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

March 30, 1993

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Health Effects Division (HED)
Carcinogenicity Peer Review Committee
Draft Document on **MELAMINE**

FROM: Esther Rinde, Ph.D. E.R.
Manager, HED Carcinogenicity Peer Review
Science Analysis Coordination Branch
Health Effects Division (H7509C)

TO: Addressees

Attached for your review is the draft document of the Carcinogenicity Peer Review Committee on **Melamine**. Please provide your comments on the draft document and return to me no later than April 16, 1993. If a reply is not received by that time, it will be presumed that you concur and have no comments.

Should you need a few extra days for a thorough review, please let me know that your comments are forthcoming.

ADDRESSEES

P. Fenner-Crisp
W. Burnam
K. Baetcke
M. Van Gemert
R. Engler
M. Copley
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L. Brunsman
J. Quest
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J. Parker
D. Mandell (2 copies)

MEMORANDUM

SUBJECT: DRAFT Carcinogenicity Peer Review of Melamine

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

and

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

TO: Phil Hutton, PM #18 and
Mike Mendelsohn, PM #17
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 1992 (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 observed (in the male rat urinary bladder), it appears that Melamine is not likely to be carcinogenic to humans under conditions of estimated human exposure associated with its pesticidal use. However, further information is needed before a definitive conclusion can be drawn concerning human hazard under the expected conditions of exposure.

A. Individuals in Attendance:

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

Penelope A. Fenner-Crisp _____

Reto Engler _____

Marcia Van Gemert _____

Karl Baetcke _____

Marion Copley _____

Julie T. Du _____

Richard Hill _____

Hugh Pettigrew _____

Esther Rinde _____

William Sette _____

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

Stephen C. Dapson[1] _____

Mike Ioannou _____

Bernice Fisher _____

Lori Brunsman _____

Lucas Brennecke[2] (Clement/PAI) _____

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 _____

Yin-Tak Woo _____

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[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.

John Quest _____

William Burnam _____

4. Other Attendees: (Observers)

Diane Mandell (Clement)

B. Material Reviewed:

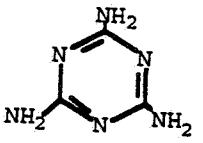
The material available for review consisted of DERs, one-liners, and other data summaries prepared by Dr. Stephen Dapson. Tables and statistical analysis were prepared by Lori L. Brunsman. The material reviewed is attached to the file copy of this report. The data reviewed are based upon studies submitted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

C. Background Information:

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

The Caswell (or Tox Chem) Number of Melamine is 861B.

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 900 0 ppm (225 or 450 mg/kg/day) of Melamine.

b. Discussion of Tumor Data

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 investigators felt that it was not due to treatment with Melamine.

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

In the rats, one low- and two high-dose males had bladder hyperplasia; one had bladder stones. Mean body weights were less than those of controls for all dosed rats after week 20. Survival of high-dose male rats was significantly decreased in comparison to controls. Females had dose-related chronic inflammation of the kidneys. In the high-dose male rats, 10/49 had bladder stones which were not found in the concurrent controls, and which were found in only 1/50 of the low-dose male rats. Nephropathy was noted in 32/45 controls, 36/50 low-dose males and 30/49 high-dose males. Females in all groups also had nephropathy.

In the mice, the mean body weights of the males were decreased after week 50. Survival of high-dose males was decreased. The male mice had intense acute and chronic inflammation (a lesion not seen in dosed 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. 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 all studies was considered to be adequate for assessing the carcinogenic potential of Melamine, based on decreased body weight gains and the development of nephropathy.

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 10000 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 tumors and benign 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 Committee, 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. This study was not reviewed by the Committee.

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, 10000, 15000, 20000, or 30000 ppm (250, 500, 750, 1000, or 1500 mg/kg/day) of Melamine in the diet. All dose groups at 15000 ppm and above had reduced body weight gain and according to the investigators, the 20000 and 30000 ppm dose groups "lost" weight. A hard crystalline solid was found in the urinary bladders of male rats at 10000 ppm and above (4/5 to 5/5), and in female rats at 20000 ppm and above (4/5).

