SULFAMETHAZINE AND ITS SODIUM SALT

1. Exposure Data

1.1   Chemical and physical data

1.1.1   *Nomenclature*

Sulfamethazine

Chem. Abstr. Serv. Reg. No.: 57-68-1
Chem. Abstr. Name: 4-Amino-*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide
IUPAC Systematic Name: N^1-(4,6-dimethyl-2-pyrimidinyl)sulfanilamide
Synonyms: 2-(4-Aminobenzenesulfonamido)-4,6-dimethylpyrimidine; 2-(*para*-aminobenzenesulfonamido)-4,6-dimethylpyrimidine; 4-amino-*N*-(2,6-dimethyl-4-pyrimidinyl)benzenesulfonamide; 4,6-dimethyl-2-sulfanilamidopyrimidine; sulfa-dimethylpyridine; 2-sulfanilamido-4,6-dimethylpyrimidine; sulfadimidine; sulfa-midine; sulphadimethylpyrimidine; sulphadimidine
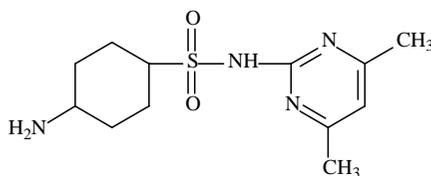
Sodium sulfamethazine

Chem. Abstr. Serv. Reg. No.: 1981-58-4
Chem. Abstr. Name: 4-Amino-*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide, monosodium salt
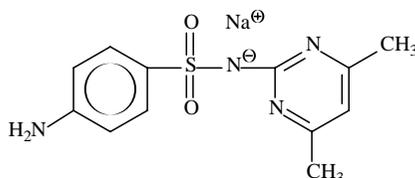*IUPAC Systematic Name*: N^1-(4,6-dimethyl-2-pyrimidinyl)sulfanilamide, mono-sodium salt
Synonyms: Sodium sulfadimidine; sulfadimidine sodium; sulfamethazine sodium; sulfamethazine sodium salt

1.1.2   *Structural and molecular formulae and relative molecular mass*

$C_{12}H_{14}N_4O_2S$ Relative molecular mass: 278.33

$C_{12}H_{13}NaN_4O_2S$ Relative molecular mass: 300.31

1.1.3 *Chemical and physical properties of the pure substance (sulfamethazine)*

(*a*) *Description*: Pale-yellow crystals (Lide & Milne, 1996)

(*b*) *Melting-point*: 198.5 °C (Lide & Milne, 1996)

(*c*) *Spectroscopy data*: Infrared [prism (423), grating (15044)], ultraviolet, nuclear magnetic resonance [proton (6671), C-13 (4417)] and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996).

(*d*) *Solubility*: Slightly soluble in water (1.5 g/L at 29 °C), acids and alkali; solubility increases rapidly with an increase in pH (Lide & Milne, 1996; Budavari, 2000)

(*e*) *Dissociation constants*: pK_1, 7.4, pK_2, 2.7 (Papapstephanou & Frantz, 1978)

1.1.4 *Technical products and impurities*

Trade names for sulfamethazine include A 502, Azolmetazin, BN 2409, Calfspan, Cremomethazine, Diazil, Diazyl, Dimezathine, Kelametazine, Mermeth, Neasina, Neazina, Pirmazin, S-Dimidine, Spanbolet, Sulfadimerazine, Sulfadimesin, Sulfadimesine, Sulfadimethyldiazine, Sulfadimethylpyrimidine, Sulfadimezin, Sulfadimezine, Sulfadimidin, Sulfadimidine, Sulfadine, Sulfamethiazine, Sulfodimesin, Sulfodimezine, Sulmet, Sulphadimethylpyrimidine, Sulphadimidine, Sulphamethasine, Sulphamethazine, Sulphamezathine, Sulphamidine, Sulphodimezine, Superseptil, Superseptyl and Vertolan.

Trade names for sulfamethazine sodium include Bovibol, Sulmet and Vesadin.

