UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 21 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Melamine

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/7/93
Senior Pharmacologist
Toxicology Branch II/Health Effects Division (H7509C)

and

Esther Rinde, Ph.D. E. Rinde
Manager, Carcinogenicity Peer Review Committee
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Phil Hutton, PM #18
Insecticide-Rodenticide Branch
Registration Division (H7505C)

The Health Effects Division Carcinogenicity Peer Review Committee (PRC) met on November 27, 1991, July 29, 1992, and on February 24 1993 (present meeting) to discuss and evaluate the weight-of-evidence for Melamine with particular reference to its carcinogenic potential. The PRC concluded that Melamine was not amenable to classification using the current Agency guidelines and chose to describe the weight-of-evidence using a narrative form. Based on a mechanistic evaluation of the only tumors seen, those that occurred at exceptionally high doses in the bladder of male rats, it appears that humans are not likely to be exposed to doses of Melamine that produce the urinary tract toxicity that precedes and seems to lead to the carcinogenic response in rats. In particular, anticipated human dietary and occupational exposure to the parent compound, Cyromazine, is estimated to produce Melamine concentrations far below the NOEL in rats for the apparent urinary tract tumor precursors (stone formation and attendant epithelial irritation). These effects are produced in rats at extremely high doses, under conditions not anticipated to occur outside of the experimental laboratory. The PRC concluded that it is unlikely that Melamine exposure would pose a carcinogenic hazard to humans from the pesticidal usage of Cyromazine. Further information would be useful to strengthen this position.

Recycled/Recyclable
Printed with Soy/Canola Ink on paper that contains at least 50% recycled fiber

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Penelope A. Fenner-Crisp	Penelope A. Fenner-Crisp
Reto Engler	Reto Engler.
Marcia Van Gemert	Marcia Van Gemert
Karl Baetcke	Karl D. Baetcke
Marion Copley	Marion Copley
Julie T. Du	Julie T. Du
Richard Hill	Richard D. Hill
Hugh Pettigrew	Hugh Pettigrew
Esther Rinde	Esther Rinde
William Sette	William Sette

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Stephen C. Dapson[1]	Stephen C. Dapson
Mike Ioannou	M. Ioannou
Bernice Fisher	Bernice Fisher
Lori Brunsman	Lori S. Brunsman
Lucas Brennecke[2] (Clement/PAI)	Lucas H. Brennecke

[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Signature indicates concurrence with pathology report.

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

William Burnam

Kerry Dearfield

George Ghali

Jean Parker

John Quest

Yin-Tak Woo

4. Other Attendees: (Observers)

Diane Mandell (Clement)

B. Material Reviewed:

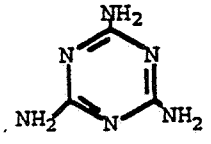
The material available for review consisted of DERs, one-liners, and other data summaries prepared by Dr. Stephen Dapson. Statistical analyses were prepared by Lori L. Brunsman. The material reviewed is attached to the file copy of this report. The data reviewed are based upon studies submitted to the Agency by Ciba-Geigy Corp.

C. Background Information:

Melamine (2,4,6-Triamino-s-triazine), is a metabolite of the pesticide Cyromazine. The PRC meetings on Cyromazine are discussed in a separate document.

The Caswell (or Tox Chem) Number of Melamine is 861B. The Chemical Abstracts Registry Number is 108-78-1.

The structure for Melamine is shown below:



D. Evaluation of Carcinogenicity Evidence:

1. Rat and Mouse Carcinogenicity Study

Reference: Carcinogenesis Bioassay of Melamine in F344/N Rats and B6C3F1 Mice (Feed Study). NTP 81-86, Publication Number 83-2501 NTP TR 245. March 1983.

a. Experimental Design

Groups of 50 male F344/N rats and 50/sex B6C3F1 mice were administered Melamine (> 95% pure) in the diet at 0, 2, 250, or 4500 ppm (0, 0.1, 12.5, or 225 mg/kg/day) for 103 weeks. Female rats were given 4500 or 9000 ppm (225 or 450 mg/kg/day) of Melamine.

b. Discussion of Tumor Data

Rats. In the male rats, there was a statistically significant ($p < 0.01$) increase in transitional cell carcinomas of the urinary bladder at the high dose level (4500 ppm). There was also a statistically significant trend for these tumors. Bladder carcinomas were found in 8/49 of the high dose males; with one exception, urinary bladder stones were observed in male rats that had the transitional cell carcinomas. There was one bladder papilloma in a high-dose male that also had stones.

One low- and one high-dose female had a papilloma; no females had stones. A statistically significant positive trend for C-cell carcinoma of the thyroid gland was found in female rats; however, the high-dose incidence (3/50) was not statistically significantly different from the historical control data; and the National Toxicology Program (NTP) investigators thought that they were not treatment-related.

Mice. In the mice of both sexes, there was no evidence of tumors due to treatment with Melamine at the dose levels tested (up to 4500 ppm).

c. Non-neoplastic Lesions

Rats. In the rats, survival of high-dose male rats was significantly decreased in comparison to controls. Mean body weights were less than those of controls for all dosed rats after week 20. One low- and two high-dose males had bladder hyperplasia; one of the two high-dose animals had bladder stones. In the high-dose male rats, 10/49 had bladder stones; they were not found in the concurrent controls, and only 1/50 of the low-dose male rats had them. Nephropathy was noted in 32/45 controls, 36/50 low-dose males and 30/49 high-dose males.

