REDACTIONS ARE MADE UNDER SECTION 41 AND 43

MODULE 2.4

NON-CLINICAL OVERVIEW

NAME OF PRODUCT:	GLYCOPYRRONIUM BROMIDE TABLETS 1 MG / 2 MG
ACTIVE SUBSTANCE:	GLYCOPYRRONIUM BROMIDE
FORMULATION:	TABLETS 1 MG / 2 MG

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ABBREVIATIONS

%	Percent
[^{3}H]-NMS	[^{3}H]-N-methylscopolamine
~	Approximately
<	Less than
>	Greater than
^{14}C	Carbon-14
ADE	Arrhythmogenic dose of epinephrine
AUC	Area under plasma concentration-time curve
b.i.d.	Twice a day
BW	Body weight
Ca^{2+}	Calcium ion
C_{max}	Maximum plasma concentration
CSF	Cerebrospinal fluid
CYP	Cytochrome P450
e.g.	*Exempli gratia* (for example)
ED_{50}	Effective dose, 50%
ET	End-tidal
FS	Foetal serum
g	Gram(s)
GLP	Good Laboratory Practice
h	Hour(s)
HHSiD	Hexahydrosiladiphenidol
hM	Human muscarinic receptor
Hz	Hertz
i.e.	*Id est* (it is)
i.m.	Intramuscular(ly)
i.p.	Intraperitoneal(ly)
i.v.	Intravenous(ly)
IC_{50}	Inhibitory concentration, 50%
IOP	Intraocular pressure
kg	Kilogram
K_i	Binding affinity constant/ antagonist dissociation constant
K_m	Michaelis constant
K_{off}	Dissociation rate constant
l	Litre
LAMAs	Long-acting muscarinic antagonists
LD_{50}	Lethal dose, 50%
mEq	Milliequivalent
mg	Milligram(s)
min	Minute(s)
ml	Millilitre
mM	Millimolar
mmHg	Millimetre of mercury
mRNA	Messenger ribonucleic acid
MS	Maternal serum
n	Number
ng	Nanogram
nM	Nanomolar
nmol	Nanomole
NOAELs	No-observed-adverse-effect-levels
NOEL	No observed effect level
p	Probability
p.o.	*Per oral*
pA_2	Negative logarithm of the antagonist dissociation constant
pCO_2	Partial pressure of carbon dioxide
pH	*Pondus hydrogenii* (negative decadal logarithm of the hydrogen ion concentration
PTP	Post-tetanic twitch potentiation
s	Second(s)
s.c.	Subcutaneous(ly)
SDRRI	Standard deviation of RR intervals
SE	Standard error
SEM	Standard error of mean
SmPC	Summary of product characteristic
$t_{1/2}$	Half-life
t_{max}	Time to reach C_{max}
UK	United Kingdom
vs.	Versus
α	Alpha
µ	Micro
µM	Micromolar
µmol	Micromole

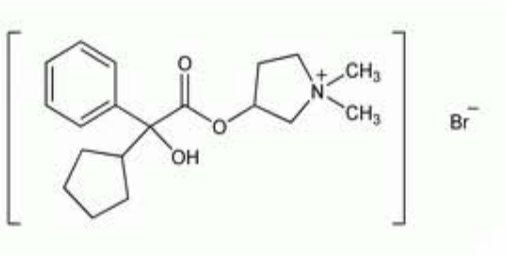
Introduction

The applicant Kinedexe limited, UK, intends to file an application for marketing authorisation of glycopyrronium bromide (glycopyrrolate) 1 and 2 mg tablets for "**use in adults as add-on therapy in the treatment of peptic ulcer**", in accordance with Article 10a of Directive 2001/83/EC, as amended.

Glycopyrrolate is a synthetic quaternary ammonium compound, has anti-muscarinic effects. It is originally synthesised in 1960 and is extensively used for the treatment of peptic ulceration ([redacted]). Glycopyrrolate has been reported effective in reducing gastric secretions and far superior to atropine and scopolamine. It has been used to control hypertonicity without completely abolishing peristalsis or causing constipation. It is remarkably safe and free from unwanted side effects. As anticholinergic therapy to control chronic duodenal ulcers, it has inhibited acid secretion, thus lessening the severity of gastric acidosis ([redacted]).

Glycopyrrolate is used during anaesthesia as an antisialgogoue and to prolong the effectiveness of some anticholinesterases such as physostigmine and neostigmine against neuromuscular blockade ([redacted]). Glycopyrrolate intensifies neuromuscular blockade, delays recovery after atracurium and is a potent antagonist of the muscarinic effects of neostigmine ([redacted], [redacted]). Glycopyrrolate produces less tachycardia than atropine ([redacted]). Glycopyrrolate is superior to atropine when used with anticholinesterases. This is due to the fact that atropine can readily cross the blood-brain barrier and produce adverse central cholinergic effects whereas glycopyrrolate minimally penetrates the blood-brain barrier and therefore, does not significantly affect the central acetylcholine pathway ([redacted])

Glycopyrronium bromide is 3-[(cyclopentylhydroxyphenylacetyl)oxy]-1,1-dimethylpyrrolidinium bromide. The molecular formula is C H BrNO and the molecular weight is 398.33 ([redacted]; [redacted]) Its structural formula is as follows:



Glycopyrrolate occurs as a white, odourless crystalline powder. It is soluble in water and alcohol and practically insoluble in chloroform and ether. It is completely ionised at physiological pH values. CAS Registry Number: 596-51-0 ([redacted])

The aim of this Non-clinical Overview is to provide a comprehensive update of preclinical information on glycopyrronium bromide and to evaluate whether the proposed summary of product characteristic (SmPC) reflects the current information on the preclinical pharmacology and toxicology of glycopyrronium bromide. It will be seen that the data are strongly supportive for the use of glycopyrronium bromide for the indications outlined in the SmPC.

In the following, the current state of knowledge on the active substance with respect to the pharmacological and preclinical information will initially be reviewed and a reflection of the relevant data will then follow in *italics*. The majority of existing reports on preclinical studies were generated either during the initial development of the drug, or were conducted following the demonstration of clinical efficacy. Nevertheless, the early studies were performed in accordance with the techniques and requirements established at the time. Due to their age, it cannot be taken for granted that all published studies cited in the overview were conducted in accordance with current GLP requirements. However, this is not thought to affect the overall conclusions of the report.

2.4.1 Overview of the Non-Clinical Testing Strategy

In order to obtain a complete and current update on the published literature that may cover any significant findings of glycopyrrolate, searches in a variety of classified databases have been performed (up to July 2015). The following databases were searched for preclinical pharmacology and toxicology data:

- Medline (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi)
- TOXLINE Special (http://toxnet.nlm.nih.gov/)
- DART Special (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?DARTETIC.htm)
- HSDB (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.htm)
- IRIS (http://www.epa.gov/iris/)
- GENETOX (Genetic Toxicology [Mutagenicity]: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?GENETOX)
- CCRIS (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS)
- TRI (http://www.epa.gov/tri/)
- CHEMID*plus* (http://chem.sis.nlm.nih.gov/chemidplus/setupenv.html)
- CAS (http://www.cas.org/)
- RTECS (http://www.ccohs.ca/products/databases/rtecs.html)

2.4.2 Pharmacology

2.4.2.1 Primary Pharmacodynamics

Mechanism of action

Glycopyrrolate is a quaternary ammonium antimuscarinic agent and like other anticholinergic agents, it inhibits the action of acetylcholine on structures innervated by postganglionic cholinergic nerves and on smooth muscles that respond to acetylcholine but lack cholinergic innervation. These peripheral cholinergic receptors are present in the autonomic effector cells of smooth muscle, cardiac muscle, the sinoatrial node, the atrioventricular node, exocrine glands and to a limited degree in the autonomic ganglia. Thus, it diminishes the volume and free acidity of gastric secretions and controls excessive pharyngeal, tracheal and bronchial secretions. Glycopyrrolate antagonises muscarinic symptoms (e.g. bronchorrhea, bronchospasm, bradycardia and intestinal hypermotility) induced by cholinergic drugs such as the anticholinesterases [redacted]

Effect on receptor affinity and potency

[redacted]) analysed the potency of glycopyrronium bromide in blocking responses mediated *via* subtypes of muscarinic receptors *in vitro*. Glycopyrronium bromide shifted to the right the curve for inhibition of the twitch response induced by the agonist McN-A-343, and the methacholine-induced curves for inhibition of rat atrial contraction, and for tonic contraction of guinea pig ileum and rabbit iris sphincter. Glycopyrronium bromide blocked with very high potency (>11, apparent -log K_B) the response in rabbit vas deferens. Its affinity was low (9.09) for the M_2 subtype, and intermediate (10.31 or 10.13) for the ileal M_3 and the atypical iris muscarinic receptor subtype, respectively. Except at the receptors in rabbit vas deferens, the blockade of agonist effect appeared to be of simple competitive type. The study concluded that glycopyrronium bromide was about 10 or 100-fold more potent in preventing a response to activation of the pre-junctional receptor in rabbit vas deferens than in blocking an M_3 or M_2 muscarinic receptor subtype, respectively, *in vitro*.

