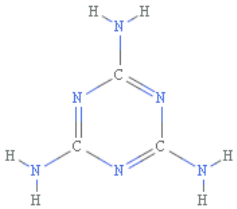
WORKPLACE ENVIRONMENTAL EXPOSURE LEVEL®

Melamine (2016)

I. IDENTIFICATION[1,2]

Chemical Name: 1,3,5-triazine-2,4,6-triamine
Synonyms: 2,4,6-triamino-1,3,5-triazine, cyanuramide
CAS Number: 108-78-1
Molecular Formula: $C_3H_6N_6$
Structural Formula:



II. CHEMICAL AND PHYSICAL PROPERTIES[2]

Physical State and Appearance: White solid
Odor Description and Threshold: Not available
Molecular Weight: 126.12
Conversion Factors: Not available
Melting Point: 354 °C (662 °F)
Boiling Point: Sublimes
Vapor Pressure: 3.59 x 10^{-10} mm Hg @ 20 °C (extrapolated)
Solubility in Water: 3.2 g/L at 20 °C (68 °F)
Log (octanol/water partitioning): -1.14
Flammability Limits: Not available
Flash Point (closed cup): >93.3 °C (200 °F)
Autoignition Temperature: >500 °C
Specific Gravity: 1.573 g/cm^3 @ 14 °C (57 °F)
Reactivity and Incompatibilities: No data available

Commercially available melamine has a mass median diameter of approximately 0.1 µm.[3] There was one government import record for ground melamine imported from Japan, and the melamine particle size was all <10 µm.

III. USES

Melamine is used mainly for the production of resins and polymers. Less than 1 % is used as a flame retardant in plastics or as a concrete additive.

IV. ANIMAL TOXICITY DATA

A. Acute Toxicity

1. Lethality Data

Species	Route	LD_{50} Or LC_{50}
Rat	Oral	3500 mg/kg (range 3161-3828)[4]
Rat	Inhalation (4-hr)	>5190 mg/m^3[3,8]
Mouse	Oral	5150 mg/kg (range 3296-7014)[4]

2. Eye Irritation

Melamine is not an eye irritant based on studies in rabbits. One study dosed rabbits with 30 gs dry powder, and the other study dosed 0.05 ml of a 10% suspension.[4]

3. Skin Absorption

Acute toxicity following skin application has not been studied for melamine. The maximum human skin permeation rate (skin in contact with saturated aqueous solution) was estimated to be 0.34 µg/cm^2/hr.[5] This estimated skin permeation rate would not likely add significantly to total systemic dose at the concentrations associated with the WEEL.

4. Skin Irritation

Melamine is not a skin irritant based on studies in rabbits. Study details are: 1 g aqueous paste/kg bw and also 0.1 g of a 4% aqueous solution/kg bw, occlusive, 18-hour exposure. A study with guinea pigs dosed with 1% melamine solution in water did not result in irritation.[4,6]

5. Skin Sensitization

Skin sensitization was not observed in guinea pigs or in human volunteers.[4,6]

6. Acute Inhalation Toxicity

Rats inhaled air saturated with melamine; no lethality was observed.[4] The estimated vapor pressure under heated conditions (at 100 °C (212 °F) is 0.157 Pa or 1.18E-3 mmHg).[7]

The concentration in air, corresponding with this vapor pressure, was 1.6 ppm or 6.6 mg/m^3.

In another study, rats were exposed for 4 hr to an aerosol of solid ultrafine grade melamine. In preparing the aerosol the material appeared to be slightly sticky. The particle distribution indicated 15 µm as median diameter and approximately 30 µm was the 90th-percentile particle size. These parameters reflect more the behavior of the aggregates than of the individual particles. No mortalities or other serious clinical signs were observed.[3,8]

B. Subacute Toxicity

1. Inhalation

No data available.

2. Oral

Male Fischer-344 (F-344) rats (40 animals per dose group) were given feed containing 2000, 4000, 7000, 10,000, 13,000, or 19,000 ppm melamine daily for 28 days. The corresponding daily doses ranged from 133 mg/kg-day to 1267 mg/kg-day. Melamine produced a dose-dependent incidence of urinary bladder calculi and urinary bladder hyperplasia. All animals with hyperplasia, except one, had calculi. Spectroscopic analysis of stones indicated the presence of melamine, phosphorus, sulfur, potassium, and chloride. The no observed adverse effect level (NOAEL) was 2000 ppm melamine in feed or a daily dose of 133 mg/kg-day.[9]

C. Subchronic Toxicity

1. Inhalation

No data available.

