SPIRONOLACTONE

This substance was considered by previous working groups, in 1980 (IARC, 1980) and 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

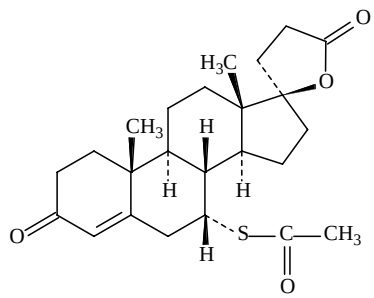
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 52-01-7
Chem. Abstr. Name: (7α,17α)-7-(Acetylthio)-17-hydroxy-3-oxopregn-4-ene-21-carboxylic acid, γ-lactone
IUPAC Systematic Name: 17-Hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone, acetate
Synonym: 3-(3-Oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl)propionic acid, γ-lactone

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{24}H_{32}O_4S$ Relative molecular mass: 416.58

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Light, cream-coloured to light-tan crystalline powder (Gennaro, 1995)

(*b*) *Melting-point*: 198–207 °C, with decomposition; occasionally shows preliminary melting at about 135 °C, followed by resolidification (US Pharmacopeial Convention, 1999)

(*c*) *Spectroscopy data*: Infrared, ultraviolet, nuclear magnetic resonance and mass spectral data have been reported (Sutter & Lau, 1975).

(*d*) *Solubility*: Practically insoluble in water; soluble in chloroform, ethanol and most organic solvents; slightly soluble in fixed oils (Gennaro, 1995; Budavari, 2000)

(*e*) *Optical rotation*: $[\alpha]_D^{20}$, –33.5° (chloroform) (Budavari, 1998)

1.1.4 *Technical products and impurities*

Spironolactone is available as 25-, 50- and 100-mg tablets (Gennaro, 1995).

Trade names for spironolactone include Aldace, Aldactone, Aldopur, Altex, Almatol, Aquareduct, Deverol, Diatensec, Dira, Duraspiron, Euteberol, Lacalmin, Lacdene, Laractone, Nefurofan, Osiren, Osyrol, Sagisal, SC-9420, Sincomen, Spiresis, Spiretic, Spiridon, Spiroctan, Spiroderm, Spirolactone, Spirolang, Spirolone, Spirone, Spiro-Tablinen, Supra-Puren, Suracton, Uractone, Urusonin, Verospiron, Verospirone and Xenalon.

1.1.5 *Analysis*

Several international pharmacopoeias specify infrared and ultraviolet absorption spectrophotometry with comparison to standards and thin-layer chromatography as the methods for identifying spironolactone; ultraviolet absorption spectrophotometry and high-pressure liquid chromatography (HPLC) with ultraviolet detection are used to assay its purity. In pharmaceutical preparations, spironolactone is identified by infrared absorption spectrophotometry and thin-layer chromatography; ultraviolet absorption spectrophotometry and HPLC with ultraviolet detection are used to assay for spironolactone content (British Pharmacopoeia Commission, 1993; Society of Japanese Pharmacopoeia, 1996; Council of Europe, 1997; US Pharmacopeial Convention, 1999).

1.2 Production

Spironolactone can be prepared by treating dehydroepiandrosterone (prepared from cholesterol or sitosterol) with acetylene to form the 17α-ethynyl-17β-hydroxy derivative, which is carbonated to the 17α-propiolic acid. Reduction of the unsaturated acid in alkaline solution yields the saturated acid, which cyclizes to the lactone on acidification. Bromination to the 5,6-dibromo compound, followed by oxidation of the

3-hydroxyl group to the ketone and dehydrobromination to the 7α-hydroxyl derivative, produces spironolactone when esterified with thiolacetic acid (Gennaro, 1995).

Information available in 2000 indicated that spironolactone was manufactured by two companies each in China and Germany and by one company each in France, Hong Kong, Hungary, Italy and the USA (CIS Information Services, 2000a).

Information available in 2000 indicated that spironolactone was manufactured and/or formulated as a pharmaceutical by 13 companies in Germany and Japan, nine companies in France, eight companies each in Austria, Italy and Switzerland, seven companies in the United Kingdom, six companies in India, five companies each in Spain, Sweden and the USA, three companies each in South Africa and Taiwan, two companies each in Argentina, Australia, Brazil, Chile, Colombia, Greece, the Islamic Republic of Iran, the Philippines, Poland, Portugal, Singapore and Thailand and one company each in Bulgaria, Canada, Denmark, Hungary, Indonesia, Ireland, Israel, Mexico, Norway, Peru, the Russian Federation, Turkey and Venezuela (CIS Information Services, 2000b).

