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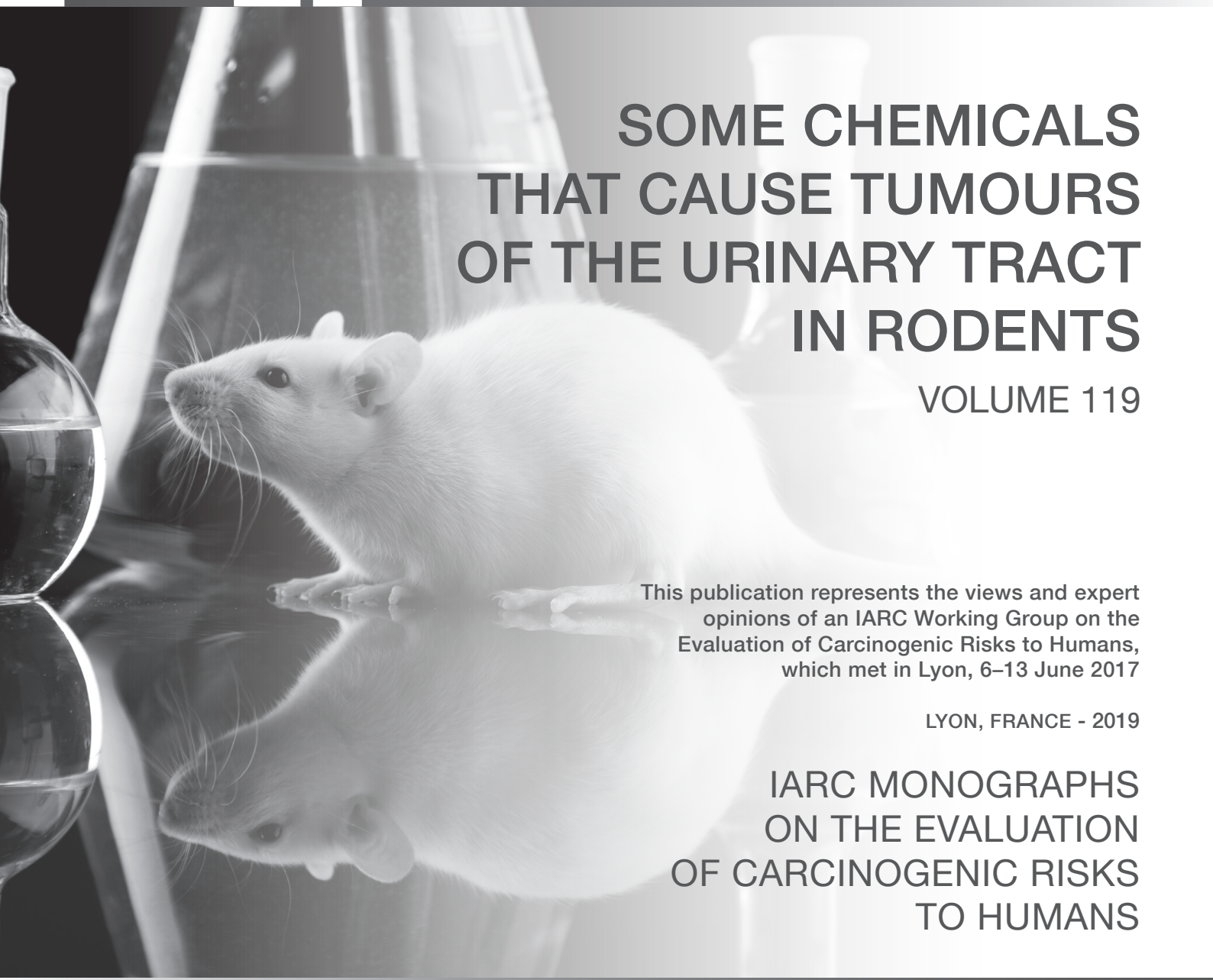


SOME CHEMICALS THAT CAUSE TUMOURS OF THE URINARY TRACT IN RODENTS

VOLUME 119

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 6–13 June 2017

LYON, FRANCE - 2019

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

MELAMINE

1. Exposure Data

This substance was considered by the Working Groups in 1985 (IARC, 1986), 1987 (IARC, 1987), and 1999 (IARC, 1999). New data have become available since that time, and these have been incorporated and taken into consideration in the present evaluation.

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 108-78-1

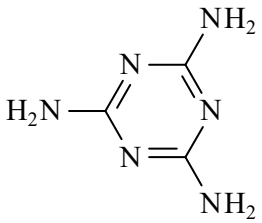
Previously used Chem. Abstr. Serv. Reg. Nos: 504-18-7; 65544-34-5; 67757-43-1; 68379-55-5; 70371-19-6; 94977-27-2

Chem. Abstr. Serv. name: 1,3,5-Triazine-2,4,6-triamine

IUPAC systematic name: Melamine

Synonyms: Cyanuramide; cyanurotriamide; cyanurotriamine; isomelamine; triaminotriazine; 2,4,6-triaminotriazine; triamino-*s*-triazine; 2,4,6-triamino-1,3,5-triazine; 2,4,6-second-triazinetriamine; 1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-triamine

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_3H_6N_6$

Relative molecular mass: 126.12

From IARC (1999); Merck Index (2013).

1.1.3 Chemical and physical properties

Description: Monoclinic prisms (Merck Index, 2013)

Melting point: 345 °C; decomposes (Lide, 1997); emits highly toxic fumes of cyanides when heated to decomposition (Sax, 1975); non-inflammable (Hawley, 1981)

Density: 1.573 g/cm^3 at 16 °C (Lide, 1997)

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

Octanol/water partition coefficient (P): log K_{ow}, –1.14 (Verschueren, 1996)

Conversion factor: 1 ppm = 5.16 mg/m^3 at normal temperature (25 °C) and pressure (103.5 kPa) (IARC, 1999)

Stability: Stable when stored under normal warehouse conditions (Crews et al., 2006)

Impurities: The purity of melamine products is highly dependent upon the manufacturing process and the level of purification employed. Melam, melem, ammeline, ammelide, ureidomelamine, and cyanuric acid have been described as impurities, generally present at less than 0.2% (WHO, 2008, 2009a).

1.2 Production and use

1.2.1 Production process

Melamine was first prepared and described by Liebig in 1834 (Liebig, 1834) and has since become an increasingly important chemical commodity (Crews et al., 2006). Until about 1960, melamine was prepared exclusively from dicyandiamide. This conversion was carried out in autoclaves at 10 MPa and 400 °C in the presence of ammonia. In the early 1940s, it was discovered that melamine could also be synthesized from urea at 400 °C, with or without catalyst. Today, melamine is produced industrially almost exclusively from urea using various low- or high-pressure processes. For details, see review in Crews et al. (2006).

1.2.2 Production volume

Melamine is listed by the Organisation for Economic Co-operation and Development (OECD) as a chemical with a high production volume. In 1970, world production capacity was estimated at 200 000 tonnes. Production in 1994 was 610 000 tonnes/year (Crews et al., 2006).

World production in 2007 was approximately 1 200 000 tonnes (WHO, 2009b). In 2013, it was more than 1 600 000 tonnes (Merchant Research & Consulting Group, 2015). In 2017, China was the biggest producer, accounting for about 50% or more of global production and exports and 41% of global consumption (IHS Markit, 2017).

The database Chem Sources International (2017) lists 46 manufacturing companies worldwide, of which 18 are located in the USA and 13 in China, including Hong Kong Special Administrative Region.

According to ECHA (2018), 100 000–1 000 000 tonnes of melamine are manufactured and/or imported in the European Economic Area per year.

1.2.3 Use

Melamine is a synthetically produced chemical that has many industrial uses, including the manufacture of melamine resins, laminates, glues and adhesives, surface coating resins, plastic moulding compounds, tarnish inhibitors, textile resins, textile finishes, permanent-press fabrics, bonding resins, flame-retardants, gypsum–melamine resin mixtures, orthopaedic casts, rubber additives and paper products, electrical equipment, construction materials such as plywood, and fertilizer urea mixtures (IARC, 1999; Hilts & Pelletier, 2009; WHO, 2009b; Tjioe & Tinge, 2010; IHS Markit, 2017; ECHA, 2018). However, it is primarily used in the production of melamine–formaldehyde resins for the manufacture of laminates, plastics, coatings (including can coatings), commercial filters, adhesives, and tableware. Important new applications are under development in the field of fire retardants for polymeric materials, especially polyurethane foams. Applications and uses of melamine differ widely among the main consumer countries or regions (Crews et al., 2006; WHO, 2009b; Castle et al., 2010).

Melamine has been used illegally to increase the nitrogen content in foods and animal feeds (Lachenmeier et al., 2009). Melamine contains about 66.6% nitrogen, and the addition of 1% melamine to protein leads to a false increase in the apparent protein content by 4.16%, if unspecific analytical methods are applied (Bisaz & Kummer, 1983). The first cases of melamine adulteration were detected in fish meal from Italy in the late 1970s (Cattaneo & Cantoni, 1979, 1982).

Most recently, a mass poisoning was reported in 2008 in China from contaminated milk and milk-based infant formula (WHO, 2009b).

1.3 Analytical methods

The most sensitive and selective analytical method to measure melamine, suitable for many matrices including milk, milk powder, and infant formula, as well as body fluids and tissues, is liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) (EFSA, 2010). Usable screening techniques for melamine include enzyme-linked immunosorbent assays (ELISA) and various spectroscopic techniques such as near infrared (NIR), Fourier transform infrared (FTIR), and nuclear magnetic resonance (NMR). The United States Food and Drug Administration (FDA) field laboratories use LC-MS/MS methods that are capable of determining melamine and cyanuric acid at concentrations of 0.25 ppm in powdered infant formula and other dairy-containing food products or ingredients, as well as a gas chromatography with mass spectrometry (GC-MS) method for melamine and its analogues (FDA, 2014; ECHA, 2016).

Several comprehensive reviews of analytical methods for melamine are available (Tyan et al., 2009; Tittlemier, 2010; Liu et al., 2012a; Lu et al., 2017; Nascimento et al., 2017; Wang et al., 2017). Representative methods for the analysis of melamine are presented in Table 1.1.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

Melamine does not occur naturally. Melamine may enter the environment from its industrial production, the processing and manufacture of resins, and from widespread use and disposal. In addition, the manufacture, use, and disposal of substances that degrade to form melamine (i.e. triazine-based herbicides, cyromazine, and trichloromelamine) may also lead to the presence of melamine in the environment (WHO, 2009a).

Melamine does not readily biodegrade; however, its bioconcentration potential is considered to be low. A biodegradation pathway with *Pseudomonas* involves the conversion of melamine to ammeline and cyanuric acid. Volatilization from moist soil and from water surfaces, biodegradation, and hydrolysis are not expected to be important environmental fate processes. Most of the melamine present in the environment is thought to be distributed in water, based on its physiochemical properties, with minor amounts being distributed to soil, sediment, biota, and air (HSDB, 2007).

Monitoring data from rivers in Japan have indicated low concentrations of melamine in water, sediment, and fish. Data from 1986–1994 indicate melamine concentrations of < 0.0001–0.0076 mg/kg in river waters, < 0.01–0.40 mg/kg in sediment, and < 0.02–0.55 mg/kg in biota (fish). These data were considered insufficient to estimate possible concentrations in drinking-water or fish in general (OECD, 1999).

In certain provinces in China, waste water and soil near melamine-manufacturing facilities (100 m) contained high concentrations of melamine. In these situations, melamine was detected in 13 of 37 waste water samples, at concentrations ranging from 0.02 to 227 mg/L. Concentrations of melamine detected in 31 of 65 soil samples near to melamine-manufacturing facilities ranged from 0.1 to 41 mg/kg. Six of 94 irrigation water samples, collected from rivers or from underground sources, contained melamine at detectable concentrations of 0.02–0.20 mg/L. Of the 124 soil samples collected from farmlands at least 150 km from melamine factories (from 14 provinces in China), only 1 contained melamine at a detectable concentration of 0.18 mg/kg (Qin et al., 2010).

Table 1.1 Representative methods for the analysis for melamine

Sample matrix	Assay procedure	Limit of detection	Reference
Animal feed	LC-MS/MS	12.3 µg/kg	Chen et al. (2009)
Beverages	HPLC-UV	50 µg/L (limit of quantitation)	Ishiwata et al. (1987)
Chicken eggs	LC-MS/MS	8 µg/kg	Wang et al. (2012)
Chinese cabbage	GC-NPD	100 µg/kg	Bardalaye et al. (1987)
Dog food	ELISA	1 mg/L	Garber (2008)
Eggs	GC-MS/MS	3.5 µg/kg	Miao et al. (2010)
Milk powder	GC-MS/MS	3.8 µg/kg	Miao et al. (2010)
Fish and shrimp	LC-MS/MS	3.2 µg/kg	Andersen et al. (2008)
Salmon	LC-MS/MS	7.4 µg/kg	Karbiwnyk et al. (2009)
Catfish, tilapia, trout, shrimp	LC-MS/MS	3.5 µg/kg	Karbiwnyk et al. (2009)
Wheat gluten, chicken feed and processed foods	SERS	0.033 µg/mL	Lin et al. (2008)
	HPLC	1 µg/mL	
Infant formula	LC-MS/MS	8 µg/kg	Braekevelt et al. (2011)
Infant formula	HPLC-VIS	0.1 µg/L	Faraji & Adeli (2017)
Infant formula powder	NIR, FTIR-ATR, FTIR-DRIFT	1 µg/kg	Mauer et al. (2009)
Infant formula and candy	SPE-LC-MS/MS	5 µg/kg	Lachenmeier et al. (2009)
	NMR 400 MHz tube	33.26 mg/kg	
	NMR 700 MHz HRMAS	0.69 mg/kg	
Kitchenware	HPLC-FD	8 µg/L	de Lourdes Mendes Finete et al. (2014)
Liquid milk	Spectrophotometry	80 µg/L	Chansuvarn et al. (2013)
Liquid milk	CE-DAD	120 µg/kg	Sun et al. (2010a)
Milk	CE-DAD	47 µg /L	Chen & Yan (2009)
Milk and dairy products	GC-MS	20 ng/kg	Jurado-Sánchez et al. (2011)
Milk and dairy products	HPLC-DAD	35–110 µg/kg	Filazi et al. (2012)
Milk and fish feed	HPCE	80 µg/L	Wen et al. (2010)
	HPLC	50 µg/L	
Milk and infant formula	LC-MS/MS	25 µg/kg	Desmarchelier et al. (2009)
Milk products and animal feed	Immunochromatographic strip test	1 mg/L	Li et al. (2011a)
Milk-based products	HPLC-MS/MS	100 µg/kg	Ibáñez et al. (2009)
Fruit juice and milk blends		10 µg/L	
Muscle tissue	HPLC-MS/MS	1.7 µg/kg	Filigenzi et al. (2007)
Nutritional food ingredients	UPLC-MS/MS	100 µg/kg	Draher et al. (2014)
Tissue and body fluids	ELISA	50 µg/L	Wang et al. (2010)
	GC-MS	1 µg/L	

Table 1.1 (continued)

Sample matrix	Assay procedure	Limit of detection	Reference
Urine	UPLC-MS/MS	6 µg/L	Cheng et al. (2009)
Urine	HPLC-MS/MS	0.66 µg/L	Panuwet et al. (2012)
Urine	LC-MS/MS	10 µg/L	Zhang et al. (2010)
Various foods	LC-MS/MS	< 20.7 µg/kg	Deng et al. (2010)
Vegetable protein products	LC-MS/MS	1 µg/L	Levinson & Gilbride (2011)
Wastewater	MLC	13 µg/L	Beltrán-Martinavarro et al. (2013)

ATR, attenuated total reflectance; CE, capillary electrophoresis; DAD, diode array detector; DRIFT, diffuse reflectance; ELISA, enzyme-linked immunosorbent assay; FD, fluorescence detection; FTIR, Fourier transform infrared spectroscopy; GC, gas chromatography; HPCE, high-performance capillary electrophoresis; HPLC, high-performance liquid chromatography; HRMAS, high-resolution magic angle spinning; LC, liquid chromatography; MLC, micellar liquid chromatography; MS/MS, tandem mass spectrometry; NIR, near-infrared spectroscopy; NMR, nuclear magnetic resonance spectroscopy; NPD, nitrogen-phosphorus detector; SERS, surface-enhanced Raman spectroscopy; SPE, solid-phase extraction; UPLC, ultra-performance liquid chromatography; UV, ultraviolet; VIS, visible detection

Melamine and other triazine compounds are used as nitrogen sources in slow-release urea-based fertilizer mixtures (Hilts & Pelletier, 2009). In China, the majority of crop samples tested, including 235/246 maize samples, 141/143 soybean samples, and 166/168 wheat samples, collected from 21 provinces between October and December in 2008, contained melamine at detectable levels. However, less than 20% of crop samples contained melamine at concentrations above 0.1 mg/kg, and only 3 samples above 1 mg/kg, with the maximum of 2.05 mg/kg measured in a wheat sample (Qin et al., 2010).

