2.4 NON-CLINICAL OVERVIEW

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Abbreviations

7α-TMS	7α-thiomethylspironolactone
8-OHdG	8-oxo-2'-deoxyguanosine
11ß-HSD2	11-beta-dehydrogenase iso-enzyme 2
ACE	Angiotensin Converting Enzyme
ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism, Excretion
AE	Adverse Event
AGEP	Acute Generalized Exanthematous Pustulosis
AGIQ	α-glycosyl isoquercitrin
Akt	Protein Kinase B
AIP	Aldosterone Induced Protein
ANS	Food Additives and Nutrient Sources
ARA	Aldosterone Receptor Antagonist
ATP	Adenosine TriPhosphate
AUC	Area Under the Curve
BDL	Bile Duct Ligation
BNF	British National Formulary
BUN	Blood Urea Nitrogen
cGMP	Cyclic Guanosine Monophosphate
CHF	Chronic Heart Failure
ChIP	Chromatin Immunoprecipitation
CI	Confidence Interval
CKD	Chronic Kidney Disease
CLIA	Chemiluminescent Assay
CNS	Central Nervous System
COX	CycloOxygenase
CS	Conditioned Stimulus
CTGF	Connective Tissue Growth Factor
CYP	Cytochrome P450
DMBA	7,12-dimethylbenz[a]-anthracene
DNA	Deoxyribonucleic Acid
DOC	Deoxycorticosterone
DRESS	Drug Rash with Eosinophilia and Systemic Symptoms
EFSA	European Food Safety Authority
eGFR	Estimated Glomerular Filtration Rate
EMA	European Medicines Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FPIA	Fluorescence Polarization Immunoassay
FSMP	Foods for Special Medical Purposes and Special Formulae
G6PD	Glucose-6-Phosphate Dehydrogenase
GRAS	Generally Regarded as Safe
GSH/GSSG	Glutathione
H	Hydrogen
hAR	Human Androgen Receptor
hERG	Human Ether-à-go-go-Related Gene

HFD	High Fat Diet
HFpEF	Heart Failure with Preserved Ejection Fraction
hGR	Human Glucocorticoid Receptor
hMR	Human Mineralocorticoid Receptor
HPLC	High Performance Liquid Chromatorgraphy
hPR	Human Progesterone Receptor
HSC	Hepatic Stellate Cells
HTMS	6β-hydroxy-7α-thiomethyl-spironolactone
I3C	Indole-3-Carbinol
i.p.	Intraperitoneal
i.v.	Intra venous
IC_{50}	Half Maximal Inhibitory Concentration
IARC	International Agency for Research on Cancer
IL	Interleukin
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K	Potassium
Kg	Kilogram
LBD	Ligand Binding Domain
LD_{50}	Lethal Dose for 50% of subjects
MAP	Mean Arterial Pressure
MCA	Manufactured Citric Acid
MEIA	Microparticle Enzyme Immunoassay
mg	Milligram
mL	Milliliter
MR	Mineralocorticoid Receptor
MRA	Mineralocorticoid Receptor Antagonist
Na	Sodium
NADPH	Nicotinamide adenine dinucleotide phosphate
NCF1	Neutrophil Cytosol Factor 1
ng	Nanogram
nM	Nanomolar
NO	Nitric Oxide
NOAEL	No Observed Adverse Effect Level
NOS	Nitric Oxide Synthase
NOX	NADPH Oxidase
NR3C2	Nuclear Receptor Subfamily3, Group C, Member 2
NSAID	Non Steroidal Anti Inflammatory Drug
NYHA	New York Heart Association
OFC	Olfactory Fear Conditioning
OR	Odds Ratio
p.o.	par os
p47phox	Neutrophil Cytosol Factor 1
Panx1	Pannexin 1
PCa	Prostate Cancer
PD	Pharmacodynamics
Ph.Eur	European Pharmacopoiea
PK	Pharmacokinetics
PKG	Protein Kinase G
RAAS	Renin-Angiotensin-Aldosterone System
RALES	Randomized Aldactone Evaluation Study

RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SCAR	Severe Cutaneous Adverse Reaction
SCCP	Scientific Committee on Consumer Products
SD	Sprague Dawley
SGK-1	Serum- and Glucocorticoid-Regulated Kinase 1
SHR	Spontaneously Hypertensive Diabetic Rats
SJS	Steven-Johnson Syndrome
SmPC	Summary of Product Characteristics
TEN	Toxic Epidermal Necrolysis
TLC	Thin Layer Chromatography
TNF	Tumour Necrosis Factor
TOPCAT	Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist Trial
TSH	Thyroid Stimulating Hormone
UDP	Uridine 5'-diphospho-glucuronosyltransferase
UGT	UDP Glucuronosyltransferase
WHO	World Health Organisation

2.4 NON-CLINICAL OVERVIEW

Spironolactone was first identified in the 1950's, and first approved by the Food and Drug Administration (FDA) and launched in the USA in 1960 by Searle Laboratories as a diuretic for the management of oedematous conditions, primary aldosteronism and essential hypertension [redacted] Spironolactone is listed as a medicine for heart failure on the World Health Organisation (WHO) Model List of Essential Medicines [redacted], which contains the medications considered to be most effective and safe to meet the most important needs in a health system.

The mineralocorticoid aldosterone is secreted by the adrenal glands and is thought to contribute to a number of pathological conditions, including myocardial fibrosis, endothelial dysfunction, and vascular inflammation. These processes are involved in the development of heart failure, which is a leading cause of morbidity and mortality throughout the world [redacted]

Spironolactone is used world-wide to block aldosterone-dependent sodium transport in the distal tubule of the kidney in order to reduce oedema and to treat essential hypertension and primary hyperaldosteronism. Spironolactone is also used commonly in the treatment of other hyperaldosterone-related diseases such as liver cirrhosis and congestive heart failure. The drug acts at the mineralocorticoid receptor (MR) level by competitively inhibiting aldosterone binding [redacted]

The inhibitory action of spironolactone gives rise to its effect as a potassium-sparing diuretic, and is thus used in the treatment of several indications, including oedema, hypertension and 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) ([redacted]).

Spironolactone is a synthetic steroid that acts as a competitive antagonist of the potent endogenous mineralocorticosteroid 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 spironolactone in combination with another diuretic. Minor uses (not included in present SmPC) 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 ([redacted]).

The activity of spironolactone in inhibiting the physiological effects of aldosterone, give rise to spironolactone affecting aspects of physiology that include cellular hypertrophy, interstitial fibrosis and endothelial dysfunction of the distal renal tubules, myocardium and vasculature ([redacted] .

The benefit of the addition of spironolactone to a heart failure treatment regimen has been clearly demonstrated in the RALES (Randomized Aldactone Evaluation Study). Results of the trial showed that spironolactone treatment significantly reduced the risk of mortality by 30% after a mean follow-up of 24 months in patients with severe heart failure (left ventricular ejection fraction < 35% and New York Heart Association (NYHA) class III or IV symptoms) as compared with placebo [redacted]

Spironolactone 10 mg/ml Oral Suspension is indicated in the management of refractory oedema associated with congestive cardiac failure; hepatic cirrhosis with ascites and oedema, malignant ascites, nephrotic syndrome, diagnosis and treatment of primary aldosteronism and essential hypertension, as detailed in the SmPC in Module 1.3.1.

For the various indications, the dose range for the treatment of adults is 25-400 mg per day, and the dose range for children is 1-3 mg/kg body weight per day.

Spironolactone 10 mg/ml Oral Suspension has therefore been developed to substitute for approved spironolactone oral tablet and capsule medicinal products, where an oral suspension provides clinical benefit, particularly in the case of young children who find swallowing tablets difficult and adolescents and adult patients who prefer liquids and patients with dysphagia.

2.4.1 Overview of the Non-Clinical Testing Strategy

Spironolactone oral tablet and capsule formulations are already authorised and marketed throughout all EU Member States for the same indications as for Spironolactone 10 mg/ml Oral Suspension.

The posology of the already licensed spironolactone tablets, Aldactone® 25 mg, 50 mg and 100 mg tablet, and Spironolactone 10 mg/ml Oral Suspension are the same.

The applicant has conducted a bioequivalence study (INV684) to compare Spironolactone 10 mg/ml Oral Suspension (test) to the reference authorised/marketed tablet formulation (Aldactone 100 mg tablet®). This was a single-dose, open-label, laboratory-blind, randomized, four period crossover, replicate study with orally administered spironolactone 100 mg conducted under fed conditions in 36 healthy males and females at a single study centre. The study was conducted in accordance with Good Clinical Practice (GCP).

The test and reference formulation were shown to be bioequivalent in terms of Area Under the Curve (AUC)0-t and AUC0-∞. However, the mean C_{max} values of spironolactone were higher for the tablet formulation, with the 90% confidence limits falling below the lower 70% limit, demonstrating that the two formulations are not bioequivalent for this parameter.

The mean AUC0-t and AUC0-∞ values of the metabolites 7α-thiomethylspironolactone (7α-TMS), 6β-hydroxy-7α-thiomethyl-spironolactone (HTMS) and canrenone were also very similar for both formulations. In contrast to spironolactone, the mean C_{max} values of each of the metabolites were higher in the test formulation compared to the reference.

There are no reasons to support the view that differences in mean C_{max} between the spironolactone oral suspension and Aldactone® will lead to an altered safety or efficacy

profile in clinical practice. Antagonism of mineralocorticoid activity is mediated through altered gene expression and hence delayed. Moreover, spironolactone is essentially a pro-drug with three active metabolites, and it is the active metabolites that are largely responsible for the pharmacological effect and its persistence. As such, the parent spironolactone's peak blood levels (C_{max}) assume less relevance, and it is the AUC that is assumed to best correlate with pharmacodynamic effect. This is further discussed in Module 2.5 Clinical Overview. In support of the present hybrid application, article 10(3) states that "results of the appropriate pre-clinical tests shall be provided". The applicant considers that non-clinical and clinical data from the scientific and medical literature are appropriate to address all sections of the non-clinical overview. Therefore, no formal non-clinical development programme was undertaken for spironolactone, in view of:

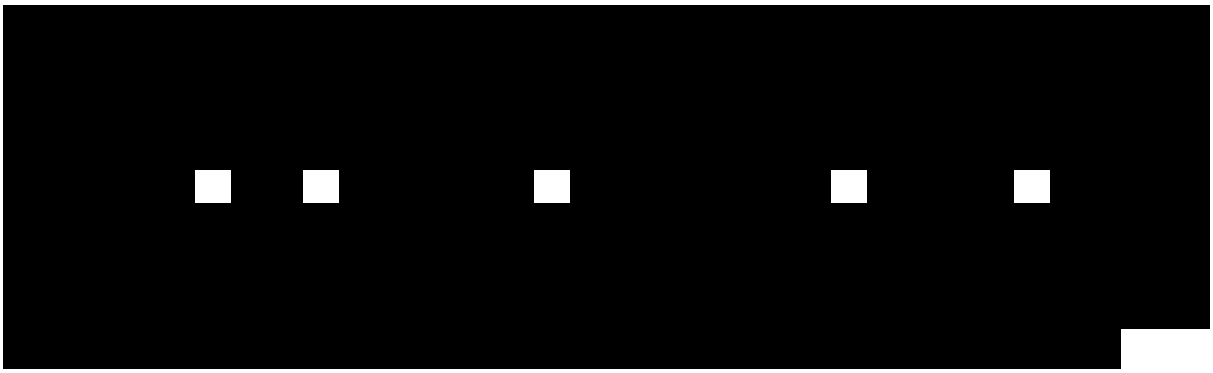
- Clinical pharmacokinetics (PK) exposure for the oral suspension as discussed above and in Module 2.5.
- Spironolactone is a clinically well-established drug substance with extensive efficacy, PK and safety data over many years' use in EU Member States for all of the indications and doses proposed in the SmPC (see SmPC in Module 1.3.1).
- Extensive nonclinical literature for spironolactone, as discussed in this nonclinical overview, which support the clinical data.
- There being no nonclinical data gaps, taking into account nonclinical and clinical data.

It is therefore considered that conducting any further nonclinical pharmacology, PK or toxicity studies of spironolactone is not appropriate.

Spironolactone is a well-established active substance which has been marketed for several decades. The specification of spironolactone drug substance used in Spironolactone 10 mg/ml Oral Suspension is in accordance with the European Pharmacopoeia (Ph. Eur.) monograph for this drug substance. All excipients used in the formulation of the drug product are well-established with a record of pharmaceutical use in adult and paediatric patients. All components of the drug product are Ph. Eur. grade, with the exception of the strawberry flavour liquid and the bitter masker flavour, which are to the manufacturers' specifications, and summarised in section 2.4.4.7.6 and also 3.2.P.

In section 4.4 of the SmPC, appropriate warnings are stated for sucrose. Impurities are addressed in section 2.4.4.7.4.

2.4.1.1 Nonclinical Literature Search Strategy



2.4.1.2 Pharmacodynamics including Safety Pharmacology

Pharmacodynamics ([PD], primary, secondary and PD interactions) and safety pharmacology data are based upon published studies in the literature.

2.4.1.3 Pharmacokinetics

The pharmacokinetics (PK) of spironolactone in humans are well characterised, and a PK study has been conducted in human volunteers using Spironolactone 10 mg/ml Oral Suspension as discussed above. *In vitro* PK studies and *in vivo* PK studies in rodents and non-rodents have been reported from the literature.

2.4.1.4 Toxicology

Single dose toxicity studies with spironolactone in the mouse, rat and rabbit have been identified in the literature and repeat dose toxicity data are available for rat, dog, marmoset and monkey by intraperitoneal or oral routes of administration.

In the two most recent [redacted], an assessment of the genotoxicity of spironolactone was not undertaken with the reason stated as being due to no data being available to the [redacted] on both occasions. However, a veterinary European Public Assessment Report evaluating the use of spironolactone in dogs reported that spironolactone did not show any mutagenic or genotoxic activity.

In the two [redacted] monographs mentioned above, it was concluded that there was *inadequate evidence* in humans for the carcinogenicity of spironolactone. Furthermore there was deemed to be *limited evidence* in experimental animals for the carcinogenicity of spironolactone. As such, the overall conclusion was that spironolactone is *not classifiable as to its carcinogenicity to humans* (Group 3) [redacted] Carcinogenic effects of spironolactone in rats after long-term exposure are reflected in Sections 5.3 of the SmPC.

A number of studies have investigated the reproductive and development toxicity of spironolactone, specifically male fertility and embryofetal development, by various routes of administration in mice, rats and rabbits. No teratogenic effects of spironolactone have been identified in any mouse study investigated. Studies showed that spironolactone reduced fertility in mice and delayed the onset of puberty when administered to young female rats. Dose-related feminizing effects were also noted in male rat offspring. Rabbits had an increased rate of resorption and a lower number of live pups.

There is no evidence of any dependence concerns for spironolactone, based on its mechanism of action and clinical experience.

The non-clinical assessment is based on the large body of published non-clinical data and clinical experience including clinical safety data, as discussed in detail in the Clinical Overview (Module 2.5) and cross referenced in this overview where applicable.

2.4.2 Pharmacology

The mineralocorticoid aldosterone is secreted by the adrenal glands and is thought to contribute to a number of pathological conditions, including myocardial fibrosis, endothelial dysfunction, and vascular inflammation. These processes are involved in the development of heart failure, which is a leading cause of morbidity and mortality throughout the world [redacted]

The MR belongs to the nuclear receptor subfamily 3, group C, member 2, (NR3C2) and is a protein that in humans is encoded by the NR3C2 gene on chromosome 4q31.1-31.2. The MR ligand is aldosterone, and the MR is 'protected' from glucocorticoids by co-localization of an enzyme, corticosteroid 11-beta-dehydrogenase iso-enzyme 2(11ß-HSD2), which converts cortisol to cortisone that does not bind to the MR. Binding of the MR by aldosterone results in its translocation to the cell nucleus, homodimerization and binding to hormone-response elements present in the promoter of certain genes [redacted]

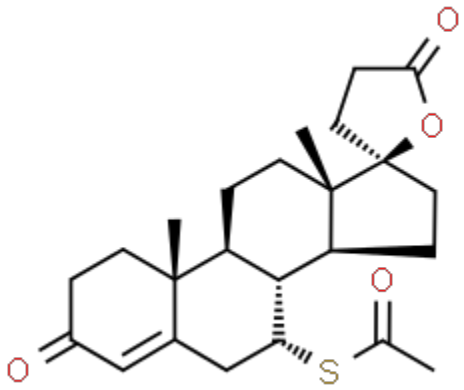
The MR is expressed in many tissues, such as kidney, colon, heart, central nervous system ([CNS], hippocampus), brown adipose tissue and sweat glands. In epithelial tissues, its activation leads to the expression of proteins regulating ionic and water transport. These proteins include the epithelial sodium channel, Na+/K+ pump, serum and glucocorticoid-regulated kinase-1 (SGK-1), resulting in sodium reabsorption and as a consequence an increase in the extracellular volume, increase in blood pressure and excretion of potassium [redacted]

The literature also shows that aldosterone also has a variety of "non-epithelial" effects, which was deduced with the discovery of MRs in multiple non-epithelial locations. The non-epithelial effects of aldosterone appear to be mediated by a second messenger system, which involves activation of the Na+/H+ transporter. Aldosterone-receptor antagonists (ARAs) such as spironolactone limit both the epithelial and non-epithelial responses to aldosterone [redacted]

In the late 1950s and early 1960s, animal and human studies demonstrated that the synthetic mineralocorticoid spironolactone (Figure 2.4.1) could antagonize the renal excretory effects of aldosterone and reduce arterial pressure in patients with hypertension [redacted] Thus, spironolactone was the first diuretic engineered expressly to block a particular renal transport process involved in sodium (Na+) handling; structurally, spironolactone contained elements of the progesterone molecule and as was to be expected, its use was accompanied by some progestogenic and antiandrogenic adverse effects [redacted]

