MELAMINE

This substance was considered by previous working groups, in 1985 (IARC, 1986) and 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

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

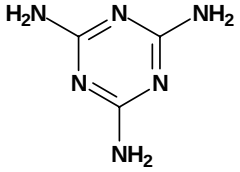
Deleted CAS Reg. Nos.: 504-18-7; 65544-34-5; 67757-43-1; 68379-55-5; 70371-19-6; 94977-27-2

Chem. Abstr. 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-*s*-triazinetriamine; 1,3,5-triazine-2,4,6(1H,3H,5H)-triimine

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_3H_6N_6$ Relative molecular mass: 126.12

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Monoclinic prisms (Budavari, 1996)

(*b*) *Melting-point*: 345°C; decomposes (Lide, 1997)

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

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

(*e*) *Octanol/water partition coefficient (P)*: log P, –1.14 (Verschueren, 1996)

(*f*) *Conversion factor*: mg/m^3 = 5.16 × ppm

1.2 Production and use

Information available in 1995 indicated that melamine was produced in 14 countries (Chemical Information Services, 1995).

Melamine forms synthetic resins with formaldehyde (Budavari, 1996). It is used in the manufacture of melamine resins, laminates, surface coating resins, plastic moulding compounds, textile resins, bonding resins, gypsum–melamine resin mixtures, orthopaedic casts, rubber additives and paper products (National Toxicology Program, 1991).

1.3 Occurrence

1.3.1 *Natural occurrence*

Melamine is not known to occur naturally.

1.3.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 1998), approximately 43 000 workers in the United States were potentially exposed to melamine. Occupational exposure to melamine may occur during its production and during its use in the manufacture of synthetic resins with formaldehyde.

1.3.3 *Environmental occurrence*

According to the Environmental Protection Agency Toxic Chemical Release Inventory for 1987, 82 000 kg melamine were released into the air, 240 000 kg were discharged into water, 11 000 kg were disposed of by underground injection and 2500 kg were released onto the land from manufacturing and processing facilities in the United States (National Library of Medicine, 1998). Exposure to melamine in the environment has been judged to be low (Environmental Protection Agency, 1988), but few quantitative data are available.

1.4 Regulations and guidelines

No international guidelines for melamine in drinking-water have been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Previous evaluation

Melamine was tested for carcinogenicity by oral administration in the diet in one study in mice and in one study in rats, and for initiating activity by skin application in one

study in mice. No neoplasm related to treatment was observed after oral administration to mice. Male rats fed diets containing melamine developed transitional-cell tumours of the urinary bladder; with one exception, all tumour-bearing animals had bladder stones probably consisting of melamine. This finding precluded a clear interpretation of the results. In a two-stage mouse-skin assay in which melamine was tested at one dose, it did not show initiating activity (IARC, 1986). [The present Working Group noted the occurrence of urinary bladder hyperplasia associated with toxicity in male mice treated with melamine in the diet.]

New studies

Oral administration

Rat: Groups of 20 male Fischer 344 rats, six weeks of age, were fed diets containing 0.3, 1 or 3% melamine (purity, > 99%) for a total of 36 weeks and were killed four weeks later. Carcinomas of the urinary bladder were observed in 0/20, 1/20 and 15/19 rats at the low, intermediate and high doses and papillomas in 0/20, 1/20 and 12/19 rats, respectively. One carcinoma and three papillomas of the ureter were also induced in 19 rats at the high dose. The findings of tumours correlated with the formation of calculi (see section 4; Okumura *et al*., 1992).

Groups of 20 male Fischer 344 rats, six weeks of age, were fed diets containing 1.0 or 3.0% melamine (purity, 99.94%), with or without 5 or 10% sodium chloride (NaCl) for a total of 36 weeks and were killed at week 40. Urinary bladder carcinomas were observed in 4/19, 18/20 and 18/20 rats given 1% melanine alone, 3% melamine alone or 3% melamine plus 5% NaCl, respectively. No carcinomas were observed in the groups receiving 3% melamine plus 10% NaCl or 1% melamine plus 5 or 10% NaCl. The incidences of papillomas were similarly decreased by NaCl. In contrast to the incidence of 10/20 in the group given 3% melamine alone, 5/20 and 3/20 rats receiving 3% melamine plus 5% NaCl or 10% NaCl, respectively, developed papillomas. Papillomas developed in 8/19 rats receiving 1% melamine alone. The occurrence of tumours correlated with calculus (melamine–uric acid salt) formation and papillomatosis (Ogasawara *et al*., 1995).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The urinary metabolites recovered after administration of hexamethylmelamine to two patients indicated that the *s*-triazine ring is very stable and does not undergo cleavage *in vivo* (Worzalla *et al*., 1974).

