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# Final Report on the Safety Assessment of Methenamine

## ABSTRACT

**Methenamine is a biocide that is used in cosmetic eye make-up preparations at concentrations of less than 1%. Methenamine, following oral administration, undergoes hydrolysis and generates formaldehyde. Methenamine is rapidly absorbed from the intestinal tract and excreted mostly unchanged in the urine. A single oral dose of 20 g/kg Methenamine did not cause mortality in rats. No untoward signs of toxicity were observed in either subchronic or chronic studies.**

**Methenamine was slightly irritating to the skin of rabbits. In ocular studies it was mildy irritating. In animal assays, Methenamine was a sensitizer when tested at a concentration of 25%, but not at 0.2%. It was neither an irritant nor a sensitizer to humans at 0.1%.**

**In a number of teratologic and reproductive studies, no teratogenic effects attributable to Methenamine were observed. Methenamine was a mutagen in *Drosophila melanogaster* but not in other *in vitro* mutagenicity assays. Methenamine did not show any carcinogenic activity, either alone or when nitrite was included in the drinking water of the test animals.**

**Methenamine is judged to be safe for non-aerosolized cosmetic products at a concentration not to exceed 0.16%. At this concentration, the released formaldehyde concentration will not exceed 0.2%.**

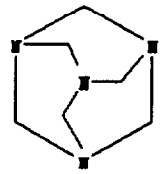
## INTRODUCTION

METHENAMINE IS AN ORGANIC amine which functions as a cosmetic biocide that is used primarily in eye make-up preparations (FDA, 1989). Methenamine undergoes hydrolysis and liberates formaldehyde, particularly at relatively low pH. The CIR final report on the safety assessment of formaldehyde can be found in the *Journal of the American College of Toxicology*, Vol. 3, No. 3 (Elder, 1984).

## CHEMISTRY

### Definition and Structure

Methenamine (CAS No. 100-97-0) is the organic amine that conforms to the following formula (Estrin et al., 1982);



Methenamine is also known as hexamethylenetetramine (Estrin et al., 1982; Windholz, 1983), hexamethyleneamine, 1,3,5,7-tetraazatricyclo[3,3,1,1]-decane (Estrin et al., 1982), 1,3,5,7-tetraazatricyclo[3.3.1.1$^{3,7}$]-decane, hexamine, 1,3,5,7-tetraazaadamantane, and hexamethylenamine (Windholz, 1983).

### Properties

Methenamine occurs as odorless crystals, granules, or powder (Kabara, 1984; Windholz, 1983), as practically odorless, colorless, lustrous crystals, or as a practically odorless white crystalline powder (Hawley, 1971; Osol, 1980). Physical and chemical properties of Methenamine are summarized in Table 1.

### Manufacture and Production

Methenamine is prepared by adding a moderate excess of ammonia water to formaldehyde solution and then evaporating it to dryness (Osol, 1980). It can also be produced by reacting aqueous formaldehyde with gaseous ammonia (Clayton and Clayton, 1981).

### Analytical Methods

Methenamine can be determined by heating a 10% solution with 2-*N*-sulfuric acid. Formaldehyde is liberated, which can be identified by its odor and by its darkening of paper moistened with silver ammonium nitrate TS. With the addition of an excess of 1 *N*-sodium hydroxide to the solution, ammonia is volatized (Heller, 1980).

The infrared absorption spectrum of a potassium bromide dispersion of Methenamine, previously dried, will exhibit maxima only at the same wavelengths as that of a similar preparation of USP Methenamine RS (Heller, 1980).

Methenamine can be determined by gas chromatography (Klinge et al., 1982; Nieminen et al., 1980).

### Impurities

After drying Methenamine over phosphorus pentoxide for 4 h, it contains not less than 99.0% of $C_6H_{12}N_4$ (Heller, 1980).

## USE

### Cosmetic

Methenamine is used as a preservative in lotions and creams (Kabara, 1984). The production formulation data that were submitted to the Food and Drug Administration in 1989 for Methenamine indicated that it is present in a total of seven cosmetic product formulations at a concentration of <1.0% (Table 2). Methenamine was used in mascara and other eye makeup preparations and in face, body, and hand skin preparations, excluding shaving preparations. The greatest reported use of Methenamine was in mascara and other eye makeup preparations, a total of six formulations.

The FDA cosmetic product formulation computer printout (FDA, 1989) is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations (1982). Ingredients are listed in preset concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the

**TABLE 1.** PROPERTIES OF METHENAMINE

| | | *References* |
|---|---|---|
| Physical Appearance | White hygroscopic powder | COLIPA, 1989 |
| | Crystals, granules, or powder | Windholz, 1987; Kabara, 1984 |
| | Colorless, lustrous crystals or white crystalline powder | Osol, 1980; Hawley, 1971 |
| Odor | None | Windholz, 1987; Kabara, 1984 |
| | Practically odorless | Osol, 1980; Hawley, 1971 |
| Taste | Slightly sweetish flavor, leaves a somewhat bitter after taste | COLIPA, 1989 |
| Molecular Formula | $C_6H_{12}N_4$ | Estrin et al., 1982 |
| Molecular Weight | 140.19 | Weast, 1982 |
| Density | 1.331 at −5°C | Weast, 1982 |
| Specific Gravity | 1.27 at 25°C | Hawley, 1971 |
| Melting Point | ≈263°C, sublimes | Windholz, 1983 |
| | 285–95°C, sublimes | Weast, 1982 |
| | 280°C, sublimes | Sax, 1979 |
| | >200°C, sublimes | Hawley, 1971 |
| Flash Point | 482°F | Sax, 1979 |
| Residue on Ignition | ≤0.1% | Heller, 1980 |
| Solubility | 1 g dissolves in 1.5 ml water, 12.5 ml alcohol, 320 ml ether, 10 ml chloroform | COLIPA, 1989 |
| | Soluble in water, alcohol, acetone, and chloroform | Weast, 1982 |
| | Very soluble in water and glycerol; soluble in chloroform, ethanol, and methanol | Clayton and Clayton, 1981 |
| | Soluble in water, alcohol, and chloroform; insoluble in ether | Hawley, 1971 |
| Flammability | Readily burns, smokeless flame | Windholz, 1983 |
| | Flammable | Clayton and Clayton, 1981 |
| Threshold Limit Value | None | Clayton and Clayton, 1981 |
| Miscellaneous | pH of 0.2 M aqueous solution = 8.4 | COLIPA, 1989 |

finished product; the actual concentration would be a fraction of that reported to the FDA. Data submitted within the framework of preset concentration ranges provide the opportunity for overestimation of the actual concentration of an ingredient in a particular product. At entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to tenfold error in the assumed ingredient concentration.

### International

Methenamine is accepted for use by the European Economic Community (EEC) as a preservative at a concentration no greater than 0.15%. Methenamine may also be added to cosmetic products at a concentration greater than 0.15% for other specific uses apparent from the presentation of the products. These other uses include deodorants in soaps or anti-dandruff agents in shampoos (EEC, 1986).

### Noncosmetic

Methenamine is used as a urinary tract anti-infective drug. The adult dosage ranges from 500 mg to 1.5 g, with the usual dose being 1 g four times a day. Children 6–12 years of age receive 500 mg four times a day; children under 6 years of age receive 50 mg/kg/day in three doses (Osol, 1980). Other noncosmetic uses of Methenamine include its use in preservation of hides, as a corrosion inhibitor for steel, as a cross-linking agent for hardening phenol-formaldehyde resin (Kabara, 1984; Windholz, 1983), as a hardener in core-molding processes (Hayakawa et al., 1988), in fuel tablets for camping stoves, and as a substance for the manufacture of explosives (Hawley, 1971; Windholz, 1983). Methenamine is also used as a food preservative (Ariens et al., 1982), as a protein modifier (Hawley, 1971), as an accelerator in rubber compounds (Fisher, 1978), as a cross-linking agent in the vulcanization of rubber, as an ingredient in adhesive, coating and sealing compounds, in the chemical detection of metals, and it is used with sodium phenate and NaOH as an absorber of poisonous gases (Windholz, 1983).