Spironolactone has a four ring structure characteristic of steroids and binds to the MR in the renal tubules blocking the synthesis of aldosterone induced proteins (AIP). This reduces Na+ reabsorption and K+ and H+ secretion by various mechanisms (Figure 2.4.2). The magnitude of diuresis produced by spironolactone depends on the plasma level of aldosterone [redacted]

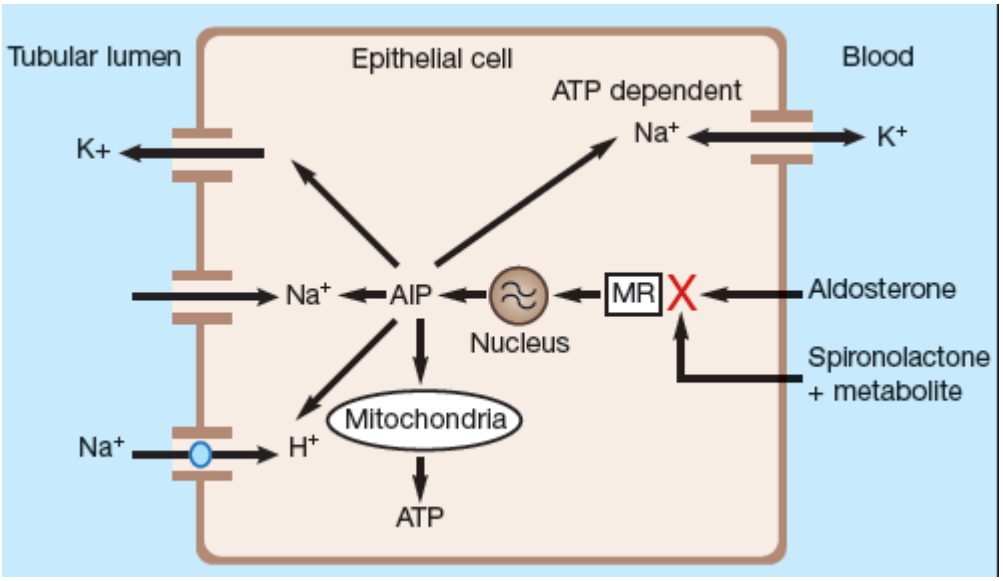
Figure 2.4.1. Structure of Spironolactone



Taken from [redacted]

Due to MR antagonism in the kidney, spironolactone results in increased excretion of sodium and water to lower fluid retention and lessen pressure on the heart. Spironolactone has been shown to reduce total and cardiovascular mortality in heart failure patients when administered along with other inhibitors of the renin–angiotensin–aldosterone (RAAS) system [redacted]

Figure 2.4.2. Mechanism of Action of Spironolactone



Taken from [redacted]

Spironolactone is well absorbed orally, is highly protein bound and extensively metabolised in the liver. It undergoes enterohepatic recirculation and has a short half-life of less than 2 hours [redacted] Due to the short half-life of spironolactone, it is considered not only as a mineralocorticoid receptor antagonist (MRA), but also as a pharmacologically active prodrug. Spironolactone undergoes metabolism in the liver to three

major metabolites: Two sulfur-containing metabolites, 7α-TMS, and HTMS and the major dethioacetylated metabolite canrenone, which have mean half-lives in healthy volunteers of 13.8, 15.0 and 16.5 h, respectively [redacted] Canrenone was long considered to be the major active metabolite of spironolactone. However, 7α-TMS was subsequently found to be the major active metabolite at steady state after oral administration of 100 mg spironolactone per day: peak absolute and relative plasma concentrations of spironolactone, canrenone, 7α-TMS and HTMS were found to be 80 (10.3), 181 (23.3), 391 (50.3) and 125 (16.1) ng/mL (%) respectively [redacted] Detailed discussion of the metabolism of spironolactone is provided in section 2.4.3.4. The comparatively long half-lives of the metabolites of spironolactone give rise to a prolonged diuretic effect [redacted]

2.4.2.1 Primary Pharmacodynamics

Due to the longevity and well established use of spironolactone clinically, there are a multitude of studies investigating spironolactone in humans (Module 2.5). Furthermore, various mechanistic studies have been performed pre-clinically. Historically, the antagonism of the MR by spironolactone has been established as its primary mode of action. However, some more recent studies have been identified in the literature, which investigate this action and the downstream events further.

The table below summarises the pharmacodynamic activities of spironolactone.

Table 2.4.1. Pharmacodynamic Summary of Spironolactone

	Spironolactone
Effect on MR stability	Destabilizes helix 12 [3,4]; does not promote aldosterone-induced MR degradation [38••]
Effect on aldosterone-induced intra-nuclear translocation of MR	Permits nuclear localization, but delayed [38••,57]
Effect on coactivator recruitment	Reduced due to helix 12 destabilization; SRC-1 recruitment intact but RNA polymerase II not recruited [58]
Epithelial ion transport	Increase urinary Na^+:K^+ ratio
Renin–angiotensin-axis	Increase renin release and aldosterone level by disrupting feedback loop
Blood pressure regulation	Little additional effect beyond 100 mg/day; maximal hypotensive effect takes 3–4 weeks [59,60]
Heart failure protection	RALES: 30% reduction in all-cause mortality; 35% reduction in hospitalization for worsening heart failure
Potassium	$K^+ > 5.5$ mmol/L in 13% (25 mg/d), 20% (50 mg/d) and 24% (75 mg/d) of patients
Diabetic kidney disease protection	53% reduction in albuminuria when combined with ARB versus 20% reduction in ACEi + ARB group (1.5 years) [62]

Taken from [redacted]

In Vitro Studies

In a study published in [redacted] sought to gain a better understanding of the molecular basis of the anti-mineralocorticosteroid actions of aldosterone antagonists. In the chick intestine cytosol, spironolactone and aldosterone bound to a common site, which was indicated by inhibition of [^{3}H]-spironolactone binding by aldosterone and Hl0E (a monoclonal anti-idiotypic) antibody. Kinetic studies revealed a clear-cut difference in the dissociation rate between aldosterone and its antagonists. The half-life of antagonist-MR complexes was ~100-fold shorter than that of aldosterone-MR complexes. In addition, spironolactone and progesterone induced a weaker interaction between Hsp90 and the steroid-binding subunit than aldosterone. The authors concluded that aldosterone antagonists dissociated rapidly from the MR, and *in vitro*, their binding facilitated the release of Hsp90 from the receptor complex. The authors noted that whether these events occurred *in vivo* and how they were involved in inactivating the receptor to promote gene transcription, remained to be established [redacted]

In a study published in [redacted] determined how agonists and antagonists interacted with the human mineralocorticoid receptor (hMR) based on a homology model of the hMR ligand-binding domain (LBD) and to understand the consequence of their interaction on the receptor transactivation function. A 3D model of the hMR-LBD was constructed. Results showed that the MR family from human and other species has a high degree of sequence conservation (82%). Comparison of MRs with human progesterone, glucocorticoid and androgen receptors (hPR, hGR and hAR) revealed a lower sequence identity (48%), but a strong sequence similarity. Study results identified key residues of the hMR involved in the recognition of agonist and antagonist ligands through alanine-scanning mutagenesis. The 3D model revealed that mutations of Gln776 and Arg817 in site I reduced the affinity of hMR for both agonists and antagonists and affected the capacity of hMR to activate transcription, suggesting that the C3-ketone group, common to all ligands, was anchored by these two residues conserved within the nuclear steroid receptor family. In the light of this hMR homology model, a new mechanism of antagonism was proposed in which the AF2-AD core region was destabilized by the loss of contacts between the antagonist and the helix H12 region [redacted] Taken together, the studies by [redacted] examining the binding of spironolactone showed that it has high affinity for the MR and binds the same site as aldosterone but dissociates more rapidly, destabilises the receptor and impairs coactivator recruitment. Furthermore the high degree of sequence conservation in the MR family and comparison of MRs with human progesterone, glucocorticoid and androgen receptors revealing a strong sequence similarity help to explain some of the secondary pharmacological effects of spironolactone, which are discussed in section 2.4.2.2.

[redacted] recently characterized the molecular mechanisms of action of spironolactone at several key steps of the MR signalling pathway. Molecular modelling and mutagenesis approaches were utilised. The studies showed that aldosterone-dependent phosphorylation and degradation of MR are inhibited by spironolactone. Their studies also showed that spironolactone treatment permitted aldosterone induced intranuclear translocation of MR, but that this effect was delayed [redacted] Studies investigating the effect of spironolactone on coactivator recruitment, using chromatin immunoprecipitation (ChIP) experiments were performed on chromatin extracts from HK-GFP-MR cells treated for 1 h with 100 nM aldosterone, 1 mM spironolactone or vehicle. Spironolactone promoted both

MR and SRC1 recruitment on the SCNN1A promoter indicating that spironolactone has partial aldosterone-like activity since treatment with aldosterone led to significant recruitment of MR but also of SRC1 and ribonucleic acid (RNA) pol II, as compared with vehicle treatment. However, spironolactone did not induce substantial RNA pol II recruitment, which may account for the repression of transcription initiation in response to spironolactone [redacted]

***In Vivo* Studies**

The effect of spironolactone on the potential for improvement in vascular reactivity by increasing glucose-6-phosphate dehydrogenase (G6PD) was evaluated *in vivo*. Genetically modified mice deficient in G6PD were infused with aldosterone for 7 days, and decreased aorta G6PD activity was confirmed. Spironolactone (20 mg/kg/day) was then administered in the drinking water during the next 7 days of aldosterone infusion. In aldosterone-treated mice administered spironolactone, there was a reduction in systolic blood pressure at 14 days compared to mice that did not receive spironolactone. In addition, spironolactone increased aorta G6PD expression, activity, and nicotinamide adenine dinucleotide phosphate (NADPH) levels leading to a decrease in reactive oxygen species (ROS) and an increase in cyclic guanosine monophosphate (cGMP) levels. Furthermore, in spironolactone-treated mice, vasodilator responses to acetylcholine and sodium nitroprusside were restored to levels observed in vehicle-infused mice [redacted] The authors concluded that *in vivo*, infusion of aldosterone decreased vascular G6PD expression and impaired vascular reactivity. These effects were abrogated by spironolactone or vascular gene transfer of G6PD.

A study was performed to evaluate whether spironolactone ameliorates nephropathy by increasing G6PD activity and reducing oxidative stress in streptozotocin-induced spontaneously hypertensive diabetic rats (SHR). The SHR rats received 50 mg/kg/day spironolactone in drinking water, or no treatment for eight weeks. Results showed plasma glucose levels and systolic blood pressure were both unaltered by spironolactone treatment. Albuminuria, fibronectin expression, 8-oxo-2'-deoxyguanosine (8-OHdG) urinary levels, lipid peroxidation and p47phox (a.k.a. NCF1 [neutrophil cytosol factor 1]) expression were higher in the diabetic rats compared with the control animals, and these levels were reduced by treatment with spironolactone. The antioxidant GSH/GSSG ratio was reduced in the diabetic rats and spironolactone treatment re-established it. Diabetes-induced SGK1 up-regulation was inhibited by spironolactone treatment. Furthermore, ROS and superoxide production induced by NADPH oxidase were increased by hyperglycemia, and were reduced with spironolactone treatment. Hyperglycemia and high glucose decreased G6PD activity, which was restored with spironolactone treatment. These results suggest that spironolactone ameliorates nephropathy in diabetic SHRs by restoring G6PD activity and diminishing oxidative stress without affecting glycaemia and blood pressure [redacted]

The transgenic TG(mRen2)27 rat (Ren2), harbouring the mouse renin gene, is an experimental model of excessive tissue local RAAS activity that, through paracrine adrenal effects, leads to increased plasma deoxycorticosterone (DOC), 18-hydroxy-corticosterone, and aldosterone levels as well as whole body and skeletal muscle insulin resistance. The impact of *in vivo* MR blockade with low-dose spironolactone on systemic insulin sensitivity was evaluated in these transgenic rats. Young insulin-resistant Ren2 rats and aged-matched Sprague-Dawley (SD) control rats (age 6 – 8 weeks) were implanted with subcutaneous time-

release pellets containing spironolactone (0.24 mg/day) or placebo over 21 days. Along with increased soleus muscle NADPH oxidase activity and ROS, results showed systemic insulin resistance and reduced muscle IRS-1 tyrosine phosphorylation, protein kinase B (Akt) phosphorylation/ activation, and GLUT4 expression in the Ren2 group (each $P < 0.05$). Despite not decreasing blood pressure, low-dose spironolactone treatment improved soleus muscle insulin signalling parameters and systemic insulin sensitivity in concert with reductions in NADPH oxidase subunit expression/activity and ROS production (each $P < 0.05$) [redacted] The potential involvement of Akt noted in this study was also noted in a study by [redacted]

In a similar model, [redacted] investigated whether protection elicited by MR blockade was through attenuation of vascular apoptosis and injury, independently of blood pressure lowering. To this end, a low dose of spironolactone (0.24 mg/day via a subcutaneous time release, matrix-driven delivery pellet) or vehicle was administered for 21 days to hypertensive transgenic Ren2 rats or Sprague-Dawley rats. To assess blood pressure-independent effects of MR antagonism on vasculature protection, a dose of spironolactone was used, which did not reduce systolic blood pressure in hypertensive Ren2 rats. Results showed that although Ren2 rats developed higher systolic blood pressure compared with Sprague-Dawley rats, low-dose spironolactone treatment did not reduce systolic blood pressure compared with untreated Ren2 rats. Ren2 rats exhibited vascular injury as evidenced by increased apoptosis, hemidesmosome-like structure loss, mitochondrial abnormalities, and lipid accumulation compared with Sprague-Dawley rats, and these abnormalities were attenuated by spironolactone treatment [redacted]

Protein kinase B activation is critical to vascular homeostasis via regulation of cell survival and expression of apoptotic genes. The aortas of Ren2 rats were isolated and investigated for Protein kinase B serine473 phosphorylation. This activity was found to be impaired in Ren2 rat isolated aortas and restored with treatment with spironolactone. *In vivo*, treatment with spironolactone promoted antiapoptotic effects by increased phosphorylation of BAD serine136 (pro-apoptotic molecule) and expression of Bcl-2 and Bcl-xL, decreased cytochrome c release and BAD expression, and suppression of caspase-3 activation. Furthermore, spironolactone treatment substantially reduced the elevated NADPH oxidase activity and lipid peroxidation, expression of angiotensin II, angiotensin type 1 receptor, and MR in Ren2 vasculature. These results demonstrated that antagonism of the MR receptor by spironolactone led to protection of the vasculature from aldosterone-induced vascular apoptosis and structural injury via rescuing protein kinase B activation, independent of blood pressure effects [redacted]

In a bile-duct-ligated rat model of cirrhosis, spironolactone was investigated to establish its effects on cirrhosis activated RhoA/ROCK-2 signalling and inhibition of nitric oxide (NO) availability, which contribute to increased intrahepatic resistance and portal hypertension. Increased RhoA/ROCK-2 reduces NO synthase activity via down-regulation of endothelial nitric oxide synthase (eNOS). NO induces vasorelaxation via activation of cGMP/protein kinase G (PKG) [redacted] Initial findings *in vitro* showed that aldosterone induced contraction of activated hepatic stellate cells (HSCs) by activation of the RhoA/ROCK-2 signalling pathway, while spironolactone and the ROCK-2 inhibitor Y27632 could suppress this effect. The next aim was to investigate the effect of chronic spironolactone treatment *in vivo* on intrahepatic RhoA/ ROCK-2 signalling and NO/PKG pathway as well as on liver

fibrosis and portal hypertension. Liver cirrhosis was induced by bile duct ligation (BDL) in male Wistar rats. Biliary hepatic fibrosis was induced by double ligation and transection of the common bile duct. Treatment with spironolactone (20 mg/kg/day, p.o.) significantly lowered portal pressure compared to vehicle (saline, p.o.), and this was associated with attenuation of liver fibrosis, intrahepatic resistance and inhibition of HSC activation. In BDL rat liver, spironolactone suppressed up-regulation of proinflammatory cytokines (tumour necrosis factor (TNF) -α and interleukin (IL) -6). Additionally, spironolactone significantly decreased ROCK-2 activity without affecting expression of RhoA and Ras. Moreover, spironolactone markedly increased the levels of eNOS, phosphorylated eNOS and PKG activity in the liver. The authors concluded that spironolactone lowered portal hypertension by improvement of liver fibrosis and inhibition of intrahepatic vasoconstriction via down-regulating ROCK-2 activity and activating NO/PKG pathway [redacted]

In a cardiomyopathy model examining the cardioprotective role of spironolactone, streptozotocin-induced diabetic male Sprague Dawley rats were utilised to explore the potential mechanisms involved. Diabetic SD rats were treated by oral gavage either with spironolactone (20 mg/kg/day) or vehicle (saline) for 12 weeks after the onset of diabetes [redacted] Treatment with spironolactone restored the mitochondrial contents and ameliorated the dysfunction of myocardial energy metabolism by elevating the gene expression of ATP5α1 and cyclooxygenase (COX) 5b (key components of the electron transport chain). Expression of ATP5α1 was significantly decreased in diabetic animals compared to nondiabetic rats, and treatment with spironolactone reversed this change. Investigation of cardiac structure suggested that spironolactone attenuated mitochondrial morphological abnormalities and sarcoplasmic reticulum enlargement in diabetic rats. Compared to the saline group, cardiac oxidative stress, fibrosis, inflammation, and mitochondrial dysfunction were improved by spironolactone treatment. Mitochondria are also involved in the production of ROS, which may also be connected to the beneficial outcomes following spironolactone treatment in this model. There was no difference between treatment groups in blood pressure or blood glucose levels, suggesting that protective effects of spironolactone in this model were independent of lowering glucose or blood pressure [redacted]