1.4.2 Occurrence in food

(a) Food-contact materials

Melamine can be present in food as a result of its use in food-contact materials, including articles made of melamine–formaldehyde plastics, can coatings, paper and paperboard, adhesives, and cellophane polymers (WHO, 2009a; Bradley et al., 2011).

Melamine has been shown to migrate into food and food simulants from melamine–formaldehyde tableware. The amount of melamine migration is dependent on temperature, acidity, contact time, and simulant used, as well as the quality of the product (Ishiwata et al., 1986; Sugita et al., 1990; Martin et al., 1992; Bradley et al., 2005; Lund & Petersen, 2006; Bradley et al., 2011; Chien et al., 2011; Chik et al., 2011). Studies demonstrated that high temperatures applied to foods or simulants strongly influenced the degradation and migration of melamine, while the duration of heating and food and/or simulant acidity had only a minor influence (Bradley et al., 2011). When kitchen utensils containing melamine were tested under boiling conditions, migration was especially high. In microwave heating, high peak temperatures ("hot spots") have been shown to result in high rates of melamine transfer despite short contact times (Bradley et al., 2010; BfR, 2011).

Melamine transfer into foods and simulants results from the migration of residual free monomers present after the manufacturing process; further transfer results from polymer breakdown, as well as chemical degradation and hydrolysis of the melamine resins (Martin et al., 1992; Lund & Petersen, 2006; Bradley et al., 2010; BfR, 2011).

Like the migration and polymer breakdown that has been observed in melamine tableware, melamine-based resins used as can coatings and on metal closure lids of glass jars in the food industry also appear to degrade. This clearly occurs by hydrolysis of the melamine cross-linked resins, resulting in the release of additional melamine during the retort canning process (Bradley et al., 2011; Magami et al., 2015).

(b) Precursor compounds that can form melamine

Melamine can occur in the environment and in food via commonly used chemicals that can form melamine (see Table 1.2).

Trichloromelamine, which decomposes to melamine, is a sanitizer and disinfectant for use on food packaging materials (except milk containers), hard food-contact surfaces, food processing equipment and utensils (except for dairy applications), and as a component of fruit and vegetable wash solutions in the USA (commercial disinfectant solutions diluted before use). It may also be used in other countries. The FDA estimated the concentration of melamine in food from disinfection to be 0.14 mg/kg based on a very conservative assumption that all disinfectants contain trichloromelamine (WHO, 2009b).

Humans are also exposed to melamine in food as a metabolite and degradation product of triazine-based pesticides, such as cyromazine (Cook & Hütter, 1981; WHO, 2009b). Cyromazine can undergo metabolism in crop plants, poultry, ruminants, and other animals to form melamine.

Table 1.2 Precursor compounds that can degrade or metabolize to form melamine

Compound	Use of compound	Conditions under which the compound converts/metabolizes to melamine	Reference
Trichloromelamine (CAS No. 7673-09-8)	Sanitizer and disinfectant for use on food-packaging materials (except milk containers); food-processing equipment and utensils (except for dairy applications); hard food-contact surfaces and as a component of fruit and vegetable washes; non-food sanitizer e.g. in hospitals; pesticide	Readily decomposes to melamine during regular use as sanitizer	EPA (2005); WHO (2009a)
Cyromazine (CAS No. 66215-27-8)	Pesticide and herbicide; veterinary drug; insecticide (feed-through larvicide in poultry, incorporated in feed of laying hens to prevent flies hatching in manure, inhibits insect growth in cattle manure)	Undergoes metabolism in crop plants via dealkylation reactions to form melamine Undergoes metabolism via dealkylation reactions in poultry, ruminants, and other animals to form melamine Aerobic degradation to melamine in soil Photolytic degradation to melamine on soil	Sancho et al. (2005); Karras et al. (2007); Hilts and Pelletier (2009); WHO (2009a)
Triazine-based compounds	Pesticides, insecticides and herbicides As nitrogen sources in slow-release urea-based fertilizer mixtures (unknown whether melamine is still used); extent of use in fertilizers unknown	Certain triazine-based compounds may undergo environmental (bacterial, fungal) degradation and/or metabolism in crop plants	Hilts and Pelletier (2009); WHO (2009b)
Prometryn (CAS No. 7287-19-6)	Used as herbicide (triazine-based) on fruit and vegetable crops, cotton, potatoes, pastures, seed crops	Not present in fruit and vegetable crops, needs to be ingested by animals to degrade and form melamine	DAFF (2008)

CAS, Chemical Abstracts Service

Cyromazine is used in many countries as an insecticide, pesticide, or veterinary drug (EFSA, 2008; WHO, 2009b). Melamine residues on the edible part of vegetables resulting from the application of cyromazine are generally expected to be less than 1 mg/kg. These levels were found in tomatoes, lettuce, and celery plants treated with cyromazine in Japan (WHO, 2009c).

(c) Animal feed

Melamine is not permitted as an additive for animal feed in the European Union or USA (Hilts & Pelletier, 2009; EFSA, 2010). However, melamine can be present as an impurity in urea-based commercial feed additives used in ruminants at concentrations of up to 50 mg/kg (EFSA, 2010). Animal feed may also contain melamine as a result of its presence in the environment from approved uses of triazine pesticides and fertilizers (WHO, 2009b). On a worldwide scale, the adulteration of animal feed or feed ingredients with melamine and derivatives has been practised since 1989 and possibly earlier (Cattaneo & Ceriani, 1988; Cianciolo et al., 2008; NTP, 2008; Cruywagen & Reyers, 2009; González et al., 2009; WHO, 2009b).

Animal feed additives such as guanidinoacetic acid or urea can contain melamine as an impurity, which would result in trace amounts of melamine in feed (European Commission, 2009).

Melamine may also enter the food chain indirectly as a result of carry-over from adulterated animal feed into products of animal origin such as milk, eggs, meat, and fish (FDA, 2007; Pittet et al., 2008; WHO, 2009b). Carry-over of melamine from animal feed into animal tissues and/or products has been demonstrated in pigs (Buur et al., 2008; Wang et al., 2014), eggs and body tissues of laying hens (Bai et al., 2010; Dong et al., 2010; Valat et al., 2011; Gallo et al., 2012; Novák et al., 2012), eggs of Japanese quail (Zhang et al., 2012), dairy cows' milk (Cruywagen et al., 2009; Battaglia et al., 2010) and body tissues (Sun et al., 2011), body tissues of sheep (Lv et al., 2010; Cruywagen et al., 2011), milk of dairy goats (Baynes et al., 2010), and fish (Andersen et al., 2008, 2011). These studies reported that melamine deposition and/or carry-over transfer rates from feed into animal products ranged from non-detectable to 3.6% in mammals and birds. Higher rates were reported in fish and shrimp.

(d) Melamine levels in food

Melamine concentrations measured in survey data submitted for consideration at a 2008 WHO Expert Meeting could not be easily distinguished as resulting from baseline contamination (levels occurring indirectly from approved uses of melamine or melamine precursors) or intentional adulteration, since a large number of samples were targeted as a result of potential adulteration. However, baseline levels are generally expected to be less than 1 mg/kg (Hilts & Pelletier, 2009; WHO, 2009b).The concentrations of melamine in non-adulterated foods are shown in Table 1.3.

1.4.3 Exposure in the general population

Exposure of the general population to melamine is thought to result primarily from the ingestion of melamine in non-adulterated food (HSDB, 2007). Estimates of exposure to melamine were provided by the OECD in the 1999 screening assessment, and are summarized in Table 1.4. This table presents exposure estimates from available studies in which some form of estimated exposure to melamine was provided. [The Working Group noted that the manner in which exposure was estimated differed between the studies, so direct comparisons could not be made.]

The WHO Expert Meeting estimated dietary exposure to melamine for scenarios using both baseline and adulterated concentrations in food (WHO, 2009b); the latter are discussed in Section 1.4.4. Baseline exposure estimates were made using data on concentrations of melamine

Table 1.3 Survey data on concentrations of melamine in non-adulterated food

Region or authority conducting survey	Foods surveyed	Limit of quantitation	Median melamine concentration (range)[a]	Fraction of total samples found to be positive (%)	Reference
Australia, Canada, New Zealand, Taiwan (China), USA	Infant formula	NR	All < 1 mg/kg	NR	WHO (2009b)
Health Canada Survey	Milk (n = 73) and soy-based (n = 19) infant formula (liquid ready-to-eat or concentrated [n = 31] and powdered [n = 63]) (total, n = 94)	4 ng/g	16 ng/g (range, 4.3–346 ng/g, "as purchased"; 5.5–69 mg/kg, calculated "as consumed")	71/94 (76%)	Health Canada (2008); Tittlemier et al. (2009); Braekevelt et al. (2011)
Health Canada Survey[b]	Domestic and imported dairy products and soy-based dairy substitutes (n = 117); milk- and soy-containing items originating from Asia (n = 91); TDS milk and yogurt composites, 2004–2007 (n = 38)	4 ng/g	13 samples from China, 51 ng/g; 9 samples from North America, 8 ng/g; individual dairy and soy products (4.35–282 ng/g); TDS composite samples (95.1–7.2 ng/g)	Individual samples positive, 28/208 (13%); TDS composites positive, 4/38 (11%)	Tittlemier et al. (2010a)
Health Canada Survey[b]	A variety of egg-containing, soy-based, vegetable or fish and shrimp products (n = 364); TDS shrimp composites, 1993–2008 (n = 14)[c]	4 ng/g	Median values not reported Egg-containing items (5.1–247 ng/g); soy-based meat substitutes (4.1–47.9 ng/g); fish and shrimp products (4.1–1100 ng/g); vegetable products (4.6–688 ng/g); (5.6–29.8 ng/g) TDS shrimp composites	98/378 (26%) samples positive 8/113 (8%) egg-containing items; 8/87 (9%) soy-based items; 32/64 (50%) fish and shrimp products; 46/100 (46%) vegetable products; 6/14 (43%) TDS shrimp composites	Tittlemier et al. (2010b)
China	Eggs collected from markets	10 ng/g	(84–206 ng/g)	6/42 (14%)	Xia et al. (2009)
Germany	Protein powder, food supplements, sports food (n = 99)	1000 ng/g	ND	0%	Lachenmeier et al. (2017)

ND, not detected; NR, not reported; TDS, Total Diet Study (Canadian market basket survey that samples various food items from four different grocery stores and fast food restaurants in a selected Canadian city over a 5-week period each year (Conacher et al., 1989); foods are prepared as for consumption, and replicate food items from the various grocery stores or restaurants visited are combined and homogenized to form a composite sample)

[a] Range of positives unless otherwise indicated

[b] Many of the food items analysed were complex multi-ingredient processed foods collected at the retail level, and thus the source of the melamine in these items cannot be easily identified

[c] Most TDS shrimp composites collected after 2001 were found to contain melamine, suggestive of a relatively recent exposure to melamine

Table 1.4 Estimates of exposure to melamine in the general population from various permitted uses and unintentional contamination, as reported from various sources

Source of exposure	Estimated daily exposure (μg/kg bw)	Comments[a]	Reference
Indirect exposure via the environment	1.1	Based on local monitoring data; used highest monitored concentration in drinking-water (0.0076 mg/L), and in fish (0.55 mg/kg); water intake, 2 L per d; fish intake, 0.115 kg per d; for 70 kg bw	OECD (1999)
	2.4	Based on modelled data for local level (EUSES)	
	0.05	Based on modelled data for regional level (EUSES)	
Overall exposure to consumer (i.e. general population)	10	Based on modelled data indirect via environment, dermal and inhalation from contact with polymers containing melamine (0.003 mg/kg bw, assumed 1% of occupational exposure), and from migration into food from melamine tableware (0.007 mg/kg bw; assuming average intake of hot food of 0.5 kg/d and 70 kg bw)	
Infant formula	0.54–1.60	Mean exposure; Health Canada occurrence dataset for baseline levels in infant formula (values < LOD = 1/2 LOD = 2 μg/kg); Institut National de Santé Publique du Québec, 2001 consumption estimates	Crossley et al. (2009); WHO (2009b)
Foods other than infant formula	0.03–0.12	Adults, mean exposure; Health Canada occurrence dataset for baseline levels in foods (values < LOD = LOD = 4 μg/kg); EFSA Concise European Food Consumption Database for 17 countries; 60 kg bw	
Disinfection in food processing	7	Adults, very conservative estimate; 0.14 mg/kg food, assumed all disinfectants contained trichloromelamine; 3 kg food consumption; 60 kg bw	
Migration from melamine-containing plastics (melamine tableware)	13	Adults, conservative estimate; assumes concentration of 1 mg/kg food; 25% of diet in contact with melamine tableware (0.25 × 3 kg = 750 g/person per d); 60 kg bw	
Migration from melamine-containing adhesives	< 0.35	Adults, conservative estimate; 3 kg food consumption; 60 kg bw	
Migration from melamine-containing paper and paperboard	0.0019	Adults, conservative estimate; 3 kg food consumption; 60 kg bw	
Residues arising from use of cyromazine as a pesticide	0.04–0.27	Adults, conservative estimate; concentration levels from STMR of the JMPR in 2007 (FAO, 2007) for cyromazine, assumed that ~10% of cyromazine residue was melamine, except for edible offal and mushrooms where assumed equal to STMR; GEMS/Food 13 cluster diets; 60 kg bw	

Table 1.4 (continued)

Source of exposure	Estimated daily exposure (µg/kg bw)	Comments[a]	Reference
Mean adult exposure from food	1.09–2.16	Using mean upper-bound melamine concentrations from industry dataset; EFSA CEFCD 19 countries, individual data	EFSA (2010)
Adult exposure from food	2.05–3.92	Using mean upper-bound melamine concentrations, 95th percentile, from industry dataset; EFSA CEFCD 19 countries, individual data	
Mean adult exposure from food	2.66–6.16	Using 95th percentile upper-bound melamine concentrations from industry dataset; EFSA CEFCD 19 countries, individual data	
Adult exposure from food	6.21–10.58	Using 95th percentile upper-bound melamine concentrations from industry dataset; EFSA CEFCD 19 countries, individual data	
Infant exposure from infant formula	1.3 (mean) and 1.8 (high)	800 g/d as mean intake, 1100 g/d as high value intake; 95th percentile upper-bound occurrence value; 6 kg bw; assume 1 part formula to 7 parts water	
Adult exposure from cyromazine use (sheep, poultry)	< 0.020 (for each)	300 g meat consumption; 60 kg bw adult	
Adult exposure from cyromazine use (eggs)	0.260–0.780	100 g egg consumption; 60 kg bw adult	
Migration from melaware (melamine tableware); scenario A; children aged 1–2 yr and 3–6 yr	Mean, 30–80; 95th percentile, 50–120	Scenario A, "typical migration levels"; assumes migration into food: 1 mg/kg acidic foods HF, 0.6 mg/kg aqueous foods HF, 0.2 mg/kg fatty foods HF, 0.05 mg/kg dry foods HF; summed exposure from all food groups; EXPOCHI consumption data, 12 Member States	
Migration from melaware (melamine tableware); scenario B; children aged 1–2 yr and 3–6 yr	Mean, 40–110; 95th percentile, 70–230	Scenario B, "high migration levels"; assumes migration into food: 5 mg/kg acidic foods, 3 mg/kg aqueous foods, 1 mg/kg fatty foods, 0.05 mg/kg dry foods; food item leading to highest exposure; EXPOCHI consumption data, 12 Member States	
Migration from coatings on metal cans and closures, infants aged 6 mo	34	Very conservative; 0.407 kg commercial baby food and drinks consumed (95th percentile) + 0.125 kg powdered infant formula; 7.8 kg bw; 0.5 mg/kg migration from coatings	
Migration from coatings on metal cans and closures, children aged 1.5 yr	92	Very conservative; 2 kg food consumed; 11 kg bw; 0.5 mg/kg migration from coatings	
Migration from coatings on metal cans and closures, adults	25	Very conservative; 3 kg food consumed; 60 kg bw; 0.5 mg/kg migration from coatings	

[a] Upper-bound values < LOD, set equal to LOD

bw, body weight; CEFCD, Concise European Food Consumption Database; d, day(s); EUSES, European Union System for the Evaluation of Substances; EXPOCHI, EFSA Article 36 project, individual food consumption data and exposure assessment studies for children; FAO, Food and Agriculture Organization of the United Nations; GEMS, Global Environment Monitoring System; HF, hot filled; JMPR, Joint FAO/WHO Meeting on Pesticide Residues; LOD, limit of detection; mo, month(s); STMR, supervised trial median residue levels; yr, year(s)

Note: the Working Group considered that it was not appropriate to sum the dietary exposure assessments from different sources within each report, as the individual exposure assessments were generally very conservative

in different foods, together with food consumption data or very conservative exposure estimates. Estimates of exposure to melamine at baseline concentrations from various sources suggested: a maximum of 13 μg/kg body weight (bw) per day from the migration of melamine from tableware products such as cups, bowls, plates, or utensils; a maximum of 7 μg/kg bw per day from disinfection in food processing; a mean exposure of 0.54–1.6 μg/kg bw per day from infant formula; and a mean exposure for adults of 0.03–0.12 μg/kg bw per day from other foods.