1.1.5 *Analysis*

Several international pharmacopoeias specify infrared absorption spectrophotometry with comparison to standards and thin-layer chromatography as the methods for identifying sulfamethazine; electrometric titration with sodium nitrite is used to assay its purity. In pharmaceutical preparations, sulfamethazine is identified by infrared absorption spectrophotometry and high-performance liquid chromatography (HPLC) with ultraviolet detection; HPLC with ultraviolet detection and electrometric titration with sodium nitrite are used to assay for sulfamethazine content (British Pharmacopoeia Commission, 1993; Council of Europe, 1997; US Pharmacopeial Convention,, 1999).

The British Pharmacopoeia specifies infrared absorption spectrophotometry with comparison to standards as the method for identifying sodium sulfamethazine; electrometric titration with sodium nitrite is used to assay its purity (British Pharmacopoeia Commission, 1993).

Methods for the analysis of sulfamethazine in animal fluids (milk, plasma, urine) and tissues (muscle, organs), eggs, bee honey, animal feeds, meat-based baby food and animal wastewater have been reported. The methods include colorimetry, biosensor immunoassay, enzyme-linked immunosorbent assay, microbiological diffusion assay, microtitre plate assay, gas chromatography with positive chemical-ionization mass spectrometry, atomic emission, electron-capture or flame-ionization detection, thin-layer chromatography, high-performance thin-layer chromatography with spectro-densitometric detection, liquid chromatography with tandem mass or thermospray spectrometry or fluorimetric detection, reverse-phase liquid chromatography, gas–liquid chromatography, HPLC with chemiluminescence, fluorescence, fluorimetric, photo-diode array or ultraviolet detection (Cieri, 1976; Belliardo, 1981; Holder *et al*., 1981; Munns & Roybal, 1982; Schwartz, 1982; Jonas *et al*., 1983; Petz, 1983; Stout *et al*., 1984; McGary, 1986; Smallidge *et al*., 1988; Weber & Smedley, 1989; Agarwal, 1990; Kruzik *et al*., 1990; Larocque *et al*., 1990; Park & Lee, 1990; Takatsuki & Kikuchi, 1990; Carignan & Carrier, 1991; Diserens *et al*., 1991; Hoffmeister *et al*., 1991; Van Poucke *et al.*, 1991; Mineo *et al.*, 1992; Weber & Smedley, 1993; Sekiguchi *et al*., 1994; Boison & Keng, 1995; Strebel & Schneider, 1995; Tsai *et al*., 1995; Casetta et al., 1996; Martínez García & Holzwarth, 1996; Nishimura *et al*., 1996; Edder *et al*., 1997; Le Boulaire *et al*., 1997; Chiavarino *et al*., 1998; Jen *et al*., 1998; Lynas *et al*., 1998; Martínez García *et al*., 1998; Nishimura *et al*., 1998; Petkov & Gechev, 1998a,b; Tsai *et al*., 1998; Watanabe *et al*., 1998; Baxter *et al*., 1999; Fránek *et al*., 1999; Gaudin & Pavy, 1999; Park, 1999; Reeves, 1999; Shaikh *et al*., 1999; Sugama *et al*., 1999; Yang, 1999; Bartolucci *et al*., 2000; Buick *et al*., 2000; Stoev & Michailova, 2000).

1.2   Production

Sulfamethazine can be prepared by reacting acetylsulfanilyl chloride with 2-amino-4,6-dimethylpyrimidine suspended in dry pyridine or in acetone and pyridine, followed by alkaline hydrolysis of the 2-(N^4-acetylsulfanilamido)-4,6-dimethylpyrimidine; the resulting salt is neutralized with SO_2. The 2-amino-4,6-dimethylpyrimidine is prepared by condensing acetylacetone with guanidine carbonate in toluene. It can also be prepared by condensation of equimolar amounts of sulfanilylguanidine and acetyl-acetone directly. It is precipitated in the presence of water in the form of very pale-yellow crystals (Papastephanou & Frantz, 1978; Gennaro, 1995).