Although spironolactone is known to act at the MR, there is increasing evidence of MR-independent effects of spironolactone. [redacted] conducted studies investigating whether pannexin 1 (Panx1) channels could be a relevant *in vivo* target of spironolactone. Clinically, the addition of spironolactone is beneficial to patients already prescribed three drugs from different classes, including a diuretic, and thus the blood pressure reduction action of spironolactone could be partially attributed to 'off-target' effects. Since Panx1 can regulate α1-adrenergic receptor-mediated vasoconstriction and blood pressure, the authors assessed whether Panx1 may contribute to the anti-hypertensive effects of spironolactone as an 'off-target' effect. Initial studies identified spironolactone as a potent inhibitor of Panx1, which was confirmed by electrophysiological analysis. Next, spironolactone was shown to inhibit α-adrenergic vasoconstriction in arterioles from mice and hypertensive humans in *ex vivo* studies, an effect dependent upon smooth muscle Panx1, but independent of the MR. Spironolactone evoked a rapid decrease in mean arterial pressure (MAP) when injected into C57BL/6 control and smooth muscle cell MR-KO, whereas spironolactone did not reduce blood pressure in smooth muscle cell Panx1-KO mice or mice with global deletion of Panx1

(Panx1−/−). The authors also tested the effect of spironolactone on spontaneously hypertensive BPH/2 mice, with elevated MAP that is largely attributed to sympathetic overdrive. In these BPH/2 mice, again, spironolactone induced a strong reduction in MAP. The authors concluded that spironolactone acutely lowered blood pressure, which was dependent on smooth muscle cell expression of Panx1 and independent of the MR [redacted]

Other work investigated whether treatment with spironolactone following the establishment of hypertension, could modify the progression of renal fibrosis in Cyp1a1Ren2 rats. The transgenic Cyp1a1Ren2 rat model allows hypertension to be reversibly induced by diet manipulation, to any desired level consistent with survival, and may more accurately represent the clinical setting. In this transgenic rat, mouse Ren2 complementary deoxyribonucleic acid (DNA) expression is controlled by an inducible Cyp1a1 promoter, integrated into the Y chromosome of Fischer 344 rats. Induction of the Cyp1a1 promoter (via dietary indole-3-carbinol (I3C)) leads to increased circulating renin levels, activation of the RAAS and a consequent increase in blood pressure. Significantly, the degree of hypertension is I3C dose dependent, allowing for tight titration of blood pressure. Removal of I3C from the diet results in rapid restoration to pretreatment levels of blood pressure [redacted]

Using male Cyp1a1Ren2 rats, hypertension was established by addition of 0.167% I3C (w/w) to the diet for 2 weeks prior to treatment. Rats were then divided into normotensive, hypertensive (H), or hypertensive with daily oral spironolactone treatment (H + SP) (human equivalent dose 50 mg/day). After 4 weeks of spironolactone treatment, there was no demonstrable effect on systolic blood pressure, proteinuria, or macrophage infiltration in the renal cortex. However, glomerulosclerosis and renal cortical fibrosis were significantly decreased. Following 12 weeks of spironolactone treatment, systolic blood pressure was lowered (albeit not back to normotensive levels), proteinuria was reduced, and the progression of glomerulosclerosis and renal cortical fibrosis was significantly blunted. This was associated with a significant reduction in macrophage and myofibroblast infiltration, as well as connective tissue growth factor (CTGF) and phosphorylated SMAD2 (pSMAD2) expression. The authors concluded that in this model of established hypertension, spironolactone significantly blunted the progression of renal fibrosis and glomerulosclerosis, and downregulated the renal inflammatory response, associated with reduced proteinuria, despite only a partial reduction in systolic blood pressure. The authors considered this to suggest a blood pressure independent effect of spironolactone on renal fibrosis [redacted].

Six patients, with similar degrees of hypertension, were given oral doses of 300 mg spironolactone daily (75 mg four times a day) over a four week period. This study found that spironolactone treatment increased the urinary sodium:potassium ratio [redacted] In another study in hypertensive patients, spironolactone dosed at 50 mg, 100 mg or 200 mg led to increased renin release and aldosterone levels by disrupting the feedback loop [redacted]

2.4.2.2 Secondary Pharmacodynamics

Aldosterone regulates Na+ and K+ transport in a variety of epithelial locations, including the distal convoluted/ connecting tubules, salivary glands, and the gastrointestinal tract. Aldosterone has demonstrable epithelial and nonepithelial actions, and its blockade by spironolactone can both lower blood pressure as well as favourably influence altered structure/function relationships in the heart, kidney, and various vascular beds [redacted]

Structurally, spironolactone contains elements of the progesterone molecule, and although spironolactone displays reduced affinity for progesterone receptors, it may still show progestin-like side effects, i.e. mastodynia (breast pain) and disturbance of the menstrual cycle in women [redacted] Indeed, the SmPC for marketed spironolactone tablets (Aldactone, and also included in Spironolactone 10 mg/ml Oral Suspension SmPC) states that menstrual irregularities and breast pain may occur, as well as other effects which are detailed below.

Hyperkalaemia/Hyponatremia

Hyperkalaemia is a well-known and common electrolyte abnormality, where serum potassium are typically >5.0 mmol/L [redacted] Hyperkalaemia is associated with an increased risk of sudden death, partly explained by hyperkalaemia-induced cardiac arrhythmia. Hyperkalaemia could also contribute to peripheral neuropathy and cause renal tubular acidosis [redacted] Furthermore, undetected hyperkalaemia may be suspected as a possible cause of sudden death in some patients treated for heart failure with spironolactone and ACE inhibitors [redacted]

As described in the Aldactone SmPC (Aldactone SmPC), patient potassium levels should be monitored while receiving spironolactone and particularly patients with complex clinical situations, e.g. patients with chronic kidney failure (CKD). The pattern of serum K+ change in heart failure, was observed in the seminal RALES, and provides a good basis for understanding the risk-to benefit ratio for spironolactone therapy [redacted] See Clinical Overview Module 2.5 for further detail.

As such, the risk of hyperkalaemia is included in the SmPC section 4.4. Furthermore spironolactone is also contraindicated in patients with existing hyperkalaemia as in section 4.3 of the SmPC. Further discussion regarding the effects of co-administration of spironolactone with ACE inhibitors, as well as other medications is provided in section 2.4.2.4.

As compared with hyperkalaemia, cases of hyponatremia are rare following treatment with spironolactone. Sonnenblick et al analysed 129 reports of patients with diuretic induced severe hyponatremia (where serum sodium levels were below 115 mEq/L). Of these cases, only one patient developed hyponatremia following spironolactone treatment alone [redacted]

Gynaecomastia/Hormonal Disturbance

Spironolactone-associated gynaecomastia has been recognized for more than 40 years [redacted] Gynaecomastia, or enlargement of male breast tissue, defined as palpable dense and mobile subareolar tissue in the male breast, is due to the

proliferation of the glandular component of the male breast. Breast pain may also occur [redacted] . Gynaecomastia occurs in <1% of persons who take anti-hypertensive medications and in most cases the incidence is no more common than that seen with placebo [redacted]

However, gynaecomastia has become more pertinent in the past 5 years with the widespread use of spironolactone for patients with congestive heart failure or resistant hypertension. In the RALES investigation, gynaecomastia and/or breast pain was reported as an adverse event by 10% of men who received spironolactone at a dose of 25 mg/day [redacted] Spironolactone-associated gynecomastia is dose-dependent and has a reported incidence of up to 52% with the use of a dose of 150 mg/day. It is also related to duration of therapy and is usually reversible after discontinuation of the drug [redacted]

Several other endocrine side effects commonly reported with the use of spironolactone include impotence, decreased libido, and menstrual abnormalities/disturbances. Gynaecomastia and these other endocrine effects are the result of an alteration of the testosterone–oestrogen ratio in favour of oestrogen. Specifically, spironolactone blocks androgen production by inhibiting enzymes in the testosterone synthetic pathway (i.e., 17α-hydroxylase and 17,20-desmolase), blocks testosterone and dihydrotestosterone from binding to their receptors, increases serum free oestradiol by displacing oestradiol from sex hormone binding globulin, and increases peripheral conversion of testosterone to oestradiol. As such, these clinical findings are reported in the SmPC.

Effects due to fluid/electrolyte imbalance (depletion)

There are many common adverse effects that have been observed following treatment with spironolactone that may be due the desired effect of spironolactone as a diuretic, which in turn may lead to fluid imbalance. These effects include somnolence/dizziness/confusional state, nausea or a general disturbance to the gastrointestinal tract may be experienced. Other effects such as muscle spasms, malaise and drug fever have also been reported (Aldactone SmPC).

Pruritus/Rash/Urticaria

Rare cases of skin conditions have also been reported, following treatment with spironolactone. In 1988, Helfer et al prospectively evaluated 26 consecutive premenopausal women with idiopathic hirsutism during spironolactone treatment. Sixteen women initially received 100 mg spironolactone twice daily on days 4–21 of their menstrual cycles. Aside from metorrhagia and scalp hair loss, the only other cause of discontinuation in one woman was due to urticaria [redacted]

Severe cutaneous adverse reactions (SCARs), including Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN), and acute generalized exanthematous pustulosis (AGEP) have been reported in the literature following co-administration of spironolactone with pembrolizumab or sorafenib respectively. SJS/TEN typically present as a rapidly developing blistering exanthema of purpuric macules and target-like lesions accompanied by mucosal and skin detachment, in which SJS involves 30%. Although rare, they are potentially fatal, with a mortality rate of 10% for SJS, 30% for SJS–TEN overlapping, and 50% for TEN. SJS/TEN also commonly causes long-term sequelae of the skin and eyes. AGEP is characterized by a sudden eruption of mainly small non-follicular pustules on a background

of erythema with systemic involvement associated with fever and neutrophilia. The course is relatively benign, but 4% of AGEP cases develop to life-threatening situations [redacted]

In a skin patch-testing study, all the tests (including components of the vehicles) were negative, except for spironolactone, which gave a strong positive reaction. Ten controls in healthy volunteers were negative. The diagnosis of drug rash with eosinophilia and systemic symptoms (DRESS) induced by spironolactone was made. This was the first report of DRESS due to spironolactone topical treatment [redacted] described DRESS associated with spironolactone as uncommon with very few cases described in the literature [redacted]. DRESS is referred to in section 4.8 of the SmPC, with its frequency associated with oral administration of spironolactone unknown.

Acute kidney injury/Impaired Renal Function

A recent study investigated the association between baseline renal function and the net benefit of spironolactone in patients with heart failure with a preserved ejection fraction (HFpEF). This investigation analysed data from patients enrolled in the [redacted] study to examine the association between the baseline estimated glomerular filtration rate (eGFR) and the primary composite outcome of cardiovascular death, heart failure hospitalization, or aborted cardiac arrest, as well as safety outcomes, including hyperkalaemia, worsening renal function, and permanent drug discontinuation for adverse events (AEs). Results supported use of spironolactone to treat HFpEF patients with advanced CKD, but only when close laboratory surveillance is possible [redacted] However, significant renal compromise is a contraindication at section 4.3 of the SmPC.

In a study of 13,349 hospitalized medical patients monitored in a drug surveillance program, 788 (5.9%) received spironolactone during one or more admissions. Adverse reactions were attributed to spironolactone in 164 patients (20.8%). Hyperkalaemia was reported in 68 patients (8.6%). Frequency increased with the level of blood urea nitrogen (BUN) and reached 20.3% in patients with BUN values of ≥50 mg/100 mL. Of patients with BUN values of ≥50 mg/100 mL who received potassium chloride, 42.1% became hyperkalemic [redacted]

Abnormal hepatic function

In some patients with pre-existing cirrhosis, a reversible hyperchloremic metabolic acidosis has been reported, as detailed in section 4.4 of the SmPC. [redacted] reported six patients with alcoholic cirrhosis developed a reversible metabolic acidosis during treatment with spironolactone. Mean serum bicarbonate concentration decreased significantly with spironolactone therapy (100 to 200 mg/day). Upon withdrawal of spironolactone, serum bicarbonate concentration significantly increased. During the development of hyperchloremic metabolic acidosis, serum potassium concentration increased, although not significantly and this reversed after cessation of spironolactone therapy. Spironolactone treatment was not associated with significant alterations in serum creatinine or sodium concentration. Even though spironolactone treatment in cirrhotic patients may prevent hypokalaemia and rapid diuresis, it may also induce or worsen hyperchloremic metabolic acidosis [redacted]

Blood and lymphatic disorders

Similar to abnormal hepatic function, blood and lymphatic disorders are rare following spironolactone treatment. However some reports have been identified in the literature (see Clinical Overview Module 2.5), and blood disorders are detailed in section 4.8 of the SmPC. Cases of idiosyncratic agranulocytosis have been reported [redacted]

Gastrointestinal disorders

In a population based case-control study in the Netherlands, results showed that within the source population of 306,645 patients, 523 cases of gastric or duodenal ulcer or upper gastrointestinal bleeding were identified and matched to 5230 controls. Current use of spironolactone was associated with a 2.7-fold (95% confidence interval [CI] 1.2 to 6.0) increased risk of a gastrointestinal event [redacted]

2.4.2.3 Safety Pharmacology

Spironolactone is an old and well-established drug with extensive clinical safety data and, as such, safety pharmacology data have been superseded by clinical experience. However some relevant safety pharmacology studies have been identified in the literature and are reported herein.

In patients with NYHA class II to IV chronic heart failure (CHF), treatment with spironolactone (50 mg/day for month) led to significantly reduced indices of QT dispersion. The reductions in QTcmax, QTd and QTcd were greatest in the morning. In addition, spironolactone had favourable autonomic effects, which were limited to the morning as well, including heart rate reduction and an improvement in heart rate variability [redacted] These findings led to a study by [redacted] to further investigate the effect of spironolactone on Human Ether-à-go-go-Related Gene (hERG) currents. Using the whole-cell patch-clamp technique in stably transfected Chinese hamster ovary cells, hERG currents were evaluated. Treatment with spironolactone decreased hERG currents in a concentration-dependent manner (IC_{50}=23.0±1.5 μmol/L) and moved the midpoint of the activation curve to more negative potentials without modifying the activation and deactivation kinetics. Spironolactone (1 μmol/L) also induced blockade at the range of membrane potentials coinciding with that of channel activation, and thereafter, it remained constant. The metabolite of spironolactone, canrenoic acid also blocked hERG channels in a voltage- and frequency-independent manner and shifted the midpoint of the activation curve and accelerated the time course of channel activation. The results demonstrate that spironolactone and its metabolite canrenoic acid directly block hERG channels. However, it should be stressed that the experiments were carried out in the absence of aldosterone, and thus, the observed effects are not attributable to antagonism of its effects at the aldosterone receptor level. Furthermore, the authors state that the clinical implications of this have not been elucidated in the study [redacted] It should be noted that in clinical studies, spironolactone has been found to improve QT dispersion [redacted] In addition, a study in 2003 found that oral potassium and spironolactone therapy led to improvement in the treatment of patients with hERG mutations and long QT syndrome. The authors considered that the oral potassium therapy led to the beneficial effect. An indirect effect of aldosterone

blockade on myocellular repolarization may have been associated with the therapeutic effect, and increased serum potassium may exert secondary effects on other ion channels critical in modulating the cardiac action potential duration by altering the resting membrane potential of cardiomyocytes [redacted]

Using an olfactory fear conditioning (OFC) task, male Wistar rats were submitted to the OFC with different training intensities. Following high intensity OFC acquisition, a set of defensive coping strategies, including avoidance and risk assessment behaviours, was elicited when subjects were exposed to the conditioned stimulus (CS) 48 h later. In addition, following either OFC acquisition or retrieval (CS-I test), a profound corticosterone secretion was also detected. Systemic administration of spironolactone (up to 20 mg/kg, s.c.) altered the behavioural coping style irrespective of whether spironolactone was administered 60 min prior to the acquisition or before the retrieval session. In conclusion, the study suggested the involvement of the MR in the fine-tuning of behavioural adaptation necessary for optimal information storage and expression, as revealed by the marked alterations in the risk assessment behaviour [redacted]

The effect of spironolactone on anaesthetic agents is discussed in section 2.4.3.6. Furthermore, in section 4.8 of the Aldactone SmPC, a confusional state and libido disorders are noted. [redacted] identified the presence of MRs in the rat brain, which may be connected to effect of spironolactone in the CNS findings as described above [redacted] Libido disorders may be related to the progestogenic and antiandrogenic activity of spironolactone.

2.4.2.4 Pharmacodynamic Drug Interactions

As spironolactone has been used widely for many years, the drug interactions are well established, as detailed in particular in the SmPC Sections 4.4 and 4.5 and in Module 2.5.

It is stated at section 4.4 in the Aldactone SmPC and Spironolactone 10 mg/ml Oral Suspension SmPC that concomitant use of spironolactone with many other agents, listed as: other potassium sparing diuretics, ACE inhibitors, non steroidal anti-inflammatory drugs (NSAID), angiotensin II antagonists, aldosterone blockers, heparin, low molecular weight heparin or other drugs or conditions known to cause hyperkalaemia, potassium supplements, a diet rich in potassium, or salt substitutes containing potassium, may lead to severe hyperkalaemia. The impact of hyperkalaemia is discussed in section 2.4.2.2 on secondary PD.

Cardiac Glycosides

Digoxin is a mildly positive inotrope (at higher concentrations) that also increases vagal tone and suppresses renin secretion from the kidneys (at lower concentrations) [redacted] A kinetic and haemodynamic study of digoxin and spironolactone, performed in six healthy subjects, showed that spironolactone reduced renal tubular secretion of digoxin and attenuated its positive inotropic effect (evaluated by systolic time intervals and echocardiography). The authors suggested that the renal handling of digoxin was influenced by the intracellular potassium concentration in the renal tubular cell [redacted] The potential for interaction between spironolactone and digoxin is detailed at section 4.5 of the SmPC.

Trimethoprim/Sulfamethoxazole (Co-Trimoxazole)

The antibiotic trimethoprim reduces urinary potassium excretion by approximately 40%. The inhibition of potassium secretion results in a dose-related antikaliuretic effect that may predispose susceptible people to clinically important hyperkalaemia. In combination with sulfamethoxazole, trimethoprim is commonly used for the treatment of urinary tract infections. Because trimethoprim-sulfamethoxazole (co-trimoxazole) and spironolactone are both widely used drugs, the likelihood of co-prescription is high [redacted] This is reflected in the SmPC at section 4.5.