In an *in vitro* study, the possible selectivity of glycopyrronium for M_2 and M_3 muscarinic receptor subtypes was investigated. Muscarinic receptor subtypes in Wistar rat ventricle and submandibular gland homogenates were characterised with [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) by ligand binding studies. Inhibition of [^{3}H]-NMS binding by non-labelled compounds showed the following order: in rat ventricle: glycopyrronium > atropine >> otenzepad > hexahydrosiladiphenidol (HHSiD) > pirenzepine; in rat submandibular gland: glycopyrronium > atropine >> HHSiD >> pirenzepine > otenzepad. These were similar to the expected order of frequency of M_2 and M_3 subtypes, respectively. Glycopyrronium showed similarly high affinities for both M_2 (K_i = 1.889 (SEM 0.049) nmol/l) and M_3 (K_i = 1.686 (0.184) nmol/l) subtypes. Glycopyrronium bound to a homogeneous population of binding sites in both tissues and showed no selectivity for M_2 or M_3 muscarinic receptor subtypes [redacted] .

In another study, a comprehensive preclinical comparison of tiotropium with other long-acting muscarinic antagonists (LAMAs), namely aclidinium bromide and glycopyrrolate was performed. The different muscarinic antagonists were characterised for their 1) affinity toward the different human muscarinic receptor subtypes expressed in Chinese hamster ovary cells and kinetics of receptor dissociation, 2) potency in inhibiting the agonist-induced activation of muscarinic receptors through measurement of second messengers, and 3) efficacy and duration of bronchoprotection, as tested in a model of acetylcholine-induced

bronchoconstriction in anesthetised dogs over a period of 24 h. All of the tested LAMAs showed high affinity and potency toward the human muscarinic M_3 (hM_3) receptor (tiotropium, pA_2 = 10.4; aclidinium, pA_2 = 9.6; and glycopyrrolate, pA_2 = 9.7) (Table 1). However, dissociation half-lives of the LAMAs from the hM_3 receptor differed significantly (tiotropium, $t_{1/2}$ = 27 h; aclidinium, $t_{1/2}$ = 10.7 h; and glycopyrrolate, $t_{1/2}$ = 6.1 h) (Table 2). In line with their kinetic properties at the hM_3, the tested LAMAs provided different levels of bronchoprotection in the *in vi vo* setting 24 h after administration (tiotropium = 35%, aclidinium = 21%, and glycopyrrolate = 0% at 24 h) when applied at equieffective doses (Table 3) ([redacted]).

Table 1: Binding affinities of different muscarinic antagonists against the five human muscarinic receptor subtypes ([redacted])

	hM_1	hM_2	hM_3	hM_4	hM_5
Atropine	9.77	9.47	9.68	9.97	9.50
NMS	10.31	10.25	10.32	10.42	9.59
Ipratropium	9.40	9.53	9.58	9.65	9.07
Pirenzepine	8.63	<7.0	7.03	7.91	7.32
Tiotropium	10.80	10.69	11.02	11.02	9.96
Aclidinium	10.78	10.68	10.74	10.84	10.26
Glycopyrrolate	10.09	9.67	10.04	10.26	9.74

NMS, N-methyl-^{3}H]scopolamine methyl chloride; hM, human muscarinic receptor

Table 2: K_{off} values (h^{-1}) and dissociation half-lives (h) for the different muscarinic antagonists against the human M_1, M_2, and M_3 receptor subtypes ([redacted])

	hM_1		hM_2		hM_3		$t_{1/2}$ ratio
	K_{off} (/h)	$t_{1/2}$(h)	K_{off} (/h)	$t_{1/2}$ (h)	K_{off} (/h)	$t_{1/2}$ (h)	M_3/M_2
Ipratropium	6.75 ± 0.15	0.1	17.7 ± 0.3	0.03	3.09 ± 0.09	0.22	7.3
Tiotropium	0.066 ± 0.01	10.5	0.26 ± 0.05	2.6	0.026 ± 0.005	27	10.4
Aclidinium	0.11 ± 0.007	6.4	0.39 ± 0.03	1.8	0.071 ± 0.01	10.7	5.9
Glycopyrrolate	0.35 ± 0.07	2	1.84 ± 0.1	0.37	0.11 ± 0.02	6.1	16.5

K_{off}, dissociation rate constant; hM, human muscarinic receptor.

Table 3: Functional properties of the muscarinic antagonists at the human M_1, M_2, and M_3 receptors ([redacted])

	hM_1		hM_2		hM_3	
	pA_2 muscarine	pA_2 carbachol	pA_2 muscarine	pA_2 carbachol	pA_2 muscarine	pA_2 carbachol
Atropine	9.11	9.10	9.53	9.68	9.23	9.21
NMS	9.34	9.41	10.30	10.28	9.43	9.58
Ipratropium	9.08	9.11	9.62	9.68	9.17	9.21
Pirenzepine	8.13	8.04	6.63	6.44	6.60	6.67
Tiotropium	10.43	10.52	11.15	11.09	10.44	10.68
Aclidinium	9.55	9.93	11.25	11.48	9.58	10.04
Glycopyrrolate	9.77	9.62	10.01	9.94	9.68	9.63

hM, human muscarinic receptor. pA_2, negative logarithm of the antagonist dissociation constant.

A study characterised the *in vitro* and *in vivo* profiles of two novel long-acting muscarinic antagonists, aclidinium bromide and glycopyrronium bromide, using tiotropium bromide and

ipratropium bromide as comparators. All four antagonists had high affinity for the five muscarinic receptor sub-types (M_1-M_5); aclidinium had comparable affinity to tiotropium but higher affinity than glycopyrronium and ipratropium for all receptors (Table 4). Glycopyrronium dissociated faster from recombinant M_3 receptors than aclidinium and tiotropium but more slowly than ipratropium; all four compounds dissociated more rapidly from M_2 receptors than from M_3 receptors (Table 5). *In vitro*, aclidinium, glycopyrronium and tiotropium had a long duration of action at native M_3 receptors (>8 h vs. 42 min for ipratropium). *In vivo*, all compounds were equipotent at reversing acetylcholine-induced bronchoconstriction. Aclidinium, glycopyrronium and ipratropium had a faster onset of bronchodilator action than tiotropium. Aclidinium had a longer duration of action than glycopyronnium (time to 50% recovery of effect [$t_{1/2}$ offset] = 29 h and 13 h, respectively); these compare with a $t_{1/2}$ offset of 64 h and 8 h for tiotropium and ipratropium, respectively. Aclidinium was less potent than glycopyrronium and tiotropium at inhibiting salivation in conscious rats (dose required to produce half-maximal effect [ED_{50}] = 38, 0.74 and 0.88 µg/kg, respectively) and was more rapidly hydrolysed in rat, guinea pig and human plasma compared with glycopyrronium or tiotropium (Table 6). These results indicate that while aclidinium and glycopyrronium are both potent antagonists at muscarinic receptors with similar kinetic selectivity for M_3 receptors vs. M_2, aclidinium has a longer dissociation $t_{1/2}$ at M_3 receptors and a longer duration of bronchodilator action *in vivo* than glycopyrronium. The rapid plasma hydrolysis of aclidinium, coupled to its kinetic selectivity, may confer a reduced propensity for systemic anticholinergic side effects with aclidinium vs. glycopyrronium and tiotropium ([redacted]).

Table 4: Binding affinity of aclidinium, glycopyrronium, tiotropium, and ipratropium for human M_1, M_2, M_3, M_4 and M_5 receptors ([redacted])

	K_i (nM)				
	M_1	M_2	M_3	M_4	M_5
Aclidinium	0.10 ± 0.00	0.14 ± 0.04	0.14 ± 0.02	0.21 ± 0.04	0.16 ± 0.01
Glycopyrronium	0.42 ± 0.02	1.77 ± 0.06	0.52 ± 0.04	0.78 ± 0.04	1.29 ± 0.09
Tiotropium	0.13 ± 0.00	0.13 ± 0.04	0.19 ± 0.04	0.30 ± 0.09	0.18 ± 0.06
Ipratropium	1.31 ± 0.15	1.12 ± 0.13	1.24 ± 0.08	1.92 ± 0.18	3.22 ± 0.15

K_i, antagonist dissociation constant.

Table 5: Dissociation half-lives of [^{3}H]aclidinium, [^{3}H]glycopyrronium, [^{3}H]tiotropium and [^{3}H]ipratropium from human M_2 and M_3 receptors([redacted])

	M_2 $t_{1/2}$ (h)	M_3 $t_{1/2}$ (h)	Relative half-life at M_3 receptor[a]	$t_{1/2}$ M_3/M_2 ratio
Aclidinium	4.69 ± 0.29	29.24 ± 0.61	62	6.2
Glycopyrronium	1.07 ± 0.20	8.10 ± 0.45	17	7.3
Tiotropium	15.11 ± 1.57	62.19 ± 2.96	132	4.1
Ipratropium	0.08 ± 0.01	0.47 ± 0.02	1	5.9

[a]Half-lives expressed relative to [^{3}H]ipratropium.
$t_{1/2}$, dissociation half-life.

