ECETOC

February, 1983

Joint Assessment of Commodity Chemicals

No. 1

MELAMINE

CAS : 108-78-1

European Chemical Industry
Ecology & Toxicology Centre

Avenue Louise 250, B. 63
B - 1050 Brussels, Belgium

THE ECETOC SCHEME FOR THE "JOINT ASSESSMENT OF COMMODITY CHEMICALS" (JACC)

This report has been produced as part of a programme for making critical reviews of the toxicology, including ecotoxicology, of selected industrial chemicals.

A number of organisations, world-wide, have produced and are continuing to produce such reviews with the aim of ensuring that, based on an up-to-date knowledge of the toxicological, and other relevant, information regarding existing chemicals they can continue to be produced and used safely. ECETOC is contributing to this activity with its JACC reviews.

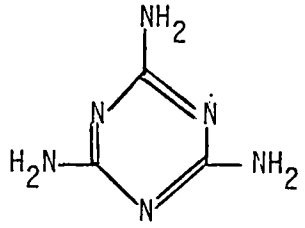
In general, commodity chemicals, ie. those produced in large tonnage by several companies and having widespread and multiple uses, are reviewed jointly by experts from a number of the companies concerned. Before it is decided to review a chemical, every effort is made to discover whether an adequate review exists already, in which case no work is necessary.

It should be noted that in a JACC review only the uses of the chemical as such are considered, ie. its occurrence as an impurity in other products is not normally taken into account.

CONTENTS

A critical assessment of the toxicology and ecotoxicology of melamine is presented. Melamine was chosen as one of four, varied chemicals on which trial exercises were carried out to aid in developing the JACC scheme.

A. CHEMICAL IDENTITY AND PHYSICAL-CHEMICAL PROPERTIES



$C_3N_6H_6$

CAS No.108-78-1

1. Physical Properties

Molecular weight	126.13
Physical form	Crystalline solid (monoclinic colourless prisms)
Boiling point	sublimes
Melting point	354°C
Specific gravity (14°C)	1.573
Vapour pressure	50 mm Hg (315°C)
Vapour density	4.34 (air=1)
Refractive index (20°C)	1.872
Solubility	Slightly soluble in water; very soluble in hot water; very slightly soluble in hot alcohol.

2. Chemical Properties

Purity of technical product : typically 99.5%.

Impurities in technical melamine :

- Guanidine
- Urea
- Ammeline
- Ammelide
- Ureidomelamine
- Melamine cyanurate

3. Monitoring

Methods for analysing melamine depend on the purpose of the analysis, which could be for the determination of :

- melamine content
- purity

- content of impurities in melamine
- content in waste water.

Melamine can be specifically and quantitatively determined by gravimetric analysis in which it is precipitated by cyanuric acid (Seiffarth and Ardelt, 1966).This method is used for the determination of melamine in urine and blood (Kechek et al.,1975), but is laborious.

Melamine can also be determined by measuring the extinction at 235 nm of a solution of melamine in ca. 1 N HCl, but the method is not specific for melamine because ammeline and ammelide also absorb at about the same wavelength (Farrow et al., 1972). Before the spectrometric determination, melamine can be separated from impurities by ion-exchange chromatography (Boitsov et al., 1969) for which both cationic and anionic exchangers can be used. Anionic exchangers are preferred because the solvents used with them are less corrosive to the equipment.

B. PRODUCTION AND USE

Melamine is produced by the catalysed decomposition of urea at elevated temperature and pressure in the presence of excess ammonia.

World production is estimated at 305,000 T/y. The installed production capacity in Europe is about 175,000 T/y. It is used mainly for the production of resins and polymers. Less than 1% is used as flame retarder in plastics or as an additive in concrete.

C. HUMAN EXPOSURE CONDITIONS

People may be occupationally exposed to melamine in production plants by direct inhalation of dust. The number of these production workers is estimated to be between 400 and 500 in the EEC countries. Data about the exposure of industrial users of melamine are not available.

Threshold limit values for melamine have not been established in Europe or the United States, possibly because of its very low toxicity. Melamine should be considered as a nuisance dust, for which the ACGIH threshold limit value of 10 mg/m^3 is applicable.

D. TOXICOLOGICAL DATA

Toxicological information about melamine has been reviewed by Patty (1981). Information on recent toxicological research sponsored by American, European and Japanese companies is also available.