Carbenoxolone

Carbenoxalone is an established ulcer healing drug and is often co-administered with spironolactone to prevent symptoms of pseudo-hyperaldosteronism, a common adverse effect of carbenoxolone [redacted] In a report from 1968, it was identified that spironolactone possessed an antagonistic effect on the action of carbenoxalone. Ulcer healing was found to be impaired in patients treated with spironolactone and carbenoxolone [redacted] This is reflected in the SmPC at section 4.5 where it states that concurrent use of carbenoxolone and spironolactone should be avoided.

ACE Inhibitors

Since ACE inhibitors decrease aldosterone production, they should not routinely be used with spironolactone, particularly in patients with marked renal impairment, which is reflected at section 4.5 of the SmPC.

The impact of hyperkalaemia is discussed in section 2.4.2.2 and the introduction to 2.4.2.4.

Ammonium chloride and Colestyramine

As detailed in section 4.5 of the SmPC, hyperkalaemic metabolic acidosis has been reported in patients given spironolactone concurrently with ammonium chloride or colestyramine.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

As detailed at section 4.5 of the SmPC, NSAIDs such as aspirin, indomethacin and mefenamic acid may attenuate the natriuretic efficacy of diuretics due to the inhibition of intra-renal synthesis of prostaglandins and have been shown to attenuate the diuretic effect of spironolactone.

Noradrenaline

Spironolactone has been reported to reduce the vascular responsiveness to noradrenaline, as detailed at section 4.5 of the SmPC. (Aldactone SmPC).

Anaesthetics

It is recommended that potassium sparing diuretics, such as spironolactone should be avoided prior to surgery due to the possibility of the development of hyperkalaemia if renal perfusion is impaired or if there is tissue damage [redacted] Hyperkalaemia is discussed in detail above.

Additional interactions between anaesthetics and spironolactone are discussed in pharmacokinetic drug interactions in section 2.4.3.6.

2.4.3 Pharmacokinetics

2.4.3.1 Methods of Analysis

In publications where analytical methods are summarised, the methods include high performance liquid chromatography (HPLC), anion exchange, radio-isotopic dilution and spectrofluorometric techniques, along with thin-layer chromatography (TLC). Fluorimetry has been used to analyse spironolactone metabolites in plasma [redacted]

It has been reported that spironolactone, potassium canrenoate, and their common metabolite canrenone cross-react with the [redacted] used for the evaluation of digoxin levels. This can lead to falsely elevated serum digoxin concentrations. In contrast, serum digoxin concentrations were falsely lowered when the [redacted] was used. No interference was observed in the presence of these compounds when a [redacted] was used for digoxin measurement. Issues with [redacted] can be mostly eliminated by monitoring free digoxin concentration or by using the [redacted] digoxin assay [redacted] In section 4.5 of the SmPC, it is stated that in fluorimetric assays, spironolactone may interfere with the estimation of compounds with similar fluorescence characteristics.

2.4.3.2 Absorption

In studies conducted at Searle Laboratories, the absorption of [22-^{14}C]-spironolactone was compared in male Charles River rats, female Beagle dogs and female Rhesus monkeys. The drug was administered at the fixed dose of 5 mg/kg p.o and i.v. For each species, plasma was separated from blood within 1 hr after collection and urine and faeces collected for 6 days after drug treatment. Results showed the gastrointestinal absorption of [^{14}C]-spironolactone, as estimated from the p.o./i.v. ratio of the AUC for total ^{14}C, was 82% in the rat, 62% in the dog, and 103% in the monkey. The absolute bioavailability of the metabolite, canrenone, estimated from the 8-hr AUC was 57% in the dog and 48% in the monkey. The blood volume collected from the rat was too small to allow analysis of the extractable radioactivity and canrenone as shown in Table 2.4.2 [redacted]

Table 2.4.2. Peak Plasma Concentrations and Bioavailability of Spironolactone Metabolites in the Rat, Dog, Monkey and Human.

Parameters	Species			
	Rat[a]	**Dog**[a]	**Monkey**[a]	**Man**[b]
Dose (mg/kg)	5	5	5	2.9
Peak plasma concentrations (µg/mL) (time to reach peak levels, hr)				
Total radioactivity	0.56±0.23 (2.0)	2.07±0.32 (0.5)	4.75 ±0.82 (2.0)	2.05 ±0.06[c] (24)
Extractable	d	1.33 (1.0)	1.63 (1.0)	0.66±0.08 (2.5)
Canrenone	d	0.36 (1.0)	0.25 (2.0)	0.40±0.05 (2.5)
AUC (µg/mL) x hr, oral				
0-24 hr total radioactivity	5.75±1.06	16.0±2.04	41.6±1.91	43.7±3.32
0-24 hr extractable	d	11.5	11.9	8.04±1.18
0-8 hr Canrenone	d	1.56	1.03	0.14
AUC (µg/mL) x hr, i.v.				
0-24 hr total radioactivity	6.98±1.46	26.0±1.80	40.3±1.32	
0-24 hr extractable	d	21.0	13.2	
0-8 hr Canrenone	d	2.72	2.14	
Bioavailibility (%)				
From plasma level data[e])				
Total radioactivity	82	62	103	
Canrenone	d	57	48	
From urinary excretion data[f]				
Total radioactivity	63	82	100	
Canrenone	102	42	119	
Total fluorogenic metabolites	91	68	117	

a Data in the dog and monkey are averages ± standard error for three animals. In the rat, plasma level data are averages of three animals and urinary excretion data averages of five animals.
b Data from previously published in which 200 mg of [20-3H]spironolactone were administered p.o. to five healthy men (average weight 68.5kg)
c A substantial quantity of the radioactivity in the serum of men was associated with tritiated water
d Blood volume was too small to allow analysis of the extractable radioactivity and canrenone
e AUC after oral dose/AUC afver i.v. dose x 100
f six-day excretion (% of dose) after oral dose/Six day excretion (% of dose) after i.v. dose x 100.
Taken from [redacted]

The disappearance of radioactivity from the plasma of all species occurred in at least two phases. The second (log-linear) phase half-life of radioactivity after the p.o. dose was 10.6, 26.2, and 16.0 hr in the rat, dog, and monkey, respectively. The plasma half-life of the metabolite canrenone also occurred in two phases. Its log-linear phase half-life was 13.5 hr in the dog (p.o. study) and 25.9 hr in the monkey (i.v. study) [redacted]

Spironolactone is poorly soluble in aqueous fluids and an i.v. formulation is not available for routine clinical use. The absolute bioavailability of spironolactone has not been determined in humans; however, mass balance experiments would suggest that the extent of absorption is in the 80–90% range for some but not all commercial preparations [redacted]

The onset of action for spironolactone is typically very slow, with a peak response sometimes occurring 48 hours or more after the first dose. This correlates with it taking several days of spironolactone dosing for its active metabolites to reach steady-state plasma levels. The natriuretic effect of spironolactone gradually decreases over a period of 48 to 72 hours. The duration of spironolactone effect may differ relative to natriuresis and antikaliuresis, as

antikaliuresis can persist for several days following discontinuation of spironolactone [redacted]

In healthy human volunteers taking spironolactone (100 mg/day) for 15 days, the mean half-lives ($t_{1/2}$) for spironolactone and its metabolites, canrenone, 7α-TMS and 6ß-hydroxy-7α-TMS were 1.4, 16.5, 13.8, and 15 hours, respectively. Thus, although unmetabolised spironolactone is present in serum, it is quickly cleared [redacted] Spironolactone is used in cirrhotic patients to induce natriuresis and in heart failure as a component of two or three-drug diuretic therapy. In cirrhotic patients, pharmacokinetic studies indicate that the $t_{1/2}$ of spironolactone and its metabolites are increased. The $t_{1/2}$ for spironolactone, canrenone, 7α-TMS and 6ß-hydroxy-7α-TMS are 9, 58, 24, and 126 hours, respectively. This demonstrates that the metabolism and excretion of spironolactone and its metabolites were impaired in cirrhotic patients [redacted]

The effect of food on the bioavailability of spironolactone has been evaluated. The influence of food intake on the bioavailability of canrenone, a metabolite of spironolactone, was explored in 8 healthy male volunteers. Spironolactone was administered as a single oral dose of 100 mg, both in the fasting state and together with a standardised breakfast. Results indicated that more canrenone is in the general circulation when spironolactone is ingested together with a meal [redacted] It should be noted that the major metabolite of spironolactone is 7α-TMS rather than canrenone (section 2.4.3.4). More recent studies found that food increased the absorption of spironolactone and possibly decreased its first-pass metabolism; however, administration of spironolactone with food in hypertensive patients results in similar therapeutic effects as when the drug is given in a fasted state [redacted]

2.4.3.3 Distribution

The distribution of [20-^{3}H]-spironolactone in the mouse was examined using autoradiography (quantitative data were not available for this method). Animals received an injection of [20-^{3}H]-spironolactone, after which they were sacrificed at 1, 30, 60, or 120 min. High levels of radioactivity were seen in the proximal segment of the small intestine, hepatic bile ducts, and gall bladder. Moderate uptake of radioactivity was also seen in the liver and kidneys [redacted]

In studies conducted at Searle Laboratories, the distribution of [22-^{14}C]-spironolactone was evaluated in male Charles River rats at a dose of 5 mg/kg p.o and i.v. Results showed uptake of total ^{14}C and canrenone in the selected tissues of the male rats at 1 hr after administration of [^{14}C]-spironolactone. Following oral administration, the ^{14}C concentrations in the liver, adrenals, and kidneys were approximately 11-, 6-, and 2-fold, respectively, higher than that in plasma. However in the heart and testes, they were similar to plasma levels. Between 55 and 92% of the radioactivity was extractable with ethyl acetate (pH 3) from the homogenates of all organs except the liver, where the extractability was between 30 and 40%. With the exception of the heart, the concentration of canrenone was similar after p.o. or i.v. administration. In the plasma, the extractable radioactivity was mostly due to canrenone, whereas more polar metabolites were present in the tissues in amounts higher than those of canrenone (Table 2.4.3; [redacted]

Table 2.4.3. Extraction of Radioactivity from the Rat and Percent Canrenone in the Extractable Fraction at 1 hr post dosing of [22-^{14}C]-spironolactone

Tissue or Fluid	Route	Concentration of Total ^{14}C (µg/g or ml)	T/P[a] Total ^{14}C	% of ^{14}C Extractable with Ethyl Acetate (pH3)[b]	% of Canrenone in Ethyl Acetate Extract[b]	Concentration of Canrenone (µg/g or ml)	T/P[a] Canrenone
Plasma	i.v.	0.82	1.00	65	54	0.29	1.00
	p.o.	1.19	1.00	66	39	0.31	1.00
Liver	i.v.	7.13	8.70	42	16	0.48	1.66
	p.o.	12.64	10.62	33	15	0.63	2.03
Adrenals	i.v.	4.01	4.89	90	32	1.15	3.97
	p.o.	6.89	5.79	92	18	1.14	3.68
Kidneys	i.v.	1.72	2.10	59	34	0.35	1.21
	p.o.	2.47	2.08	73	24	0.43	1.39
Heart	i.v.	0.79	0.96	88	59	0.41	1.41
	p.o.	1.22	1.03	65	24	0.19	0.61
Testes	i.v.	0.98	1.20	56	18	0.10	0.34
	p.o.	1.08	0.91	63	18	0.12	0.39

a T/P = Concentration in tissues µg/g / Concentration in plasma (µg/ml)
b The extraction efficiencies of radioactivity (mean ± standard error, n=3) when [^{14}C]canrenone was added to the control plasma and tissue homogenates were as follows: plasma, 82±0.7%; liver, 89±0.3%; adrenals, 92±0.6%; kidneys, 90±0%; heart, 90±0%; testes, 86±0.9%. TLC analysis showed that between 86 and 87% of the extractable radioactivity in all cases was canrenone.
Taken from [redacted]

Plasma and tissue concentrations of total-^{14}C at 1 and 24 hr after 5 mg/kg of [22-^{14}C]-spironolactone (i.v. and p.o.) were evaluated in male rats. One hour after i.v. injection, the highest level of radioactivity was in the gastrointestinal tract. Total ^{14}C concentrations were between 2 and 9 times higher in the testes, kidneys, adrenals, and liver, compared to that in the plasma, while total ^{14}C concentrations in the skeletal muscle, heart, lungs, and salivary glands were similar to plasma, and in the brain and thymus, the ^{14}C concentrations were lower than that in plasma. The 1 hr tissue-to-plasma concentration ratios were similar to those at 24 hr in all tissues except for the gastrointestinal tract, salivary glands and adrenals. Evidence for retention of radioactive materials was obtained only in the adrenals (Table 2.4.4). The nature of metabolites identified in the plasma and the endocrine organs was also examined. Canrenone was the major extractable constituent in the plasma and although canrenone was still detected in the tissues, large amounts of at least six other metabolites more polar than canrenone were also present. The proportion of these polar metabolites relative to canrenone was low in the kidneys and heart, and high in the liver, testes, and adrenals [redacted]

Table 2.4.4. Tissue-to-Plasma Concentration Ratios[a] of Total-^{14}C Materials in Male Rats at 1 and 24 hr Following 5 mg/kg Ora1 or Intravenous Doses of [20-^{14}C]-spironolactone

	Time after drug administration			
	Oral		Intravenous	
Tissues or Fluid	**1 hr**	**24 hr**	**1 hr**	**24 hr**
Brain	0.50	0.31	0.46	0.29
Skeletal Muscle	0.74	0.79	1.6	0.70
Thymus	0.68	0.36	0.59	0.35
Blood	0.75	0.98	0.66	0.55
Testes	1.1	0.77	2.0	0.71
Heart	1.2	0.62	1.2	0.54
Kidneys	3.8	3.0	2.7	2.8
Lungs	2.6	0.99	1.3	0.65
Salivary Glands	3.1	4.9	1.7	3.7
Spleen	3.5	0.88	0.95	1.0
Adrenals	8.1	11.0	4.4	8.2
Liver	19.0	13.0	8.4	7.4
Stomach	50.0	1.1	11.0	2.8
Small intestine	47.0	29.0	40.0	21.0
Large intestine	9.1	1.5	68.0	43.0

a Tissue-to-Plasma concentration ratio = Total-^{14}C concentration (µg/g) in tissue / Total-^{14}C concentration (µg/ml) in plasma

Each value is a mean from three animals.
Taken from [redacted]

Another study also investigated the distribution of spironolactone in the rat, where [20-^{3}H]-spironolactone was administered intravenously and liver analysed one hour post injection. Highest radioactivity levels were found in the microsomal fraction of the liver, with significant radioactivity levels also seen in the nucleus, mitochondria, and lysosomes (cited from [redacted]

The tissue distribution of total radioactivity in the rat at 1 and 24 hr after a single i.v. dose of 10 mg/kg dose of [20-^{3}H]-potassium canrenoate was found to be substantial in the liver, kidneys, and adrenals, where tissue-to-blood concentration ratios at 1 and 24 hr in the single dose study were: liver, 12.7 and 15.4; kidneys, 5.6 and 13.0; and adrenals, 7.3 and 11.7. In a multiple-dose study where i.v. dose of 10 mg/kg dose of [20-^{3}H]-potassium canrenoate was given once a day for 4 days, the ratios at 4 and 24 hr after the final dose were: liver, 14.7 and 12.3; kidneys, 11.3 and 14.4; and adrenals, 9.1 and 11.9 [redacted]

[redacted] studied the tissue distribution of potassium canrenoate and its metabolites in the dog. One hour after i.v. administration of 20 mg/kg of [22-^{14}C]-potassium canrenoate, levels of radioactivity in the skeletal muscle, fat, and liver accounted for 50 to 60% of the injected radioactivity. The ^{14}C concentration in most tissues at 1 hr after injection was similar to the concentration in the plasma. However, in the kidneys, liver, fat, and adrenals, there was some accumulation. In most tissues, canrenone was the major radioactive constituent, but more polar metabolites were found, particularly in the liver, kidneys, adrenals, and testes [redacted]

2.4.3.4 Metabolism

[redacted]

Despite some of the early erroneous findings, it remains relevant to note that these early studies also identified differences in the metabolic pathways of spironolactone between species [redacted]

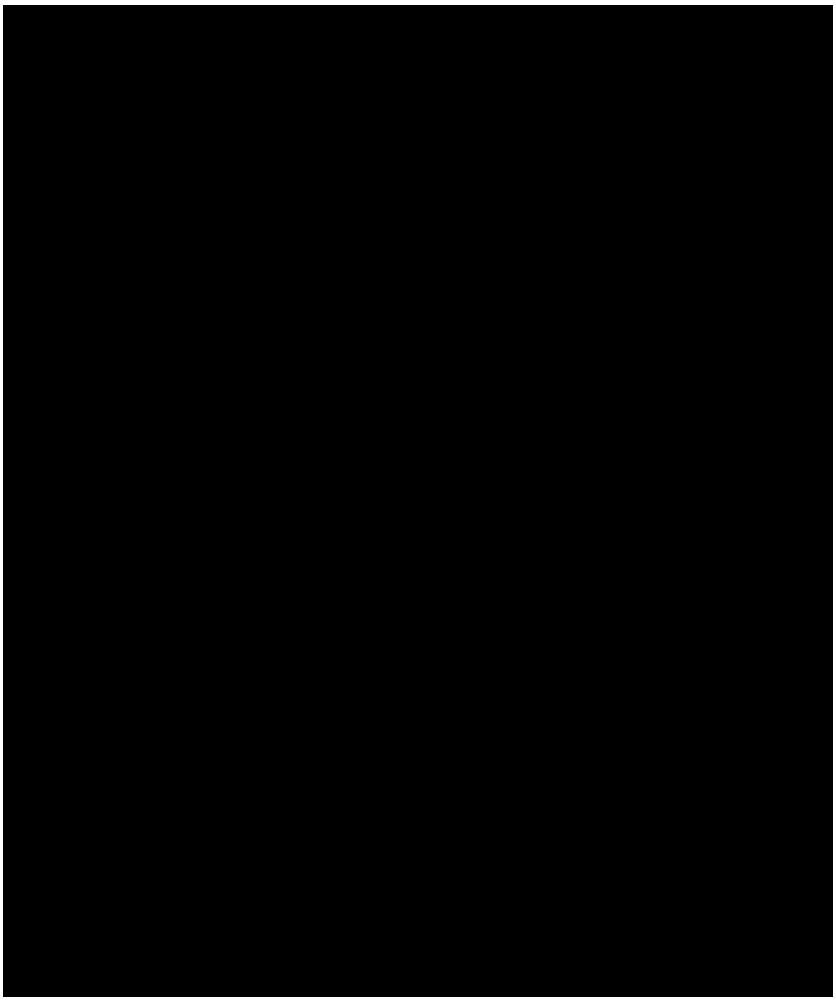
The disposition of [^{14}C]spironolactone was studied in male rats, female dogs and female monkeys after i.v. or oral administration of 5 mg/kg bw. Gastrointestinal absorption was estimated to be 82% in rats, 62% in dogs and 103% in monkeys, as described in section 2.4.3.2. In all animals tested, spironolactone was extensively metabolized and the metabolites were excreted primarily in the urine and faeces [redacted] However, species differences were also noted in the biotransformation of spironolactone.