## GENERAL BIOLOGY

### Antimicrobial Spectrum

Methenamine does not itself have antimicrobial activity. Antimicrobial activity is derived from the formaldehyde released under hydrolytic conditions in an acidic environment (Kabara, 1984).

**TABLE 2.** PRODUCT FORMULATION DATA FOR METHENAMINE

| *Product category* | *Total no. of formulations in category* | *Total no. containing ingredient* | *No. of product formulations within each concentration range (%) <1.0* |
|---|---|---|---|
| Mascara and other eye makeup preparations | 439 | 6 | 6 |
| Face, body, and hand skin care preparations (excluding shaving preparations) | 1121 | 1 | 1 |
| 1989 Totals | | 7 | 7 |

*Source:* FDA, 1989.

## Absorption, Distribution, Metabolism, Excretion

Methenamine, after oral administration, undergoes hydrolysis and generates formaldehyde (Ariens et al., 1982). The hydrolysis takes place fastest at relatively low pH (e.g., in the stomach and the urine). Methenamine is rapidly absorbed from the intestinal tract and is excreted, for the most part, unchanged in the urine. Methenamine can pass the placenta and is detectable in the amniotic fluid and in milk. Methenamine's renal clearance is slightly lower than that of creatinine; its plasma half-life is 4 h. Approximately 10–30% of an oral dose of methenamine is converted to formaldehyde in the stomach. A pH of 5–6 produces the peak concentration of formaldehyde in the urine. At a urinary pH of 6 and a urine volume of 1000–1500 ml per 24 h, a 2 g dose of Methenamine produces a formaldehyde concentration of 18–60 μg/ml of urine. One mole of methenamine produces 6 moles of formaldehyde. In *in vitro* studies, formaldehyde reacting with HCl forms a highly carcinogenic compound, bis(chloromethyl) ether; this process might also occur in the stomach. Although this reaction occurs readily in the gaseous phase, it is much less likely to occur in liquid phase (Conning, 1973). In aqueous medium, bis(chloromethyl)ether is highly unstable; at ambient temperature, it has an approximate half-life of 10–40 s (Tou and Kallas, 1974).

Ten healthy volunteers, 6 women and 4 men, were used to study the absorption, metabolism, and excretion of Methenamine (Klinge et al., 1982). The subjects were administered two different formulations of 1 g Methenamine hippurate tablets. The first formulation was administered for 8 days, followed by a week nontreatment period, and then the second formulation was administered for 8 days. The subjects were given one tablet at 8:00 a.m. on the first day of each administration, and at 8:00 a.m. and 8:00 p.m. on days 2 through 6. On the seventh day of administration, the last tablet was given at 8:00 a.m. Subjects were fasted the night before administration of the morning tablets on days 1, 6, and 7, and they did not eat until 2 h after taking the tablet. There were no dietary restrictions on any other days.

A number of blood samples were taken on days 1, 6, and 7 after ingestion of the tablet, and urine samples were also collected at various intervals on these days. On days 6 and 7, a blood sample was also taken prior to ingestion of the morning tablet.

Methenamine in the samples was determined using a gas chromatographic method modified from Nieminen et al. (1980). The maximum serum concentration was achieved in approximately 1 h. The distribution volume was approximately 0.56 L/kg; this value was similar to the value obtained by Allgren et al. (1979). The renal clearance was approximately 1.46 ml/min/kg and the total clearance was about 1.59 ml/min/kg (Klinge et al., 1982). The individual elimination half-lives ranged from 2.8 to 6.0 h, with a mean of 4.3 h. Based on urinary excretion rates, an approximate estimation of elimination half-life after a single dose was 3.9 h for the first formulation and 3.5 h for the second formulation. On day 7, this value was 3.8 h for both formulations. The value obtained pertaining to elimination half-life was similar to the value obtained by Anderson et al. (1976), Allgren et al. (1979), and Ball (1980), but considerably greater than the value obtained by Jackson and McLeod (1974) and Ritschel (1980).

The hippurate salt was rapidly and well absorbed from an empty stomach. Following a single dose, approximately 82% of the Methenamine was recovered in the urine in 24 h. During continuous b.i.d. administration, approximately 88% of the dose was excreted within a 12 h dosing period. The predicted steady-state minimum and maximum serum concentration values of 4.3 and 35.2 mg/ml, respectively, based on the data obtained after a single dose, were in agreement with the measured values.

These results, combined with the fact that the drug accumulation index was 0.48, indicated that no accumulation took place.

Due to large interindividual variations in urine volumes, Methenamine concentrations varied considerably within each urine fraction; the differences between renal clearances were small. The average minimum concentration did not go below 150 mg/L. According to Hamilton-Miller and Brumfitt (1977), the rate of hydrolysis of Methenamine was remarkedly increased when the pH was kept between 5 and 6; this did not prove true in all the subjects. At steady state, the urine was not signficantly more acidic than following a single dose.

## ANIMAL TOXICOLOGY

### Acute Oral Toxicity

Two groups of five adult rats each received either 10 or 20 g/kg of Methenamine as an 80% aqueous solution by oral intubation (Della Porta, 1966). No mortality was observed.

### Intravenous

Fourteen male and female Wistar rats received a single intravenous (i.v.) injection of Methenamine as an 80% aqueous solution (Della Porta, 1966). Ten mg/kg of Methenamine was used. No evidence of a toxic effect was observed and none of the rats died.

In 1977, the "Profiles on Occupational Hazards for Criteria Document Priorities" determined the i.v. $LD_{50}$ for rats to be 9200 mg/kg. Malorny et al. (1965) reported this same value.

### Intraperitoneal

The following treatments gave these results after a single intraperitoneal (i.p.) injection: 200 ml/kg of 0.25 M Methenamine, buffered in 0.3 M glucose to pH 6.0, with 0.0165 M sodium phosphate killed all mice (number not stated) in 2–4 h; 200 ml/kg of 0.20 M Methenamine, buffered in 0.3 M glucose to pH 6.0, with 0.0135 M phosphate killed all mice (number not stated) in 4–20 h; 50 ml/kg of 1.0 M Methenamine, buffered in 0.3 M glucose to pH 6.0, with 0.09 M phosphate killed 50% of the mice (number not stated) in 2 h; and 200 ml/kg of 0.25 M Methenamine, buffered in 0.15 M glucose to pH 6.0, with 0.0165 M phosphate killed 6 of 14 mice. Signs of toxicity were trembling, weakness of the hind quarters, and terminal convulsions (Stevens and Lehman, 1973).

### Subcutaneous

The subcutaneous $LD_{50}$ for rats was 200 mg/kg (Kabara, 1984). A subcutaneous injection of 50 ml/kg of 3.5 M Methenamine and a simultaneous i.p. injection of 100 ml/kg of 0.3 M glucose killed 3 of 15 mice. Signs of toxicity were trembling, weakness of the hind quarters, and terminal convulsions (Stevens and Lehman, 1973).

### Short-Term Oral Toxicity

Mice were fed Methenamine for 10 days at doses up to 5.0 g/kg (Krasovskii and Fridlyand, 1967). No toxicity was observed.

## Dermal Toxicity

Two groups of rabbits, 6 males per group, were used in a short-term toxicity test (COLIPA, 1989). Two ml of 0.20% Methenamine in distilled water were applied to one group of rabbits; the other group served as a control group. The test animals received applications of test solution 5 days a week for a period of 6 weeks; the applications were not under occlusive patches. The animals, general behavior, hair growth, and weight gain was the same in both the control and test groups. No erythema, edema, scratching, or variation of the cutaneous fold was observed in the treated group when compared to the controls.