Information available in 2000 indicated that sulfamethazine was manufactured by 23 companies in China, two companies in India and one company each Egypt, Mexico and Spain, while sulfamethazine sodium was manufactured by four companies in

China and one company each in Egypt, Mexico and Spain (CIS Information Services, 2000a).

Information available in 2000 indicated that sulfamethazine was formulated as a pharmaceutical by 26 companies in Italy, 20 companies in India, 13 companies in Canada, 12 companies in the USA, 11 companies in Mexico, seven companies each in Argentina and the United Kingdom, five companies in South Africa, four companies in China, three companies each in Australia, Egypt and New Zealand, two companies each in Colombia and Spain and one company each in the Czech Republic, Germany, Greece, Hungary, Ireland, the Islamic Republic of Iran, Malta, Peru, Poland, Taiwan and Thailand. The same source indicated that sulfamethazine sodium was formulated as a pharmaceutical by 14 companies in Canada, eight companies in Mexico, five companies each in Colombia, the United Kingdom and the USA, four companies each in Italy and South Africa, three companies each in Australia, Austria and the Netherlands, two companies in Argentina and one company each in Belgium, France, India, Ireland, the Islamic Republic of Iran, Peru, Spain, Switzerland and Turkey (CIS Information Services, 2000b).

1.3   Use

Sulfamethazine is a sulfonamide used to treat a variety of bacterial diseases in humans and other species. It has been used since the late 1950s to treat respiratory disease and promote growth in food-producing animals (cattle, sheep, pigs and poultry). It is a short-acting sulfonamide drug with similar properties to those of sulfamethoxazole (see monograph in this volume). There is currently no single-entity dosage form of sulfamethazine, and it is used only in combinations. It has been used with trimethoprim and with other sulfa drugs, particularly sulfadiazine and sulfamerazine. The sulfamethazine sodium salt may be given orally (2 g initial dose followed by 0.1–1.0 g every 6–8 h) or parenterally. The usual adult dose of a combination with equal amounts of sulfadiazine and sulfamerazine (trisulfapyrimidines) has been 6–8 g/day (Gennaro, 1995; WHO, 1995; American Hospital Formulary Service, 1997; Food & Drug Administration, 1988; Royal Pharmaceutical Society of Great Britain, 2000).

1.4   Occurrence

1.4.1   *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 2000), about 17 000 workers, including 12 400 food production workers, 4000 agricultural service workers (2000 veterinarians) and 700 health service workers, were potentially exposed to sulfamethazine in the USA.

1.4.2   *Environmental occurrence*

No data were available to the Working Group.

1.5   Regulations and guidelines

Sulfamethazine is listed in the pharmacopoeias of China, the Czech Republic, France, Germany and the USA and in the European and International pharmacopoeias (Council of Europe, 1997; US Pharmacopeial Convention, 1999; Royal Pharmaceutical Society of Great Britain, 2000; Swiss Pharmaceutical Society, 2000), and sodium sulfamethazine is listed in the pharmacopoeias of Austria, the Czech Republic and the United Kingdom and in the International Pharmacopoeia (Royal Pharmaceutical Society of Great Britain, 2000; Swiss Pharmaceutical Society, 2000).

In 1994, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an acceptable daily intake (ADI) of 0–50 µg/kg bw. The Codex Committee on Food Additives and Contaminants recommended maximum residue limits (MRLs) for sulfamethazine of 100 µg/kg in muscle, liver, kidney and fat of cattle, sheep, pigs and poultry; and 25 µg/L in milk. Sulfamethazine should not be used in laying hens, and an MRL was not recommended for eggs (WHO, 1995).

2.   Studies of Cancer in Humans

No data were available to the Working Group.