2. Oral

Fischer-344 rats and $B6C3F_1$, mice were exposed to 750, 1500, 3000, 6000, 9000, 12,000, 15,000, or 18,000 ppm melamine in their diet for 90 days. In male rats, there was a dose-dependent increase in the incidence of urinary bladder stones starting at the feeding level of 750 ppm, the lowest observable adverse effect level (LOAEL). In female rats, bladder stones were observed at a dietary level of 15,000 ppm and higher. In mice, bladder stones were found at a dietary level of 12,000 ppm and higher. Hyperplasia of the bladder epithelium was observed, when bladder stones were also noted.[10]

The NOAELs for bladder stones in this study were: male mice 2,800 mg/kg-day, female mice 3,500 and female rats 1,200 mg/kg-day. In male rats, the LOAEL (lowest dose) was 72 mg/kg-day.

D. Chronic Toxicity/Carcinogenicity

1. Inhalation

No data available.

2. Oral

In an NTP chronic study, groups of 50 F-344 rats and 50 $B6C3F_1$ mice per dose level per sex received melamine through their food intake for 2 years. The dietary melamine exposure levels were 0, 2250, or 4500 ppm for male rats and mice of each sex, and 0, 4500, or 9000 ppm for female rats. The survival rate in the high dose group of male rats was decreased significantly from week 101 compared with the male control group. Survival did not differ in the other rat dose groups. Food consumption and mean body weight did not differ significantly from the control groups.

In each of the male rats of the high dose group, transitional-cell carcinomas of the urinary bladder were found, but in all other dose groups of mice and rats these tumors did not occur.[10] Bladder stones were not found in female rats. There were no male rats with bladder stones in the control group, but in the low and high dose groups 1 and 10 rats, respectively, were found with urinary bladder stones. With one exception, bladder stones were observed in all rats having transitional-cell carcinoma. The bladder stones consisted mainly of melamine. The LOAEL is 2250 ppm, but an absolute value for a NOAEL could not be established. The survival in the high dose group of mice was decreased significantly compared with the controls. Food consumption and body weight were not significantly affected by melamine dosing. In male mice there was a marked increase in the incidence of urinary bladder stones (40 of 47 mice examined in the low dose group, and 41 of 44 examined in the high dose group) as well as acute and chronic inflammation and epithelial hyperplasia of the urinary bladder. Urinary bladder stones were observed in 4 of 50 high dose female mice. There were no other lesions (neoplastic or non-neoplastic) associated with the administration of melamine in male or female mice.

To clarify the findings in the NTP study cited above, and to establish a NOAEL, a lifetime feeding study was conducted in rats. Groups of 55 male F-344 rats were fed a diet containing 0, 100, 500, or 1000 ppm melamine for 123 weeks, and groups of 55 female F-344 rats were fed a diet containing 0, 100, 1000, or 2000 ppm for 130 weeks. No differences in survival, body

weight, food consumption, gross and clinical pathology, and histopathology were observed between the control and treated groups to the end of the exposure periods.[11]

The incidences of bladder transitional cell carcinomas and papillomas, and the occurrence of hyperplasia of the papilla in the kidneys, is suppressed dose-dependently by a simultaneous NH_4Cl treatment.[12] NH_4Cl co-treatment reduces calculi formation. Therefore, the authors of the study concluded that the irritative stimulation of calculi, not molecular interaction, caused the urinary tract lesions.

The highest NOAEL in male rats (the most sensitive of the tested species and sexes), was 1000 ppm in the food. This dietary concentration corresponds to a daily dose of 80 mg/kg-day (based on food intake of 0.03 kg/day and body weight of 0.38 kg).

Neither the literature reviewed nor the scientific agencies whose focus is on identifying carcinogens support the classification of melamine as carcinogenic in humans.[13,14]

E. Reproductive/Developmental Toxicity

Three groups of pregnant Wistar rats (ages approximately 6 months) were dosed intraperitoneally (i.p.) with 70 mg/kg-day melamine. Group 1 animals were dosed on gestational days 4 and 5 (N = 21), group 2 animals were dosed on gestational days 7 and 8 (N = 26), and group 3 animals on gestational days 11 and 12 (N = 26). Maternal animals were euthanized and litters examined on gestational day 21. Litters were examined for success of implantation, fetal weight, length and viability as well as litter weight and gross malformations. The authors reported that there were no significant effects of melamine treatment on any of these parameters. The group treated on gestational days 4 and 5 did have fewer successful implants, fewer live fetuses and more resorptions than the other two treatment groups. The authors state that the findings in the first group were not statistically different from controls but data for control animals are not provided in the publication, so this cannot be verified. The overall conclusion of the authors was that melamine "is harmless to the rat fetus."[15]

