 males and females served as controls. The first tumour appeared at 57 weeks. Histology indicated that five of 17 (29%) animals showed the presence of bladder tumours. Three animals were reported to be "still alive" (Hicks & Chowaniec, 1977).

A group of 20 female Wistar rats, 10 weeks of age, received 2-naphthylamine (purity not specified) at a dose of 300 mg/kg bw in arachis oil by gastric intubation once a week for 57 weeks. Animals were killed when moribund, or after 100 weeks. A group of 20 animals served as arachis-oil controls. Nineteen animals survived 57 weeks or longer. The bladders of 18 rats were examined histologically: eight of 18 (44%) had urothelial hyperplasia, and four of these eight had also carcinomas (Hicks *et al.*, 1982).

3.1.3 *Hamster*

A group of 30 male and 30 female Syrian golden hamsters, nine weeks of age, received 1% or 0.1% (w/w) 2-naphthylamine (purity unspecified) in the diet for life. The estimated intake was 600 mg 2-naphthylamine/week. Bladder carcinomas were observed in 10/23 (43%) effective males and in 8/16 (50%) effective females treated with the 1% concentration, with the first tumours appearing after 45 weeks. One male and one female hamster also developed hepatomas. Dietary concentrations of 0.1% did not produce carcinomas in 60 animals after 97 weeks. Repeated administration of the chemical by gavage (10 mg, twice weekly for 40 weeks) did not produce any tumours. In two control groups of 60 and 40 animals, fed the diet only, no bladder carcinomas were observed (Saffiotti *et al.*, 1967).

3.1.4 *Rabbit*

Oral doses (200 mg, twice weekly) of 2-naphthylamine (purity unspecified) given to six rabbits (age and sex unspecified) for more than five years induced a transitional-cell bladder papilloma at 4.75 years in one animal, and epithelial hyperplasia with downgrowth in the bladder in another after 5.25 years (Bonser *et al.*, 1952).

3.1.5 *Dog*

A group of 16 medium-sized to large female mongrel dogs, weighing 8–20 kg, received daily subcutaneous injections of commercial 2-naphthylamine (4 mg/day for dogs ≤ 12 kg: 5 mg/day for larger dogs) for 14 weeks. The dose was doubled during the next 12 weeks and tripled during the subsequent 63 weeks. During the last 54 weeks of this period, the dogs also received a daily dose of 2-naphthylamine (by capsule) in the food. The number of dogs on this dual regimen was gradually increased, as was the oral dose (starting with 100 mg/day). By the time the subcutaneous injections were terminated, all dogs received a daily oral dose of 300 mg of 2-naphthylamine. During the first five months of this experimental period, eight dogs received sodium bicarbonate (100 mg/kg bw) to alkalinize the urine. Four control dogs received no treatment. Over a total treatment period of 20–26 months, 13 of the 16 (81%) dogs had developed papillomas and carcinomas in the bladder (Hueper *et al.*, 1938). [This evidence was based partly on autopsy and partly on cystoscopy and biopsy.]

A group of four medium-sized mongrel Airedale dogs (one female and three males) received 2-naphthylamine (BDH; purified by distillation and crystallization) at 150 mg per day in milk for three weeks. The dose was then reduced to 100 mg/day (for an unspecified period). The dose was gradually increased to 700 mg per day after the end of the first year. One male dog died after one year of treatment. Multiple papillomas in the bladder were seen in the three other dogs after 3.7–5 years of treatment. In one animal, one anaplastic carcinoma was found. There were no metastases, and the renal pelves, ureters and urethra were tumour-free, as were all other organs (Bonser, 1943).

A group of four female mongrel dogs received 200 mg 2-naphthylamine (purity unspecified) orally in a gelatin capsule on six days per week for six months. The daily dose was then increased to 600 mg and continued for up to two years. The maximum cumulative dose of 2-naphthylamine given to a dog was 310 g. One dog died after 14.5 months of treatment without neoplastic changes in the bladder. Another dog received 2-naphthylamine for one year and was killed nine months later with no neoplastic changes in the bladder epithelium. Multiple transitional-cell tumours, some of which were histologically malignant, were seen in the bladder of the remaining two dogs. One of these died after two years of treatment; the other was killed one year later. The latter dog had developed an invasive bladder carcinoma (Bonser *et al.*, 1956a).

A group of four female mongrel dogs, each weighing 12–14 kg, were fed 400 mg 2-naphthylamine [purity unspecified] daily for two years. Bladder tumours were noted and confirmed by microscopic examination of biopsy sections in all four animals within 9–18 months after the start of the experiment. The dogs received intravenous and intracavitary therapy with 5-fluorouracil, whereupon transient tumour regression was observed. All dogs were then kept on standard diet and followed for 23 to 55 additional months until death or sacrifice. Metastases of the bladder carcinomas were seen in the lungs of two dogs and in the kidney in one (Harrison *et al.*, 1969).

A group of 34 beagle dogs, 8–10 months of age, received 2-naphthylamine [purity unspecified] mixed with lactose, orally in gelatin capsules on 6 days per week in doses of 6.25, 12.5, 25, and 50 mg/kg bw (dose groups A, B, C, D) per day for 2–26 months. Two male and two female control dogs received capsules with lactose only. Within 30 months, bladder tumours were induced across all dose groups in 24/34 (70%) dogs: invasive transitional-cell carcinomas were seen in two dogs each in dose groups A, B, and D, and in five dogs in dose group C; invasive squamous-cell carcinomas were observed in one, two, three and two dogs in dose groups A, B, C and D, respectively; papillary carcinomas were noted in one, three and four dogs in dose groups B, C and D, respectively. Overall, carcinomas were present in 9/11 (81%) dogs that had received a total of 100–200 g 2-naphthylamine and in 6/23 (26%) dogs receiving a total amount of < 100 g. No tumours were seen in the controls. The total amount of 2-naphthylamine required for tumour induction was less for dose group A after 24–30 months than for dose groups C and D after 9–18 months (Conzelman & Moulton 1972).