## Subchronic Toxicity

By multiple routes of administration. Three groups of rats were administered Methenamine (Brendel, 1964). The first group, 5 males and 5 females, received 400 mg/day methenamine by gavage for 90 days. The second group, 5 males and 5 females, received intramuscular injections of 200 mg/day for 90 days. The third group, consisting of 15 males and 15 females, received 400 mg/day methenamine by gavage for 333 days. No toxic effects were observed in any group. The only clinical sign observed was a citrus-yellow discoloration of the hair coat.

## Chronic Oral Toxicity

Two-month-old Wistar rats were assigned to either a test or a control group, 16 males and 16 females per group, to assess the toxicity of Methenamine (Natvig et al., 1971). Control animals were fed a standard diet; test animals were fed a standard diet containing 0.16% Methenamine. In a palatability test, no real preference was found for feed with or without Methenamine. On an average, rats in the treatment group had a daily intake of 100 mg/kg Methenamine. The animals were kept on study until they died. Body weights were taken at approximately 3-month intervals and at death.

No significant differences in body weight were observed at any stage of the study. Voluntary muscular activity was measured using the method of Wulff Rasmussen (1953, 1957) on day 11 and after 3, 7, and 14 months; no significant difference was observed between treated and control groups. The average life span was 6–9% longer for controls than the test animals; this difference was associated with slightly lower terminal body weights in the test group, especially for males.

At the time of death, all animals, with the exception of ten for which it was not possible, were necropsied. There was no significant difference in organ to body weight ratios for the liver, kidneys, adrenal glands, or gonads. A yellow staining of the hairs of the perineum was observed in one male and three females in the test group. No other signs due to the administration of Methenamine were observed.

Observance of the yellow staining of these hairs is in agreement with the findings by Brendel (1964) and Della Porta et al. (1968). Kewitz and Welsch (1966) explained it as a reaction between formaldehyde present in the urine from the treated rats and kynurenine, a normal constituent of rat hair. Except for a few rats in both groups, the majority of deaths were attributed to pneumonia.

A group of cats, two males and three females, ate feed containing approximately 50,000 ppm of Methenamine as a dose of 1250 mg/kg/day (Kewitz, 1966). Another group of cats, one male and three females, ate feed containing approximately 15,000

ppm of formaldehyde as a dose of 375 mg/kg/day. Animals were fed treated feed for 2 years. A third group, 3 males and 3 females, served as the controls. One female in the formaldehyde group died of pleuritis in the seventh month and a female in the Methenamine group died of a pyrogenic infection of the nasal cavity and paranasal sinuses in the twenty-third month. Neither Methenamine nor formaldehyde affected feed consumption, weight gain, or appearance. No differences in microscopic lesions were observed between the control and treated groups.

## Dermal Irritation

Methenamine was topically applied to the flanks of 6 male rabbits (COLIPA, 1989). The test compound was administered in distilled water at a concentration of 0.20%; a volume of 0.5 ml was applied under an occlusive patch. Both intact and abraded sites were used. The test solution was kept in contact with the skin for 24 h; the time of observation was 72 h. Methenamine was slightly irritating to the skin of rabbits.

## Ocular Irritation

An ocular irritation test using nine New Zealand White albino rabbits was performed with a mascara that contained 0.1% Methenamine (Stillmeadow, Inc., 1980). The eyes of the rabbits were examined prior to treatment and 100 mg of test material were placed in the conjunctival sac of one eye of each rabbit. The eyes of 6 rabbits, three males and three females, were not rinsed after application of the test material while the eyes of three male rabbits were rinsed with deionized water 30 s after application. The treated eyes of all nine animals were examined 24, 48, and 72 h after application and after 4 and 7 days. Fluorescein sodium ophthalmic solution, 0.2%, was used only when necessary in grading ocular reactions.

Maximum irritation scores of 6.2 for unrinsed eyes and 2.7 for rinsed eyes were obtained; both of these scores were categorized as "mildly irritating." For unrinsed eyes a marginal test result was obtained, with two of six animals having a positive reaction. According to the protocol, if a marginal test result occurs, the material could be retested to obtain a definite classification. The material was not retested in unrinsed eyes, therefore the test material was not classified in unrinsed eyes. The test material was classified as a nonirritant in rinsed eyes because none of the animals with rinsed eyes had a positive reaction.

Methenamine was applied once to the conjunctival sac of the eyes of 6 male rabbits (COLIPA, 1989). The concentration used was 0.20% Methenamine in distilled water; 0.10 ml of solution was administered. The solution was not rinsed from the eye. Neither conjunctival irritancy, iris alteration, nor corneal lesions were observed after administration of the 0.20% aqueous solution. Methenamine was non-irritating.

## Sensitization

The sensitization potential of Methenamine was evaluated by performing a guinea pig maximization test (Magnusson and Kligman, 1969). Two groups of Dunkin-Hartley albino guinea pigs, five males and five females per group, were used (COLIPA, 1989). The first group was a control group and the second group was administered Methenamine in distilled water at a concentration of 0.20%. Application was made using an occlusive patch, and the volume applied was the amount of solution that saturated a

Whatman paper. When used, Freund's adjuvant was at 0.50% concentration in saline. The test group received one application of 0.20% Methenamine solution which was left in contact with the skin for 48 h. The animals were not treated for 14 days and then received a challenge application of the test solution. No erythema or edema was observed in the treated group. After microscopic examination of skin, no differences were observed between treated and control groups.

Two compounds, AH26 and zinc oxide-eugenol (ZOE), were assessed for sensitization potential using the guinea pig maximization test (Magnusson and Kligman, 1969) that included the use of Freund's complete adjuvant (Kallus et al., 1983). Thirty female Dunkin-Hartley guinea pigs were used, 10 per group. All animals were weighed at the beginning of the study and on days 24, 36, and 57; no statistically significant difference was observed in weight gain between the treated and control groups. AH26, which was composed of 25% Methenamine, 10% silver powder, 60% bismuth oxide, and 5% titanium dioxide, was prepared as a saline extract and was mixed at a powder/liquid ratio of 1.75/1. Saline was used as a negative control.

At the beginning of the study, the guinea pigs were injected with either AH26, ZOE, or saline. The shoulder region was clipped and shaved, and the first group received the following three injections on each side of the midline of the shoulder region: 0.1 ml AH26 extract, 0.05 ml AH26 extract emulgated in 0.05 ml Freund's complete adjuvant, and 0.1 ml Freund's complete adjuvant. For the second and third groups, the AH26 was replaced with ZOE and saline, respectively. On day 6, the test region was reshaved and the skin was rubbed with 10% sodium lauryl sulfate in petrolatum to enhance skin penetration. On day 7, AH26 or ZOE in petrolatum was applied to the test groups and pure petrolatum was applied to the control group at the previous site of injection using occlusive bandages. The bandages were removed after 48 h.

The challenge was performed on day 22 by mixing the test materials in petrolatum, 1/10 (w/w), and applying them to the shaved left flank using an occlusive bandage. The control group received applications of petrolatum only. The patches were removed on day 23, the challenge sites evaluated on day 24, and re-evaluated on day 25.

Nine of the 10 animals were sensitized to AH26 and eight of the ten were sensitized to ZOE. In the AH26 group, most animals had moderate skin challenge responses; in the ZOE group, a slight skin reaction was observed for most animals. The controls had no reaction.

Polyethylene tubes, open on both ends with a circular window in the center, were used as material carriers for subcutaneous implants. Each animal, while anesthetized, received one tube of each test material. The implants were placed 2 cm from the midline on the caudal half of the back, an area that was previously clipped and shaved. Tubes containing AH26 were implanted on day 29 and tubes containing ZOE were implanted on day 30. Both implants were left *in situ* until day 58 when the animals were killed. Observations as to the animals' general condition and skin reactions were observed 24 h after each implant.