F. Genotoxicity/Mutagenicity

1. In vitro

The genotoxic potential of melamine has been evaluated in numerous assays. The weight of evidence suggests that melamine is not genotoxic. No evidence of genotoxicity was reported in Ames test with *Salmonella typhimurium* strain hisg 46, TA 1530, TA 1531, TA 1532, TA 1534, TA 1535, TA 1537, TA 1538, TA 98, and TA 100 and *Saccharomyces cerevisiae* D4.[16,17] No genotoxicity was seen in DNA damage and repair assay in rat hepatocyte primary culture or in *Escherichia coli* WP2uvrA, W3110/polyA+, and p3478/polyA.[16,18]

2. In vivo

Melamine was also evaluated and found negative in HGPRT forward mutation assay with Chinese hamster ovary (CHO) cells.[19] Mouse lymphoma assay with mouse lymphoma cells L5178Y[20] and sister-chromatid exchange (SCE) assay with CHO cells[19] were negative too. Melamine also yielded negative results in sex-linked recessive lethal (SLRL) test with *Drosophila melanogaster* (oral feed contained up to 1% melamine)[21] and micronucleus assay with CD-I mice (single application of 1000 mg/kg; administration route not listed).[19]

G. Metabolism/Pharmacokinetics

Melamine was administered as a single dose to rats (250 mg/kg) and dogs (125 mg/kg). Of the quantity ingested, 50% and 61.3%, respectively, was recovered in the urine within 6 hr.[22] From this result, a biological half-life of 6 hr in rats and 4.4 hr in dogs was estimated based on assumed first-order kinetics. These data suggest that melamine does not accumulate in tissues.

The metabolism, excretion, and disposition of melamine were determined after administration of a single oral dose of 0.025 mCi (0.38 mg, 1.3 mg/kg/bw) [^{14}C] melamine to adult male F344 rats. Within the first 24 hr, 90% of the administered dose was excreted in the urine. Negligible radioactivity appeared in the breath and feces. Blood, liver, or plasma concentrations were found to be similar, suggesting that melamine distributes in body water. Much higher levels of radioactivity than in plasma were found only in the kidney and the bladder. The bladder level was by far the highest. According to the authors of the study, this finding is probably due either to back diffusion from urine or to contamination of bladder tissue with urine. Virtually no residual radioactivity was observed in tissues examined at 24 hr or later. The elimination phase half-life calculated from plasma data (2.7 hr) agreed with the urinary-excretion half-life of 3 hr. The renal clearance of melamine was 2.5 mL/min. Radioactivity in plasma or urine co-chromatographed with that of the dosing solution, indicating that melamine is not metabolized in the male F-344 rat.[20]

V. HUMAN USE AND EXPERIENCE

The use of melamine has not been associated with reports of occupational morbidity or mortality. Recent occurrences of renal and bladder stones in humans following ingestion of contaminated food confirm the ability of melamine to induce such effects in humans. Public health organizations have recommended safe doses for humans with a range of 0.2 to 0.5 mg/kg-day.[23,24]

VI. RATIONALE

Melamine has a very low acute toxicity. It is not genotoxic, not a skin or eye irritant, nor a skin sensitizer. In the single developmental toxicity study identified, melamine did not induce teratogenic effects in rats. The primary adverse effect associated with melamine based on dietary administration in rats and mice is the formation of bladder stones. The formation of bladder stones was associated with the occurrence of urinary bladder tumor formation. Since melamine is not genotoxic and is not tissue reactive or metabolized, the cause of bladder inflammation, hyperplasia, and tumors has been hypothesized to result from irritation of the bladder tissue by bladder stones when the solubility of melamine in urine is exceeded[12]. No workplace studies were available that identified adverse effects in humans from occupational exposure to melamine. There are recent human exposure case studies from melamine-contaminated food. For example, nearly 53,000 individuals (primarily infants and small children) reportedly developed kidney stones after melamine adulterated milk was consumed in China in 2008.[25] Concentrations of melamine in the milk ranged from 0.1 to 2,500 mg/L, but concentrations in the brand of infant formula most strongly associated with the disease outbreak (Sanlu) had a mean melamine concentration of 1212 mg/L.[25,26] Some international public health agencies have used these case studies and the NTP rat study to estimate a safe non-occupational melamine dose to avoid bladder stones. In 2008, the World Health Organization and the Food and Agricultural Organization of the United Nations recommended a safe human dose of 0.2 mg/kg-day to lower the risk of bladder stones.[23,24] General population exposure limits set by other agencies range from 0.2 to 0.5 mg/kg-day. Analyses have suggested the levels of melamine exposure in the Chinese cases were up to 200 times higher than the estimated safe dose.[27]

