ortho-TOLUIDINE

This substance was considered by previous working groups, in June 1977 (IARC, 1978), February 1981 (IARC, 1982) and March 1987 (IARC, 1987a). Since that time, new data have become available, and these have been incorporated in the monograph and taken into consideration in the evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

ortho-Toluidine

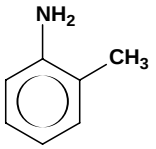
Chem. Abstr. Serv. Reg. No.: 95-53-4
Chem. Abstr. Name: 2-Methylbenzenamine
IUPAC Systematic Name: *ortho*-Toluidine
Synonyms: 1-Amino-2-methylbenzene; 2-amino-1-methylbenzene; 2-aminotoluene; *ortho*-aminotoluene; 2-methyl-1-aminobenzene; 2-methylaniline; *ortho*-methylaniline; *ortho*-methylbenzenamine; 2-methylphenylamine; *ortho*-tolylamine

ortho-Toluidine hydrochloride

Chem. Abstr. Serv. Reg. No.: 636-21-5
Chem. Abstr. Name: 2-Methylbenzenamine hydrochloride
IUPAC Systematic Name: *ortho*-Toluidine hydrochloride
Synonyms: 1-Amino-2-methylbenzene hydrochloride; 2-amino-1-methylbenzene hydrochloride; 2-aminotoluene hydrochloride; *ortho*-aminotoluene hydrochloride; 2-methyl-1-aminobenzene hydrochloride; 2-methylaniline hydrochloride; *ortho*-methylaniline hydrochloride; *ortho*-methylbenzenamine hydrochloride; 2-methylphenylamine hydrochloride; *ortho*-tolylamine hydrochloride

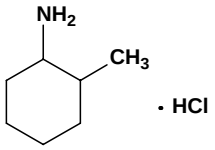
1.1.2 *Structural and molecular formulae and relative molecular mass*

***ortho*-Toluidine**



C_7H_9N Relative molecular mass: 107.16

***ortho*-Toluidine hydrochloride**



$C_7H_9N.HCl$ Relative molecular mass: 143.62

1.1.3 *Chemical and physical properties of the pure substances*

***ortho*-Toluidine**

(*a*) *Description*: Colourless to light yellow liquid becoming reddish brown on exposure to air and light (Verschueren, 1996; Budavari, 1998)
(*b*) *Boiling-point*: 200.3 °C (Lide & Milne, 1996)
(*c*) *Melting-point*: –16.3 °C (Lide & Milne, 1996)
(*d*) *Density*: 0.9984 g/cm^3 at 20 °C (Lide & Milne, 1996)
(*e*) *Spectroscopy data*: Infrared (prism [1543]; grating [8160]), ultraviolet [442], nuclear magnetic resonance (proton [107]; C-13 [225]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)
(*f*) *Solubility*: Slightly soluble in water (15 mg/L at 25 °C) (Verschueren, 1996); miscible with carbon tetrachloride, diethyl ether and ethanol (Lide & Milne, 1996)
(*g*) *Volatility*: Vapour pressure, 0.013 kPa at 20 °C; relative vapour density (air = 1), 3.72 (Verschueren, 1996)
(*h*) *Stability*: Flash-point (closed cup), 85 °C; oxidizes with prolonged exposure to air and light (Budavari, 1998)
(*i*) *Octanol/water partition coefficient (P)*: log P, 1.32 (Hansch *et al*., 1995)
(*j*) *Conversion factor*[1]: mg/m^3 = 4.38 × ppm

[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

***ortho*-Toluidine hydrochloride**

From IARC (1982), National Toxicology Program (1991), New Jersey Department of Health and Senior Services (1994) and Chemfinder (2000), except where otherwise indicated.

(*a*) *Description*: Colourless to white crystalline solid
(*b*) *Boiling-point*: 242.2 °C
(*c*) *Melting-point*: 215 °C
(*d*) *Spectroscopy data*: Infrared (prism [6330]; grating [29769], ultraviolet [1740], nuclear magnetic resonance (proton [10946]) (Sadtler Research Laboratories, 1980) and mass spectral data (Mass Spectrometry Data Centre, 1974) have been reported.
(*e*) *Solubility*: Very soluble in water (< 1 g/L at 22 °C); soluble in ethanol and dimethyl sulfoxide; insoluble in benzene, and diethyl ether
(*f*) *Volatility:* Vapour pressure, 0.13 kPa at 43.9 °C
(*g*) *Stability*: Sensitive to exposure to light and moisture
(*h*) *Conversion factor*: $mg/m^3 = 5.87 \times ppm$

1.1.4 *Technical products and impurities*

ortho-Toluidine from one source is available with the following specifications: purity, 99.5% min.; *meta*-toluidine, 0.4% max.; *para*-toluidine, 0.1% max; sum of *meta*- and *para*-toluidine, 0.5% max.; and water, 0.1% max. (Bayer Organic Chemicals, 1995). *ortho*-Toluidine hydrochloride is available from another source as 98% pure (Chemfinder, 2000).

1.1.5 *Analysis*

Selected methods for the analysis of *ortho*-toluidine in various matrices are given in Table 1.

ortho-Toluidine has also been determined in biological samples (urine, blood) as the free amine or in derivatized form by gas chromatography (GC) or high-performance liquid chromatography with electrochemical or electron capture detection (El-Bayoumy *et al.*, 1986; Teass *et al.*, 1993; Brown *et al.*, 1995; Riffelmann *et al.*, 1995; Ward *et al.*, 1996). Food and beverage samples have been analysed by GC with flame ionization detection or mass spectrometry (Vitzthum *et al.*, 1975; Neurath *et al.*, 1977).

1.2 Production

Reacting *ortho*-chlorotoluene with sodium in liquid ammonia generates a mixture of 67% *ortho*-toluidine and 33% *meta*-toluidine (Lin & Krishnamurti, 1993). *ortho*-Toluidine can also be produced by reduction of *ortho*-nitrotoluene or obtained mixed with *para*-toluidine by reduction of crude nitrotoluene (Lewis, 1993).

Table 1. Selected methods for the analysis of *ortho*-toluidine

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Adsorb (silica gel); desorb (ethanol)	GC/FID	0.01 mg/sample	Eller (1994) [Method 2002]
Wastewater, municipal and industrial	Add isotope-labelled analogue; extract with dichloromethane; dry over sodium sulfate; concentrate	GC/MS	10 µg/L	Environmental Protection Agency (1999a) [Method 1625]
Solid waste matrices	Concentrate by azeotropic distillation	GC/MS	13 µg/L	Environmental Protection Agency (1996a) [Method 8260B]
Air sampling media, water samples, solid waste matrices, soil samples	Liquid–liquid extraction or water dilution	GC/MS	10 µg/L (aqueous) (EQL)	Environmental Protection Agency (1996b) [Method 8270C]
Ground and surface water	Solvent extraction or direct injection	GC/FID	NR	Environmental Protection Agency (1996c) [Method 8015B]

GC, gas chromatography; FID, flame ionization detector; MS, mass spectrometry; EQL, estimated quantitation limit (the EQL of Method 8270 for determining an individual compound is approximately 660 µg/kg (wet weight) for soil/sediment samples, 1–200 mg/kg for wastes (dependent on matrix and method of preparation), and 10 µg/L for groundwater samples); NR, not reported

ortho-Toluidine was first produced commercially in the United Kingdom in 1880. It has been produced commercially in the United States for over 70 years and commercial production of the hydrochloride salt was first reported in 1956. Production of *ortho*-toluidine in the United States in the late 1970s was reported to range from 500 to 5000 tonnes per year (IARC, 1982); production ranged from 6600 to 12 800 tonnes in the early 1990s (Environmental Protection Agency, 1999b).

Information available in 1999 indicated that *ortho*-toluidine was manufactured by 16 companies in China, 11 companies in India, six companies in the United States, three companies each in Germany and Russia, two companies each in Japan and Poland, and one company each in Brazil, France, Italy, Mexico, Romania and Spain, and that *ortho*-toluidine hydrochloride was manufactured by one company each in Germany and India (Chemical Information Services, 1999).

1.3 Use

The major uses of *ortho*-toluidine and its hydrochloride salt are as intermediates in the manufacture of over 90 dyes and pigments, including acid-fast dyestuffs, optical brighteners, synthetic rubber and rubber chemicals, pharmaceuticals and pesticides (IARC, 1982; Bayer Organic Chemicals, 1995; American Conference of Governmental Industrial Hygienists, 1999).

1.4 Occurrence

1.4.1 *Natural occurrence*

ortho-Toluidine has been detected in tea (Vitzthum *et al.*, 1975) and possibly in some fresh vegetables (Neurath *et al.*, 1977).

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999), as many as 30 000 workers in the United States were potentially exposed to *ortho*-toluidine and its hydrochloride salt (see General Remarks). Occupational groups included workers in the chemical industry, laboratory workers, machine operators and cleaners and janitors. Ninety laboratory workers, health-care workers and university teachers were identified as exposed to *ortho*-toluidine or its salts in the Finnish Register of Employees Exposed to Carcinogens in 1997 (Savela *et al.*, 1999). National estimates of workers exposed were not available from other countries.

In the former USSR (Khlebnikova *et al.*, 1970), workers at a plant manufacturing *ortho*-toluidine from *ortho*-nitrotoluene were exposed to concentrations of *ortho*-toluidine in the air (n = 215) generally exceeding the maximum permissible concentrations [of 3 mg/m^3, IARC, 1982] by 2–7 times . The highest airborne exposure levels were observed during distillation and extraction processes (25–28.6 mg/m^3). Concurrently, *ortho*-nitrotoluene levels in the air in 80–90% of the samples exceeded the maximum permissible concentration of 1 mg/m^3; levels up to approximately 5 mg/m^3 were also reported (the highest levels were observed during extraction and heating of the reduction reaction). Dermal deposition of 0.01–0.03 mg *ortho*-toluidine per square decimetre of skin was measured by collecting 1% acetic acid washes from wrists, chest and back of individuals at the end of work-shifts (n = 168). After post-shift showers, dermal levels of *ortho*-toluidine decreased by a factor of 10. Patches of cloth placed on the workers' overalls collected about 0.10 mg *ortho*-toluidine per square decimetre (n = 46).

Measurements in the 1940s in a United States dye production plant indicated that the concentration of *ortho*-toluidine was < 0.5 ppm [2.19 mg/m^3] in the workroom air in the breathing zone and area samples and from < 0.3 ppm to 1.7 (mg/L) ppm in the urine of workers engaged in the production of thioindigo. In addition to inhalatory

exposure, there was concern about exposure from ingestion and skin contact (Ott & Langner, 1983).

Exposure to *ortho*-toluidine was reported to occur in an Italian plant producing fuchsin (magenta) and safranine T-based dyes (Rubino *et al.*, 1982), in a German plant producing 4-chloro-*ortho*-toluidine (Stasik, 1988) and in a plant producing rubber chemicals in the United Kingdom (Sorahan *et al.*, 2000), but no data on exposure levels were provided.

ortho-Toluidine, aniline, hydroquinone and toluene were used to synthesize a rubber antioxidant in a United States chemical plant. Despite low air concentrations (< 1 ppm) [4.38 mg/m^3], elevated levels of *ortho*-toluidine were detected in the urine of exposed workers (mean, 104 μg/L post-shift), suggesting dermal exposure, in measurements carried out in 1990 (Ward *et al.*, 1991; Teass *et al.*, 1993). A more recent exposure study showed similar levels of *ortho*-toluidine (mean, 99 μg/L post-shift) in the urine of workers in the rubber chemicals department of the plant. Exposed workers also had significantly increased levels of *ortho*-toluidine–haemoglobin adducts in blood (Ward *et al.*, 1996) (see Section 2.2 for further details).

1.4.3 *Environmental occurrence*

The production of *ortho*-toluidine and its use as an intermediate in the production of dyes and pigments, rubber chemicals and other products may result in its release to the environment through various waste streams. The primary routes of potential human exposure to *ortho*-toluidine and its hydrochloride salt are inhalation and dermal contact. Consumer exposure may occur from residues present in commercial dyes and on textiles and via smoking (IARC, 1982; Department of Health and Human Services, 1982; Environmental Protection Agency, 1984, 1997; Department of Health and Human Services, 1999).

(*a*) *Air*

According to the Toxics Release Inventory (Environmental Protection Agency, 1997), air emissions of *ortho*-toluidine from 23 industrial facilities were approximately 5260 kg in 1995 in the United States.

(*b*) *Water*

Surface water discharges of *ortho*-toluidine from 23 industrial facilities in the United States in 1995 amounted to 116 kg, as reported in the Toxics Release Inventory (Environmental Protection Agency, 1997), reduced from a value of 242 kg in 1994 (Environmental Protection Agency, 1996d).

ortho-Toluidine has been reported in surface water samples taken from three rivers in Germany at levels of 0.3–1 μg/L (Neurath *et al.*, 1977).

ortho-Toluidine has been identified in one secondary effluent from seven industrial and publicly owned treatment works (Ellis *et al.*, 1982) and in wastewaters from

synthetic fuel production (Leenheer *et al*., 1982; Stuermer *et al*., 1982). It has also been detected in effluents from refineries and chemical production facilities, in process water, river water, groundwater and seawater in the United States (Shackelford & Keith, 1976; Environmental Protection Agency, 1984; Department of Health and Human Services, 1999).

(*c*) *Soil*

Soil discharges of *ortho*-toluidine from 23 industrial facilities in 1995 in the United States amounted to only 5.5 kilogrammes, as reported in the Toxics Release Inventory (Environmental Protection Agency, 1997). Additionally, an estimated 10 000 kg of *ortho*-toluidine were released via underground injection.

(*d*) *Food and beverages*

Unspecified isomers of toluidine were found in samples of kale and celery (1.1 mg/kg) and carrots (7.2 mg/kg) (Neurath *et al*., 1977). *ortho*-Toluidine has been identified in the volatile aroma components of black tea (Vitzthum *et al*., 1975).

(*e*) *Tobacco smoke*

ortho-Toluidine has been reported to be present in mainstream cigarette smoke at 32–162 ng per cigarette and at 3 μg per cigarette in sidestream smoke (IARC, 1986).

ortho-Toluidine was identified in the urine of both smokers and nonsmokers at levels of 6.3 ± 3.7 μg/24 h in all 10 subjects tested and 4.1 ± 3.2 μg/24 h in all nine subjects tested, respectively, suggesting that sources other than cigarette smoke contribute significantly to *ortho*-toluidine exposures (El-Bayoumy *et al*., 1986). In a biological monitoring study of occupationally exposed workers, significantly higher concentrations of *ortho*-toluidine were also found in the urine of smokers (1.7 ± 1.6 μg/L) who were in the control (non-exposed) study group, in comparison with nonsmokers (0.0 ± 0.0 μg/L) in control groups (Riffelmann *et al*., 1995).

1.5 Regulations and guidelines

Occupational exposure limits and guidelines for *ortho*-toluidine are presented in Table 2.