On day 30, following AH26 implantation, the AH26-sensitized animals had signs of general malaise, including fatigue, dozing, shivering, nervousness, and refusing to eat. These signs lasted only a day, were not apparent in any other group, and did not appear after ZOE implantation. By day 30, the AH26-sensitized animals developed redness and swelling around the AH26 implantation site; these lesions regressed with time. No lesions were observed around the ZOE implantation sites or around the AH26 implantation sites in ZOE-sensitized animals or in nonsensitized animals. AH26-sensitized animals developed severe to extreme tissue responses to AH26 and moderate

or severe responses to ZOE. Moderate or severe tissue responses to both materials were observed in ZOE-sensitized and in nonsensitized animals. The more severe tissue response was to AH26 rather than to ZOE by the AH26-sensitized animals and the difference was statistically significant.

The correlation between tissue response versus skin challenge results was assessed using Spearman's rank correlation coefficient ($r_s$) at the 5% level. A statistically significant correlation was found between tissue responses to AH26 implants and skin challenge results in the AH26-sensitized animals. The researchers stated that based on the skin challenge result, "AH26 should be rated as an extreme sensitizer." Both AH26 and ZOE produced a high degree of tissue toxicity; histologically, it was difficult to discern additive immunologic effects.

## Reproductive/Teratology

Results of the reproductive and teratology studies are summarized in Table 3.

Fifty-one female beagles divided into five groups were fed either 600 or 1250 ppm Methenamine in feed, 125 or 375 ppm of a 40% solution of formaldehyde in feed, or control feed (Hurni and Ohdar, 1973). Test compound was administered on days 4–56, inclusive, after mating. Animals were fasted each Saturday and the amount of feed given was reduced one week before term. No feed was given on the day of parturition. Pups of inadequately lactating mothers were given reconstituted cow's milk.

Eleven dogs were mated in the control and 125 ppm formaldehyde groups. Nine control dogs became pregnant and nine had litters; 10 bitches of the 125 ppm formaldehyde group became pregnant and all had litters. Ten bitches of the 375 ppm formaldehyde group were mated, nine became pregnant and had litters; 9 bitches of the 600 ppm Methenamine group were mated and 8 became pregnant and had litters. Of the 1250 ppm Methenamine group, 10 bitches were mated, 9 became pregnant, and 8 had litters; one pregnant dog was severely injured and eliminated from the study.

The bitches were weighed at weekly intervals and throughout lactation; no differences were observed between treated and control groups. The pups were weighed at birth and twice weekly; starting at week 4 of age, they received control feed. Pups were observed for visible defects at birth and after 8 weeks. There was a retardation of growth and an increase in mortality of the pups from the 1250 ppm Methenamine group during the first month. This group also had a lower percentage of pups surviving to weaning when compared to the other treated groups and the control group. Any stillborn pups or pups lost before weaning were necropsied and examined for internal and skeletal abnormalities; no malformations were found. Pregnancy rate, weight gain during pregnancy, and length of gestation were not affected by treatment. Mean litter size was within normal range for all groups. The percentage of stillborn pups was greater in the 1250 ppm Methenamine group than in any other group; in one litter of this group, only two of nine pups were born alive.

Of the pups weaned in this study, 212 were observed for a longer period of time. All of these dogs were normal in behavior, motility, and muscular coordination. After almost 2 years of observation, neither the dogs used in breeding colonies nor their litters had any abnormalities or reproductive disorders.

Within the chronic toxicity diet study by Natvig et al. (1971) that was described earlier in this report, a reproductive diet study was performed. After month 3 of the study the age of the rats was 5 months. The males and females of the control and test groups

**Table 3.** Reproductive and Teratogenicity Studies Using Methenamine

| *Concentration* | *Number and species of animal* | *Method* | *Results and comments* | *Reference* |
|---|---|---|---|---|
| 600 or 1250 ppm Methenamine in feed or 125 or 375 ppm of 40% formaldehyde solution in feed | 51 female beagles | Bitches were fed treated diet on days 4–56 inclusive after mating. Bitches were fasted every Saturday. | No difference in body weight was observed between the bitches of the treated and control groups. During the first month, a retardation of growth and increase in mortality was observed in the pups of the 1250 ppm Methenamine group. The percentage of stillborn pups was higher in the 1250 ppm Methenamine group than in any other group. 212 pups were kept for observation, with 18 of them being used for breeding. After 2 yrs, all were normal in behavior, motility, and muscular coordination. Neither the bitches used for breeding nor their pups had any abnormalities or reproductive disorders. | Hurni and Ohcler, 1973 |
| 0.16% Methenamine in feed | 16 male and 16 female Wistar rats<br>16 male and 16 female offspring | Males and females ate treated diet for 3 mos and then were mated. 16 male and 16 female offspring were chosen and fed the same diet as their parents at the time of weaning. | No significant difference was observed between the test and control parent groups' fertility. At 6 wks of age, the offspring were tested for voluntary muscular activity. Body weights were taken at 7 and 15 wks. After 123 days, half the offspring were killed and necropsied. There was no significant difference in muscular activity, body weight, or relative organ weight when the treated and control groups were compared. The only gross observation was a yellow coloration of the hair around the perineum of some rats. | Natvig et al., 1971 |

**TABLE 3.** REPRODUCTIVE AND TERATOGENICITY STUDIES USING METHENAMINE (*CONTINUED*)

| *Concentration* | *Number and species of animal* | *Method* | *Results and comments* | *Reference* |
|---|---|---|---|---|
| 1% Methenamine in drinking water | 6 male and 12 female Wistar rats | Two wks before mating, rats were given treated water. Females were treated during pregnancy and lactation. 24 offspring of each sex were given 1% Methenamine in their drinking water for 20 wks. | No malformations were observed. Up to 9 wks for male pups and 20 wks for female pups, test animal body weights were significantly lower than control body weights. At the end of offspring dosing, the animals were killed. No differences were observed in organ weight, gross observations, or histopathologic results between the treated and control groups. | Della Porta et al., 1970 |
| 1% Methenamine in drinking water | 6 male and 12 female rats (strain unspecified) | Rats were given treated water before mating.<br>24 offspring/gender were kept and given 1% Methenamine in drinking water. | No difference was observed in fertility, litter size, or lactation performance between the treated and control groups. No difference in body weight, growth, or organ appearance was observed when test offspring were compared to controls. | Della Porta, 1966 |
| 5 or 50 mg/kg Methenamine daily in drinking water | rats (no. and strain unspecified) | In a 5 generation study lasting 3.5 yrs, rats were given treated water. Animals used for pathological study, including pregnant dams, were selected at half-yearly intervals after 1.5 yrs. | No changes due to Methenamine were observed in test animals, fetuses, or placentae.<br>Neoplasms were observed in 3 of 48 animals in the high dose group. | Malorny, 1966 |

| *Concentration* | *Number and species of animal* | *Method* | *Results and comments* | *Reference* |
|---|---|---|---|---|
| 400, 800, or 1600 mg/kg Methenamine in feed | 10 male and 10 female rats (strain unspecified) | Rats ate treated feed for 2 yrs. The 10 pairs were mated at age 20, 28, and 35 weeks. | No differences were observed in growth rate, survival, reproduction, offspring viability, or pathological lesions between the treated and control groups. | Berglund, 1966 |
| 1250–1875 mg/kg Methenamine in feed or 125–375 mg/kg formaldehyde in feed | 2 male and 4 female mongrel dogs fed Methenamine<br>4 male and 4 female mongrel dogs fed formaldehyde | Animals ate treated feed for 32 mos. 3 male and 2 female offspring from the treated groups and 2 male and 2 female control offspring were kept. Methenamine offspring ate 1250 mg/kg Methenamine in feed and formaldehyde offspring ate 375 mg/kg formaldehyde in feed. | No differences in feed consumption, growth, reproduction, litter number, or litter size were observed between the treated and control groups. No differences were found during monthly observations of blood chemistry, cell total and differential counts, or urinalysis. Of the 30 litters in the Methenamine-treated group, 66.7% were noted as unusual due to the presence of stillborn and cannibalized animals; 5 animals had malformations. In the formaldehyde treated group, 60% of the 34 litters had stillborn pups, and 10 pups had malformations. In the control group, 7.7% of the 16 litters had stillborn pups; no malformations were observed. | Kewitz, 1966 |