In a study designed to investigate the morphology of 2-naphthylamine-induced bladder tumours, eight female dogs, weighing 18–30 kg, were treated with 2-naphthylamine (5–30 mg/kg bw, 4–6 days per week, for 7.5 months and 30 mg/kg bw, 4–6 days per week, for 8.5 months). After 55 months since the beginning of the experiment, bladder carcinomas developed in seven (87%) animals (Romanenko & Martynenko, 1972).

A group of 15 female dogs, weighing 16–22 kg, received an oral capsule containing 500–600 mg 2-naphthylamine [purity not specified] daily for 20–26 months. All dogs had haematuria, and all developed bladder carcinoma (Rigotti *et al.*, 1977).

Eight female pure-bred beagle dogs were given a daily dose of 2-naphthylamine (5 mg/kg bw; source and purity not specified; route of administration not given) for 30 days. Four dogs then started on a daily supplement of 6 g D,L-tryptophan admixed into 300 g dog chow during the rest of the experiment. The other four dogs were kept on standard diet. Four additional dogs received only the dietary supplement but no 2-naphthylamine. After three years, all 12 dogs were killed and the bladders were examined grossly and by histology. The bladders of dogs that received only 2-naphthylamine showed no abnormal effects. The dogs that were given tryptophan with 2-naphthylamine showed gross and histological changes, such as papillary tumours and epithelial hyperplasia. The bladder mucosa of dogs given tryptophan alone showed similar hyperplastic changes (Radomski *et al.*, 1977).

Five male purebred beagle dogs received capsules with 2-naphthylamine in corn oil orally in doses of 25 mg/kg bw, daily on five days per week for 26 weeks. In a second experiment, groups of four dogs (two females, two males) were similarly treated for one, six or 36 weeks, then promptly killed. In a third experiment, eight dogs (four females, four males) were treated with the same regimen for 26 weeks, then four dogs were killed and the others kept without further treatment for three years. Loss of bladder luminal membrane, hyperplasia of the bladder epithelium, and lymphocyte infiltration of the submucosa were seen after one and six weeks of treatment in some dogs, and to a more severe degree after 36 weeks of treatment in all dogs. Two of the four dogs that were kept for three years after a 26-week treatment period had papillary carcinomas of the bladder (Rdomski *et al*., 1978).

A group of two female and three male beagle dogs, approximately nine months of age, received 400 mg pure 2-naphthylamine daily on five days per week for 34 months. The chemicals were given as tablets enclosed in a gelatin capsule. Control dogs (four males and four females) received capsules with lactose. All treated dogs developed transitional cell carcinomas of the bladder within 34 months. No neoplasia of the bladder was found in the controls (Purchase *et al.*, 1981).

3.1.6 *Monkey*

Twenty-four young male and female rhesus monkeys (*Macaca mulatta*; weighing 2–3 kg) received 2-naphthylamine mixed with lactose, orally in gelatin capsules on six days per week in doses of 6.25, 12.5, 25, 50, 100, 200, and 400 mg/kg bw per day for 33–60 months. Some animals received a fixed dose during the entire experiment; others received different doses over the course of five years. Papillary carcinoma, transitional-cell carcinoma or carcinoma in situ were observed in the bladder of nine treated monkeys, the earliest of these tumours appearing after 33 months. The majority of tumours occurred in animals given high doses of 2-naphthylamine. No other types of tumour were observed, and no tumours occurred in three control monkeys (Conzelman *et al.* 1969).

3.2 Subcutaneous or intra-peritoneal injection

3.2.1 *Mouse*

Swiss albino mice, approximately 12 weeks of age, received 0.1 ml of a freshly made 3% solution of 2-naphthylamine (BDH; purified by distillation and crystallization) in arachis oil by subcutaneous injection, twice weekly for 50 weeks. None of 13 mice surviving 33 weeks of treatment had subcutaneous sarcomas; two of four mice (50%; all females) surviving > 77 weeks had hepatomas. In a similar experiment with 2-naphthylamine (RCH; purified by gradient sublimation), local subcutaneous sarcomas were seen in two of 12 mice (17%; at 37 and 41 weeks, respectively) and one hepatoma at 52 weeks. When a 3% solution of 2-naphthylamine (BDH; purified by distillation and crystallization) was allowed to stand for four weeks and injected subcutaneously into Swiss albino mice (0.1 ml per mouse; twice weekly for 50 weeks), 10 of 16 (62%) mice that survived ≥ 20 weeks had subcutaneous injection-site sarcomas, and four of five (80%) mice that survived ≥ 80 weeks had hepatomas. No sarcomas or hepatomas developed in 17 male and female controls treated with arachis oil only (Bonser *et al.*, 1956a).

CBA mice, 12 weeks of age, received 0.1 mL of a freshly made 3% solution of 2-naphthylamine-HCl (BDH; purified by distillation and crystallization) by subcutaneous injection, twice weekly for six months and then once weekly for a further four months. No sarcomas were seen in 11 mice surviving ≥ 56 weeks of treatment, and hepatomas were observed in four of 11 (36%) mice dying after 82 weeks. In a similar experiment with 2-naphthylamine-HCl purified by gradient sublimation, no subcutaneous sarcomas were seen in ten mice surviving ≥ 58 weeks of treatment, and hepatomas were observed in four of 11 (36%) mice dying after 82 weeks. One (7%) hepatoma developed in 15 male and female control animals (Bonser *et al.*, 1956a).