Table 6: Potency and duration of action of aclidinium, glycopyrronium, tiotropium, and ipratropium at native M_2 receptors (isolated guinea pig left atria) and M_3 receptors (isolated guinea pig trachea) (n = 3-13) ([redacted])

	M_2		M_3	
	EC_{50} (nM)	$t_{1/2}$ offset (min)	EC_{50} (nM)	$t_{1/2}$ offset (min)

Aclidinium	17.4 ± 1.1	102	5.3 ± 1.6	>480
Glycopyrronium	17.3 ± 1.2	30	4.2 ± 0.3	>480
Tiotropium	11.8 ± 1.1	184	3.0 ± 0.6	>480
Ipratropium	19.9 ± 1.1	4	3.0 ± 0.4	42
EC_{50}, concentration required to produce 50% inhibition of the maximum carbachol-induced relaxation (M_2) or 50% inhibition of electrically stimulated contractions (M_3); $t_{1/2}$ offset, time to 50% recovery of the maximum carbachol-induced relaxation (M_2) or to 50% recovery of electrically stimulated contractions (M_3).				

Another study evaluated the pharmacological profile of the muscarinic antagonist glycopyrrolate in guinea-pig and human airways in comparison with the commonly used antagonist ipratropium bromide. Glycopyrrolate and ipratropium bromide inhibited electrical field stimulation-induced contraction of guinea-pig trachea and human airways in a concentration-dependent manner. Glycoprrolate was more potent than ipratropium bromide. The onset of action (time to attainment of 50% of maximum response) of glycopyrrolate was similar to that obtained with ipratropium bromide in both preparations. In guinea-pig trachea, the offset of action (time taken for response to return to 50% recovery after wash out of the test antagonist) for glycopyrrolate ($t_{1/2}$ offset = 26.4 ± 0.5 min) was less than that obtained with ipratropium bromide (81.2 ± 3.7 min). In human airways, however, the duration of action of glycopyrrolate ($t_{1/2}$ offset >96 min) was significantly more prolonged compared to ipratropium bromide ($t_{1/2}$ offset = 59.2 ± 17.8 min). In competition studies, glycopyrrolate and ipratropium bromide bind human peripheral lung and human airway smooth muscle muscarinic receptors with affinities in the nanomolar range (K_i = 0.5-3.6 nM). Similar to ipratropium bromide, glycopyrrolate showed no selectivity in its binding to the M_1-M_3 receptors. Kinetics studies, however, showed that glycopyrrolate dissociates slowly from human airway smooth muscle muscarinic receptors (60% protection against [N-methyl-^{3}H]-scopolamine binding at 30 nM) compared to ipratropium bromide. The study concluded that glycopyrrolate bind human and guinea-pig airway muscarinic receptors with high affinity. The slow dissociation profile of glycopyrrolate might be the underlying mechanism by which this drug accomplishes its long duration of action ([redacted]).

Effect of gastric secretion

In a study, the effect of glycopyrrolate on gastric secretion and terminal antral motor activity in vagally innervated and denervated gastric pouch dogs were investigated. Glycopyrrolate, as the methobromide salt, was given orally to the female mongrel dogs (n = 4) at a dose of 1, 100 or 1000 µg/kg body weight (BW) 30 min prior to the feeding of a 400 g test meal. Low doses of the drug (100 µg/kg p.o.) markedly inhibited food-stimulated secretion, with little or no effect on antral motility. At a higher dose (1000 µg/kg p.o.), gastric secretion and antral activity were decreased to a significant degree. The study concluded that glycopyrrolate produced similar effects in both types of surgically prepared pouch preparations ([redacted]).

In another study, glycopyrrolate (0.75 g/l) dissolved in drinking water was given to the male adult Wistar rats. The treatment lasted for 5 months. A secretion study was then performed in gastric fistula-bearing animals, infused for 24 h with normal saline or histamine. A significant decrease of the secretory function of parietal and zymogenic cells was found in treated rats, as compared with untreated control animals. The study concluded that long term administration of an anticholinergic drug glycopyrrolate in drinking water produced gastric hypo-secretion in rats ([redacted]). The results are summarised in Table 7.

Table 7: Effect of prolonged treatment of rats with glycopyrrolate on 24-h gastric secretion* (n = 11/group) ([redacted])			
Group	**Glycopyrrolate-**	**Infused**	**24-h gastric secretion**

	treated	intravenously	Volume (ml)	pH	HCl/meq	Pepsin/mg
1	+	Histamine	8.0	1.9	0.55	191
2	+	Saline	5.7	2.2	0.41	104
3	-	Histamine	28.0	1.1	3.30	563
4	-	Saline	11.9	1.1	1.20	278
p			0.01†	0.01‡	0.01†	0.01†

*Histamine dihydrochloride 100 mg/kg body weight was infused during the collection of gastric content.
p values are for 24-h outputs.
†Third and fourth vs. other groups.
‡First and second vs. third and fourth groups.

In a study, glycopyrrotate, was given in a dosage of 0.1 mg/kg 4 times daily for a period of 11 weeks to 4 dogs equipped with Heidenhain pouches and 2 intact dogs in which maximal histamine tests were performed weekly 1 h after glycopyrrolate administration. A steadily diminishing acid secretory response was found in both groups throughout the periods of drug administration. Four weeks elapsed following the drug discontinuation before control secretory function was regained. Serial pouch and mucosal biopsies examined by light and phase microscopy, revealed no departures from the normal state. In a third group of 4 intact dogs, parietal cell counts performed after the 11 weeks of glycopyrrolate were sharply lower than those obtained in 2 intact, untreated controls. Ultramicroscopic examination of the mucosa in this group revealed in approximately 25% of the parietal cells alterations consisting of collapse of the intracellular canaliculi, marked dilatation of the endoplasmic reticulum, and transformation of endoplasmic reticulum from a smooth to a granular type. The study concluded that long term administration of glycopyrrolate diminished gastric acid secretory function ([redacted] .

In another study, atropine sulphate (0.65 mg, given orally 4 times daily to a group of 4 dogs) and propantheline bromide (15 mg, given orally to a second group of 4 dogs) were found to induce alterations in the nucleus, mitochondria, cytoplasmic vesicles and vacuoles, and intracellular canaliculi of parietal cells when examined by electron microscopy at the end of 7 weeks of treatment. Vagotomy (performed in a third group of 4 dogs) failed to induce parietal cell alterations when examined after 7 weeks. Giycopyrrolate (2 mg, given orally 4 times daily to a fourth group of dogs) was found to induce profound cytoplasmic vacuolisation, in addition to producing alterations of the parietal cell nucleus, mitochondria and intracellular canaliculus. Similar changes developed in the parietal cells of the vagotomised dogs after 7 weeks of glycopyrrolate administration. Atropine was accompanied by the most profound mitochondrial derangements. Glycopyrrolate administration was marked by the most extensive cytoplasmic vacuolisation ([redacted]) reported that anticholinergic drugs such glycopyrrolate and atropine diminished gastric acidity and volume increased elicited by methacholine chloride.

A study evaluated the effects of hydromorphone, hydromorphone and glycopyrrolate, medetomidine, and butorphanol premedication on the difficulty and time required to pass an endoscope into the stomach and duodenum of female cats (n = 8) anaesthetised with ketamine and isoflurane. Each cat was premedicated and anaesthetised 4-times with an interval of at least 7 days between procedures. Cats were premedicated with hydromorphone, hydromorphone and glycopyrrolate, medetomidine, or butorphanol administered i.m.. Twenty minutes after premedication, sedation was assessed by use of a subjective ordinal scale. Cats received ketamine administered i.m., and 10 min later a cuffed orotracheal tube was placed and anaesthesia maintained with isoflurane. Cats breathed spontaneously

throughout the procedure. When end-tidal isoflurane concentration was stable at 1.4% for 15 min, endoscopy was begun. The times required to pass the endoscope through the cardiac and pyloric sphincters were recorded, and the difficulty of endoscope passage was scored by use of a subjective ordinal scale. No significant differences in difficulty or time required to pass the endoscope through the cardiac and pyloric sphincters were found among premedicant groups. Premedication with medetomidine resulted in the greatest degree of sedation and longest time to return to sternal recumbency. The study concluded that hydromorphone, hydromorphone and glycopyrrolate, medetomidine, and butorphanol at the doses tested can be used satisfactorily to premedicate cats prior to general anaesthesia for gastroduodenoscopy ([redacted]).

Another study evaluated. 1) the effect of two anticholinergic agents glycopyrrolate (0.1 and 0.2 mg/25 lb BW) and atropine (0.02 mg/lb) as pre-anaesthetics (dogs, n = 20), and 2) the effect of each agent (glycopyrrolate 0.1 mg/25 lb and atropine 0.02 mg/lb) when administered as adjunct medication during anaesthetic maintenance (dogs, n = 10). Gastric secretions averaged above 2.5 pH for all groups, with a mean of 5.26 ± 1.90 for glycopyrrolate at 0.2 mg/25 lb and 3.73 ± 0.1 for 0.1 mg/25 lb level, indicating 0.2 mg/25 lb as being more effective for maintaining high gastric pH. With atropine at 0.2 mg/lb the pH of gastric secretion was 3.5 ± 0.27. Glycopyrrolate had a longer lasting effect and at a dosages used, gave more smooth muscle relaxation with little cardiovascular adverse effect when compared with atropine. Glycopyrrolate was effective in preventing aspiration of gastric secretion and the pulmonary complications associated with Mendelsons syndrome, not only for producing a higher pH of gastric secretions but in the reduction of intestinal smooth muscle activity and thus likelihood of regurgitation ([redacted]).