Table 2. Occupational exposure limits and guidelines for *ortho*-toluidine[a]

Country	Year	Concentration (mg/ m^3)	Interpretation[b]
Australia	1993	9 (sk, ca)	TWA
Belgium	1993	9 (sk, ca)	TWA
Canada	1994	9 (sk, susp.h.ca)	TWA
Czech Republic	1993	5	TWA
		20	STEL
Denmark	1993	9 (sk, ca)	TWA
Finland	1998	22 (sk)	TWA
		44	Ceiling
France	1993	9 (ca)	TWA
Germany	1999	(2, sk), 0.5	TRK
Ireland	1997	9 (sk)	TWA
Japan	1998	4.4 (sk, ca)	TWA
Philippines	1993	22 (sk)	TWA
Poland	1998	3 (sk)	TWA
		9	STEL
Russian Federation	1993	0.5 (sk, ca)	TWA
		1	STEL
Sweden	1993	none (ca)	
Switzerland	1993	9 (sk, ca)	TWA
Turkey	1993	22 (sk)	TWA
United Kingdom	1993	0.9 (sk)	TWA
United States			
ACGIH[c]	1999	9 (sk, A3)	TWA
NIOSH	1999	lfc (sk, ca)	TWA
OSHA	1999	22 (sk)	TWA

[a] From Finnish Ministry of Social Affairs and Health (1998); American Conference of Governmental Industrial Hygienists (ACGIH) (1999); Deutsche Forschungsgemeinschaft (1999); United Nations Environment Programme (1999); Occupational Safety and Health Administration (OSHA) (1999)

[b] TWA, time-weighted average; STEL, short-term exposure limit; 2 (Germany), Substances that are considered to be carcinogenic for man because sufficient data from long-term animal studies or limited evidence from animal studies substantiated by evidence from epidemiological studies indicate that they can make a significant contribution to cancer risk. Limited data from animal studies can be supported by evidence that the substance causes cancer by a mode of action that is relevant to man and by results of *in vitro* tests and short-term animal studies; TRK (Germany), technical exposure limit; A3 (ACGIH), confirmed animal carcinogen with unknown relevance to humans; ca, carcinogen; lfc, lowest feasible concentration; sk, skin notation; susp.h.ca, suspected human carcinogen

[c] These countries follow the recommendations of the ACGIH threshold limit values: Bulgaria, Colombia, Jordan, Republic of Korea, New Zealand, Singapore and Viet Nam.

2. Studies of Cancer in Humans

2.1 Case reports

One case report pertaining to *ortho*-toluidine has been published since the previous review (IARC, 1982). Conso and Pontal (1982) reported three cases of bladder cancer occurring among 50 workers employed in a factory where *para*-toluenediamine (IARC, 1987b) was synthesized from *ortho*-toluidine and *ortho*-aminoazotoluene (see IARC, 1987c). [The Working Group noted that although a formal estimate of expected numbers of bladder cancer cases was not provided, three bladder cancers among 50 persons is likely to represent a substantial excess. The relationship of these bladder cancers to *ortho*-toluidine exposure cannot be determined because of co-exposure to *ortho*-aminoazotoluene, which has been classified as possibly carcinogenic to humans (IARC, 1975, 1987c).]

2.2 Cohort studies (see Table 3)

Among the epidemiological studies of cancer in humans identified in the previous IARC review (1982), only the study by Rubino *et al*. (1982) provides adequate information on the study population and methods. In that study, 906 men first employed in a dyestuff factory in northern Italy between 1922 and 1970 were followed from 1946 to 1976. Mortality was compared with that of the Italian male population as a whole. Follow-up was 95.8% complete and, among those traced (868), 260 (30%) had died. Thirty-six deaths from urinary bladder cancer were observed, with 1.23 expected. Thirty-one of these deaths occurred among 610 men with exposure to benzidine, 1-naphthylamine, 2-naphthylamine or several of these, but five (with 0.08 expected) occurred among 53 men engaged solely in the manufacture of fuchsin (magenta; see IARC, 1993) and safranine T, involving exposure either to a combination of toluene, *ortho*-nitrotoluene, *ortho*-toluidine and 4,4′-methylene bis(2-methylaniline) (three deaths from bladder cancer) or to a combination of *ortho*-toluidine, 4,4′-methylene bis(2-methylaniline), *ortho*-nitrotoluene, 2,5-diaminotoluene, *ortho*-aminoazotoluene, aniline, fuchsin and safranine T (two deaths from bladder cancer). The standardized mortality ratio (SMR) for bladder cancer in this group was 62.5 [95% confidence interval (CI), 20.3–145.6]. No quantitative exposure measurements or data on smoking were available. [The Working Group noted that the excess of bladder cancer could not be attributed with certainty specifically to exposure to *ortho*-toluidine or to any one of the other compounds involved.]

Three pertinent epidemiological studies have been published since the last IARC review. Ott and Langner (1983) studied the mortality of 342 employees assigned to three aromatic amine-based dye production areas from 1914 to 1958 in the United States. One of these areas, the bromoindigo and thioindigo production area, involved potential exposure to *ortho*-toluidine, 4-chloro-*ortho*-toluidine and 4-chloroacetyl-*ortho*-toluidine.

Table 3. Summary of cohort studies of workers exposed to *ortho*-toluidine

Reference	Rubino *et al.* (1982)	Ott & Langner (1983)	Stasik (1988)	Ward *et al.* (1991); Prince *et al.* (2000)	Sorahan *et al.* (2000)
Country	Italy	USA	Germany	USA	UK
Industry	Dyestuff production	Dye production	4-Chloro-*ortho*-toluidine production and processing	Rubber chemicals	Rubber chemicals
Size of the total study	906 men	275 men	116 men	1749 (1643 men)	2160 men
Cohort definition	First employed 1922–70	Employed any time during 1940–58	Employed before 1970	Employed any time during 1946–88	Employed six months between 1955–84
Period of follow-up	1946–76	1940–75	< 1967–86	1973–88	1955–96 (mortality) 1971–92 (incidence)
Deaths					
All causes					
N	260	98	19	[190]	1131
SMR (95% CI)	1.5 [1.4–1.7]	1.0 [0.8–1.2]	1.12 (0.68–1.7)	[0.9] [0.8–1.0]	1.01 (0.96–1.1)
All cancers					
N	96	23	5	[49]	305
SMR (95% CI)	2.6 $p < 0.001$	1.3 [0.8–2.0]	1.4 (0.5–3.4)	[1.0] [0.7–1.3]	1.02 (0.91–1.1)
Bladder cancer mortality					
N	36	0		[2]	
SMR (95% CI)	29.3 $p < 0.001$	1.2 expected		[2.1] [0.3–7.6]	17 SMR, 1.4 (0.8–2.2)
Bladder cancer incidence					
N			8	13	19
SIR (95% CI)			72.7 (31.4–143.3)	3.6 [1.92–6.2]	SIR, 1.1 (0.6–1.7)

Table 3 (contd)

Reference	Rubino *et al.* (1982)	Ott & Langner (1983)	Stasik (1988)	Ward *et al.* (1991); Prince *et al.* (2000)				Sorahan *et al.* (2000)
Subgroup exposed to *ortho*-toluidine								
				Duration of exposure (y)				
Size	53	117	Same as full cohort	Total	< 5	5–9.99	≥ 10	53
				708	584	51	73	
Bladder cancer mortality								
N	5	0						3
SMR (95% CI)	62.5 [20.3–145.6]							SMR, [15.8] [3.3–46.4]
								RR (internal analysis)
								1–4 years: *n* = 2;
								6.7 (1.6–28.4)
Bladder cancer incidence								≥ 5 years; *n* = 1;
N				7	0	1	6	7.6 (1.0–56.9)
SIR (95% CI)				6.5 [2.6–13.3]	–	8.8 [0.2–49.0]	27.2 [10.0–59.3]	[SIR 7.0; 95% CI, 1.4–20.4]
Co-exposures[a]	4,4′-Methylene bis (2-methylaniline) (2B) Magenta (2B) Safranine T *ortho*-Nitrotoluene (3) 2,5-Diaminotoluene (3) Aniline (3) *ortho*-Amino-azotoluene (2B)	Multiple exposures including 4-chloro-*ortho*-toluidine and 4-chloroacetyl-*ortho*-toluidine	*N*-Acetyl-*ortho*-toluidine 6-Chloro-*ortho*-toluidine 4-Chloro-*ortho*-toluidine (2A)	Aniline (3) Hydroquinone (3) Toluene (3) Carbon disulfide Sulfur Benzothiazole 4-Aminobiphenyl (contaminant) (1) 2-Mercaptobenzothiazole (Ward *et al.*, 1996) (Proprietary chemical)				Aniline (3) 2-Mercaptobenzothiazole Phenyl-β-naphthylamine (3)

[a] Previous IARC overall evaluations of carcinogenicity are given in parentheses.
N, number; SMR, standardized mortality ratio; SIR, standardized incidence ratio

ortho-Toluidine concentrations measured in breathing zone and area samples were consistently below 0.5 ppm [2.19 mg/m^3] and urinary levels measured in 1948 ranged from undetected (< 0.3 mg/L) to 1.7 mg/L in workers engaged in the production of thioindigo. In order to be included in the study, employees had to be currently working as of 1 January 1940 or hired after that date. Mortality was followed from 1 January 1940 through 31 December 1975; US white male referent rates were used to calculate expected number of deaths. Individuals not known to be deceased based on company records and social security follow-up were assumed to be alive. Mortality was analysed separately for 275 individuals not exposed to arsenicals, vinyl chloride or asbestos. Among 98 deaths identified, 94 death certificates (95.9%) were obtained. No deaths due to bladder cancer were observed, with 1.2 deaths expected from malignant neoplasms of the urinary organs. There were 23 deaths due to malignant neoplasms (17.5 expected; SMR, 1.3; [95% CI, 0.8–2.0]), 10 of which were coded to digestive organs (5.7 expected; SMR, 1.8; [95% CI, 0.8–3.2]). The expected number of bladder cancers in the subcohort of 117 workers exposed to *ortho*-toluidine in the production of bromoindigo and thioindigo was not provided. There were no data on the smoking habits of the cohort. [The Working Group noted that the conclusions of this study were limited by the small size of the population exposed to *ortho*-toluidine.]

In a historical mortality study of 335 male employees involved in the production and processing of 4-chloro-*ortho*-toluidine between 1929 and 1982 in Essen, Germany, no deaths from bladder cancer were identified. Four monocyclic amines had been used at the plant: *N*-acetyl-*ortho*-toluidine, 6-chloro-*ortho*-toluidine, *ortho*-toluidine and 4-chloro-*ortho*-toluidine; exposure to 4-chloro-*ortho*-toluidine was reported to be predominant. Quantitative exposure data were not available. Urothelial carcinomas were noted in eight of the employees, between 1967 and 1985, two of whom had died as of December 1986. All eight had been employed in the 4-chloro-*ortho*-toluidine production plant before improvements in industrial hygiene were made in 1970. As a result of this discovery, an incidence study was initiated and the vital status ascertainment for a subcohort of 116 subjects who had been employed in the 4-chloro-*ortho*-toluidine production plant before 1970 was extended through 1986. The expected number of incident bladder cancers in the cohort of 116 men, calculated based on the 1983 rates from the Saarland Cancer Registry, was 0.11. The standardized incidence ratio (SIR) based on eight observed cases was 72.7 (95% CI, 31.4–143) (Stasik, 1988). [The Working Group noted that the definition of the subcohort was made *a posteriori*, but this was justified by the comment that improvements in industrial hygiene were introduced in 1970. It was also unclear in what year the follow-up started. The excess of bladder cancer could not be attributed with certainty specifically to *ortho*-toluidine or to any one of the other compounds present.]

A bladder cancer incidence study was conducted among workers exposed to *ortho*-toluidine at a plant manufacturing rubber chemicals in New York State, United States. The study was initiated at the request of the union representing workers at the plant, who had noted a number of bladder cancers among workers in a department where an

antioxidant and an accelerator were produced. Among the major reactants used in these processes were two primary aromatic amines, *ortho*-toluidine and aniline; other reactants and intermediates included 2-mercaptobenzothiazole (Ward *et al.*, 1996), hydroquinone, toluene, carbon disulfide, sulfur, benzothiazole and a proprietary chemical which was introduced into the process in 1970 (Ward *et al.*, 1991). 4-Aminobiphenyl was identified as a potential low-level contaminant (< 1 ppm) [4.38 mg/m^3] in some bulk samples of process chemicals in 1990 (Ward & Dankovic, 1991). A study cohort was identified from personnel records of the chemical plant, which opened in 1946 for production of poly(vinyl chloride). The antioxidant was produced in a separate building that opened in 1957 and in 1970 an expansion of this building was completed and production of a new accelerator was begun. There were 1749 (1643 male) individuals employed in the plant between 1946 and 1988, 708 of whom had been assigned to the department where the accelerator and antioxidant were produced (and were considered to be definitely exposed to *ortho*-toluidine and aniline); 288 had been assigned to departments such as maintenance in which possible exposure was considered to have occurred, and 753 of whom had never worked in either definitely or possibly exposed jobs. Within the exposed department, it was not possible to separate individuals with exposure to *ortho*-toluidine from individuals with exposure to other chemicals. Vital status was identified from company records and records of the United States social security administration. Bladder cancer cases were identified from company and union records and confirmed through medical records, or through matching with the cancer registry in the state where the plant was located. Cancer incidence rates were compared with bladder cancer incidence rates in the same state. Person–years at risk began on 1 January 1973 (the first year in which matching with the registry through social security number was possible) or on the date of starting employment, whichever occurred later. Follow-up was from 1973 to 1988. Race was recorded in the personnel records for only 670 subjects, among whom 91% were white. There were 13 cases of bladder cancer in 1973–88 in the cohort overall (SIR, 3.6; [95% CI, 1.9–6.2]), seven of which occurred in the definitely exposed group (SIR, 6.5; [95% CI, 2.6–13.3]) and four in the possibly exposed group (SIR, 3.7; [95% CI, 1.0–9.4]). The SIR was not elevated among workers in the probably unexposed group (two observed; SIR, 1.4; [95% CI, 0.17–5.0]). Six of the seven bladder cancer cases occurred among workers employed in the exposed department for over 10 years; the SIR for this group was 27.2 [95% CI, 10.0–59.2]. Data on smoking were available for 143 members of the cohort and showed that cohort members were slightly more likely to be current or former smokers than the general United States population. It was estimated, using a method of indirect adjustment (Axelson & Steenland, 1988), that differences in smoking habits between the cohort and the United States general population would account for an SIR of 1.05 for bladder cancer. A subsequent mortality analysis of the same cohort followed through 1994 (Prince *et al.*, 2000) showed no elevation in all-cause, all-cancer or bladder cancer mortality in the total cohort (Table 3).

The carcinogenicity of some of the reactants, intermediates and end-products present in the previous cohort has been evaluated by IARC. Aniline is not known to induce bladder cancer in humans or animals (IARC, 1987d).

4-Aminobiphenyl, which was present as a potential contaminant, is classified by IARC in Group 1 and is known to be a highly potent human bladder carcinogen (IARC, 1972, 1987e). Potential for 4-aminobiphenyl contamination arose from use of diphenylamine as a reactant intermittently from 1972 to 1985 (Ward *et al.*, 1994) (commercial diphenylamine was contaminated with low levels of 4-aminobiphenyl in the 1970s and earlier (Safe *et al*., 1977)) and from its possible formation in one of the reactions in the antioxidant process. 2-Mercaptobenzothiazole has not been reviewed by IARC, but has shown some evidence of carcinogenicity in rats and equivocal evidence of carcinogenicity in mice (National Toxicology Program, 1988).

An exposure assessment study was conducted at the plant in February–March 1990 (Ward *et al*., 1996). This study demonstrated substantially higher urinary concentrations and levels of haemoglobin adducts of aniline and *ortho*-toluidine among exposed workers compared with in-plant controls. Levels of 4-aminobiphenyl adducts were much lower than those of *ortho*-toluidine and aniline and did not differ between the exposed and unexposed groups (Table 4). Haemoglobin adducts reflect only recent exposures (Hemminki, 1992), and it is therefore possible that higher levels of 4-aminobiphenyl contamination existed in the past. [The Working Group noted that, while *ortho*-toluidine was a plausible cause of the bladder cancer excess, the contribution of other chemicals cannot be ruled out. The presence of a proprietary chemical was noted, for which the Working Group had no data on carcinogenicity.]