1.3 Use

Spironolactone is a potassium-sparing diuretic used mainly in the treatment of oedema and hypertension. It is used in particular in the treatment of primary hyperaldosteronism (e.g. associated with adrenal adenomas or bilateral adrenal hyperplasia) and in the treatment of refractory oedema associated with secondary aldosteronism (cardiac failure, hepatic cirrhosis, nephrotic syndrome, severe ascites).

Spironolactone is a synthetic steroid that acts as a competitive antagonist of the potent endogenous mineral–corticosteroid aldosterone. It has a slower onset of action than triamterene or amiloride, but its natriuretic effect is slightly greater during long-term therapy. By blocking the sodium-retaining effects of aldosterone on the distal convoluted tubule, it corrects one of the most important mechanisms responsible for the production of oedema, but spironolactone is effective only in the presence of aldosterone. It is a relatively weak diuretic and usually is used as an adjunct to other diuretics, such as the thiazides. When used in this combined manner, it enhances the excretion of sodium and decreases the excretion of potassium. Further increase in diuresis may be obtained by the use of a glucocorticoid with this drug in combination with another diuretic. Minor uses include the treatment of hirsutism in women with polycystic ovary syndrome or idiopathic hirsutism, and in controlling acne or other defects of familial precocious puberty (Gennaro, 1995; Hardman *et al.*, 1996; American Hospital Formulary Service, 2000; Royal Pharmaceutical Society of Great Britain, 2000).

The usual adult oral dose of spironolactone is 25 mg four times a day; the usual range of doses is 25–200 mg/day. The usual paediatric oral dose of spironolactone is 3.3 mg/kg bw per day in divided doses. If a satisfactory diuretic effect is not achieved within 5 days, a thiazide diuretic is added to the regimen (Gennaro, 1995).

Spironolactone ranked 94th out of the 200 generic drugs most commonly sold by prescription in the USA in 1999 (Anon., 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 900 pharmacists in the USA were potentially exposed to spironolactone.

1.4.2 *Environmental occurrence*

No data were available to the Working Group.

1.5 Regulations and guidelines

Spironolactone is listed in the pharmacopoeias of China, the Czech Republic, France, Germany, Italy, Japan, Poland, the United Kingdom and the USA and in the European and International pharmacopoeias (Society of Japanese Pharmacopoeia, 1996; Royal Pharmaceutical Society of Great Britain, 2000; Swiss Pharmaceutical Society, 2000). It is registered for human use in Finland, Ireland, the Netherlands, Norway, Portugal, Spain and Sweden (Instituto Nacional de Farmacia e do Medicamento, 2000; Irish Medicines Board, 2000; Medical Products Agency, 2000; Medicines Evaluation Board Agency, 2000; National Agency for Medicines, 2000; Norwegian Medicinal Depot, 2000; Spanish Medicines Agency, 2000).

2. Studies of Cancer in Humans

2.1 Case reports

Stierer *et al*. (1990) described 15 cases of cancer of the breast in men treated between 1972 and 1988 in Vienna, Austria. Seven of the patients (47%) had used spironolactone.

2.2 Cohort studies

Danielson *et al*. (1982) evaluated the relation between breast cancers and use of selected non-estrogenic drugs among 283 000 members of the Group Health Cooperative of Puget Sound in Washington State, USA. The information on the 302 new cases of breast cancer diagnosed between 1977 and 1980 in women aged 35–74 years was linked with an automated file of all drugs dispensed to individual members

since July 1976. On the basis of 184 438 women-years of observation, the crude rate of breast cancer in non-users of spironolactone (1.6/1000) was compared with that in users (0/1000), to give an age-adjusted relative risk of 0.0 (90% confidence interval [CI], 0.0–3.0). There was a total of 634 women–years of exposure to spironolactone.

Three publications have summarized the findings at screening during follow-up periods of up to 7 years (Friedman & Ury, 1980), up to 9 years (Friedman & Ury, 1983) and up to 15 years (Selby *et al*., 1989) of a cohort of 143 574 members of the Kaiser Permanente Medical Care Program, whose computerized pharmacy records were available from 1969 to 1973. As of December 1984, 1 370 000 person–years of follow-up had been accumulated, and 68 695 persons (48% of the original cohort) remained active members of the Program (Selby *et al.*, 1989). A total of 1475 users of spironolactone were identified, and 155 cancer cases were observed versus 127.4 expected (standardized morbidity ratio [SIR], 1.22; $p < 0.05$). Of the SIRs for 54 specific cancer sites and two combinations of sites examined, the only one that was significantly elevated was for 'pharynx unspecified' (20.5; $p < 0.01$; two cases observed, 0.1 expected).