Groups of 45 male and 45 female mice (from a local dealer, strain and age not specified) were given 2-naphthylamine (BDH, purified by distillation and crystallization via subcutaneous injection (0.1 mL of a freshly prepared or four-week-old 30-mg/ml suspension in arachis oil) twice weekly for 52 weeks (total dose, 312 mg per mouse). A total of 12 mice had subcutaneous sarcomas, seven mice had hepatomas, and two mice had intestinal tumours (one benign, one malignant) (Bonser *et al.*, 1956b). [The Working Group noted the lack of concurrent controls.]

Groups of 10 male and 10 female inbred A/St mice, 6–8 weeks of age, received intraperitoneal injections of 2-naphthylamine in tricaprylin. The injections were given three times per week for eight weeks. Doses per injection were 62.5, 125 or 250 mg/kg bw. At 24 weeks after the first injection the surviving mice were killed and the lungs were excised. The average number of lung tumours per mouse (1.38 ± 0.30) was statistically different ($P < 0.01$) from that in the corresponding solvent control (0.19 ± 0.10) in the mice treated with the highest dose of 2-naphthylamine (total dose 6 g/kg bw). The tumour yield in mice treated with 2-naphthylamine was not significantly different from that in the controls (Theiss *et al.*, 1981).

Three groups of 16 females and 16 males A/J mice, 6–8 weeks of age, received 2-naphthylamine (purity not specified) in tricaprylin by intraperitoneal injection three times per week for eight weeks, resulting, respectively, in total doses per animal of 120, 300 and 600 mg/kg bw. At 24 weeks, the incidence of lung tumours varied from 27% to 47%, which was not significantly different from the lung tumour rate in the vehicle controls (Stoner *et al.*, 1986).

A group of 91 newborn BALB/c mice received a single 0.02-mL subcutaneous injection containing 50 µg 2-naphthylamine (purity unspecified) in 1% aqueous gelatine on the first day of life. Among 71 surviving mice that were killed between the 36th and 43rd weeks, 15 (21%) had lung tumours [not significant], and one had a hepatoma. In a concurrent control group, 2/21 (10%) mice developed lung tumours (Roe *et al.*, 1963).

Twelve groups of 50–60 newborn BALB/c mice received subcutaneous injections of 100 µg 2-naphthylamine in 20 µL arachis oil once within 24 hours after birth, or once daily on the first five days of life. After 40 weeks, the numbers of pulmonary adenomas in the surviving mice in the two groups were not statistically different from those in the controls. In a second experiment, newborn BALB/c mice (number not specified) received subcutaneous injections of 100 µg 2-naphthylamine in 20 µl 3% aqueous gelatin once daily on the first five days of life. Surviving mice were killed at 50 weeks of age. Lung adenomas were seen in 9/41 (22%) surviving mice and in 4/48 (8%) controls, which was not significant. No other tumours were found. The failure of 2-naphthylamine to induce tumours in newborn mice was ascribed to immaturity of their metabolic system (Walters *et al.*, 1967).

A group of 68 newborn Swiss mice (sex unspecified) ($n = 68$) were given a single subcutaneous injection of 30 µL commercial, recrystallized 2-naphthylamine as 3% suspension in gelatin [purity unspecified] within 24 hours after birth. Ten months later, bronchogenic adenomas were found in six of 63 (10%) autopsied mice [not significant], and no tumours in 57 controls (Radomski *et al.*, 1971).

A group of 28 males and 23 females newborn Swiss mice were given subcutaneous injections of 30 µL commercial, recrystallized 2-naphthylamine as 3.3-mg/mL suspension in 3% gelatin (100 µg per dose) on the first, third and fifth day of life. Twelve months later, 2/26 (8%) autopsied male mice had hepatomas, and one of the 21 (5%) autopsied females had a lymphosarcoma. Among the controls (20 males, 30 females) one male mouse had a lymphosarcoma (Radomski *et al.*, 1971).

3.2.2 *Rat*

A group of 16 male Chester Beatty random inbred albino rats, weighing 200 g, received intraperitoneal injections of 50 mg/kg bw 2-naphthylamine freshly suspended in arachis oil twice weekly for three months. Thereafter, the rats were maintained until tumours were palpable or until death (survival period, 123–622 days). Of 14 rats examined, three had tumours: two (14%) had sarcomas, one (7%) had a salivary gland tumour (Boyland *et al.*, 1963). [The Working Group noted the absence of control rats.]

3.3 Topical application

3.3.1 *Mouse*

A group of twenty-five IF mice (sex not specified), weighing on average 25 g, received a dorsal dose of a saturated solution of 2-naphthylamine in benzene (applied by skin-painting; no details given) once a week for 99 weeks. No skin tumours or liver tumours were found (Bonser *et al.*, 1952). [The Working group noted the absence of control mice.]

3.4 Intravesicular implantation or instillation

3.4.1 *Mouse*

A group of 89 albino mice received an intravesicular implant of 2-naphthylamine (dose unclear) in paraffin wax. Forty-one mice received the compound after purification as in Bonser (1943), fourty-eight mice were given the compound after purification by gradient sublimation (Henson *et al*., 1954). After 40 weeks, eight mice (9%) had developed bladder carcinomas, four of which were invasive. This tumour yield was not significantly different from that in the control mice implanted with paraffin wax alone, but, according to the authors, the tumours were better established histologically (Bonser *et al.*, 1956c). In contrast, among 74 stock mice that survived 40 weeks after having received an intravesicular implant of a stearic-acid pellet containing 2-naphthylamine (dose unclear), no bladder adenomas, papillomas or carcinomas were found (Boyland *et al.*, 1964).