Sorahan *et al*. (2000) updated a study of workers exposed to several aromatic amines in a factory manufacturing chemicals for the rubber industry in the United Kingdom (Sorahan & Pope, 1993). All subjects had at least six months' employment in the factory and some employment in the period 1955–84. Mortality was examined for the period 1955–96 and cancer incidence was examined for the period 1971–92. The updated study included 2160 male production workers, 605 of whom had been exposed to aniline, 2-mercaptobenzothiazole, phenyl-β-naphthylamine or *ortho*-toluidine. There were nine bladder cancer deaths observed and 3.25 expected among the 605 individuals (SMR, 2.8; 95% CI, 1.3–5.3). A total of 30 incident bladder cancers were identified, of which nine occurred among [357] individuals who had been exposed to mercaptobenzothiazole [SIR, 2.6; 95% CI, 1.2–4.9], five occurred among 94 individuals who had been exposed to phenyl-β-naphthylamine [SIR, 3.8; 95% CI, 1.2–8.8], seven occurred among individuals exposed to aniline [SIR, 1.5; 95% CI, 0.6–3.1] and three occurred among 53 individuals who had been exposed to *ortho*-toluidine [SIR, 7.0; 95% CI, 1.4–20.4]. All of the bladder cancer cases among workers exposed to *ortho*-toluidine had also had exposure to one or more of the other chemicals. Poisson regression analyses revealed no association between estimated duration of mercaptobenzothiazole exposure and risk of bladder cancer, some evidence for an exposure–response relationship with duration of exposure to *ortho*-toluidine (RR internal analysis: 1–4 years, n = 2, RR, 6.7; 95% CI,

Table 4. Air and urine levels and haemoglobin adduct levels measured in 1990 among chemical workers employed at a plant where excess bladder cancer incidence was observed

Exposure matrix	Exposure group	No.	Mean of total group (SD)	*p* value
Aniline levels in air (μg/m^3)	Exposed	28	187 (181)	
ortho-Toluidine levels in air (μg/m^3)	Exposed	28	412 (366)	
Aniline levels in post-shift urine (μg/L)	Unexposed Exposed	25 42	3.9 (2.8) 29.8 (25.7)	0.0001
ortho-Toluidine in post-shift urine (μg/L)	Unexposed Exposed	25 42	2.8 (1.4) 98.7 (119.4)	0.0001
Aniline adducts (pg/g Hb)	Unexposed Exposed	27 46	3163 (1302) 17 441 (8867)	0.0001
ortho-Toluidine adducts (pg/g Hb)	Unexposed Exposed	27 46	3515 (6036) 40 830 (32 518)	0.0001
4-Aminobiphenyl adducts (pg/g Hb)	Unexposed Exposed	27 42	74.5 (63.8) 81.7 (106.1)	0.48

From Ward *et al.* (1996)
Hb: haemoglobin

1.6–28.4; ≥ 5 years, *n* = 1, RR, 7.6; 95% CI, 1.0–56.9), and the strongest evidence for an exposure–response relationship with duration of exposure to phenyl-β-naphthyl-amine (RR internal analysis: 1–4 years, *n* = 1, RR, 1.3; 95% CI, 0.17–9.3; ≥ 5 years, *n* = 4, RR,7.5; 95% CI, 2.6–21.5).

[The Working Group made two observations relevant to the interpretation of all five cohort studies. Individual smoking habits had not been taken into account in the analysis of most of the reported studies; however, the excesses of bladder cancer reported in the four positive studies were much too large to have been due to smoking alone.

Negative results on mortality from bladder cancer might be caused by limited power due to high survival from this disease. Therefore, differences between results of analyses based on mortality and morbidity data might reflect the lower sensitivity of the former.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 25 male and 25 female Swiss CD-1 mice, four to six weeks of age, were treated with *ortho*-toluidine hydrochloride (purity, 97–99%) in the diet at dose levels of 16 000 or 32 000 mg/kg diet (ppm) for three months and then at levels of 8000 or 16 000 ppm for a further 15 months. Animals were kept without treatment for an additional three months and then killed. The doses were chosen on the basis of preliminary tests, the higher being the maximum tolerated dose. A simultaneous control group of 25 untreated mice of each sex was used, plus additional controls used for the other compounds tested in the study, and tumour incidences of matched and pooled controls were compared statistically (both separately and together) with those of treated groups. Animals that died during the first six months of the study were discarded without necropsy. In male mice, the incidence of vascular tumours (haemangiomas and haemangiosarcomas combined, observed in abdominal viscera) was 0/14, 5/99, 5/14 ($p < 0.025$, Fisher's exact test) and 9/11 ($p < 0.025$, Fisher's exact test) in concurrent controls, pooled controls, low-dose and high-dose groups, respectively. In female mice, the incidence of vascular tumours (haemangiomas and haemangiosarcomas combined, observed in abdominal viscera) was 0/15, 9/102, 5/18 ($p < 0.05$, Fisher's exact test) and 9/21 ($p < 0.025$, Fisher's exact test) in concurrent controls, pooled controls, low-dose and high-dose groups, respectively [the separate incidences for haemangiomas and haemangiosarcomas were not reported] (Weisburger *et al*., 1978).

Groups of 50 male and 50 female B6C3F_1 mice, six weeks of age, were administered *ortho*-toluidine hydrochloride (purity, > 99%) in the diet at 1000 or 3000 ppm for 102–103 weeks. Concurrent control groups consisted of 20 male and 20 female untreated mice. Mean body weights of both treated males and females were lower than those of the corresponding controls. Mortality was not significantly related to treatment in either sex. In male mice, the incidence of haemangiomas or haemangiosarcomas (combined, all sites, mainly observed in the abdominal viscera) was increased: 1/19, 2/50 and 12/50 ($p < 0.002$, Cochran–Armitage trend test) in control, low-dose and high-dose groups, respectively. In female mice, the incidence of hepatocellular adenomas or carcinomas (combined) was increased: 0/20, 4/49 and 13/50 ($p < 0.007$, Fisher's exact test; $p = 0.001$ trend test) in control, low-dose and high-dose groups, respectively (National Cancer Institute, 1979).

3.1.2 *Rat*

Groups of 25 male Sprague-Dawley CD rats, four to six weeks of age, were treated with *ortho*-toluidine hydrochloride (purity, 97–99%) in the diet at dose levels of 8000 or 16 000 ppm for three months and then at levels of 4000 or 8000 ppm for a further

15 months. Animals were kept without treatment for an additional six months and then killed. The doses were chosen on the basis of preliminary tests, the higher being the maximum tolerated dose. A concurrent control group of 25 untreated male rats was used, plus additional controls used for the other compounds tested in the study, and tumour incidences of matched and pooled controls were compared with those of treated groups. Animals that died during the first six months of the study were discarded without necropsy. The incidence of subcutaneous fibromas and fibrosarcomas (combined) was 0/16, 18/111, 18/23 ($p < 0.025$, Fisher's exact test) and 21/24 ($p < 0.025$, Fisher's exact test, compared with all controls) in simultaneous controls, pooled controls, low-dose and high-dose groups, respectively. A non-statistically significant increase in the incidence of transitional-cell carcinomas of the urinary bladder was also observed: 0/16, 5/111, 3/23 and 4/24 in simultaneous controls, pooled controls, low-dose and high-dose groups, respectively (Weisburger *et al*., 1978).

Groups of 30 male Fischer 344 rats, eight weeks of age, were treated with *ortho*-toluidine hydrochloride [purity not specified, recrystallized product] in the diet at a concentration of 4000 ppm (0.028 mol/kg of diet) for 72 weeks. A control group of 30 untreated male rats was used. The experiment was terminated at 93 weeks. Mean body weights were lower in the treated than in the control group. The incidence of fibromas of the skin was 1/27 and 25/30 [$p < 0.001$, Fisher's exact test]; that of fibromas of the spleen was 0/27 and 10/30 [$p < 0.001$, Fisher's exact test]; that of mammary fibroadenomas was 0/27 and 11/30 [$p < 0.001$, Fisher's exact test]; and that of peritoneal sarcomas was 0/27 and 9/30 [$p < 0.01$, Fisher's exact test] in control and treated groups, respectively (Hecht *et al*., 1982).

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were administered o*rtho*-toluidine hydrochloride (purity, > 99%) in the diet at concentrations of 3000 or 6000 ppm for 101–104 weeks. Matched control groups consisted of 20 male and 20 female untreated rats. Mean body weights of treated male and female rats were lower than those of the corresponding controls. Mortality was significantly affected by treatment of male and female rats ($p < 0.001$, Tarone test for positive trend). In males, the incidence of sarcomas, fibrosarcomas, angiosarcomas or osteosarcomas (combined) of multiple organs (mainly subcutis and spleen or bone) was 0/20, 15/50 ($p = 0.003$, Fisher's exact test) and 37/49 ($p < 0.001$, Fisher's exact test); that of subcutaneous integumentary fibromas was 0/20, 28/50 ($p < 0.001$, Fisher's exact test) and 27/49 ($p < 0.001$, Fisher's exact test); and that of mesotheliomas of multiple organs or tunica vaginalis was 0/20, 17/50 ($p < 0.001$, Fisher's exact test) and 9/49 ($p = 0.036$, Fisher's exact test) in control, low- and high-dose groups, respectively. In females, the incidence of transitional cell carcinomas of the urinary bladder was 0/20, 9/45 ($p = 0.028$, Fisher's exact test) and 22/47 ($p < 0.001$, Fisher's exact test); that of sarcomas, fibrosarcomas, osteosarcomas or angiosarcomas (combined) of multiple organs (mainly subcutis and spleen or bone) was 0/20, 3/50 and 21/49 ($p = 0.001$, Fisher's exact test); and that of mammary adenomas and fibroadenomas (combined) was 7/20, 20/50 and 35/49

($p = 0.006$) in control, low- and high-dose groups, respectively (National Cancer Institute, 1979).

Male Fischer 344/N rats, 45 days of age, were administered *ortho*-toluidine hydrochloride at a concentration of 5000 ppm in the diet for 13 weeks and then kept for observation for a further 13 weeks, when animals were killed. Mesotheliomas in the epididymis were observed in 2/20 male rats exposed to *ortho*-toluidine hydrocloride. No mesotheliomas were seen in concurrent controls (0/10) (National Toxicology Program, 1996).

3.2 Subcutaneous injection

Hamster: Groups of 15 male and 15 female Syrian golden hamsters, eight weeks of age, were given subcutaneous injections of 1.9 mmol/kg bw (2 mg/kg bw) *ortho*-toluidine (free base) [purity not specified, recrystallized product] in peanut oil once per week for 52 weeks. Control groups of 15 male and 15 female hamsters were given 52 subcutaneous injections of peanut oil vehicle. The experiment was terminated at 87 weeks. Mean body weights in the treated groups were similar to those of the control groups. Mean survival times were shorter in the treated groups, being 61.3 and 57.8 weeks in male and female treated hamsters, respectively, compared with 75.5 and 68.7 weeks in male and female controls, respectively. The incidence of tumours in the treated groups was not significantly different from that in the control groups [details on the incidence of specific tumours not reported] (Hecht *et al.*, 1983). [The Working Group noted the small number of animals, low dose and short duration of treatment.]

3.3 Carcinogenicity of metabolites

Rat: Groups of 30 male Fischer 344 rats, eight weeks of age, were treated with *ortho*-nitrosotoluene [purity not specified, recrystallized product] in the diet at a concentration of 3380 ppm (0.028 mol/kg of diet) for 72 weeks. A control group of 30 untreated male rats was used. The experiment was terminated at 93 weeks. Mean body weights were lower in the treated than in the control group. The incidence of fibromas of the skin was 1/27 and 19/29 [$p < 0.001$, Fisher's exact test]; that of fibromas of the spleen was 0/27 and 14/29 [$p < 0.001$, Fisher's exact test]; that of hepatocellular carcinomas was 0/27 and 18/29 [$p < 0.001$, Fisher's exact test]; and that of urinary bladder tumours was 0/27 and 15/29 [$p < 0.01$, Fisher's exact test] in control and treated groups, respectively (Hecht *et al.*, 1982).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

ortho-Toluidine is absorbed via the respiratory tract and skin. It can be inhaled as dust, fumes or vapour (ILO, 1971). Ward *et al*. (1996), while not able to determine the relative contributions of airborne versus dermal exposure, found strong evidence that *ortho*-toluidine was absorbed in an exposed group of workers at a chemical manufacturing facility (see Section 2.2). While *ortho*-toluidine is a component of cigarette smoke (IARC, 1986), El-Bayoumy *et al*. (1986) found that the levels in urine of smokers and nonsmokers were not significantly different (see Section 1.4.3(*e*)). The authors suggested that nitrobenzene, nitrotoluenes and dietary factors may be major contributors to *ortho*-toluidine levels in human urine, which might in part explain the marked variability (~ 100-fold) in *ortho*-toluidine–haemoglobin adducts reported in unexposed groups between nonsmokers and smokers in three studies (Stillwell *et al*., 1987; Bryant *et al*., 1988; Ward *et al*., 1996). In two of these studies (Stillwell *et al*., 1987; Bryant *et al.*, 1988), smoking significantly increased the level of *ortho*-toluidine–haemoglobin adducts, whereas, in the study by Ward *et al*. (1996), adduct levels were similar in smokers (40 494 pg/g Hb) and nonsmokers (41 028 pg/g Hb) in the exposed group, as well as in the unexposed group in smokers (3510 pg/g Hb) and nonsmokers (3518 pg/g Hb). They concluded that the possibility of some exposure to *ortho*-toluidine in the 'unexposed' workers could not be ruled out.

4.1.2 *Experimental systems*

The tissue distribution of radioactivity in male Fischer 344 rats 48 h after subcutaneous injection of 50 or 400 mg/kg bw *ortho*-[*methyl*-^{14}C]toluidine was mainly in the liver, kidney, lung, spleen, colon and bladder. The major excretory route was via the urine, with ~ 84% of the 400-mg/kg bw dose being eliminated via this pathway within the first 48 h. Approximately 1% of the 400-mg/kg bw dose was eliminated via the lungs and ~ 3% in the faeces (Son *et al*., 1980). Following oral administration of a single 50-mg/kg bw dose of *ortho*-[*methyl*-^{14}C]toluidine to male Sprague-Dawley rats, > 92% of the administered radiolabel was eliminated in the urine within 72 h. The amount of unchanged compound appearing in the urine within 24 h varied according to the isomer of toluidine administered at 500 mg/kg bw: 21% for *ortho*-toluidine, 2.5% for *meta*-toluidine and 2.5% for *para*-toluidine. Such differences may help to explain why only the *ortho*-isomer causes tumours in the urinary bladder of rats (Cheever *et al.*, 1980). However, in contrast to this finding, after subcutaneous doses of 400 mg/kg bw (Son *et al.*, 1980) or 0.82 mmol/kg [88 mg/kg bw] (Kulkarni *et al.*, 1983), Fischer 344 rats

excreted only approximately 5% of the dose in the urine as *ortho*-toluidine over 48 and 6 h, respectively.