In vitro guinea pig hepatic and renal microsomal preparations showed that spironolactone was rapidly converted to 7α-TMS as the only metabolite. However, *in vivo*, in the guinea pig, both 7α-TMS and canrenone were identified as circulating metabolites [redacted]

Canrenone was the principal extractable metabolite in the rat [redacted] 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 investigated species, canrenone was a principal constituent. Notable species differences in the metabolites of spironolactone in the faeces were found, where the pattern of metabolites in dog faeces was different from that in rats, monkeys or humans. The disposition and metabolism of spironolactone in monkeys was found to be the closest to that in humans [redacted]

Spironolactone undergoes enterohepatic recirculation, but no unchanged drug appears in [redacted]

[redacted]



From [redacted]

Overall, spironolactone has several active metabolites, which vary in quantity across species [redacted] The toxicological effects of spironolactone as well as potassium canrenoate have been reported in section 2.4.4. As discussed above, canrenone and canrenoate are in equilibrium with one another. Canrenone undergoes hydrolysis of its γ-lactone ring to canrenoate, and is reformed by lactonization of the γ-hydroxy acid.

It is now considered that it is the longer half-life of 13-17 hours of these spironolactone metabolites that provide the activity, as compared to the short half-life of spironolactone itself (less than 2 hrs). Due to the long half-life of these metabolites, spironolactone itself has been considered a prodrug [redacted]

Cytochrome P450

In older studies reported [redacted] spironolactone was found to be metabolized by microsomal monooxygenases and converted to a reactive metabolite that destroyed microsomal cytochrome P450 (CYP) and decreased steroid hydroxylase activity. This was only seen in animals that produced cortisol rather than corticosterone and in those that had high activity of the adrenal steroid, 17α-hydroxylase [redacted] In studies where spironolactone was incubated with guinea-pig hepatic, adrenal, renal or testicular

microsomal preparations, 7α-thiospironolactone was produced which destroyed adrenal and testicular CYP. In guinea-pig microsomes, 7α-thiospironolactone was an obligatory intermediate in the action of spironolactone on adrenal monooxygenases, but required further metabolism for its toxicity. However, hepatic microsomal CYP in guinea-pigs was not inhibited by spironolactone, apparently because 7α-thiospironolactone was not further metabolized [redacted] Species differences in the effects of spironolactone on CYP showed inhibition in adrenal microsomes from guinea-pigs and dogs, but not in adrenal microsomes from rats or rabbits [redacted] Spironolactone was reported to inactivate dexamethasone-inducible rat hepatic CYP in a suicidal manner [redacted] In guinea-pig adrenal glands, a good correlation was found between covalent binding and CYP destruction, which was consistent with the hypothesis that 7α-thiospironolactone is a suicide inhibitor of adrenal CYP and that covalent binding to protein is involved in the degradation of these isozymes [redacted]

Further information regarding spironolactone acting as an inducer of hepatic microsomal drug metabolizing enzymes is provided in section 2.4.3.6 on pharmacokinetic drug interactions.

2.4.3.5 Excretion

In studies conducted at Searle Laboratories, the excretion of [22-^{14}C]-spironolactone was compared in male Charles River rats, female Beagle dogs and female Rhesus monkeys. [22-^{14}C]-spironolactone was administered at 5 mg/kg p.o. or i.v.

Results showed urinary excretion of either the radioactivity or the fluorogenic metabolites was lowest in the rat and highest in the monkey, irrespective of the route of administration (Table 2.4.5). The oral bioavailability of spironolactone determined from urinary excretion of total ^{14}C in the three species was 63% (rat), 82% (dog), and 100% (monkey). The cumulative average excretion of radioactivity in the urine as percentage of the oral dose in 6 days was 4.69% in the rat, 18.5% in the dog, and 46.0% in the monkey (Table 2.4.5) [redacted].

About half of the radioactivity in the urine of all species was extractable with chloroform at pH 3, and between 19% and 28% of this radioactivity in all species was due to canrenone. The remainder of the extractable radioactivity was distributed among several more polar metabolites. The percentage of the water-soluble conjugated metabolites that was hydrolysed on alkaline treatment was low in the urine of the rat and dog, but high in that of the monkey. Canrenone and a new polar metabolite (R_F 0.68) were the principal aglycones released from monkey urine, which constituted approximately 35 and 23%, respectively, of the hydrolysed fraction. In dog urine, 10% of the radioactivity in the hydrolysed fraction was due to canrenone and the remainder distributed among several more polar metabolites. In rat urine, canrenone constituted only 2%, with the remainder being a highly polar material that remained near the origin of the TLC plate and was the principal constituent of the hydrolysed fraction [redacted]

Table 2.4.5. Summary of Excretion of Radioactivity and Fluorogenic Metabolites in the Urine and Faeces of the Rat, Dog, Monkey and Human After Administration of Radiolabelled Spironolactone

Time after drug administration	Oral dose				Intravenous dose		
	Rat[a] (n=5)	Dog (n=3)	Monkey (n=3)	Human (n=5)	Rat[a] (n=5)	Dog (n=3)	Monkey (n=3)
				% of dose			
Urine							
0.24hr							
A[b]	0.49±0.07	0.59±0.14	5.58±0.77	3.22±0.90	0.44±0.06	1.41±0.23	4.53±1.12
B[b]	0.72±0.10	1.39±0.29	6.24±0.86	4.55±0.79	0.55±0.07	1.85±0.25	5.73±0.82
C[b]	0.76±0.10	1.48±0.29	12.01±1.95	8.03±0.90	0.61±0.07	2.02±0.28	10.14±2.86
D[c]	4.20±0.14	9.23±0.27	41.3±6.54	19.2±1.51	6.49±0.60	10.74±1.17	39.10±6.32
0-6 days							
A[b]	0.65±0.08	0.82±0.15	5.86±0.76	4.43±1.17	0.61±0.06	1.95±0.23	4.92±0.91
B[b]	0.94±0.13	1.77±0.34	6.42±0.83	6.02±1.06	0.92±0.06	2.61±0.23	6.22±0.63
C[b]	1.13±0.15	1.91±0.34	12.14±2.09	12.19±1.45	1.18±0.08	2.82±0.27	10.41±2.76
D[c]	4.69±0.17	18.51±0.59	46.03±6.39	31.6±2.63	7.46±0.64	22.54±1.92	46.33±5.14
Faeces							
0-3 days							
D[c]	73.96±2.73	66.00±4.64	33.70±5.00	16.80±5.24	89.14±2.08	59.90±1.41	48.90±0.61
E[d]	43.3	22.5	13.4	16.5[f]	50.1	19.1	16.3
F[e]	5.1	1.9	1.1	1.8[f]	3.9	1.0	0.5
0-6 days							
D[c]	74.19±2.73	69.25±3.55	40.10±4.10	22.7±6.31	90.18±2.09	65.93±0.89	55.27±1.30

Animals received a 5 mg/kg dose of [22-^{14}C]spironolactone orally (in solution) and i.v. Human data were taken from previously described study. Data represent means ±standard error.

a After 6 days<0.1% of the administered radioactivity remained in the carcass of the rat after oral and 0.91± 0.09% after i.v. administration.
b Method A measures canrenone (VI), B measures VI + canrenoic acid (VIII), and C measures VI+VIII+canrenoate and/or other fluorogenic ester conjugates.
c Total radioactive materials.
d Chloroform-extractable radioactive materials from 0-3 day pooled faeces. The extraction efficiency (mean±standard error, n=3) of radioactivity when [^{14}C]canrenone was added to control rat faeces was 76 ± 0.3%, and >80% of this radioactivity was due to canrenone.
e Materials with R_F of spironolactone and canrenone in 3-3 day pooled faeces.
f This value was obtained from one subject. Taken from [redacted]

Results from faecal excretion showed that the highest proportion of radioactivity was excreted in the faeces of the rat and lowest in the monkey, irrespective of the route of administration. The corresponding excretion values were 74.2 (rat), 69.3 (dog) and 40.1 % (monkey). The high excretion of radiolabel in the faeces of rats (90%) after intravenous administration shows the importance of biliary excretion for that species, as shown in Table 2.4.5 [redacted] Approximately half of the radioactivity in 0- to 3-day pooled faeces of different species was extractable with chloroform, and compounds with the R_F of spironolactone and of canrenone represented less than 6% of the administered dose in every case with either route of administration. The extractable radioactivity in the faeces of the rat and monkey contained a complex mixture of several metabolites with widely different polarities. Most of them were highly polar and remained at or near the origin of the TLC plate. The extractable faecal radioactivity in the dog mainly contained a material that was slightly more polar (R_F 0.65) than canrenone (R_F 0.78). Further studies have shown this material to contain at least three closely related compounds in which the Δ^4-3-oxo group of spironolactone had been reduced [redacted]

Since the total recovery of radioactivity in the rat was less than 100% (Table 2.4.5), the radioactivity that remained in the carcass after 6 days was examined. Irrespective of the route of administration, less than 1% of the radioactive dose was found to be in the carcass [redacted]

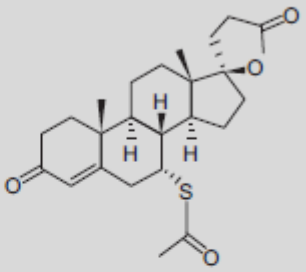
In summary, spironolactone was extensively metabolized in rats, dogs and monkeys, although differences existed in the composition of the metabolites in their plasma, urine and faeces. The amount of radioactivity that was excreted in the urine and faeces was not affected by route of administration. In monkeys, as in humans, the amounts excreted in urine and faeces were approximately equal, while faecal excretion predominated in rats and dogs as a result of biliary excretion. Comparison of animal data with those published for humans indicated that the disposition and metabolism of spironolactone in the rhesus monkey, rather than those in the rat or the dog was closest to that in human [redacted]

The excretion of radioactive metabolites in the urine and faeces of healthy human subjects was examined after orally administering 200 mg of [20-^{3}H]-spironolactone to five subjects. Averages of 31.6% (range 23 to 38%) and 22.7% (range 2.6 to 41.1%). of the radioactivity were excreted in the urine and faeces, respectively [redacted] This is similar to [redacted] who found an average of 52.8% (range 47 to 58%) of the radioactivity to be excreted in the urine of five human male subjects and 36.2% (range 35 to 38%) in the faeces of two subjects after 500 mg oral dose of [20-^{3}H]-spironolactone. The fractionation of urinary metabolites showed approximately half of the urinary radioactivity to be in the form of chloroform extractable materials that contained canrenone (5.0% of dose) and 6 P-OH-sulfoxide (6.2% of dose) as the major metabolites. The principal water-soluble metabolite was canrenoate ester glucuronide (6.2% of dose). 6β-OH-7α-methylsulfonyl-spirolactone was also identified as the major lipophilic urinary metabolite by [redacted] Another study by the same authors demonstrated that the radioactivity detected in faeces was not due to the poor oral absorption of [20-^{3}H]-spironolactone, but to the biliary excretion of spironolactone metabolites. After administering 300 mg of [20-^{3}H]-spironolactone orally to eight patients with biliary fistulas, 5.4 to 33% of the radioactivity was recovered in the bile in 4 days. Enterohepatic cycling of spironolactone

metabolites was demonstrated, and differences were noted in the composition of the urinary metabolites between normal subjects and patients with biliary fistulas [redacted]

The table below provides a summary of the pharmacokinetics of spironolactone.

Table 2.4.6. Pharmacokinetic Summary of Spironolactone

	Spironolactone
Structural features	Based on progesterone; γ-lactone ring as substituent at C-17 
Oral bioavailability	80–90%
Plasma protein binding	88% (bound to albumin) [56]
$T_{1/2}$ (h), parent drug	1.3–2
Metabolic pathways	Hepatic, deacetylation and dethiolation
Active metabolites	7α-Thiomethylspironolactone and canrenone
$T_{1/2}$ (h), active metabolites	16.5 (canrenone) 13.8 (7α-thiomethylspironolactone) 15.0 (6β-hydroxy-7α-thiomethyl spironolactone)
CYP enzyme inducer	Yes
Tissue distribution (based on quantitative whole-body autoradiography in rodents)	Renal concentration six-fold higher than cardiac concentration

Taken from [redacted]

2.4.3.6 Pharmacokinetic Drug Interactions

As discussed above, spironolactone possesses activity as an inducer of hepatic microsomal drug metabolizing enzymes. This and other pharmacokinetic drug interactions are detailed in this section.

Anaesthetics (local or general)

As detailed at section 4.5 of the SmPC, caution should be exercised in the management of patients subjected to regional or general anaesthesia while they are being treated with Spironolactone.

In the [redacted] the following study by [redacted] was summarised and it was stated that:

> *"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".*

Solymoss and colleagues described studies in the rat, where pretreatment with spironolactone, norbolethone, or ethylestrenol increased the oxidation of pentobarbital by liver microsomes, enhanced its disappearance from blood and proportionally decreased the depth of anaesthesia [redacted]

In male and female Swiss mice, a single dose of spironolactone (100 mg/kg i.p.) decreased hexobarbital (120 mg/kg i.p.) induced sleeping time and increased substrate metabolism, liver weights and CYP content. The authors suggested that spironolactone was exerting its effects via increasing activity of drug metabolising microsomal enzymes and electron transport. The results of kinetic studies suggested that the activity of spironolactone may be mediated by increased amount of enzyme rather than changes in the affinity of the enzyme [redacted]

Antipyrine (phenazone)

As detailed at section 4.5 of the SmPC, spironolactone enhances the metabolism of antipyrine.

Antipyrine (phenazone) is a NSAID and has been linked to spironolactone with respect to an enhanced rate of metabolism and excretion of antipyrine when co-administered with spironolactone. In a 1973 study, spironolactone treatment was associated with a decrease in the plasma antipyrine half-life in all 9 volunteers studied. This was associated with an increased excretion of 4-OH antipyrine, but not 3-hydroxymethyl antipyrine in their urine. The plasma antipyrine disappearance rate correlated with the excretion rate of 4-OH antipyrine in the urine. Spironolactone also increased the excretion of 6-βOH cortisol in the urine. These data provided indirect evidence that spironolactone is an inducer of hepatic drug hydroxylation in humans [redacted]

Opioids

Co-administration of spironolactone with opioids was conducted to investigate whether spironolactone enhanced the antinociceptive effect of opioids via pharmacodynamic and pharmacokinetic mechanisms. The effect of spironolactone (100 mg/kg, i.p.) on acute oxycodone-induced (0.75 mg/kg, s.c.) and morphine-induced (3 mg/kg, s.c.) antinociception using tail-flick and hot plate tests in male Sprague Dawley rats was evaluated. Spironolactone was administered 30 min. before the opioids, and behavioural tests were performed 30 and 90 min. after the opioids. In the hot plate test at 30 and 90 min., spironolactone significantly enhanced the antinociceptive effect (% of maximum possible effect) of oxycodone from 10% to 78% and from 0% to 50%, respectively, and that of morphine from 12% to 73% and from 4% to 83%, respectively. Spironolactone alone did not elicit any antinociceptive effects. The results suggest that spironolactone enhanced the antinociceptive effect of both oxycodone and morphine by increasing their concentrations in the CNS, quantified by mass spectrometry [redacted]

Lithium salts

Lithium has long been used and is one of the most effective mood stabilisers for people with a mood disorder [redacted] However, reports of interactions between spironolactone and lithium have been identified in the literature for over 30 years. Indeed reports in the literature state that spironolactone reduces the clearance of lithium and concomitant use is not recommended [redacted]). This is reflected in the SmPC at section 4.5, where it states that concurrent use of lithium salts and spironolactone should be avoided.

2.4.3.7 Other Pharmacokinetic Interactions

Spironolactone (200 mg/kg bw/day for 14 days) induced hepatic Uridine 5'-diphospho-glucuronosyltransferase (UDP)-glucuronosyltransferase (UGT) activity in rats [redacted] Spironolactone was found to be a more specific and effective inducer of hepatic microsomal bilirubin UGT activity in rats than phenobarbital [redacted] Spironolactone induced β-glucuronidase activity in the liver of female rats [redacted].

The effect of spironolactone pretreatment on glutathione S-transferase activity and on the relative content of the principal subunits (Ya, Yc, Yb1, Yb2 and Yp) was studied in rat liver, jejunum and colon. Male Wistar rats were given spironolactone i.p. at daily doses of 50, 100 or 200 µmol/kg for 3 consecutive days. Results showed a dose-dependent increase in enzyme activity in liver only. Immunoblot analysis revealed more Ya and Yp protein in liver (140 and 118 % increase respectively) and jejunum (45 and 145 % increase respectively) from treated rats. The authors concluded that spironolactone increased glutathione S-transferase activity mainly by induction of the Ya subunit in liver and Yp subunit in jejunal mucosa [redacted]

2.4.4 Toxicology

Spironolactone has a comprehensive safety profile, as the clinical use of spironolactone has been well-established for several decades, and its pharmacology and toxicology has been extensively tested. It is known that the mode of action is as an antagonist of aldosterone at the MR, giving rising to a potassium-sparing diuretic pharmacological activity. Off-target effects are also discussed in this section, as well as in section 2.4.2.