The LOAEL in the male rat (the most sensitive species and sex) is 75 mg/kg-day. A typical 70-kg worker is estimated to inhale 10 m^3 of air during a typical work day. Based on breathing rate and body-weight assumptions, the dose in rats associated with urinary bladder effects corresponds to an air concentration of 525 mg/m^3. It is noteworthy that the standards for general good industrial hygiene practice of 10 mg/m^3 would yield a total dose well below the limit of urine solubility, suggesting limited concern for calculi formation and resulting secondary effects in the urinary bladder. A WEEL of 3 mg/m^3 will provide an additional margin of safety to account for extrapolation from animal studies and incidences of shorter duration than lifetime worker exposures. This WEEL value is consistent with the range of melamine exposure limits established by various regulatory agencies intended to protect members of the general public.

VII. RECOMMENDED WEEL

8-hr time-weighted average (TWA): 3 mg/m^3 inhalable fraction and vapor.

This WEEL value was originally established in 1999. No significant new literature was identified since 1999 that supports a change to the recommended WEEL value.

VIII. REFERENCES

(1) Clayton, G. D.; Clayton, F. E.; Cralley, L. J.; Cralley, L. V.; Harris, R. X.; Bus, J. S. Melamine. In *Patty's Industrial Hygiene and Toxicology. Vol. 2E, Chap. 35 [CD-ROM]*.; John Wiley & Sons: New York, 1996; Vol. Vol. 2E, Chap. 35 [CD-ROM]., pp. 3402–3405.

(2) PubChem. Melamine- Compound Summary for CID 7955. U.S. National Library of Medicine (NLM), Bethesda, MD. https://pubchem.ncbi.nlm.nih.gov/compound/7955#section=Top (accessed Feb 5, 2016).

(3) DSM Corporate Safety, Environment, Health & Technology. *Acute Inhalation Toxicology of Melamine Solid Ultrafme Grade. April 20, 1998. [Correspondence from W. Ten Berge to F. van Yperen and J. OpdenKamp-Oosterhof, Made Available to the AIHA WEEL Committee.]*; 1998.

(4) European Chemicals Bureau (ECB). *Melamine — International Uniform Chemical Information Database (IUNID) Data Sheet.*; 1996.

(5) Wilschut, A.; ten Berge, W. F.; Robinson, P. J.; McKone, T. E. Estimating Skin Permeation. The Validation of Five Mathematical Skin Permeation Models. *Chemosphere* **1995**, *30*, 1275–1296.

(6) Schaffer, C.B. *Personal Communication. As Cited in Reference # 1; P. 3405.*; American Cyanimid Company, 1955.

(7) Meylan, W. *MPBPWIN, Ver. 3.16 (computer Program). Copyright 1994-1995*; Syracuse Research Corporation: Syracuse, NY, 1995.

(8) Arts, J. H. E. *Acute (4~hour) Inhalation Toxicity Study with Melamine in Rats (TNO Report No. V9S.420)*; TNO Nutrition and Food Institute: AJ Zeist, The Netherlands, 1998.

(9) Friedman, M. A. *Supplemental Information on Melamine. Unpublished Data.*; American Cyanimid Company: Wayne, NJ, 1982.

(10) Melnick, R. L.; Boorman, G. A.; Haseman, J. K.; Montali, R. J.; Huff, J. Urolithiasis and Bladder Carcinogenicity of Melamine in Rodents. *Toxicol. Appl. Pharmacol.* **1984**, *72*, 292–303.

(11) Rao, G. N.; Giesler, P. J.; Palmers, T. E.; Mast, R. W.; Friedman, M. A.; Shaffer, C. B. Chronic Toxicity of Melamine in Fischer-344 Rats — Abstracts of the 1982 Annual Conference of the Society of Toxicology (Abstract 626). *The Toxicologist* **1982**, *2*.