Three 13-week rat feeding studies were conducted. The first used diets containing 6000, 9000, 12000, 15000, or 18000 ppm (300, 450, 600, 750, or 900 mg/kg/day). Mean body weight gain in the 12000 ppm dose group and above was depressed by more than 8% when compared to controls. Stones were found in "most" dosed 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 18000 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 12000 ppm (37.5, 75, 150, 300, 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 12000 ppm, with no similar effect in females. The incidence of stones in the urinary bladders of male rats was considered dose-related [1/10 (controls), 2/10, 5/10, 7/10, 9/10, 9/9], with stones present in the 750 ppm dose group and control group. 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 of the females. 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 18000 ppm 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).

A 14-day mouse feeding study used doses of 5000, 7500, 10000, 12500, 15000, or 30000 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 30000 ppm.

A 13-week study was conducted in mice receiving 6000, 9000, 12000, 15000, or 18000 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 dose of Melamine out of 7 doses between 2000 and 19000 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

Metabolism study in the rat

Characterization and Identification of ^{14}C-Cyromazine and Metabolites in Rats, Hazelton 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.

^{14}C-Cyromazine was administered by oral gavage or intravenous injections to rats and the urine and fecal

metabolites were analyzed. A comparison of oral and i.v. disposition found Cyromazine to be well absorbed after oral administration. The investigators found rapid excretion with the low dose tested (3 mg/kg) with radioactivity eliminated in the urine by 24 hours, but noted an apparent delay in the urinary excretion at the high dose tested (300 mg/kg). Fecal elimination was equivalent among dose groups except for the high dose males, where a greater percentage was eliminated by this route. The origin of fecal radioactivity was apparently through biliary elimination. The majority of the radioactivity (82 - 92%) was in the urine, with lesser amounts (3 - 7%) in the feces. The investigators found minimal residual radioactivity in the tissues. Melamine, hydroxyCyromazine, methylCyromazine and unmetabolized Cyromazine were identified as metabolites.

Identified Metabolites
Percent of Urinary or Fecal Radioactivity

	Urinary	Fecal
methylCyromazine	1.8-3.5	4.7-12.5 (hydroxy- included)
Melamine	2.8-12.1	4.7-7.6
hydroxyCyromazine	4.6-16.3	above
Cyromazine	55.8-83.6	68-76
unidentified	2.1-8.7	-

3. Mutagenicity

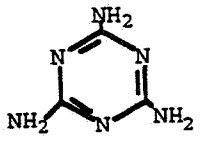
Open literature data from mutagenicity studies provided in the NTP report stated that Melamine was not mutagenic for Drosophila melanogaster; not mutagenic for Salmonella typhimurium G46, TA 98, TA 100, TA 1530, TA 1531, TA 1532, TA 1534, TA 1535, and TA 1538 both with and without metabolic activation; 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. NTP assays were negative for chromosomal aberrations in a questionable test for sister chromatid exchanges in CHO cells, and negative for gene mutations in mouse lymphoma cells.

7. Structure-Activity Correlations

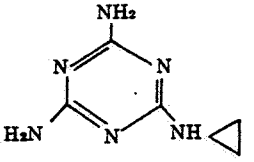
Melamine is related to the triazine class of chemicals although most of the triazines are used as herbicides. A NLM Chemline search found 6073 triazines, of these 49 were classified as agricultural chemicals. A further reduction of the initial 6073 triazines found in the database search in reference to specific

tumor data available in the open literature found the following pesticide chemicals: atrazine, propazine, simazine, cyanuric acid, melamine, and anilazine. Structures for related compounds are shown below.