Females had statistically significant ($p < 0.001$) dose-related chronic inflammation of the kidneys; the incidence of this finding was 8% in controls, 34% in low- and 41% in high-dose animals. Females in all groups also had nephropathy. None of the treated females had bladder stones.

Mice. In male mice, mean body weights were decreased after week 50 and survival was decreased at the highest dose tested (HDT). The male mice had intense acute and chronic bladder inflammation (a lesion not seen in dosed male rats) and very mild epithelial hyperplasia of the urinary bladder (without the histological features of pre-neoplastic lesions). Bladder stones were found in 40/47 of the low-dose males and 41/44 of the high-dose males, but in only 2/45 controls.

Body weights in treated female mice were comparable to controls throughout the study. In the high-dose females, 4/50 had stones, and a few had bladder hyperplasia without stones.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing in both studies was considered to be adequate for assessing the carcinogenic potential of Melamine, based on decreased body weight gains. At the high dose, the male rats had a 11% decrease in body weight gain at week 100, the females had a 4% decrease. The male mice had a 21% decrease in body weight gain at week 100, the female mice had a 6% decrease. Female rats in all groups also had nephropathy.

2. Chronic feeding study in rats

Reference: American Cyanamid Co. 1953. Chronic Feeding Studies in Rats with Melamine. Wayne, N.J.

a. Experimental Design

Groups of 10 Carworth Farm rats were fed 0, 1000 or 10,000 ppm (0, 50 or 500 mg/kg) of Melamine for 2 years.

b. Discussion of Tumor Data

In the high-dose group, 4/10 males and 2/10 females developed bladder papillomas; all tumor-bearing animals had bladder stones.

c. Non-neoplastic Lesions

No compound-related effects were seen in the low-dose group. No other data were provided.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The original study was not reviewed by the PRC, but was referred to in the NTP report on Melamine. However, it was noted that the study used a low number of animals.

3. Chronic feeding study in rats

Reference: 49 FR 18120. 1984. Chronic Feeding Study in Rats. Conducted by American Cyanamid Co. Submitted by Ciba-Geigy.

In a chronic feeding study (29-30 months) in F344 rats, males were given diets with 100 to 1000 ppm of Melamine, and females were given 100 to 2000 ppm. No bladder tumors or stones were reported among any of the animals in the study. This study was not reviewed by the PRC.

4. Subchronic feeding study in rats

Reference: Okumura, M., R. Hasegawa, T. Shirati, M. Ito, S. Yamada, and S. Fukushima. 1992. Relationship between calculus formation and carcinogenesis in the urinary bladder of rats administered the non-genotoxic agents, thymine or Melamine. Carcinogenesis 13: 1043-5.

Groups of 20 male F344 rats were administered Melamine in the diet at 3000, 10,000, or 30,000 ppm for 35 weeks followed by 4 weeks without the chemical. Bladder tumors showed a significant dose-related increase in frequency. No tumors were found in the control and low-dose males, while in the mid-dose there was 1 papilloma and 1 carcinoma. Among high-dose males 12 had papillomas and 15 had carcinomas. Exploratory laparotomy had been performed at 36 weeks on 10 animals of each dose group and bladder calculi were found in 0, 7, and 10 of the low-, mid-, and high-dose animals, respectively; none were found in controls. The association between bladder stones upon laparotomy and bladder tumors among the 30 dosed animals was highly significant ($p = 0.007$). In the ureters of animals receiving 30,000 ppm Melamine, 3 papillomas and 1 carcinoma were found with accompanying calculi.

E. Additional Toxicology Data

1. Subchronic Toxicity Studies

Reference: Carcinogenesis Bioassay of Melamine in F344/N Rats and B6C3F1 Mice (Feed Study). NTP 81-86, Publication Number 83-2501 NTP TR 245. March 1983.

A 14-day rat feeding study used doses of 5000, 10,000, 15,000, 20,000, or 30,000 ppm (250, 500, 750, 1000, or 1500 mg/kg/day) of Melamine in the diet. All dose groups at 15,000 ppm and above had reduced body weight gain and according to the investigators, the 20,000 and 30,000 ppm dose groups "lost weight". A hard crystalline solid was found in the urinary bladders of male rats at 10,000 ppm and above (4/5 to 5/5), and in female rats at 20,000 ppm and above (4/5).

Three 13-week rat feeding studies were conducted. The first used diets containing 6000, 9000, 12,000, 15,000, or 18,000 ppm (300, 450, 600, 750, or 900 mg/kg/day). Mean body weight gain in the 12,000 ppm dose group and above was depressed by more than 8% when compared to controls. Stones were found in most Melamine-exposed male rats [0/12 (controls), 6/12, 8/12, 12/12, 10/12, 12/12] and were dose-related. Also, 25% or more females of the two highest dosed groups had bladder stones (3/12, 5/12). There was diffuse epithelial hyperplasia of the urinary bladder in 80% of the males and 20% of the females in the 18,000 ppm dose group, and 10% of the males and none of the females in the 6000 ppm dose group (other groups were not examined).

The second study was conducted in rats to find a no-effect level for the urinary bladder stones using doses of 750, 1500, 3000, 6000 or 12,000 ppm (37.5, 75, 150, 300 or 600 mg/kg/day) in the diet. Male mean body weights were depressed more than 10% when compared to controls for those treated with 6000 and 12,000 ppm, with no similar effect in females. The incidence of stones in the urinary bladders of male rats was significantly dose-related [1/10 (controls), 2/10, 5/10, 7/10, 9/10, 9/9]; the incidence of stones was significantly elevated above control among males receiving 3000 ppm and above. No stone formation was noted in the female rats, but there was a dose-related incidence of calcareous deposits in the straight segments of the proximal tubules. Hyperplastic epithelial changes were only found among male rats with bladder stones [0/10 (controls), 0/10, 0/10, 1/10, 3/10, 9/9].