In 2010, the European Food Safety Authority identified legitimate potential sources of melamine in food, including from food-contact materials, and estimated the associated dietary exposures (Table 1.4). Data submitted by industry, after excluding a small number of samples related to the adulteration incident, were used as the basis for dietary exposure assessment. For adult consumers of high concentrations, the dietary exposure estimates for melamine using the Concise European Food Consumption Database upper-bound occurrence values were less than 11 μg/kg bw per day (EFSA, 2008). For infants fed solely formula, the dietary exposure estimates were all less than 2 μg/kg bw per day. These estimates were considered to be conservative because many of the occurrence data were upper-bound values for samples in which melamine was found to be below the limit of detection (EFSA, 2010).

1.4.4 Exposure to melamine from contaminated food

(a) Humans

The largest incident of melamine poisoning occurred in China, beginning in the spring of 2008 (Chen, 2009). Relatively pure melamine was used in the illegal adulteration of raw milk that was subsequently used in the manufacture of infant formula and other foods (WHO, 2009b; Dorne et al., 2013; Wang et al., 2013a). Because of globalization and the worldwide trade of food, melamine-contaminated foods containing milk products from China were detected in a large number of countries, including North America and the European Union (Lachenmeier et al., 2009).

Table 1.5 presents estimates of exposure to melamine in infants and young children from the adulterated infant formula. Based on the median melamine concentration (1000 mg/kg), estimates of melamine exposure for Chinese children exposed to adulterated infant formula ranged from 8.6 to 23.4 mg/kg bw per day (Jia et al., 2009; WHO, 2009b). Based on the mean melamine concentration (1212 mg/kg), 90th percentile concentration (2600 mg/kg), and maximum concentration (4700 mg/kg), dietary exposure estimates of melamine for children aged 3–24 months were 10.4–28.4 mg/kg bw per day, 22.3–61.0 mg/kg bw per day, and 40.3–110.2 mg/kg bw per day, respectively (Jia et al., 2009).

Dietary exposure to melamine from foods (other than infant milk formula) containing adulterated milk powder (e.g. ice cream, yoghurt, meal replacements, biscuits, chocolates) was also estimated during the WHO Expert Meeting. Using a conservative approach, assuming melamine was present in all food groups with the highest reported result for a food in that group and an average body weight of 60 kg, a dietary exposure of 0.16–0.70 mg/kg bw per day was estimated for adults consuming products adulterated with melamine (WHO, 2009b).

While limits were implemented (see Section 1.5) and food surveillance strengthened following this crisis, melamine contamination was still occasionally reported for protein-rich foods and food supplements in some countries (Gabriels et al., 2015; Deldicque & Francaux, 2016). Probably due to tightened importation and market controls in Germany, no contamination was found in these products in Karlsruhe (Lachenmeier et al., 2017).

Table 1.5 Estimates of exposure to melamine from adulterated infant formula in 2008 and associated urolithiasis in infants and young children, as reported from various sources in China

Age of affected child	Age of screened child	Duration of exposure[a]	Exposure history[b]	Region	Reference
1–96 mo (mean, 25 mo)	1–126 mo (mean, 28 mo)	1.3–84 mo (mean, 19.5 mo)	12–2563 mg/kg (mean, 1295.3 mg/kg) in serum	Anhui	Hu et al. (2010); Hu et al. (2013)
≤ 3 yr	≤ 3 yr	≥ 30 d	High content (> 500 mg/kg): *n* = 23; moderate content (< 150 mg/kg): *n* = 19; no melamine: *n* = 8	Beijing	Guan et al. (2009)
17.5 ± 9.3 mo (stones) vs 16.9 ± 9.0 mo, mean (SD)	≤ 3 yr	Median, 6 mo (stones) vs 1 mo	0.77 (stones) vs 0.04 mg/kg bw per d, median exposure using current body weight; 2.35 (stones) vs 0.13 mg/kg bw per d, median exposure using birth weight; range of exposures, 0–51.2 mg/kg bw per d using current body weight, 0–102.4 mg/kg bw per d using birth body weight Four adulterated infant formula brands (12, 53.4, 150, and 2563 mg/kg)	Beijing	Li et al. (2010)
5–72 mo (median, 15 mo)	Mostly infants, also children	2–30 mo (mean, 13.7 ± 7.4 mo)		Gansu	Nie et al. (2013)
3 mo–4 yr; 91.7% < 3 yr (mean, 10 mo)	≤ 4 yr	1–24 mo	Melamine concentration of formula: 955–2563 mg/kg (consumed by 11 patients with stones); 6.2–17 mg/kg (consumed by 1 patient with stones). All consumed other foods or breast milk in addition to adulterated formula	Guang Dong	Zhu et al. (2009)
≤ 36 mo (mean, 19.8 mo)	≤ 36 mo	3 mo	36–220 mg/d (mean, 116 mg/d)	Yuanshi county	Liu et al. (2010b)
2–96 mo (median, 27 mo; geometric mean, 24 mo)	2–96 mo	2–96 mo (median, 20 mo; geometric mean, 17 mo)	0.01–62.67 mg/kg bw per d (median, 0.9; geometric mean, 1.28)	Shandong	Chen et al. (2009)
< 3 yr (77/79 children, 97.47%) (mean, 13.52 ± 10.13 mo)	4–72 mo (median, 15 mo)	With stones: 0.5–45 mo (mean, 12.53 ± 8.47 mo; median, 12 mo), 79 screened Without stones: mean, 8.65 ± 3.4 mo, 103 screened	5.17 ± 4.53 mg/kg bw per d (those with stones); 2.38 ± 3.39 mg/kg bw per d (103 screened without stones); exposed to 1–3 different brands of contaminated formula (those with stones)	Shandong	Sun et al. (2010b)
≤ 6 yr	≤ 6 yr	1–36 mo	High (Sanlu brand, 162–2563 mg/kg); medium (Sanlu and other brands); low (other brands, 0.09–150 mg/kg)	Shanghai	Gao et al. (2011)

Table 1.5 (continued)

Age of affected child	Age of screened child	Duration of exposure[a]	Exposure history[b]	Region	Reference
2–138 mo (median, 27.4 ± 25.5 mo)	Infants and children	1–54 mo (mean, 13.3 mo), stones; 1–96 mo (mean, 11.5 mo), no stones	Sanlu and Nanshan brand formula, > 5500 mg/kg; other formula, < 200 mg/kg; those with stones: 30.9% fed formula, 69.1% fed breast milk and formula; those without stones: 39% and 61%, respectively Those with stones: 56.7% fed Sanlu only, 13.4% fed Sanlu + others/Nanshan ± others, 29.9% fed other brands (< 200 mg/kg) Those without stones: 0.18% fed Sanlu only, 19% fed Sanlu + others/Nanshan ± others, 80.8% fed other brands	Sichuan	Wang et al. (2011)
1–60 mo (median, 16 mo) for 326 children with stones who had detailed data	1–180 mo (mean, 22 mo)	Mean, 15.7 ± 12.84 mo (stones); mean, 12.53 ± 9.49 mo (without stones)	Highest melamine concentration for brands: Sanlu, 2563 mg/kg; Shengyuan, 150 mg/kg; Yashili, 53.40 mg/kg; Shien, 17 mg/kg; Yili, 12 mg/kg	Zhejiang	Zhang et al. (2009)
88.6% ≤ 36 mo	1 mo–15 yr (mean, 22 mo)	≥ 1 mo	0.09–2563 mg/kg in formula (22 brands)	Zhejiang	He et al. (2009)
< 3 yr	NR	High exposure group: 0.67–36 mo (mean, 7.2 mo); low exposure group: 3–48 mo (mean, 17.4 mo)	High exposure: > 2.5 mg/kg; low exposure: 0.05–2.5 mg/kg; control exposure: < 0.05 mg/kg (LOD)	Taiwan, China	Wang et al. (2009a)
1.3–9 yr; high exposure: 1.3–4.8 yr; low exposure: 2.5–4 yr; control exposure: 1.9 and 9 yr	0–16 yr	High exposure: median, 12 mo (3.3–24.0); control exposure: median, 6 mo (4.0–7.0)	High exposure: > 2.5 mg/kg; low exposure: 0.05–2.5 mg/kg; control exposure: < 0.05 mg/kg (LOD)	Taiwan, China	Wang et al. (2009b)
NR	0.1–12.9 yr (mean, 6.4 yr)	≥ 1 mo	0.01–0.21 mg/kg bw per d (stones or renal deposits); 0.25 to > 1.5 L formula consumed daily; 68 mg/kg, highest concentration of melamine-adulterated formula	Hong Kong SAR	Lam et al. (2008)
3.5–32 mo	NR	3–24 mo; median, 12 mo	0.87–2002 µg/mmol creatinine (median, 21) urinary melamine levels; 1–3 brands melamine-contaminated infant formula consumed, 20–210 g daily Controls: 0.08–37 µg/mmol creatinine (median, 6.6) urinary melamine levels	Hong Kong SAR	Lam et al. (2009)

Table 1.5 (continued)

Age of affected child	Age of screened child	Duration of exposure[a]	Exposure history[b]	Region	Reference
6.7 yr, renal stones; 9.5 yr, renal deposits; 7 yr, suspected renal deposits	≤ 12 yr	26, 47, 24 pack mo	68 mg/kg, highest concentration in melamine-adulterated formula	Hong Kong SAR	Lau et al. (2012)

d, day(s); LOD, limit of detection; mo, month(s); NR, not reported; SAR, Special Administrative Region; SD, standard deviation; vs, versus; yr, year(s)

[a] Those with stones unless indicated otherwise; indicated and reported as a range unless otherwise indicated

[b] Those with stones unless indicated otherwise

There may be overlap between studies; cases reported by some authors may also be among the cases described by other authors in consideration of the institutional affiliation of some of the authors

(b) Companion animals

After an investigation by the FDA, it was determined in 2007 that wheat flour, presented as wheat gluten and rice protein, imported from China as pet food ingredients and subsequently incorporated into pet food manufactured in North America, had been contaminated with melamine and its analogues, cyanuric acid, ammeline, and ammelide. Melamine had been deliberately added to the wheat flour to falsely elevate the measured protein levels, in order to claim that the product was wheat gluten (Brown et al., 2007; Dobson et al., 2008; Hilts & Pelletier, 2009; WHO, 2009b; Dorne et al., 2013). The estimated number of deaths of dogs and cats attributable to exposure to pet food contaminated with melamine and cyanuric acid ranged between 2000 and 7000 (Dorne et al., 2013).

1.4.5 Biomonitoring data and biomarkers of exposure

Melamine is poorly metabolized and is mainly excreted in urine (IARC, 1999; WHO, 2009b). The estimated half-life for urinary elimination of melamine in humans is approximately 6 hours (Wu et al., 2013, 2015a).

Urine samples were analysed in the general population of the USA; 76% of 492 urine samples contained melamine at detectable levels (limit of detection, 0.66 ng/mL). The geometric mean and 95th percentile concentrations were 2.4 ng/mL and 12 ng/mL [approximately 0.24 µg/mmol creatinine and 1.2 µg/mmol creatinine], respectively (Panuwet et al., 2012).

Lin et al. (2013) analysed 87 urine samples from 22 children aged 6–10 years and 70 urine samples from their parents in a community in Taiwan, China, and detected melamine in 98.7% of the samples. The median (and interquartile range) of melamine concentrations from the children's urine were 0.93 (0.49–1.30) µg/mmol creatinine for the first spot samples, and 1.73 (0.84–2.74) µg/mmol creatinine for the second spot samples 24 hours later. For their parents, the corresponding melamine concentrations were 0.84 (0.51–1.97) and 0.87 (0.36–1.44) µg/mmol creatinine for the fathers (n = 22), and 0.87 (0.58–2.36) and 1.21 (0.65–2.14) µg/mmol creatinine for the mothers (n = 22).

In 2007–2008, a population survey was conducted in Hong Kong Special Administrative Region to examine the prevalence of metabolic syndrome in schoolchildren. The melamine concentrations in spot urine tests of the 502 children examined ranged from undetectable to 1467 µg/mmol creatinine (median, 0.8 µg/mmol creatinine; 58% of samples had concentrations above the limit of detection) (Kong et al., 2011, 2013). Similarly, Wu et al. (2015b) found that melamine was detectable in about two thirds of 264 urine samples from 88 university students in Taiwan, China. The geometric mean concentration and the highest measures were 6.5 ng/mL and 219 ng/mL [approximately 0.6 µg/mmol creatinine and 21.9 µg/mmol creatinine], respectively.

In humans, melamine reacts with uric acid to form melamine–urate crystals in the kidney (Cruywagen et al., 2011). In a study in Taiwan, China, in 211 adult patients diagnosed with calcium urolithiasis and 211 age- and sex-matched controls, urinary levels of melamine ranged from below the limit of detection to 192 ng/mL (62.1% detectable) [~19.2 µg/mmol creatinine] in case patients, and from below the limit of detection to 56 ng/mL (20.4% detectable) in controls [~5.6 µg/mmol creatinine] (Liu et al., 2011). In another study in 11 adults with uric acid urolithiasis, 22 adults with calcium urolithiasis, and 22 age- and sex-matched controls, measured median urinary concentrations of melamine were 0.50, 0.14, and 0.06 µg/mmol creatinine, respectively (Wu et al., 2010a).

1.4.6 Occupational exposure

Melamine has in the past been widely considered to be a low-toxicity dust except if decomposed by heat, when it emits highly toxic fumes of nitrogen oxides and hydrogen cyanide (PubChem, 2018). There are very few published exposure measurements for this substance, although occupational exposure could potentially occur by inhalation and inadvertent ingestion from hand-to-mouth contacts. Occupational exposure to melamine is most likely from its use in synthetic resins. Workers exposed to melamine may also be exposed to wood dust, phenol, formaldehyde, urea, and other hazardous substances (Blair et al., 1990a).

Wu et al. (2015a) investigated exposure to melamine among 44 workers in a small study at two factories manufacturing melamine tableware in Taiwan, China. Workers were involved in manufacturing and moulding, grinding and polishing, packing, and administration. In addition, a group of 105 non-exposed control workers was recruited from a neighbouring factory. Personal and area air samples at the worksite were obtained daily over 1 week for the workers exposed to melamine; pre- and post-shift spot urine samples were also acquired on each workday, as well as one spot urine sample each weekend morning and the following Monday morning. A single spot urine sample was collected on Friday morning from the control group. A blood sample was also obtained from all cases and controls. All samples were analysed for melamine. Air samples were also collected to measure exposure to formaldehyde. Exposure to melamine in the manufacturing and moulding group was consistently highest (mean personal air concentration, 97 μg/m^3; urine, 84.4 μg/mmol creatinine; and serum, 7.2 ng/mL) compared with the administrative workers (mean personal air concentration, 0.5 μg/m^3; urine, 4.6 μg/mmol creatinine; and serum, 1.7 ng/mL). Grinders and polishers, and packers had, on average, intermediate exposures to melamine. The control group had the lowest average urinary concentrations of melamine (0.7 μg/mmol creatinine). There was a high correlation between urinary and serum melamine concentration for 39 workers in the melamine tableware plants (Spearman correlation coefficient $r = 0.808$; $P < 0.001$).