2.3 Case–control studies

Ron *et al*. (1987) carried out a case–control study of 159 cases of thyroid cancer and 285 population controls between 1978 and 1980 in Connecticut, USA. Use of diuretics drugs was not associated with thyroid cancer. Use of spironolactone was reported by two patients and one control (relative risk, 4.3; 95% CI, 0.3–120).

Mellemgaard *et al*. (1994) conducted a study of 368 cases of renal-cell carcinoma and 396 population controls in Denmark between 1989 and 1992. The response rates were 76% among cases and 79% among controls. The relative risks for use of potassium-sparing diuretics (including agents such as spironolactone) were 2.2 (95% CI, 0.4–13) in men and 0.8 (95% CI, 0.3–2.2) in women, after adjustment for hypertension.

Another population-based case–control study on renal-cell cancer (Weinman *et al*., 1994) (206 cases and 292 controls) was carried out among members of the Kaiser Permanente Northwest Plan in the USA between 1980 and 1991 (men) or 1960 and 1991 (women). Data on potassium-sparing drugs were abstracted from medical records, and 46 users were identified among cases and 49 among control subjects, yielding a relative risk of 2.1 (95% CI, 1.3–3.6). There was no trend in risk with duration of use. According to the authors, it was impossible to disentangle the effect of potassium-sparing diuretics from that of other anti-hypertensive medications or from the effect of hypertension.

McLaughlin *et al*. (1995) studied 1732 patients with renal-cell cancer and 2309 non-hospital controls in five countries who were interviewed between 1989 and 1991. The response rates were 72% and 75% among cases and controls, respectively. The relative risks for use of potassium-sparing diuretics, after adjustment for hypertension, were 1.2 (95% CI, 0.7–1.8), 1.0 (95% CI, 0.6–1.6), 1.0 (95% CI, 0.6–1.7) and 0.6 (95% CI, 0.3–1.2) across four increasing levels of life-time consumption.

Yuan *et al*. (1998) conducted a case–control study on regular use of 58 diuretic and hypertensive drugs among 1204 cases of renal-cell carcinoma and 1204 neighbourhood controls in Los Angeles, USA, between 1986 and 1994 . There was no evidence that potassium-sparing diuretics were associated with renal-cell carcinoma among normotensive subjects (relative risk for heavy use versus no use, 1.1; 95% CI, 0.1–8.5). Hypertension was a strong risk factor, with a relative risk of 2.2 (95% CI, 1.8–2.6). Among subjects with hypertension, the relative risks for light and heavy use of potassium-sparing diuretics were 2.6 (1.5-4.3) and 1.8 (1.1-3.0) in comparison with subjects with no hypertension and no use of these drugs.

Shapiro *et al*. (1999) studied 238 cases of renal-cell carcinoma and 616 controls in Washington State, USA, between 1980 and 1995. The use of anti-hypertensive drugs was not associated with risk after adjustment for hypertension. Any use of potassium-sparing diuretics was associated with a relative risk of 2.0 (95% CI, 1.0-4.0) in men and 0.9 (0.4-1.7) in women, with no clear dose–response relationship. The relative risks after adjustment for hypertension were not presented.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Rat: Groups of 36 male and 36 female Sprague-Dawley rats, 7 weeks of age, received diets providing a dose of 50, 150 or 500 mg/kg bw per day spironolactone for 78 weeks. Groups of 72 males and 72 females served as untreated controls. In a second experiment reported by the same authors, groups of 30 male and 30 female rats received diets providing a dose of 0 (control), 10, 30 or 100 mg/kg bw per day of spironolactone for 104 weeks. Both studies were terminated at the end of treatment. In the 78-week study, an excess of thyroid adenomas in both males and females and of interstitial-cell adenomas in the testis was observed at the two higher doses. The incidences of thyroid tumours in controls and at the three doses were 0/59, 4/33, 15/31 and 18/28 for males and 1/62, 1/31, 12/34 and 13/34 for females, respectively. Interstitial (Leydig-cell) tumours of the testis occurred in 0/72, 0/36, 5/36 and 12/36 males at the same doses, respectively. No increased tumour incidence was seen at these organ sites in the 104-week study at doses up to 100 mg/kg bw per day (Lumb *et al*., 1978). [The Working Group noted inconsistencies in the effective numbers for different organ sites.]