3.4.2 *Dog*

Four beagle dogs (age and sex unspecified) were given 10 mg commercial 2-naphthylamine (recrystallized before use) dissolved in 5 ml DMSO once every two weeks for 30 months. The solution was instilled by catheter, directly into the bladder lumen. No tumours were seen when the dogs were sacrificed at 45 months (Radomski *et al.*, 1971).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, elimination

4.1.1 *Humans*

Because of the known carcinogenicity of 2-naphthylamine in humans, few metabolic studies have been carried out. However, *N*-(2-naphthyl)-hydroxylamine and bis-(2-amino-

1-naphthyl) phosphate have been identified in the urine of some hospital patients given small doses of 2-naphthylamine (Troll & Nelson, 1961; Troll *et al.*, 1963).

Grimmer *et al.* (2000) analysed the amounts of the aromatic amines 1- and 2-naphthylamine and 2- and 4-aminobiphenyl in the urine of 48 German smokers and non-smokers. The results indicate that both groups excrete these four aromatic amines, with smokers excreting approximately twice as much (736 ng/24 hours *vs* 303 ng/24 hours). Similar amounts of urinary 2-naphthylamine and 4-aminobiphenyl were found in the two groups. The excreted aromatic amines decompose in the urine within a few hours, which explains why aromatic amines are difficult to detect in this matrix (in this study *para*-toluidine was added to the urine as a stabilizer). The origin of the aromatic amines found in the urine of non-smokers is unknown at present. Based on the cotinine levels in the urine of non-smokers, environmental tobacco smoke can be excluded as a major source of aromatic amines. In addition, neither diesel exhaust-related nitroarenes, nor the corresponding amino-derivatives to which they may be metabolically converted, were detected. The aromatic amines in urine arising from sources other than tobacco smoke or diesel exhaust may play a role in the etiology of bladder cancer in non-smokers.

4.1.2 *Animals*

Boyland (1958) and Boyland & Manson (1966) identified 24 metabolites of 2-naphthylamine in the urine of rats, rabbits, dogs or monkeys. Metabolism occurs along four main pathways: (i) *N*-hydroxylation followed by conversion to 2-amino-1-naphthyl-mercapturic acid, 2-nitrosonaphthalene and rearrangement to 2-amino-1-naphthol; (ii) oxidation at positions C5 and C6 to an arene oxide, which rearranges to 5-hydroxy-2-naphthylamine, reacts with water to form a 5,6-dihydroxydihydro derivative and forms a 5-hydroxy-6-mercapturic acid; (iii) conjugation of the amino group with acetic, sulphuric or glucuronic acid; (iv) secondary conjugation of the hydroxyl group with phosphate sulphuric or glucuronic acid. The proportion of these metabolites excreted in the urine of various experimental animals is different (Bonser *et al.*, 1951; Boyland & Manson, 1966; Conzelman *et al.*, 1969), although in most species 2-amino-1-naphthyl sulfate is the predominant metabolite. In early studies it was proposed that the *ortho*-hydroxylation metabolite 2-amino-1-naphthyl glucuronide was hydrolysed by β-glucuronidase present in urine, yielding 2-amino-1-naphthol (Allen *et al.*, 1957), which may act as the proximate carcinogen. The latter compound may be oxidized to form 2-amino-1-naphthoquinone, which can form DNA adducts (Beland & Kadlubar, 1985; Yamazoe *et al.*, 1985).

In dogs a small percentage of 2-naphthylamine was metabolized to bis-(2-amino-1-naphthyl) hydrogen phosphate (Boyland *et al.*, 1961; Troll *et al.*, 1963). *N*-Oxidation of 2-naphthylamine leads to more reactive metabolites (Deichmann & Radomski, 1969). Radomski & Brill (1971) showed that after a single oral dose of 70 mg/kg bw of both the carcinogenic 2-isomer and the weakly active or inactive 1-isomer of naphthylamine, the proportion of the dose converted into the corresponding *N*-hydroxylamine and nitroso

compound and excreted in the urine was about the same. When a dose of 5 mg/kg bw was given, the proportion of the 2-isomer converted to these metabolites in the urine remained about the same (0.2%), whereas the 1-isomer gave rise to barely detectable traces of these compounds. The unstable *N*-hydroxy metabolites are excreted in the urine as glucuronic acid conjugates and hydrolysed by acid or β-glucuronidase. These conjugates have been considered as the carcinogenic urinary metabolites of aromatic amines in the dog (Radomski *et al.,* 1973). Methaemoglobinaemia, which is a measure of *N*-hydroxy compounds in the blood, was higher in the dog with the 2-isomer at the 70-mg/kg bw dose (Radomski & Brill, 1971).

4.1.3 *In-vitro systems*

Dermal penetration of 2-naphthylamine and *ortho*-toluidine through human skin was studied by use of diffusion cells. Both compounds penetrate rapidly (lag time approximately 1.2 and 0.8 hours, respectively) and in high percentages (54 and 50%, respectively, of the applied dose within 24 hours) (Lüersen *et al.*, 2006).

Duverger-van Bogaert *et al.* (1991) evaluated the ability of the cytosol from human red blood cells to activate aromatic amines using the Ames test with *S. typhimurium* strain TA98 under liquid preincubation conditions. While negative results were obtained with 1-naphthylamine, a slight response was observed for 2-naphthylamine.