2.4.4.1 Single Dose Toxicity

Single dose toxicity data following administration of spironolactone are summarized in the table below (Table 2.4.7):

Table 2.4.7. Spironolactone Single Dose Toxicity Studies Summary

Study	Species/Sex/Number	Dose/Route	LD_{50} (mg/kg)
[redacted]	Mouse, NS	p.o.	>1000
	Rat, NS	p.o.	>1000
	Rabbit, NS	p.o.	>1000
	Mouse, NS	i.p.	356 ± 93.6
	Rat, NS	i.p.	786 ±125.4
	Rabbit, NS	i.p.	866 ± 155.6

LD_{50} Lethal dose sufficient to kill 50% of the population
i.p. Intraperitoneal
p.o. *per os (L*; by mouth)
NS Not stated.

2.4.4.2 Repeat Dose Toxicity

Repeat dose toxicity studies following administration of spironolactone are summarized in the table below (Table 2.4.8), with each study summarized further in text below the table:

Table 2.4.8. Spironolactone Repeat Dose Toxicity Studies Summary

Study	Species/Sex/Number	Dose/Route	Duration	Noteworthy Findings
[redacted]	Rat, Male n=10 /group	6, 50, 200 mg/kg bw/day, dietary.	13 weeks	↑ thyroid gland weight, ↑concentration of TSH, but ↓ T3 and T4 concentrations, histologically, the follicles were smaller, with diminished colloid, epithelial cells were taller with some swollen, ↑liver weight and UGT activity, ↑ thyroid gland weight
[redacted]	Rat, Sprague Dawley n=20-25 / sex/group	120, 300, 700 ppm for first 3 weeks, then 150, 500 or 2000 ppm for weeks 4-26, dietary	Up to 26 weeks	↑liver weight, thyroid gland weight, histologically, the follicles were smaller, with diminished colloid, epithelial cells were taller with some swollen
[redacted]	Rat, Sprague Dawley n=36-72 / sex/group	50, 150, 500 mg/kg bw/day, dietary	78 weeks	↑liver weight, adrenal gland weight, thyroid gland weight, histologically, the follicles were smaller, with diminished colloid, epithelial cells were taller with some swollen ↓prostate weight, slight arrest of testis maturation
[redacted]	Rat, Sprague Dawley n=30 / sex/group	10, 30, 100 mg/kg bw/day, dietary	104 weeks	↑liver weight, thyroid gland weight, histologically, the follicles were smaller, with diminished colloid, epithelial cells were taller with some swollen
[redacted]	Dog, Beagle, n=2/sex/group	12, 30, 70 mg/kg bw/day for 6 weeks then 12, 30, 100 mg/kg bw/day for weeks 7-9, then 12, 30, 250 mg/kg bw/day for weeks 10-13	Up to 13 weeks	There were no significant findings

Study	Species/Sex/Number	Dose/Route	Duration	Noteworthy Findings
[redacted]	Marmoset, Callithrix jacchus	30, 100 mg/kg bw/day	4 weeks	Follicular cell hypertrophy and ↓T4 concentration; ↑hepatic CYP and T4-UGT activity
[redacted]	Monkey, *Macaca mulatta* n=6-12 /group, males only	125 mg/kg bw/day	26 weeks	There were no significant findings
[redacted]	Monkey, *Macaca mulatta* n=4/sex/group	20, 50, 125 mg/kg bw/day for first 9 weeks, then 20, 50, 250 mg/kg bw/day for weeks 10-52	Up to 52 weeks	↑ liver weight in males, ↓ testis weight and depression of maturation, ↑cellular activity in acini of mammary gland

NS Not stated , TSH Thyroid stimulating hormone

Subchronic Toxicity Studies

Male Sprague Dawley rats were given diets containing spironolactone at concentrations resulting in doses of 0, 6, 50 or 200 mg/kg bw per day (n=30/group) 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 of thyroid, thyroid iodine uptake and organification, and UGT activity. After 13 weeks of treatment, thyroid gland weights 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 was 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 UGT activity were significantly increased. The authors suggested that the transient increase in TSH and decrease in T3 and T4 levels led to thyroid hypertrophy as a compensatory mechanism to lowered thyroid hormone levels. The authors then evaluated the effect of spironolactone at 200 mg/kg/day in the diet (n=10/group) on thyroidal iodide uptake and organification for 2 weeks in male Sprague Dawley rats. This part of the study revealed that T4 levels were decreased but that this was not caused by reduced synthesis, as iodide uptake, organification and UGT activity were also significantly increased following spironolactone treatment. The results suggest 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 [redacted] This is a well-established mechanism in the rat.

A 13 week study was conducted in Beagle dogs (n=2/sex/group) in which initial doses of 0, 12, 30 or 70 mg/kg bw per day spironolactone were given in a capsule for 6 weeks and then increased to 12, 30 or 100 mg/kg bw per day for weeks 7–9 and to 12, 30 or 250 mg/kg bw per day for the last 4 weeks. No mammary abnormalities were noted in the dogs. In contrast to the rat, there were no changes in the thyroid gland. In further contrast to the rat, there were no changes to liver weight or associated histological changes in the liver. There was a slight

decrease in testes weight, but this was not statistically significant and there were no associated histological abnormalities [redacted]

Marmosets (*Callithrix jacchus*) were given spironolactone at 0, 30 or 100 mg/kg bw per day (n=3 males per dose per group, n=6 males in vehicle control group), for 4 weeks. Spironolactone was suspended in 0.5% carboxymethylcellulose-sodium solution. Results showed that spironolactone caused thyroid follicular-cell hypertrophy. Plasma T4 levels were maintained at almost pretreatment or control levels after spironolactone treatment. Treatment with spironolactone also increased hepatic CYP P450 content and T4-UGT activity [redacted]

Chronic Toxicity Studies

In a 26-week study (with interim sacrifices at weeks 5 and 13), Sprague-Dawley 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, groups of 36/sex Sprague-Dawley rats (7 weeks of age; 72/sex for control group) were given diets that provided a dose of 0, 50, 150 or 500 mg/kg bw per day. The rats 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. The authors considered that the species-specific changes in the liver were attributed to the differences in metabolism between species. Dogs, monkeys and humans metabolise spironolactone only partially in the liver and excrete approximately 50% via the kidney. However, the rat metabolises spironolactone in the liver and the majority is excreted in the bile [redacted]

Liver weights were increased following 13 weeks of treatment and onwards. Liver nodules were observed on gross examination in some animals, but significance was noted at 150 and 500 mg/kg/day dose levels at 78 weeks and 104 weeks. These nodules tended to be more frequent in males than females. There was no correlation between the gross observation of nodules and the nodular hyperplasia observed microscopically. Most of the gross nodules were areas of non-specific inflammatory infiltration associated with focal necrosis. On microscopic examination, cellular hypertrophy was noted at the mid and high dose groups only. However, this was not seen in the 104 week rat study. Hepatocellular carcinomas were noted in one male in the mid and one male in the high dose group only. The diagnosis of carcinoma was made based on the presence of cellular atypia and bizarre cellular arrangement. Mitotic figures were not a feature and there was no evidence of invasiveness at tumour edges, nor were there metastases. Although there appears to be a slight increase in the incidence of carcinoma of the liver and mid and high doses (1 in mid and 2 in high dose), the numbers were considered very small and not statistically significant compared to the control group.

In all dose groups, localised areas of the thyroid which formed microscopic islands or foci of cells, occasionally bilateral were noted. These foci were not encapsulated, and there was no evidence of invasiveness. These were also noted in the 104 week study as noted below. They were categorised as adenomas and showed a dose related increase in incidence at the 150 and 500 mg/kg/day dose level. The “adenomas” consisted of irregular groups of follicles of mixed appearance; some were compact with high epithelial cells and no colloid, while immediately adjacent were larger, cystic follicles, some of which had papillary projections. Occasionally,

colloid was present. Where the papillary processes predominated, the term "papilliferous adenoma" was used and where the composition was principally of simple follicles, the term "simple adenoma" was used. Six parafollicular cell nodules or adenomas were noted which showed no sex or dose relationship. These were microscopic foci of calcitonin cells with no capsule and no evidence of invasiveness of surrounding tissue. Papillary cystadenocarcinomas of the thyroid were noted in one male at 50 mg/kg/day and one male at 500 mg/kg/day and there was no evidence of invasiveness or metastases [redacted]

At the mid dose level only, female rats had a slightly increased incidence of glomerulonephritis compared with controls. This was not seen at the highest dose, and therefore the authors concluded that the finding was not of biological significance. All other findings regarding organ weights and histology were comparable in control and test animals. A moderate degree of chronic glomerulonephritis, the type normally seen in an aged rat population was described in most groups. Uric acid, BUN, chloride and potassium/sodium ratios were all normal. There no significant pituitary weight changes between groups. Dose-dependent decreases in prostate weights were seen in males at all doses and a slight arrest of maturation in the testis (an increased number of immature spermatozoal precursors) was noted in the two higher dose groups. However, as there were no accompanying abnormal histological findings the authors only noted a slight generalized atrophy [redacted]

In a 104-week study, Sprague-Dawley rats received a dose of 0, 10, 30 or 100 mg/kg/day. As also seen in the 78 week study, liver weights were increased at all doses, and the thyroid gland weights were increased in males and females at the high dose. Although phaeochromocytomas were seen in all groups in this study, the incidence was not dose-related, leading the authors to conclude that the findings were not spironolactone-related. Papillary cystadenocarcinomas of the thyroid were noted in one female at 10 mg/kg/day with no evidence of invasiveness or metastases [redacted] A summary table of the tumour incidence in rats is shown below.

[redacted]

In all the rat studies conducted by Lumb et al, 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. Although gynaecomastia occurs in male human patients treated with spironolactone, no mammary abnormalities were noted in rats [redacted]

In male rhesus monkeys (*Macaca mulatta*), a 26-week study was conducted in which the animals received a dose of 0 or 125 mg/kg bw per day No further study details or results are stated [redacted]

A 52-week study was conducted in rhesus monkeys (*Macaca mulatta*) 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. There no weight changes or histological findings observed in females, but males had a slight increase in liver weight at the high dose with no associated histological changes. There were no changes in kidney weights or histological differences between control and test groups. Serum uric acid, potassium/sodium ratios and BUN were unaffected. A slight decrease in testis weight and a slight depression of testicular maturation was observed at the two higher doses. A slight depression of maturation activity was indicated in the medium and high doses, manifested by increased numbers of immature spermatozoal precursors. However, there was no evidence of germinal epithelial cell arrest or abnormality. Although gynaecomastia occurs in male human patients treated with spironolactone, and no mammary abnormalities were noted in rats or dogs, male monkeys showed a treatment-related increase in cellular activity in the acini of the mammary gland. This change was deemed to be a type of adenosis that was regular in nature, and without cellular aberrations or stroma changes. Nodules or tumours were not observed. In contrast to the rat, but similar to the dog, there were no changes to the thyroid gland [redacted]

Thyroid follicular cell hypertrophy was noted in rodent species following 13 week administration (up to 200 mg/kg/day; [redacted] and marmosets following 4 weeks (up to 100mg/kg/day; [redacted] treatment with spironolactone. It is considered unusual for this form of hypertrophy to be seen in non-rodent species. However, it should be noted that both rats and marmosets lack thyroxine binding globulin which may be related to these effects [redacted] Furthermore, thyroid follicular cell hypertrophy was not seen in dog (up to 100 mg/kg/day, 13 weeks, [redacted] or rhesus monkey (up to 250 mg/kg/day, 52 weeks, [redacted] studies. Lumb et al discuss a report where in 17 normal adults receiving up to 400 mg Aldactone daily for six months, no abnormalities were noted in serum T4 or thyroid stimulating hormone levels. Indeed, in humans, no hypertrophic or hyperplastic change in the thyroid caused by spironolactone has been reported in humans [redacted] As such it is considered that the rat and marmoset are not representative of humans in this aspect.

2.4.4.3 Genotoxicity

In the two most recent [redacted] where spironolactone has been evaluated [redacted], there has not been any assessment of the genotoxicity of spironolactone due to no data being available to the [redacted] on both occasions.

However, a more recent veterinary European Public Assessment Report (EPAR) [redacted] states the following:

> *Two studies conducted in 2003 were described, an in vitro bacterial reverse mutation test in Salmonella typhimurium and an in vivo study (bone marrow micronucleus test in mice). The submitted tests were GLP-compliant and in accordance with VICH guidance. Spironolactone did not show mutagenic activity in the bacterial reverse mutation test under the experimental conditions of the test. In the second experiment with and without metabolic activation, moderate to strong toxicity was noted but there was no significant increase in revertants in any of the 5 strains. There was no evidence of an increase of micronuclei in the micronucleus test and spironolactone did not induce damage to the chromosomes or mitotic apparatus of mice bone marrow cells.*
>
> *The CVMP noted that the two studies indicated that spironolactone had no mutagenic activity; however, a third study was required to conclusively assess clastogenicity. In addition, a further in vivo genotoxicity test using a target tissue other than bone marrow was required to confirm whether spironolactone is genotoxic or not.*
>
> *Data from two new GLP-compliant studies conducted in 2006 were provided; an in vitro mouse lymphoma assay and in vivo/in vitro liver UDS assay. The results show that spironolactone does not show any mutagenic or genotoxicity activity.* [redacted]

In mutagenicity studies, potassium canrenoate was not mutagenic in tests using bacteria and yeast, or in an in vivo mammalian system. It was mutagenic in vitro tests in mammalian cells following metabolic activation (Aldactone SmPC).

At concentrations of 10-90 µM in primary cultures of hepatocytes from rat and human donors, potassium canrenoate induced dose-dependent DNA fragmentation detected by the Comet assay and of DNA repair synthesis measured by quantitative autoradiography. In rat hepatocytes, the DNA fragmentation and repair were more marked after 3 hours than after 20 hours exposure, and in cultures from females more than males. However, in human hepatocytes, the extent of DNA fragmentation was similar in males and females, whilst DNA repair was detected in cultures from only two of the same three donors, and was to a less extent than that seen in rat hepatocytes. A statistically significant increase in micronucleated cells was observed in primary cultures of replicating rat hepatocytes following exposure to 10 or 30 µM potassium canrenoate after 48 hours incubation. However, no [redacted] formation was seen in human hepatocytes. The authors concluded that potassium canrenoate induced DNA-damaging reactive species in rat hepatocytes to a greater extent than seen in human hepatocytes [redacted]

[redacted]

[redacted]

[redacted] as referred to in section 2.4.4.4 below, compared the metabolic fates of potassium canrenoate and spironolactone for the rat *in vivo* and *in vitro*. Approximately 18% of an in vivo dose of spironolactone was metabolized to canrenone and related compounds in the rat. While *in vitro*, 20-30% of spironolactone was dethioacetylated to canrenone and its metabolites by rat liver S9, the major route of spironolactone metabolism in the rat was considered to be via the retention of the sulfur moiety in the molecule. In contrast, potassium canrenoate was metabolized by rat hepatic S9 to 6α, 7α- and 6β, 7β-epoxy-canrenone. The β-epoxide was further metabolized to its 3α- and 3β-hydroxy derivatives and its glutathione (GSH) conjugate. Both 3α- and 3β-hydroxy-6β-epoxy-canrenine and 7β-epoxy-canrenone were direct acting mutagens in the mouse lymphoma assay. Notably, these mutagenic metabolites, their precursor epoxides and their GSH conjugates were not formed from spironolactone under identical conditions. This difference in metabolic profile between spironolactone and potassium canrenoate may be due to inhibition of metabolism of canrenone formed from spironolactone by spironolactone and/or its S-containing metabolites, since the in vitro metabolism of potassium canrenoate by rat hepatic microsomes was appreciably reduced in the presence of spironolactone. The authors' hypothesized mechanism(s) for this inhibition was that spironolactone and its S-containing metabolites specifically inhibited an isozyme of hepatic CYP or spironolactone is a preferred substrate over potassium canrenoate/canrenone for the metabolizing enzymes. Absence of the canrenone epoxide pathway in the metabolism of spironolactone provides a possible explanation for the observed differences in the toxicological profiles of the two compounds. Overall, the authors concluded that the mechanisms of metabolism of spironolactone and potassium canrenoate differed, yielding mutagenic metabolites that are unique to potassium canrenoate [redacted]

Therefore, it can be inferred that a genotoxic risk may be presented by potassium canrenoate, but not by spironolactone. These findings are reflected in section 5.3 of the SmPC.

2.4.4.4 Carcinogenicity

[redacted]

[redacted]

[redacted]

It should be noted that no carcinogenic potential was observed with spironolactone in chronic dog and monkey studies [redacted]

However additional studies with potassium canrenoate in the early 1980s demonstrated a tumour association in rats. Carcinogenic findings for rat are detailed also in the SmPC at section 5.3.

[redacted] Such tumours were not found with potassium canrenoate in toxicology studies performed in dogs and monkeys.

As also discussed in section 2.4.4.3, investigations of the metabolism of spironolactone and canrenoate by Searle scientists resolved the phenomenon of the different toxicological findings: potassium canrenoate is metabolized to different epoxy-canrenone derivatives, which were found to be direct mutagens in the mouse lymphoma assay. Importantly, these mutagenic metabolites or their precursor epoxides were not formed from spironolactone [redacted] Therefore, the occurrence of myelocytic leukemia in long-term studies with canrenoate in rats can be explained by the formation of these mutagenic metabolites [redacted]

Uncommon reports of benign breast neoplasms in males have been reported following treatment with spironolactone, as detailed in section 4.8 of the SmPC.