were mated. No significant difference was observed in fertility. In both groups, 16 male and 16 female offspring were chosen and fed the same diet as their parents at the time of weaning. Their body weights were taken at the age of 7 and 15 weeks. When approximately 6 weeks old, they were tested for voluntary muscular activity over a period of 11 days; again the method of Wulff Rasmussen (1953, 1957), as in the chronic diet study, was used. When the offspring were 123 days old, half were randomly selected for necropsy. No significant differences were observed in body weight, mean voluntary activity, or relative organ weight between the treated and control groups. The only gross observation made was that of a yellow coloration of the hair around the perineum of some rats.

Two weeks before being mated, two groups of Wistar rats, 12 females and six males per group, were given either 1% Methenamine in their drinking water or untreated water (Della Porta et al., 1970). The females received treated water during pregnancy and lactation. Twelve treated females and 11 control females had litters; no malformations were observed. From the pups that were born, 24 of each sex were given 1% Methenamine in drinking water until 20 weeks of age. Up to week 9 of age for males and week 20 of age for females, the body weights of the treated animals were significantly lower than the controls. At the termination of treatment, both groups were killed and no differences were observed between the treated and control groups in respect to organ weight and gross and microscopic alterations.

Twelve female rats were given 1% Methenamine in their drinking water and mated with males which were also treated (Della Porta, 1966). Another group of 12 females were untreated and served as controls. No diferences were observed between the treated and control groups in regard to fertility, litter size, or lactation. A group of 24 males and 24 females was selected from the litters of the treatment group and was treated for 20 weeks. The control group consisted of 24 males and 24 females selected from the control litters. No differences in body weight, growth, or appearance of organs were observed.

Groups of 80, 80, and 245 rats were given 0, 5, and 50 mg/kg/day Methenamine in their drinking water in a five generation study that lasted 3.5 years (Malorny, 1966). Animals to be used for study of lesions, including pregnant dams, were selected from each group at half-yearly intervals starting at 1.5 years. From that time on, no lesions due to Methenamine were found in the test animals, fetuses, or placentae. Neoplasms were observed in three of 48 animals in the high-dose group.

Groups of rats, 10 males and 10 females per group, were fed diet containing 0, 400, 800, or 1600 mg/kg Methenamine for 2 years (Berglund, 1966). The ten pairs were mated at the age of 20, 28, and 35 weeks. No differences were observed in growth rate, survival, reproduction, offspring viability, or lesions between treated and control groups.

Two male and four female mongrel dogs were fed 1250–1875 mg/kg Methenamine in their feed, four male and female mongrels were fed 125–375 mg/kg formaldehyde in their feed, and two males and one female were fed untreated diet and served as controls (Kewitz, 1966). The test groups ate treated feed for 32 months. Three male and two female offspring from the Methenamine and formaldehyde groups' litters and two males and two females from the control group's litter were kept for testing. The Methenamine offspring received 1250 mg/kg Methenamine and the formaldehyde offspring received 375 mg/kg formaldehyde in their feed. The animals were fed for 22 months. At the termination of dosing, the test animals were fed control diet and the controls were fed the 1250 mg/kg Methenamine diet for 1 year.

No effects due to either Methenamine or formaldehyde were produced on feed consumption, growth, reproduction, litter number, or litter weights. Also, no differences were found during monthly observations of hematological and blood chemical values and urinalysis. Of the 30 liters in the Methenamine-treated group, approximately 20 had a few stillborn and cannibalized pups, and 5 pups were born with abnormalities. Of the 34 litters in the formaldehyde group, 60% were considered unusual because of stillborn pups, and 10 pups had abnormalities. In the control group, 7.7% of the 16 litters were considered unusual because of stillborn pups; no malformations were observed.

## MUTAGENICITY

### *In Vitro*

Results of the mutagenicity studies are summarized in Table 4.

An Ames test was performed on Methenamine using *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 in the presence of S9 (Crebelli et al., 1985). A dose range of 200–5000 μg/plate was used. Triplicate plates were used; the test was repeated at least once. Dimethylsulfoxide was used as the solvent; 2-aminoanthracene, sodium azide, and 4-nitro-*o*-phenylenediamine were used as positive controls. No mutagenic effects were produced by Methenamine.

An *S. typhimurium* reversion test using strains TA98 and TA100 was performed using Methenamine (Crebelli et al., 1984). An Ames test was carried out with and without metabolic activation. Preincubation at 37°C for 20 min was used with metabolic activation; this step was to improve the sensitivity to mutagenic nitrosamines. Methenamine was tested at doses of 0, 0.2, 1, and 5 mg/plate. All tests were repeated at least once. Sodium azide, 4-nitro-*o*-phenylenediamine, and 2-aminoanthracene were used as positive controls. A nitrosation reaction was carried out following the method described by De Flora and De Flora (1981). Following *in vitro* nitrosation, Methenamine was tested with and without S9 at concentrations of 0, 0.01, 0.025, 0.05, and 0.075 mg/plate. Before nitrosation, negative results were obtained for both strains with and without metabolic activation. After nitrosation, Methenamine was mutagenic toward strain TA98 with metabolic activation at concentrations of 0.025, 0.05, and 0.075 mg/plate and at concentrations of 0.05 and 0.075 mg/plate toward strain TA100.

An Ames test was performed with the *Salmonella*/microsome assay using *S. typhimurium* strains TA98 and TA100 (Orstavik and Hongslo, 1984). The test was performed with and without S9 metabolic activation. The positive control was benzo(a)pyrene with S9 and 1-nitropyrene without S9. AH26, a powder composed of 25% Methenamine, 10% silver powder, 60% bismuth oxide, 5% titanium dioxide, and an epoxy-bis-phenol resin, was the material tested. Mutagenic activity toward strain TA100, but not toward TA98, was found. The mutagenic activity was dose-dependent and occurred to a greater extent without metabolic activation. The test was repeated and similar results were obtained. The individual components of AH26 were tested for mutagenicity and only the epoxy-*bis*-phenol resin had mutagenic activity and it followed the same characteristics of mutagenicity observed with the AH26 formulation.