3.   Studies of Cancer in Experimental Animals

3.1   Oral administration

Mouse: Groups of 96 male and 96 female B6C3F$_1$ mice, 3–4 weeks of age, were fed diets containing sulfamethazine (purity, 97–99%) at 300, 600, 1200, 2400 or 4800 mg/kg for 24 months, while 192 males and 192 female controls received basal diet. Additional groups of 24 male and 24 female mice were included for necropsy at 12 and 18 months. No deaths occurred. A statistically significant ($p < 0.001$) increase in the incidence of follicular-cell adenomas of the thyroid gland was observed in mice at the highest dietary concentration killed after 24 months. The incidences were 2/184, 0/95, 1/92, 4/88, 4/94 and 31/93 for males, and 5/180, 1/91, 1/93, 0/95, 2/94 and 23/89 for females in the controls and at the five concentrations, respectively. One male at 2400 mg/kg of diet and one female each at 600 and 4800 mg/kg had one follicular-cell carcinoma. Diffuse and focal thyroid follicular-cell hyperplasia was also observed at the three highest concentrations in males and at the two highest concentrations in females.

Marginally significant but inconsistent, non-dose-related increases in the incidence of hepatocellular tumours were also reported in female mice (Littlefield *et al*., 1989). [The Working Group analysed the data for liver tumours by Fisher's exact test for differences between groups and a Cochrane-Armitage test for trend and found no statistically significant increase in liver tumour incidence.]

Rat: Groups of 90 male and 90 female Fischer 344 rats were fed diets containing sulfamethazine (purity > 99%) at a concentration of 10, 40, 600, 1200 or 2400 mg/kg from weaning for 24 months. The rats were derived from parents fed diets containing the same concentration of sulfamethazine for at least 80 days before mating. At weaning, the offspring were allocated to different groups so that littermates were housed separately. A group of 180 male and 180 female controls received basal diet, and additional groups of 15 males and 15 females were included for necropsy at 3, 12 and 18 months. After 2 years on the study, the mortality rate of the female controls was approximately 41%, while the rates were 36, 35, 26, 19 and 19% at the five concentrations of sulfamethazine, respectively. The corresponding figures in males were 37% for controls and 24–28% for the treated groups. One male at 1200 mg/kg and one at 2400 mg/kg that were killed at 12 months had a follicular-cell adenoma of the thyroid gland. The incidences of combined thyroid gland follicular-cell adenomas and adenocarcinomas at 24 months were 0, 2, 0, 5, 5 and 11% (0/180, 2/87, 0/90, 4/88, 4/83 and 10/87) in males and 4, 0, 1, 5, 10 and 9% (6/170, 0/90, 1/85, 4/84, 9/87 and 8/88) in females in controls and at the five dietary concentrations, respectively. The corresponding incidences of follicular-cell adenocarcinomas were 0/180, 2/87, 0/90, 2/88, 2/83 and 7/87 for males and 1/170, 0/90, 0/85, 0/84, 6/87 and 6/88 for females. The differences in the incidence of thyroid neoplasia were statistically significant at the two higher doses in both males and females when compared with controls ($p < 0.05$). There were no other treatment-related neoplasms. Thyroid follicular-cell hyperplasia, described as focal, multifocal or diffuse, was observed at the three higher doses (Littlefield *et al*., 1990).

4.   Other Data Relevant to an Evaluation of Carcinogenicity and Its Mechanisms

4.1   Absorption, distribution, metabolism and excretion

4.1.1   *Humans*

Sulfamethazine acetylation phenotypes were determined in 19 healthy adults (aged 17–46 years; 15 men, four women; nine white, nine oriental, one black) given a single oral dose of 20 mg/kg bw sulfamethazine in 200 mL of water. The results showed a well-defined trimodal pattern for acetylation clearance and for overall elimination or metabolic rate constants and confirmed that the fast acetylator phenotype can be subdivided

into intermediate and rapid acetylator groups. The average acetylation clearance rate for rapid acetylators (1.34 mL/min per kg bw) was 8.8 times the estimated clearance for slow acetylators (0.15 mL/min per kg bw) and 1.8 times that for intermediate acetylators (0.75 mL/min per kg bw). The average percentage of an absorbed dose excreted as acetylsulfamethazine in 72-h urine was 93.7 for rapid acetylators, 87.7 for intermediate acetylators and 65.6 for slow acetylators (Chapron *et al.*, 1980). A trimodal distribution of sulfamethazine acetylator phenotypes was also indicated by measurements of the percentage acetylation of sulfamethazine in plasma samples obtained from 49 persons in South India 6 h after ingestion of a single dose of 10 mg/kg bw (51% slow, 12% intermediate, 37% rapid acetylators) (Peters *et al.*, 1975).