3.2 Administration with known carcinogens

Rat: In an initial experiment, 70 female Sprague-Dawley rats, approximately 50 days of age (weight, 150–180 g), received a single dose of 40 mg 7,12-dimethylbenz[*a*]-anthracene (DMBA) dissolved in 2 mL of corn oil by oral gavage. Of these rats, 20 also

received spironolactone (pharmaceutical-grade) at a dose of 100 mg/kg bw in 1 mL of distilled water by oral gavage twice daily for 7 days, starting 4 days before DMBA administration. The study was terminated 150 days after DMBA treatment, and the mammary tumour incidence determined by palpation. The incidence of palpable mammary tumours was reduced from 21/24 in the group receiving DMBA alone to 3/14 in that given DMBA plus spironolactone. In a second experiment, 80 female Sprague-Dawley rats received an intravenous injection into the jugular vein of 2 mg of DMBA in 0.4 mL of oil emulsion once daily on days 1, 4 and 7. Two days before the first DMBA injection, 40 of these rats received spironolactone (pharmaceutical-grade) at a dose of 100 mg/kg bw in 1 mL of distilled water by oral gavage twice daily for 12 consecutive days. On termination of the study 147 days after the start of DMBA treatment, mammary tumours were found at necropsy in 32/32 rats receiving DMBA alone and 23/36 rats receiving DMBA plus spironolactone ($p < 0.001$) (Kovacs & Somogyi, 1970).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

At the time spironolactone was introduced for clinical use, its bioavailability was inadequate, and this was improved by preparing the drug in finely powdered or micronized form. The absolute bioavailability was indirectly estimated at approximately 73%, which was enhanced in the presence of food. Nearly all absorbed spironolactone (> 90 %) is bound to plasma proteins and, with repeated dosing, a steady state is achieved within 8 days. After oral intake of a 100-mg dose, the plasma half-time of spironolactone was 1–2 h, the time to maximum plasma concentration was 2–3.2 h, the maximum blood concentration was 92–148 ng/mL, the area under the concentration–time (0–24 h) curve was 1430–1541 ng/mL per h and the elimination half-time was 18–20 h (Overdiek & Merkus, 1987).

Spironolactone is rapidly and extensively metabolized to compounds that are excreted in the urine and faeces. It undergoes enterohepatic recirculation, but no unchanged drug appears in urine or faeces (Sadée *et al*., 1973, 1974a; Karim *et al*., 1976a,b; Overdiek *et al*., 1985; Overdiek & Merkus, 1987; Gardiner *et al*., 1989).

The metabolites of spironolactone can be divided into two main groups: those in which the sulfur moiety is retained and those in which the sulfur is removed by dethioacetylation. For many years, it was thought that the dethioacetylated metabolite, canrenone, was the major metabolite; however, with more specific analytical methods such as HPLC, 7α-thiomethylspirolactone was recognized as the major metabolite of spironolactone (Overdiek *et al*., 1985; Gardiner *et al*., 1989). This metabolite is

formed by hydrolysis of the thioacetate group to form 7α-thiospirolactone (as an intermediate), followed by *S*-methylation to 7α-thiomethylspirolactone. This can then be hydroxylated to form 6β-hydroxy-7α-thiomethylspirolactone and oxidized to form 7α-methylsulfinyl- and 7α-methylsulfonylspirolactone or via sulfoxidation to form 6β-hydroxy-7α-methylsulfinyl- and 6β-hydroxy-7α-methylsulfonylspirolactone.

For formation of the group of metabolites in which sulfur is removed, 7α-thiomethylspirolactone is also dethioacetylated to canrenone, which is further metabolized by three pathways: hydrolysis of the γ-lactone ring to form canrenoate, which is excreted in the urine as a glucuronic ester, and, next, hydroxylation to form 15α-hydroxy-canrenone or reduction to produce several di-, tetra- and hexa-hydro derivatives. Canrenone and canrenoate are in equilibrium with one another.

Not only spironolactone but several of its metabolites have biological activity; in decreasing order of potency, these are 7α-thiospirolactone, 7α-thiomethylspirolactone and canrenone.