A third study was conducted in rats using 18,000 ppm Melamine in the diet both in the presence and absence of 1% ammonium chloride in the drinking water. The investigators stated that, according to the literature, ammonium chloride added to the drinking water inhibited stone and tumor formation in the urinary bladders of mice fed diets containing 4-ethylsulfonylnaphthalene-1-sulfonamide; however, in this study, ammonium chloride had no effect on bladder stone formation in rats. The rats had decreased body weight gains relative to controls which received water acidified with hydrochloric acid. Bladder stones were seen in all males (1/10 controls, 10/10 treated) and 30% of the female rats (0/10 controls, 3/10 treated). Bladder stones were found to contain mainly Melamine and protein with traces of uric acid, oxalate and phosphate. The

water solubility of Melamine is in excess of the concentrations reached in the urine of treated rats.

A 14-day mouse feeding study used doses of 5000, 7500, 10,000, 12,500, 15,000 or 30,000 ppm of Melamine in the diet (100, 150, 200, 250 or 300 mg/kg/day). The only reported finding was a hard crystalline solid found in the bladder in all treated male mice (5/5) and in 2/5 of the female mice fed 30,000 ppm.

A 13-week study was conducted in mice receiving 6000, 9000, 12,000, 15,000 or 18,000 ppm in the diet (120, 180, 240, 300 or 360 mg/kg/day). The investigators found that the mean body weight gain of all treated groups relative to controls was decreased by 9% or more. They also found that the incidence of mice with bladder stones increased in a dose-related manner and was more prevalent in males (0/10, 0/10, 0/10, 6/10, 9/10, 7/10) than females (0/10, 0/10, 0/10, 1/10, 3/10, 7/10). There was also ulceration of the urinary bladder epithelium with 60% of the mice with bladder ulcers also having urinary bladder stones. The bladder ulcers were multifocal or associated with inflammation (cystitis). However, according to the investigators, the results did not provide any evidence for an association between ulceration and bladder stones in either sex.

Reference: Heck, H.D. and R.W. Tyl. 1985. The induction of bladder stones by terephthalic acid, dimethylterephthalate, and Melamine (2,4,6-triamino-s-triazine) and its relevance to risk assessment. Reg. Toxicol. Pharmacol. 5: 294-313.

In a 4-week dietary study, male rats were given 1 of 7 doses of Melamine between 2000 and 19,000 ppm. Bladder stones increased significantly with doses above 2000 ppm. Hyperplasia was noted at doses of 7000 ppm and above, and 93/94 had stones. This study was not reviewed by the PRC.

2. Metabolism

a. Metabolism study in the rat

References: Mast, R.W., A.R. Jeffcoat, B.M. Sadler, R.C. Kraska, and M.A. Friedman. 1983. Metabolism, disposition, and excretion of ^{14}C Melamine in male Fischer 344 rats. Food Chem. Toxicol. 21: 807-10.

Worzalla, J., B. Kaiman, B. Johnson, G. Ramirez, and G. Bryan. 1974. Metabolism of hexamethylmelamine ring-^{14}C in rats. Cancer Res. 34: 2669-75.

It appears that Melamine distributes in the body water, with the kidney and bladder being the only organs showing higher levels of radioactivity than in the plasma. The elimination phase t½ from plasma is about 3 hours (Mast et al. 1983).

Urinary metabolites following dosing with hexamethylmelamine to rats and to two human subjects indicate that the triazine ring of Melamine is resistant to cleavage in the body (Worzalla et al. 1974).

b. Metabolism study (Cyromazine administration) in the rat

Characterization and Identification of ^{14}C-Cyromazine and Metabolites in Rats, Hazleton Laboratories America, Inc., Laboratory Project ABR-89108 (January 1990) and Metabolism of 14C-Cyromazine in Rats, HLA 6117-160 (October 1989), MRID No. 414421-01.

The study details are described more fully in the PRC document on Cyromazine, however a brief synopsis follows.

Oral gavage administration of Cyromazine resulted in rapid absorption and excretion of this compound in rats; Melamine, hydroxyCyromazine, methylCyromazine and unmetabolized Cyromazine were identified as metabolites. Melamine was present as 2.8-12.1% of urinary radioactivity and 4.7-7.6 % of fecal radioactivity.

3. Genotoxicity

Open literature data and studies conducted by NTP for genotoxicity that were provided in the NTP report concluded that Melamine was not mutagenic for Drosophila melanogaster; not mutagenic for Salmonella typhimurium G46, TA 98, TA 100, TA 1530, TA 1531, TA 1532, TA 1534, TA 1535, and TA 1538 both with and without metabolic activation; not mutagenic for the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells in the presence or absence of metabolic activation; negative in tests for induction of chromosomal aberrations in CHO cells; negative in a sister chromatid exchange study in CHO cells; negative in an unscheduled DNA synthesis using a primary culture of rat hepatocytes; oral administration of Melamine to mice did not produce a significant increase in the number of micronuclei in polychromatic erythrocytes or induce chromosome aberrations in vivo; a dominant lethal test was negative.