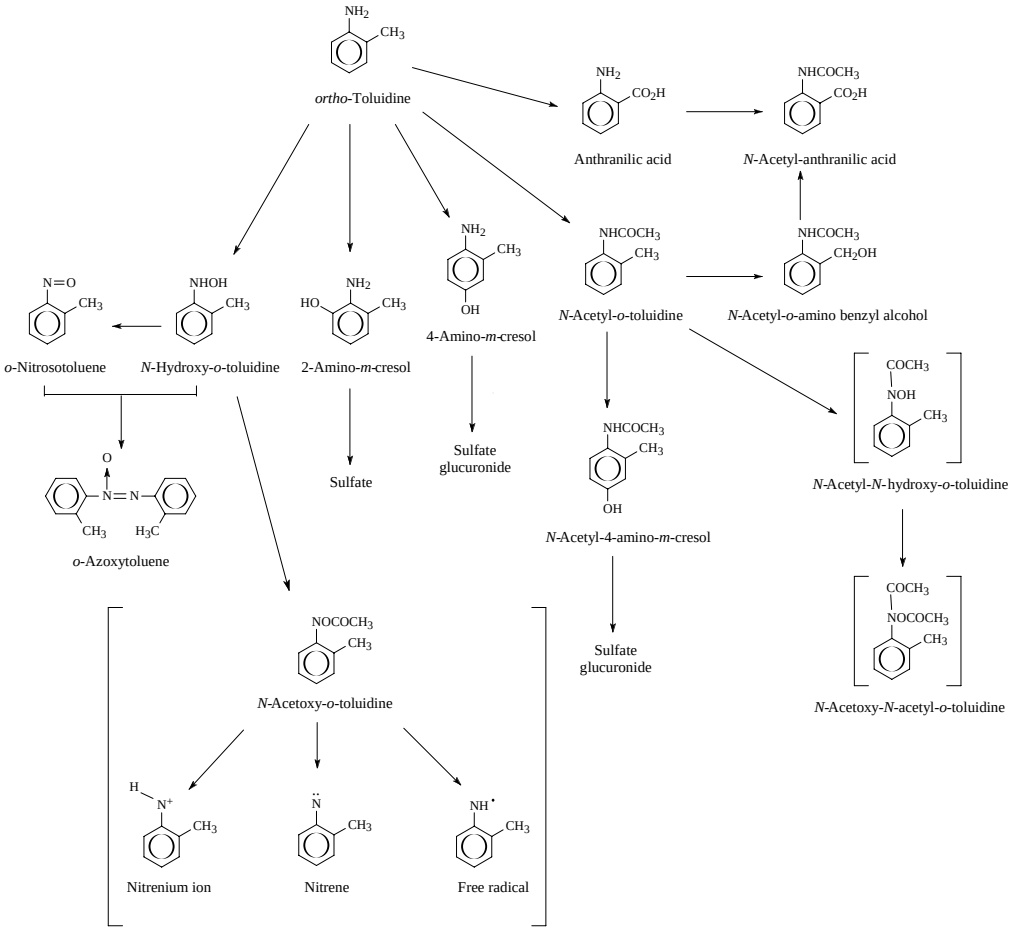
The metabolism of *ortho*-toluidine in rats proceeds primarily through ring hydroxylation with subsequent conjugation (Cheever *et al*., 1980; Son *et al*., 1980). *N*-Acetylation of *ortho*-toluidine is also a major metabolic pathway in male Fischer 344 rats. The major ether-extractable urinary metabolites of a 400-mg/kg dose of *ortho*-toluidine are azoxytoluene, *ortho*-nitrosotoluene, *N*-acetyl-*ortho*-toluidine, *N*-acetyl-*ortho*-aminobenzyl alcohol, *N*-acetyl-4-amino-*meta*-cresol, 4-amino-*meta*-cresol, anthranilic acid and *N*-acetylanthranilic acid (Son *et al*., 1980). *N*-Hydroxy-*ortho*-toluidine is also a urinary metabolite of male Fischer 344 rats (Kulkarni *et al*., 1983). After a 400-mg/kg bw dose of *ortho*-[*methyl*-^{14}C]toluidine administered subcutaneously to male Fischer 344 rats, 51% of urinary metabolites were conjugates, with sulfate conjugates of 4-amino-*meta*-cresol (27.8% of the dose), *N*-acetyl-4-amino-*meta*-cresol (8.5% dose) and 2-amino-*meta*-cresol (2.1%), dominating over glucuronides of 4-amino-*meta*-cresol (2.6%), *N*-acetyl-4-amino-*meta*-cresol (2.8%) and *N*-acetyl-*ortho*-aminobenzyl alcohol by a ratio of 6:1. A double acid conjugate of 4-amino-*meta*-cresol was also identified. *ortho*-Toluidine can be oxidized to yield *N*-hydroxy-*ortho*-toluidine and *ortho*-nitrosotoluene (Son *et al*., 1980) (see Figure 1).

On the basis of data from the *Salmonella*/mammalian microsomal mutagenicity assay, Gupta *et al*. (1987) proposed that *N*-hydroxy-*ortho*-toluidine undergoes further metabolic activation via an acetylation reaction catalysed by *N*-acetyltransferase to form *N*-acetoxy-*ortho*-toluidine. The latter metabolite could undergo non-enzymatic breakdown to yield a highly reactive nitrenium ion, nitrene or free radical that can covalently bind to tissue macromolecules (Figure 1). The same authors also postulated that *N*-acetyl-*ortho*-toluidine can be metabolized to produce *N*-acetyl-*N*-hydroxy-*ortho*-toluidine, which could undergo further acetylation to form *N*-acetoxy-*N*-acetyl-*ortho*-toluidine. However, there are no in-vivo data to substantiate the existence of *N*-acetyl-*N*-hydroxy-*ortho*-toluidine or *N*-acetoxy-*N*-acetyl-*ortho*-toluidine in rats fed *ortho*-toluidine; the suggestion that they are formed is based on results with other aromatic amines and amides (Thorgeirsson *et al*., 1983; Gupta *et al*., 1987). The secondary metabolite *ortho*-azoxytoluene is postulated to be formed by an interaction between *ortho*-nitrosotoluene and *N*-hydroxy-*ortho*-toluidine (Son *et al*., 1980). Another putative secondary metabolite, *ortho*-azotoluene, is proposed to be formed through a reaction of *ortho*-toluidine with *ortho*-nitrosotoluene, but no data on its in-vivo formation exist (Gupta *et al*., 1987).

Cheever *et al*. (1980) also reported finding 4-amino-3-methylphenol in the urine of rats fed *ortho*-toluidine. Leslie *et al*. (1988) have shown that administration of *ortho*-toluidine to male Sprague-Dawley rats induces various metabolic activities associated with the cytochrome P450 system.

In male Crl:CD rats, after a single oral dose of 500 mg/kg bw *ortho*-toluidine, binding to hepatic DNA and RNA appeared to be maximal 24–48 h after dosing (Brock *et al*., 1990). The highest levels of binding were found in the blood, spleen, kidney and liver.

Figure 1. Metabolic disposition of *ortho*-toluidine in rats



Adapted from Cheever *et al.* (1980); Son *et al.* (1980); Kulkarni *et al.* (1983) and Gupta *et al.* (1987)
Brackets indicate postulated proximate or reactive metabolites of *ortho*-toluidine, based on data from the metabolism of other aromatic amines.

ortho-Toluidine forms haemoglobin adducts in female Wistar rats and female B6C3F$_1$ mice after administration of a single oral dose of 0.47–0.6 mmol/kg bw [50.4–64.3 mg/kg bw] (Birner & Neumann, 1988). In male Sprague-Dawley rats given intraperitoneal doses of [^{14}C]*ortho*-toluidine ranging from 10 to 100 mg/kg bw, peak albumin binding occurred after 4 h at 50 mg/kg bw and peak haemoglobin binding at 24 h following a dose of 100 mg/kg bw (DeBord *et al.*, 1992). A linear dose–response relationship was seen for [^{14}C]*ortho*-toluidine binding to haemoglobin. The biological half-lives of [^{14}C]*ortho*-toluidine bound to albumin and haemoglobin were calculated to be 2.6 and 12.3 days, respectively. The route of [^{14}C]*ortho*-toluidine administration

significantly affected binding to haemoglobin, with rats injected intraperitoneally having approximately twofold higher [^{14}C]*ortho*-toluidine–haemoglobin levels than animals treated orally.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

The following intraperitoneal LD_{50} values have been reported for *ortho*-toluidine hydrochloride: 179 mg/kg bw in male mice, 113 mg/kg bw in female mice, 164 mg/kg bw in male rats and 246 mg/kg bw in female rats (Weisburger *et al*., 1978). Administration of 225 mg/kg bw *ortho*-toluidine per day by gavage (estimated oral LD_{50}, 900 mg/kg bw) to male Fischer 344 rats for either five, 10 or 20 days led to splenic congestion, increased haematopoiesis, haemosiderosis and bone marrow hyperplasia (after 10 days) consistent with enhanced erythrocytic destruction. Histopathological examination of the livers did not reveal any overt hepatotoxic effect (Short *et al*., 1983). In both male and female Fischer 344 rats, 3000 and 6000 ppm (mg/kg) *ortho*-toluidine hydrochloride given in the diet over 104 weeks induced proliferative mesenchymal lesions in the spleen, including capsular and parenchymal fibrosis (Goodman *et al*., 1984).

ortho-Toluidine given to male Wistar rats by intraperitoneal injection (75 mg/kg bw for three consecutive days) increased the microsomal arylhydrocarbon hydroxylase activity, presumably reflecting CYP1A isoenzymes, in the liver, kidney and lung. The activity of NADPH-cytochrome c reductase and the content of cytochrome b_5 were enhanced only in the liver. No significant effect was observed on epoxide hydrolase or glutathione *S*-transferase activity using benzo[*a*]pyrene 4,5-oxide and 1-chloro-2,4-dinitrobenzene, respectively, as substrates (Gnojkowski *et al*., 1984).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

[The Working Group noted that many aromatic amines induce methaemoglobinaemia (Watanabe *et al*., 1976; Coleman & Coleman, 1996). The effect of methaemoglobinaemia on fetal development has not been well studied, but may be associated with suboptimal fetal outcome (Fan & Steinberg, 1996; Kilpatrick & Laros, 1999).]

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Tables 5 and 6 for references)

The genetic toxicology of *ortho*-toluidine was extensively studied in two international collaborative trials for evaluation of short-term tests for carcinogens (de Serres & Ashby, 1981; Ashby *et al*., 1985). A review (Danford, 1991) summarizes the conclusions of these trials. The genetic toxicology of *ortho*-toluidine has also been reviewed more briefly, in the context of carcinogenesis, by Sellers and Markowitz (1992). There are some difficulties in evaluating the significance of much of the available data, since there seems to be substantial variation in results between different laboratories and minor variations in protocols.

By far the majority of the data from bacterial or bacteriophage assay systems show negative or, at most, weakly positive results. *ortho*-Toluidine gave positive results for induction of bacteriophage lambda, but only when tested in the presence of exogenous metabolic activation. However, it failed to induce SOS activity in *Salmonella typhimurium* TA1535/PSK1002. At very high concentrations (20 mg per plate), it was differentially toxic towards *Escherichia coli* strains differing in capacity for recombinational repair, in the absence of S9 mix. However, this result was not reproduced in two further studies carried out in other laboratories, using lower concentrations. *ortho*-Toluidine gave positive results for forward mutation in recombination-deficient strains of *Bacillus subtilis*. A large series of studies have been reported using *S. typhimurium* strains TA100, TA102, TA1535, TA1537, TA1538, TA98 and TA97. Almost all of the results were negative, although there are sporadic reports of positive responses, only in the presence of S9 mix, with strains TA100, TA98, TA1535 and TA1538. Uniformly negative results were found for reverse mutation in *E. coli* strains WP2 or WP2 *uvrA*. Where positive response have been seen in microbial assays, they have generally required variations to the standard test procedures, including the use of the fluctuation protocol, or incorporating the addition of norharman or lithocholic acid. High concentrations of S9 mix, or special types of S9 mix may also be important.

In *Saccharomyces cerevisiae*, treatment with *ortho*-toluidine resulted in differential toxicity between repair-proficient and -deficient strains. However, inconsistent data were seen in all other assays with this species of yeast. Of eight assays for gene conversion carried out in different laboratories, one positive result was reported only when exogenous metabolic activation was present and another only when it was absent. Although *ortho*-toluidine caused a recombinogenic event leading to deletion (in either the presence or absence of exogenous metabolic activation), it failed to cause intrachromosomal recombination in the same yeast strain. It was a mitochondrial ‘petite’ mutagen, but failed to give a positive response for forward mutation in a nuclear gene.