4.1.2 *Experimental systems*

The disposition of [^{14}C]spironolactone was studied in male rats, female dogs and female monkeys after intravenous or oral administration of 5 mg/kg bw. Gastrointestinal absorption was estimated to be 82% in rats, 62% in dogs and 103% in monkeys. Spironolactone was extensively metabolized in all three species, and the metabolites were excreted primarily in the urine and faeces. The amount of radiolabel excreted in urine or faeces of all three species was similar after intravenous and after oral dosing. In monkeys, as in humans, the amounts excreted in urine and faeces were about equal, while faecal excretion predominated in rats and dogs as a result of biliary excretion. After the oral dose, the percentage of urinary excretion was 4.7% in rats, 18% in dogs and 46% in monkeys. The high excretion of radiolabel in the faeces of rats (90%) after intravenous administration shows the importance of biliary excretion for that species (Karim *et al.*, 1976c). Species differences were also noted in the biotransformation of spironolactone. Canrenone was a principal extractable metabolite in rat (Sadée *et al.*, 1974b) and dog plasma, whereas in monkeys and humans, both canrenone and a very polar, unidentified metabolite were the major constituents. In the urine of all four species, canrenone was a principal constituent. Notable species differences in the metabolites of spironolactone in the faeces were found, the pattern of metabolites in dog faeces being markedly different from that in rats, monkeys or humans. Overall, it was concluded that the disposition and metabolism of spironolactone in monkeys, rather than that in rats or dogs, is closest to that in humans.

Spironolactone is metabolized by microsomal monooxygenases and is converted to a reactive metabolite that destroys microsomal cytochrome P450 (CYP) and decreases steroid hydroxylase activity. This effect is seen only in animals that produce cortisol rather than corticosterone and that have a high activity of adrenal steroid 17α-hydroxylase (Menard *et al.*, 1974a,b, 1976, 1979). When spironolactone was incubated with

guinea-pig hepatic, adrenal, renal or testicular microsomal preparations, 7α-thiospirolactone, a potent mineralocorticoid antagonist, was produced which destroyed adrenal and testicular CYP. In guinea-pig microsomes, the 7α-thiospirolactone metabolite was determined to be an obligatory intermediate in the action of spironolactone on adrenal monooxygenases, but required further metabolism for its toxicity. In contrast, hepatic microsomal CYP in guinea-pigs was not inhibited by spironolactone, apparently because 7α-thiospirolactone was not further metabolized (Sherry *et al.*, 1986). Further studies of the species difference in the effects of spironolactone on CYP showed inhibition in guinea-pigs and dogs but not in adrenal microsomes from rats or rabbits (Sherry *et al.*, 1988). Spironolactone was reported to inactivate dexamethasone-inducible rat hepatic CYP in a suicidal manner (Decker *et al.*, 1986, 1989). In adrenal glands, a good correlation was found between covalent binding and CYP destruction, consistent with the hypothesis that 7α-thiospirolactone is a suicide inhibitor of adrenal CYP and that covalent binding to protein is involved in the degradation of these isozymes (Colby *et al.*, 1991).

4.2 Toxic effects

4.2.1 *Humans*

Spironolactone is an aldosterone antagonist that acts on the mineralocorticoid receptor. It is a potassium-sparing diuretic, and hyperkalaemia is the most common and potentially serious complication of therapy. Impaired kidney function appears to increase this risk, as does supplementation with potassium chloride. Excessive diuresis can also lead to dehydration and hyponatraemia (Greenblatt & Koch-Weser, 1973). A number of endocrine effects have also been reported, the most common of which is gynaecomastia, with a dose-related incidence of 7–52%. This side-effect is reversible and disappears upon discontinuation of therapy (Jeunemaitre *et al.*, 1988; Nielsen, 1990; Thompson & Carter, 1993). Other endocrine effects include loss of sexual potency in men and menstrual irregularity, amenorrhoea, breast engorgement and chloasma in women. These effects are probably due to interaction of spironolactone with the antrogen receptor.

There are a few isolated case reports of idiosyncratic drug reactions, including one case of hepatitis (Shuck *et al.*, 1981) and several cases of agranulocytosis (Stricker & Oei, 1984; Jivraj *et al.*, 1987; Ferguson *et al.*, 1993; Whitling *et al.*, 1997; van der Klauw *et al.*, 1998). Approximately 10 cases have been reported of allergic contact dermatitis after topical application of spironolactone for various dermal indications involving its antiandrogen activity (Corazza *et al.*, 1996).

In an assessment of pituitary–thyroid function in spironolactone-treated hypertensive women, high doses for 4 weeks did not affect the basal serum concentrations of triiodothyronine (T3), thyroxine (T4) or thyroid-stimulating hormone (TSH) in six euthyroid, hypertensive women. It did augment the pituitary TSH and thyroid T3

response to thyrotropin-releasing hormone (Smals *et al.*, 1979). Spironolactone also had no consistent effect on basal serum concentrations of T3, T4 or TSH in healthy men who had taken similar doses of spironolactone for as long as 24 weeks (Caminos-Torres *et al.*, 1977).