After 10 male and two female healthy volunteers were given oral doses of sulfamethazine of 12–17 mg/kg bw, 10–20% of the dose was excreted in the urine as free and conjugated hydroxylated metabolites and 61–81% as N^4-acetylsulfamethazine. Six of the individuals were considered to be fast acetylators and six slow acetylators. The plasma concentration–time curve for sulfamethazine in the fast acetylators was biphasic, with half-times of 1.7 and 5.4 h, respectively, whereas in the slow acetylators it was monophasic, with a half-time of 7.6 h (Vree *et al.*, 1986).

Five healthy men received an oral dose of 10 or 40 mg/kg bw sulfamethazine approximately 14 days apart in a non-randomized cross-over study. Non-linear kinetics was determined at the high dose, as a dose-dependent decrease in absorption rate was observed in some subjects, whereas apparent metabolic clearance decreased with increasing dose in all subjects (Du Souich *et al.*, 1979).

4.1.2 *Experimental systems*

Suspensions of parenchymal, but not non-parenchymal cells, isolated from male Wistar rats metabolized sulfamethazine by acetylation and other pathways (Mørland & Olsen, 1977; Olsen & Mørland, 1981). Hydroxylation of sulfamethazine in isolated hepatocytes from male Wistar rats was significantly greater than in hepatocytes from female or castrated male rats. Acetylation activity was higher in females than males (Van't Klooster *et al.*, 1993).

After a single intravenous administration of 20 mg/kg bw sulfamethazine to seven male and eight female Wistar rats, male rats showed faster clearance from the plasma than females and excreted larger amounts of the hydroxy metabolites and smaller amounts of its N^4-acetylated metabolite (Witkamp *et al.*, 1992). Two phenotypes of sulfamethazine acetylation in rats — a high and a low percentage of acetylsulfamethazine in urine — were described in females, but not males, of five inbred strains and two random-bred stocks (Zídek & Janku, 1976).

4.1.3   *Comparison of animals and humans*

Sulfamethazine is metabolized similarly in animals and humans, with N^4-acetylation dominating. A trimodal pattern of sulfamethazine acetylation is seen in humans. Differences in acetylation rates were observed between male and female rats and among females of different strains.

4.2   **Toxic effects**

4.2.1   *Humans*

The toxic side-effects of sulfamethazine are expected to be similar to those of other sulfonamides, which include disorders of the haematopoietic system and hypersensitivity reactions.

4.2.2   *Experimental systems*

Sulfamethazine inhibited the iodination of tyrosine catalysed by porcine thyroid peroxidase in a reversible manner; the approximate median inhibitory concentration was 0.5 mmol/L, and the K_i was 0.42 mmol/L (Doerge & Decker, 1994).

Groups of 12 male and female B6C3F$_1$ mice and Fischer 344 rats were fed either a control diet or a diet containing 300, 600, 1200, 2400 or 3600 mg/kg sulfamethazine for 90 days. In the mice, no treatment-related lesions were seen grossly or by light microscopy. Thyroid gland enlargement was seen in one of 24 rats fed the diet containing 2400 mg/kg and in 12 of 24 rats at the highest dietary concentration. Thyroid gland hyperplasia was evident in all treated rats but was more pronounced and occurred at a higher incidence in rats at the higher concentrations (Heath & Littlefield, 1984a,b).

In B6C3F$_1$ mice that received diets containing sulfamethazine at a concentration of 0, 300, 600, 1200, 2400 or 4800 mg/kg for 24 months, non-neoplastic dose-related lesions were observed in both males and females, including follicular-cell hyperplasia (diffuse and focal) of the thyroid gland (Littlefield *et al.*, 1989).