4. Structure-Activity Correlations

Melamine is related to the triazine class of chemicals although most of the triazines are used as herbicides. An NLM Chemline search found 6073 triazines, of these 49 were classified as agricultural chemicals. A further reduction of the initial 6073 triazines in reference to specific tumor data available in the open literature found the following pesticide chemicals: Cyanazine, Hexazinone, Prometryn, Terbuthylazine, Atrazine,

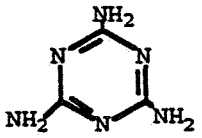
Terbutryn, Propazine, Simazine, S-Cyanuric acid (Trihydroxytriazine), Anilazine, and Cyromazine.

Toxicological information and structures for related compounds are shown below.

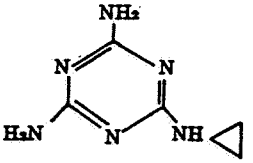
- Cyanazine produced malignant mammary gland tumors at doses ≥ 25 ppm in a chronic rat toxicity/carcinogenicity study.
- Hexazinone produced systemic toxicity but no carcinogenic response in a chronic rat study at doses up to 2500 ppm.
- Prometryn has a very weak data base with no evidence of carcinogenicity (at dose levels up to 1250 ppm) or mutagenicity.
- Terbuthylazine led to significantly increased mammary gland carcinomas in high-dose females (750 ppm) in a chronic rat toxicity/carcinogenicity study. In another chronic study toxicity was noted at the HDT (30 ppm), but there was no evidence of carcinogenicity.
- Atrazine-treated rats showed an increase in mammary tumors (fibroadenomas at the HDT (1000 ppm), an increase in adenocarcinomas plus carcinosarcomas at the three highest dose levels (70, 500, and 1000 ppm), and an increase in testicular interstitial cell tumors in male rats at the HDT (1000 ppm) in a 2 year chronic toxicity/oncogenicity study.
- Terbutryn produced an increase in mammary gland adenomas in female rats and testicular interstitial cell adenomas in male rats at the HDT (3000 ppm) in a chronic toxicity/carcinogenicity study.
- Propazine treatment resulted in increase in total mammary gland tumors at 1000 ppm in a chronic rat toxicity/carcinogenicity study.
- Simazine produced an increase in carcinomas and fibroadenomas at 1000 ppm in a chronic rat toxicity/carcinogenicity study.
- S-Cyanuric acid (trihydroxytriazine) did not produce evidence of carcinogenicity at levels tested (up to 5375 ppm) in rats or mice.
- Anilazine did not produce any treatment related effects in a chronic rat study at doses up to 2000 ppm.
- Cyromazine was classified as Group C - possible human carcinogen, based on a statistically significant increase in mammary tumors in the female mouse, at a dose (3000 ppm) that may have been insufficient for an adequate assessment of carcinogenic potential, and to a lesser degree, the same tumor type in the rat.

None of these substances can be considered completely adequate structural and functional analogs of Melamine, since they all have critical components, such as chlorine or hydrocarbon moieties which are not present in Melamine. Although the substances may be structurally related, they cannot be used to predict the activity of Melamine. Melamine is not considered to have any structural components that would alert one to potential carcinogenicity (Ashby and Tennant 1991).

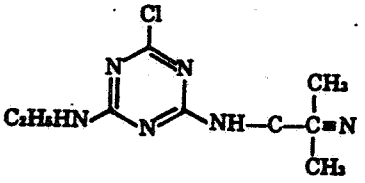
Structural Analogs of Melamine



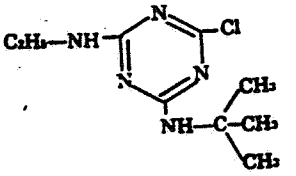
Melamine



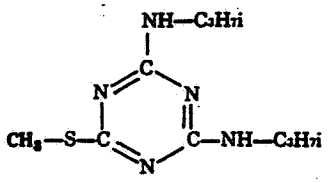
Cyromazine



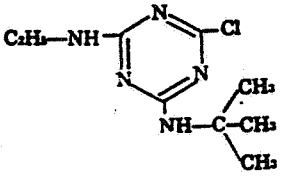
Cyanazine



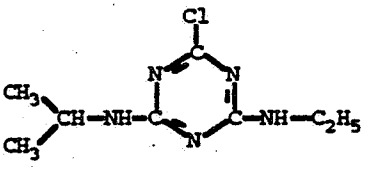
Hexazinone



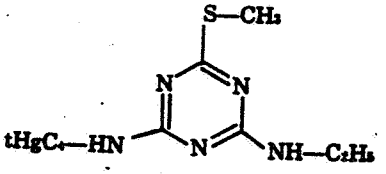
Prometryn



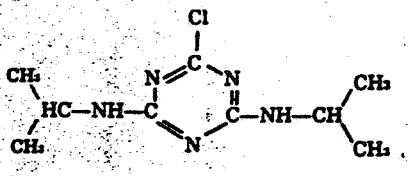
Terbuthylazine



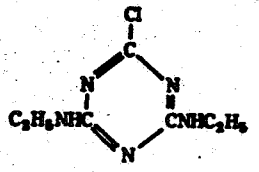
Atrazine



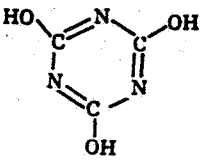
Terbutryn



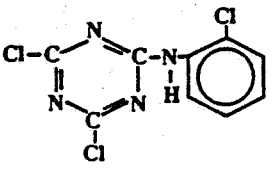
Propazine



Simazine



Trihydroxytriazine
also called S-Cyanuric Acid



Anilazine

F. Weight of Evidence Considerations:

The PRC considered the following facts regarding the toxicology data on Melamine to be of importance in a weight-of-evidence determination of carcinogenic potential:

1. In carcinogenicity studies conducted by the NTP, Melamine induced rare transitional cell tumors of the urinary bladder in male rats following dietary exposure to 4500 ppm for two years, a dose level at which mortality was significantly increased. No tumors were observed in female rats at dose levels up to and including 9000 ppm or in male rats at a dose level of 250 ppm. Stones of the urinary bladder were found in 7/8 tumor-bearing males. In a second rat chronic study, 4/10 males and 2/10 females were found to have benign bladder tumors with urinary stones after the administration of 10,000 ppm in the diet. In a third chronic feeding study in the rat, dose levels in males up to 1000 ppm and dose levels in females up to 2000 ppm did not induce bladder stones or bladder tumors when administered in the diet for 29 to 30 months.

2. In a 13-week rat feeding study, stone formation with diffuse epithelial hyperplasia of the urinary bladder occurred in 10% of the males (and 0% of the females) given 6000 ppm and in 80% of the males (and 20% of the females) given 18,000 ppm of Melamine in the diet. Hyperplasia in the urinary bladder was reported in 4% of the males (and 0% of the females) receiving 4500 ppm in the NTP carcinogenicity study. In 4-week and 13-week studies in the rat, the proportion of males with stones increased at doses above 2000 to 3000 ppm. Bladder hyperplasia was noted at doses of 6000 to 7000 ppm, and essentially all such animals had stones. Female rats did not show stones until doses of 15,000 ppm and greater. Although one male rat in the NTP chronic study was found to have a urinary bladder tumor without the observation of stones, it is likely that stones were present and were either lost during the necropsy or passed through the urethra during the in-life phase of the study. In a second two-year study in the rat, all tumor-bearing animals of both sexes had urinary stones. The incidence of stone formation with Melamine is clearly dose-related. Neither hyperplasia nor neoplasia are found at dose levels which are not also associated with stone formation.

3. Chronic administration of Melamine in the diet at dose levels of up to 4500 ppm did not induce tumors at any site in the mouse. In male mice, stones (93%) and very mild epithelial hyperplasia (30%) without features of pre-neoplastic lesions were observed frequently at 4500 ppm. Stones and hyperplasia were observed less commonly at 250 ppm in male and female mice. Intense inflammation of the urinary bladder, a lesion not noted in the rat, was commonly observed in the presence of urinary stones in mice.

4. Urinary stones following Melamine administration have been characterized as being composed of Melamine and protein with traces of oxalate, uric acid and phosphate. Although Melamine is soluble in water at higher concentrations than those resulting in stone formation in rats, the presence of proteins and urinary ions may enhance Melamine precipitation from the urine of this species. Possibly urinary constituents (e.g. proteins) or the narrower and longer urethra of the male rodent may be the basis for the greater sensitivity of males to stone formation.

5. There is convincing evidence that stones are associated with bladder lesions. After 4 weeks of dosing, 93/94 male rats with hyperplasia had stones. Whereas about half the male rats with bladder stones developed bladder lesions in the 13-week NTP studies, all animals with hyperplasia had stones. In the chronic NTP study, 10/12 males with bladder lesions (hyperplasia, papillomas, or carcinomas) had stones; only 1 had stones without a lesion. It is likely that Melamine-induced bladder hyperplasia and tumors in rats result from mechanical irritation caused by stones which results in the stimulation of cell division. This conclusion is consistent with the demonstration that the implantation of wax pellets in the rat bladder induces tumor formation (Chapman et al. 1974) and the observation that the presence of stones frequently leads to the development of transitional cell hyperplasia and tumors of the urinary bladder in rats (Appendix 1).

6. Extensive genotoxicity testing of Melamine for point mutations in bacteria and cultured mammalian cells, and for chromosome aberrations in cultured mammalian cells and in vivo have been uniformly negative. Tests in vitro for sister chromatid exchange and unscheduled DNA synthesis are also negative.

7. Melamine is rapidly excreted unchanged in the urine of the rat with a plasma half-life of about 3 hours. Administration of hexamethyl Melamine to humans and rats found that the s-triazine ring is not cleaved.

8. The association between bladder stones and human bladder cancer is limited (see Appendix 2). The human bladder appears to be much less sensitive than the rat to the induction of bladder tumors by urinary stones.

G. Human Exposure Considerations

Melamine is not a pesticide but is a metabolite of the pesticidal agent Cyromazine. In the body about 10% of Cyromazine is metabolized to Melamine. Although about 50% of the Cyromazine residue on treated commodities is in the form of Melamine [FR 50: 20379, May 15, 1984], this residue only results in a human

exposure that is about one million-fold less than that which produces bladder stones and tumors in male rats. The PRC did not consider the mammary tumors in the rat resulting from exposure to Cyromazine, the parent compound of Melamine, to be applicable to evaluating the carcinogenic potential of Melamine. (Cyromazine is discussed more fully in a separate document.)

H. Characterization of the Human Carcinogenic Potential:

Criteria contained in the current EPA Guidelines [51FR: 33992-34003, 1986] were considered. The PRC recognized that on the one hand, the guidelines emphasize the use of a weight-of-the-evidence evaluation in determining the carcinogenic hazard of an agent; but on the other hand, they also lay out rather rigid criteria for the evidence associated with each of the categories in the classification system. Due to these different emphases, the PRC presents a description of the hazard potential of Melamine in a narrative form that is not associated with the categories per se in the EPA guidelines.