4.2.2 *Experimental systems*

The acute toxicity of spironolactone was determined after administration via the oral and intraperitoneal routes in rats, mice and rabbits. The oral LD_{50} was > 1000 mg/kg bw in all species, and the intraperitoneal LD_{50} was 786 mg/kg bw, 356 mg/kg bw and 866 mg/kg bw in rats, mice and rabbits, respectively (Lumb *et al.*, 1978). Spironolactone can exert antiandrogenic effects by several mechanisms: it can destroy testicular CYP and decrease 17α-hydroxylase activity, resulting in decreased testosterone synthesis (Menard *et al.*, 1974b); and it can inhibit 5α-dihydrotestosterone binding to cytosolic androgen receptor in the prostate (Pita *et al.*, 1975; Rifka *et al.*, 1978).

Studies of toxicity were conducted in Sprague-Dawley-derived rats, beagle dogs and rhesus monkeys (*Macaca mulatta*). In a 26-week study, rats were given diets containing spironolactone at a concentration of 0, 120, 300 or 700 mg/kg for the first 3 weeks and 0, 150, 500 or 2000 mg/kg for the remaining 23 weeks. In a 78-week study, rats were given diets that provided a dose of 0, 50, 150 or 500 mg/kg bw per day; and in a 104-week study, the animals received a dose of 0, 10, 30 or 100 mg/kg bw per day. In dogs, a 13-week study was conducted, in which initial doses of 0, 12, 30 and 70 mg/kg bw per day were given in a capsule for 6 weeks and then increased to 100 mg/kg bw per day for weeks 7–9 and to 250 mg/kg bw per day for the last 4 weeks. In rhesus monkeys, a 26-week study was conducted in which the animals received a dose of 0 or 125 mg/kg bw per day in a banana sandwich, and a 52-week study was conducted with doses of 0, 20, 50 and 125 mg/kg bw per day for 9 weeks followed by 250 mg/kg bw per day for 43 weeks (Lumb *et al.*, 1978).

Rats treated for 78 weeks showed a dose-related increase in the weight of the liver at all doses, increased adrenal gland weights in males at the two higher doses and increased thyroid gland weights in males at the high dose and in all treated females. Dose-dependent decreases in prostate weights were seen in males at all doses. Similarly, in the 104-week study, liver weights were increased at all doses, and the thyroid gland weights were increased in males and females at the high dose. A slight arrest of maturation in the testis (an increased number of immature spermatozoal precursors) was noted in the 78-week study at the two higher doses. In monkeys, a slight decrease in testis weight and a slight depression of maturation was observed at the two higher doses. Although gynaecomastia occurs in male human patients treated with spironolactone, no mammary abnormalities were noted in rats or dogs, but male monkeys showed a treatment-related increase in cellular activity in the acini of the mammary gland (Lumb *et al.*, 1978).

These studies revealed marked species differences in effects on the thyroid gland. No changes were seen in dogs or monkeys treated for 3 months or up to 1 year, respectively. In rats, thyroid changes were seen as early as 13 weeks at the high dose. The thyroid gland weights were increased, and, histologically, the follicles were smaller than normal, with diminished colloid, and the epithelial cells were taller and in some cases swollen. Species differences were also noted in the liver. Rats showed increased liver weights with no histological changes, while dogs had no increase in organ weight or histological changes. In monkeys, no weight changes or histological findings were observed in females, but males had a slight increase in liver weight at the high dose with no associated histological changes (Lumb *et al*., 1978).

Male rats were given diets containing spironolactone at concentrations that resulted in a dose of 0, 6, 50 or 200 mg/kg bw per day, for 13 weeks. Ten rats per group were killed after 2, 4 and 13 weeks of treatment for assessment of TSH, T4 and T3 concentrations, thyroid gland weights, histological appearance, thyroid iodine uptake and organification, and UDP glucuronosyltransferase (UGT) activity. After 13 weeks of treatment, the weights of the thyroid gland were significantly increased at all doses and the concentration of TSH was increased at the two higher doses. T3 and T4 concentrations were significantly decreased at the high dose at 2 and 4 weeks but had returned to normal by week 13. Thyroid iodine uptake and binding or organification were significantly increased at the high dose. Histologically, the follicular size patterns were altered in treated rats. The follicles were generally small to medium-sized, and the few remaining large follicles were lined with taller, wider follicular epithelial cells. In addition, the liver weights and *para*-nitrophenol UGT activity were significantly increased. The results of this study support the conclusion that spironolactone at high doses increases the hepatic clearance of T4 by inducing microsomal UGT activity. This causes a decrease in the serum concentrations of thyroid hormones, which activates a compensatory increase in pituitary TSH secretion resulting in increased thyroid gland weights and follicular-cell hypertrophy and hyperplasia (Semler *et al.*, 1989).