1.5 Regulations and guidelines

There are no approved uses for the direct addition of melamine to food (WHO, 2009b). In the USA, melamine is an indirect food additive for use only as a component of adhesives (21 Code of Federal Regulations (CFR) 175.105). [According to the FDA, indirect food additives are substances that may come into contact with food as part of packaging or processing equipment, but are not intended to be added directly to food (FDA, 2018a).]

Melamine is approved for paper and paperboard and cellophane polymers in the USA (WHO, 2009a). Regulations for melamine–formaldehyde resins include 21 CFR sections 175.300 (resinous and polymeric coatings), 175.320 (resinous and polymeric coatings for polyolefin films), 176.170 (components of paper and paperboard in contact with aqueous and fatty foods), 176.180 (components of paper and paperboard in contact with dry food), 177.1010 (acrylic and modified acrylic plastics, semirigid and rigid), 177.1200 (cellophane), 177.1460 (melamine–formaldehyde resins in moulded articles), 177.1630 (polyethylene phthalate polymers), 177.2260 (filters, resin-bonded), and 177.2470 (polyoxymethylene copolymer) (WHO, 2009a). Melamine at the maximum allowed use level of 0.2% by weight as a stabilizer in polyoxymethylene copolymers is regulated in 21 CFR 177.2470, destined for use in the manufacture of repeat-use articles that may contact food (FDA, 2018b).

In Europe, melamine is approved for use as a monomer and as an additive in plastics (European Commission, 2002; WHO, 2009b). The current

specific migration limit laid down in European Union legislation for plastics was lowered from 30 mg/kg food (EFSA, 2010) to 2.5 mg/kg food in 2011 (European Commission, 2011). In China, the migration standard for food containers is 1.2 mg/L or 0.2 mg/dm^2 (Ling et al., 2016).

In 2008, after findings of high levels of melamine in infant milk and milk products in China, the European Commission required Member States to check all consignments of feed and food containing milk products, soya, or soya products from China. An action level of 2.5 mg/kg was established by the European Commission to distinguish between the unavoidable background presence of melamine (from food-contact materials, pesticide use, etc.) and possible adulteration (EFSA, 2010).

WHO established a tolerable daily intake (TDI) of 0.2 mg/kg bw (WHO, 2009b), which was supported by EFSA (2010). WHO (2009b) suggested that the limits for melamine in powdered infant formula (1 mg/kg) and in other foods (2.5 mg/kg) provided a sufficient margin of safety for dietary exposure relative to the TDI of 0.2 mg/kg bw.

In 2012, the Codex Alimentarius Commission, which is jointly run by WHO and the United Nations Food and Agriculture Organization (FAO), adopted the following maximum levels for melamine: liquid infant formula, 0.15 mg/kg; powdered infant formula; 1 mg/kg; and other foods and animal feed, 2.5 mg/kg (United Nations News, 2012). In Europe, the maximum levels for powdered infant formula (1 mg/kg) and for other foods were implemented in Regulation No. 1881/2006 (Lachenmeier et al., 2017).

No occupational exposure limits for melamine were available.

2. Cancer in Humans

Melamine is often used in industry in conjunction with formaldehyde, and no occupational cohorts that were exposed to melamine and not to formaldehyde were identified by the Working Group; however, some pertinent data were available from a study of mortality among 25 619 workers in 10 industrial plants in the USA where formaldehyde was used. Exposure to formaldehyde was the focus of a series of publications based on this cohort (Blair et al., 1990b; Hauptmann et al., 2003, 2004; Beane Freeman et al., 2009, 2013), although 28% of the workers were ever exposed to melamine (Hauptmann et al., 2003). In the follow-up of this cohort to 1980, a trend in mortality from cancer of the lung with the duration of exposure to melamine was observed; this trend was statistically significant without consideration of latency ($P \leq 0.05$), but non-significant when a latency of 20 years or longer was assumed. Standardized mortality ratios (SMRs) for cancer of the lung were 1.3, 1.5, 1.9, and 2.0 for < 1, 1 to < 10, 10 to < 20, and ≥ 20 years of exposure, respectively (Blair et al., 1990b). Similar trends with standardized mortality ratios for cancer of the lung were seen for exposure to urea, and non-significant trends were seen for exposures to phenol and wood dust (which were used together with melamine in the production of resins and/or moulding compounds). No data were reported for associations between other cancers and exposure to melamine. Associations between duration of exposure to melamine and mortality from cancer of the nasopharynx, and between duration of exposure to melamine, dyes, plasticizers, and pigments [it was unclear whether exposure was to these agents in combination or separately] and mortality from all leukaemias, were reported in the text of a later publication based on further follow-up of the same cohort until 1994, but no estimates of risk or precision were given

(Hauptmann et al., 2003, 2004). Exposure to melamine was analysed as a potential confounder of associations between leukaemia or cancer of the nasopharynx and exposure to formaldehyde in subsequent publications, based on extended follow-up of this cohort (Beane Freeman et al., 2009, 2013), and as a co-exposure in a re-analysis (Marsh et al., 1992), but associations for melamine were not reported. [The Working Group noted that no quantitative exposure data were available for melamine, and that the analysis was not adjusted for co-exposure to other chemicals, notably formaldehyde, or for tobacco smoking.]

Developmental and clinical effects of exposure to melamine as a contaminant in infant milk formula (melamine content, 0.1–2500 ppm) were examined in follow-up studies of fewer than 200 children in China who developed urinary stones after consuming adulterated infant milk formula. Clinical examinations carried out during 4 years of observation included ultrasound screening for cancer of the urinary system, which did not detect any tumours (Wen et al., 2011; Yang et al., 2013). [The Working Group noted that this study included only children who had developed urinary stones and that the follow-up period was very short. The Working Group considered that the sensitivity of this study was low.]

3. Cancer in Experimental Animals

Melamine was previously evaluated by the Working Group with respect to its carcinogenicity in experimental animals (IARC, 1986, 1999). In its evaluation in 1999 (IARC, 1999), the Working Group concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of melamine under conditions in which it produces bladder calculi.

See Table 3.1.

3.1 Mouse

3.1.1 Oral administration

In a study by the National Toxicology Program (NTP), groups of 50 male and 50 female B6C3F$_1$ mice (age, 6 weeks) were fed diets containing technical-grade melamine (purity, 97% [impurities were not further characterized]) at a concentration of 0, 2250, or 4500 ppm [0%, 0.225%, or 0.45%], at a dose of 0, 327, and 688 mg/day per kg bw for males and 0, 523 and 1065 mg/day per kg bw for females, ad libitum for 103 weeks, followed by a basal diet for 2 weeks (NTP, 1983). Mean body weights of males at the higher dose were slightly lower than those of the controls after week 50 of the study; mean body weights of males at the lower dose and of both treated groups of females were comparable to those of their respective controls throughout the study. The survival of males at the higher dose was significantly reduced when compared with that of the controls; survival at termination of the study was: controls, 39/49; lower dose, 36/50; and higher dose, 28/50 for males; and controls, 37/50; lower dose, 43/50; and higher dose, 41/50 for females.

No treatment-related increase in the incidence of tumours was observed in males or females. In male mice, treatment-related increases were observed in the incidence of (i) urinary bladder stones [composition unspecified]: controls, 2/45 (4.4%); lower dose, 40/47 (85%); and higher dose, 41/44 (93%); (ii) acute and chronic inflammation of the urinary bladder: controls, 0/45; lower dose, 25/47 (53%); and higher dose, 24/44 (55%); and (iii) "very mild" epithelial hyperplasia [not further specified] of the bladder: controls, 1/45 (2%); lower dose, 11/47 (23%); and higher dose, 13/44 (30%). Non-significant increases in the incidences of urinary bladder stones [composition unspecified] (4/50, 8%) and "very mild" epithelial hyperplasia (4/50, 8%) were also seen in females at the higher dose compared with controls

Table 3.1 Studies of carcinogenicity with melamine in rodents

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6 wk 105 wk NTP (1983)	Oral Melamine, 97% Diet 0, 2250, 4500 ppm Ad libitum for 103 wk 49, 50, 50 39, 36, 28	No significant increase in tumour incidence in treated animals	NS	Principal strengths: studies in males and females, well-conducted study Urinary bladder stones were observed: 2/45 (4.4%) controls, 40/47 (85%) lower dose, 41/44 (93%) higher dose Urinary bladder hyperplasia (epithelial) was observed: 1/45 (2.2%) controls, 11/47 (23%) lower dose, 13/44 (30%) higher dose
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 105 wk NTP (1983)	Oral Melamine, 97% Diet 0, 2250, 4500 ppm Ad libitum for 103 wk 50, 50, 50 37, 43, 41	No significant increase in tumour incidence in treated animals	NS	Principal strengths: studies in males and females, well-conducted study Urinary bladder stones were observed: 0/42 controls, 0/49 lower dose, 4/50 (8.0%) higher dose Urinary bladder hyperplasia (epithelial) was observed: 0/42 controls, 0/49 lower dose, 4/50 (8.0%) higher dose

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Carcinogenicity with other modifying factor Mouse, BALB/c (M+F) (combined) NR (weanling) 22 wk Cremonezzi et al. (2001)	Oral Melamine, NR Diet 0, 1.2% Ad libitum 22, 27 20, 27	*Urinary bladder* Dysplasia or carcinoma in situ (combined): 0/21, 9/27 (33%)* *Ureter* Dysplasia or carcinoma in situ (combined): 0/21, 7/27 (26%)* *Renal pelvis* Dysplasia or carcinoma in situ (combined): 1/21 (4.8%), 4/27 (15%)	 NS *[$P = 0.0030$, Fisher exact test] NS *[$P = 0.0136$, Fisher exact test] NS	Principal limitations: limited number of animals, use of a single dose, data combined for sexes, limited number of organs examined, number of mice of each sex NR, short exposure duration This study was conducted to investigate the effect of dietary polyunsaturated fatty acids on mouse urinary bladder lesions induced by melamine Urinary bladder stones were observed in all groups treated with melamine (60–85% incidences, composition unspecified). No bladder stones were observed in the group without melamine In the group fed a diet containing 1.2% melamine plus 6% corn oil, the incidence of dysplasia/carcinoma in situ was significantly increased ($P < 0.05$, vs melamine group) in the urinary bladder and ureter at 13/23 (57%) and 10/23 (43%), respectively. In the group fed a diet containing 1.2% melamine plus 6% olein, the incidence of dysplasia/carcinoma in situ was significantly increased ($P < 0.05$, vs melamine group) in the urinary bladder, ureter, and renal pelvis at 11/18 (61%), 9/18 (50%), and 10/18 (56%), respectively
Initiation–promotion (tested as initiator) Mouse, CD-1 (F) 8 wk 31 wk Perrella & Boutwell (1983)	Skin application Melamine, NR Acetone 0 µmol (followed by TPA), 1 µmol (followed by TPA), single skin application 20, 20 20, 20	*Skin* Papilloma: 14%, 19% Tumour multiplicity: 0.14, 0.25	 NS NS	Principal limitations: limited number of animals, use of a single dose, only one sex, low dose of application, limited number of organs examined, histopathology of tumours NR In both groups, single administration of melamine or acetone was followed by skin applications of 10 nmol TPA in 0.2 mL acetone twice weekly for 31 wk

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 6 wk 105 wk NTP (1983)	Oral Melamine, 97% Diet 0, 2250, 4500 ppm Ad libitum for 103 wk 49, 50, 50 30, 30, 19	*Urinary bladder* Transitional cell carcinoma: 0/45*, 0/50, 8/49 (16%)**	*$P < 0.001$, Cochran-Armitage and life-table trend tests; $P = 0.002$, incidental tumour trend test **$P = 0.003$, relative to control, life-table test; $P = 0.016$, relative to control, incidental tumour test; $P = 0.002$ relative to control, Fisher exact test	Principal strengths: studies in males and females, well-conducted study Urinary bladder stones were observed: 0/45 controls, 1/50 (2%) lower dose, 10/49 (20%) higher dose Urinary bladder transitional cell hyperplasia was observed: 0/45 controls, 1/50 (2%) lower dose, 2/49 (4.1%) higher dose
		Transitional cell papilloma or carcinoma (combined): 0/45*, 0/50, 9/49 (18%)**	*$P < 0.001$, Cochran-Armitage, life-table, and incidental tumour trend tests **$P = 0.002$, relative to control, life-table and Fisher exact tests; $P = 0.008$, relative to control, incidental tumour test	
		Transitional cell papilloma: 0/45, 0/50, 1/49 (2.0%)	NS	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 6 wk 105 wk NTP (1983)	Oral Melamine, 97% Diet 0, 4500, 9000 ppm Ad libitum for 103 wk 50, 50, 50 34, 30, 27	*Urinary bladder* Transitional cell carcinoma: 0/49, 0/49, 0/47 Transitional cell papilloma: 0/49, 1/49 (2.0%), 1/47 (2.1%) *Thyroid gland* C-cell carcinoma: 0/50*, 0/49, 3/50 (6%)[a] C-cell adenoma or carcinoma (combined): 0/50, 2/49 (4.1%), 3/50 (6.0%) C-cell adenoma: 0/50, 2/49 (4.1%), 0/50	NS NS *P = 0.038, Cochran-Armitage trend test; P = 0.025, life table and incidental tumour trend tests NS NS	Principal strengths: studies in males and females, well-conducted study Neither urinary bladder stones nor transitional cell hyperplasia were observed in any group. No historical control data were provided for urinary bladder tumours in females [a] The incidence at the higher dose was not significantly different from the historical incidence of this thyroid tumour in untreated female F344/N rats at the same laboratory (14/689, 2.0%) or throughout the NTP bioassay programme (98/3544, 2.8%; range, 0–10%)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344 (M) 6 wk 40 wk Okumura et al. (1992)	Oral Melamine, > 99% Diet 0, 0.3, 1.0, 3.0%, ad libitum for 36 wk, followed by 4-wk basal diet period 20, 20, 20, 20 20, 20, 20, 19	*Urinary bladder* Transitional cell carcinoma: 0/20, 0/20, 1/20 (5%), 15/19 (79%)* Transitional cell papilloma: 0/20, 0/20, 1/20 (5%), 12/19 (63%)* *Ureter* Carcinoma: 0/20, 0/20, 0/20, 1/19 (5.3%) Papilloma: 0/20, 0/20, 0/20, 3/19 (16%)	*$P < 0.01$ compared with the control group, Fisher exact test *$P < 0.01$ compared with the control group, Fisher exact test NS NS	Principal strengths: multiple dose study Principal limitations: only one sex, limited number of animals and organs examined, short duration of the study Urinary bladder stones were observed: 0/20 controls, 4/20 (20%) lowest dose, 9/20 (45%) intermediate dose, 8/19 (42%) highest dose
Carcinogenicity with other modifying factor Rat, F344 (M) 6 wk 40 wk Ogasawara et al. (1995)	Oral Melamine, 99.9% Diet 0, 1, 3%, ad libitum for 36 wk, followed by 4-wk basal diet period 10, 20, 20 10, 19, 20	*Urinary bladder* Transitional cell carcinoma: 0/10, 4/19 (21%), 18/20 (90%)* Transitional cell papilloma: 0/10, 8/19 (42%)*, 10/20 (50%)**	*[$P < 0.0001$, Fisher exact test] *[$P < 0.03$, Fisher exact test]; **[$P < 0.02$, Fisher exact test]	Principal strengths: multiple dose study Principal limitations: only one sex, limited number of animals and organs examined, short duration of the study Urinary bladder stones (melamine–uric acid salt) were observed: 0/10 controls, 7/19 (37%) lower dose, 6/20 (30%) higher dose In the group fed diets containing 3% melamine plus NaCl at 5% or 10%, the incidence of transitional cell carcinoma in the urinary bladder was 18/20 (90%) and 0/20, respectively In the group fed diets containing 1% melamine plus NaCl at 5% or 10%, no carcinomas were observed in the urinary bladder (0/19 and 0/19)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Carcinogenicity with other modifying factor Rat, Wistar (M+F) (combined) NR (weanling) 36–40 wk Cremonezzi et al. (2004)	Oral Melamine, NR Diet 0, 1.5%, ad libitum 36, 20 NR, NR	*Urinary bladder* Dysplasia: 0/36, 0/20 *Renal pelvis* Dysplasia: 0/36, 2/20 (10%)	NS NS	Principal limitations: limited number of animals, dose groups, and organs examined; data combined for males and females; number of rats of each sex NR; short duration of the study This study was conducted to investigate the effect of dietary polyunsaturated fatty acid on rat urinary bladder lesions induced by melamine. In groups fed 1.5% melamine plus 6% olein diet or 6% of a mixture containing mainly stearic acid, the incidence of dysplasia in the renal pelvis was significantly increased ($P < 0.05$, vs melamine group) at 10/18 (56%) or 16/26 (62%), respectively
Carcinogenicity with other modifying factor Rat, Wistar (M+F) (combined) NR (weanling) 22–25 wk Cremonezzi et al. (2004)	Oral Melamine, NR Diet 0, 1.5%, ad libitum 22, 21 NR, NR	*Urinary bladder* Dysplasia: 0/22, 0/21 *Renal pelvis* Dysplasia: 0/22, 1/21 (4.8%)	NS NS	Principal limitations: limited number of animals, dose groups, and organs examined; data combined for males and females; number of rats of each sex NR; short exposure duration This study was conducted to investigate the effect of dietary polyunsaturated fatty acids on rat urinary bladder lesions induced by melamine Urinary bladder stones were not observed In the groups fed 1.5% melamine plus 6% corn-oil diet or 6% of a mixture containing mainly stearic acid, the incidence of dysplasia in the renal pelvis was significantly increased ($P < 0.05$, vs melamine group) at 11/20 (55%) or 14/21 (67%), respectively

F, female; M, male; NaCl, sodium chloride; NR, not reported; NS, not significant; NTP, National Toxicology Program; ppm, parts per million; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; vs, versus; wk, week(s)

(0/42 and 0/42, respectively) (NTP, 1983; Melnick et al., 1984). [The Working Group considered that the strengths of this well-conducted study included the evaluation of multiple dose levels, the use of both males and females, and the study duration including most of the lifespan.]