Fischer 344 rats received diets containing sulfamethazine at a concentration of 0, 10, 40, 600, 1200 or 2400 mg/kg for 24 months, and interim sacrifices were carried out after 3, 12 and 18 months. The incidences of non-neoplastic lesions of the thyroid gland were significantly higher among treated animals than among controls and included follicular-cell hyperplasia, follicular cellular change and multilocular cysts (Littlefield *et al.*, 1990).

Groups of 120 male and 120 female Fischer 344 rats were fed diets containing 10, 40, 600, 1200 or 2400 mg/kg sulfamethazine. The control group consisted of 210 males and 210 females. Serum samples were analysed for concentrations of thyroid-stimulating hormone (TSH), total thyroxine, total triiodothyronine and triiodothyronine uptake after 12, 18 or 24 months of continuous exposure. There was no statistically significant difference in triiodothyronine concentration or percentage uptake in animals

of either sex after any length of exposure. Serum TSH concentrations were not statistically significantly altered, although there was a trend for increasing concentrations in animals receiving 600 mg/kg of diet or more (703 ± 206 ng/100 mL with 2400 mg/kg, 575 ± 133 ng/100 mL in controls at 12 months; 420 ± 184 ng/100 mL with 2400 mg/kg, 217 ± 81 ng/100 mL in controls at 18 months). At each sacrifice time, rats at 1200 and 2400 mg/kg of diet had significantly heavier thyroid glands than controls (Fullerton *et al.*, 1987). [The Working Group noted that any change in thyroid hormone homeostasis occurring before 12 months would not have been revealed in this study. The high, dose-related increase in the variance of the values for TSH was also noted.]

Groups of 15 Sprague-Dawley CR/CD rats [sex not specified] were fed diets containing 0, 20, 40, 80, 160, 400, 800, 1600, 3300, 8000 or 12 000 mg/kg sulfamethazine for 4 weeks. This range of concentrations spanned the doses that induced thyroid tumours in rodents. A characteristic log dose–response relationship was observed in thyroid weight increases, decreased serum thyroxine concentration, decreased serum triiodothyronine concentration and increased serum TSH concentration. There were no significant effects at low concentrations, but a sharp, relatively linear rise was seen at higher concentrations: thyroid weights increased and serum thyroxine and triiodothyronine concentrations decreased at ≥ 3300 mg/kg of diet, and the serum TSH concentration increased at ≥ 1600 mg/kg of diet. All the morphological changes seen in the thyroid gland were reversible after withdrawal of sulfamethazine treatment. Supplemental dietary administration of thyroid hormone completely inhibited the functional and morphological changes observed with sulfamethazine at concentrations that normalized but did not suppress TSH. Further, no detectable effects on the thyroid gland were observed in hypophysectomized rats treated with sulfamethazine [experimental details not given]. *In vitro*, sulfamethazine did not increase cell proliferation in FRTL-5 cells in the absence of TSH [experimental details not given]. No effect on thyroid gland function was observed in cynomolgus monkeys (*Macaca fascicularis*) at doses of up to 300 mg/kg bw per day for 13 weeks [no further experimental details given] (McClain, 1995).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

Heinonen *et al.* (1977) reported no increase in malformation rates in the offspring of 47 women treated with sulfamethazine during the first four lunar months of pregnancy.

4.3.2 *Experimental systems*

The effect of sulfamethazine on fertility was assessed in three groups of 20 male and 20 female Swiss CD-1 mice given diets containing the drug at 0.25, 0.5 or 1.0% (equivalent to 0, 313, 625 and 1250 mg/kg bw per day) and compared with a control group of 38 males and 38 females. The mice were exposed to sulfamethazine continuously