A *Salmonella*/mammalian–microsome mutagenicity test was used to assay for the mutagenic potential of Methenamine (Andrews et al., 1979). An Ames test was

**Table 4.** Mutagenicity Studies Using Methenamine

| *Test* | *Organism* | *Strain* | *Method* | *Results* | *Reference* |
|---|---|---|---|---|---|
| Ames Test | *Salmonella typhimurium* | TA1535, TA98, TA1537, TA100 | Concentrations of 200–5000 µg/plate were tested with S9. Triplicate plates were used; the tests were repeated. Positive and negative controls were used. | Negative | Crebelli et al., 1985 |
| Reversion Test | *S. typhimurium* | TA98, TA100 | Ames test performed with and without metabolic activation at concentrations of 0, 0.2, 1, and 5 mg/plate. Plates were preincubated in presence of S9. All tests were repeated. Following *in vitro* nitrosation, concentrations used were 0, 0.01, 0.025, 0.05, and 0.075 mg/plate with and without metabolic activation. Positive controls were used. | Negative before nitrosation. After nitrosation, positive in the presence of S9 at 0.025, 0.05, and 0.075 mg/plate with TA98 and at 0.05 and 0.075 mg/plate with TA100. | Crebelli et al., 1984 |
| *Salmonella*/Microsome Assay | *S. typhimurium* | TA98, TA100 | Ames test performed with and without metabolic activation testing AH26, which contained 25% Methenamine. The tests were run twice. The individual components were also assayed. Positive controls were used. | AH26 had mutagenic activity with TA100, but not with TA98. Activity was dose-dependent and occurred to a greater extent without S9. When components were tested individually, Methenamine did not have mutagenic activity. | Orstavik and Hongslo, 1984 |
| *Salmonella*/mammalian-microsome mutagenicity test | *S. typhimurium* | TA98, TA1535, TA100, TA1537, TA1538 | Ames test was performed with and without metabolic activation. Tested using 1000 µg in both plate and liquid preincubation tests. After nitrosation with nitrite in acetic acid, 0.1 ml of resuspended reaction mixture was diluted to 10% and assayed for mutagenicity. | Prior to nitrosation, negative. After nitrosation, mutagenic with TA98 with and without metabolic activation. | Andrews et al., 1980 |

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|---|---|---|---|---|---|
| *In Vivo* | *Drosophila melanogaster* | | | Mutagen in *Drosophila*. According to Nafei et al. (1964), mutations only occur in the larval spermatocytes and the conditions necessary for change exist only for a short time. Stumm-Tegethoff (1964) demonstrated the causative agent to be formic acid, an impurity of formaldehyde. | Auerbach, 1951 |
| Urinary Mutagenicity Assay | *S. typhimurium* | TA1535, TA98, TA100 | Urine samples were taken from 72 workmen, 44 who smoked, in a tire plant where Methenamine was used. 23 clerks, 16 who smoked, were used as controls. Urine concentrations were incubated with 100 μg/ml of *Escherichia coli* β-glucuronidase and then prepared for absorption onto XAD-2 resin via the method of Yamasaki and Ames (1977). An Ames test and microtitre fluctuation test were performed using S9. Concentrates equivalent to 10 ml of urine were used. A positive control was used. | Results of Ames testing indicated only weak mutagenic activity. No significant difference was observed between exposed and unexposed employees. An excess of mutagenic samples were observed when workmen who smoked were compared to clerks that smoked. Smokers had a highly significant excess of mutagenic urine concentrates when compared to non-smokers. Results from the microtitre fluctuation tests were the same as the Ames test results. | Crebelli et al., 1985 |

performed with and without metabolic activation using *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100. Methenamine was tested at a concentration of 1000 μg in both plate and liquid preincubation tests; it was not mutagenic. Methenamine was then treated with nitrite in acetic acid and 0.1 ml of resuspended reaction mixture was diluted to 10% and assayed for mutagenicity. Methenamine was mutagenic toward TA98 with and without metabolic activation.

### *In Vivo*

Methenamine acted as a mutagen in a test using *Drosophila melanogaster* (Auerbach, 1951). Stumm-Tegethoff (1964) demonstrated the causative agent of the mutagenic effect was formic acid, which was present as an impurity in formaldehyde. According to Nafei and Auerbach (1964), the mutations only occur in the larval spermatocytes and that the conditions necessary for change exist for only a short time.

### *In Vivo/In Vitro*

Urine samples were collected from 72 workmen, 44 of whom smoked, who worked in a tire plant where methenamine was a workplace pollutant (Crebelli et al., 1985). Twenty-three clerks, 16 of whom smoked, were used as controls. The urine concentrates were incubated overnight with 100 μg/ml of *Escherichia coli* β-glucuronidase and then prepared by absorption on to XAD-2 resin following the methods of Yamasaki and Ames (1977). An Ames test and a microtitre fluctuation test were carried out with S9 using *S. typhimurium* strains TA1535, TA98, and TA100. The amounts of concentrate used were equivalent to 10 ml of urine. The positive control was 2-aminoanthracene.

The results obtained from the Ames test were those of a weak mutagen. No significant difference was observed when exposed employees were compared with unexposed employees. When workmen who smoked were compared to control clerks who smoked, an excess of mutagenic samples were observed among the workmen; this difference was not statistically significant. Smokers had a highly significant excess of urine concentrates which were mutagenic when compared to non-smokers. In the microtitre fluctuation test, no significant excess of mutagenic samples was found when comparing the workmen to the controls.

## CARCINOGENICITY

Results of the carcinogenicity studies are summarized in Table 5.

A transformation assay using mycoplasma-free neonatal hamster kidney cells (BHK cells) was used to screen for carcinogenic potential of Methenamine (Plesner and Hansen, 1983). The assay was performed following the methods of Styles (1977). Sterile distilled water served as both the solvent and negative control; cyclophosphamide was used as the positive control. The test was performed with metabolic activation; however, the toxicity and transforming activity of Methenamine was not influenced by metabolic activation. In initial experiments, a concentration dose range of 0.025–250 μg/ml Methenamine was used. A dose-dependent increase in the number of transformed colonies and a negligible toxic effect was observed. The concentration range was then increased to 1–10,000 μg/ml. When the test cultures were compared to the

**Table 5.** Carcinogenicity Studies Using Methenamine

| *Concentration* | *Number and species of animal* | *Method* | *Results and comments* | *Reference* |
|---|---|---|---|---|
| 0.025–250 μg/ml Methenamine the concentration was increased to 1–10,000 μg/ml | Mycoplasma-free neonatal hamster kidney cells | A transformation assay was performed with S9 by the method of Styles (1977). Negative and positive controls were used. | Initially, a dose-dependent increase in the number of transformed colonies and a negligible toxic effect was observed. At higher concentrations, a dose-dependent and significant increase in the number of transformations was observed; transforming activity was observed at a nontoxic or very weakly toxic dose. When test cultures were compared to non-exposed cultures, 80% of the cells survived the maximum concentration. | Plesner and Hansen, 1983 |
| 0.1% Methenamine in drinking water or 0.1% Methenamine with 0.2% sodium nitrite in drinking water | 15 male and 15 female Sprague-Dawley rats per group | 5 days/wk, 60 ml of solutions were given to each cage, 3 rats/cage; tap water was given on the other 2 days. Animals were treated for 50 wks and kept until they died or were killed due to moribund condition. Each rat received a total of 5 g Methenamine over 50 wks. Rats given sodium nitrite received a total of 10 g of nitrite over 50 wks. A complete necropsy and histopathologic examination was performed. | No significant difference was observed in survival between any of the groups. No neoplasms were induced due to Methenamine alone or in combination with sodium nitrite. | Lijinsky and Taylor, 1977 |