The cytosol from nine fresh autopsy specimens of human bladder tissue was analysed for the presence of *N*-acetyltransferase (NAT) activity towards *para*-aminobenzoic acid, 4-aminobiphenyl, 2-aminofluorene, and 2-naphthylamine. Apparent Km values indicated little difference in NAT affinity (100–300 microM) for any of the substrates between the nine individual bladders. However, the apparent V_{max} values showed that the bladders could be divided in rapid or slow acetylator phenotypes, based on their NAT activity towards 4-aminobiphenyl, 2-aminofluorene, and 2-naphthylamine. Four of the bladder cytosols had mean activities significantly higher (approximately 10-fold, $P < 0.01$) than the mean NAT activities of the other five bladder cytosols towards each arylamine carcinogen. However, no significant difference was detected in their NAT activities when *para*-aminobenzoic acid ws used as a substrate. The human bladder cytosols were also tested for their capacity to activate *N*-hydroxy-3,2'-dimethyl-4-aminobiphenyl to a DNA-binding electrophile through direct *O*-acetyltransferase (OAT)-mediated catalysis. The *N*-hydroxyarylamine OAT activity also discriminated between two levels of activation, being significantly higher (about twofold, $P = 0.0002$) in the rapidly *N*-acetylating bladder cytosols, which correlated ($r = 0.94$) with the measured levels of NAT activity in each cytosol. These results suggest that NAT activity and OAT activity of the human bladder vary concordantly with *N*-acetylator phenotype. The polymorphic expression of these acetylation activities may be important risk factors in human susceptibility to bladder cancer from arylamine carcinogens (Kirlin *et al.*, 1989).

The metabolic activation of 4-aminobiphenyl, 2-naphthylamine, and several heterocyclic amines has been shown to be catalysed by rat cytochrome P450ISF-G

[CYP1a2] and by its human ortholog, cytochrome P450PA [CYP1A2] (Butler *et al.*, 1989a). In humans, hepatic microsomal caffeine 3-demethylation is the initial major step in caffeine biotransformation, which is selectively catalysed by cytochrome P450PA. Caffeine 3-demethylation was highly correlated with 4-aminobiphenyl *N*-oxidation ($r = 0.99$; $P < 0.0005$) in hepatic microsomal preparations obtained from 22 human organ donors, and both activities were similarly decreased by the selective inhibitor 7,8-benzoflavone. A rabbit polyclonal antibody raised against human cytochrome P450PA strongly inhibited these activities as well as the *N*-oxidation of the carcinogen 2-naphthylamine and other amines. Human liver cytochrome P450PA also catalysed caffeine 3-demethylation, 4-aminobiphenyl *N*-oxidation, and phenacetin *O*-deethylation. Thus, estimation of caffeine 3-demethylation activity in humans may be useful in the characterization of arylamine *N*-oxidation phenotypes and to assess whether or not the hepatic levels of cytochrome P450PA, as affected by environmental or genetic factors, contribute to inter-individual differences in susceptibility to arylamine-induced cancers (Butler *et al.*, 1989b).

Moore *et al.* (1984) investigated the metabolism of benzidine and 2-naphthylamine in organ cultures of human and rat bladder. There was little oxidative metabolism of either carcinogen in either species. In particular, *N*-hydroxy-2-naphthylamine, a proximate carcinogen of 2-naphthylamine could not be detected. In contrast, large amounts of the acetylated metabolites, *viz*. *N*-acetylbenzidine, *N,N*-diacetylbenzidine and *N*-acetyl-2-naphthylamine were formed both in rat and human bladder cultures.

Microsomal enzyme preparations from dog liver, kidney, and bladder were used to assess the prostaglandin H synthase-catalysed activation of carcinogenic aromatic amines to bind covalently to proteins and nucleic acids. Bladder transitional epithelial microsomes activated *ortho*-dianisidine, 4-aminobiphenyl, and 2-naphthylamine to bind to protein and tRNA, and benzidine and *ortho*-dianisidine to bind to DNA. Co-substrate and inhibitor specificities were consistent with activation by prostaglandin H synthase. Binding of benzidine to protein was not observed with either hepatic or renal cortical microsomes upon addition of arachidonic acid or reduced nicotinamide adenine dinucleotide phosphate. Prostaglandin H synthase and mixed-function oxidase-catalysed binding of 2-naphthylamine to protein and to tRNA were compared by use of liver and bladder microsomes. Only mixed-function oxidase-catalysed binding was observed in liver, and only prostaglandin H synthase-catalysed binding was seen in bladder. The rate of binding catalysed by bladder microsomes was considerably greater than that catalysed by hepatic microsomes. In addition, the bladder content of prostaglandin H synthase activity was approximately 10 times that in the inner medulla of the kidney, a tissue reported to have a relatively high content of this enzyme in other species. These results are consistent with involvement of bladder transitional epithelial prostaglandin H synthase in the development of primary aromatic amine-induced bladder cancer (Wise *et al.*, 1984)