In a study to investigate the effect of dietary polyunsaturated fatty acids on mice urinary bladder lesions induced by melamine, male and female homozygous weanling BALB/c mice [age not reported] were randomly distributed to several groups of 18–27 animals. In the group fed 1.2% melamine only [purity not reported; food intake data not provided] for 22 weeks, the incidence of dysplasia or carcinoma in situ [not further specified] (combined) was increased in the urinary bladder (9/27, 33%) [$P = 0.0030$] and the ureter (7/27, 26%) [$P = 0.0136$] compared with that in the control group receiving basal commercial diet (0/21, 0/21). Dysplasia or carcinoma in situ were also observed in the renal pelvis of mice in the group receiving melamine (4/27, 15%) and in the control group (1/21, 4.8%), without statistically significant differences. In another group receiving a diet containing melamine plus 6% corn oil for 22 weeks, there were significant ($P < 0.05$) increases in the incidence of dysplasia or carcinoma in situ (combined) of the urinary bladder (13/23, 57%) and ureter (10/23, 43%) compared with the group receiving melamine only. Finally, in a group receiving a diet containing melamine plus 6% olein for 22 weeks, there were significant ($P < 0.05$) increases in the incidence of dysplasia or carcinoma in situ (combined) of the urinary bladder (11/18, 61%), ureter (9/18, 50%), and renal pelvis (10/18, 56%) compared with the group receiving melamine only. Urinary bladder stones [composition unspecified] were observed in all groups (range, 60–85% [no further information provided]) treated with melamine (Cremonezzi et al., 2001). [The Working Group noted some limitations of the study, including the small number of animals, unspecified numbers of males and females, use of a single dose, short duration of exposure, small number of organs examined, and lack of controls for the modifying factors used in this study, as well as the fact that only representative samples of the urothelium were investigated.]

3.1.2 Initiation–promotion

A group of 20 female CD-1 mice (age, 8 weeks) received a single topical application of melamine [purity not reported] of 1 µmol in 0.2 mL of acetone on shaved back skin, followed by twice-weekly applications of 10 nmol of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 0.2 mL of acetone for 31 weeks. A control group of 20 female mice received a single application of acetone alone, followed by applications of TPA. At 31 weeks, no significant increase in the incidence of skin papilloma was observed in melamine-treated mice (19%) when compared with controls (14%) (Perrella & Boutwell, 1983). [The Working Group noted the low dose of melamine used and the limited description of clinical observations and histopathology of observed tumours.]

3.2 Rat

3.2.1 Oral administration

In a study by the NTP, groups of 49–50 male and 50 female Fischer 344/N rats (age, 6 weeks) were fed diets containing technical-grade melamine (purity, 97% [impurities were not further characterized]) at a concentration of 0, 2250, or 4500 ppm for males (0, 126, and 263 mg/day per kg bw), and 0, 4500, or 9000 ppm for females (0, 262, and 542 mg/day per kg bw), ad libitum for 103 weeks, followed by a basal diet for 2 weeks (NTP, 1983). Mean body weights of male and female treated rats were lower than those of the controls after week 20 of the study. The survival of males at the higher dose was significantly reduced when compared with that of the controls;

the survival at termination of the study was: controls, 30/49; lower dose, 30/50; and higher dose, 19/50 for males; and controls, 34/50; lower dose, 30/50; and higher dose, 27/50 for females.

The incidence of transitional cell carcinoma of the urinary bladder in males was: controls, 0/45; lower dose, 0/50; and higher dose, 8/49 (16%) (control vs higher dose, $P \leq 0.016$; P (trend) ≤ 0.002). There was also a dose-related increase in the incidence of bladder stones in male rats: controls, 0/45; lower dose, 1/50 (2%); and higher dose, 10/49 (20%). Of 49 male rats at the higher dose, 7 (14%) had transitional cell carcinoma of the urinary bladder and urinary bladder stones, 1 (2%) had a carcinoma without stones, and 3 (6%) had stones without carcinoma (1 of these rats had a papilloma of the urinary bladder and 1 had epithelial hyperplasia). A statistically significant association ($P \leq 0.001$) was found between the presence of bladder stones and bladder tumours. No urinary bladder stones were reported in female rats, while one female at the lower dose and one at the higher dose had a papilloma of the urinary bladder; no data on historical controls were provided for this tumour. There was also a small but significant ($P = 0.038$) positive trend in the incidence of thyroid C-cell carcinoma in females (0/50, 0/49, 3/50); the incidence of this tumour in the group at the higher dose (3/50, 6%) was not significantly different from the historical incidence of this tumour at the laboratory (14/689, 2.0%) or throughout the NTP bioassay programme (98/3544, 2.8%; range, 0–10%) [the Working Group performed statistical tests and confirmed the lack of significance by pairwise comparison] (NTP, 1983; Melnick et al., 1984). [The Working Group noted that the strengths of this well-conducted study included the use of multiple dose levels and both males and females, and that the duration included most of the lifespan. The Working Group also noted that there may have been a relationship between the presence of stones and tumours of the urinary bladder. See also Sections 4.2 and 4.5.]

Four groups of 20 male Fischer 344 rats (age, 6 weeks) were fed diets containing melamine (purity, > 99%) at a concentration of 0% (control), 0.3%, 1.0%, or 3.0% (food intake, 15.3, 15.0, 14.7, and 11.7 g/rat per day) for 36 weeks, followed by a basal diet for 4 weeks. Mean body weight of rats at the highest dose was significantly lower than that of the controls ($P < 0.001$). Transitional cell carcinomas of the urinary bladder were observed in 0/20, 0/20, 1/20 (5%), and 15/19 (79%) ($P < 0.01$, increase) rats at the control, low, intermediate, and highest doses, and transitional cell papillomas in 0/20, 0/20, 1/20 (5%), and 12/19 (63%) ($P < 0.01$, increase) rats, respectively. One (5.3%) rat at the high dose developed a carcinoma of the ureter and 3 (16%) rats at the high dose developed papillomas of the ureter. The findings of tumours correlated ($P = 0.0065$) [correlation coefficient not provided] with the formation of urinary bladder calculi [composition unspecified] (Okumura et al., 1992). [The Working Group noted the short duration of the study, the use of one sex only, the small number of animals at start, and the small number of organs examined.]

In a study in which the effects of urinary volume on melamine-induced urinary bladder calculi formation were examined by administration of a diet supplemented by sodium chloride (NaCl), six groups of 20 male Fischer 344 rats (age, 6 weeks) were fed diets containing melamine (purity, 99.9%) at a concentration of 1% or 3% (food intake, 14.8 or 12.2 g/rat per day for the rats receiving melamine at 1% or 3%, respectively), with or without NaCl at 5% or 10% for 36 weeks, followed by a basal diet for 4 weeks. No transitional cell papillomas or carcinomas of the urinary bladder were observed in 10 control rats fed only the basal diet (food intake, 15.0 g/rat per day). Transitional cell carcinomas of the urinary bladder were observed in 4/19 (21%), 18/20 (90%) [$P < 0.0001$, increase compared with basal diet controls], and 18/20 (90%) rats given 1% melamine only, 3% melamine only, or 3%

melamine plus 5% NaCl, respectively. No transitional cell carcinomas of the urinary bladder were observed in the groups receiving 3% melamine plus 10% NaCl (0/20), or 1% melamine plus 5% (0/19) or 10% NaCl (0/19). The incidence of transitional cell papilloma of the urinary bladder was similarly decreased by NaCl. The incidence of transitional cell papilloma of the urinary bladder was 10/20 (50%) [$P < 0.02$, increase compared with basal diet controls] in the group given 3% melamine only, but 5/20 (25%) and 3/20 (15%) in the rats receiving 3% melamine plus 5% NaCl or 10% NaCl, respectively. Transitional cell papillomas of the urinary bladder developed in 8/19 (42%) rats receiving 1% melamine only [$P < 0.03$, increase compared with basal diet controls]. The occurrence of tumours correlated with calculus (melamine–uric acid salt, determined by high-performance liquid chromatography) formation and papillomatosis. The total combined contents of melamine and uric acid in the calculi obtained from four rats in the group treated with 1% melamine only were 61.1–81.2%, and the molar ratios of uric acid to melamine were 0.99–1.05 (Ogasawara et al., 1995). [The Working Group noted the use of males only, the short duration of the study, the small number of animals at the start, and the small number of organs examined.]

In a study to investigate the effect of dietary polyunsaturated fatty acids on lesions of the urinary bladder induced by melamine, male and female weanling Wistar rats [age not reported] were randomly distributed into several groups of 18–36 animals. In two groups fed diets containing melamine [purity not reported] at a concentration of 1.5% [food intake data not provided] for 22–25 weeks or 36–40 weeks, dysplasia [not further specified] was observed in the renal pelvis of 1/21 (4–8%) and 2/20 (10%) rats, respectively, but not in the urinary bladder. No dysplasia of the renal pelvis or urinary bladder was observed in the respective control groups receiving basal commercial diet (0/22 and 0/36, respectively). In other groups treated with melamine, additional dietary administration of 6% corn oil or 6% of a mixture containing mainly stearic acid for 22–25 weeks significantly increased ($P < 0.05$) the incidence of dysplasia in the renal pelvis (11/20 (55%) and 14/21 (67%), respectively). Additional administration of 6% olein or 6% of a mixture containing mainly stearic acid for 36–40 weeks significantly increased ($P < 0.05$) the incidence of dysplasia in the renal pelvis (10/18 (56%) and 16/26 (62%), respectively). Urolithiasis was not observed in any group (Cremonezzi et al., 2004). [The Working Group noted the small number of animals, the unspecified number of males and females, the use of a single dose, the short duration of the study, the short exposure duration, the small number of organs examined, the lack of controls for the modifying factors used in this study, and the fact that only representative samples of the urothelium were investigated.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Absorption

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In rats and monkeys, melamine was rapidly absorbed after oral administration (Liu et al., 2010a; Jacob et al., 2012).

The apparent efficiency of absorption of melamine was 76% in eight Dohne Merino rams. After a 10-day period during which all animals received a forage-based diet supplemented with control pellets, six rams received pellets containing melamine and two rams received control pellets

for 8 days. Melamine intake for the treated rams was 0.69 g/day (Cruywagen et al., 2011).

4.1.2 Distribution

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Monkeys

When given to three rhesus monkeys as a single oral dose at 1.4 mg/kg bw, melamine was rapidly absorbed and cleared, and mainly distributed in body fluids. The maximum concentration of melamine in plasma was 1767 ± 252 µg/L. The time to maximum concentration was 2.67 ± 1.16 hours, and the half-life of melamine in plasma was 4.41 ± 0.43 hours (Liu et al., 2010a).

(ii) Rats

In several studies in rats, melamine was distributed to the kidney and urinary bladder, among other organs. In Fischer 344 rats given a single oral dose of [^{14}C]-labelled melamine (0.025 mCi; ~1.3 mg/kg bw), the only organs showing concentrations of radiolabel much higher than those in the plasma were the kidney and bladder (Mast et al., 1983). In Sprague-Dawley rats treated with melamine (50 mg/kg, gavage), melamine concentrations were highest in the bladder, while almost no melamine was found in the brain (Wu et al., 2010b). In groups of six Sprague-Dawley rats randomly assigned to receive a single oral dose of melamine at 5 mg/kg, or a single intravenous dose at 2 mg/kg, melamine was predominantly restricted to blood or extracellular fluid and was not extensively distributed to organ tissues (Yang et al., 2009).

When administered at a daily dose of 40 or 400 mg/kg bw by gavage on days 13–20 of gestation in pregnant female F344 rats, melamine passed the placental barrier to reach the fetus in a dose-dependent manner (Jingbin et al., 2010). Similarly, in pregnant and neonatal Sprague-Dawley rats treated with melamine at a single oral dose of 21.4 mg/kg per day, melamine was able to pass through the placenta and reach the fetus, and to accumulate in the lactating mammary gland and neonatal kidney. Moreover, melamine was eliminated via the kidneys for the neonates and via the placenta for the fetus, and later excreted into the amniotic fluid (Chu et al., 2010).

(iii) Pigs

Melamine residues were detected in the brain, duodenum, liver, heart, muscle, and kidney of fattening pigs given a diet supplemented with melamine at a concentration of 500 or 1000 mg/kg diet. Tissue concentrations declined 5 days after the withdrawal of melamine from the diet, to less than 2.5 mg/kg (Wang et al., 2014).

In five weanling pigs given melamine intravenously at a dose of 6.13 mg/kg bw, with plasma samples being collected for 24 hours, the data best fitted a one-compartment model with a half-life of 4.04 (± 0.37) hours, clearance of 0.11 (± 0.01) L/h per kg, and volume of distribution of 0.61 (± 0.04) L/kg (Baynes et al., 2008).