The PRC concluded that Melamine has been adequately tested for carcinogenicity in rats and mice, and that the only tumor type which is associated with compound administration under the conditions of the bioassay, that of the urinary bladder (and possibly the ureter) in rats, appears to be due to urinary stone formation, epithelial irritation, and proliferation of urothelium (see Appendix 1). Male rats are more sensitive than female rats to stone formation and bladder tumor development; females develop both but at doses above those that produce effects in males. Mice developed stones, intense inflammation of the bladder not seen in treated rats, and epithelial hyperplasia without features of pre-neoplastic lesions in chronic studies; no tumors were found.

Besides stone formation, there is no other readily apparent cause for the induction of bladder (and possible ureteral) tumors in rats. Melamine was negative for gene and chromosomal mutations in short term tests.

In summary, Melamine induces a rare tumor of the bladder (and possibly the ureteral) epithelium in rats but not mice. Bladder stones are induced in both species, but only those in rats go on to produce tumors. It appears that stone formation is a prerequisite for toxic effects in the bladder of rats and for tumor formation. Thus, it follows that exposures far below those that induce stones would not be associated with cancer potential. Residues of Melamine are present in the human diet from the use of the pesticide Cyromazine; however, the resulting Melamine exposures are only about one millionth of the dose that produces bladder stones and tumors in male rats. In addition, only about 10% of Cyromazine is converted to Melamine <u>in vivo</u>. Since the

upper dose limit for adequate chronic testing of Cyromazine in rats is about 3000 ppm and since the dose of Melamine needed to produce stones is about 2000 ppm to 3000 ppm, it is unlikely that chronic exposure to Cyromazine could ever result in concentrations of Melamine that would induce bladder stones, much less bladder cancer. Thus, the PRC concluded that it is unlikely that Melamine exposure would pose a carcinogenic hazard to humans from the pesticidal usage of Cyromazine.

There are uncertainties which preclude a definitive conclusion concerning the carcinogenic hazard to humans. The most important uncertainty is the extent to which direct genetic damage may influence the development of tumors. Although Melamine is not positive in short-term tests for point and chromosomal mutations, no information is available on the target cells in the rat urinary bladder. In addition, questions remain concerning the precise mechanism of stone formation in rats. Further information which addresses the above uncertainties would permit a more definitive conclusion concerning human carcinogenic hazard potential under expected conditions of pesticidal exposure.

References

Ashby, J. and R.W. Tennant. 1991. Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. Mut. Res. 257: 229-306.

Chapman, W.H., D. Kirchheim, and J.W. McRoberts. 1973. Effect of the Urine and Calculus Formation on the Incidence of Bladder Tumors in Rats Implanted with Paraffin Wax Pellets. Canc. Res. 33: 1225-9.

Appendix 1- Bladder stones in rodents

The etiology of transitional cell tumors of the urinary bladder has been subject to extensive investigation in laboratory animals. Studies over the last quarter of a century have led to the knowledge that urinary stones can lead to irritation of urothelium, increases in urinary epithelial cellular proliferation, cellular hyperplasia, and benign and malignant neoplasms. It appears that it is the presence of a physical body (and urine) in the bladder, rather than its chemical composition which is essential for tumor induction; that is, the tumors are secondary to the presence of stones. Examples are available which typify this type of carcinogenic process in the bladder and serve to elucidate the various mechanisms by which tumors are formed in treated animals.

It has long been known that the implantation of inert material such as glass beads or wax pellets into the urinary bladder of rodents leads to events terminating in bladder cancer development (Chapman et al. 1973). The presence of mechanical irritation for at least 6 months appears to be necessary for the induction of neoplasia (Roe 1964). The surface characteristics of the foreign body also influence the likelihood of tumor development; rough glass beads result in a higher tumor incidence than do smooth glass beads (DeSesso 1989).

A number of other factors have been identified as influencing the induction of bladder tumors. Increased incidences are observed with some chemicals at a high urinary pH and lowering of urinary pH decreases or eliminates carcinogenic activity e.g. saccharin (Cohen and Ellwein 1990) and sodium ortho-phenyl phenol (Fujii et al. 1987). Uric acid and cysteine may precipitate and form stones at low pHs, while calcium and phosphorus-containing stones form in an alkaline medium. Elevated sodium ion concentration has also been shown to increase the rate of cellular proliferation and resultant tumor formation following the administration of ortho-phenyl phenol to rats (Shibata et al. 1989). Decreased osmolality and increased urine volume have also been associated with increased bladder tumor formation (Munro et al. 1975).

Male rodents appear to be more susceptible to stone formation than do female rodents (Teelman and Nieman 1979). This may be due, in part, to the longer and narrower urethra of the male rodent which results in retention of calculi and stasis of the urine. The chemical constitution of urine also differs between the sexes and the presence of higher protein levels and possibly other constituents in male urine may also serve to catalyze the formation of calculi. The rat appears to be more sensitive to the induction of bladder tumors by mechanical irritation than do other species such as the mouse and guinea pig. The basis for this difference in species sensitivity is as

yet unexplained. Humans appear to be relatively insensitive to tumor induction by urinary stones (see Appendix 2).