Table 5. Genetic and related effects of *ortho*-toluidine

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Prophage induction, SOS repair, DNA strand breaks or cross-links	NT	+	2500	Thomson (1981)
Prophage induction, SOS repair, DNA strand breaks or cross-links (*Salmonella typhimurium* TA1535/pSK1002)	–	–	1670	Nakamura *et al*. (1987)
Escherichia coli pol A/W3110-P3478, differential toxicity (liquid suspension tests)	–	–	250 µg/plate	Rosenkranz *et al*. (1981)
Escherichia coli rec strains, differential toxicity	+	NT	20 µL/disc	Rosenkranz & Poirier (1979)
Escherichia coli rec strains, differential toxicity	?	–	2500	Green (1981)
Escherichia coli rec strains, differential toxicity	–	–	1000	Tweats (1981)
Bacillus subtilis rec strains, forward mutation	+	+	20 µL/disc	Kada (1981)
Salmonella typhimurium TM677, forward mutation	NT	–	500	Skopek *et al*. (1981)
Salmonella typhimurium TM677, forward mutation	–	–	500	Liber (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10 000 µg/plate	McCann *et al*. (1975)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1000 µg/plate	Simmon (1979)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	1000 µg/plate	Tanaka *et al*. (1980)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	10 µL/plate	Baker & Bonin (1981)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA92, TA98, reverse mutation	–	–	2000 µg/plate	Brooks & Dean (1981)
Salmonella typhimurium TA100, TA98, TA1537, reverse mutation	–	–	5000 µg/plate	MacDonald (1981)
Salmonella typhimurium TA100, TA1537, TA98, reverse mutation	–	–	1000 µg/plate	Nagao & Takahashi (1981)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	10 000 µg/plate	Richold & Jones (1981)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2000 µg/plate	Rowland & Severn (1981)
Salmonella typhimurium TA100, reverse mutation	NT	–	200 µg/plate	Sugimura *et al.* (1982)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, G46, C3076, reverse mutation	–	–	1000 µg/mL agar	Thompson *et al.* (1983)
Salmonella typhimurium TA100, TA98, TA97, TA102, reverse mutation	–	–	10 000 µg/plate	Baker & Bonin (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1000 µg/mL	Falck *et al.* (1985)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	2000 µg/plate	Matsushima *et al.* (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	5000 µg/plate	Rexroat & Probst (1985)
Salmonella typhimurium TA100, reverse mutation	–	+[c]	2000 µg/plate	Zeiger & Haworth (1985)
Salmonella typhimurium BA13 (L-arabinose resistance), forward mutation	NT	+	480 µg/plate	Dorado & Pueyo (1988)
Salmonella typhimurium TA1535, TA1538, reverse mutation	–	–	250 µg/plate	Rosenkranz & Poirier (1979)
Salmonella typhimurium TA1535, TA1537, TA98, reverse mutation	–	–	300 µg/mL	Gatehouse (1981)
Salmonella typhimurium TA1535, reverse mutation	–	–	1000 µg/plate	Zeiger & Haworth (1985)
Salmonella typhimurium TA1538, reverse mutation	–	–	100 µg/plate	Ferreti *et al.* (1977)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA1538, reverse mutation	–	–	100 µg/plate	Garner & Nutman (1977)
Salmonella typhimurium TA1538, reverse mutation	–	+[d]	10 µg/mL	Gatehouse (1981)
Salmonella typhimurium TA98, reverse mutation	NT	–	200 µg/plate	Nagao *et al*. (1977)
Salmonella typhimurium TA98, reverse mutation	NT	–	50 µg/plate	Nagao *et al.* (1978)
Salmonella typhimurium TA98, reverse mutation	NT	+	2.5 µg/plate	Kawalek *et al*. (1983)
Salmonella typhimurium TA98, TA97, reverse mutation	–	–	1000 µg/plate	Zeiger & Haworth (1985)
Escherichia coli WP2 *uvrA*, reverse mutation	–	–	1000	Gatehouse (1981)
Escherichia coli WP2 and WP2 *uvrA*, reverse mutation	–	–	1000	Thompson *et al*. (1983)
Escherichia coli WP2 *uvrA*, reverse mutation	–	–	1000	Falck *et al*. (1985)
Aspergillus nidulans, forward mutation	–	NT	504	Carere *et al*. (1985)
Aspergillus nidulans, genetic crossing-over	–	NT	2520	Carere *et al*. (1985)
Saccharomyces cerevisae, DNA repair-deficient strains, differential toxicity	+	+	300	Sharp & Parry (1981a)
Saccharomyces cerevisiae, gene conversion	–	–	333 µg/plate	Jagannath *et al.* (1981)
Saccharomyces cerevisiae, gene conversion	+	NT	50	Sharp & Parry (1981b)
Saccharomyces cerevisiae, gene conversion	NT	–	2 µL/mL	Zimmermann & Scheel (1981)
Saccharomyces cerevisiae, gene conversion, reverse mutation	–	–	500	Arni (1985)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Saccharomyces cerevisiae, gene conversion	–	–	2000	Brooks *et al.* (1985)
Saccharomyces cerevisiae, gene conversion, forward/reverse mutation	–	–	1000	Inge-Vechtomov *et al.* (1985)
Saccharomyces cerevisiae, gene conversion, reverse mutation	–	–	500	Parry & Eckardt (1985a)
Saccharomyces cerevisiae, deletion assay	+	+	1000	Carls & Schiestl (1994)
Saccharomyces cerevisiae, interchromosomal recombination	–	–	5000	Carls & Schiestl (1994)
Saccharomyces cerevisiae, forward 'petite' mutation	+	NT	2500	Ferguson (1985)
Saccharomyces cerevisiae, strain XV185-14C, reverse mutation	–	–	2222	Mehta & von Borstel (1981)
Saccharomyces cerevisiae, reverse mutation	–	–	21.2	Harrington & Nestmann (1985)
Saccharomyces cerevisiae strain D7-144, gene conversion	–	+[e]	378	Mehta & von Borstel (1985)
Saccharomyces cerevisiae strain XV185-14C & RM52, reverse mutation	+	+[e]	378	Mehta & von Borstel (1985)
Saccharomyces cerevisiae, aneuploidy	+	+	50	Parry & Sharp (1981)
Saccharomyces cerevisiae, aneuploidy	+	+	NR	Parry & Eckardt (1985b)
Saccharomyces cerevisiae, aneuploidy	–	NT	1.5 µL/mL	Zimmermann *et al*. (1985)
Drosophila melanogaster, genetic recombination	+		2 mM in feed	Vogel (1985)
Drosophila melanogaster, genetic crossing-over, somatic mutation or recombination	+		0.94 mM in feed[f]	Würgler *et al.* (1985)
Drosophila melanogaster, somatic mutation (and recombination)	(+)		10 700	Fujikawa *et al*. (1985)
Drosophila melanogaster, somatic mutation (and recombination)	+		1 mM in feed	Vogel (1985)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Drosophila melanogaster, somatic mutation (and recombination)	+		1 mM in feed	Batiste-Alentorn *et al*. (1991)
Drosophila melanogaster, somatic mutation	–		2 mM in feed	Batiste-Alentorn *et al*. (1994)
Drosophila melanogaster, somatic mutation (and recombination), wing-spot test	+		5 mM in feed	Batiste-Alentorn *et al*. (1995)
DNA strand breaks, cross-links or related damage, rat hepatocytes *in vitro*	+	NT	319	Bradley (1985)
DNA strand breaks, cross-links or related damage, Chinese hamster ovary cells *in vitro*	+	+	4280	Douglas *et al*. (1985)
DNA strand breaks, cross-links or related damage, Chinese hamster ovary cells *in vitro*	–	(+)	2140	Lakhanisky & Hendrickx (1985)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	54	Thompson *et al.* (1983)
Unscheduled DNA synthesis, Sprague-Dawley rat primary hepatocytes *in vitro*	–	NT	10.7	Kornbrust & Barfknecht (1984)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	53.5	Probst & Hill (1985)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	10	Williams *et al*. (1985)
Unscheduled DNA synthesis, Sprague-Dawley rat primary hepatocytes *in vitro*	–	NT	10.7	Barfknecht *et al.* (1987)
Unscheduled DNA synthesis, golden Syrian hamster primary hepatocytes *in vitro*	–	NT	10.7	Kornbrust & Barfknecht (1984)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Unscheduled DNA synthesis, golden Syrian hamster primary hepatocytes *in vitro*	–	NT	10.7	Barfknecht *et al.* (1987)
Gene mutation, *Hprt* locus, ouabain resistance, Chinese hamster ovary cells *in vitro*	–	–	500	Zdzienicka & Simons (1985)
Gene mutation, Chinese hamster lung V79 cells *Hprt* locus *in vitro*	–	NT	2000	Fox & Delow (1985)
Gene mutation, Chinese hamster lung V79 cells, *Hprt* locus *in vitro*	(+)	–	500	Kuroda *et al*. (1985)
Gene mutation, Chinese hamster lung V79 cells *Hprt* locus *in vitro*	–	–	NR	Lee & Webber (1985)
Gene mutation, Chinese hamster lung V79 cells, oubain resistance *in vitro*	–	–	535	Kuroki & Munakata (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	800	Amacher & Turner (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus and *Hprt* locus *in vitro*	–	–	1.3 µL/mL	Knaap & Langebroek (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	NR	Lee & Webber (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	NT	300	Myhr *et al*. (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	500[g]	Oberly *et al*. (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	(+)	–	1004	Styles *et al*. (1985)
Gene mutation, mouse lymphoma L5178Y cells, ouabain or thioguanine resistance *in vitro*	–	+	200	Garner & Campbell (1985)
Gene mutation, mouse lymphoma L5178Y cells, ouabain or trifluorothymidine resistance, *in vitro*	–	–	1004	Styles *et al.* (1985)
Gene mutation, Balb/c 3T3 cells, ouabain resistance *in vitro*	NT	(+)	250	Matthews *et al*. (1985)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	–	300	Perry & Thomson (1981)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	1070	Douglas *et al.* (1985)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	50	Gulati *et al.* (1985)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	–	500	Lane *et al.* (1985)
Sister chromatid exchange, Chinese hamster cells *in vitro*	–	–	2140	Natarajan *et al.* (1985)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	268	van Went (1985)
Sister chromatid exchange, RL_4 rat liver cells *in vitro*	+	NT	21.8	Priston & Dean (1985)
Micronucleus formation, Chinese hamster ovary cells *in vitro*	–	–	1070	Douglas *et al.* (1985)
Micronucleus formation, Syrian hamster embryo cells *in vitro*	+	NT	NR	Fritzenschaf *et al.* (1993)
Chromosomal aberrations, Chinese hamster CH1-L liver fibroblasts *in vitro*	+	NT	12	Danford (1985)
Chromosomal aberrations, Chinese hamster cells *in vitro*	+	+	250	Gulati *et al.* (1985)
Chromosomal aberrations, Chinese hamster cells *in vitro*	–	+	1000	Ishidate & Sofuni (1985)
Chromosomal aberrations, Chinese hamster cells *in vitro*	–	–	2140	Natarajan *et al.* (1985)
Chromosomal aberrations, Chinese hamster cells *in vitro*	–	(+)	300	Palitti *et al.* (1985)
Chromosomal aberrations, RL_4 rat liver cells *in vitro*	+	NT	700	Priston & Dean (1985)
Aneuploidy, Chinese hamster CH1-L liver fibroblasts *in vitro*	+	NT	60	Danford (1985)
Cell transformation, C3H/10T½ mouse cells	–	(+)	600	Lawrence & McGregor (1985)
Cell transformation, BALB/c3T3 mouse cells	–	+[h]	150	Matthews *et al.* (1985)
Cell transformation, C3H/10T½ mouse cells	+	NT	500	Nesnow *et al.* (1985)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	1	Barrett & Lamb (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	100	Sanner & Rivedal (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	750	Kerckaert *et al.* (1998)
Cell transformation, baby hamster kidney BHK-21 cells	+	+	NR	Daniel & Dehnel (1981)
Cell transformation, baby hamster kidney BHK-21 cells	NT	+	25	Styles (1981)
Cell transformation, Chinese hamster ovary cells	–	–	500	Zdzienicka *et al.* (1985)
Cell transformation, RLV/Fischer rat embryo cells	(+)	NT	10	Suk & Humphreys (1985)
Cell transformation, SA7/Syrian hamster embryo cells	(+)	NT	965	Hatch & Anderson (1985)
DNA strand breaks (Comet assay), MCL-5 cells	+	NT	454	Martin *et al.* (1999)
Gene mutation, human TK6 cells *in vitro*	–	+	450	Crespi *et al.* (1985)
Gene mutation, human AHH-1 cells *in vitro*	+	NT	300	Crespi *et al.* (1985)
Sister chromatid exchange, human lymphocytes *in vitro*	?	–	600	Obe *et al.* (1985)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	21.4	Lindahl-Kiessling *et al*. (1989)
Micronucleus formation, human lymphocytes *in vitro*	+	–	214	Vian *et al*. (1993)
Body fluids from Sprague-Dawley rats (urine), microbial mutagenicity (*S. typhimurium* TA98)	–	+[c]	300 po × 1	Tanaka *et al*. (1980)
Body fluids from WAG/Rij rats (plasma), sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+		400 ip × 1	Darroudi & Natarajan (1985)
DNA strand breaks, cross-links or related damage, animal cells *in vivo*	+		100	Cesarone *et al*. (1982)
Sister chromatid exchange, B6C3F$_1$ mouse bone marrow cells *in vivo*	(+)		200	Neal & Probst (1983)
Sister chromatid exchange, animal cells *in vivo*	+		600	McFee *et al*. (1989)
Micronucleus formation, B6C3F$_1$ mice *in vivo*	–		338 ip × 2	Salamone *et al*. (1981)
Micronucleus formation, CD-1 mice *in vivo*	–		160 ip × 2	Tsuchimoto & Matter (1981)
Chromosomal aberrations, B6C3F$_1$ mice bone marrow cells *in vivo*	–		300 ip × 1	McFee *et al*. (1989)
Micronucleus formation, B6C3F$_1$ mice bone marrow cells *in vivo*	–		300 ip × 1	McFee *et al*. (1989)
Micronucleus test, *Pleurodeles waltl in vivo*	+		20 µg/mL	Fernandez *et al*. (1989)
Binding (covalent) to DNA, RNA or protein, Crl:CD rat liver *in vivo*	+		500 po × 1	Brock *et al*. (1990)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Inhibition of intercellular communication, Chinese hamster V79 cells rodent *in vitro*	+	NT	5	Elmore *et al.* (1985)
Inhibition of intercellular communication, Chinese hamster V79 cells rodent *in vitro*	+	NT	5	Scott *et al.* (1985)
Inhibition of intercellular communication, Chinese hamster V79 cells rodent *in vitro*[i]	–	NT	535	Umeda *et al.* (1985)
Sperm morphology, (CBA × BALB/c)F_1 mice *in vivo*	?		250 ip × 5	Topham (1981)
Sperm morphology, (CBA × BALB/c)F_1 mice *in vivo*	–		400 ip × 5	Topham (1980)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive; NR, not reported
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro test, µg/mL; in-vivo test, mg/kg bw/day; ip, intraperitoneal; po, oral
[c] Active only with 30% hamster liver S9; not with rat liver S9
[d] S9 from phenobarbital-treated rats
[e] Activity detected in YEPD medium
[f] Acute feeding
[g] Toxicity higher in the presence of S9
[h] Activation by co-cultivation with X-irradiated primary rat hepatocytes
[i] Growth of V79 (T2-14) 6-thioguanine-resistant cells

Table 6. Genetic and related effects of metabolites of *ortho*-toluidine

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
***N*-Hydroxy-*ortho*-toluidine**				
Salmonella typhimurium TA100, reverse mutation	–	+	0.16 µg/plate	Gupta *et al*. (1987)
Salmonella typhimurium TA98, reverse mutation	–	–	0.62 µg/plate	Gupta *et al*. (1987)
***N*-Acetyl-*ortho*-toluidine**				
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	3.75 µg/plate	Gupta *et al*. (1987)
***N*-Acetyl-*N*-hydroxy-*ortho*-toluidine**				
Salmonella typhimurium TA100, reverse mutation	–	–	4.1 µg/plate	Gupta *et al*. (1987)
Salmonella typhimurium TA98, reverse mutation	–	+	2.1 µg/plate	Gupta *et al*. (1987)
***N*-Acetoxy-*N*-acetyl-*ortho*-toluidine**				
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	5.2 µg/plate	Gupta *et al*. (1987)

[a] +, positive; –, negative
[b] LED, lowest effective dose; HID, highest ineffective dose

It gave a positive result in one out of seven assays for reverse mutation. However, it caused aneuploidy in two out of three assays. Assays for forward mutation or genetic crossing-over in *Aspergillus nidulans* gave completely negative results, as did a forward mutation assay in *Schizosaccharomyces pombe*.

ortho-Toluidine caused DNA strand breakage in various animal cell lines *in vitro*, apparently in the absence of exogenous metabolic activation. The alkaline single-cell gel electrophoresis ('comet') assay revealed DNA breakage after *ortho*-toluidine treatment in a metabolically competent human mammary cell line, MCL-5, as well as in primary cultures of cells isolated from human breast milk. The response was substantially increased when the cells were incubated in the presence of the DNA repair inhibitors, hydroxyurea and cytosine arabinoside. Only one out of eight studies showed that treatment with *ortho*-toluidine could lead to unscheduled DNA synthesis. A single study suggested a weak positive effect in gene mutation at the *Hprt* locus in V79 Chinese hamster cells, although two other similar studies gave negative results. *ortho*-Toluidine failed to cause mutation to ouabain resistance in V79 Chinese hamster cells. Two out of five studies suggested a positive response at the *Tk* locus but not usually at other loci in mouse lymphoma L5178Y cells. However, there are isolated reports of *ortho*-toluidine increasing gene mutations at loci other than *Tk* in L5178Y cells or in other animal cells *in vitro*, but only in the presence of exogenous metabolic activation.

There have been occasional reports of *ortho*-toluidine causing chromosomal aberrations or micronuclei in various cultured cell lines. Manifestation of these effects seems to require incubation times longer than 3 h. In some of these studies, S9 mix was required, while in others it appeared to reduce the effect. Most studies of effects on sister chromatid exchanges, in either animal or human cells, have revealed positive results, even in the absence of exogenous metabolic activation. *ortho*-Toluidine caused aneuploidy in mammalian cells *in vitro*, and increased cell transformation in all but one of 11 studies. The latter effects did not generally appear to require exogenous metabolic activation, although it should be noted that the cell types have some endogenous metabolic capability. In two out of three studies, *ortho*-toluidine inhibited intracellular communication.