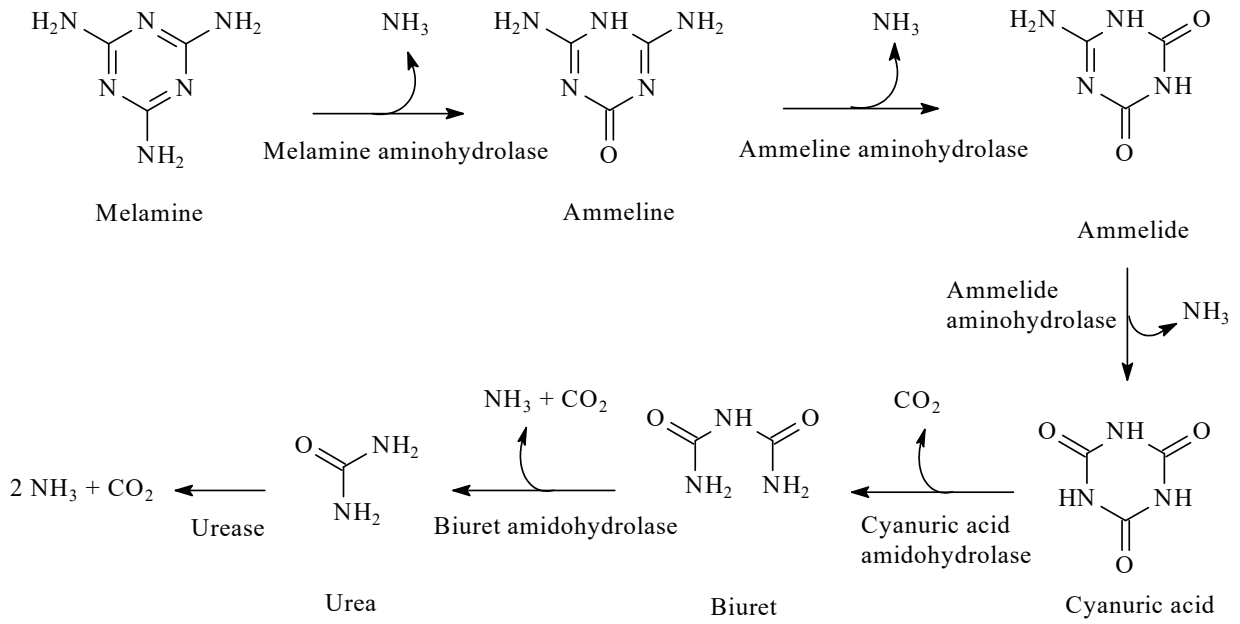
(iv) Sheep

In a study involving rams fed pellets containing melamine (described in Section 4.1.1(b)), melamine was detected in the urine, blood, muscle, and fat tissue of all rams that received melamine. Melamine concentrations reached 5.4 mg/kg in serum on day 8 of the collection period, and 9.6 mg/kg in meat (Cruywagen et al., 2011).

(v) Goats

Five lactating goats were given melamine as a single oral dose at 40 mg/kg bw. Blood samples were collected for 144 hours. The apparent plasma half-life (11.12 hours) was 3 times as long in these ruminants compared with monogastrics such as pigs and rodents (Baynes et al., 2010).

Fig. 4.1 The bacterial degradation of melamine



Source: Eaton & Karns (1991), amended with permission from the American Society for Microbiology.

4.1.3 Metabolism

Multiple studies, including in rats and in non-human primates, have indicated that melamine is not metabolized in mammalian tissue (e.g. Mast et al., 1983; Yang et al., 2009; Liu et al., 2010a). Comparable data were not available for humans, but a similar lack of metabolism is recognized from inferences that can be made (Wu & Zhang, 2013).

Melamine may be metabolized by bacteria, such as *Klebsiella terrigena* or *Pseudomonas*, to several metabolites, including cyanuric acid (see Fig. 4.1).

(a) Humans

Zheng et al. (2013) reported a correlation between melamine-induced toxicity in humans (Liu et al., 2010b) and the incidence of *K. terrigena* colonization in humans. [The Working Group noted that metabolism of melamine in the human gut has not been shown to be mediated by *K. terrigena* or other bacteria.]

(b) Experimental systems

Rats that had been colonized with *K. terrigena* exhibited exacerbated melamine-induced nephrotoxicity. Melamine-induced toxicity in rats was attenuated, and melamine excretion increased, after antibiotic suppression of gut microbial activity. Cyanuric acid was detected in the kidney of rats given melamine only, and the concentration was increased after *K. terrigena* colonization (Zheng et al., 2013). [The Working Group noted that the role of *K. terrigena* in the metabolism of melamine in the human gut has not been established.]

4.1.4 Excretion

See Section 4.5 for a discussion of the formation of precipitates containing melamine in the urinary tract.

(a) Humans

Urinary concentrations of melamine have been measured in children not specifically known to have been exposed to melamine.

In 2007–2008 in Hong Kong Special Administrative Region, 502 schoolchildren aged 6–20 years participated in a primary and secondary school survey that used a cluster sampling method. A high urinary level of melamine was defined as urine melamine/creatinine ratio > 7.1 μg/mmol. In 213 children (42%), melamine was undetectable. In 47 children (9%), urinary levels of melamine were high. The median urine melamine/creatinine ratio for all the schoolchildren tested was 0.76 μg/mmol (Kong et al., 2011).

Melamine was detectable in all urine samples collected from schoolchildren aged 6–10 years (7 girls and 16 boys) in Taiwan, China. The median melamine concentrations in one-spot overnight urine samples on the mornings of the first and second day were 0.93 and 1.73 μg/mmol creatinine, respectively. Melamine concentrations on the second morning were highly correlated with the total melamine excretions in urine during the previous 8 and 24 hours (Lin et al., 2013).

In a pilot study, 16 healthy volunteers (age range, 20–27 years) consumed 500 mL of hot noodle soup (initial temperature, 90 °C) served in melamine bowls. Postconsumption mean urinary melamine concentrations (corrected for urinary creatinine) initially increased sharply, peaked at 4–6 hours, and then declined (sharply for 2 hours, and then less steeply) until 12 hours after consumption. In another experiment in the same study, groups of three men and three women fasted for 8 hours before consuming 500 mL of hot noodle soup (initial temperature, 90 °C) served in either melamine bowls or ceramic bowls. Total urinary excretion of melamine in the urine over 12 hours was 8.35 ± 1.91 μg for those who were served soup in melamine bowls and 1.31 ± 0.44 μg for those who were served soup in ceramic bowls ($P < 0.001$) (Wu et al., 2013).

(b) Experimental systems

(i) Monkeys

In three rhesus monkeys given melamine as a single oral dose at 1.4 mg/kg bw, melamine was rapidly excreted, mainly through urinary clearance (Liu et al., 2010a).

(ii) Rats

In adult male Fischer 344 rats, more than 90% of a single oral dose of [^{14}C]-labelled melamine (0.025 mCi; ~1.3 mg/kg bw) was excreted within 24 hours via urine, exhaled air, and faeces, with 99% total recovery after 96 hours. The elimination half-life, urinary-excretion half-life, and renal clearance for melamine were 2.7 hours, 3.0 hours, and 2.5 mL/min, respectively. No residual radiolabel was observed in the blood or plasma after 24 hours. At this time point, residual radiolabel in the liver and kidney was 1.8 and 1.3 μg equivalents/kg tissue, respectively; radiolabel concentrations were much higher in the bladder and ureter (31 and 12 μg equivalents/kg tissue, respectively) (Mast et al., 1983).

In pregnant Sprague-Dawley rats given a single dose of melamine at 21.4 mg/kg bw by gavage at day 16–18 of gestation, 80% of the administered dose was found in the dams' serum at 0.5 hours. The peak melamine concentration of 7.15 ppm was reported in the fetuses after 2 hours, with 4.36 ppm reported in amniotic fluid after 3 hours. In the lactating rats, 40% of maternal intake of melamine was transferred to the milk, with peak concentrations at 3 hours (Chan et al., 2011).

(iii) Sheep

In a study in rams fed pellets containing melamine, urine was the major excretion route, accounting for 53.2% of ingested melamine; faeces accounted for 23.3% (Cruywagen et al., 2011; described in Section 4.1.1(b)).

Table 4.1 Genetic and related effects of melamine in exposed humans

End-point	Tissue	Cell type, if specified	Description of exposed and controls	Response[a], significance	Comments	Reference
DNA oxidation	Urine	NA	Infants exposed to melamine in contaminated powdered formula Four exposure groups: high: > 90% intake from contaminated formula; moderate: 50–90% intake from contaminated formula; low: < 50% of intake from contaminated formula; reference group, > 90% intake from imported milk powdered formula not containing melamine	–	Groups 1–3 are the observation groups, and Group 4 is the reference group	Ke et al. (2010)

[a] –, negative
NA, not applicable

(iv) Cows and goats

Melamine was shown to distribute to the milk in lactating goats (Baynes et al., 2010) and in lactating cows (e.g. Cruywagen et al., 2009; Sun et al., 2012).

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

The data on tests for genotoxicity with melamine were reviewed previously by the NTP (1983) and IARC (1999). New data have become available since then, and these have been incorporated into this Section.

(a) Humans

(i) Exposed humans

See Table 4.1.

Urinary levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) were measured in a cross-sectional study in China of 73 male and 66 female infants (age, 0.5–1.5 years) who presented urinary problems and who were grouped by intake of melamine-contaminated infant formula milk (Ke et al., 2010). Even in the group with highest exposure (infants who received more than 90% of their intake from contaminated formula), no increases in 8-OHdG levels were seen. [The Working Group noted that the study did not evaluate the relationship between 8-OHdG levels and the occurrence of urinary tract stones.]

(ii) Human cells in vitro

No evidence of malignant transformation was observed in a human liver cell line, L02, up to 6 months after treatment with melamine (doses up to 4000 μM) (Zhang et al., 2011).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.2.

In male Sprague-Dawley rats treated by gavage, melamine did not induce DNA damage (as measured by the comet assay) in urothelial bladder cells or liver, even in the presence of some histopathological changes suggestive of cytotoxicity (Wada et al., 2014). In male F344 rats given drinking-water containing melamine, there was no increase in levels of γH2AX, a marker of DNA double-strand breaks, in the urinary bladder (Toyoda et al., 2015). No increases in the

Table 4.2 Genetic and related effects of melamine in rodents in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Chromosomal damage	Mouse, B6C3F$_1$ (M)	Bone marrow (PCE)	–	2000 mg/kg bw per day for 3 day	i.p.		Shelby et al. (1993)
Chromosomal damage	Mouse, NIH (M and F)	Bone marrow (PCE)	–	1600 mg/kg bw per day for 2 days	i.p., sampling 6 h after last dose		Zhang et al. (2011)
Chromosomal damage	Mouse, Kunming (M and F)	Bone marrow	–	Melamine + cyanuric acid, 294.5 mg/kg bw	Gavage; 2 doses, 24 h interval	Dose levels not clearly described	Liu et al. (2014)
DNA strand breaks (comet assay)	Rat, Sprague-Dawley (M)	Urothelial bladder and liver cells	–	2000 mg/kg bw	Gavage, 2 doses on 2 consecutive days		Wada et al. (2014)
DNA damage	Rat, F344 (M)	Urinary bladder epithelial cells	–	3% or ~2089 mg/kg bw per day	Diet, 4 wk with and without a 2-wk recovery period		Toyoda et al. (2015)
Mutation	Rat, Sprague-Dawley (M)	Peripheral blood	–	2000 mg/kg bw per day for 3 days	Gavage; sampling at 15, 29, and 60 days after treatment		Tu et al. (2015)
Mutation	Rat, Crl:CD(SD) (M)	Peripheral blood	–	2000 mg/kg bw	Gavage; 1×, sampling after 1, 2, or 4 wk		Kyoya et al. (2016)
Chromosomal damage	Mouse, B6C3F$_1$ (M)	Bone marrow	±	300 mg/kg bw	i.p.; 1×, sampling at 36 h after injection		NTP (2017a)
Chromosomal damage	Mouse, B6C3F$_1$ (M)	Bone marrow	+	87.5 mg/kg bw	i.p.; 1×, sampling at 23 and 42 h after injection		NTP (2017b)

[a] +, positive; –, negative; ±, equivocal (variable response in several experiments within an adequate study); the level of significance was set at $P < 0.05$ in all cases

bw, body weight; F, female; h, hour(s); HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose (units as reported); M, male; PCE, polychromatic erythrocytes; wk, week(s)

Table 4.3 Genetic and related effects of melamine in rodent cells in vitro

End-point	Species, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Results without metabolic activation	Results with metabolic activation			
Chromosomal aberrations	CHO	–	–	300 µg/mL	Highest dose, non-toxic; limited by solubility	Galloway et al. (1987)
Sister-chromatid exchange	CHO	±	–	225 µg/mL	Highest dose limited by solubility	Galloway et al. (1987)
Gene mutation	Mouse, L5178Y *Tk*$^{+/-}$ lymphoma cells	–	–	160 µg/mL		McGregor et al. (1988)
Chromosomal aberrations	CHO	–	–	4 mM		Zhang et al. (2011)
Micronucleus formation	CHO-K1	–	–	300 µg/mL		Tu et al. (2015)

[a] –, negative; ±, equivocal (variable response in several experiments within an adequate study); the level of significance was set at $P < 0.05$ in all cases

CHO, Chinese hamster ovary; HIC, highest ineffective concentration; LEC, lowest effective concentration

frequency of *Pig-a* mutations or of micronucleus formation were seen in male Sprague-Dawley rats given melamine as three daily doses (up to 2000 mg/kg bw) by gavage (Tu et al., 2015; Kyoya et al., 2016).

No induction of micronucleus formation was observed in bone marrow cells of male B6C3F$_1$ or NIH mice after intraperitoneal injection of melamine (Shelby et al., 1993; Zhang et al., 2011). In bone marrow cells of male B6C3F$_1$ mice given a single intraperitoneal injection of melamine, both chromosomal aberrations and sister-chromatid exchange were reported (NTP, 2017a, b). In the test for induction of chromosomal aberrations, a significant increase was observed 36 hours after administration of melamine at the intermediate dose (300, but not 150 or 600 mg/kg bw); the trend test was not significant ($P = 0.358$) (NTP, 2017a). In the test for sister-chromatid exchange, significant increases were seen in two trials (four mice per group) at 23 hours (but not at 42 hours) after injection. In the first trial, only the group given an intermediate dose (175 mg/kg bw) gave a positive result; in the second trial, the groups given a low dose (87.5 mg/kg bw) and intermediate dose (175 mg/kg bw) gave positive results (NTP, 2017b).

(ii) Non-human mammalian cells in vitro

See Table 4.3.

No induction of gene mutation was observed in mouse lymphoma L5178Y *Tk*$^{+/-}$ cells (McGregor et al., 1988). No induction of chromosomal aberrations, or of micronucleus formation, was observed in Chinese hamster ovary cells exposed to melamine with or without metabolic activation from induced rat liver S9 (Galloway et al., 1987; Zhang et al., 2011; Tu et al., 2015). In Chinese hamster ovary cells tested in the absence of metabolic activation, one of two trials yielded a small increase in the frequency of sister-chromatid exchange; no increases in the frequency of sister-chromatid exchange were seen in a single trial with rat liver S9 (Galloway et al., 1987).

(iii) Non-mammalian systems

See Table 4.4.

Table 4.4 Genetic and related effects of melamine in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Agent, concentration (LEC or HIC)	Comments	Reference
		Results without metabolic activation	Results with metabolic activation			
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	Melamine, 1111 µg/plate	Tested in four strains in two laboratories	Haworth et al. (1983)
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	Cyanuric acid, 10 000 µg/plate		Haworth et al. (1983)
Drosophila melanogaster Canton-S	Sex-linked recessive lethal mutations	±		Melamine, 1000 ppm	Feeding administration, equivocal results; injection, negative results	Foureman et al. (1994)
Salmonella typhimurium TA97, TA98, TA100, TA102	Reverse mutation	–	–	Melamine, 5000 µg/well		Zhang et al. (2011)
Salmonella typhimurium TA97, TA98, TA100, TA102	Reverse mutation	–	–	Melamine and cyanuric acid in combination, 500 µg/plate	Highest dose was limited by toxicity	Liu et al. (2014)
Salmonella typhimurium TA97a, TA98, TA100, TA102, TA1537	Reverse mutation	–	–	Melamine, 1000 µg/well	Highest concentration limited by solubility	Tu et al. (2015)

[a] –, negative; ±, equivocal (variable response in several experiments within an adequate study)
HIC, highest ineffective concentration; LEC, lowest effective concentration; ppm, parts per million

The results of a test for sex-linked recessive lethal mutation in *Drosophila melanogaster* fed with melamine were equivocal; a second sex-linked recessive lethal assay using injection as the route of exposure yielded negative results (Foureman et al., 1994).

Although in cell-free systems melamine interacted with native DNA via minor groove binding by hydrogen bonds (Shen et al., 2011; Xie et al., 2015), melamine (doses up to 5000 µg/plate) did not induce reverse mutation in any of several strains of *Salmonella typhimurium* in the presence or absence of exogenous metabolic activation (Haworth et al., 1983; Zhang et al., 2011; Tu et al., 2015).