1. Human Data

In the scientific literature almost no data about the toxicity of melamine to man have been reported , and only information on irritation and sensitization is available. Human subjects were exposed to melamine by means of patch tests but it did not cause irritation or sensitization (Shaffer, 1955).

2. Experimental Data

2.1. Acute toxicity

The acute oral LD_{50} for mice is 4500 mg/kgbw. Signs of toxicity accompanying the administration of lethal doses to mice included lachrymation, dyspnea, intermittent tremors and coma preceding death. Vasodilatation in the tail and ears, and paralysis of the forequarters were also observed (Schaffer, 1955). The acute oral LD_{50} for male and female rats is 3160 and 3850 mg/kgbw, respectively (NTP,1982).Melamine produced neither local irritation nor systemic toxicity when applied as a paste in water to the skin of rabbits at doses as high as 1 g/kgbw. A 1% solution in water, applied under a rubber cuff to guinea pig skin, produced little or no irritation (Shaffer, 1955). Sensitization in guinea pigs could not be induced (Fassett and Roudabush, 1981).

Introduction of melamine powder into the rabbit eye caused mild transient irritation, but a ten percent suspension in water was without any effect (Shaffer, 1955).

2.2. Subacute toxicity

When rabbits and dogs were fed 126 mg/kgbw daily for 1 to 4 weeks, no effects were found either macroscopically or microscopically (Lipschitz and Stockey, 1945).

Crystalline deposits were found in the renal tubules of rats given five successive intraperitoneal doses of 500 mg/kgbw. No symptoms were observed except for moderate transient weight loss, and histological

examinations revealed no differences from the controls. (Philips and Thiersch,1951).

Pre-chronic studies (NTP,1982) showed the formation of bladder stones in rats dosed with high concentrations of melamine in their feed. In a 14-day study, most of the males were given 10,000 ppm melamine or more, and most of the females 20,000 ppm or more. In two 13-week studies there was a dose-related incidence from 750 to 18,000 ppm in males, and at levels of 15,000 and 18,000 ppm in females. In the 13-week studies these findings were accompanied by epithelial hyperplasia of the urinary bladder which increased in gravity and frequency, in a dose-related fashion, in males dosed with 3,000 ppm to 18,000 ppm, and in 2 of 10 females dosed with 18,000 ppm. The addition of 1% ammonium chloride to the drinking water had no apparent effect on the incidence of urinary bladder stones related to the feeding of melamine. Kidney changes in male rats were minimal. Dose-related calcareous deposits were observed in the proximal tubules in female rats. In these studies, males showed a decrease in weight-gain at doses of 3,000 ppm above, and females at 12,000 ppm and above (NTP, 1982).

2.3. Chronic toxicity including carcinogenicity

Dogs were fed with melamine for 1 year at a dietary level of 30,000 ppm. After 60 to 90 days the dogs showed melamine crystalluria, which continued until the end of the treatment. At these levels there were no gross pathological and histopathological effects which could be attributed to the feeding of melamine (Shaffer, 1955).

Rats were fed melamine for two years at a dietary level of 1,000 and 10,000 ppm. Bladder stones associated with benign papillomata were found in about one-third of the animals at the higher dose level. These papillomata are interpreted as a typical response of the bladder mucosa to the presence of bladder stones. From later experiments it appeared that the bladder stones consisted of melamine. No other gross pathological and histopathological effects were found at either dose level compared to the controls (Shaffer,1955).

A two-year feeding study in Fischer 344 rats and B6C3F1 mice was finished in 1980 (NTP, 1982). Male rats, and female and male mice, were fed a diet containing 0, 0.225 and 0.45% , and female rats a diet

containing 0, 0.45 and 0.90% of melamine. In 8 of the 49 higher-dosed male rats, transitional cell carcinomas in the urinary bladder were observed, but none were found in any other treatment group. The occurrence of bladder tumours was accompanied by the finding of bladder stones consisting mainly of melamine (Mast, 1982; Rao et al.,1982). These tumours are assumed to be caused by irritation of the bladder mucosa by the bladder stones. The final NCI report is expected at the end of 1982.