during the 7-day pre-mating and 98-day co-habitation periods. At the conclusion of this phase of the study, cross-over matings were performed with the parental mice, consisting of control male × control female; high-dose (1% sulfamethazine) male × control female; control male × high-dose female. The effects observed in the F_0 group receiving 1% sulfamethazine included significant decreases in the number of litters produced and in the number of live pups per litter and a significant increase in the proportion of live male pups per total live pups per litter. No significant difference was found in the percentage of motile sperm, sperm concentration or percentage of abnormal sperm in the cauda epididymis in the group fed 1% sulfamethazine versus the control group. The cross-over part of the study showed that fertility was affected in animals of each sex, the average number of live pups per litter being significantly decreased. No treatment-related histopathological effects were observed in the pituitary or reproductive organs of male or female mice in the group fed 1% sulfamethazine. Exposure of mice to 0.25 or 0.5% sulfamethazine in the diet during the continuous breeding phase of the study had no effect on fertility or reproductive performance (Reel *et al.*, 1992).

4.4   Effects on enzyme induction or inhibition and gene expression

4.4.1   *Humans*

No data were available to the Working Group.

4.4.2   *Experimental systems*

Intraperitoneal administration of various doses of sulfamethazine to adult male Wistar rats for 3 or 5 days and to Hubbard chickens for 3 days significantly increased the electron transport components (rats only) and the activities of aminopyrine *N*-demethylase and aniline hydroxylase at a dose of 150 mg/kg bw. A dose of 300 mg/kg bw produced a significant decrease in cytochrome P450 content and in the activity of aminopyrine *N*-demethylase in the rats and of aniline hydroxylase in the chickens. Administration of sulfamethazine to young male rats resulted in significant induction of electron transport components and drug-metabolizing enzymes at both 150 and 300 mg/kg bw. However, treatment of old rats produced significant decreases in electron transport components and aminopyrine *N*-demethylase activity at both doses. A significant increase in electron transport components was observed with 150 mg/kg bw sulfamethazine in female rats. These studies suggest that sulfamethazine is a substrate of the mixed-function oxidase system, and induction is dependent on the dose and on the age and sex of the animals. Intraperitoneal administration of a single dose of 300 mg/kg bw sulfamethazine to rats pretreated with intraperitoneal doses of 80 mg/kg bw per day phenobarbital for 3 days decreased microsomal protein, electron transport components and drug-metabolizing enzyme activities to a greater extent than phenobarbital alone (Kodam & Govindwar, 1995; Kodam *et al.*, 1996; Kodam & Govindwar, 1997).

4.5   Genetic and related effects

4.5.1   *Humans*

No data were available to the Working Group.

4.5.2   *Experimental systems* (see Table 1 for references)

Sulfamethazine did not induce mutations in *Salmonella typhimurium* and it did not induce unscheduled DNA synthesis in human fibroblasts in culture. [The Working Group was aware of data from three different laboratoires, showing that sulfamethazine (*a*) did not induce gene mutation at the *Hprt* locus (at concentrations up to 7 µg/mL) or chromosomal aberrations (at up to 5000 µg/mL) in Chinese hamster ovary cells in the absence or presence of exogenous metabolic activation, (*b*) gave rise to sister chromatid exchange in these cells (at concentrations up to 1500 µg/mL) in the absence but not in the presence of an exogenous metabolic activation systems, and (*c*) did not induce chromosomal aberrations in bone-marrow cells of rats treated with a single oral dose of 3000 mg/kg bw (WHO, 1994).]

4.6   Mechanistic considerations

Sulfamethazine is considered not to be genotoxic, since it did not induce mutagenicity in bacterial or mammalian cells *in vitro* and was not clastogenic in mammalian cells *in vitro* or *in vivo.*

The available data indicate that thyroid hormone imbalance plays a role in the development of follicular-cell neoplasia caused by sulfamethazine in rats and mice. The drug altered thyroid hormone homeostasis in rats treated with doses spanning the range that induced thyroid tumours in this species, and it produced thyroid gland enlargement (goitre) in rats and follicular-cell hypertrophy and hyperplasia in rats and mice. The mechanism is based on reversible inhibition of thyroid peroxidase, as with other sulfonamides.