Studies of experimental bladder carcinogenesis have suggested an interaction of genotoxic and nongenotoxic events, including cell division, as critical components (Cohen and Ellwein 1989 1990). For a review of some of the genetic events associated with human bladder cancer, see Raghavan et al. (1990). Some chemical substances that have produced bladder tumors in rodents appear both to produce mutations and stimulate cell division (e.g., N-[4-(5-nitro-2-furyl)2-thiazolyl]-formamide), whereas others, like saccharin, seem to influence only cell division. In the latter case, genetic events might be considered to be "spontaneous" and not associated with saccharin per se. Certainly the finding of bladder tumors following the implantation of inert materials like glass or wooden beads or wax pellets would also fall into this category, since they irritate the bladder wall and stimulate cell turnover without having genotoxic activity.

The chemicals which induce carcinogenesis through irritation of the urothelium at high levels of exposure are expected to present little or no increase in tumor incidence until a fixed level of exposure is exceeded (Cohen and Ellwein 1989). The disruption of homeostasis in the rat at high levels of exposure to such compounds results in the presence of calculi, which induce hyperplasia of the urothelium and neoplasia after prolonged exposure. Exposure to levels of these chemicals which do not induce calculi would not be expected to result in either hyperplasia or neoplasia.

REFERENCES FOR APPENDIX 1

Chapman, W.H., D. Kirchheim, and J.W. McRoberts. 1973. Effect of the urine and calculus formation on the incidence of bladder tumors in rats implanted with paraffin wax pellets. Cancer Res. 33: 1225-9.

Cohen, S.M. and L.B. Ellwein. 1989. Cell growth dynamics in bladder carcinogenesis: Implications for risk assessment. J. Am. Col. Toxicol. 8: 1103-13.

Cohen, S.M. and L.B. Ellwein. 1990. Proliferative and genotoxic cellular effects in 2-acetylaminofluorene bladder and liver carcinogenesis. Biological modeling of the ED01 study. Toxicol. Appl. Pharmacol. 104: 79-93.

DeSesso, J.M. 1989. Confounding factors in direct bladder exposure studies. Comments Toxicol. 3: 317-34.

Ellwein, L.B. and S.M. Cohen. 1988. A cellular dynamics model of experimental bladder cancer: Analysis of the effects of sodium saccharin in the rat. Risk Anal. 8: 215.

Fujii, T., K. Nakamura, and K. Hiraga. 1987. The effects of pH on the carcinogenicity of o-phenyl phenol and sodium o-phenylphenate in the rat urinary bladder. F. Chem. Toxicol. 25: 359-62.

Munro, I.C., C.A. Moodie, D. Krewski, and H.C. Grice. 1975. A carcinogenicity study of commercial saccharin in the rat. Toxicol. Appl. Pharmacol. 32: 513-26.

Raghavan D., W.U. Shipley, M.B. Garnick, et al. 1990. Biology and management of bladder cancer. N. Eng. J. Med. 322: 1129-38.

Roe, F.C.J. 1964. An illustrated classification of the proliferative and neoplastic changes in mouse bladder epithelium in response to prolonged irritation. Br. J. Urol. 36: 238-53.

Shibata, M.A., S. Tamano, Y. Kurata, A. Hagiwara, and S. Fukushima. 1989. Participation of urinary Na+, K+, pH, and L-ascorbic acid in the proliferative response of the bladder epithelium after the oral administration of various salts and/or ascorbic acid to rats. Food Chem. Toxicol. 27: 403-13.

Teelmann, K. and W. Niemann. 1979. The short term fate of dischargeable glass beads implanted surgically in the mouse urinary bladder. Arch. Toxicol. 42: 51-61.

Appendix 2 - Human bladder cancer

The urinary bladder is lined with transitional epithelium as are the renal calyces and pelves and the ureters. Under certain stresses like inflammation, the epithelium undergoes metaplasia to stratified squamous epithelium. Cancer statistics are not collected in a manner that would allow for an inclusive evaluation of all of these urinary structures. Consequently, the urinary bladder is usually investigated separately from the upper part of the urinary system. However, urinary stones (calculi) can form anywhere along the urinary collection system from the calyces and pelvis to the ureter and bladder.

Stones of the urinary tract are quite common and are seen in about 1% of autopsies (Smith 1982). Unless they produce stasis by occluding urinary flow or causing infection, they are usually clinically silent. About 1 in 1000 adults is hospitalized annually with stones. The incidence of stones varies significantly in different geographic regions of the world, probably due to dietary and other factors (Smith 1982). In the U.S. most stones are found in the upper urinary tract, with fewer in the urinary bladder. Males are much more frequently affected than females. Over 90% of stones are composed of calcium or in some cases magnesium along with oxalate and phosphate. Most of the remainder are organic in composition and contain uric acid or cysteine (Cheng 1980, Smith 1963).

Most bladder cancers in humans are transitional cell carcinomas (over 90%) while the remainder are squamous cell carcinomas and other types (Silverman et al. 1992). Bladder cancer constitutes 7% of all cancer cases in men and 4% in women, while bladder cancer deaths as a part of total cancer deaths are 2% and 1%, respectively (Boring et al. 1991).

Bladder cancer is largely a disease of the elderly: about 2/3 of the cases occur at 65 years or older. Among race-sex groups in the U.S., the lifetime risk of bladder cancer is highest among white males (nearly 3%), and the male to female ratio is about 3 to 4 (Silverman et al. 1992).

A number of epidemiologic studies have identified risk factors in the development of bladder cancer. Many of the associations involve exposure to genotoxic chemicals, notably the aromatic amines and compounds in cigarette smoke. Occupational associations include workers exposed to dyes, working in the leather and rubber industries, being a professional painter and being a professional truck driver. Certain other associations have not yet been conclusively established, like those for coffee drinking and artificial sweeteners (Silverman et al. 1992).