Effect on antral motility

The effect of atropine, and glycopyrrolate on the antral motility was investigated in eight dogs (four Beagles and four Labradors) using passive telemetry. Both anticholinergics induced a pronounced and lasting reduction of the intensity and frequency of the contractions. A definite dose-related inhibition of the antral motility was seen in Beagles, similar for both active substances. Low doses of atropine (0.02 mg/kg BW i.m.) and glycopyrrolate (0.005 mg/kg BW, i.m.) completely inhibited the gastric motility for at least 30 min, whereas higher doses (0.04 or 0.01 mg/kg BW) caused a cessation of activity for more than 3 h. In Labradors, the effects of both active substances were not so dose related and the effect of glycopyrrolate lasted at least 6 h, whereas the effect of atropine gradually decreased after 3 h. The study reported that anticholinergica mainly inhibit the emptying by reducing the intensity and frequency of gastric contractions ([redacted]).

2.4.2.2 Secondary Pharmacodynamics

Effect on neuromuscular transmission

Effects of atropine and glycopyrrolate on neuromuscular transmission and on muscle contraction, were studied, in the male Sprague-Dawley rat phrenic nerve-diaphragm preparation, by analysing their effects on the indirectly (and directly)-elicited twitch (0.2 Hz), tetanic (50 Hz for 20 s duration), post-tetanic twitch responses (at 5 s after the tetanus), and on the phenomenon of post-tetanic twitch potentiation (PTP), which is thought to be of a presynaptic origin, i.e. due to increased transmitter release. Atropine (0.001-10 µM) increased the indirectly-elicited twitch tension by 22 ± 2.1% (control 0.9 ± 0.1 g, $p < 0.02$), the tetanus by 15 ± 1.1% (control 3.9 ± 0.7 g, $p < 0.05$), the post-tetanic twitch response by 33 ± 3.1% (control 1.2 ± 0.1 g, $p < 0.01$) and the PTP value by 36 ± 1.9% (control 33± 2.3%, $p < 0.01$,

mean ± SEM = 6). Atropine (0.001-10 µM) had little effect on the directly-elicited twitch tension, but in high concentrations (e.g. 20 µM), it blocked the twitch tension. In contrast, glycopyrrolate (0.1-100 µM) had little effect on the twitch tension (direct or indirect), but it significantly reduced the tetanus (by 38 ± 3.5%, $p < 0.01$), the post-tetanic twitch response (by 17 ± 1.2%, $p < 0.05$) and the PTP values (by 24 ± 3.1%, $p < 0.02$). In the presence of hemicholinium (1.3 µM), the responses to atropine and glycopyrrolate were altered (decreased), indicating a possible action on presynaptic mechanism of transmission. It was concluded that atropine and glycopyrrolate produce different (opposite) effects at the rat neuromuscular junction, atropine enhances whereas glycopyrrolate depresses neuromuscular transmission. The effects of these two antimuscarinic drugs may be exerted at the presynaptic nerve terminals, i.e. on presynaptic muscarinic receptors, which are involved in the feedback mechanism of transmitter release ([redacted] .

The effect of glycopyrrolate (10 µmol/l) and neostigmine (1 µmol/l) on atracurium (0.1-100 µmol/l)-induced neuromuscular blockade was studied in the rat isolated phrenic nerve-diaphragm preparation, to see if glycopyrrolate intensified the neuromuscular blockade produced by atracurium in this preparation. Atracurium had a rapid onset of blockade, reaching a complete block in 30-40 s. Glycopyrrolate had no significant effect on indirectly-elicited twitch (0.2 Hz) tension, whereas it significantly increased atracurium-induced depression of twitch tension and shortened the time needed to a complete block by 10 s. Combinations of glycopyrrolate + neostigmine, only slightly reversed atracurium-induced blockade, if compared to the reversal by neostigmine alone. The mean concentrations to produce 50% depression of twitch tension were: 1.6 ± 0.1 (atracurium), 0.3 ± 0.1 (atracurium + glycopyrrolate), 4.8 ± 0.2 (atracurium + neostigmine) and 2.7 ± 0.1 µmol/l (atracurium + glycopyrrolate + neostigmine) (means ± SEM, n = 6, $p < 0.001$, with respect to control value of atracurium alone). It was concluded that glycopyrrolate enhanced atracurium-induced neuromuscular blockade in the rat diaphragm preparation, and that this effect should be noted when dosing glycopyrrolate in man ([redacted] .

Cardiovascular effect

A study reported that glycopyrrolate had biphasic effects on the contraction of guinea-pig atrium. At concentrations between 0.4 and 20 µM, glycopyrrolate induced a small but consistent increase in the contraction force. Further increase in the concentration of glycopyrrolate produced a concentration dependent reduction in the force of contraction with a EC_{50} of 0.24 mM. This negative ionotropic effect was opposed by a Ca^{2+}-channel agonist, Bay K 8644. Glycopyrrolate also antagonised potently the depressant effects of carbacbol and acetylcholine in guinea-pig atrium ([redacted] .

Another study evaluated the effectiveness of glycopyrrolate (0.005 or 0.01 mg/kg BW) in anesthetised dogs (n = 40) for reversal of bradycardia (<65 beats/min). Following random i.v. treatment, heart rate was determined at 5 min and, if it was < or = 70 beats/min, the lower dose was repeated. A 2-way analysis of variance considered dose and animal size (< or = 10 kg, >10 kg) effects ($p < 0.05$). Glycopyrrolate produced a significant increase in heart rate and infrequent tachycardia (< or = 150 beats/min), which was not dose-related. The size of the dog produced a significant effect on baseline heart rate (higher in small), rate following the first dose (lower in small), and requirement for retreatment (47% in small, 13% in large). In a separate group of anesthetised dogs (n = 20), the blood pressure effect of glycopyrrolate (0.01 mg/kg BW, i.v.) treatment of bradycardia (65-85 beats/min, weight-adjusted) was studied. A significant increase in systolic, diastolic, and mean blood pressure was produced. The study

concluded that the effective dose of glycopyrrolate treatment was size-related and produced a beneficial effect on blood pressure (Dyson DH et al., 1999).

Autonomic and cardiovascular changes were studied when neuromuscular blockade was antagonised in 96 dogs with one of eight anticholinesterase-antimuscarinic drug combinations. Neostigmine (50 or 100 µg/kg) was administered before or after atropine (40 µg/kg) or glycopyrrolate (10 µg/kg). The high dose of neostigmine (100 µg/kg) caused bradyarrhythmias, salivation, and signs of bronchosecretion when used with either antimuscarinic agent and irrespective of the administration sequence. The heart rate increased, but not significantly, when atropine was injected before either dose of neostigmine. This did not occur when this administration sequence was reversed. Arrhythmias and cardiovascular and autonomic changes did not occur when glycopyrrolate was injected before or after neostigmine at 50 µg/kg ([redacted]).

A study evaluated and compared the haemodynamic changes caused by the administration of i.v. atropine or glycopyrrolate after i.v. xylazine in isoflurane-anesthetised dogs. Six healthy beagles (male = 2, female = 4; 8.2-10.7 kg) were used in two trials separated by 7 days. Anaesthesia was induced and maintained with isoflurane in 100% oxygen with controlled ventilation. Once constant end-tidal isoflurane (1.8%) and arterial partial pressure of carbon dioxide (35-45 mmHg) values were reached, baseline data were recorded and xylazine (0.5 mg/kg, i.v.) was given. In trial 1 atropine (0.1 mg/kg, i.v.) was given 5 min after xylazine, and in trial 2 glycopyrrolate (0.025 mg/kg, i.v.), was given 5 min after xylazine. Haemodynamic variables were recorded 3 min after xylazine and 3 min after anticholinergic administration. In trial 2, bilateral vagotomies were performed 10 min after glycopyrrolate, and haemodynamic variables were recorded 3 min later. Heart rate, cardiac index, and stroke index decreased; arterial pressure and systemic vascular resistance increased after xylazine. Heart rate, cardiac index, and rate pressure product increased after anticholinergic administration. Significant differences between atropine and glycopyrrolate were not observed in any of the haemodynamic parameters. Similarly, significant differences between glycopyrrolate and bilateral vagotomy were not observed. The authors concluded that i.v. atropine and glycopyrrolate had equivalent haemodynamic actions during the increased pressure phase after i.v. xylazine in isoflurane-anesthetised dogs; that i.v. atropine and glycopyrrolate produced comparable increased in heart rate and that both may increase the risk of myocardial hypoxia associated with an increase in rate pressure product; and that vagal blockade produced by high-dose glycopyrrolate (0.025 mg/kg, i.v.) was similar to that produced by bilateral vagotomy[redacted]).