Hammons *et al.* (1985) studied the in-vitro hepatic metabolism of 2-aminofluorene (2-AF), 2-naphthylamine and 1-naphthylamine by use of high-pressure liquid chromatography. Hepatic microsomes from rats, dogs, and humans were shown to

catalyse the *N*-oxidation of 2-AF and of 2-naphthylamine, but not of 1-naphthylamine; and the rates of *N*-oxidation of 2-AF were 2- to 3-fold greater than the *N*-oxidation rate of 2-naphthylamine. In each species, rates of 1-hydroxylation of 2-naphthylamine and 2-hydroxylation of 1-naphthylamine were comparable and were 2- to 5-fold greater than 6-hydroxylation of 2-naphthylamine or 5- and 7-hydroxylation of 2-AF. Purified rat hepatic monooxygenases, cytochromes P450UT-A, P450UT-H, P450PB-B, P450PB-D, P450BNF-B, and P450ISF/BNF-G but not P450PB-C or P450PB/PCN-E, catalysed several ring oxidations as well as the *N*-oxidation of 2-AF. Cytochromes P450PB-B, P450BNF-B, and P450ISF/BNF-G were most active; however, only cytochrome P450ISF/BNF-G, the isosafrole-induced isozyme, catalysed the *N*-oxidation of 2-naphthylamine. The purified porcine hepatic flavin-containing monooxygenase is known to carry out the *N*-oxidation of 2-AF, but only ring-oxidation of 1-naphthylamine and 2-naphthylamine was detected. No *N*-oxidation of 1-naphthylamine was found with any of the purified enzymes, which is in line with the fact that no carcinogenicity is observed with this compound. Furthermore, carcinogenic arylamines appear to be metabolized similarly in humans and experimental animals. Enzyme mechanisms accounting for the observed product distributions were evaluated by Hückel molecular-orbital calculations on neutral, free-radical, and cation intermediates. The authors proposed a reaction pathway that involves two consecutive one-electron oxidations to form a paired substrate cation-enzyme hydroxyl-anion intermediate that collapses to ring- and *N*-hydroxy products (Hammons *et al.*, 1985; see also Sasaki *et al.*, 2002).

Boyd and Eling (1987) examined the oxidation of the bladder carcinogen 2-naphthylamine by prostaglandin H synthase (PHS) *in vitro*. Oxygen-uptake studies of 2-naphthylamine oxidation in the presence of glutathione, as well as extensive product analysis yielded data that were consistent with a one-electron mechanism of 2-naphthylamine oxidation by PHS. The formation of 2-nitroso-naphthalene was not observed under any condition. Metabolism studies with a purified PHS preparation confirmed that 2-naphthylamine oxidation is dependent on the peroxidase activity of the enzyme complex, and that a variety of organic hydroperoxides may support the reaction. Horseradish peroxidase oxidized 2-naphthylamine to the same products but, depending on the pH, in very different proportions from those obtained with PHS. Oxidation of 2-naphthylamine by a one-electron chemical oxidant resulted in a product profile similar to that obtained in the enzymatic systems. These results are consistent with a one-electron mechanism of 2-naphthylamine oxidation by PHS. The metabolism data also provide evidence for the formation of two types of potentially reactive electrophile: 2-imino-1-naphthoquinone and a free-radical species.

Poupko *et al.* (1983) studied microsome-mediated *N*-hydroxylation of 4-aminobiphenyl in mucosal tissue of bovine and canine bladder relative to the activity in liver. Bovine bladder microsomes mediated the *N*-hydroxylation of this amine at an exceptionally high rate, whereas no detectable activity was found with bovine liver microsomes. Dog-bladder microsomes were 40–100 times less active than bovine bladder microsomes and contained approximately one third the amount of cytochrome P450

(CYP). Dog liver microsomes were as active as dog bladder microsomes per nanomole CYP, and an order of magnitude more active when normalized to microsomal protein. Rat liver microsomes contained the highest level of CYP of all the preparations studied, and *N*-hydroxylase activity was approximately twice that in dog liver. Metabolic conversion of 4-ABP, 2-naphthylamine, and 1-naphthylamine into mutagens with S9 from bovine bladder mucosa was investigated in *Salmonella typhimurium* and found to parallel the carcinogenic potency of these compounds. These results demonstrate considerable tissue-, species-, and compound-specificity for the metabolic activation of aromatic amines, and provide further evidence in support of activation of the amines in the bladder as a mechanism of aromatic amine-induced bladder cancer (Poupko *et al.*, 1983).

4.2 Genetic and related effects

4.2.1 *Experimental systems*

(a) DNA adducts of 2-naphthylamine

Three DNA adducts are formed by the reaction of *N*-hydroxy-2-naphthylamine with DNA *in vitro* at pH 5.0 (Beland *et al.*, 1983; Beland & Kadlubar, 1985). The major adduct has been characterized as an imidazole ring-opened derivative of *N*-(deoxyguanosin-8-yl)-2-naphthylamine (50% of the total adducts); there were smaller amounts of 1-(deoxyguanosin-N^2-yl)-2-naphthylamine (30% of total adducts) and 1-(deoxyadenosin-N^6-yl)-2-naphthylamine (15% of total adducts). These same three DNA adducts were formed in target (urothelium) and non-target (liver) tissues of dogs two days after the oral administration of 2-naphthylamine (Beland & Kadlubar, 1985). A four-fold higher binding level of 2-naphthylamine was found in urothelial DNA compared with that in the liver. The major adduct in both tissues was the ring-opened derivative of *N*-(deoxyguanosin-8-yl)-2-naphthylamine; there were smaller amounts of 1-(deoxy-adenosin-N^2-yl)-2-naphthylamine and 1-(deoxyguanosin-N^2-yl)-2-naphthylamine. The N^2-deoxyguanosine adduct persisted in the dog liver, and both this adduct and the ring-opened C8-deoxyguanosine adduct persisted in the bladder. The differential loss of adducts indicates that active repair processes are ongoing in both tissues, and the relative persistence of the ring-opened C8-deoxyguanosine adduct in the target but not the non-target tissue suggests that this adduct is a critical lesion for the initiation of urinary bladder tumours.