In a study by [redacted] it was concluded that for breast, uterus, ovarian and cervical cancer, the authors found there was no evidence of increased risk with spironolactone use [redacted]

In a recent [redacted] studied the risk of cancer among users of spironolactone. A pharmacoepidemiological propensity score-matched cohort study was performed to assess the effect of spironolactone exposure on cancer incidence. The pre-specified primary outcomes were the first incidence of ovarian, endometrial, pancreatic, colorectal, prostate, renal cell, pharyngeal and thyroid cancers and myelomonoblastic/-cytic leukaemias. The results of the study showed that there was no evidence of an increased risk of any cancer associated with spironolactone use [redacted]

A retrospective study, found that there was no evidence of interactions between 5α-reductase inhibitors and spironolactone with endocrine therapies used in breast cancer. Sex hormone alteration with 5α-reductase inhibitor or spironolactone use was variable. Overall, most patients did not have a significant alteration in the level of oestrogen when using 5α-reductase inhibitors or spironolactone. No consistent evidence of increased risk of female breast cancer while on spironolactone was reported in 3 studies including 49,298 patients. The authors concluded that most patients did not show increased oestrogen levels with spironolactone and there was no data suggesting increased risk of breast cancer [redacted]

In addition, it is considered worth noting that very recent studies published from 2018 onwards, have suggested a protective potential of spironolactone in terms of carcinogenicity. Some examples are included below.

In [redacted] evaluated the potential of spironolactone to inhibit chronic liver diseases for which NADPH oxidases (NOX) may play a role based on the previously observed ability of spironolactone in affecting NOX. The effects of spironolactone alone or in combination with the antioxidant α-glycosyl isoquercitrin (AGIQ) on hyperlipidemia-related and steatosis-related precancerous lesions in high-fat diet (HFD)-fed rats subjected to a two-stage hepatocarcinogenesis model were investigated. The results indicated that spironolactone in combination with AGIQ had the potential of suppressing hyperlipidemia-related and steatosis-related early hepatocarcinogenesis through the reduced expression of NOX subunits [redacted]

[redacted] found that in a cancer stem cell model, spironolactone did not induce DNA damage, but rather impaired DNA repair in cancer as well as in cancer stem cells and spared healthy cells. Notably, spironolactone was toxic to both cancer stem cell-like and stem-like glioma cells, while it was safe for healthy cells. In vivo, a cancer stem cell like system was used in mice, which showed that spironolactone was able to slow down tumour growth and reduce cancer stem cell content in the tumours [redacted]

As discussed in this overview, spironolactone has antiandrogenic effects through its non-selective binding to steroid receptors, and hence may affect prostate cancer (PCa) risk. Beckmann et al investigated the association between spironolactone use and PCa risk. Multivariable analyses indicated reduced risk of PCa among those ever exposed to spironolactone (odds ratio [OR] 0.83; 95% CI: 0.76–0.89), with a stronger association for current users (OR: 0.77, 95% CI: 0.69–0.86) than past users (OR: 0.88; 95% CI: 0.79–0.97) and decreasing risk with increasing dose (p-trend < 0.001). The authors acknowledged that biases due to differences in prescribing patterns or frequency of prostate specific antigen (PSA) testing may have influenced these findings. The authors concluded that their study found PCa risk was reduced among men exposed to the diuretic spironolactone and noted that further investigation regarding the chemopreventive potential of spironolactone is warranted ([redacted]

2.4.4.5 Reproductive and Developmental Toxicity

As described in section 2.4.2, spironolactone has structural similarities to hormones such as progesterone and androgen giving rise to anti-androgenic activity. Furthermore, the MR, that which is the target of spironolactone, when compared with human progesterone,

glucocorticoid and androgen receptors (hPR, hGR and hAR) reveals a lower sequence identity (48%) but a strong sequence similarity [redacted] It is possible that these receptor similarities give rise to the reproductive and developmental effects of spironolactone.

A number of studies have investigated the reproductive and development toxicity of spironolactone and potassium canrenoate (potassium canrenaote, as discussed in earlier sections, follows a different metabolic pathway compared to spironolactone) [redacted], by various routes of administration in mice, rats and rabbits and are summarised in the table below:

[redacted]

Appropriate wording is included in the SmPC at section 4.6 for pregnancy and breast-feeding on the basis of the data as discussed in the following sections.

2.4.4.5.1 Fertility and Early Embryonic Development

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 [redacted]

A group of Wistar rats with regular oestrous 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 oestrus was accompanied by a reduction in plasma oestradiol of 48%. These effects were associated with retarded ovarian follicle development and a reduction in circulating oestrogen levels, impaired mating, fertility and litter size (fecundity) [redacted]

A three-litter reproduction study, female rats (strain or duration of study not stated) received 15 or 500 mg/kg/day spironolactone in the diet. Results showed no effects on mating and fertility [redacted]

Potassium Canrenoate

An increased resorption rate was found in rats that received 100 mg/kg bw per day for various periods before day 6 of gestation [redacted]

2.4.4.5.2 Embyro-Fetal Development

[redacted]

Detailed information from rabbit studies has not been yielded from any primary sources of literature. However information from previous SmPC and FDA labels of Aldactone have described that a dose of 20 mg/kg of spironolactone (route of administration or duration not

stated) have resulted in an increased rate of resorption and a lower number of live fetuses in rabbits (Aldactone SmPC, Aldactone Pfizer label, 2021).

[redacted]

A summary of studies identified by [redacted] is shown below in Table 2.4.12 where the top table shows cases of animal studies and lower table shows cases of human males, exposed to spironolactone *in utero*.

[redacted]

[redacted]

2.4.4.5.3 Prenatal and Postnatal Development (Including Maternal Function)

Some findings from fetal prenatal exposure to spironolactone have been discussed in section 2.4.4.5.2.

Pregnant Wistar rats 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 or body 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 follicle-stimulating hormone, luteinizing hormone, testosterone and 5α-dihydrotestosterone were normal, but those of prolactin were decreased. In females, the oestrus 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, oestradiol and progesterone were comparable to those of controls; however, the concentrations of luteinizing hormone were increased [redacted]

With respect to the passage of spironolactone from nursing mother to infant, levels of canrenone were evaluated. In a 17 days postpartum woman, who was being administered 25 mg spironolactone, four times a day, the levels of canrenone in her milk two hours post dose were 104 µg/L and at 14.5 hours post dose were 47 µg/L. It was estimated that the nursing infant would receive approximately 0.2% of the mother's total daily dose of spironolactone in the form of canrenone [redacted]

2.4.4.5.4 Juvenile Toxicity

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 onset of puberty was not prevented in any control animals. When female rats were treated simultaneously with spironolactone (100 mg/kg bw per day) and oestradiol (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 oestradiol [redacted]

2.4.4.6 Local Tolerance

As detailed at section 4.8 of the SmPC, gastrointestinal disorders in patients are classified as common (nausea) and of unknown frequency (gastrointestinal disorder) and the full extent of gastrointestinal disorders due to the secondary PD and drug interaction of spironolactone are discussed elsewhere (section 2.4.2.2).

2.4.4.7 Other Toxicity Studies

2.4.4.7.1 Antigenicity/Immunotoxicity

As discussed in detail in section 2.4.2.2, in a skin patch-testing study, all the tests (including components of the vehicles) were negative, except for spironolactone, which gave a strong positive reaction. A diagnosis of drug rash with eosinophilia and systemic symptoms (DRESS) was made ([redacted]). As also mentioned in section 2.4.2.2, this finding is considered extremely rare, with only one other case identified in the literature in 2020 [redacted]

2.4.4.7.2 Dependence

No specific information on the dependence of spironolactone is believed to have been reported in the literature, and there is no evidence from clinical experience. This is expected given the mechanism of action.

2.4.4.7.3 Metabolites

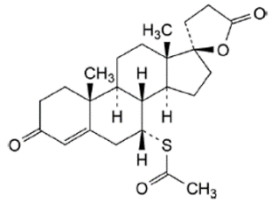
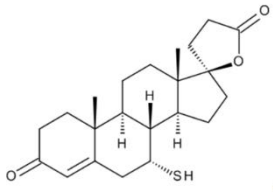
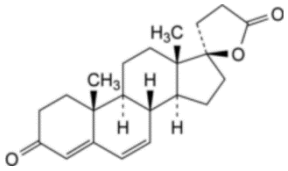
The pharmacological and toxicological effects of the metabolites of spironolactone are discussed in detail in sections 2.4.3.4 on Metabolism, 2.4.3.6 on Pharmacokinetic Drug Interactions, 2.4.4.3 on genotoxicity, 2.4.4.4 on carcinogenicity and 2.4.4.5 on reproductive and developmental toxicity.

2.4.4.7.4 Impurities (Including degradation products

There are no impurity issues for the drug substance, spironolactone, which has an EDQM Certificate of Suitability, demonstrating that the specifications of the current version of the monograph SPIRONOLACTONE no. 688 of the European Pharmacopoeia have been met.

The structures of spironolactone and its degradation products are provided in Table 2.4.13.

Table 2.4.13. Structures of spironolactone and potential degradation products

Structure	Name	Formation
	Spironolactone (CAS: 446-86-6)	Parent molecule
	Desacetylspironolactone Also known as 7-thiospironolactone or as 7α-thiospironolactone (CAS: 38753-76-3)	Key degradant formed under acidic and oxidative conditions
	Canrenone Also known as Ph. Eur Impurity F (CAS: 976-71-6)	Key degradant formed in drug product stability studies

Desacetylspironolactone and canrenone are metabolites of spironolactone in animals and humans [redacted] as further discussed in 2.4.3.4.

Since they are significant animal and human metabolites, they are toxicologically qualified taking into account of the low level of canrenone and the potential formation at very low levels, if at all present, of desacetylspironolactone (also known as 7-thiospironolactone or as 7α-thiospironolactone).

As discussed in 3.2.P.5.5, there are also no elemental impurities or nitrosamine impurities risks for Spironolactone 10 mg/ml Oral Suspension. There are also no residual solvent risks for Spironolactone 10 mg/ml Oral Suspension.

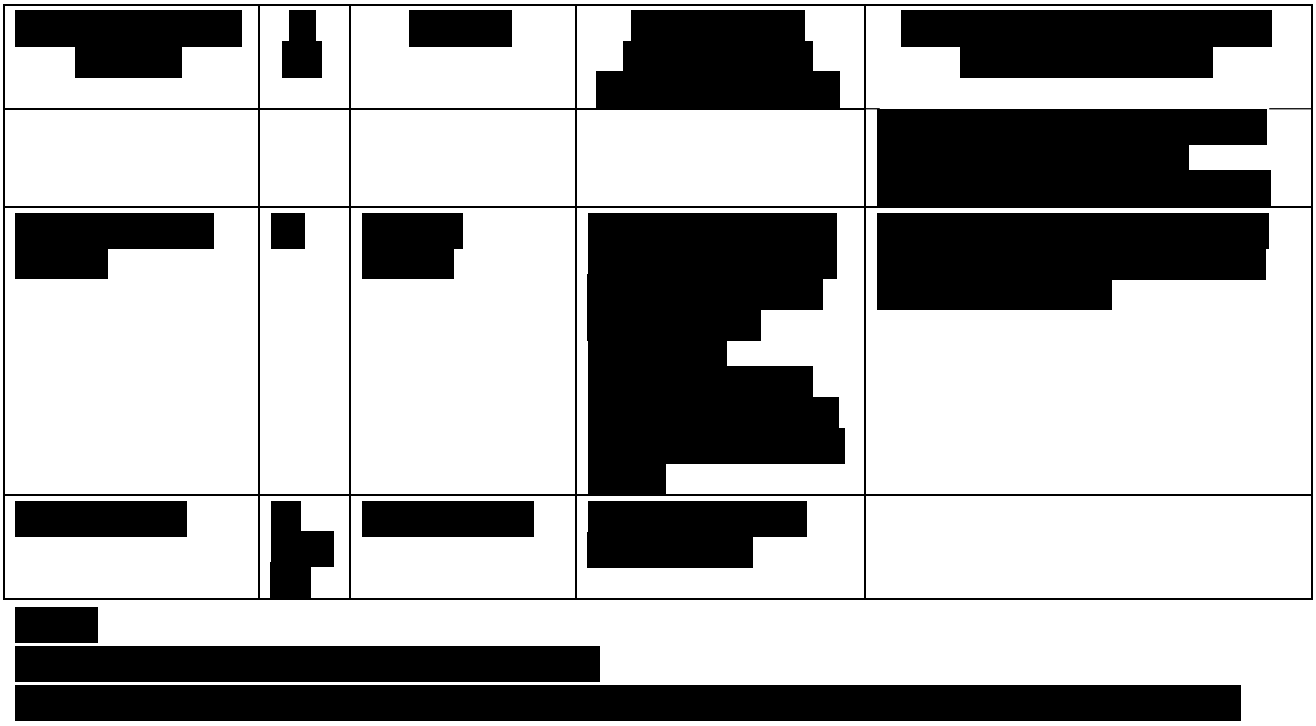
2.4.4.7.5 Phototoxicity

No specific concerns for any potential phototoxicity of spironolactone have been identified, taking into account the extensive clinical experience with spironolactone.

2.4.4.7.6 Excipients

All of the excipients in the proposed formulation are well established and meet pharmacopoeial specifications. They are also used in oral liquid preparations (including paediatric formulations) currently on the market and so there is no reason to anticipate any issue with oral local tolerance of the formulation, Spironolactone 10 mg/ml Oral Suspension.

The proposed formulation contains no alcohol or colouring agents. The excipients listed are commonly used in pharmaceutical formulations including those formulated for children, and are widely accepted. No evidence of any incompatibility between the excipients or between the active substance and the excipients has been found. Further details on each of the excipients are given below.



Sodium Benzoate E211)

Sodium benzoate is used primarily as an antimicrobial preservative in pharmaceuticals, as well as in food and cosmetics. It is generally used in concentrations of 0.02 – 0.5% in oral medicines and up to 0.5% in parenteral products and cosmetics. Sodium benzoate occurs as a white granular or crystalline slightly hygroscopic powder.

Sodium benzoate is found as an excipient in some medicinal products administered orally, topically or injected, used as a bacteriostatic antiseptic. In accordance with the "Questions and answers on benzoic acid and benzoates used as excipients in medicinal products for human use" EMA document Furthermore, sodium benzoate is also administered orally or intravenously as an active substance to infants and children for the treatment of hyperammonaemia related to urea cycle disorders.

With regards to safety, Spironolactone 10 mg/ml Oral Suspension is intended to be available to the paediatric population through to adults, as such it is prudent to note that the main point of concern surrounding the use of sodium benzoate is its ability to displace bilirubin from albumin. It is of particular concern only in pre-term and full-term neonates where immaturity of metabolic enzymes until 8 weeks of age may result in accumulation of benzoic acid. Neonatal unconjugated hyperbilirubinemia and resultant clinical jaundice affect up to 85% of newborns, though this condition is usually benign

However, the displacement of bilirubin from albumin leads to hyperbilirubinaemia which may cause serious brain injury in some neonates with jaundice. Thus, acute bilirubin encephalopathy may evolve to kernicterus (bilirubin-induced brain dysfunction) if left untreated. This risk exists with oral, parenteral and also cutaneous preparations [redacted]

The WHO acceptable daily intake (ADI) of total benzoates calculated as benzoic acid has been estimated at up to 5mg/kg body weight.

Sodium benzoate is listed as *generally regarded as safe* (GRAS), and is accepted as a food additive in Europe. It is also included in the FDA Inactive Ingredients database (for dental preparations, intramuscular, intravenous injections, oral capsules, solutions and tablets, rectal, and topical preparations). It is also included in nonparenteral medicines licensed in the UK and is included in the Canadian Natural Health Products Ingredients Database [redacted]

According to the opinion of the [redacted] the ADI for benzoic acid and its salts has been established to 0–5 mg/kg bw in agreement with the [redacted]. Young children (< 3 years old) may not be sufficiently mature to metabolise and eliminate benzoic acid as efficiently as adults. Therefore the upper limit of the ADI should be considered with caution in this age group [redacted]

[redacted]

In consideration of the above and the labelling recommendations in [redacted] which states: "Benzoic acid/benzoate salt may increase jaundice (yellowing of the skin and eyes) in newborn babies (up to 4 weeks old)", it is deemed appropriate to include wording to this effect in the SmPC and Package Leaflet for Spironolactone 10 mg/mL Oral Suspension.

<u>Sucrose</u>

Sucrose, chemical name β-D-fructofuranosyl-α-D-glucopyranoside [redacted] is widely used in oral pharmaceutical formulations. Sucrose has many synonyms including sugar, cane sugar, beet sugar, refined sugar, saccharose, saccharosum, saccharum, sucrosum and α-D-glucopyranosyl-β-D-fructofuranoside [redacted]

Sucrose syrups, as well as being used in tableting as a binding agent for wet granulation and as tablet-coating agents, are also widely used as vehicles in oral liquid dosage forms to enhance palatability or to increase viscosity. In addition, sucrose is widely used in foods and confectionary [redacted]

Sucrose is a sugar obtained from sugar cane, sugar beet and other sources. It contains no added substances. Sucrose occurs as colourless crystals, as crystalline masses or blocks, or as white crystalline powder. It is odourless and has a sweet taste [redacted]

Sucrose is hydrolysed in the small intestine by the enzyme sucrase to yield dextrose and fructose, which are then absorbed. When administered intravenously, sucrose is excreted unchanged in the urine. Although sucrose is very widely used in foods and pharmaceutical formulations, sucrose consumption is a cause of concern and should be monitored in patients with diabetes mellitus or other metabolic sugar intolerance. [redacted]

Sucrose is also considered to be more cariogenic than other carbohydrates since it is more easily converted to dental plaque [redacted]

Sucrose is listed as GRAS and is included in the FDA Inactive Ingredients Database (injections; oral capsules, solutions, syrups, and tablets; topical preparations). Sucrose is also included in nonparenteral and parenteral medicines licensed in the UK and in the Canadian Natural Health Products Ingredients Database [redacted] Within this publication, EFSA noted that consumption of sugar-containing foods is frequent and can increase risk of dental caries, especially when oral hygiene and fluoride prophylaxis are insufficient. However, available data do not allow the setting of an upper limit for intake of (added) sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by frequency of consumption, oral hygiene, exposure to fluoride, and various other factors ([redacted]

In section 4.4 of the SmPC, appropriate warnings are stated for sucrose.