Another study determined the influence of cholinergic blockade produced by glycopyrrolate on arrhythmogenic dose of epinephrine (ADE) in 1.5 minimum alveolar concentration halothane- and isoflurane-anesthetised dogs. Eight dogs (male = 5, female = 3; weighing between 12.5 and 21.5 kg) were randomly assigned to four treatment groups and each treatment was replicated 3-times. Anaesthesia was induced and maintained with halothane (1.31%, end-tidal [ET]) or isoflurane (1.95%, ET) in oxygen. Ventilation was controlled (carbon dioxide [pCO_2] 35-40 mmHg, ET). Glycopyrrolate was administered 10 min before ADE determination at a dose of 22 µg/kg (isoflurane 11 µg/kg, i.v. and isoflurane 11 µg/kg, i.m.). The ADE was determined by i.v. infusion of epinephrine at sequentially increasing rates of 1.0, 2.5, and 5.0 µg/kg/min; and defined as the total dose of epinephrine producing at least four ectopic ventricular contractions within 15 s during a 3 min infusion and up to 1 min after the end of the infusion. Total dose was calculated as the product of infusion rate and time to arrhythmia. Data were analysed using a randomised complete block analysis of variance. When significant ($p < 0.05$) F values were found a least significant difference test was used to

compare group means. Values are reported as means ± SE. The ADE (µg/kg) for halothane, halothane + glycopyrrolate, isoflurane, and isoflurane + glycopyrrolate were 1.53 ± 0.08, 3.37 ± 0.46, 1.61 ± 0.21, and >15.00, respectively. Heart rates (beats/min) and systolic pressures (mmHg) at the time of arrhythmia formation for halothane, halothane + glycopyrrolate, isoflurane, and isoflurane + glycopyrrolate were (60.3 ± 4.0 and 142.0 ± 7.6), (213.0 ± 13.1 and 239.2 ± 7.1), (62.9 ± 4.5 and 151.9 ± 6.3), and (226.3 ± 6.1 and 323.5 ± 3.4), respectively. The halothane and isoflurane ADE were not different. The halothane + glycopyrrolate ADE was significantly less than the isoflurane + glycopyrrolate ADE. The halothane and isoflurane ADE were significantly less than the halothane + glycopyrrolate and isoflurane + glycopyrrolate ADE. The authors concluded that (1) two distinct mechanisms were responsible for the development of arrhythmias, and (2) cholinergic blockade produced by glycoprrolate significantly increased ADE but was associated with higher rate pressure products and myocardial work ([redacted]).

Cardiopulmonary effect

In a study, nine groups of rats (n = 5/group) received an i.m. injection of one of the following drugs or drug combinations: saline, atropine (0.05 mg/kg), glycopyrrolate (0.5 mg/kg), ketamine:xylazine (85:15 mg/kg), ketamine:detomidine (60:10 mg/kg), atropine:ketamine:xylazine (0.05:85:15 mg/kg), glycopyrrolate:ketamine:xylazine (0.5:85:15 mg/kg), atropine:ketamine:detomidine (0.05:60:10 mg/kg) or glycopyrrolate:ketamine:detomidine (0.5:60:10). Similarly six groups of rabbits (n = 5) received an i.m. injection of either saline, atropine (0.2 mg/kg), atropine (2 mg/kg), glycopyrrolate (0.1 mg/kg), ketamine:xylazine (35:10 mg/kg) or glycopyrrolate:ketamine:xylazine (0.1:35:10 mg/kg). In rats, atropine sulphate (0.05 mg/kg) and glycopyrrolate (0.5 mg/kg) produced an increase in heart rate for 30 and 240 min, respectively. In rabbits atropine sulphate at either 0.2 or 2.0 mg/kg did not induce a significant increase in heart rate, but glycopyrrolate (0.1 mg/kg) elevated the heart rate above saline treated animals for over 50 min. Both atropine and glycopyrrolate provided protection against a decrease in heart rate in rats anesthetised with ketamine:xylazine (85:15 mg/kg) or ketamine:detomidine (60:10 mg/kg); however, glycopyrrolate was significantly more effective in maintaining the heart rate within the normal range. Glycopprrolate also prevented a decrease in heart rate in rabbits anaesthetised with ketamine:xylazine (35:5 mg/kg). Neither glycopyrrolate nor atropine influenced respiration rate, core body temperature or systolic blood pressure when used alone or when combined with the injectable anaesthetic. The study concluded that glycopyrrolate is an effective anticholinergic agent in rabbits and rodents and more useful as a pre-anaesthetic agent than atropine sulphate in these animals ([redacted]).

Mydriatic effect

In a study, glycopyrrolate 0.5% and atropine 1.0% were instilled separately in the eyes of albino rabbits. Pupil diameter and intra-ocular pressure were monitored. Mydriasis was noted within 5 min of glycopyrrolate instillation, reached near-maximal level at 15 min and persisted for 1 week. Glycopyrrolate 0.5% showed a faster, stronger and more persistent mydriatic effect than atropine 1.0%. Administration of glycopyrrolate 0.5% solution b.i.d. for 1 week did not affect intra-ocular pressure or produce any adverse reaction. The study concluded that glycopyrrolate solution has the potential to deliver an ocular anticholinergic effect without causing associated central anticholinergic hazards ([redacted]).

2.4.2.3 Safety Pharmacology

Gastrointestinal effect

[redacted] studied: Phase I, in which 12 mixed-breed dogs (male/female = 6/6), were used to evaluate the effect of three different dosages (0.0055, 0.011, and 0.022 mg/kg, respectively) of glycopyrrolate on sialorrhea, and intestinal peristalsis, and Phase II, in which glycopyrrolate (0.011 mg/kg) was given as a pre-anaesthetic to 35 dogs representing a variety of breeds. Four dogs were included that received no anticholinergic as a pre-anaesthetic. The final clinical evaluation included the effects of glycopyrrolate as an anticholinergic agent used as a pre-anaesthetic in 5 cats. The cats received 0.011 mg/kg glycopyrrolate i.m. about 10 min before general anaesthesia was induced. In Phase I, glycopyrrolate at 0.0055 mg/kg did not completely prevent sialorrhea and intestinal peristalsis. But levels of 0.011 and 0.022 mg/kg did suppress sialorrhea and intestinal peristalsis. In Phase II, glycopyrrolate was given to animals anesthetised in the clinic for performance of a variety of surgical procedures. All of these animals responded satisfactorily. No salivation or bronchial secretions were noted during surgery and recovery. All 4 dogs that did not receive an anticholinergic agent as a pre-anaesthetic exhibited sialorrhea. Glycopyrrolate as a pre-anaesthetic agent in cats controlled salivary and bronchial secretion during surgery and recovery.

Cardiovascular effect

In a study, placental transfer and maternal and foetal haemodynamic effects of atropine and glycopyrrolate were compared in chronically and in acutely instrumented, unanaesthetised pregnant ewes. Administration of either atropine (0.05 mg/kg) or glycopyrrolate (0.025 mg/kg) increased maternal heart rate by 25% without changing maternal arterial pressure, foetal arterial pressure, foetal heart rate, or beat-to-beat variability. Maternal and foetal blood gas tensions also did not change. Placental transfer of atropine was significantly greater than that of glycopyrrolate throughout the entire experimental period. The peak foetal/maternal ratios observed 4 h after injection were 1.0 for atropine and 0.13 for glycopyrrolate. On the basis of placental transfer data only, it is possible to postulate that the use of glycopyrrolate may be preferable to atropine. However, in view of the absence of circulatory effects in the foetal lamb after either atropine or glycopyrrolate, it was concluded that neither agent is preferable to the other insofar as foetal effects are concerned during ovine pregnancy ([redacted]).

The effects of i.v. administered atropine (0.2 mg/kg) and glycopyrrolate (0.01 mg/kg) on heart rate were studied in 10 conscious mature female goats (n = 10). In a drug cross-over fashion, either atropine, glycopyrrolate, or 0.9% saline solution was administered using the same volume (0.05 ml/kg). Atropine and glycopyrrolate caused a significant increase in heart rate ($p < 0.05$), whereas saline solution (0.09%) did not. The mean percent changes in heart rate from baseline were similar for atropine and glycopyrrolate up to 14 min after administration. Thereafter, glycopyrrolate had a significantly greater mean change in heart rate than atropine, i.e., up to 29 min ($p < 0.05$). Within the atropine group, the mean percentage changes in heart rate became significantly lower compared with the initial increase (1 min) starting at 11 min. For the glycopyrrolate group, the mean percent changes became significantly lower starting at 27 min. Glycopyrrolate and atropine had a mean percentage change in heart rate of greater than 1.0%, up to 31 and 22 min, respectively. At the doses used, glycopyrrolate had longer duration of action than atropine but the magnitude of increase was similar ([redacted]) studied whether the ventricular rhythm pattern during atrial fibrillation is, in part, modulated by vagal activity. Vagal oscillations were forced at 0.15 Hz by neck suction in 12 Yorkshire pigs with sustained atrial fibrillation with and without glycopyrrolate

(0.15 µg/kg, i.v.) vagal blockade. Vagal activity was evaluated using time- and frequency-domain heart rate variability measures. The standard deviation of RR intervals (SDRRI) was significantly increased during vagal activation compared with baseline ($p = 0.006$). Moreover, SDRRI correlated significantly with spectral power at 0.15 Hz during baseline ($r = 0.90$, $p < .001$) and vagal activation ($r = 0.86$, $p < 0.05$). Glycopyrrolate blocked the increase in SDRRI ($p < 0.001$) and blunted spectral power at 0.15 Hz ($p < 0.05$). These results indicated that: (1) power spectral analysis may be used to assess parasympathetic regulation during atrial fibrillation, and (2) vagal oscillations produced an entrainment of the ventricular rhythm during atrial fibrillation in pigs.