Peroxidative enzymes, such as prostaglandin H synthase (PHS), catalyse both the *N*-oxidation and ring-oxidation of 2-naphthylamine, a major ring-oxidation product being 2-amino-1-naphthol (Yamazoe *et al.*, 1985). When PHS was used to catalyse the binding of 2-naphthylamine to DNA, the same three adducts arising from *N*-hydroxy-2-naphthylamine were detected. In addition there were three other adducts, which appeared to be formed from 2-imino-1-naphthoquinone, the oxidative product of 2-amino-1-naphthol. The major product was characterized as N^4-(deoxyguanosin-N^2-yl)-2-amino-1,4-naphthoquinone-imine; two minor products were tentatively identified as

N[4]-(deoxyadenosin-*N*[6]-yl)-2-amino-1,4-naphthoquinone-imine and a deoxyguanosin-*N*[2]-yl adduct of a naphthoquinone-imine dimer (Beland & Kadlubar, 1985; Yamazoe *et al.*, 1985). These DNA adducts, formed via peroxidation, accounted for approximately 60% of the total DNA binding that was observed by incubation of 2-naphthylamine with PHS *in vitro*. *In vivo*, in dogs, the DNA adducts derived from 2-imino-1-naphthoquinone accounted for approximately 20% of the 2-naphthylamine bound to urothelial DNA, but they were not detected in liver DNA (Yamazoe *et al.*, 1985). The remaining adduction products were derived from *N*-hydroxy-2-naphthylamine. Thus, PHS expressed in the bladder could play a significant role in bioactivation of arylamines directly in the bladder and could contribute to carcinogenesis of 2-naphthylamine and other arylamines that serve as substrates of PHS.

(b) Genotoxicity of 2-naphthylamine

Most of the genetic effects of 2-naphthylamine have been reviewed (Mayer 1982) and are summarized in Supplement 7 of the IARC Monographs (IARC, 1987).
2-Naphthyl-amine exhibits weak mutagenicity in the microbial assays and in some mammalian systems *in vitro*. Results for the mutagenicity of 2-naphthylamine in yeast assays have been inconsistent. In the assays *in vivo*, 2-naphthylamine was positive in the sex-linked recessive lethal assay with *Drosophila melanogaster*, but it failed to induce sister chromatid exchange in the mouse. Several micronucleus tests and sperm-abnormality assays with mice yielded inconclusive results. In the bone-marrow micronucleus assay, very high doses of 2-naphthylamine (200–800 mg/kg bw) were required to elicit effects in C57BL6 male mice (Mirkova & Ashby, 1988). 2-Naphthylamine was positive in the mammalian spot test in T stock mice given nine doses of 2-naphthylamine (50 to 100 mg/kg bw), but there was no clear dose-response effect (Chauhan *et al.*, 1983). Few other data exist about the mutagenic effects of 2-naphthylamine in whole organisms, although it is known to induce DNA fragmentation in rodent liver after treatment *in vivo* (Parodi *et al.*, 1981).

(i) *Bacterial mutagenesis*

The metabolic conversion of 2-naphthylamine (5–50 µg/plate) to a bacterial mutagen in *S. typhimurium* TA100 was catalysed much more efficiently by the hamster than by any of the other species tested (human, pig, rat, mouse). Mouse preparations displayed the weakest activity (Phillipson & Ioannides, 1983). In two other studies, the activities of liver-S9 preparations from rats pre-treated with PCBs or 3-methylcholanthrene (3-MC) were comparable to the activities of liver-S9 obtained from guinea-pigs pre-treated with the same CYP450 inducers in bio-activation of 2-naphthylamine (2.5–10 µg/plate) to a bacterial mutagen in strain TA100 (Baker *et al.*, 1980). Liver-S9 preparations from rats pre-treated with PCB or 3-MC also displayed comparable activity in bio-activation of 2-naphthylamine (10–50 µg/plate) in strain TA1535 (Bock-Hennig *et al.*, 1982). Bovine urinary bladder cells, but not hepatocytes, were able to activate 2-naphthylamine to bacterial mutagens in *S. typhimurium* TA98; the minimal amount of 2-naphthylamine

required to increase the level of revertants over background was 20 µg/plate (Hix *et al.*, 1983). Bovine bladder cells were also able to bioactivate 2-naphthylamine (20–80 µg/plate) to a mutagen in *S. typhimurium* strain TA100, with the activity being about six- to eight-fold higher than in TA98; however, bladder cells did not activate 2-naphthylamine (20 µg/ml) to a mutagen in hamster V79 cells when resistence to 6-thioguanine was used as a genetic marker (Oglesby *et al.*, 1983). The finding of bacterial mutagenicity of 2-naphthylamine (4–5000 µg/ml) was corroborated by bioactivation with rat-liver S9 (PCB-pretreatment) in the Ames II assay (Flückiger-Isler *et al.*, 2004).

Recombinant human CYPs 1A1, 1A2 and 1B1 were unable to activate 2-naphthylamine (5 µM) to a DNA-damaging agent, when the induction of *umu* was used as an endpoint, in *S. typhimurium* strain NM2009, which expresses multiple copies of bacterial *O*-acetyltransferase (Shimada *et al.*, 1996). 2-Naphthylamine (up to 100 µM) also failed to induce the *umu* response in *S. typhimurium* tester strains expressing human CYP1A1, 1A2, 1B1, 2C9, 2D6, 2E1 or 3A4 with bacterial *O*-acetyltransferase (Oda *et al.*, 2001). However, 2-naphthylamine (≥ 1 µM) in the presence of recombinant human CYP1A2 did induce the *umu* response in *S. typhimurium* tester strains NM6001 and NM6002, which expressed recombinant human acetyltransferase NAT1 and NAT2 isoforms, respectively, but not in strain NM6000, which is *O*-acetyltransferase-deficient (Oda 2004). The level of *umu* induction by 2-naphthylamine was about twofold higher in strain NM6001 than in NM6002 (Oda 2004). Purified rat CYP4B1 was the most efficient haemoprotein among 10 different CYP450s in bioactivating 2-naphthylamine (10 µM) and inducing the *umu* response in *S. typhimurium* NM2009 (Imaoka *et al.*, 1997).