Sodium Citrate Dihydrate

Sodium citrate dihydrate, chemical name trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate [redacted] is widely used in pharmaceutical formulations. Sodium citrate dihydrate is colourless, odourless and occurs as monoclinic crystals or a white crystalline powder with a cooling, saline taste.

Sodium citrate is prepared by adding sodium carbonate to a solution of citric acid until effervescence ceases. The resulting solution is filtered and evaporated to dryness. Sodium citrate dihydrate is a stable material. After ingestion, sodium citrate is absorbed and metabolised to bicarbonate. Although it is generally regarded as a non-toxic, and non-irritant excipient, excessive consumption may cause gastrointestinal discomfort. Therapeutically, in adults, up to 15g daily of sodium citrate dihydrate may be administered orally in divided doses as an aqueous solution to relieve the irritation caused by cystitis.

Sodium citrate dihydrate is listed as GRAS and is accepted for use as a food additive in Europe, included in the FDA inactive ingredients database (inhalations, injections, ophthalmic products, oral solutions: suspensions, syrups and tablets, nasal, otic, rectal, topical, transdermal and vaginal preparations). Sodium citrate dihydrate is also included in nonparenteral and parenteral

medicines licensed in the UK. It is also included in the Canadian Natural Health Products Ingredients Database [redacted]

[redacted] there is no limit on the ADI of sodium citrate dihydrate [redacted]

[redacted] published a scientific opinion on the substantiation of health claims related to sodium and potassium salts of citric acid and maintenance of normal bone [redacted] This opinion addressed the scientific substantiation of health claims in relation to sodium and potassium salts of citric acid and maintenance of normal bone.

The food constituents that are the subject of the health claim are sodium and potassium salts of citric acid. [redacted] considered that sodium and potassium salts of citric acid were sufficiently characterised. The claimed effect was “acid-base balance and bone health”. The target population was assumed to be the general population. In the context of the proposed wordings, the [redacted] assumed that the claimed effect referred to the maintenance of normal bone by maintaining acid-base balance. [redacted] considered that maintenance of normal bone is a beneficial physiological effect. In weighing the evidence, [redacted] took into account that the results from the two human intervention studies provided which investigated the effects of potassium citrate on bone mineral density in post-menopausal women were conflicting, and that the adequately powered intervention study of longer duration using a higher dose of potassium citrate did not show an effect on bone mineral density. On the basis of the data presented, the Panel concluded that a cause and effect relationship had not been established between the dietary intake of potassium or sodium salts of citric acid and maintenance of normal bone [redacted]

Citric Acid

Citric acid, chemical name 2-hydroxy-1,2,3-propanetricarboxylic acid monohydrate [redacted] is widely used pharmaceutical formulations and food products primarily for the adjustment of the pH of solutions. Citric acid monohydrate occurs as a colourless or translucent crystal, or as a white crystalline efflorescent powder. It is odourless and has a strong acidic taste.

Citric acid occurs naturally in a number of plant species including lemons and pineapple waste. Citric acid is found naturally in the body, mainly in the bones and is commonly consumed as part of a normal diet.

Citric acid is listed as GRAS and is included in the FDA Inactive Ingredients database (inhalations; intramuscular, intravenous and other injections; ophthalmic preparations; oral capsules, solutions, suspensions and tablets; topical and vaginal preparations. It is also included in nonparenteral and parenteral medicines licensed in Japan and the UK. It is also included in the Canadian Natural Health Products Ingredients Database [redacted]

Despite this well-established knowledge on citric acid, a recent study in the literature in 2018 reported that it is not the naturally occurring citric acid, but the manufactured citric acid (MCA) that is used extensively as a food and beverage additive. Approximately 99% of the world's production of MCA is carried out using the fungus Aspergillus niger since 1919. Aspergillus niger is a known allergen and in 2016, 2.3 million tons of MCA were produced, predominantly in China, and approximately 70% is used as a food or beverage additive. There have been no identified scientific studies performed to evaluate the safety of MCA when ingested in substantial amounts and with chronic exposure [redacted] evaluate four case reports of patients with a history of significant and repetitive inflammatory reactions including respiratory symptoms, joint pain, irritable bowel symptoms, muscular pain and enervation following ingestion of foods, beverages or vitamins containing MCA. The authors conclude that ingestion of the MCA may lead to a harmful inflammatory cascade which manifests differently in different individuals based on their genetic predisposition and susceptibility [redacted]

[redacted]

"Citric acid may be produced by recovery from sources such as lemon or pineapple juice or fermentation of carbohydrate solutions or other suitable media using Candida spp. or non-toxicogenic strains of Aspergillus niger"

[redacted] Information provided with the citric acid monohydrate powder includes a mytotoxin/aflatoxin certificate providing further information that the presence of mould or contamination is unlikely (Mycotoxin-Aflatoxin Certificate). The applicant can confirm that as part of the preservative efficacy test, 6 microorganisms are tested for as per the anti-microbial effectiveness test (Ph Eur 5.1.3) and the sodium benzoate is demonstrated to have been effective. Furthermore a test for Aspergillus brasiliensis (fungi) which is also known as *Aspergillus niger* is also conducted. These studies are described in Module 3.2.P.2.5. As such, it is anticipated that the citric acid present in Spironolactone 10 mg/ml Oral Suspension does not pose a risk.

Strawberry Flavour Liquid

Strawberry liquid flavour [redacted] is synthetic flavour [redacted] The strawberry flavour is mixture of various natural and synthetic flavouring substances along with other ingredients. [redacted]

[redacted]

[redacted]

[redacted]

The strawberry flavour is a synthetic flavour and the synthetic flavours are routinely employed in pharmaceutical preparations to improve the palatability of the formulation. There are number of marketed products intended for children's use which employ the same flavours e.g Hydroxycarbamide oral solution [redacted] or similar flavours e.g. Paracetamol oral suspension [redacted] and Amoxicillin Paediatric suspension [redacted]

Bitter Masker Flavour

Bitter masker flavour [redacted] is a synthetic flavour [redacted] The bitter masker flavour is mixture of various synthetic favouring substance. The composition of bitter masker flavour is listed in Table 2.4.17 below.

[redacted]

[redacted]

[redacted]

[redacted] There are number of marketed product currently approved in EU, including for the paediatric population, employing similar synthetic flavours [redacted]

The strawberry flavour in combination with the bitter masker flavour and sucrose significantly improves the palatability of the formulation. It also helps mask the inherent bitter taste of spironolactone drug substance.

Polysorbate 80

Polysorbate 80, or polyoxyethylene 20 sorbitan monoleate, is abbreviated from polyoxyethylene sorbitan fatty acid esters, and are a series of partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20, 5 or 4 moles of ethylene oxide for each mole of sorbitol and its anhydrides. The resulting product is therefore a mixture of molecules of varying sizes rather than a single uniform compound. Polysorbates are used in a wide variety of pharmaceutical formulations including oral, otic, ophthalmic, topical and parenteral preparations. Polysorbate 80 is the most commonly used surfactant in FDA-approved parenteral products. Polysorbates are also widely used in cosmetics and food products. Polysorbates have a characteristic odour and a bitter taste. At 25ºC, polysorbate appears as a yellow oily liquid [redacted]

Polysorbates are widely used in cosmetics, food products, and oral, parenteral and topical pharmaceutical formulations and are generally regarded as nontoxic and nonirritant materials. There have, however, been occasional reports of hypersensitivity to polysorbates following their topical and intracumuscular use. Polysorbates have also been associated with serious adverse effects, including some deaths in low birth weight infants following intravenous administration of a vitamin E preparation containing a mixture of polysorbates 20 and 80. When heated to decomposition, the polysorbates emit acrid smoke and irritating fumes. The WHO have set an estimated ADI for polysorbate 80 (but also other polysorbates) calculated as total polysorbate esters, at up to 25 mg/kg body weight. In animal studies, polysorbate 80 administered to rats and mice led to the following LD_{50} values [redacted]

Mouse, i.p. 7.6 g/kg

Mouse i.v. 4.5 g/kg

Mouse, oral 25 g/kg

Rat, i.p. 6.8 g/kg

Rat i.v. 1.8 g/kg

The [redacted] re-evaluated the safety of polysorbate 80 (as well as other polysorbates) as food additives. [redacted] erived an ADI of 25 mg/kg body weight (bw)/day (group ADI for polysorbates 20, 40, 60, 65 and 80) and the [redacted] derived a group ADI of 10 mg/kg bw/day. Small amounts of polyoxyethylene sorbitans are absorbed. The [redacted] considered that similar toxicokinetics would be expected for all polysorbates based on their similarities in structure and metabolic fate. The acute toxicity was deemed to be very low. There was no concern regarding genotoxicity, carcinogenicity or developmental

toxicity. From a limited number of studies, there was considered to be no indication of reproductive toxicity. The [redacted] considered the long-term carcinogenicity study in rats with a No Observed Adverse Effect Level (NOAEL) equivalent to 2 500 mg/kg bw/day – consistent with the NOAEL defined in subchronic studies – as the key study and allocated a group ADI of 25 mg/kg bw/day using an uncertainty factor of 100 [redacted]

The [redacted] did note that the estimated exposure of toddlers at the highest level in non-brand loyal scenario remained very close to the ADI (24.5 mg/kg bw/day). The [redacted] was aware that for three food categories no reported uses had been obtained and that other dietary sources of exposure to polysorbates could not be considered in their current opinion and therefore more data (usage and analytical data) would be needed to decrease uncertainties in the refined exposure assessment scenario used [redacted]

Overall, polysorbate 80 is listed as GRAS and is also accepted as a food additive in Europe. Polysorbate 80 is included in the FDA inactive ingredients database (intra-muscular, intra-venous, oral, ophthalmic, otic, rectal, topical and vaginal preparations). Polysorbates are included in parenteral and nonparenteral medicines licensed in the UK. Polysorbate 80 is listed in the Canadian Natural Health Products Ingredients Database [redacted]

<u>Simethicone 30% Emulsion</u>

Simethicone (USAN) is mixture of polydimethylsiloxane (dimethicone) and silicone dioxide. It is also known as by its international non-proprietary name simeticone (British Pharmaocopoeia, Ph.Eur) and is widely used as an active ingredient to relive symptoms of excessive gas in the gastrointestinal track at the maximum dose of up to 250 mg/day for the infants less than 2 years in age and up to 480 mg/day in children greater than 2 years age [redacted]

Simethicone is extensively used in pharmaceutical, cosmetic and food products as an antifoaming agent at the concentration of 1 to 50 ppm. It generally regarded as nontoxic and non-irritant when used an excipient. Due to its limited water solubility it is often used [redacted] Therefore, simethicone 30% emulsion is used in the proposed formulation [redacted]

The safety of simethicone as antifoaming agent was evaluated by the [redacted] and proposed the ADI of up to 1.5 mg/kg bw. It is listed GRAS by FDA and also included in the FDA inactive ingredient database. It is widely used in marketed pharmaceutical preparations intended for paediatric population in EU and US [redacted]

[redacted]

Xanthan Gum [redacted]

Xanthan gum is an exocellular heteropolysaccharide produced by fermentation. The polymer backbone consists of 1,4 linked β-D-glucose to which trisaccharide side chains are attached. Xanthan gum solutions are highly pseudoplastic, exhibiting a high viscosity, gel-like structure at rest which effectively keeps dispersed particles in suspension.

Xanthan gum ([redacted] E415) is a commonly used component of both pharmaceutical products (see Table 2.4.14) and foodstuffs. It has been extensively tested in animals and accepted as a food additive in Europe and USA in the FDA inactive Ingredients Database (oral solutions, suspensions and tablets; rectal and topical preparations. It is also included in the nonparenteral medicines licensed in the UK and in the Canadian Natural Health Products Ingredients Database [redacted]

Xanthan gum is employed as a suspending agent to reduce the rate of API sedimentation. Xanthan gum is widely used in marketed formulations in paediatric populations including Calpol Infant paracetamol suspension. The [redacted] provided a scientific opinion re-evaluating the safety of xanthan gum (E 415) as food additive.

Based on the reported use levels, a refined exposure of up to 64 mg/kg bw per day in children for the general population, 38 mg/kg bw per day for children consumers only of food supplements at the high level exposure and 115 mg/kg bw per day for infants consuming foods for special medical purposes and special formulae (FSMPs), were estimated. It was considered that xanthan gum (E 415) is unlikely to be absorbed intact and is expected to be fermented by intestinal microbiota. No adverse effects were reported at the highest doses tested in chronic and carcinogenicity studies and there is no concern with respect to the genotoxicity. Repeated oral intake by adults of xanthan gum up to 214 mg/kg bw per day for ten days was well tolerated, but some individuals experienced abdominal discomfort, an undesirable but not adverse effect. The ANS Panel concluded that there is no need for a numerical ADI for xanthan gum, and that there is no safety concern for the general population at the refined exposure assessment of xanthan gum as a food additive. Considering the outcome of clinical studies and post-marketing surveillance, the ANS Panel concluded that there is no safety concern from the use of xanthan gum in FSMPs for infants and young children at concentrations reported by the food industry [redacted]

[redacted]

Water

Water for irrigation is purchased sterile and complies with the requirements of the European Pharmacopoeia Monograph for “Preparations for Irrigation”. [redacted]

2.4.5 Integrated Overview and Conclusions

Spironolactone 10 mg/ml Oral Suspension is indicated in the management of refractory oedema associated with congestive cardiac failure; hepatic cirrhosis with ascites and oedema, malignant ascites, nephrotic syndrome, diagnosis and treatment of primary aldosteronism, essential hypertension. Spironolactone oral tablet formulations are already registered and marketed throughout the EU for the same indications.

The posology of the already licensed spironolactone tablet, Aldactone® 25mg, 50mg and 100mg tablet, and Spironolactone 10 mg/ml Oral Suspension are the same, where the maximal dose is up to 400 mg/day.

The applicant has conducted a bioequivalence study (INV684) to compare Spironolactone 10 mg/ml Oral Suspension (test) to the reference marketed tablet formulation (Aldactone 100 mg tablet®). The test and reference formulation were shown to be bioequivalent in terms of AUC_{0-t} and $AUC_{0-\infty}$. However, the mean C_{max} values of spironolactone were higher for the tablet formulation, with the 90% confidence limits falling below the lower 70% limit, demonstrating that the two formulations are not bioequivalent for this parameter. [redacted]

A comprehensive literature review was conducted to investigate pharmacology, ADME and toxicology characteristics of spironolactone, in addition to the extensive clinical experience and use for several decades of spironolactone.

There have been some comprehensive reviews including [redacted] which have contributed significantly to the general information available in evaluating spironolactone.

Pharmacology

Spironolactone has a four ring structure characteristic of steroids and binds to the MR in the renal tubules blocking the synthesis of AIP. This reduces Na+ reabsorption and K+ and H+ secretion by various mechanisms. The magnitude of diuresis produced by spironolactone depends on the plasma level of aldosterone.

Due to MR antagonism in the kidney, spironolactone results in increased excretion of sodium and water to lower fluid retention and lessen pressure on the heart. Spironolactone has been shown to reduce total and cardiovascular mortality in heart failure patients when administered along with other inhibitors of the RAAS system.

Spironolactone is not associated with obvious adverse effects on behaviour or respiratory endpoints. Due to the desired pharmacology, electrolytes should be monitored as discussed in detail in the relevant sections above.

Pharmacokinetics

Spironolactone is well absorbed orally, is highly protein bound and extensively metabolised in the liver. It undergoes enterohepatic recirculation and has a short half-life of less than 2 hours. Distribution of spironolactone has been found across the whole body, with higher concentrations in the liver, stomach, large and small intestines and kidneys. Due to the short half-life of spironolactone, it is considered not only as a mineralocorticoid receptor antagonist, but also as a pharmacologically active prodrug. [redacted] In monkeys, as in humans, the amounts excreted in urine and faeces were approximately equal, while faecal excretion predominated in rats and dogs as a result of biliary excretion.

Toxicology

The full safety profile of spironolactone is well-known, as spironolactone tablet and capsule formulations are marketed throughout the EU and clinical use of spironolactone has been well established for several decades. It is known that the mode of action is as an antagonist of aldosterone at the MR, giving rising to a potassium-sparing diuretic pharmacological activity. As detailed previously in this document (section 2.4.2.4), there are several drug-drug interactions due to the activity and prescription patterns associated with spironolactone use, which should be considered as well as the toxicology.

It has been shown that the potential risks associated with spironolactone are due to effects connected to the desired pharmacology. Effects of spironolactone on the thyroid and related hormones were considered to be species-specific. The genotoxic and carcinogenic risks presented by spironolactone are considered to be low. Recent studies suggest a possible protective effect of spironolactone in terms of carcinogenicity. Spironolactone has affected fertility, fecundity in toxicity studies, and feminization on external genitalia of male rat fetuses have been observed as a result of the anti-androgenic activity of spironolactone, and this is

reflected in the SmPC at section 4.6. Breast-feeding of infants should be avoided, as detailed in the SmPC at section 4.6, on the basis of the metabolite canrenone being detected in breast milk.

Conclusions

Spironolactone has been used clinically for several decades.

The reports in the literature surrounding the possible toxicity of spironolactone must be considered in the context of the clinical benefit, and as such careful benefit-risk analysis of using spironolactone must always be conducted prior to use. In this respect and all other respects, the SmPC for Spironolactone 10 mg/ml Oral Suspension is aligned with the already licensed spironolactone tablet, Aldactone® 25mg, 50 mg and 100 mg tablet, and with other approved oral tablets and capsules containing spironolactone.

In the clinic, when administered appropriately in accordance with the SmPC, spironolactone is used with significant clinical benefit, and the risks associated with this drug substance are well-established and appropriate warnings and precautions are stated.

In view of the human pharmacokinetics of Spironolactone 10 mg/ml Oral Suspension, as discussed in section 2.4.1 and in Module 2.5, the risks associated with this new formulation are comparable with oral tablet and capsule formulations of spironolactone which have been approved in EU member states for many years, with the same indications, posology and labelling.

2.4.6 Literature References

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]