Intercurrent urologic conditions also have been investigated as potential risk factors for bladder cancer without developing a

clear cut position. There is some evidence suggesting that urinary stasis from various causes, such as stones and infection (bacteria and Schistosoma haematobium) may be related to cancer, but more work is needed in these areas (Matanoski and Elliott 1981, Silverman et al. 1992). For instance, as to the role of stones in the induction of all types of bladder cancer, several studies fail to show any link (Morin and Hemminger 1962, Thompson 1959, Waller and Hamer 1950). However, based on a review of the clinical literature, there may be some associations between urinary tract stones and cancer. In these studies, stones appear to be associated with the development of squamous metaplasia and squamous cell carcinoma, an uncommon form of urinary tract cancer (Peterson 1992).

The existing epidemiologic literature fails to establish stones as a significant risk factor. In a hospital bladder cancer case control study with 350 cases and an equal number of controls, bladder stones were noted in 4.6% of the cancer patients and 2.3% of controls, a suggestive but non-significant difference (Wynder et al. 1963). The average time between observation of the stones and the cancer was 15 years. Supposedly these patients had not had bladder infections. In a follow-up study, these authors questioned the influence of stones on cancer development (Wynder and Goldsmith 1977). Three other epidemiologic studies with about 300 bladder cancer cases each also fail to show a significant relationship between bladder stones and bladder cancer: Dunham et al. (1968) had relative risks of 2.4 and 2.1 for males and females, Kjaer et al. (1989) showed a relative risk of 1.5, and La Vecchia et al. (1991) showed a relative risk of 1.0.

The only positive epidemiologic indicator of a potential role for stones as a risk factor for bladder cancer in humans comes from an analysis of data on nearly 3000 new bladder cancer cases and double that number of controls. Subjects were administered a questionnaire that requested information on urinary stones and infections that had occurred more than 1 year before the interview. Relative risks were significantly increased for bladder stones, with or without infection (RR=2.0 and RR=1.8, respectively). The time between the finding of stones and cancer was not given. Kidney stones showed no increased risk for bladder cancer (Kantor et al. 1984).

In summary, the accumulated evidence for urinary bladder stones as a significant factor in bladder cancer risk is marginal. In the U.S. most urinary tract stones are not found in the bladder. Epidemiologic studies provide little evidence for a major contribution by stones; from the clinical literature, it appears that stones may be associated with squamous cell cancer, an uncommon form of bladder cancer. Thus, the overall effect of stones on bladder cancer seems minimal. Certainly humans appear

to be much less sensitive to the impact of stones on bladder carcinogenesis than are laboratory rodents.

REFERENCES FOR APPENDIX 2

Boring, C.C., T.S. Squires, and T. Tong. 1991. Cancer statistics: 1991. Ca-Cancer J. Clin. 41: 19-36.

Cheng, L. 1980. Urinary tract calculi in man and laboratory animals: Incidence, composition, and etiology. J. Environ. Pathol. Toxicol. 4: 317-49.

Dunham, L.J., A.S. Rabson, H.L. Stewart, A.S. Franke, and J.L. Young. 1968. Rates interview and pathology study of cancer of the urinary bladder in New Orleans, Louisiana. J. Natl. Cancer Inst. 41: 683-709.

Kantor, A.F., P. Hartge, R.N. Hoover, R.N., A.S. Narayand, J.W. Sullivan, and J.R. Fraumeni, Jr. 1984. Urinary tract infection and risk of bladder cancer. Am. J. Epidemiol. 119: 510-5.

Kjer, S.K., J.B. Knudsen, B.L. Sorenson, and O. Moller-Jensen. 1989. The Copenhagen case-study of bladder cancer. V. Review of the role of urinary-tract infection. Acta Oncolog. 28: 631-636.

LaVecchia, C., E. Nagri, B. D'Avunzo, R. Savodelli, and S. Franceschi. 1991. Genital and urinary tract diseases and bladder cancer. Cancer Res. 51:629-631.

Matanoski, G.M. and E.A. Elliot. 1981. Bladder cancer epidemiology. Epidemiol. Rev. 3: 203-29.

Morin, L.J., and C.H. Hemmenger. 1962. Bladder tumors: a ten year review. J. Urol. 87: 368-372.

Peterson, R.O. 1991. Urologic pathology. 2nd ed. Philadelphia, PA: J.B. Lippincott.

Silverman, D.T., P. Hartge, A.S. Morrison, and S.S. Devesa. 1992. Epidemiology of bladder cancer. Hematol. Oncol. Clinico (In press)

Smith, D.R. 1975. General urology. 8th ed. Los Altos, CA: Lange Medical.

Smith, L.H. 1982. Urinary calculi. *In* R. Berkow, ed. Merck Manual of Diagnosis and Therapy. 14th ed. Rahway, NJ. Merck, Sharp and Dome. pp. 1597-9.

Thompson, N. 1959. Carcinoma of the bladder. Br. J. Urol. 31: 287-97.

Walley, J.I. and H.G. Hamer. 1950. Bladder tumors: A survey of 373 cases. J. Urol. 64: 651-6.

Wynder, E.L., J. Onderdone, and N. Mantel. 1963. An epidemiological investigation of cancer of the bladder. Cancer 16: 1388-1407.

Wynder, E.L. and R. Goldsmith. 1977. The epidemiology of bladder cancer: A second look. Cancer 40: 1246-68.