In the *E. coli* K-12 *uvrB/recA* DNA-repair host-mediated assay in male NMRI mice, 2-naphthylamine (i.p. 200 mg/kg bw) elicited differential killing in *E. coli* retrieved from the kidney and testicles, but no activity was observed in blood, liver or lung. Surprisingly, 1-naphthylamine (i.p. 33 and 100 mg/kg bw) showed a quite different pattern, inducing differential killing of *E. coli* retrieved from blood, liver, lung and kidneys (Hellmér & Bolcsfoldi, 1992).

The results of studies on bacterial mutagenesis of 2-naphthylamine with recombinant CYP450 enzymes are inconsistent. This may be due to varying assay conditions and the presence of different phase-II enzymes that modulate the metabolism of 2-naphthylamine.

(ii) *Mammalian mutagenesis*

2-Naphthylamine (50 µg/mL and 100 µg/mL) in the presence of liver microsomes from PCB-pretreated rats was shown to increase the level of mutations by up to tenfold over background levels at five independent genetic loci in Chinese hamster ovary (CHO) cells (Gupta & Singh, 1982). Mutation induction was also observed at these loci in the absence of a metabolic activation system, indicating that the CHO cells are capable of bioactivating 2-naphthylamine (Gupta & Singh, 1982). However, 2-naphthylamine (20 µg/ml) was not mutagenic in hamster V79 cells, based on selection of 6-thioguanine-resistant mutants in the presence of bovine urinary bladder cells for bioactivation (Oglesby *et al.*, 1983).

(iii) *DNA damage induced by human carcinogens in cell-free assays*

2-Naphthylamine (0.2–1 mM) induced DNA damage—measured by ^{32}P-postlabelling—when calf-thymus DNA was co-incubated with 2-naphthylamine and liver microsomes from rats pretreated with phenobarbital or β-naphthoflavone as CYP inducers. However, the bioactivated 2-naphthylamine did not induce DNA fragmentation (Adams *et al.*, 1996).

5. Summary of Data Reported

5.1 Exposure data

2-Naphthylamine was formerly used as a dye intermediate and in the rubber industry; it is no longer used commercially and its manufacture has been banned in the European Union since 1998. 2-Naphthylamine can be formed by pyrolysis of nitrogen-containing organic matter. Recent analytical methods permit detection at the parts-per-billion level.

Occupational exposure occurred during production, during its use in the manufacturing of dyes, and when present as a contaminant in antioxidants used in rubber production. In workers exposed primarily to aniline and 4-chloroaniline, urinary levels of 2-naphthylamine of both smokers and non-smokers were higher than in non-smoking non-exposed workers. The rubber antioxidant *N*-phenyl-2-naphthylamine can be contaminated with 2-naphthylamine and may also be metabolised to 2-naphthylamine.

The major sources of exposure for the general population are environmental exposure to 2-naphthylamine and 2-nitronaphthalene, tobacco smoke and fumes from cooking oil.

5.2 Human carcinogenicity data

Numerous case series and 11 cohort studies (four in the USA, two in the United Kingdom, two in Japan, and one each in Italy, Poland and the Russian Federation) are available concerning bladder-cancer risks in workers engaged in the manufacture and use of 2-naphthylamine. All of these case series and cohort studies indicate that markedly elevated bladder-cancer risks are associated with the manufacture and use of 2-naphthylamine. In some of these studies, it was not possible to quantify the relative contributions of benzidine and 2-naphthylamine exposures in the overall excess risks. However, a United Kingdom study on bladder-cancer risks in the British rubber industry showed that the elimination of the antioxidants contaminated with 2-naphthylamine (without other changes in industrial practices) was enough to eliminate the excess risk for bladder cancer in the industry.

5.3 Animal carcinogenicity data

2-Naphthylamine was tested for carcinogenicity by oral administration in mice, rats, hamsters, rabbits, dogs and monkeys. Following oral administration, it induced bladder neoplasms in monkeys, dogs and hamsters, and liver tumours in mice. A low incidence of bladder carcinomas was observed in rats after oral administration. In lung-adenoma bioassays in mice by oral and intraperitoneal injection, 2-naphthylamine produced an increase multiplicity in lung tumours.

5.4 Other relevant data

2-Naphthylamine undergoes bioactivation by human and animal CYP450s to produce *N*-hydroxy-2-naphthylamine, which reacts with DNA to form several adducts. A second bioactivation pathway occurs with peroxidases to form 2-amino-1-naphthol. This metabolite can undergo oxidation to produce 2-imino-1-naphthoquinone, which reacts with DNA to form a unique adduct in the urothelium of experimental animals. Liver and bladder tissues from various species activate 2-naphthylamine to a genotoxicant *in vitro* in bacteria and mammalian cells. Both CYPs and peroxidases may play a significant role in inducing genetic damage of 2-naphthylamine in the urothelium.

6. Evaluation

6.1 Cancer in humans

There is *sufficient evidence* in humans for the carcinogenicity of 2-naphthylamine. 2-Naphthylamine causes bladder cancer in humans.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2-naphthylamine.

6.3 Overall evaluation

2-Naphthylamine is *carcinogenic to humans (Group 1)*.

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