A life-span rat feeding study was carried out at Raltech Scientific Services in the USA, sponsored by a number of American, European and and Japanese producers (Rao et al, 1982). Groups of 55 males were fed a diet containing 0, 100, 500 and 1,000 ppm, and groups of 55 females a diet containing 0, 100, 1,000 and 2,000 ppm of melamine. This mortality and morbidity study was terminated in December 1981 after exposure of the males for 123 weeks and of the females for 130 weeks. No differences in survival body weight, feed consumption, gross and clinical pathology and histopathology were observed between control and treated groups up to the end of the exposure period. A final report is still expected.

3. Metabolism and Pharmacokinetics

When a single oral dose of melamine was given to rats and dogs, 50 and 61.3% respectively of the quantity introduced was recovered in the urine within 6 hours (Lipschitz and Stokey, 1945). From this, a biological half-life of 6 hours for rats and of 4.4 hours for dogs can be estimated, if first-order kinetics are assumed. These data suggest that melamine does not accumulate in the organism.

4. Mutagenicity

Jagannath and Brusick (1977) investigated the mutagenicity of melamine in microbial systems, according to Ames and Slater, at a maximum applied dose of 0.5 mg per plate. The test criteria were the number of revertants of Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100 and Escherichia coli strain WP 2 uvrA; the number of convertants of Saccharomyces cerevisiae strain D4; and the size of the zone of growth-inhibition of Escherichia coli strains W 3110/Pol A^+ and p3478/Pol/A^-. All tests were carried out in the presence and absence of a metabolic activation system. Melamine demonstrated no mutagenic activity

in any of these assays and was considered non-mutagenic under the test conditions.

Mast et al.(1982-a) observed no mutagenic activity in the Ames test with Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100 and in the CHO/HGPRT forward mutation assay, both with and without metabolic activation.. Exposure of CHO-cells to melamine failed to produce a dose-dependent increase of sister chromatid exchanges in the presence or absence of metabolic activation. The maximum applied dose was 5 mg per plate in the Ames test and 1 mg per ml in the CHO-tests. In addition, the results for melamine were negative in the rat hepatocyte primary culture/DNA repair test up to 6 mg/ml, the highest concentration tested (Pharmakon Res. Int., 1982). The authors interpret the results to mean that melamine is not mutagenic.

Mast et al.(1982-b) carried out a mouse micro-nucleus assay involving one single oral dose of 1000 mg/kgbw, or two oral doses of 1,000 mg/kgbw separated by 24 hours. There was no increase in the number of micro-nuclei/1,000 polychromatic erythrocytes compared to the negative controls, i.e. under the test conditions melamine was not mutagenic.

5. Reproductive Toxicity

Female rats were given an intraperitoneal injection of 70 mg/kgbw melamine on two successive days between the 4th and 12th day of gestation. By this route, and at this level of dosing, melamine appeared to be non-teratogenic (Thiersch, 1957).

E. ECOTOXICOLOGICAL DATA

1. Environmental Distribution

The use pattern of melamine is exclusively industrial and therefore opportunities for it to reach the natural environment, except near production sites, are limited. Data regarding its occurrence in water or soil are lacking, but in view of the above there seems no urgent need for them.

2. Biodegradability

Melamine has a very low biodegradability. Swope and Kenna (1950) measured the standard BOD_5 for melamine as 0.006 g O_2/g melamine. The theoretical oxygen demand is 3.04 if complete nitrification occurs. Without nitrification, oxygen consumption will not occur. Hauck and Stephenson (1964) studied the nitrification of triazine-nitrogen, especially of cyanuric acid and melamine in soil. From their results, a half-life of melamine in soil of 2 to 3 years can be calculated.

3. Aquatic Toxicity

3.1. Toxicity to Daphnia magna

Adema (1978) investigated the acute and chronic toxicity of melamine to laboratory-cultured daphnids less than 24 hours old . The LC_{50}-48 hr was > 2,000 $mg.l^{-1}$. In the chronic test, daphnids were exposed to melamine solutions of 0, 5.6, 10, 18, 32 and 56 $mg.l^{-1}$ during 21 days. At 56 $mg.l^{-1}$ all daphnids died, but at 32 $mg.l^{-1}$ the survival was hardly less than that of the controls. Reproduction was not influenced at 32 $mg.l^{-1}$, and 18 $mg.l^{-1}$ was considered as a no-observed-effect concentration.