Cardiopulmonary effect

[redacted] studied: Phase I, in which 12 mixed-breed dogs, 6 males and 6 females, were used to evaluate the effect of three different dosages (0.0055, 0.011, and 0.022 mg/kg, respectively) of glycopyrrolate on heart rate, respiration rate, and blood pressure, and Phase II, in which glycopyrrolate (0.011 mg/kg) was given as a pre-anaesthetic to 35 dogs representing a variety of breeds. Four dogs were included that received no anticholinergic as a pre-anaesthetic. The final clinical evaluation included the effects of glycopyrrolate as an anticholinergic agent used as a pre-anaesthetic in 5 cats. The cats received 0.011 mg/kg glycopyrrolate i.m. about 10 min before general anaesthesia was induced. In Phase I, glycopyrrolate at 0.0055, 0.011, and 0.022 mg/kg was effective in keeping heart rate, respiration rate, and blood pressure within normal limits. In Phase II, none of the animals demonstrated cardiac or respiratory abnormalities. In dogs whose blood pressures were monitored, blood pressures were normal. Two of these 4 developed bigeminal pulses while they were anesthetised. Both of these dogs required an anticholinergic and 1 required oxygen and positive ventilation. A third dog required an anticholinergic agent to correct bradycardia. Glycopyrrolate as a pre-anaesthetic agent in cats did not show cardiac or respiratory abnormalities. Heart rates and respiratory rates remained within normal limits. The authors concluded that glycopyrrolate was a safe and effective pre-anaesthetic agent.

Ocular effect

In a randomised, blinded, placebo-controlled study, the effect of i.m. glycopyrrolate (0.01 mg/kg) on pupil diameter and intraocular pressure (IOP) in unanesthetised normal dogs was investigated. Treatment with glycopyrrolate did not change pupil diameter or IOP from baseline, nor were there differences between glycopyrrolate and saline-treated (control) dogs. In addition, the authors retrospectively reviewed the medical records of 2,828 dogs undergoing general anaesthesia between April 1987 and September 1990 to determine if there was an association between parenteral anticholinergic medication and post-anaesthetic elevation in IOP. The authors also determined the frequency of bradycardia requiring anticholinergic therapy during anaesthesia in dogs with glaucoma. Of the 2,828 cases reviewed, the records of 46 dogs coded for glaucoma were examined in detail. The 46 dogs underwent 62 episodes of anaesthesia, with 23 episodes including exposure to an anticholinergic drug. An increase in IOP from pre-anaesthetic to post-anaesthetic measurement occurred in 3 dogs. One of these dogs received anticholinergic medication for bradycardia during anaesthesia. The post-anaesthetic elevation in IOP in this dog was probably not drug related. Pre-anaesthetic anticholinergic administration did not affect the incidence of anticholinergic administration for bradycardia during the anaesthetic episode. Anticholinergic therapy during anaesthesia was more frequent when the pre-anaesthetic medication included an opiate drug. These studies did not indicate an association between parenteral anticholinergic administration and elevations in IOP ([redacted]

2.4.2.4 Pharmacodynamic Drug Interactions

Radioligand binding studies showed that glycopyrrolate displaced pirenzepine and AF-DX 384 from their binding to the M_1 and M_2 muscarinic receptors in guinea-pig brain membranes respectively. The respective binding constants were 0.60 and 0.03 nM. The Hill coefficient value for glycopyrrolate against [^{3}H]pirenzepine was larger than unity, suggesting positive cooperativity at the receptor complex. In contrast, the coefficient value of agonist [^{3}H]AF-DX 384 was not different from unity, indicating simple competitive inhibition [redacted]., [redacted]

Glycopyrrolate is a synthetic quaternary ammonium compound, has anti-muscarinic effects. It inhibits the action of acetylcholine on structures innervated by postganglionic cholinergic nerves and on smooth muscles that respond to acetylcholine but lack cholinergic innervation. Long-acting muscarinic antagonists showed high affinity and potency toward the human muscarinic M_3 receptor (tiotropium, pA_2 = 10.4; aclidinium, pA_2 = 9.6; and glycopyrrolate, pA_2 = 9.7). However, dissociation half-lives of the long-acting muscarinic antagonists from the human muscarinic M_3 receptor differed significantly (tiotropium, $t_{1/2}$ = 27 h; aclidinium, $t_{1/2}$ = 10.7 h; and glycopyrrolate, $t_{1/2}$ = 6.1 h). Glycopyrrolate reduces gastric acid secretions and antral activity. It is superior to atropine and scopolamine. Glycopyrrolate has potential to depress neuromuscular transmission. Glycopyrrolate alters cardiac (increased heart rate, conduction, and force of contraction) and respiratory function. Glycopyrrolate has more persistent mydriatric effect than atropine.

Glycopyrrolate controls salivary and bronchial secretion during surgery and recovery. Glycopyrrolate did not show cardiac or respiratory abnormalities. Heart rates and respiratory rates remained within normal limits. Thus, it is a safe and effective pre-anaesthetic agent. Glycopyrrolate did not change pupil diameter or intraocular pressure.

2.4.3 Pharmacokinetics

2.4.3.1 Methods of Analysis

Liquid chromatography-mass spectrometry methods were used to evaluate the concentration of glycopyrrolate in plasma of mice, rats, rabbits and dogs (AusPAR, Glycopyrronium bromide, 2013; EPAR, Glycopyrronium bromide, 2012).

2.4.3.2 Absorption

The applicability of the assay for kinetic studies in human was studied by determining the plasma concentrations in three gynaecological surgical patients, who received 8 µg/kg of glycopyrrolate as a premedication i.m.. Tritiated N-methyl scopolamine was used to label the muscarinic cholinergic receptors in the membrane preparation obtained from the rat brain. The limit of detection of the assay was 70 ng/l in plasma, 2 µg/l in urine and 140 ng/l in cerebrospinal fluid (CSF). There was no evidence of cross-reactivity of glycopyrrolate derivatives in clinical concentrations. A very rapid absorption was found with a mean maximum plasma concentration (C_{max}) of 14.26 (range 12.02-16.97) µg/l and mean t_{max} (time to C_{ma}) of 13.3 (range 10-15) min. The CSF levels of glycopyrrolate were under detection limit ([redacted]).

The absolute bioavailability of inhaled glycopyrrolate was approximately 40% in humans, which is considerably lower than that observed in rats following intratracheal application (96%). The absolute oral bioavailability of glycopyrrolate was low in both rats and humans (≤5%). This means that the majority of the swallowed dose fraction following inhalation of glycopyrrolate in humans is either subject to extensive first-pass metabolism or not absorbed and excreted in the faeces. No significant systemic accumulation was seen with repeat daily dosing in animals (AusPAR, Glycopyrronium bromide, 2013; EPAR, Glycopyrronium bromide, 2012).

Half-life ($t_{1/2}$) after i.v. administration of glycopyrrolate was similar in mice, dogs and humans (approximately 4, 4.4 and 6.2 h, respectively) but longer in rats (23 h) (AusPAR, Glycopyrronium bromide, 2013).

2.4.3.3 Distribution

Following i.v. administration of radio-labelled glycopyrrolate, total radio-labelled components were located extravascularly and rapidly distributed throughout the body. High levels of radioactivity were observed in highly perfused tissues (kidney, liver, small intestine and various glands). Although glycopyrrolate-related material was eliminated fast from most tissues; several tissues (eye, brown fat, Harderian gland, kidney and liver) had a significantly slow elimination (AusPAR, Glycopyrronium bromide, 2013; EPAR, Glycopyrronium bromide, 2012).

Glycopyrrolate binds weakly to plasma proteins (range 23-44%) in all species tested (i.e., mice, rats, rabbits, dogs, and humans). Generally, the plasma protein binding was comparable between the species although the values were slightly higher for rabbits and humans. Hence, the free drug concentrations are expected to be slightly higher in the toxicological species than in humans. Binding studies conducted with purified human serum albumin and α_1-acid glycoprotein indicated that the observed plasma protein binding in humans cannot solely be explained by binding to these two proteins. Although a slight concentration-dependency in plasma protein binding was observed in all species, it is considered without any relevance for

the interpretation of the safety data (AusPAR, Glycopyrronium bromide, 2013; EPAR, Glycopyrronium bromide, 2012).

No or limited placenta transfer was observed in pregnant mice, rabbits, dogs and humans. Glycopyrrolate and its metabolites distributed well into milk from lactating rats and generally reached higher concentrations in milk when compared with those observed in plasma (up to 11.3-times). Its significance to humans is unknown (AusPAR, Glycopyrronium bromide, 2013; EPAR, Glycopyrronium bromide, 2012).

Minimal amounts of glycopyrrolate cross the blood brain barrier. Both animal and human studies show that placental transfer is limited ([redacted]).