(12) Ogasawara, H.; Imaida, K.; Ishiwata, H.; Toyoda, K. Urinary-Bladder Carcinogenesis Induced by Melamine in F344 Male-Rats - Correlation between Carcinogenicity and Urolith Formation. *Carcinogenesis* **1995**, *16*, 2773–2777.

(13) IARC. *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Chemicals Used in Plastics and Elastomers, Vol. 39.*; International Agency for Research on Cancer (IARC): Lyon, France, 1986.

(14) NTP. 13th Report on Carcinogens. National Toxicology Program, U.S. Dept. of Health and Human Services. Washington, DC. http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html (accessed Feb 8, 2016).

(15) Thiersch, J. B. Effect of 2,4,6, Triamino-"S"-Triazine (TR), 2,4,6 "Tris" (Ethyleneimino)-"S"-Triazine (TEM) and N, N', N''-Triethylenephosphoramide (TEPA) on Rat Litter in Utero. *Exp. Biol. Med.* **1957**, *94*, 36–40.

(16) Litton Bionetics, Inc. *Mutagenicity Evaluation of Melamine (2,4,6-Triamine-S-Triazine), Final Report (LBI Project No. 2838). September 1977. [Unpublished Report, Prepared for DSM Research and Patents.]*; Litton Bionetics, Inc: Kensington, MD, 1977.

(17) Mast, R. W.; Friedman, M. A.; Finch, R. A. Mutagenicity Testing of Melamine — Abstracts of the 1982 Annual Conference of the Society of Toxicology (Abstract 602). *The Toxicologist* **1982**, *2*, 172.

(18) Pharmakon Research International, Inc. *ACHO/SCE In Vitro Sister Chromatid Exchange in Chinese Hamster Ovary Cells (CHO) C Melamine (PH319-AC-002-82). May 1982.*; Pharmakon Research International, Inc: Waverly, PA, 1982.

(19) Mast, R. W.; Naismith, R. W.; Friedman, M. A. Mouse Micronucleus Assay of Melamine. In *Abstracts of the 13th Annual Meeting of the Environmental Mutagen Society*; Environmental Mutagenesis: Boston, 1982; Vol. 4, pp. 297–344.

(20) Mast, R. W.; Jeffcoat, A. R.; Sadler, B. M.; Kraska, R. C.; Friedman, M. A. Metabolism, Disposition and Excretion of [14C]melamine in Male Fischer 344 Rats. *Food Chem. Toxicol.* **1983**, *21*, 807–810.

(21) Lüers, H.; Röhrborn, G. The Mutagenic Activity of Ethyleneimino Derivatives with Different Numbers of Reactive Groups. *Genet Today Proc Int Congr* **1963**, *1*, 64–65.

(22) Lipschitz, W. L.; Stokey, E. The Mode of Action of Three New Diuretics: Melamine, Adenine and Formoguanamine. *J. Pharmacol. Exp. Ther.* **1945**, *83*, 235–249.

(23) World Health Organization (WHO). WHO Expert meeting to review toxicological aspects of melamine and cyanuric acid. Food and Agriculture Organization of the United Nations (FAO). Rome, Italy. http://www.fao.org/food/food-safety-quality/a-z-index/melamine/en/ (accessed Feb 8, 2016).

(24) EFSA. Scientific Opinion on Melamine in Food and Feed. European Food Safety Authority, Parma, Italy. http://www.efsa.europa.eu/en/efsajournal/pub/1573 (accessed Feb 8, 2016).

(25) Center for Food Safety and Applied Nutrition. Chemical Contaminants - Interim Safety and Risk Assessment of Melamine and its Analogues in Food for Humans. U.S. Food and Drug Administration (FDA). Washington, DC. http://www.fda.gov/Food/Foodborneillnesscontaminants/chemicalcontaminants/ucm164522.htm (accessed Feb 8, 2016).

(26) Hilts, C.; Pelletier, L. *Background Paper on Occurrence of Melamine in Foods and Feed Prepared for the WHO Expert Meeting on Toxicological and Health Aspects of Melamine and Cyanuric Acid. In Collaboration with FAO Supported by Health Canada Health Canada, Ottawa, Canada 1–4 December 2008*; World Health Organization (WHO): Geneva, 2009.

(27) Lawley, R.; Curtis, L.; Davis, J. Non-Biological Chemical Contaminant: Melamine. In *The Food Safety Hazard Guidebook*; Royal Society of Chemistry, 2015; pp. 397–401.