**TABLE 5.** CARCINOGENICITY STUDIES USING METHENAMINE (*CONTINUED*)

| *Concentration* | *Number and species of animal* | *Method* | *Results and comments* | *Reference* |
|---|---|---|---|---|
| 1% or 2% Methenamine in drinking water | Rats (strain unspecified)<br>$F_1$ gen.: 13 males & 13 females<br>$F_2$ gen.: 15 males & 11 females<br>$F_3$ gen.: 12 males & 12 females<br>Offspring from 2% Methenamine-treated parents: 16 males & 16 females (no. of parents not specified) | For 3 successive generations, rats were given 1% Methenamine-treated water. The $F_1$ and $F_2$ generations were treated up to 40 wks and the $F_3$ generation was treated up to 20 wks. Offspring taken from parents given water treated with 2% Methenamine received 2% Methenamine-treated water for 59 wks. All groups were observed for 2 yrs. | No evidence of carcinogenicity was found for any group. | Della Porta et al., 1970 |
| Orally:<br>0.5%, 1.0%, or 5.0% Methenamine in drinking water or<br>Subcutaneously:<br>5 g/kg Methenamine | 0.5% Oral: mice<br>50 male & 50 female CTM<br>1.0% Oral: mice<br>96 male & 102 female CTM<br>29 male & 27 female SWR<br>49 male & 44 female C3Hf<br>rats:<br>48 male & 48 female Wistar<br>5.0% Oral: mice<br>29 male & 50 female CTM<br>rats:<br>12 male & 12 female Wistar<br>Subcutaneous: mice<br>39 male & 44 female CTM<br>rats:<br>20 male & 20 female Wistar | Oral Treatment Duration:<br>60 wks — 0.5% and 1.0% CTM mice, SWR mice, C3Hf mice<br>30 wks — 5.0% CTM mice<br>104 wks — 1.0% Wistar rats<br>2 wks — 5.0% Wistar rats<br>Subcutaneous Treatment:<br>Methenamine administered as a 30% solution in water dosed on 5 alternate days for a total of 25 g/kg. Dosing started at day 10 of age.<br>After dose termination, all mice were observed up to 110–130 weeks of age. Rats in the 1% oral group were observed up to | A yellow discoloration of the coats of treated rats was observed; this was not observed for mice. 5% Methenamine caused 50% mortality in rats within 2 wks of treatment initiation; surviving rats recovered rapidly and had no lasting effects. A slight reduction in growth rate and survival was observed for CTM mice given 5% Methenamine in water. SWR mice in the 1% Methenamine group had a slight retardation of growth. No evidence of carcinogenic activity was seen in any group. | Della Porta et al., 1968 |

| | | | | |
|---|---|---|---|---|
| | | 3 yrs and rats in the 5.0% oral and the subcutaneous group were up to 2 yrs. Gross and histopathological observations were made. Animals dying before 20 wks of age, with 2 exceptions, and 15 other animals were excluded when calculating neoplasm incidence. | | |
| 1% Methenamine in feed or 0.15% formaldehyde in feed | 30 male and 30 female NMRI albino mice per group | Animals ate treated feed for 2 yrs. | 20 neoplasms were found in the Methenamine-treated group, 12 in the formaldehyde group, and 11 in the controls. With the exception of 1 control and 2 Methenamine-treated males, all neoplasms occurred in females. | Kewitz, 1966 |
| 0.1%, 0.5%, or 1.0% Methenamine in feed | 50 female mice per group (strain unspecified) | Because increased neoplasm incidence due to Methenamine could not be ruled out in the previous study, a second study was conducted. Animals ate treated feed for 31 wks. | No difference in neoplasm incidence was observed among groups. | Kewitz, 1966 |
| 10% Methenamine in chloroform or 1.5% aqueous formaldehyde or chloroform | 13 mice per group (sex and strain unspecified) | Dermal application of test article for 300 days. | No malignant neoplasms were observed in any group. | Kewitz, 1966 |

non-exposed cultures, 80% of the cells survived the maximum concentration. A dose-dependent and significant increase in the number of transformations was observed; transforming activity was observed at a nontoxic or a very weakly toxic concentration.

Methenamine was administered in water to two groups of Sprague-Dawley rats, 15 males and 15 females per group (Lijinsky and Taylor, 1977). Each group received either 0.1% Methenamine in water with 0.2% sodium nitrite added or 0.1% Methenamine alone. The total dose of Methenamine per rat was 5 g over a 50 week period for each group. The group with nitrite added received a total of 10 g of nitrite over a 50 week period. For 5 days/week, 60 ml of solution was given to each cage, three rats per cage; on the other 2 days tap water was given. The historical control group consisted of 26 males and 30 females which were given 0.2% sodium nitrite solution for 2 years in a previous study by Taylor and Lijinsky (1975). Animals were treated for 50 weeks and then kept until they died or were terminated when moribund. Necropsy and histopathologic evaluation were performed on each animal. No significant difference was observed in survival rate between any of the groups. No neoplasms were induced due to Methenamine alone or in combination with sodium nitrite.

For three successive generations, rats, strain unspecified, received 1% Methenamine in their drinking water (Della Porta et al., 1970). The $F_1$ and $F_2$ generations were treated up to 40 weeks of age and the $F_3$ generation was treated up to 20 weeks of age. The $F_1$ group consisted of 13 males and seven females, the $F_2$ group consisted of 15 males and 11 females, and the $F_3$ group consisted of 12 males and 12 females. One group of test animals was treated with 2% Methenamine; 16 male and 16 female offspring were taken from this group and also given 2% Methenamine for a period of 59 weeks. A group of 48 males and 48 females served as a control group. All groups were observed for 2 years and no evidence of carcinogenicity was found in any of the Methenamine-treated groups.

To assess the carcinogenic potential of Methenamine, outbred 10-week-old CTM mice, inbred 5-week-old C3Hf/Dp mice, inbred 7-week-old SWR/Dp mice and outbred 10-week-old Wistar rats were used in an oral study and 10-day-old CTM mice and Wistar rats were used in a subcutaneous study (Della Porta et al., 1968).

In the oral study, Methenamine was administered in the drinking water, *ad libitum*. The CTM mice groups were as follows: 99 males and 100 females served as controls and received untreated drinking water, 50 males and 50 females received 0.5% Methenamine in their water, 96 males and 102 females received 1.0% Methenamine, and 29 males and 50 females received 5.0% Methenamine in their water. The 0.5% and 1.0% Methenamine groups were treated for 60 weeks and the 5.0% group was treated for 30 weeks. The SWR mouse controls consisted of 45 males and 30 females. The treated SWR mouse group consisted of 29 males and 27 females which received 1.0% Methenamine in their water for 60 weeks. Thirty male and 63 female C3Hf mice received untreated water. Forty-nine male and 44 female C3Hf mice received 1.0% Methenamine for 60 weeks. Three groups of Wistar rats were used: 48 males and 48 females served as controls, 48 males and 48 females received 1.0% Methenamine in their water, and 12 males and 12 females received 5.0% Methenamine. The 1.0% group was treated for 104 weeks and the 5.0% group was treated for 2 weeks.

The subcutaneous portion of the study used 39 male and 44 female 10-day-old CTM mice and 20 male and 20 female Wistar rats. Both the rats and mice received a 5 g/kg dose of a 30% solution of Methenamine in water on 5 alternate days. Dosing started on day 10 of age. Daily observations were made and body weights were taken

every 2 weeks; observations may have been made more frequently at the beginning of the study.

After the termination of treatment, mice were observed up to 110–130 weeks of age in both studies. Rats were observed from up to 3 years in the control and 1% group, of the oral study, and for up to 2 years in the other oral test groups and the subcutaneous group. Necropsy was performed on all animals that died on study or were killed at termination. Tissues and lesions taken at necropsy were evaluated microscopically. Animals dying before the age of 20 weeks, with the exception of an 18-week-old mouse and a 17-week-old rat with lymphosarcoma, were excluded from the effective number used for the calculation of neoplasm incidence. Also excluded were 11 mice and four rats that died on study and were lost due to either cannibalism or decomposition.

A yellow discoloration of the coats of treated rats were observed; this was not observed in the mice. The SWR mice that received 1% Methenamine orally had a slight retardation of growth. Oral administration of 5% Methenamine resulted in a slight reduction in growth rate and survival for CTM mice; however, it caused a 50% mortality in rats within 2 weeks of daily treatment. Rats from the 5% oral group that did not die recovered rapidly and did not have any lasting ill effects. Water intake was comparable between the treated and test groups.

No significant difference was found in neoplasm incidence between treated and control mice. For rats, the incidence was essentially the same in the control and 1% groups, which were kept for up to 3 years; neoplasm incidence was lower in the 5% oral group and the subcutaneous group, which were kept for up to 2 years. The percentage of neoplasm-free animals were greater in the treated groups than in the controls. No evidence of carcinogenic activity by Methenamine was found in any groups of this study.