Marmosets (*Callithrix jacchus*) were given spironolactone at 30 or 100 mg/kg bw per day, phenobarbital (see monograph in this volume) at 50 mg/kg bw per day or methimazole (see monograph in this volume) at 10 or 30 mg/kg bw per day for 4 weeks. Spironolactone caused follicular-cell hypertrophy, but less severely than methimazole and with less reduction of T4 concentration. Spironolactone and phenobarbital, but not methimazole, increased hepatic CYP and T4-UGT activity (Kurata *et al.*, 2000).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

The anti-androgenic effects of spironolactone are discussed in section 4.2.

4.3.2 *Experimental systems*

No defects were produced in the offspring of rats and mice given intraperitoneal doses of up to 80 mg/kg bw per day potassium canrenoate, which is a metabolite of spironolactone in humans (Sadée *et al*., 1973; Funder *et al*., 1974) on days 8–14 (rats) or 7–13 (mice) of gestation, although at 80 mg/kg bw per day some resorptions occurred in mice (Miyakubo *et al*., 1977). An increased resorption rate was found in rats that received 100 mg/kg bw per day for various periods before day 6 of gestation (Selye *et al.*, 1971).

Mature virgin female CD-1 mice were caged with fertile males for 2 weeks, during which time they were given an intraperitoneal injection of 100 mg/kg bw spironolactone daily. There was no effect on mating (8/15 treated versus 22/30 controls), but the number of mice that became pregnant was reduced (3/8 versus 19/22), and fewer embryos per pregnant mouse were observed (mean, 4.3 versus 13.3). Similar results were obtained when mice were injected intraperitoneally with 100 mg/kg bw spironolactone twice daily. It was shown that the anti-fertility effect of spironolactone was mediated by inhibition of both ovulation and implantation, since the number of implants in ovulating animals could be increased by injection of estradiol on day 3 after mating (Nagi & Virgo, 1982).

A group of Wistar rats with regular estrous cycles received daily intraperitoneal injections of 100 mg/kg bw spironolactone for 7 days. The time spent in diestrus was significantly increased from 2 to 4 days, and, during the 14 days after treatment, 12 days were spent in diestrus; none of the animals had a complete cycle. Absence of estrus was accompanied by a reduction in plasma estradiol of 48%. In a group of 15 female rats treated with spironolactone from day 21 to day 45 of age, the onset of puberty was prevented in 47% of the animals, whereas all controls were postpubertal by that time. When female rats were treated simultaneously with spironolactone (100 mg/kg bw per day) and estradiol (1 μg/kg bw per day) on days 21–45 of age, vaginal opening and uterine development were normal, showing that spironolactone did not inhibit the peripheral actions of estradiol (Nagi & Virgo, 1982).

Pregnant Wistar rats, weighing 130 g, were given daily subcutaneous injections of 10 or 20 mg spironolactone on days 14–20 of gestation and were then allowed to deliver their pups and rear them normally. At 70–80 days of age, some of the animals were killed while in the basal state or after injection of gonadotropin-releasing hormone plus thyrotropin-releasing hormone, and blood and tissue samples were taken for analysis. The offspring showed no changes in the external genitalia, body weight or testis weight after spironolactone treatment *in utero*, but males showed a dose-related decrease in ventral prostate and seminal vesicle weight. The basal and stimulated plasma concentrations of follicule-stimulating hormone, luteinizing hormone, testosterone and 5α-dihydrotestosterone were normal, but those of prolactin were decreased. In females, the estrus cycle was unaffected, but the weights of the ovaries and uterus were significantly increased in those given 20 mg spironolactone,

and the plasma concentrations of follicle-stimulating hormone, prolactin, estradiol and progesterone were comparable to those of controls; however, the concentrations of luteinizing hormone were increased (Jaussan *et al.*, 1985).