3.2. Toxicity to algae

The toxicity to fresh-water green algae, Scenedesmus pannonicus, was determined in a growth-inhibition test (Oldersma and Hanstveit, 1982). Melamine was found to impair the growth of the algae at concentrations higher than 320 $mg.l^{-1}$. With respect to the number of active growing cells in the inoculum, the EC_{50} was found to be 940 $mg.l^{-1}$, with a 95% confidence interval of 880 to 1000 $mg.l^{-1}$. The no-observed-effect concentration was found to be 320 $mg.l^{-1}$.

3.3. Toxicity to fish

Jansen (1978) carried out an acute toxicity test with guppies. After 96 hours exposure to a saturated melamine solution in water (4.4 $g.l^{-1}$) the mortality was less than 10%, and it appeared to be impossible to establish an LC_{50}. No information is available on the bioaccumulation, in fish, of melamine from water. However, from the solubility of melamine in water it is possible to predict the bioconcentration factor from a regression equation, derived by Chiou et al.(1977):

$$\log BCF = 3.41 - 0.508 \log WS$$

BCF = bioconcentration factor = ratio between concentration

in fish and in water, at equilibrium.

WS = water solubility in $\mu Mol.l^{-1}$.

This gives a BCF of about 15, a value which is small enough to indicate that there is no need for further bioaccumulation studies.

4. Adsorption on soil

Quantitative data about the adsorption of melamine on soil have not been reported. The adsorption on soil of most organic compounds is directly dependent on the soil organic-carbon content and correlates strongly with solubility in water or octanol-water partition coefficient. On the basis of these relationships, as derived for 106 organic compounds by Kenaga and Goring (1980), an estimation of the adsorption of melamine on soil organic-carbon can be made from the regression equation :

$$\log Koc = 3.64 - 0.55 (\log WS)$$

Koc = Soil sorption coefficient =

$$\frac{\mu g \text{ adsorbed/g soil organic-carbon}}{\mu g \text{ dissolved/g solution}}$$

WS = Water solubility in $mg.l^{-1}$

At a concentration of melamine in water of 3000 $mg.l^{-1}$ a soil sorption coefficient (Koc) of 53 can be estimated. For comparison, the Koc values of two known compounds are mentioned (Hamaker and Thompson, 1972) :

Koc (DDT) = 243,000

Koc (urea) = 14.3

This indicates that the adsorption of melamine onto soil is not important. Thus, a soil with 2% organic carbon in contact with a 0.1 ppm (100 $\mu g.l^{-1}$) solution will adsorb melamine to give approximately 0.1µg/g soil.

F. SUMMARY AND CONCLUSIONS

Melamine has a very low acute toxicity and is not a mutagen, a skin or eye irritant or a skin-sensitiser. It has shown no teratogenic effects in rats. Exposure of laboratory animals to unrealistically high doses leads to the formation of bladder stones which may in turn, by chronic irritation, cause bladder tumours. On the basis of the available toxicological information, almost wholly from animal studies, no harmful acute or chronic effects are to be expected from the occupational exposure of humans to melamine.

Melamine has a very low acute toxicity towards species in the aqueous environment. The high values of the no-observed effect level for growth-inhibition in algae and for the reproduction of daphnids suggests that although data on chronic effects on higher aquatic organisms are lacking, there is no pressing need to assess such effects.

G. BIBLIOGRAPHY

- Adema, D.M.M.(1978). The acute and chronic toxicity of melamine to Daphnia magna. TNO-report, prepared on behalf of DSM.

- Boitsov, E.N., Mushkin, Yu.I. and Karlik, V.M.(1969). Analysis of cyanamide derivates in mixtures, separated in ion-exchange resins. Zavodskaya Laboratoriya, 35,(7), 790.

- Chiou, C.T., Freed, V.H., Schmedding, D.W. and Kohnert, R.L. (1977). Partition coefficient and bioaccumulation of selected organic chemicals. Env. Sci. Techn., 11,(5), 475.

- Farrow, S.G., Hill, S.R. and Skinner, J.M.(1972). High precision spectrophotometry, part II. The determination of ammelide, ammeline and melamine in the thermal decomposition products of urea. The Analyst, 97, 42.