4.1.2 *Experimental systems*

Fifty per cent of a single oral dose of 250 mg/kg bw melamine was recovered from the urine of rats within 6 h (Lipschitz & Stokey, 1945).

The urinary metabolites, including melamine, recovered after administration of hexamethylmelamine to rats indicated that the *s*-triazine ring is very stable and does not undergo cleavage *in vivo* (Worzalla *et al*., 1974).

After administration of a single oral dose of 0.38 mg [^{14}C]-melamine to adult male Fischer 344/N rats, 90% of the administered dose was excreted in the urine within the first 24 h. An elimination half-life of 3 h and a renal clearance of 2.5 mL/min were calculated. Most of the radiolabel was concentrated in the kidney and bladder, and negligible amounts were detected in exhaled air and faeces. Virtually no residual radiolabel was observed in tissues after 24 h. Chromatography of the radiolabelled material found in plasma and urine indicated that melamine was not metabolized in rats (Mast *et al*., 1983).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

The LD_{50} values for melamine given in corn oil by gavage were reported to be 3.3 and 7.0 g/kg bw in male and female B6C3F$_1$ mice, respectively, and 3.2 and 3.8 g/kg bw in male and female Fischer 344/N rats (National Toxicology Program, 1983).

Male and female Fischer 344 rats and B6C3F$_1$ mice were fed diets containing 5000–30 000 ppm [250–1500 mg/kg bw per day for rats and 750–4500 mg/kg bw per day for mice] melamine (purity, 97%) for 14 days. A hard crystalline solid was found in the urinary bladder in most male rats receiving 10 000 ppm or more and in all treated male mice; in females, this solid was found at doses of ≥ 20 000 ppm in all rats and in 2/5 mice given 30 000 ppm. In a subsequent study, diets containing 0, 6000, 9000, 12 000, 15 000 or 18 000 ppm melamine were fed to groups of 12 male and 12 female rats and to groups of 10 male and 10 female mice for 13 weeks. Stones were found in the urinary bladders of most male rats in a dose-related manner and in the bladders of some female rats receiving ≥ 15 000 ppm. Bladder stones were observed in both male and female mice receiving ≥ 12 000 ppm. Ulceration of the urinary bladder was also seen in treated mice of each sex fed ≥ 12 000 ppm; 60% of the mice that had bladder ulcers also had stones. The distribution of bladder ulcers and stones was not considered to provide evidence for an association between ulceration and bladder stones in animals of either sex. In another study of the same duration, diets containing 750, 1500, 3000, 6000 or 12 000 ppm [37.5, 75, 150, 300 and 600 mg/kg bw per day] melamine were fed to rats. Hyperplasia of the bladder epithelium was noted in male rats receiving ≥ 3000 ppm, but in none of the female rats. Urinary bladder stones were not observed in treated or control female rats, but the incidence among male rats increased in a dose-related manner from the lowest dose (2/10) to the highest (9/9) (National Toxicology Program, 1983; Melnick *et al*., 1984).

Male Fischer 344 rats and B6C3F$_1$ mice were fed diets containing 0, 2250 or 4500 ppm melamine and female rats received 0, 4500 or 9000 ppm melamine in the diet for 103 weeks. Twenty per cent of the males at the high dose, only 2% at the low dose and none of the controls had bladder stones. Seven of the eight urinary bladders with transitional-cell carcinomas and three of the remaining 41 bladders without neoplasms had stones. There was therefore a statistically significant ($p < 0.001$) correlation between the presence of bladder stones and bladder tumours. Although 4% of the controls and 85 and 93% of the treated male mice had bladder stones, none developed bladder tumours (National Toxicology Program, 1983; Melnick *et al*., 1984).

Groups of 20 male Fischer 344 rats were fed diets containing 0, 0.3, 1 or 3% melamine in the diet for 36 weeks followed by a four-week recovery period. Ten animals per group underwent exploratory laparotomy at the end of week 36, and all animals were killed at week 40. The weight of the bladder was threefold greater in rats receiving 3% in the diet than in controls. The incidences of papillary or nodular hyperplasia were 0 in controls, 5% with 0.3% melamine, 30% with 1% melamine and 63% with 3% melamine; those of papillomatosis were 0, 0, 25 and 89% and those of calculi were 0, 0, 70 and 100% at 36 weeks and 0, 20, 45 and 42% at 40 weeks. The correlation between calculus formation at week 36 and tumour incidence at week 40 was highly significant ($p = 0.0065$) (Okumura *et al*., 1992).