The levels of ^{14}C-glycopyrrolate, a quaternary ammonium anticholinergic agent, appearing in CSF and serum following a single i.v. dose of 0.1 mg/kg were determined in mongrel dogs during barbiturate anaesthesia and compared with levels reached in dogs treated with similar doses of ^{3}H-atropine. Presence of each anticholinergic drug in the peripheral circulation was confirmed by antagonism of the depressor response to i.v. administered acetylcholine. Ten minutes after drug injection, CSF levels of ^{3}H-atropine averaged 10.3 ± 3.1 ng/ml, whereas ^{14}C-glycopyrrolate CSF levels were restricted to 0.9 ± 0.4 ng/ml. Peak CSF/serum concentration ratios for ^{3}H-atropine averaged 0.87 vs. a mean ratio of 0.1 for ^{14}C-glycopyrrolate within the 4 h observation period. Peripheral anticholinergic activity produced by glycopyrrolate was of the same order as that seen with the equivalent dose of atropine. In pregnant barbiturate-anesthetised dogs peak mean foetal serum (FS) levels of 13 ± 1.5 ng/ml occurred 10 min after a single i.v. dose of 0.1 mg/kg ^{3}H-atropine administered to the mother, and represented 30% of the corresponding maternal serum (MS) concentration. ^{14}C-glycopyrrolate treated dogs showed peak mean FS levels of 0.63 ± 0.07 ng/ml 4 h after administration. The maximum FS/MS concentration ratio observed within the 4 h post-drug period for ^{3}H-atropine was 1.0 vs. 0.04 for ^{14}C-glycopyrrolate. Anticholinergic effects in the mother were significantly greater with glycopyrrolate than with identical doses of atropine. The study concluded that glucopyrrolate is more resistant than atropine to penetration across the blood-brain and placental barriers ([redacted]).

2.4.3.4 Metabolism

Studies of the metabolism of glycopyrrolate in animals indicate the major metabolic pathway to be hydroxylation of the cyclopentyl ring and oxidation of the hydroxyl group in the mandelic acid residue. These metabolites have been mainly detected in the liver and kidney ([redacted]).

Metabolism of glycopyrrolate involved oxidation of the cyclopentyl and phenyl ring moieties, subsequent dehydrogenation of the cyclopentyl ring, and hydrolysis of the ester linkage. It was this latter pathway that was the most prominent (especially after p.o. administration), generating the sole major metabolite, M9. *In vitro* experiments with mouse, rat, rabbit, dog and human hepatocytes showed more extensive metabolism in the laboratory animal species, and the formation of no unique human metabolites. No metabolism was observed in incubations with lung microsomes (rat, dog and human). Experiments with recombinant human CYPs (cytochromes) identified low metabolism by CYP2D6, and trace metabolism by CYPs 1A2, 2B6, 2C9, 2C18, 2C19 and 3A4 (AusPAR, Glycopyrronium bromide, 2013; EPAR, Glycopyrronium bromide, 2012).

2.4.3.5 Excretion

The excretion of glycopyrrolate was investigated in mice and rats (including bile duct-cannulated) following intratracheal (rat only), i.v., and p.o. administration. Following i.v. administration, the glycopyrrolate was mainly eliminated *via* urinary excretion (46-68%) and to a lesser extent *via* bile/faeces (<40%). The radioactivity could mainly be ascribed to unchanged drug in both faeces and urine accounting for about 50-60% and 25-65% of the total detected radioactivity, respectively. These data indicate that both the metabolic and biliary elimination are less important elimination pathways as compared to the urinary excretion. However, following intratracheal and p.o. administration, glycopyrrolate was mainly excreted *via* faeces (>50% and >90%, respectively). It is likely that the majority of the swallowed dose is not absorbed (and hence directly excreted in the faeces) considering 1) the low biliary excretion in bile duct-cannulated rats following i.v. administration (~7%), 2) the low oral bioavailability observed in rats and humans following p.o. administration, and 3) the fact that the majority of the radioactivity in faeces from rodents could be ascribed to unchanged drug (>60% of the detected radioactivity) following p.o. administration (AusPAR, Glycopyrronium bromide, 2013; EPAR, Glycopyrronium bromide, 2012).

The applicability of the assay for kinetic studies in human was studied by determining the renal excretion in three gynaecological surgical patients, who received 8 µg/kg of glycopyrrolate as a premedication i.m.. Tritiated N-methyl scopolamine was used to label the muscarinic cholinergic receptors in the membrane preparation obtained from the rat brain. The study reported that approximately 50% of the dose administered was excreted into the urine within 3 h ([redacted] .

A review study reported that following oral administration to mice, 7.6% was excreted in the urine and about 79% in the faeces ([redacted] .

2.4.3.6 Pharmacokinetic Drug Interactions

Glycopyrrolate was shown to be a very weak inhibitor of CYP2D6, acting with an IC_{50} of 100 µM. This is almost 200,000 times greater than the clinical C_{max}, (0.166 ng/ml; 0.52 nM) and no clinical relevance is attached to the finding. IC_{50} values for other CYPs were weaker still (>200 µM). Glycopyrrolate did not induce CYP isoforms, UGT1A1, MDR1 or MRP2 in cultures of primary human hepatocytes (≤50 nM; approximately 100-times the clinical C_{max}), and was found not to be an inhibitor of the MDR1, MXR or MRP2 transporters (≤300 µM). The drug was identified as an inhibitor/substrate of OCT1 and OCT2 (IC_{50}, 17-41 µM; Km, >100 µM) and a substrate of MATE1 (K_m [Michaelis constant] not determined; and not a substrate of MATE2K). Based on the margins between these concentrations and the clinical C_{max}, no clinical relevance is anticipated (AusPAR, Glycopyrronium bromide, 2013; EPAR, Glycopyrronium bromide, 2012).

Glycopyrrolate is poorly absorbed from the gastrointestinal tract. No significant systemic accumulation has been reported with repeat daily dosing in animals. Glycopyrrolate binds weakly to plasma proteins (range 23-44%). Minimal amounts of glycopyrrolate cross the blood brain barrier. Limited placenta transfer in pregnant mice, rabbits, dogs and humans have been reported. Glycopyrrolate and its metabolites distributed well into milk from lactating rats and generally reached higher concentrations in milk when compared with those observed in plasma (up to 11.3-times). Its significance to humans is unknown.

Glycopyrrolate is mainly metabolised by hydroxylation of the cyclopentyl ring and oxidation of the hydroxyl group in the mandelic acid residue. These metabolites have been mainly detected in the liver and kidney. Glycopyrrolate is mainly excreted via *faeces (79%) and to lesser extent* via *urine (7.6%).*

2.4.4 Toxicology

The species selected for the toxicology of glycopyrrolate were mice, rats, rabbits, and dogs. The oral, i.v., i.m., i.p., s.c., and topical routes of administration were involved in acute, sub-chronic, and chronic, reproduction, teratologic, and irritation studies. Toxicity data generated from the mouse, rat, rabbit and dog is deemed the most relevant because of the similarity of their metabolic profiles to the human ([redacted])

2.4.4.1 Single-Dose Toxicity

Acute toxicity of glycopyrrolate was studied in mice, rats, and rabbits. The LD_{50} values are summarised in Table 8.

Table 8: L D_{50} value of gl ycoprrolate (Canada P roduct M onograph, G lycopyrrolate injection, 2005; ChemIDplus, 2015; [redacted])

Species	Sex	Route	LD_{50} (mg/kg)
Mouse	Male	oral	550
	-		570
	Male	i.v.	15
	Male	i.p.	112
	Female		107
	-		90
	-	s.c.	122
Rat	Male	oral	1150
	-		709
	Female	i.v.	15
	Female	i.p.	196
	-	s.c.	833
Rabbit	-	oral	2360
	Male, Female	i.v.	~25

Higher doses by all routes in mice and rats caused mydriasis, cycloplegia, xerostomia, tremors, and tonic and clonic convulsions. Death usually followed the convulsions and apparently resulted from respiratory failure. In rabbits, all animals exhibited mydriasis, tachycardia and prostration. All survivors appeared normal at 72 h. No outstanding gross pathological changes attributable to glycopyrrolate were found in the survivors or the animals that died. (Canada Product Monograph, Glycopyrrolate injection, 2005; [redacted] .

2.4.4.2 Repeat-Dose Toxicity

In a 4-weeks study, the changes in the larynx consisted of squamous metaplasia, hyperplasia and keratosis accompanied by inflammation in the sub mucosa which was necrotising in males exposed to 1.3 mg/kg/day. Moreover, hyaline inclusions and degeneration of the olfactory respiratory epithelium were observed. Based on gene expression analysis, the epithelial hypertrophy noted at the bronchiolalveolar junction appears to be correlated with Clara cells. Similar findings were not made in the respiratory tract of dogs. The innate sensitivity of the upper respiratory tract of rodents to the pathologic effects of inhaled compounds is a well-recognised phenomenon and is probably related to differences in airflow dynamics as well as regional epithelial sensitivity in comparison with non-rodents and humans. Moreover, taking the estimated deposited mass and lung weight into consideration, the local lung exposure at the no-observed-adverse-effect-levels (NOAELs) established in the

rat studies were 24 to 194-fold higher than that anticipated in humans at the proposed therapeutic dose. Hence, the observed changes in the respiratory tract of rats do not represent a clinical risk (EPAR, Glycopyrronium bromide, 2012).