A number of in-vivo studies have been conducted. *ortho*-Toluidine gave a positive result in a host-mediated assay for bacterial mutagenesis. It increased somatic mutation but not genetic crossing-over in *Drosophila melanogaster*, and enhanced sister chromatid exchanges in rodent models. Only one out of four studies in mice, but one study in a newt model, suggested that it enhanced micronucleus frequency. Studies on sperm morphology have given equivocal data.

Metabolites of ortho-*toluidine*

Gupta *et al*. (1987) synthesized the various *N*-oxidized putative metabolites and esters of *ortho*-toluidine and tested them for mutagenic activity in the *Salmonella* microsome mutagenicity assay. On the basis of these data, they proposed a metabolic

pathway involving nitrenium ion/nitrene/free radicals which could bind covalently to DNA (see Table 6).

4.5 Mechanistic considerations

ortho-Toluidine undergoes extensive metabolism *in vivo* and, like other aromatic amines, *N*-hydroxylation is thought to be the first step in its metabolic activation.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

ortho-Toluidine and its hydrochloride salt have been widely produced commercially throughout the twentieth century for use in manufacture of dyestuffs, pigments, optical brighteners, rubber chemicals, pharmaceuticals and pesticides. Human exposure has been reported during its use in production of dyestuffs and rubber chemicals. Non-occupational exposure to *ortho*-toluidine may result from its occurrence in certain foods and from exposure to tobacco smoke.

5.2 Human carcinogenicity data

Five studies were available for evaluation. Two mortality studies were conducted in the 1980s among dye production workers in Italy and in the United States. In each case, the subgroups of workers exposed to *ortho*-toluidine were small. Two recent cohort studies in Germany, the United Kingdom and a larger study in the United States among workers in 4-chloro-*ortho*-toluidine production and in rubber chemical manufacturing looked at bladder cancer incidence. Of these five studies, four observed a very high excess of bladder cancer among *ortho*-toluidine-exposed workers. The fifth study had limited power to detect a risk. In the two studies with data on duration of exposure to *ortho*-toluidine, the highest risk was observed in the subgroup with the longest duration of exposure. In none of these studies, however, could confounding by concomitant exposure to various other potential bladder carcinogens be ruled out with confidence, although co-exposures differed between studies.

5.3 Animal carcinogenicity data

ortho-Toluidine was tested for carcinogenicity as its hydrochloride salt in two experiments in mice and in three experiments in rats and as the free base in one limited experiment in hamsters. After oral administration to mice, it induced an increased incidence of haemangiomas and haemangiosarcomas and hepatocellular carcinomas

or adenomas. In rats, oral administration of *ortho*-toluidine increased the incidence of tumours in multiple organs, including fibromas, sarcomas, mesotheliomas, mammary fibroadenomas and transitional-cell carcinomas of the urinary bladder.

5.4 Other relevant data

ortho-Toluidine undergoes extensive metabolism *in vivo*, with the bulk of the dose being excreted in the urine within 24 h. Like other aromatic amines, it is thought to undergo metabolic activation initially via *N*-hydroxylation, leading to covalent binding to tissue macromolecules. Evidence that *ortho*-toluidine undergoes metabolic activation *in vivo* is supported by the fact that it forms both haemoglobin and albumin adducts in laboratory animals and haemoglobin adducts in humans.

In rats, repeated administration of *ortho*-toluidine led to haemosiderosis, splenic congestion, bone marrow and splenic proliferation and splenic fibrosis consistent with a response to erythrocyte destruction.

Bacterial or bacteriophage assay systems showed negative or inconsistent data or, at most, weakly positive results. In the yeast *Saccharomyces cerevisiae*, *ortho*-toluidine caused reverse mutation at some loci and occasionally recombinational events. It caused gain or loss of whole chromosomes and mutation of mitochondrial DNA. In cultured mammalian cells, it generally caused sister chromatid exchanges and sometimes also increased gene mutations, chromosomal aberrations and micronuclei. It induced aneuploidy and increased cell transformation in such cells. *ortho*-Toluidine may inhibit intercellular communication. It has been demonstrated to be a mutagen but not a recombinogen in *Drosophila melanogaster*. In rodent models *in vivo*, it enhanced sister chromatid exchanges but gave equivocal results for micronuclei and sperm morphology.

5.5 Evaluation

There is *limited evidence* in humans for the carcinogenicity of *ortho*-toluidine.

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

Overall evaluation

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

6. References

Amacher, D.E. & Turner, G.N. (1985) Tests for gene mutational activity in the L5178Y/TK assay system. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 487–496

American Conference of Governmental Industrial Hygienists (1999) *TLVs and other Occupational Exposure Values—1999* [CD-ROM], Cincinnati, OH, ACGIH®

Arni, P. (1985) Induction of various genetic effects in the yeast *Saccharomyces cerevisiae* strain D7. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 217–224

Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds (1985) *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro Assays*, Amsterdam, Elsevier

Axelson, O. & Steenland, K. (1988) Indirect methods of assessing the effects of tobacco use in occupational studies. *Am. J. ind. Med.*, **13**, 105–118

Baker, R.S.U. & Bonin, A.M. (1981) Study of 42 coded compounds with the *Salmonella*/-mammalian microsome assay. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 249–260

Baker, R.S.U. & Bonin, A.M. (1985) Tests with the Salmonella plate-incorporation assay. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 177–180

Barfknecht, T.R., Naismith, R.W. & Kornbrust, D.J. (1987) Variations on the standard protocol design of the hepatocyte DNA repair assay. *Cell Biol. Toxicol.*, **3**, 193–207

Barrett, R.H. (1985) Assays for unscheduled DNA synthesis in HeLa S3 cells. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 347–352

Barrett, J.C. & Lamb, P.W. (1985) Tests with the Syrian hamster embryo cell transformation assay. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 623–628

Batiste-Alentorn, M., Xamena, N., Creus, A. & Marcos, R. (1991) Genotoxicity studies with the unstable *Zeste-White (UZ)* system of *Drosophila melanogaster*: Results with ten carcinogenic compounds. *Environ. mol. Mutagen.*, **18**, 120–125

Batiste-Alentorn, M., Xamena, N., Creus, A. & Marcos R. (1994) Further studies with the somatic *White-Ivory* system of *Drosophila melanogaster*: genotoxicity testing of ten carcinogens. *Environ. mol. Mutag*., **24**, 143–147

Batiste-Alentorn, M., Xamena, N., Creus, A. & Marcos, R. (1995) Genotoxic evaluation of ten carcinogens in the *Drosophila melanogaster* wing spot test. *Experientia*, **51**, 73–76

Bayer Organic Chemicals (1995) *Technical Information: o-Toluidine*, Pittsburgh, PA

Birner, G. & Neumann, H.-G. (1988) Biomonitoring of aromatic amines. II: Hemoglobin binding of some monocyclic aromatic amines. *Arch. Toxicol*., **62**, 110–115

Bradley, M.O. (1985) Measurement of DNA single-strand breaks by alkaline elution in rat hepatocytes. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 353–357

Brock,W.J., Hundley, S.G. & Lieder, P.H. (1990) Hepatic macromolecular binding and tissue distribution of *ortho*- and *para*-toluidine in rats. *Toxicol. Lett*., **54**, 317–325

Brooks, T.M. & Dean, B.J. (1981) Mutagenic activity of 42 coded compounds in the *Salmonella*/microsome assay with preincubation. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 261–270

Brooks, T.M., Gonzalez, L.P., Calvert, R. & Parry, J.M. (1985) The induction of mitotic gene conversion in the yeast *Saccharomyces cerevisiae* strain JD1. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro Assays*, Amsterdam, Elsevier Science, pp. 225–228

Brown, K.K., Teass, A.W., Simon, S. & Ward, E.M. (1995) A biological monitoring method for *o*-toluidine and aniline in urine using high performance liquid chromatography with electrochemical detection. *Appl. occup. environ. Hyg*., **10**, 557–565

Bryant, M.S., Vineis, P., Skipper, P.L. & Tannenbaum, S.R. (1988) Hemoglobin adducts of aromatic amines: Associations with smoking status and type of tobacco. *Proc. natl Acad. Sci. USA*, **85**, 9788–9791

Budavari, S., ed. (1998) *The Merck Index*, 12th Ed., Version 12:2 [CD-ROM], Whitehouse Station, NJ, Merck & Co.

Carere, A., Conti, G., Conti, L. & Crebelli, R. (1985) Assays in *Aspergillus nidulans* for the induction of forward-mutation in haploid strain 35 and for mitotic nondisjunction, haploidization and crossing-over in diploid strain P1. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 307–312

Carls, N. & Schiestl, R.H. (1994) Evaluation of the yeast DEL assay with 10 compounds selected by the International Program on Chemical Safety for the evaluation of short-term tests for carcinogens. *Mutat. Res.*, **320**, 293–303

Cesarone, C.F., Bolognesi, C. & Santi, L. (1982) Evaluation of damage to DNA after in vivo exposure to different classes of chemicals. *Arch. Toxicol.*, **5** (Suppl.), 355–359

Cheever, K.L., Richards, D.E. & Plotnick, H.B. (1980) Metabolism of ortho-, meta-, and para-toluidine in the adult male rat. *Toxicol. appl. Pharmacol.*, **57**, 361–369

Cheever, K.L., DeBord D.G., Swearengin, T.F. & Booth-Jones, A.D. (1992) ortho-Toluidine blood protein adducts: HPLC analysis with fluorescence detection after a single dose in the adult male rat. *Fundam. appl. Toxicol.*, **18**, 522–531

Chemfinder (2000) ortho-*Toluidine Hydrochloride* [http://www.chemfinder.com/result.asp]

Chemical Information Services (1999) *Directory of World Chemical Producers* (Version 99.1.0) [CD-ROM], Dallas, TX

Coleman, M.D. & Coleman, N.A. (1996) Drug-induced methaemoglobinaemia. Treatment issues. *Drug Saf.*, **14**, 394–405

Conso, F. & Pontal, P. (1982) [Urinary bladder amino-tumours. Possible role of occupational exposure to *o*-toluidine and *o*-aminoazotoluene.] *Arch. Mal. prof.*, **43**, 273–319 (in French)

Crespi, C.L., Ryan, C.G., Seixas, G.M., Turner, T.R. & Penman, B.W. (1985). Tests for mutagenic activity using mutation assays at two loci in the human lymphoblast cell lines TK6 and AHH-1. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 497–516

Dambly, C., Toman, Z. & Radman, M. (1981) Zoro test. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative program*, Amsterdam, Elsevier Science, pp. 219–223

Danford, N. (1985) Tests for chromosome aberrations and aneuploidy in the Chinese hamster fibroblast cell line CH1-L. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 397–411

Danford, N. (1991) The genetic toxicology of *ortho*-toluidine. *Mutat. Res.*, **258**, 207–236

Daniel, M.R. & Dehnel, J.M. (1981) Cell transformation test with baby hamster kidney cells. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 626–637

Darroudi, F. & Natarajan, A.T. (1985) Cytostatic drug activity in plasma, a bioassay for detecting mutagenicity of directly and indirectly acting chemicals, an evaluation of 20 chemicals. *Mutat. Res.*, **143**, 263–269

DeBord, D.G., Swearengin, T.F., Cheever, K.L., Booth-Jones, A.D. & Wissinger, L.A. (1992) Binding characteristics of *ortho*-toluidine to rat hemoglobin and albumin. *Arch. Toxicol.*, **66**, 231–236

Department of Health and Human Services (1982) *The Health Consequences of Smoking: Cancer—A Report of the Surgeon General* (DHHS (PHS) Publ. No. 82-50179), Rockville, MD, Office of Smoking Health, Public Health Service

Department of Health and Human Services (1999) *Report on Carcinogens*, 8th Ed., Research Triangle Park, NC, National Toxicology Program, pp. 202–203

Deutsche Forschungsgemeinschaft (1999) *List of MAK and BAT Values 1999*, Report No. 35, Weinheim, Wiley-VCH, pp. 100, 137

Dorado, G. & Pueyo, C. (1988) L-Arabinose resistance test with *Salmonella typhimurium* as a primary tool for carcinogen screening. *Cancer Res.*, **48**, 907–912

Douglas, G.R., Blakey, D.H., Liu-Lee, V.W., Bell, R.D.L. & Bayley, J.M. (1985) Alkaline sucrose sedimentation, sister-chromatid exchange and micronucleus assays in CHO cells. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 359–366

El-Bayoumy, K., Donahue, J.M., Hecht, S.S. & Hoffmann, D. (1986) Identification and quantitative determination of aniline and toluidines in human urine. *Cancer Res.*, **46**, 6064–6067

Eller, P.M., ed. (1994) *NIOSH Manual of Analytical Methods* (DHHS (NIOSH) Publ. No. 94-113), 4th Ed., Cincinnati, OH, National Institute for Occupational Safety and Health [Method 2002]

Ellis, D.D., Jone, C.M., Larson, R.A & Schaeffer, D.J. (1982) Organic constituents of mutagenic secondary effluents from wastewater treatment plants. *Arch. environ. Contam. Toxicol.*, **11**, 373–382

Elmore, E., Korytynski, E.A. & Smith, M.P. (1985) Tests with the Chinese hamster V79 inhibition of metabolic cooperation assay. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 597–612

Environmental Protection Agency (1984) *Chemical Hazard Information Profile:* ortho-*Toluidine*, ortho-*Toluidine Hydrochloride*, Washington DC, Office of Pesticide Program and Toxic Substances

Environmental Protection Agency (1996a) *Method 8260B—Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)*, Washington DC

Environmental Protection Agency (1996b) *Method 8270C—Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)*, Washington DC

Environmental Protection Agency (1996c) *Method 8015B—Nonhalogenated Organics Using GC/FID*, Washington DC

Environmental Protection Agency (1996d) *1994 Toxic Release Inventory* (EPA 745-R-96-002), Washington DC, Office of Pollution Prevention and Toxics, p. 264

Environmental Protection Agency (1997) *1995 Toxic Release Inventory* (EPA 745-R-97-005), Washington DC, Office of Pollution Prevention and Toxics, p. 48

Environmental Protection Agency (1999a) Method 1625. *Code Fed. Reg.*, **40** Part 136, Appendix A, Washington DC

Environmental Protection Agency (1999b) High production volume (HPV) chemicals list, Washington, DC [http://www.epa.gov/]

Falck, K., Partanen, P., Sorsa, M., Suovaniemi, O. & Vainio, H. (1985) Mutascreen®, an automated bacterial mutagenicity assay. *Mutat. Res.*, **150**, 119–125

Fan, A.M. & Steinberg, V.E. (1996) Health implications of nitrate and nitrite in drinking water: an update on methemoglobinemia occurrence and reproductive and developmental toxicity. *Regul. Toxicol. Pharmacol*., **23**, 35–43

Ferguson, L.R. (1985) Petite mutagenesis in *Saccharomyces cerevisiae* strain D5. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 229–234

Fernandez, M., Gauthier, L. & Jaylet, A. (1989) Use of newt larvae for *in vivo* genotoxicity testing of water: results on 19 compounds evaluated by the micronucleus test. *Mutagenesis*, **4**, 17–26

Ferreti, J.J., Lu, W. & Liu, M.-B. (1977) Mutagenicity of benzidine and related compounds employed in the detection of hemoglobin. *Am. J. clin. Pathol.*, **67**, 526–527

Finnish Ministry of Social Affairs and Health (1998) *Finnish Occupational Exposure Limits 1998: Concentrations known to be harmful* [http://www.occuphealth.fi]

Fox, M. & Delow, G.F. (1985) Tests for mutagenic activity at the HGPRT locus in Chinese hamster V79 cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 517–523