Three groups of NMRI/Han albino mice, 30 males and 30 females per group, were fed either control feed, feed treated with 1% Methenamine, or feed treated with 0.15% formaldehyde for two years (Kewitz, 1966). Twenty neoplasms were found in the Methenamine group, 12 in the formaldehyde group, and 11 in the control group. With the exception of one control male and two males that received Methenamine, all neoplasms occurred in females. Based on these data, the author concluded that an increased neoplasm incidence attributable to Methenamine could not be ruled out and a second study was conducted. Groups of 50 female mice were administered Methenamine at concentrations of 0, 0.1, 0.5, and 1% in diet. After 31 weeks, no difference in neoplasm incidence was observed between the groups.

Three groups of mice, 13 per group, received dermal applications of either chloroform, 10% Methenamine in chloroform, or 1.5% aqueous formaldehyde (Kewitz, 1966). The animals were dosed for 300 days. No malignant neoplasms were observed in any group.

## CLINICAL ASSESSMENT OF SAFETY

### Sensitization

Methenamine is a known sensitizer; skin irritation may develop from contact with the chemical, its vapors, or its solutions (DuPont, 1972). In previously sensitized individuals, inhalation of Methenamine may cause an asthma-like condition (Kabe, 1971).

In several instances, people who developed allergic contact dermatitis due to formaldehyde in antiperspirants experienced persisting axillary dermatitis due to the use of rubber dress shields that contained Methenamine (Fisher, 1978).

Some patients sensitized by external exposure to formaldehyde may develop an eczematous contact-type dermatitis medicamentosa by ingesting medicines that contain Methenamine (Sulzberger, 1940).

## Irritation/Sensitization

A maximization test, following the method of Kligman and Epstein (1975), was conducted to determine the contact-sensitizing potential of a mascara which contained 0.1% Methenamine (Ivy Research Laboratories, Inc., 1980). A pretest for irritation potential of the test material was conducted using 25 subjects to determine whether sodium lauryl sulfate pre-treatment would be necessary. An occlusive patch of test material was applied to the volar aspect of the forearm for 48 h and the material was non-irritating. The maximization test was performed using 25 adults, 8 men and 17 women.

An occlusive patch, to which 0.3 g of test material was applied, was covered with a 15 mm aluminum chamber and applied to the volar aspect of the forearm. The patch was applied to the same site for five 48 h periods. Sodium lauryl sulfate, 1%, was used during the induction because the test material was found to be nonirritating during the pretest. A challenge was performed following a 10-day non-treatment period. An occlusive patch was applied to a new site for 48 h using the same procedure as before. A 5% aqueous solution of sodium lauryl sulfate was used. Observations were made upon removal of the patch and after 24 h. Neither irritation during induction nor sensitization following the challenge was observed for any of the subjects.

## Case Report

A case study was reported in which a worker involved in a core molding process developed itchy eruptions on his hands, neck, and shoulders which were aggravated by sweating (Hayakawa et al., 1988). Open patch testing was performed by applying acetoaldehyde, acetone, benzene, *n*-hexane, *o*-xylene, *p*-xylene, and toluene to the inner side of the upper arm for 20 min. Negative results were obtained for all of the mentioned chemicals. In closed patch tests, chemicals were applied for 48 h and readings were taken 1 h and 24 h after removal. Only Methenamine produced a positive reaction with erythema and papules. A questionable positive result was observed at the 48 h reading with 2% aqueous formaldehyde; this site was negative at the 72 h reading.

A control patch test was performed on nine colleagues using 1% Methenamine in petrolatum; negative results were obtained for all nine. Eight months later a retest was performed using Methenamine and formaldehyde and negative results were obtained with 2% aqueous formaldehyde. Positive controls for formaldehyde were performed using formaldehyde-sensitized patients; a two plus positive reaction was obtained.

## SUMMARY

Methenamine is an organic amine that occurs as colorless or white crystals, granules, or powder. Methenamine is a cosmetic biocide and is used primarily in eye

make-up preparations; it is also used as a preservative in lotions and creams. In 1989, it was reported to the FDA that Methenamine was used in seven ingredient formulations. It is used in mascara and other eye make-up preparations and face, body, and hand skin care preparations, excluding shaving preparations, at concentrations of less than 1%. A non-cosmetic application of Methenamine is its use as an urinary tract anti-infective drug.

Methenamine, following oral administration, undergoes hydrolysis and generates formaldehyde; the hydrolysis takes place rapidly at relatively low pH. It is from the released formaldehyde that antimicrobial activity is derived. Methenamine is rapidly absorbed from the intestinal tract and excreted mostly unchanged in the urine. Methenamine can pass the placenta and is detectable in amniotic fluid and in milk. Methenamine has a renal clearance that is slightly lower than creatinine and it has a plasma half-life of 4 h. In one study, the renal clearance was approximately 1.46 ml/min/kg and the total clearance was approximately 1.59 ml/min/kg; no Methenamine accumulated.

A single oral and i.v. dose of 10 g/kg and single oral dose of 20 g/kg Methenamine did not cause mortality in rats. An i.v. $LD_{50}$ for rats was 9200 mg/kg Methenamine. An i.p. $LD_{50}$ for 1.0 M Methenamine buffered in 0.3 M glucose to pH 6.0, with 0.0135 M phosphate was 50 ml/kg. The subcutaneous $LD_{50}$ for rats was 200 mg/kg.

In a short-term diet toxicity study, 5.0 g/kg did not produce any toxic effects in mice. A short-term dermal toxicity test using aqueous Methenamine at a concentration of 0.20% did not produce any toxic effects in rabbits. In a subchronic toxicity test, no signs of toxicity were observed; the only clinical sign was a citrus-yellow discoloration of the hair. No toxicity was observed in chronic dietary studies using cats or rats; the only clinical observation was a yellow staining of the hairs of the perineum of some rats.

Methenamine was slightly irritating to the skin of rabbits. In ocular studies, Methenamine was, at most, mildly irritating. Methenamine is a known sensitizer. AH26, which is 25% methenamine, was rated a strong or potent sensitizer. However, in a guinea pig maximization test, a 0.20% solution of Methenamine did not produce any erythema or edema.

In a number of teratologic and reproductive studies, no teratogenic effects attributable to methenamine were observed.

Methenamine did not have any mutagenic potential in a number of *in vitro* mutagenicity assays; however, following nitrosation, mutagenic activity was observed. Methenamine was a mutagen in *Drosophila melanogaster*.

In six carcinogenicity studies, Methenamine did not show any carcinogenic activity. Methenamine together with nitrite in the drinking water of test animals did not induce a significantly different incidence rate of neoplasms when compared with control animals.

In a maximization test with 25 panelists using sodium lauryl sulfate, a mascara containing 0.1% Methenamine was nonirritating and did not cause sensitization.

## DISCUSSION

The CIR Expert Panel based their conclusion for Methenamine, in part, on the fact that Methenamine decomposes to ammonia and formaldehyde. Formaldehyde was previously reviewed by CIR (Elder, 1984) and it was concluded by the Panel that the maximum concentration of formaldehyde considered safe for cosmetic use was 0.2%.

Methenamine was approved for cosmetic use at a concentration not to exceed 0.3% so that the released formaldehyde concentration would not exceed 0.2% in formulation. An additional restriction on Methenamine is that it should not be used in products intended to be aerosolized since it was not concluded that formaldehyde is safe in aerosolized products.

## CONCLUSION

On the basis of the animal and clinical data presented in this report, the CIR Expert Panel concludes that Methenamine is safe for cosmetic use at concentrations not to exceed 0.16% in formulation. It cannot be concluded that Methenamine is safe for use in cosmetic products intended to be aerosolized.

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