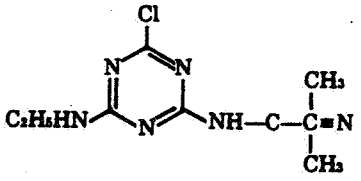
Structural Analogs of Melamine



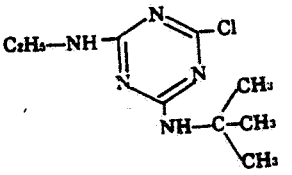
Melamine



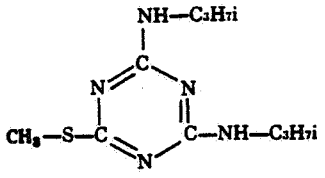
Cyromazine



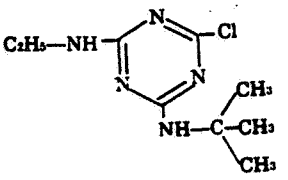
Cyanazine



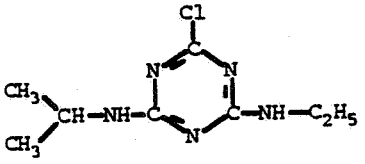
Hexazinone



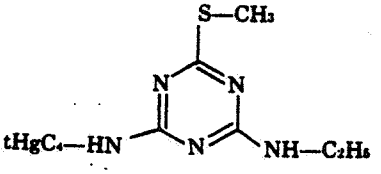
Prometryn



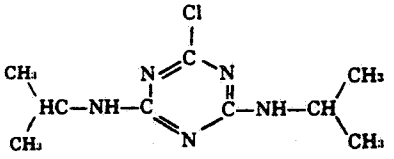
Terbuthylazine



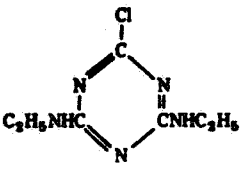
Atrazine



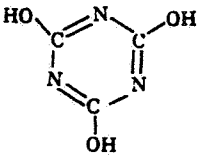
Terbutryn



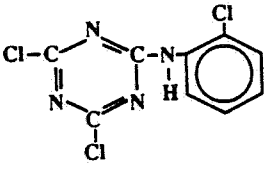
Propazine



Simazine



Trihydroxytriazine
also called S-Cyanuric Acid



Anilazine

F. Weight of Evidence Considerations:

The Committee 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 National Toxicology Program (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 2250 ppm. Stones of the urinary bladder were found in 8/9 tumor-bearing males. In a second chronic feeding study in the rat, dose levels ≥1000 ppm did not induce bladder stones or bladder tumors when administered in the diet for 29 to 30 months. In a third rat chronic study, 4/10 males and 2/10 females were found to have benign bladder tumors with urinary stones after the administration of 10000 ppm in the diet.

2. In rats, 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 (20% of the females) given 18000 ppm of Melamine in the diet for 90 days. 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 15000 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 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 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. 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 tumors in rats result from mechanical irritation caused by stones via 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 tumors of the urinary bladder in male rats. Other chemicals which lead to the formation of stones in the rat urinary bladder also induce hyperplasia, and upon chronic administration, neoplasia (See Appendix 1).

6. Genotoxicity testing of Melamine for point mutations in bacteria and cultured mammalian cells, and for chromosome aberrations and sister chromatid exchange in cultured mammalian cells have been uniformly negative.

7. Melamine is rapidly excreted unchanged in the urine of the rat with a plasma half-life of 2.7 hours. Administration of hexamethyl Melamine to humans and rats found that the s-triazine ring is not cleaved.

8. Although about 50% of the residue of cyromazine in treated commodities is in the form of Melamine [FR 50(90):20379, May 15, 1984], human exposure to Melamine residues is about one million-fold less than that which induces bladder tumors in male rats. Urinary stone formation, which is thought to be a necessary event for Melamine tumor induction in the male rat (see #5, above), is therefore not expected to occur in humans consuming residues of Melamine.

9. Even if stone formation in humans did result from Melamine exposure, the association between bladder stones and human cancer is equivocal at best (see Appendix 2). The human bladder appears to be much less sensitive than the rat to the induction of bladder tumors by urinary stones.

10. Based upon the above considerations, the tumors of the urinary bladder found in male rats after the administration of high dose levels (4500 ppm) of Melamine are not considered to be predictive of potential human carcinogenicity of Melamine.

G. Classification of Carcinogenic Potential:

Criteria contained in the current EPA Guidelines [51FR: 33992-34003, 1986] were considered. After taking into account all information and carefully examining the potential classifications in the EPA guidelines, the PRC concluded that Melamine is not amenable to classification using the 1986 Guidelines and, rather than forcing Melamine into one of the existing classifications categories, it was more appropriate to describe the weight-of-evidence in a narrative form.

The PRC concluded that this chemical 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 in the male rat, appears to be due to bladder stone formation, bladder wall irritation and profound proliferation of bladder epithelium (see Appendix 1). Male rats are more sensitive than female rats to stone formation, and mice had inflammation and epithelial hyperplasia without features of pre-neoplastic lesions in chronic studies.

Besides stone formation, there is no other apparent cause for the induction of bladder tumors in male rats. Melamine is also negative for gene and chromosomal mutations in short term tests.