4.4 Effects on enzyme induction or inhibition and gene expression

Spironolactone decreased the anaesthetic effects of pentobarbital and progesterone and a number of other compounds in female rats, and this was shown to be due to increase hepatic metabolism (Selye *et al.*, 1969). In male and female mice, spironolactone decreased hexobarbital sleeping time and increased substrate metabolism, liver weights and CYP content (Feller & Gerald, 1971). In spironolactone-pretreated rats, although substrate metabolism was increased, the content of CYP decreased, and the induction was sex-dependent, with greater induction of more substrates in female than male rats (Fujita *et al.*, 1982; Chung & Buhler, 1994).

In isolated hepatocytes from male Wistar rats pretreated with spironolactone, a dose-related increase in UGT activity was observed (Guibert *et al.*, 1983). Spironolactone increased *para*-nitrophenol UGT activity in male rats treated for 2 weeks at 200 mg/kg bw per day. It also induced bilirubin glucuronosyltransferase activity in rats and increased the plasma clearance and biliary excretion of bilirubin (Semler *et al.*, 1989). Spironolactone was found be a more specific and effective inducer of bilirubin UGT activity in rats than phenobarbital (Mottino *et al.*, 1989, 1991). The drug induced β-glucuronidase activity in the liver of female rats (Kourounakis & Tani, 1995) and glutathione *S*-transferase activity in the liver and jejunum, but not the colon, of male rats (Catania *et al.*, 1998).

4.5 Genetic and related effects

No data were available to the Working Group.

4.6 Mechanistic considerations

No data on the genotoxicity of spironolactone were available.

Spironolactone is a microsomal enzyme inducer and has been shown to increase UGT activity in rat liver. Studies on thyroid function in rats have shown decreased concentrations of thyroid hormones, increased concentrations of TSH, increased thyroid gland weight and follicular-cell hypertrophy and/or hyperplasia.

Increased pituitary secretion of TSH in response to increased thyroid hormone disposition is the likely mode of action for the production of thyroid neoplasms in rats. However, in view of the lack of data on genotoxicity, no definitive conclusion could be reached on the mechanism of spironolactone-induced carcinogenesis.

The increased incidence of Leydig-cell tumours of the testis may be related to the anti-androgenic effects of spironolactone.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Spironolactone is a steroidal potassium-sparing diuretic used in the treatment of oedema, hypertension and hyperaldosteronism.

5.2 Human carcinogenicity data

Spironolactone was mentioned specifically in two cohort studies and one case–control study. In one cohort study carried out in the USA, an excess risk for pharyngeal cancer was found, which persisted with longer follow-up. No evidence for an association with breast cancer was found in the other cohort study, and use of spironolactone was not associated with thyroid cancer in one case–control study. All three studies were based on small numbers of cases.

In five case–control studies of renal-cell carcinoma, use of potassium-sparing diuretics was not clearly identified as a risk factor independently of hypertension.

5.3 Animal carcinogenicity data

Spironolactone was tested by oral administration in one study in rats. Increased incidences of thyroid follicular-cell adenomas and Leydig-cell tumours of the testis were reported. Spironolactone reduced the incidence of 7,12-dimethylbenz[*a*]-anthracene-induced mammary tumours in rats.

5.4 Other relevant data

No data were available on the genotoxicity of spironolactone.

The metabolic pathway of spironolactone is complex and can be divided into two main routes: those in which the sulfur moiety is retained and those in which the sulfur moiety is removed by dethioacetylation.

Hyperkalaemia is the most common side-effect of exposure to spironolactone in humans, and a number of endocrine effects have been observed, the most common of which is gynaecomastia in men.

Spironolactone is transformed to a reactive metabolite that can inactivate adrenal and testicular cytochrome P450 enzymes. It also has anti-androgenic activity.

Spironolactone is a microsomal enzyme inducer. Studies on thyroid function have shown increased hepatic activity of uridine diphosphate-glucuronosyl transferase, decreased plasma triiodothyronine and thyroxine concentrations, increased thyroid-stimulating hormone concentrations and increased thyroid weights and follicular-cell hypertrophy and/or hyperplasia.

At relatively high doses, spironolactone induced resorption of embryos in rats and mice when given during the second week of gestation. Spironolactone reduced fertility in mice and delayed the onset of puberty when administered to young female rats. Prenatal treatment of rats with spironolactone caused a reduction in the weight of the prostate and seminal vesicles in male offspring.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of spironolactone.

There is *limited evidence* in experimental animals for the carcinogenicity of spironolactone.

Overall evaluation

Spironolactone is *not classifiable as to its carcinogenicity to humans (Group 3).*

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