On the basis of this information, which meets the criteria laid out in the IARC consensus report (Capen *et al.*, 1999), sulfamethazine would be expected not to be carcinogenic to humans exposed to concentrations that do not lead to alterations in thyroid hormone homeostasis. In addition, no effects on thyroid gland function were found in cynomolgus monkeys treated with sulfamethazine.

The hyperplasia induced by sulfamethazine in the thyroid gland is diffuse, in analogy with the morphological changes induced by TSH stimulation, rather than only multifocal, as would be induced by a genotoxic thyroid carcinogen (Hard, 1998). Further support for the absence of an effect of sulfamethazine in primates comes from a study by Takayama *et al.* (1986), who examined species differences between male Sprague-Dawley rats and male squirrel monkeys (*Saimiri sciureus*) treated with sulfamonomethoxine, a prototype goitrogenic sulfonamide. Whereas sulfamonomethoxine

Table 1. Genetic and related effects of sulfamethazine

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella thyphimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	333 µg/plate	Mortelmans *et al.* (1986)
Unscheduled DNA synthesis, human fibroblasts	–	NT	100	Allred *et al.* (1982)

[a] –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL

decreased the serum triiodothyronine concentration, increased the serum TSH concentration and caused thyroid follicular-cell hypertrophy and hyperplasia with accompanying thyroid enlargement in the rats, no such changes were seen in the monkeys. The median inhibitory concentration for sulfamethazine on thyroid peroxidase isolated from rats was 2.2×10^{-7} mol/L, whereas that for monkeys was $> 10^{-4}$ mol/L.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Sulfamethazine is a sulfonamide drug that has been used to treat bacterial diseases in human and veterinary medicine and to promote growth in cattle, sheep, pigs and poultry.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Sulfamethazine was tested by oral administration in one study in mice and in one study in rats that included exposure *in utero*. It produced thyroid follicular-cell adenomas in mice and follicular-cell adenomas and carcinomas in rats. No statistically significant increase was seen in the incidence of tumours at other sites in mice or rats.

5.4 Other relevant data

Sulfamethazine shows a trimodal pattern of polymorphic acetylation in humans. It caused thyroid gland enlargement (goitre) in rats and diffuse hypertrophy and hyperplasia in rats and mice. Administration of sulfamethazine to rats under bioassay conditions that caused tumours resulted in alteration of thyroid hormone homeostasis, including increased secretion of thyroid-stimulating hormone and morphological changes in the thyroid consistent with this increase. The underlying mechanism for these changes is reversible inhibition of thyroid peroxidase activity. A study in which cynomolgus monkeys were given sulfamethazine did not result in alterations in thyroid gland function.

In a continuous breeding study in mice, sulfamethazine reduced fertility in both males and females but did not change sperm parameters.

No data were available on the genetic and related effects of sulfamethazine in humans. The compound did not induce chromosomal aberrations in bone-marrow cells of rats treated *in vivo* or in Chinese hamster cells. It did induce sister chromatid

exchange in Chinese hamster cells in the absence but not in the presence of an exogenous metabolic system in one experiment. It did not induce DNA damage or mutations in mammalian cells *in vitro* or in bacteria. Sulfamethazine is considered not to be genotoxic *in vitro* or *in vivo*.

5.5   Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of sulfamethazine.
There is *sufficient evidence* in experimental animals for the carcinogenicity of sulfamethazine.

Overall evaluation

Sulfamethazine is *not classifiable as to its carcinogenicity to humans (Group 3).*
Sulfamethazine produces thyroid tumours in mice and rats by a non-genotoxic mechanism, which involves inhibition of thyroid peroxidase resulting in alterations in thyroid hormone concentrations and increased secretion of thyroid-stimulating hormone. Consequently, sulfamethazine would be expected not to be carcinogenic to humans exposed to doses that do not alter thyroid hormone homeostasis.
Evidence from epidemiological studies and from toxicological studies in experimental animals provide compelling evidence that rodents are substantially more sensitive than humans to the development of thyroid tumours in response to thyroid hormone imbalance.

6.   References

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