Fritzenschaf, H., Kohlpoth, M., Rusche, B. & Schiffmann, D. (1993) Testing of known carcinogens and noncarcinogens in the Syrian hamster embyro (SHE) micronucleus test in vitro; correlations with in vivo micronucleus formation and cell transformation. *Mutat. Res.*, **319**, 47–53

Fujikawa, K., Ryo, H. & Kondo, S. (1985) The Drosophila reversion assay using the unstable *zeste-white* somatic eye color system. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 319–324

Garner, R.C. & Campbell, J. (1985) Tests for the induction of mutations to ouabain or 6-thioguanine resistance in mouse lymphoma L5178Y cells. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 525–529

Garner, R.C. & Nutman, C.A. (1977) Testing of some azo dyes and their reduction products for mutagenicity using Salmonella typhimurium TA1538. *Mutat. Res.*, **44**, 9–19

Gatehouse, D. (1981) Mutagenic activity of 42 coded compounds in the 'Microtiter' fluctuation test. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 376–386

Glauert, H.P., Kennan, W.S., Sattler, G.L. & Pitot, H.C. (1985) Assays to measure the induction of unscheduled DNA synthesis in cultured hepatocytes. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 371–373

Gnojkowski, J., Baer-Dubowska, W., Klimek, D. & Chmiel, J. (1984) Effect of toluidines on drug metabolizing enzymes in rat liver, kidney and lung. *Toxicology*, **32**, 335–342

Goodman, D.G., Ward, J.M. & Reichardt, W.D. (1984) Splenic fibrosis and sarcomas in F344 rats fed diets containing aniline hydrochloride, *p*-chloroaniline, azobenzene, *o*-toluidine hydrochloride, 4,4′-sulfonyldianiline, or D & C Red No. 9. *J. natl Cancer Inst.*, **72,** 265–273

Green, M.H.L. (1981) A differential killing test using an improved repair-deficient strain of *Escherichia coli*. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 183–194

Gulati, D.K., Sabharwal, P.S. & Shelby, M.D. (1985) Tests for the induction of chromosomal aberrations and sister chromatid exchanges in cultured Chinese hamster ovary (CHO) cells. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 413–426

Gupta, R.L., Gupta, A.K., Pathak, D.P. & Juneja, T.R. (1987) Mutagenic studies of *ortho*-toluidine and its potential metabolites. *Indian J. exp. Biol*., **25**, 618–622

Hansch, C., Leo, A. & Hoekman, D. (1995) *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*, Washington DC, American Chemical Society, p. 32

Harrington, T.R. & Nestmann, E.R. (1985) Tests for mutagenic activity in growing cells of the yeast *Saccharomyces cerevisiae* strain XV185-14C. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 257–260

Hatch, G.G. & Anderson, T.M. (1985) Assays for enhanced DNA viral transrormation of primary Syrian hamster embryo (SHE) cells. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 629–638

Hecht, S.S., El-Bayoumy, K., Rivenson, A. & Fiala, E. (1982) Comparative carcinogenicity of *o*-toluidine hydrochloride and *o*-nitrosotoluene in F-344 rats. *Cancer Lett*., **16**, 103–108

Hecht, S.S., El-Bayoumy, K., Rivenson, A. & Fiala, E.S. (1983) Bioassay for carcinogenicity of 3,2′-dimethyl-4-nitrosobiphenyl, *o*-nitrosotoluene, nitrosobenzene and the corresponding amines in Syrian golden hamsters. *Cancer Lett.*, **20**, 349–354

Hemminki, K. (1992) Significance of DNA and protein adducts. In: Vainio, H., Magee, P.N., McGregor, D.B. & McMichael, A.J., eds, *Mechanisms of Carcinogenesis in Risk Identification* (IARC Scientific Publications No. 116), Lyon, IARC*Press*, pp. 525–534

Hubbard, S.A., Green, M.H.L., Bridges, B.A., Wain, A.J. & Bridges, J.W. (1981) Fluctuation test with S9 and hepatocyte activation. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 361–370

IARC (1972) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 1, *Some Inorganic Substances, Chlorinated Hydrocarbons, Aromatic Amines, N-Nitroso Compounds, and Natural Products*, Lyon, IARC*Press*, pp. 74–79

IARC (1975) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 8, *Some Aromatic Azo Compounds*, Lyon, IARC*Press*, pp. 61–69

IARC (1978) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 16, *Some Aromatic Amines and Related Nitro Compounds—Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals*, Lyon, IARC*Press*, pp. 349–366

IARC (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 27, *Some Aromatic Amines, Anthraquinones and Nitroso Compounds and Inorganic Fluorides Used in Drinking-water and Dental Preparations*, Lyon, IARC*Press*, pp. 155–175

IARC (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 38, *Tobacco Smoking*, Lyon, IARC*Press*, p. 108

IARC (1987a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, IARC*Press*, pp. 362–363

IARC (1987b) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, IARC*Press*, p. 61

IARC (1987c) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, IARC*Press*, pp. 56

IARC (1987d) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, IARC*Press*, pp. 99–100

IARC (1987e) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, IARC*Press*, pp. 91-92

IARC (1993) *IARC Monographs on the Evaluation of of Carcinogenic Risks to Humans*, Vol. 57, *Occupational Exposures of Hairdressers and Barbers and Personal Use of Hair Colourants: Some Hair Dyes, Cosmetic Colourants, Industrial Dyestuffs and Aromatic Amines*, Lyon, IARC*Press*, pp. 215–234

Ichinotsubo, D., Mower, H. & Mandel, M. (1981) Mutagen testing of a series of paired compounds with the Ames *Salmonella* testing system. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 298–301

ILO (International Labour Office) (1971) *Encyclopaedia of Occupational Health and Safety*, Vol. 1, New York, McGraw-Hill, p. 96

Inge-Vechtomov, S.G., Pavlov, Y.I., Noskov, V.N., Repnevskaya, M.V., Karpova, T.S., Khromov-Borisov, N.N., Chekuolene, J. & Chitavichus, D. (1985) Tests for genetic activity in the yeast *Saccharomyces cerevisiae*: study of forward and reverse mutation, mitotic recombination and illegitimate mating induction. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 243–255

Ishidate, M., Jr & Sofuni, T. (1985) The in vitro chromosomal aberration test using Chinese hamster lung (CHL) fibroblast cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 427–432

Jagannath, D.R., Vultaggio, D.M. & Brusick, D.J. (1981) Genetic activity of 42 coded compounds in the mitotic gene conversion assay using *Saccharomyces cerevisiae* strain D4. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 456–467

Kada, T. (1981) The DNA-damaging activity in 42 coded compounds in the rec-assay. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 175–182

Kawalek, J.C., Hallmark, R.K. & Andrews, A.W. (1983) Effect of lithocholic acid on the mutagenicity of some substituted aromatic amines. *J. natl Cancer Inst.*, **71**, 293–298

Kerckaert, G.A., LeBoeuf, R.A. & Isfort, R.J. (1998) Assessing the predictiveness of the Syrian hamster embryo cell transformation assay for determining the rodent carcinogenic potential of single ring aromatic/nitroaromatic amine compounds. *Toxicol. Sci.*, **41**, 189–197

Khlebnikova, M.I., Gladkova, E.V., Kurenko, L.T., Pshenitsyn, A.V. & Shalin, B.M. (1970) [Problems of industrial hygiene and health status of workers engaged in the production of *o*-toluidine.] *Gig. Tr. prof. Zabol.*, **14**, 7–10 (in Russian)

Kilpatrick, S.J. & Laros, R.K. (1999) Maternal hematologic disorders. In: Creasy, R.K. & Resnik, R., eds, *Maternal–fetal Medicine*, Philadelphia, W.B. Saunders, pp. 935–963

Knaap, A.G.A.C. & Langebroek, P.B. (1985) Assays for the induction of gene mutations at the thymidine kinase locus and the hypoxanthine guanine phosphoribosyltransferase locus in L5178Y mouse lymphoma cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 531–536

Kornbrust, D.J. & Barfknecht, T.R. (1984) Comparison of 7 azo dyes and their azo reduction products in the rat and hamster hepatocyte primary culture/DNA-repair assays. *Mutat. Res.*, **136**, 255–266

Kulkarni, B., Fiala, E.S. & Weisburger, J.H. (1983) Estimation of N-hydroxy-*o*-toluidine, a urinary metabolite of *o*-toluidine and *o*-nitrosotoluene, by high performance liquid chromatography with electrochemical detection. *Carcinogenesis*, **4**, 1275–1279

Kuroda, Y., Yokoiyama, A. & Kada, T. (1985) Assays for the induction of mutations to 6-thioguanine resistance in Chinese hamster V79 cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 537–542

Kuroki, T. & Munakata, K. (1985) Assays for the induction of mutations to ouabain resistance in V79 Chinese hamster cells in culture with cell- or microsome-mediated metabolic activation. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 543–545

Lakhanisky, Th. & Hendrickx, B. (1985) Induction of DNA single-strand breaks in CHO cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 367–370

Lane, A.M., Phillips, B.J. & Anderson, D. (1985) Tests for the induction of sister chromatid exchanges in Chinese hamster ovary (CHO) cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 451–455

Lawrence, N. & McGregor, D.B. (1985) Assays for the induction of morphological transformation in C3H/10T½ cells in culture with and without S9-mediated metabolic activation. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 651–658

Lee, C.G. & Webber, T.D. (1985) The induction of gene mutations in the mouse lymphoma L5178Y/TK$^{+/-}$ assay and the Chinese hamster V79/HGPRT assay. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro Assays*, Amsterdam, Elsevier Science, pp. 547–554

Leenheer, L.A., Noyes, T.I. & Struber, H.A. (1982) Determination of polar organic solutes in oil-shale retort wastes. *Environ. Sci. Technol*., **16**, 714–723

Leslie, C., Reidy, G.F., Murray, M. & Stacey, N.H. (1988) Induction of xenobiotic biotransformation by the insecticide chlordimeform, a metabolite 4-chloro-*o*-toluidine and a structurally related chemical *o*-toluidine. *Biochem. Pharmacol*., **37**, 2529–2535

Lewis, R.J., Jr (1993) *Hawley's Condensed Chemical Dictionary*, 12th Ed., New York, Van Nostrand Reinhold

Liber, H.L. (1985) Mutation tests with Salmonella using 8-azaguanine resistance as the genetic marker. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro Assays*, Amsterdam, Elsevier Science, pp. 213–216

Lide, D.R. & Milne, G.W.A. (1996) *Properties of Organic Compounds*, Version 5.0 [CD-ROM], Boca Raton, FL, CRC Press

Lin, H.C. & Krishnamurti, R. (1993) Chlorocarbons, -hydrocarbons (toluenes). In: Kroschwitz, J.I. & Howe-Grant, M., eds, *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th Ed., Vol. 6, New York, John Wiley, pp. 101–113

Lindahl-Kiessling, K., Karlberg, I. & Olofsson, A.-M. (1989) Induction of sister-chromatid exchanges by direct and indirect mutagens in human lymphocytes, co-cultured with intact rat liver cells. Effect of enzyme induction and preservation of the liver cells by freezing in liquid nitrogen. *Mutat. Res.*, **211**, 77–87

Loprieno, N., Boncristiani, G., Forster, R. & Goldstein, B. (1985) Assays for forward mutation in *Schizosaccharomyces pombe* strain P1. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 297–306

MacDonald, D.J. (1981) *Salmonella*/Microsome tests on 42 coded chemicals. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 285–297

Martin, F.L., Cole, K.J., Orme, M.H., Grover, P.L., Phillips, D.H. & Venitt, S. (1999) The DNA repair inhibitors hydroxyurea and cytosine arabinoside enhance the sensitivity of the alkaline single-cell gel electrophoresis ('comet') assay in metabolically-competent MCL-5 cells. *Mutat. Res.*, **445**, 21–43

Mass Spectrometry Data Centre (1974) *Eight Peak Index of Mass Spectra*, 2nd ed., Reading, UK, Atomic Weapons Research Establishment

Matsushima, T., Muramatsu, M. & Haresaku, M. (1985) Mutation tests on *Salmonella typhimurium* by the preincubation method. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 181–186

Matthews, E.J., DelBalzo, T. & Rundell, J.O. (1985) Assays for morphological transformation and mutation to ouabain resistance of Balb/c-3T3 cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 639–650

McCann, J., Choi, E., Yamasaki, E. & Ames, B.N. (1975) Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. *Proc. Natl Acad Sci USA*, **72**, 5135–5139

McFee, A.F., Jauhar, P.P., Lowe, K.W., MacGregor, J.T. & Wehr, C.M. (1989) Assays of three carcinogen/non-carcinogen chemical pairs for in vivo induction of chromosome aberrations, sister chromatid exchanges and micronuclei. *Environ. mol. Mutagen.*, **14**, 107–220

Mehta, R.D. & von Borstel, R.C. (1985) Tests for genetic activity in the yeast *Saccharomyces cerevisiae* using strains D7-144, XV185-14C and RM52. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 271–284

Mehta, R.D. & von Borstel, R.C. (1981) Mutagenic activity of 42 encoded compounds in the haploid yeast reversion assay, strain XV185-14C. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 414–423

Miller, J.A. (1970) Carcinogenesis by chemicals: an overview—G.H.A. Clowes Memorial Lecture. *Cancer Res.*, **30**, 559–576

Myhr, B., Bowers, L. & Caspary, W.J. (1985) Assays for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 555–568

Nagao, M., Yahagi, T., Honda, M., Seino, Y., Matsushima, T. & Sugimura, T. (1977) Demonstration of mutagenicity of aniline and o-toluidine by norharman. *Proc. jpn. Acad. Ser. B. Phys. Biol. Sci.*, **53**, 34–37

Nagao, M., Yahagi, T. & Sugimura, T. (1978) Differences in effects of norharman with various classes of chemical mutagens and amounts of S-9. *Biochem. biophys. Res. Commun.*, **83**, 373–378

Nagao, M. & Takahashi, Y. (1981) Mutagenic activity of 42 coded compounds in the *Salmonella*/microsome assay. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 302–313

Nakamura, S.-I., Oda, Y., Shimada, Y., Oki, I. & Sugimoto, K. (1987) SOS-inducing activity of chemical carcinogens and mutagens in *Salmonella typhimurium* TA1535/pSK1002: examination with 151 chemicals. *Mutat. Res.*, **192**, 239–246

Natarajan, A.T., Bussmann, C.J.M., van Kesteren-van Leeuwen, A.C., Meijers, M. & van Rijn, J.L.S. (1985) Tests for chromosome aberrations and sister-chromatid exchanges in Chinese hamster ovary (CHO) cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 433–437

National Cancer Institute (1979) *Carcinogenesis Bioassay of o-Toluidine Hydrochloride for Possible Carcinogenicity* (Tech. Rep. Series No.153; DHEW Publ. No. (NIH) 79-1709), Bethesda, MD

National Toxicology Program (1988) *Toxicology and Carcinogenesis Studies of 2-Mercaptobenzothiazole (CAS No. 149-30-4) in F344 N Rats and B6C3F$_1$ Mice* (*Gavage studies*) (Tech. Rep. No. 332; DHHS Publ. No. (NIH) 88-2588), Research Triangle Park, NC

National Toxicology Program (1991) *o-Toluidine Hydrochloride*, Washington DC, Department of Health and Human Services [http://ntp-db.niehs.nih.gov]

National Toxicology Program (1996) *NTP Technical Report on Comparative Toxicity and Carcinogenicity Studies of o-Nitrotoluene and o-Toluidine Hydrochloride (CAS Nos. 88-72-2 and 636-21-5) Administered in Feed to Male F344/N Rats* (NTP Toxicity Report Series No. 44; NIH Publ. No. 96-3936), Research Triangle Park, NC