(iv) Metabolites

Haworth et al. (1983) reported that cyanuric acid (doses up to 10 000 µg/plate) gave negative results in bacterial assays for mutagenicity in several strains of *S. typhimurium*, with and without metabolic activation with a preincubation protocol. Liu et al. (2014) also reported negative results in bacterial assays for mutagenicity in *S. typhimurium* and in tests for micronucleus formation in mouse bone marrow in vivo when cyanuric acid was administered in fixed combinations with melamine.

4.2.2 Inflammation

(a) Humans

After an outbreak of melamine-associated renal stones in children in 2008 in China, Lau & Tu (2013) examined clinical differences between children who had been highly exposed to contaminated infant formula milk in Sichuan and children who had been less exposed in Hong Kong Special Administrative Region. Lau & Tu (2013) reported that children exposed to milk that was highly contaminated with melamine were younger, were diagnosed with more numerous and larger renal stones, and showed a significantly higher urinary interleukin-8 (IL-8)/creatinine ratio than children exposed to milk that was less contaminated. However, after a 12-month follow-up, the urinary IL-8/creatinine ratio for highly exposed children declined, reaching levels similar to those in children whose renal stones had been completely passed in urine. WHO reported that the contaminant in the milk associated with this outbreak was primarily melamine; however, samples of infant formula collected at random from homes in 2008 showed that although 93% contained melamine (150–4700 mg/kg), 73% also contained cyanuric acid (0.4–6.3 mg/kg) (WHO, 2009b). [The Working Group noted that the ratio of melamine to cyanuric acid was much lower than the 1:1 mixtures examined in studies in experimental systems.]

(b) Experimental systems

(i) Non-human mammals in vivo

The association between melamine-induced renal or bladder inflammation and carcinogenicity in experimental animals is not clear. For example, in a 2-year study of carcinogenicity, the kidney from female F344 rats and the bladder from male $B6C3F_1$ mice showed evidence of chronic inflammation with no significant increase in the incidence of neoplasms. Conversely, the degree of inflammation of the kidneys of male F344 rats was not significantly different from that of controls, but a significant increase in the incidence of bladder neoplasms was observed (NTP, 1983).

More recently, the inflammatory effects of short-term exposure to melamine have been further investigated. For example, melamine (60, 300, or 600 mg/kg bw per day in drinking-water for 3 months) induced an overexpression of inflammatory markers in male Sprague-Dawley rats. Specifically, treatment-related increases in bone morphogenic protein 4 (BMP4) and cyclooxygenase-2 (COX-2) were observed in the kidneys and renal arteries of treated rats at all doses (Tian et al., 2016). Proteomic analyses of urinary bladder stones from male Sprague-Dawley rats fed diets containing 2% melamine (~1000 mg/kg bw per day) for 13 weeks suggested that most of the proteins in the bladder stones were from damaged or dead cells, and some were associated with an inflammatory response (Liu et al., 2012b).

Pregnant Sprague-Dawley rats exposed to melamine at 800 mg/kg bw per day by gavage on days 6–20 of gestation showed inflammatory cells in the renal tubules associated with tubular necrosis or degeneration (Kim et al., 2011). The kidney tissue of male Sprague-Dawley rat offspring, who were exposed in utero (dams exposed at a dose of 600 mg/kg bw per day from 2 weeks before mating until gestation) and at 600 mg/kg bw per day in drinking water for 3 months after parturition, showed increased mRNA expression of chemokine ligand 2 (CCL2), tumour necrosis factor (TNF), and interleukin-1 β (IL1β) (Tian et al., 2016).

In a study of male Sprague-Dawley rats given melamine and cyanuric acid (1.26:1; 0.0315–315 mg/kg bw per day for 7 days by gavage), crystal formation in the kidneys was associated with tubular damage and secondary inflammation (Choi et al., 2010). Similarly, female Sprague-Dawley rats exposed to melamine and

cyanuric acid (1:1) during days 6–19 of gestation showed an increase in the incidence of inflammatory cells in the renal tubules and tubular necrosis or degeneration when dams were exposed at 30 mg/kg bw per day. No changes were seen in the kidneys of pups (Kim et al., 2013).

Twelve of thirteen cats exposed to pet food contaminated with melamine and cyanuric acid for 4–6 days showed histopathological signs of renal tubular necrosis and perivascular inflammation (indicated by the presence of neutrophils, macrophages, eosinophils, and lymphocytes) involving the renal subcapsular veins (Cianciolo et al., 2008). Similarly, pigs exposed to contaminated feed containing melamine and various derivatives showed evidence of chronic inflammation (indicated by infiltrates of macrophages, lymphocytes, plasma cells, and multinucleated, foreign-body-type giant cells) associated with crystals in the cortex and medulla of the kidneys, which caused flattening of the renal tubular epithelial cells (González et al., 2009). Due to the inadvertent nature of the poisonings, the amount of melamine and cyanuric acid consumed by the cats and pigs was uncertain.

(ii) Non-human mammalian cells in vitro

Indicators of inflammation have been measured in murine macrophages (Kuo et al., 2013) and canine kidney cells (Choi et al., 2010) exposed to melamine. Similarly, indicators of inflammation have been measured in canine kidney cells exposed to melamine and cyanuric acid (1.26:1) (Choi et al., 2010).

(iii) In silico

Using docking and molecular dynamics simulation, Rajpoot et al. (2016) showed that melamine may bind with some known arachidonic acid-binding sites of albumin.

4.2.3 Oxidative stress

(a) Humans

No significant increase in levels of oxidative DNA damage, as measured by urinary 8-OHdG concentrations, was observed in a cross-sectional study of infants exposed to powdered formula contaminated with melamine (Ke et al., 2010; see Section 4.2.1). [The Working Group noted that the study did not evaluate the relationship between 8-OHdG levels and the occurrence of urinary tract stones.]

(b) Experimental systems

(i) In vivo

Oral exposure to melamine affects various parameters associated with oxidative stress in rat kidneys (El Rabey et al., 2014; Al-Seeni et al., 2015). For instance, exposure to melamine decreased glutathione *S*-transferase (GST) activity and increased lipid peroxidation (malondialdehyde; MDA) in the kidney tissue homogenate of male rats (*Rattus norvegicus*) fed a diet containing melamine at 20 000 ppm (~1000 mg/kg bw per day) for 28 days. Compared with controls, melamine induced a decrease of approximately 35% in GST activity and a 53% increase in MDA concentration. Signs of impaired kidney function were also apparent (Al-Seeni et al., 2015). Similarly, El Rabey et al. (2014) showed that melamine significantly decreased GST activity and increased MDA concentration in the kidney tissue homogenate of male Wistar rats fed diets containing melamine at 30 000 ppm (~1500 mg/kg bw per day). Compared with controls, melamine induced a decrease in GST activity of approximately 47% and an increase in MDA concentration of approximately 49%.

In the ovary of Sprague-Dawley rats, melamine (20 or 40 mg/kg bw per day in corn oil, for 28 consecutive days, via oral gavage) decreased mRNA expression of superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (GPx1), and

glutathione peroxidase 2 (GPx2) in the granulosa cells (Sun et al., 2016a). Oxidative stress was also induced in the hippocampus of male Wistar rats exposed to melamine at 300 mg/kg bw per day orally for 28 consecutive days (An et al., 2012).

Similarly, melamine plus cyanuric acid (1:1) was shown to affect parameters associated with oxidative stress in rodent kidney (Lv et al., 2013a, b; Li et al., 2015), testis (Lv et al., 2013b), and ovary (Sun et al. 2016b).

(ii) In vitro

In rodent kidney cells in vitro, combined treatment with trolox, a water-soluble analogue of vitamin E, significantly attenuated the effects of melamine on intracellular production of reactive oxygen species (ROS), SOD and GPx activities, and MDA concentrations Guo et al. (2012). Similarly, Wang et al. (2015) showed that an ROS scavenger (i.e. *N*-(mercaptopropionyl)-glycine) can attenuate melamine-induced (1980 μg/mL) increases in intracellular hydrogen peroxide production in rat mesangial cells (HBYZ-1). Indicators of oxidative stress have also been measured in rat pheochromocytoma cells exposed to melamine (e.g. Han et al., 2011).

4.2.4 Immunosuppression

(a) Humans

After an outbreak of melamine-associated renal stones in Chinese children in 2008, Zhou et al. (2010) investigated the effects of melamine-contaminated milk on the cellular immunity of a cohort of exposed children. Young children (age, 1–3 years) exposed to heavily contaminated milk and who presented with renal stones had decreased levels of circulating CD3+ and CD4+ lymphocytes compared with children without stones, but the CD4/CD8 ratio for children with stones was within a normal functioning range (~2) and not significantly different from that for children without stones. Additionally, with the exception of IgM (higher in infants with stones than infants without), Zhou et al. (2010) did not observe any difference in humoral immunity (i.e. IgA, IgG, C3, or C4) between children with and without stones.

(b) Experimental systems

Evidence of toxicity has been observed in the organs of the immune system of experimental systems in vivo. For example, rats (Choi et al., 2010) and mice (Yin et al., 2014, 2016; Abd-Elhakim et al., 2016) exposed orally to melamine have shown evidence of altered immune parameters and/or histopathology. In particular, Abd-Elhakim et al. (2016) showed that exposure of male Swiss mice to melamine at 50 mg/kg bw per day, by gavage for 60 days, induced hyperplasia in the white pulp and degeneration of megakaryocytes in the red pulp of the spleen. These histopathological effects were accompanied by an increased presence of splenic CD4+ and CD8+ cells, decreased circulating leukocytes, lymphocytes, and basophils, and significantly decreased IgM, IgG, phagocytic indices of the circulating leukocytes, and lysozyme activity. Similarly, mice (Yin et al., 2014, 2016) and rats (Choi et al., 2010) exposed orally to 1:1 mixtures of melamine plus cyanuric acid have shown evidence of altered immune parameters and/or histopathology.

4.2.5 Altered cell proliferation or death

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

Melamine has been shown to have effects on cell proliferation and apoptosis in the urinary tract (NTP, 1983; Kim et al., 2011; Early et al., 2013; Toyoda et al., 2015; Tian et al., 2016), testis (Yin et al., 2013; Chang et al., 2014), ovary (Sun et al., 2016b), and spleen (Yin et al., 2014) of

experimental animals. For instance, in male and female Sprague-Dawley rats treated with melamine at a dose of ≥ 700 mg/kg bw per day by gavage for 14 consecutive days, melamine induced hyperactive regeneration of the renal tubular epithelium associated with multifocal necrosis and degeneration. Moderate tubular degeneration or regeneration was also observed in 1 out of 3 monkeys exposed to melamine at a dose of 700 mg/kg bw per day for 13 weeks (Early et al., 2013).

Similarly, melamine plus cyanuric acid has been shown to affect cell proliferation and apoptosis in the urinary tract (Lu et al., 2012), testis (Yin et al., 2013; Chang et al., 2014), and ovary (Sun et al., 2016b) of exposed rodents. Melamine plus cyanuric acid (1:1) increased the number of apoptotic renal tubular cells in the cortex and medulla of male Sprague-Dawley rats fed a diet containing melamine at a dose of 250 mg/kg bw per day for 4 weeks. The increase in apoptosis in male rats was accompanied by crystal formation and tubular necrosis (Lu et al., 2012). Co-exposure with melamine and sodium citrate has been shown to significantly attenuate crystal formation and proliferating cell nuclear antigen (PCNA) levels in Sprague-Dawley rats (Chen et al., 2013).

Male rats appear to be more sensitive to proliferative changes induced by melamine in the urinary tract than are female rats or male and female mice. For example, in studies by the NTP, melamine induced hyperplasia of the bladder epithelium in most male F344 rats fed diets containing melamine at a concentration of 750–18 000 ppm (~37.5–900 mg/kg bw per day) for 13 weeks. Conversely, female F344 rats and male and female B6C3F$_1$ mice did not show hyperplasia of the bladder epithelium after feeding with diets containing melamine at concentrations of 12 000 ppm or less (~600 mg/kg bw per day for rats; 1560 mg/kg bw per day for mice) for 13 weeks (NTP, 1983). The incidence of epithelial hyperplasia of the urinary bladder was higher in male and female mice treated with melamine for 2 years than in concurrent controls (NTP, 1983). [The Working Group noted that hyperplasia of the transitional epithelium is a common response to mechanical irritation from a foreign body in the urinary bladder of rats or mice, and that there is a very strong correlation between the occurrence of hyperplasia and the presence of bladder stones in weanling male F344 rats exposed to melamine at dietary concentrations ranging from 0.2% to 1.9% for 4 weeks (Heck & Tyl, 1985).]

(ii) Non-human mammalian cells in vitro

Melamine caused dose-dependent suppression of cell proliferation and/or increased apoptosis in rodent kidney (e.g. Guo et al., 2012; Wang et al., 2015), canine kidney (Choi et al., 2010), porcine kidney (Yiu et al., 2017), rodent pheochromocytoma (e.g. Han et al., 2011), and rodent testis (Chang et al., 2017) cells. Similarly, melamine plus cyanuric acid caused dose-dependent suppression of cell proliferation and/or increased apoptosis in canine kidney cells (Choi et al., 2010).

4.2.6 Other mechanisms

Few studies were available concerning receptor-mediated effects, immortalization, or DNA repair. Regarding epigenetic effects, melamine decreased DNA methylation in the ovary of female ICR mice given drinking-water containing melamine at a dose of 10 or 50 mg/kg bw per day for 8 weeks (Duan et al., 2015).

4.3 Data relevant to comparisons across agents and end-points

For the results of high-throughput screening assays of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA, see Section 4.3 of the *Monograph* on 1-*tert*-butoxypropan-2-ol in the present volume.

4.4 Susceptibility to cancer

No data were available to the Working Group. Susceptibility to stone formation in the urinary tract is described in Section 4.5.1.

4.5 Other adverse effects

4.5.1 Humans

In 2008 in China, an incident in which infant milk formula was deliberately adulterated with melamine caused illness in approximately 300 000 infants, including 50 000 hospitalizations and 6 confirmed deaths (WHO, 2009b). The concentrations of melamine found in 111 samples of infant formula produced by one of the main manufacturers in China ranged from < 0.05 mg/kg to 4700 mg/kg (median, 1000 mg/kg), with an estimated infant exposure of 8.6–110.2 mg/kg bw per day (WHO, 2009b). Cyanuric acid was also found in samples of infant formula from this manufacturer, albeit at much lower levels (range, 0.4–6.3 mg/kg; median, 1.2 mg/kg) (WHO, 2009b).

The exposed infants presented symptoms indicative of pathology of the urinary tract, including dysuria, haematuria, proteinuria, and passage of "sand-like" precipitates in the urine (Zhu et al., 2009; Sun et al., 2010c). Nephrolithiasis and hydronephrosis were approximately 3.1 times as frequent in male infants younger than 1 year compared with female infants of that age, but this difference was not observed in older infants (Liu et al., 2010b). [The Working Group noted that the increased incidence of nephrolithiasis and hydronephrosis was not necessarily attributable to age, but could instead have been caused by differences in the amount of formula consumed with age.] The nephroliths (stones) were primarily located in the ureter and kidney, often bilaterally, and varied considerably in gross morphology and colour, ranging from sand- or granule-like shapes to stones exceeding 15 mm in diameter. A detailed analysis of these stones revealed the presence of variable proportions of melamine (0.2–339 mg/g), uric acid, ammonium urate, ammonium magnesium phosphate, and calcium carbonate apatite (Chang et al., 2012). In a surgically resected stone, calcification increased from the core to the surface of the stone (Li et al., 2011b). In biopsy samples obtained from the kidney of a boy aged 8 months who had received melamine-contaminated formula and who had complete obstruction of the right ureter, there was evidence of generalized lymphocytic infiltration, sclerosis, and fibrosis of the glomeruli, and swelling of the tubular cells, with crystal accumulation observed in the lumen (Sun et al., 2010d). [The Working Group noted that there was epidemiological evidence that cancer of the urinary tract in humans is associated with a history of calculi in the bladder (Capen et al., 1999).]

In a survey of 589 children in China in 2009, as melamine content in formula increased, the percentage of infants with stones in the urinary tract increased (Guan et al., 2009; see Table 4.5).

In a 4-year follow-up study of 45 infants with melamine-related urinary stones who underwent conservative treatment for urolithiasis, 34 infants had no detectable stones at the end of the study period, 6 infants had stones that had partially dissolved, 4 infants had stones that had not changed in size, and a single infant had a stone that had increased in size (Yang et al., 2013).