The incidence and composition of urinary bladder calculi after exposure for 36 weeks to melamine with and without additional NaCl in the diet was studied in five-week-old male male Fischer 344/DuCrj rats. Water intake, as a surrogate for urinary output, was increased in groups exposed to 3% melamine with or without 5 or 10% NaCl, in groups exposed to 1% melamine with 5 or 10% NaCl and in a group exposed to 10% NaCl only; water intake was not increased in animals exposed to 1% melamine only. The incidences of calculi and papillomatosis were 30 and 75% with 3% melamine, 75 and 85% with 3% melamine plus 5% NaCl, 30 and 10% with 3% melamine plus 10% NaCl, 37 and 47% with 1% melamine, 11 and 11% with 1% melamine plus 5% NaCl, 5 and 0% with 1% melamine plus 10% NaCl. No calculi or papillomatosis were reported in controls or with 10% NaCl alone. Therefore, the addition of NaCl to 1% melamine decrease the incidences of calculi and papillomatosis, in parallel with a decrease in the incidence of neoplasia. With 3% melamine, NaCl did not affect the induction of calculi or papillomatosis but decreased the incidence of neoplasia. Thus, with the lower concentration of melamine NaCl appeared to increase urinary output and decrease the incidences of hyperplasia, calculus formation and neoplasia. Chemical analysis of the calculi showed that they contained approximately equal amounts of melamine and uric acid on a molar basis, which together accounted for 61–81% of the weight (Ogasawara *et al*., 1995).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Melamine induced λ prophage in *Escherichia coli* WP2s(λ) but did not induce reverse mutation in *Salmonella typhimurium* in the presence or absence of an exogenous metabolic activation system. Sex-linked recessive lethal mutations were not induced in *Drosophila melanogaster*. Melamine did not induce gene mutation in mouse lymphoma L5178Y $tk^{+/-}$ cells.

It was reported in abstracts that melamine did not induce gene mutation in *S. typhimurium*, sister chromatid exchange in Chinese hamster ovary cells *in vitro* or micronuclei in mouse bone marrow *in vivo* (Mast *et al*., 1982a,b).

4.5 Mechanistic considerations

No biotransformation of melamine has been reported. A highly statistically significant, consistent relationship between bladder neoplasia in male rats and calculus formation has been found: in one bioassay, all but one rat with urinary bladder tumours also had bladder stones. The ability of rodents to eliminate bladder stones may account for the absence of stones in the one rat. The bladder stones contained melamine and uric acid in equal molar amounts, as well as other components. Melamine also produced a dose-related increase in the incidence of urinary bladder hyperplasia and papillomatosis but only at doses that also produced calculi. The addition of NaCl to the diets, which increased water consumption, inhibited the formation of bladder calculi, bladder hyperplasia and neoplasia in male rats exposed to 1% [500 mg/kg bw per day] melamine, which is similar to the dose that produces bladder tumours (Teelmann & Niemann, 1979). Although bladder tumours related to calculus formation are not considered to be species-specific, they are related to administration of high doses (Capen *et al.*, 1999).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to melamine may occur during its production and use in the manufacture of synthetic resins with formaldehyde.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Melamine has been studied for carcinogenicity in mice and rats of each sex by oral administration. It produced urinary bladder and ureteral carcinomas in male rats but only

Table 1. Genetic and related effects of melamine

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Prophage induction, *Escherichia coli* WP2s (λ)	+	+	78 μg/well	Rossman *et al.* (1991)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, reverse mutation	–	–	1110 μg/plate	Haworth *et al.* (1983)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		1% feed	Röhrborn (1962)
Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	160 μg/mL	McGregor *et al.* (1988)

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

urinary bladder hyperplasia in male mice. The occurrence of urinary bladder tumours in male rats correlated strictly with calculus formation and exposure to high doses. The dose dependence was confirmed by subsequent studies in male rats in which concomitant administration of sodium chloride to increase urinary output resulted in a decreased tumour yield.

5.4 Other relevant data

There is no evidence that melamine undergoes biotransformation. The urinary bladder tumours seen in male rats exposed to high doses of melamine appear to be produced by a non-DNA-reactive mechanism involving epithelial hyperplasia secondary to the presence of melamine-containing bladder stones. Consequently, bladder tumours would not be expected in either rodents or humans except at doses that produce bladder calculi.

No data were available on the reproductive or developmental toxicity of melamine.

No data were available on the genetic and related effects of melamine in humans. It was not genotoxic in experimental systems.

5.5 Evaluation

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

There is *sufficient evidence* in experimental animals for the carcinogenicity of melamine under conditions in which it produces bladder calculi.

Overall evaluation

In making its overall evaluation, the Working Group noted that the non-DNA-reactive mechanism by which melamine produced urinary bladder tumours in male rats occurred only under conditions in which calculi were produced.

Melamine is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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