Thus, Melamine induces a rare tumor type in male rats which is significant by pair-wise comparison and by the trend test; rats and mice responded differently to the stone-induced irritation, and the response of the human urothelium to melamine-induced calculi is not known with certainty. Residues of melamine are present in the human diet, however the level of intake which would be associated with stone formation in the human is not known; and it is possible that the presence of stones in animals with tumors is coincidental rather than causative since only rats appear to develop tumors in the presence of stones.

There are uncertainties which preclude a more 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 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.

The PRC concluded that Melamine could not be classified according to the usual Agency guidelines, because there is inadequate evidence for a determination of Group E- evidence of non-carcinogenicity for humans. The descriptor for Group E in the EPA cancer guidelines states that this grouping applies to chemicals that show no evidence of cancer in at least two animal species, and Melamine demonstrates tumor effects in male rats. In addition, there are probable rat/human differences in carcinogenicity mechanisms for bladder tumors.

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 the subject of extensive investigation in laboratory animals. Studies over the last quarter of a century have led to the knowledge that urinary stones commence steps which can include increases in urinary epithelial cellular proliferation, cellular hyperplasia, benign and malignant neoplasms. It appears that it is the presence of a physical body (and urine) in the bladder, rather than the chemical composition of the physical body which is essential for tumor induction in these cases i.e. the tumors are secondary to the presence of stones. A series of examples are available which typify this type of carcinogenic process in the bladder and which 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 resulted in a higher tumor incidence than did smooth glass beads (DeSesso 1989).

A number of other factors have been identified as influencing the induction of bladder tumors. Increased incidences of urinary bladder tumors are observed with some chemicals at a high pH; lowering of urinary pH decreases or eliminates carcinogenic activity. Saccharin appears to induce urinary bladder tumors via the production of an abrasive silicate crystal in male rats (Cohen and Ellwein 1990) and sodium ortho-phenyl phenol induces tumors through the production of calculi in the bladder (Fujii et al. 1987). The induction of tumors by each of these chemicals is pH-dependent. However, the induction of bladder tumors by other chemicals is not enhanced by decreased pH and this is most likely due to the nature of the urinary precipitate, for example, precipitation of phosphate salts or silicate salts may occur at a low pH whereas other chemicals such as uric acid or cysteine may precipitate at a higher pH. 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 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 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 as critical components (Cohen and Ellwein 1989, 1990). Stimulation of cell division is one of the elements, while genotoxic events represent the second. 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, but possibly to other factors or exposures to the chemical which has induced cell division, a metabolite of that chemical or a natural constituent of the diet or its metabolite. Certainly the finding of bladder tumors following the implantation of glass or wooden beads or wax pellets would also fall into this category, since they seem to 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 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 male 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 are not expected to result in either hyperplasia or neoplasia.

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Appendix 2- Human bladder cancer

The urinary bladder is lined with transitional epithelium as are the renal calyces and pelvis and the ureters. Under certain stresses 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 these structures; therefore, urinary bladder is usually investigated separately from the upper part of the urinary system. Urinary stones (calculi) can form anywhere along the urinary collection system from the calyces and pelvis to the ureters and bladder. Stones are quite common and are seen in about 1% of autopsies (Smith 1982). Unless they produce stasis by occluding urinary flow or cause 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). 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 cystine (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 the total are 2% and 1%, respectively (Boring et al. 1991). It is largely a disease of the elderly, with about 2/3 of the cases occurring at 65 years of age 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).

Many 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. These associations include such things as smoking and certain occupations like dye, leather, and rubber workers, painters and truck drivers. Certain other associations have not been 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 (Matanoski and Elliott 1981, Silverman et al. 1992). There is some evidence suggesting that urinary stasis from various causes, stones and infection (bacteria and Schistosoma haematobium) may be related to cancer, but more work is needed in these areas. For instance, as to the role of stones

per se in cancer, several studies fail to show any link (Morris and Hemminger 1962, Thompson 1959, Waller and Hamer 1950). 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).

The other indication of a potential role of 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 who 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).

The accumulated evidence for urinary stones being a significant factor in bladder cancer risk is marginal. Given the high frequency of stones in the population and the limited evidence of an association, it would seem that bladder stones play only a minor role, if any, in conditioning human bladder cancer. Certainly humans appear to be much less sensitive to the impact of stones on bladder carcinogenesis than are laboratory rodents.

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