Neal, S.B. & Probst, G.S. (1983) Chemically-induced sister-chromatid exchange in vivo in bone marrow of Chinese hamsters. An evaluation of 24 compounds. *Mutat. Res.*, **113**, 33–43

Nesnow, S., Curtis, G. & Garland, H. (1985) Tests with the C3H/10T½ clone 8 morphological transformation bioassay. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 651–658

Neurath, G.B., Dünger, M., Pein, F.G., Ambrosius, D. & Schreiber, O. (1977) Primary and secondary amines in the human environment. *Food Cosmet. Toxicol.*, **15**, 275–282

New Jersey Department of Health and Senior Services (1994) *Hazardous Substance Fact Sheet*—o-*Toluidine Hydrochloride*, Trenton, NJ

NOES (1999) *National Occupational Exposure Survey 1981–83*. Unpublished data as of July 1999. Cincinnati, OH, Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health

Obe, G., Hille, A., Jonas, R., Schmidt, S. & Thenhaus, U. (1985) Tests for the induction of sister-chromatid exchanges in human peripheral lymphocytes in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 439–442

Oberly, T.J., Bewsey, B.J. & Probst, G.S. (1985) Tests for the induction of forward mutation at the thymidine kinase locus of L5178Y mouse lymphoma cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 569–582

Occupational Safety and Health Administration (1999) Occupational safety and health standards. *Code Fed. Reg.*, **29**, Chap. 17, part 1910.1000, p. 17

Ott, M.G. & Langner, R.R. (1983) A mortality survey of men engaged in the manufacture of organic dyes. *J. occup. Med.*, **25**, 763–768

Palitti, F., Fiore, M., De Salvia, R., Tanzarella, C., Ricordy, R., Forster, R., Mosesso, P., Astolfi, S. & Loprieno, N. (1985) Tests for the induction of chromosomal aberrations in Chinese hamster ovary (CHO) cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 443–450

Parry, J.M. & Sharp, D.C. (1981) Induction of mitotic aneuploidy in the yeast strain D6 by 42 coded compounds. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 468–480

Parry, E.M. (1985) Tests for effects on mitosis and the mitotic spindle in Chinese hamster primary liver cells (CH1-L) in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 479–485

Parry, J.M. & Eckardt, F. (1985a) The detection of mitotic gene conversion, point mutation and mitotic segregation using the yeast *Saccharomyces cerevisiae* strain D7. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 261–269

Parry, J.M. & Eckardt, F. (1985b) The induction of mitotic aneuploidy, point mutation and mitotic crossing-over in the yeast *Saccharomyces cerevisiae* strains D61-M and D6. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 285–295

Perry, P.E. & Thomson, E.J. (1981) Evaluation of the sister chromatid exchange method in mammalian cells as a screening system for carcinogens. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 560–569

Prince, M.M., Ward, E.M., Ruder, A., Salvan, A. & Roberts, D.R. (2000) Mortality among rubber chemical manufacturing workers. *Am. J. ind. Med.*, **38,** 1–9

Priston, R.A.J. & Dean, B.J. (1985) Tests for the induction of chromosome aberrations, polyploidy and sister-chromatid exchanges in rat liver (RL_4) cells. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro Assays*, Amsterdam, Elsevier Science, pp. 387–395

Probst, G.S. & Hill, L.E. (1985) Tests for the induction of DNA-repair synthesis in primary cultures of adult rat hepatocytes. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 381–386

Rexroat, M.A. & Probst, G.S. (1985) Mutation tests with Salmonella using the plate-incorporation assay (Chapter 14). In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science Publishers, pp. 201–212

Richold, M. & Jones, E. (1981) Mutagenic activity of 42 coded compounds in the *Salmonella*/microsome assay. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 314–322

Riffelmann, M., Müller, G., Schmieding, W., Popp, W. & Norpoth, K. (1995) Biomonitoring of urinary aromatic amines and arylamine, hemoglobin adducts in exposed workers and nonexposed control persons. *Int. Arch. occup. environ. Health*, **68**, 36–43

Rosenkranz, H.S. & Poirier, L.A. (1979) Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and noncarcinogens in microbial systems. *J. natl Cancer Inst.*, **62**, 873–892

Rosenkranz, H.S., Hyman, J. & Leifer, Z. (1981) DNA polymerase deficient assay. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 210–218

Rowland, I. & Severn, B. (1981) Mutagenicity of carcinogens and noncarcinogens in the *Salmonella*/microsome test. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 323–332

Rubino, G.F., Scansetti, G., Piolatto, G. & Pira, E. (1982) The carcinogenic effect of aromatic amines: an epidemiological study on the role of *o*-toluidine and 4,4′-methylene bis(2-methylaniline) in inducing bladder cancer in man. *Environ. Res.*, **27**, 241–254

Sadtler Research Laboratories (1980) *Sadtler Standard Spectra*, 1980 Cumulative Index (Molecular Formula Index), Philadelphia, p. 165

Safe, S., Hutzinger, O., Crocker J.F.S. & Digout, S.C. (1977) Identification of toxic impurities in commercial diphenylamine. *Bull. environ. Contam. Toxicol.*, **17**, 204–207

Salamone, M.F., Heddle, J.A. & Kate, M. (1981) Mutagenic activity of 41 compounds in the in vivo micronucleus assay. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 686–697

Sanner, T. & Rivedal, E. (1985) Tests with the Syrian hamster embryo (SHE) cell transformation assay. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 665–671

Savela, A., Vuorela, R. & Kauppinen, T. (1999) *ASA 1997* [Reviews 140], Helsinki, Finnish Institute of Occupational Health, pp. 47–48 (in Finnish)

Scott, J.K., Davidson, H. & Nelmes, A.J. (1985) Assays for inhibition of metabolic cooperation between mammalian cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 613–618

Sellers, C. & Markowitz, S. (1992) Reevaluating the carcinogenicity of ortho-toluidine: a new conclusion and its implications. *Regul. Toxicol. Pharmacol.*, **16**, 301–316

de Serres, F.J. & Ashby, J., eds (1981) *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier

Shackelford, W.M. & Keith, L.H. (1976) *Frequency of Organic Compounds Identified in Water* (EPA-600/4-76-062), Athens, GA, Environmental Research Laboratory, pp. 35, 226

Sharp, D.C. & Parry, J.M. (1981a) Use of repair-deficient strains of yeast to assay the activity of 40 coded compounds. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 502–516

Sharp, D.C. & Parry, J.M. (1981b) Induction of mitotic gene conversion by 41 coded compounds using the yeast culture *JD1* (Chapter 44). In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 491–501

Short, C.R., King, C., Sistrunk, P.W. & Kerr, K.M. (1983) Subacute toxicity of several ring-substituted dialkylanilines in the rat. *Fundam. appl. Toxicol.*, **3**, 285–292

Simmon, V.F. (1979) In vitro mutagenicity assays of chemical carcinogens and related compounds with Salmonella typhimurium. *J. natl Cancer Inst.*, **62**, 893–899

Skopek, T.R., Andon, B.M., Kaden, D.A. & Thilly, W.G. (1981) Mutagenic activity of 42 coded compounds using 8-azaguanine resistance as a genetic marker in *Salmonella typhimurium*. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 371–375

Son, O.S., Everett, D.W. & Fiala, E.S. (1980) Metabolism of *o*-[*methyl*-^{14}C]toluidine in the F344 rat. *Xenobiotica*, **10**, 457–468

Sorahan, T. & Pope, D. (1993) Mortality study of workers employed at a plant manufacturing chemicals for the rubber industry: 1955–86. *Br. J. ind. Med.*, **50**, 998–1002

Sorahan, T., Hamilton, L. & Jackson, J.R. (2000) A further cohort study of workers employed at a factory manufacturing chemicals for the rubber industry, with special reference to the chemicals 2-mercaptobenzothiazole (MBT), aniline, phenyl-beta-naphthylamine and o-toluidine. *Occup. environ. Med.*, **57**, 106–115

Stasik, M.J. (1988) Carcinomas of the urinary bladder in a 4-chloro-o-toluidine cohort. *Int. Arch. occup. environ. Health*, **60**, 21–24

Stillwell, W.G., Bryant, M.S. & Wishnok, J.S. (1987) GC/MS analysis of biologically important aromatic amines. Application to human dosimetry. *Biomed. environ. Mass Spectrom.*, **14**, 221–227

Stuermer, D.H., Ng, D.J. & Morris, C.J. (1982) Organic contaminants in groundwater near an underground coal gasification site in northeastern Wyoming. *Environ. Sci. Technol*., **16**, 582–587

Styles, J.A. (1981) Activity of 42 coded compounds in the BHK-21 cell transformation test. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 638–646

Styles, J.A., Clay, P. & Cross, M.F. (1985) Assays for the induction of gene mutations at the thymidine kinase and the Na^+/K^+ ATPase loci in two different mouse lymphoma cell lines in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 587–596

Sugimura, T., Nagao, M. & Wakabayashi, K. (1982) Metabolic aspects of the comutagenic action of norharman. *Adv. Exp. Med. Biol.*, **136B**, 1011–1025

Suk, W.A. & Humphreys, J.E. (1985) Assay for the carcinogenicity of chemical agents using enhancement of anchorage-independent survival of retrovirus-infected Fischer rat embryo cells. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 673–683

Tanaka, K., Marui, S. & Mii, T. (1980) Mutagenicity of extracts of urine from rats treated with aromatic amines. *Mutat. Res*., **79**, 73–176

Teass, A.W., DeBord, D.G., Brown, K.K., Cheever, K.L., Stettler, L.E., Savage, R.E., Weigel, W.W., Dankovic, D. & Ward, E. (1993) Biological monitoring for occupational exposures to o-toluidine and aniline. *Int. Arch. occup. environ. Health*, **65**, S115–S118

Thomson, J.A. (1981) Mutagenic activity of 42 coded compounds in the lambda induction assay. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative program*, Amsterdam, Elsevier Science, pp. 224–235

Thompson, C.Z., Hill, L.E., Epp, J.K. & Probst, G.S. (1983) The induction of bacterial mutation and hepatocyte unscheduled DNA synthesis by monosubstituted anilines. *Environ. Mutagen.*, **5**, 803–811

Thorgeirsson, S.S., Glowinski, I.B. & McManus, M.E. (1983) Metabolism, mutagenicity and carcinogenicity of aromatic amines. *Rev. Biochem. Toxicol*., **5**, 349–387

Topham, J.C. (1980) Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? *Mutat. Res.*, **74**, 379–387

Topham, J.C. (1981) Evaluation of some chemicals by the sperm morphology assay. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 718–720

Tsuchimoto & Matter, B.E. (1981) Activity of coded compounds in the micronucleus test. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 705–711

Tweats, D.J. (1981) Activity of 42 coded compounds in a differential killing test using *Escherichia coli* strains WP2, WP67 (*uvrA polA*), and CM871 (*uvrA lexA recA*). In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 199–209

Umeda, M., Noda, K. & Tanaka, K. (1985) Assays for inhibition of metabolic cooperation by a microassay method. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 619–622

United Nations Environment Programme (1999) *Recommendations and Legal Mechanisms* [http://dbserver.irptc.unep.ch:8887/irptc/owa/lg.get_search]

Verschueren, K. (1996) *Handbook of Environmental Data on Organic Chemicals*, 3rd Ed., New York, Van Nostrand Reinhold, pp. 1735–1737

Vian, L., Bichet, N. & Gouy D. (1993) The in vitro micronucleus test on isolated human lymphocytes. *Mutat. Res*., **291**, 93–102

Vitzthum, O.G., Werkhoff, P. & Hubert, P. (1975) New volatile constituents of black tea aroma. *J. agric. Food Chem*., **23**, 999–1003

Vogel, E.W. (1985) The Drosophila somatic recombination and mutation assay (SRM) using the *white-coral* somatic eye color system. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 313–317

Ward, E. & Dankovic, D.A. (1991) Bladder cancer in workers exposed to aniline (Letter to the Editor). *J. natl Cancer Inst.*, **83**, 1507–1508

Ward, E., Carpenter, A., Markowitz, S., Roberts, D. & Halperin, W. (1991) Excess number of bladder cancers in workers exposed to *ortho*-toluidine and aniline. *J. natl Cancer Inst*., **83**, 501–506

Ward, E.M., Roberts, D., Dankovic, D., Flesch, J., Reed, L. & Fajen, J. (1994) A re-examination of the cause of excess bladder cancers in chemical plant workers (Letter to the Editor). *J. natl Cancer Inst.*, **86**, 60–62

Ward, E.M., Sabbioni, G., DeBord, D.G., Teass, A.W., Brown, K.K., Talaska, G.G., Roberts, D.R., Ruder, A.M. & Streicher, R.P. (1996) Monitoring of aromatic amine exposure in workers at a chemical plant with a known bladder cancer excess. *J. natl Cancer Inst*., **88**, 1046–1052

Watanabe, T., Ishihara, N. & Ikeda, M. (1976) Toxicity of and biological monitoring for 1,3-diamino-2,4,6-trinitrobenzene and other nitro-amino derivatives of benzene and chlorobenzene. *Int. Arch. occup. environ. Health*, **37**, 157–168

Weisburger, E.K., Russfield, A.B., Homburger, F., Weisburger, J.H., Boger, E., Van Dongen, C.G. & Chu, K.C. (1978) Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. *J. environ. Pathol. Toxicol.*, **2**, 325–356

van Went, G.F. (1985) The test for sister-chromatid exchanges in Chinese hamster V79 cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 469–477

Williams, G.M., Tong, C. & Ved Brat, S. (1985) Tests with the rat hepatocyte primary culture/DNA-repair test. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 341–345

Winters, K., O'Donnell, R., Batterson, J.C. & Van Baalen, C. (1976) Water-soluble components of four fuel oils: chemical characterization and effects on growth micro algae. *Marine Biol*., **36**, 269–276

Würgler, F.E., Graf, U. & Frei, H. (1985) Somatic mutation and recombination test in wings of *Drosophila melanogaster*. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 325–340

Zdzienicka, M.Z. & Simons, J.W.I.M. (1985) Assays for the induction of mutations to 6-thioguanine and ouabain resistance in Chinese hamster ovary (CHO) cells in culture. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 583–586

Zdzienicka, M.Z. de Kok, A.J. & Simons, J.W.I.M. (1985) Assays for the induction of cell transformation in Chinese hamster ovary (CHO) cells and in Syrian hamster embryo (SHE) cells. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 685–688

Zeiger, E. & Haworth, S. (1985) Tests with a preincubation modification of the Salmonella/microsome assay. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 187–199

Zimmermann, F.K. & Scheel, I. (1981) Induction of mitotic gene conversion in strain *D7* of *Saccharomyces cerevisiae* by 42 coded chemicals. In: de Serres, F.J. & Ashby, J., eds, *Progress in Mutation Research*, Volume 1, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier Science, pp. 481–490

Zimmermann, F.K., Heinisch, J. & Scheel, I. (1985) Tests for the induction of mitotic aneuploidy in the yeast *Saccharomyces cerevisiae* strain D61.M. In: Ashby, J., de Serres, F.J., Draper, M., Ishidate, M., Jr, Margolin, B.H., Matter, B.E. & Shelby, M.D., eds, *Progress in Mutation Research*, Volume 5, *Evaluation of Short-Term Tests for Carcinogens. Report of the International Programme on Chemical Safety's Collaborative Study on in vitro assays*, Amsterdam, Elsevier Science, pp. 235–242