Follow-up studies examining the incidence of cancer in children with melamine-related urinary stones are described in Section 2.

Few data were available on the toxic effects of melamine in organs other than those of the urinary tract of the infants. While some studies reported alterations in clinical chemistry markers of liver function in infants exposed to melamine (e.g. Hu et al., 2013), other studies did not report such alterations (e.g. Wang et al., 2013b). The occurrence of liver lesions, hepatomegaly, and

Table 4.5 Characteristics of children exposed to infant formula contaminated with melamine, according to the presence or absence of stones in the urinary tract

Presence of stones in the urinary tract	Age (years)			Sex		Birth type[a]		Melamine content in formula[b]		
	0 to ≤ 1 (*n* = 160)	> 1 to ≤ 2 (*n* = 224)	> 2 to ≤ 3 (*n* = 205)	Male (*n* = 341)	Female (*n* = 248)	Preterm (*n* = 36)	Term (*n* = 431)	High (*n* = 121)	Moderate (*n* = 300)	None (*n* = 168)
No. with stones (%)	11 (6.9)	24 (10.7)	15 (7.3)	30 (8.8)	20 (8.1)	7 (19.4)	29 (6.7)	23 (19.0)	19 (6.3)	8 (4.8)
No. with suspected stones (%)	30 (18.8)	36 (16.1)	46 (22.4)	64 (18.8)	48 (19.4)	7 (19.4)	87 (20.2)	30 (24.8)	58 (19.3)	24 (14.3)
No. without stones (%)	119 (74.4)	164 (73.2)	144 (70.2)	247 (72.4)	180 (72.6)	22 (61.1)	315 (73.1)	68 (56.2)	223 (74.3)	136 (81.0)

[a] Birth type was known for only 467 of the 589 children studied

[b] High melamine content was defined as > 500 ppm and moderate content was defined as < 150 ppm

Adapted from Guan et al. (2009). Melamine-contaminated powdered formula and urolithiasis in young children, Volume No. 360, issue 11, page no 1069. Copyright © (2009) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

gallstones in children who had been exposed to melamine-contaminated infant formula has been reported, but no comparison with unexposed children was included (e.g. Hu et al., 2013).

4.5.2 Experimental systems

In a study in male and female cynomolgus monkeys (*Macaca fascicularis*) dosed daily with melamine at 60, 200, or 700 mg/kg bw per day by the nasogastric route for 13 weeks, the primary target organ for toxicity was the kidney (Early et al., 2013). The range of observations, including kidney hypertrophy, renal tubular degeneration or regeneration, tubular dilatation, and tubular necrosis observed at the highest dose, was consistent with observations reported in the kidney of Sprague-Dawley rats exposed to melamine. No adverse histopathological changes were observed at the lowest dose. Several extrarenal effects were noted, including an elevation in alanine aminotransferase activity suggestive of hepatocellular injury, and pericarditis, interpreted to be secondary to uraemia.

The NTP conducted 13-week and 103-week studies in F344 rats and B6C3F$_1$ mice fed diets containing melamine (NTP, 1983; Melnick et al., 1984). In the 103-week study, the accumulation of stones was observed in the urinary bladder in male (but not female) rats; a statistically significant correlation was observed between the formation of stones and transitional cell carcinoma of the urinary bladder in male rats (see Table 3.1, Section 3). In contrast, despite a substantial increase in the incidence of stones and of epithelial hyperplasia in the urinary bladder in male mice, there was no increase in the incidence of tumours of the urinary bladder. In female mice, stones and epithelial hyperplasia in the urinary bladder were only reported in the group fed the diet with the higher dose.

In the 103-week study, there was an increase in the incidence of chronic inflammation in the kidney of female rats receiving melamine at the lowest (4500 ppm; 262 mg/kg bw per day) and highest (9000 ppm; 542 mg/kg bw per day) doses. In addition, a dose–response relationship was reported for the deposition of "calcareous deposits" in the straight segments of the proximal tubules of the kidney of female rats in one of the 13-week studies (dose range: 750–12 000 ppm; 560–1600 mg/kg bw per day). There were no reported effects in the kidney of male rats, or male and female mice (Melnick et al., 1984).

After incidents involving the adulteration of pet food with melamine and derivatives in the USA in 2007, and the adulteration of infant milk formula in China in 2008, a renewed interest in the toxicology of melamine led to a re-evaluation of archived histology slides from the NTP 103-week and 13-week studies with melamine in F344 rats (Hard et al., 2009). In contrast to the previous histopathological evaluation, the results of the re-evaluation indicated a range of kidney lesions, extending from the papilla to the cortex, and included tubule dilatation and basophilia at the 13-week end-point in male and female rats. The incidence and severity of these lesions were higher in male rats than in female rats in the 13-week studies. After exposure to melamine for 103 weeks, fibrotic scars and tubule loss were noted from the superficial cortex into the medulla of the kidney.

Several other studies have investigated the effects of melamine in the kidney of Sprague-Dawley, CD IGS, F344, and Wistar rats. Renal inflammation, fibrosis, tubular dilation, necrosis, degeneration, regeneration, transitional cell hyperplasia, ischaemic changes, hypertrophy, and elevated levels of blood urea nitrogen (BUN) and serum creatinine have been reported, without any clear evidence of strain-dependent susceptibility (Ogasawara et al., 1995; Kim et al., 2011; Wong et al., 2013; Bandele et al., 2014; El Rabey et al., 2014; Stine et al., 2014; Tian et al., 2016).

In rats, stones were not detected in the kidney after exposure to melamine, but small crystals

or crystal clusters accumulated in the lumen of the renal tubules (Bandele et al., 2014; Stine et al., 2014). The term "stone" is often misused in the literature to classify small intratubular crystals derived from melamine, as noted by Reimschuessel & Puschner (2010). While the stones formed in the kidney of infants exposed to melamine ranged from "sand-like" in size to more than 15 mm in diameter (Chang et al., 2012), the crystals formed in the renal tubules of rats treated with melamine were substantially smaller, in the order of only tens of micrometres. These crystals are soluble in formalin, and thus the true extent of their accumulation can only be ascertained in non-fixed kidney tissue with a wet-mount technique (Stine et al., 2014). Unlike the stones found in infants exposed to melamine, the precipitates formed in the kidneys of Sprague-Dawley rats exposed to melamine for 4 weeks were reported to be devoid of uric acid, and composed essentially of melamine (Cong et al., 2014); however, in F344 rats exposed for 36 weeks, the stones consisted of melamine and uric acid in equal molar ratio (Ogasawara et al., 1995).

Melamine has been studied in pigs [a model relevant for human renal physiology]. In one study in a male Yorkshire-cross pig (age, ~16 weeks) treated orally with melamine at a dose of 400 mg/kg bw per day for 3 days, there were no signs of nephrotoxicity according to blood clinical chemistry and there was no evidence of accumulation of crystals in the kidney, or of any histopathological lesion (Reimschuessel et al., 2008). In contrast, small numbers of crystals were found in the kidney of one of two weanling cross-bred Barrow pigs treated orally with melamine at a dose of 200 mg/kg bw per day for 7 days. Mass spectral analysis revealed that the crystals were composed of melamine and cyanuric acid at a ratio of approximately 1:1. In a subsequent study reported in the same publication, no crystals were detected in the kidney of eight pigs treated orally with melamine at a dose of 200 mg/kg bw per day for 28 days (Stine et al., 2011).

Nephrotoxicity (in some instances accompanied by the accumulation of renal crystals) has also been observed after exposure to melamine in other species, including sheep (Clark, 1966), broiler chickens (Brand et al., 2012), and Jinding laying ducks (Gao et al., 2010). In contrast, cats (Puschner et al., 2007) and fish (Reimschuessel et al., 2008) treated with melamine failed to show signs of nephrotoxicity under the experimental conditions used.

Although the kidney seems to be the primary organ affected by toxicity associated with exposure to melamine, toxicity has also been reported in the reproductive organs (Yin et al., 2013; Sun et al., 2016b), spleen (Yin et al., 2014), and immune system (Yin et al., 2014, 2016; Abd-Elhakim et al., 2016) of rodents.

In an incident in the USA in 2007, the adulteration of pet food ingredients with "scrap melamine" (an industrial residue from the production of melamine) containing melamine and other oxytriazines, including cyanuric acid, led to kidney disease in and the death of large numbers of cats and dogs (WHO, 2009b). Early research demonstrated that cats and F344 rats fed diets containing both melamine and cyanuric acid showed an accumulation of crystalline spherulites of a highly insoluble complex of melamine cyanurate in the lumen of the nephron, leading to obstructive nephropathy and potentially renal failure (Puschner et al., 2007; Dobson et al., 2008). A considerable number of studies have since investigated the effects of combined exposure to melamine and cyanuric acid in F344 rats (Gamboa da Costa et al., 2012; Yasui et al., 2014), Sprague-Dawley rats (Choi et al., 2010), Wistar rats (Xie et al., 2010), Kunming mice (Chang et al., 2014, 2015), C57BL/6 mice (Peng et al., 2012), pigs (Reimschuessel et al., 2008; Stine et al., 2011), and fish (Reimschuessel et al., 2008). These studies indicated that the kidney is the primary target organ of toxicity in

a broad range of species, and that the mechanism of toxicity involves the formation and accumulation of melamine cyanurate crystals in the lumen of the renal tubules. The range of reported effects associated with the obstructive nephropathy stemming from co-exposure to melamine and cyanuric acid were in general qualitatively comparable to those reported after exposure to melamine alone; however, the nephrotoxic potency of the mixture was higher than that of melamine alone, and more intense nephrotoxic effects were observed at lower doses in rats (Jacob et al., 2011; Son et al., 2014), mice (Peng et al., 2012; Chang et al., 2014), pigs (Reimschuessel et al., 2008), and fish (Reimschuessel et al., 2008) exposed to a 1:1 mixture than to melamine alone. As a result of the kinetics of absorption, distribution, and renal elimination of melamine and cyanuric acid, the timing of administration of melamine and cyanuric acid, and the mode of administration (gavage vs feed) can modulate the intensity of the nephrotoxicity of the combination in rats (Sprando et al., 2012). There was a greater accumulation of melamine cyanurate crystals in the kidney in male rats than in female rats exposed to melamine and cyanuric acid at the same doses (Gamboa da Costa et al., 2012).

Although the kidney seems to be the primary organ of toxicity associated with combined exposure to melamine and cyanuric acid, toxicity has also been reported in the gastrointestinal tract and liver (Chang et al., 2015), the reproductive organs (Yin et al., 2013), and immune system (Yin et al., 2016) of rodents. [The Working Group noted that effects were reported in studies where acute kidney toxicity, and in some instances animal mortality, was observed.]

5. Summary of Data Reported

5.1 Exposure data

Melamine has been available commercially since the late 1930s and it is primarily used in the production of certain plastic materials, including coatings, filters, adhesives, and tableware. Melamine is a chemical with a high production volume, and has world production of more than 1 million tonnes. Melamine has been used to illegally adulterate foods and animal feeds in order to increase the apparent protein content. Exposure of the general population comes from the environment, migration from food-contact materials, and from the degradation of some pesticides or disinfectants. Background exposure is generally less than 0.1 mg/kg body weight (bw) per day. Average exposures of 10–30 mg/kg bw per day have been estimated in Chinese children exposed to infant milk formula adulterated with melamine. WHO has established a tolerable daily intake (TDI) of 0.2 mg/kg bw. Specific limits for melamine have been established in several pieces of legislation as migration limits for plastic food-contact materials, and the WHO/FAO Codex Alimentarius adopted maximum levels for several food categories and for animal feeds. Occupational exposure to melamine may occur by inhalation of melamine dust during its production and its use in the manufacture of laminates, surface coatings, moulding compounds, and textiles.

5.2 Human carcinogenicity data

Two studies of cancer in humans exposed to melamine were available. A large cohort study in the USA of cancer among workers exposed to formaldehyde also identified workers exposed to other chemicals, including melamine. A positive trend in mortality attributable to cancer of the lung and duration of exposure to melamine was

observed; however, the quantitative level of exposure to melamine was not measured, and the analysis was not adjusted for tobacco smoking or exposure to other chemicals. Positive associations were also reported for leukaemia and cancer of the nasopharynx, but these also lacked adjustment for chemical exposures or other risk factors and were not reported in subsequent follow-up of the cohort.

A study of a small cohort of children who developed urinary stones after exposure to infant milk formula adulterated with melamine was considered uninformative because of its small size and short follow-up period.

5.3 Animal carcinogenicity data

In one well-conducted 103-week feeding study in male and female rats, melamine significantly increased the incidence (with a significant positive trend) of transitional cell carcinoma and of transitional cell papilloma or carcinoma (combined) of the urinary bladder in males.

In two feeding studies in male rats, melamine significantly increased the incidence of transitional cell carcinoma and of transitional cell papilloma of the urinary bladder.

In one feeding study in male and female mice (combined), melamine significantly increased the incidence of dysplasia or carcinoma in situ (combined) of the urinary bladder, and of dysplasia or carcinoma in situ (combined) of the ureter.

One well-conducted 103-week feeding study in male and female mice gave negative results. One feeding study in male and female rats (combined) gave negative results.

One initiation–promotion study in which melamine was tested as an initiator in female mice treated by skin application gave negative results.

5.4 Mechanistic and other relevant data

No data are available on the absorption or distribution of melamine in humans. In non-human primates, farm animals, and rodents, melamine is rapidly and widely distributed.

Melamine is not metabolized by mammalian tissue. Melamine is metabolized by bacteria, with production of multiple intermediates including ammeline, ammelide, cyanuric acid, and ultimately urea. Relevant bacteria, including *Klebsiella* species, are found in the human gut. Melamine has been detected in the urine of children not known to have been exposed to adulterated infant formula. In a variety of animal species, melamine is rapidly excreted.

There is evidence that melamine is not genotoxic. In the single study conducted in humans, no differences in urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels were seen. In experimental animals, melamine did not induce DNA damage, γH2AX and *Pig-a* gene mutations, or micronucleus formation. In mammalian cells in vitro, melamine did not induce gene mutations, micronucleus formation, or chromosomal aberrations. Melamine interacted with DNA in cell-free systems, but was not mutagenic in bacterial assays.

There is *strong* evidence that melamine induces chronic inflammation in the urinary tract. In human infants, a biomarker for renal inflammation was elevated and resolved after the cessation of exposure. Inflammation was seen in the urinary tract of rats and mice in the 103-week feeding studies, and in additional studies of shorter-term exposure; however, only male rats developed tumours of the bladder in the 103-week feeding study.

Oxidative stress was not observed in the single available study in exposed infants. Oral exposure to melamine in rodents induces oxidative stress in various organs, including kidney, ovary, and

the hippocampus. More pronounced effects were seen with melamine plus cyanuric acid.

There is *weak* evidence that melamine is immunosuppressive. Oral exposure to melamine induced immunosuppression in a few rodent studies.

Although no data in humans were available, melamine induced cell proliferation and increased apoptosis in the urinary tract of monkeys and rodents, with more pronounced effects with melamine plus cyanuric acid.

In infants exposed to melamine in milk formula, stones composed primarily of melamine and uric acid were found in the kidney, ureter, and urinary bladder; in most cases these stones resolved upon cessation of exposure. Nephrotoxicity was observed in some of these children. In the 103-week feeding study, male (but not female) rats developed stones in the urinary bladder (but not in the kidney), and the incidence of these stones was associated with the incidence of transitional cell carcinoma of the urinary bladder. Male mice also developed stones in the urinary bladder, accompanied by epithelial cell hyperplasia but not tumours. Unlike human infants, rats exposed to melamine do not accumulate kidney stones but instead accumulate substantially smaller intratubular crystals that are composed primarily of melamine.

Melamine was nephrotoxic in experimental animals, including cynomolgus monkeys, pigs, and rats. In a range of mammalian and fish species, co-exposure to melamine and cyanuric acid induced a nephrotoxic response at lower exposure levels than exposure to melamine alone.

Precipitates and inflammation of the urinary tract were observed in highly exposed humans and in experimental animals. Overall, inconsistent findings of inflammation, stones, and carcinogenesis were seen in different rodent sexes and species.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of melamine.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of melamine.

6.3 Overall evaluation

Melamine is *possibly carcinogenic to humans (Group 2B)*.

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