- Fassett, D.W. and Roudabush, R.L.(1981). Laboratory of industrial medicine. Eastman Kodak Co., unpublished observations, cited by Reinhardt, C.F. and Briselli M.R. in Patty, F.A., Industrial Hygiene and Toxicology, Vol.2A, 2769.

- Hamaker, J.W. and Thompson, J.M.(1972). Adsorption of organic chemicals in the soil environment. Ed., Goring, C.A.I. and Hamaker, J.W., Marcel Dekker, Inc., N.Y.

- Hauck, R.D. and Stephenson, H.F.(1964). Nitrification of triazine nitrogen. J.Agric. Food Chem.,12,(2), 147.

- Jagannath, D.R. and Brusick, D.J.(1977). Mutagenicity evaluation of melamine. Report of Litton Bionetics, prepared on behalf of DSM.

- Jansen, B.(1978). Unpublished results, DSM.

- Kechek, Yu.A. and Karhoyan, D.N.(1975). Method for determining the melamine level in urine and blood. Gig. Tr. Prof. Zabol.,6,56.

- Kenaga, E.E. and Goring, C.A.I.(1980). Relationship between water solubility, soil sorption, octanol-water partitioning and concentration of chemicals in biota. Aquatic Toxicology, ASTM STP 707. Ed., Eaton, J.G., Parrish, P.R. and Hendricks, A.C., American Society for Testing and Materials, 78.

- Lipschitz, W.L. and Stokey, E.(1945). The mode of action of three new diuretics : melamine, adenine and formoguanamine. J. Pharmacol. Exp. Ther., 83, 235.

- Mast, R.W., Friedman, M.A. and Finck, R.A.(1982-a). Mutagenicity testing of melamine. The Toxicologist, 2, 172.

- Mast, R.W., Naismith, R.W. and Friedman, M.A.(1982-b). Mouse micronucleus assay of melamine. Abstracts of the 13th annual meeting of the Environmental Mutagen Society, 103.

- Mast, R.W.(1982). Personal communication.

- NTP (1982). Draft report on the carcinogenesis bioassay of melamine in F 344/N rats and B6 C3 F1/N mice. March 24, 1982. NTP-81-086. NIH Publication No. 82-2501.

- Oldersma, H. and Hanstveit, A.O.(1982). The effect of the product melamine on the growth of the green alga Scenedesmus pannonicus. TNO-Report no.81/188.

- Patty, F.A.(1981). Industrial Hygiene and Toxicology. Volume 2A, Toxicology, John Wiley and Sons, 2769.

- Pharmakon Research International Inc. (May, 1982). CHO/SCE in vitro sister chromatid exchange in Chinese hamster ovary cells (CHO). PH319-AC-002-82. Melamine.

- Philips, F.S. and Thiersch, J.B.(1951). The nitrogen mustard-like actions of 2,4,6-tris (ethylenimino)-S-triazine and other bis(ethylenimines). J. Pharmacol. Exp. Ther.,100, 398.

- Rao, G.N., Giesler, P.J., Palmers, T.E., Mast, R.W., Friedman, M.A. and Shaffer, C.B.(1982). Chronic toxicity of melamine in Fischer 344 rats. Abstracts of the 1982 annual conference of the Society of Toxicology, The Toxicologist, 2, no.1.

- Seiffarth, K. and Ardelt, H.W.(1966). Zur Bestimmung des Melamines. Plaste und Kautschuk, 13,(1), 13.

- Shaffer, C.B.(1955). Melamine : acute and chronic toxicity. American Ganamia Company, Central Medical Department, Wayne, N.J.

- Swope, H.G. and Kenna, M.(1950). Effect of organic compounds on biochemical oxygen demand. Sewage and Industrial Wastes Engineering, 21, 467.

- Thiersch, J.B.(1957). Effect of melamine, triethylenemelamine and triethylenephosphoramide on rat litter in utero. Proc. Soc. Exp. Biol. Med.,94, 36.

H. MEMBERS OF THE JACC MELAMINE WORKING GROUP

Dr. W. ten Berge	Corporate Department for Safety and Environmental Methods D.S.M. - Heerlen
Mr. J.P. Pied	C.D.F. Chimie Paris
Dr. H.T. Hofmann	Industrial Hygiene and Toxicology Division BASF AG - Ludwigshafen