The i.v. administration of glycopyrrolate at 2.0 or 0.4 mg/kg/day 5-days a week for 4-weeks caused no signs of toxicity in Beagle dogs (Canada Product Monograph, Glycopyrrolate injection, 2005; [redacted]).

A 13-week oral gavage dose range-finding (0, 30, 100, and 300 mg/kg/day) toxicity study of glycopyrrolate was performed in male and female CD-1 mice. The study reported that survival was reduced in both genders at 100 and 300 mg/kg/day; survival at scheduled sacrifice in main study groups was 10/10, 10/10, 7/10, and 3/10 for males and 9/10, 9/10, 8/10, and 1/10 for females in the control, 30, 100, and 300 mg/kg/day groups, respectively. In addition, 0/3, 0/24, 4/24, and 13/24 toxicokinetic males and 0/3, 1/24, 3/24, and 16/24 toxicokinetic females were found dead or euthanised in extremis in the control, 30, 100, and 300 mg/kg/day groups, respectively. Pupil dilation and pilo-erection were noted in all test article-treated groups. This was presumably a pharmacological response to the test article, and was observed in a dose-related manner. Mean weight gain over days 0-91 was reduced by more than 10% in both genders in all treatment groups, although mean BW *per se* was reduced by less than 10% in the low dose and medium dose groups. The cause(s) of the treatment-related mortality and reduced BW gain were unclear. There were no remarkable effects on haematology or clinical chemistry, mean organ weights, or gross pathology. Histopathological changes were of minor severity, and did not appear to be clinically meaningful. Glycopyrrolate appeared to be reasonably well tolerated at 30 mg/kg/day, these data suggest that 30 mg/kg/day may have only slightly exceeded the maximum tolerated dose. A dosage of 30 mg/kg/day in mice equates to a human-equivalent dose of approximately 2.4 mg/kg/day (Pharmacological Reviews, Glycopyrrolate oral solution, 2010).

A 13-week oral gavage dose range-finding toxicity study of glycopyrrolate in male and female Sprague-Dawley rat was performed. This study involved oral dosing of male and female rats with glycopyrrolate for 13 weeks at dosages of 0, 40, 120, and 360 mg/kg/day. There were no effects on survival. The only relevant clinical sign was pupil dilation, which was noted in all test article-treated groups in relation to dosage. Reduced mean BW and weight gain were observed in all treatment groups, in relation to dosage; the mean weight gain differed significantly from controls for all treatment groups except low-dose males. There were no remarkable effects on haematology or clinical chemistry. Mean urine volume was increased in both genders in proportion to dosage. There were no effects on mean organ weights or gross pathology. Histopathological effects were of minor severity, and did not appear to reflect dose-limiting toxicity. A dosage of 40 mg/kg/day was reasonably well tolerated by both genders; this dosage in rats equates to a human-equivalent dose of approximately 6.5 mg/kg/day (Pharmacological Reviews, Glycopyrrolate oral solution, 2010).

The major treatment effect on gene expression following glycopyrronium bromide treatment of rats for 13 and 26-weeks was a reversible increase in expression of genes related to xenobiotic metabolism as well as to the bronchial mucosa. The changes in mucosa associated genes were localised in the terminal and respiratory bronchioles, corresponding to the site of morphological change reported by histopathology (hypertrophy of the bronchioloalveolar junction). They comprised genes expressed by associated to mucus/Clara cells. The increase in mRNA expression of the mucus/Clara cells signature was dose dependant, already visible after 3 months of treatment, not stronger after 6 months of treatment and fully recoverable

after 4 weeks of recovery. No signs of inflammation could be detected at the molecular level (EPAR, Glycopyrronium bromide, 2012).

Repeat-dose inhalation toxicity studies were conducted in Wistar rats and Beagle dogs with treatment durations of up to 26- and 39-weeks, respectively. The studies were performed using dry powder formulations containing considerably higher levels of glycopyrronium (2-8%), magnesium stearate (0.25-1%) and lactose monohydrate (91-97.75%) than applied clinically. Moreover, the applied mass median aerodynamic diameters were of a size allowing inhalation into the lung. Generally, the findings made could either be ascribed the muscarinic anticholinergic mode of action of glycopyrronium or the local irritation of the airways caused by prolonged inhalation exposure. High safety margins were obtained. Hence, the NOAELs were established in rats and dogs at AUC exposures at least 22-fold and 10-fold higher, respectively, than is observed clinically at a therapeutic dose of 50 µg/day (EPAR, Glycopyrronium bromide, 2012).

Tachycardia was recorded in dogs at doses ≥0.077 mg/kg/day which gives rise to an AUC based safety margin of 16 to 21-fold in male and female dogs, respectively. This finding is most likely the results of an exaggerated pharmacodynamic effect on the cardiovascular system since glycopyrronium will reduce the parasympathetic effect on the heart (EPAR, Glycopyrronium bromide, 2012).

Chronic oral administration glycopyrrolate at doses of 4, 16, and 64 mg/kg for up to 27 weeks in dogs produced mydriasis, cycloplegia, xerostomia, emesis, occasional lacrimation, injection of sclera and rhinorrhoea. There were no changes in organ weight and histopathology, showed no drug-related changes ([redacted]).

2.4.4.3 Genotoxicity

Glycopyrronium was neither induced gene mutations in bacteria (Ames test), gene mutations in mammalian cells *in vitro* (human peripheral lymphocyte test) nor chromosomal aberrations *in vivo* (rat bone marrow micronucleus test) (Pharmacological Reviews, Glycopyrrolate oral solution, 2014; EPAR, Glycopyrronium bromide, 2012).

No exposure to the bone marrow was observed in the whole-body autoradiography distribution studies conducted in rats following p.o. administration. Furthermore, no significant increase in tumour incidences were observed in the carcinogenicity study conducted in a transgenic mice model (i.e., rasH2 mice) (EPAR, Glycopyrronium bromide, 2012).

The results are summarised in Table 9.

Table 9: Results of genotoxicity (EPAR, Glycopyrronium bromide, 2012)

Type of test	Test system	Concentration range/ Metabolising system	Results
Gene mutations in bacteria	*Salmonella* strains TA98, TA100, TA1535, TA1537, TA102	1.6 to 5000 µg/plate +/- S9	**Negative** Toxicity observed following introduction of a S9 pre-incubation step
Gene mutations in mammalian cells	Cultured human peripheral blood lymphocytes	2039 to 3983 µg/ml (10 mM) +/- S9	**Negative** Up to 37% mitotic inhibition was seen at the highest dose

Chromosomal aberrations *in vivo*	Wistar rat (6 males/group), micronuclei in bone marrow	250, 500, 1000 mg/kg/day p.o. for two days*	**Negative** No toxicity to the bone marrow. Mean plasma C_{max} of 4800 ng/ml
*Animals were sampled 24 hours following the last dosing.			

The potential genotoxicity of glycopyrrolate was investigated in the standard battery of tests. The conduct of the studies was in accordance with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines. Concentrations/doses used were appropriate. A suitable set of *S. typhimurium* strains was used in the bacterial mutation assay. The upper dose level used in the *in vivo* assay for clastogenicity - conducted by the oral route in rats - produced a high multiple of the clinical C_{max} (>3200 times), bone marrow suppression and weight loss. The use of only male animals is acceptable given the absence of notable sex differences in toxicity. All assays were appropriately validated and returned negative results for glycopyrrolate (AusPAR, Glycopyrronium bromide, 2013).

2.4.4.4 Carcinogenicity

In a 26-week oral carcinogenicity study, glycopyrronium treatment did not increase the incidence of neoplastic findings in CByB6F1-Tg (HRAS)2Jic transgenic mice at plasma exposure levels (AUC) up to 50-fold higher than is observed in patients receiving the recommended daily dose (EPAR, Glycopyrronium bromide, 2012).

No treatment-related neoplastic findings were identified in a 104-week rat inhalation carcinogenicity study at glycopyrronium plasma exposure levels of up to 79-fold higher than is observed in patients inhaling 50 µg/day. In the uterus, imbalances in the incidence of benign endometrial polyps were recorded between control and treated groups. However, the slightly increased incidence of benign endometrial polyps observed at the highest dose level did not exceed the range of historical control incidences and further statistical analysis demonstrated that the increase in incidence was not statistically significant. Altogether, the slightly increased incidence of benign endometrial polyps is most likely of no clinical relevance (EPAR, Glycopyrronium bromide, 2012).

The carcinogenic potential of glycopyrrolate was investigated in a 6 month study by the oral route in transgenic mice (rasH2) and a 2 year inhalational study in rats. The transgenic mouse study included a positive control group and also additional groups of wild-type animals (vehicle control and high dose). Dual control groups were used in the rat study. Group sizes were adequate and dose selection was adequate. The highest dose levels exceeded the maximum tolerated dose (based on inhibition of BW gain that exceeded 10%), but survival was either unaffected (rats) or not affected to such a level as to impact study validity (transgenic mice). Very high multiples of the clinical AUC were obtained in both studies. No carcinogenic effect was seen for the drug in either species. Relative systemic exposure was ≤71 in male mice (75 mg/kg/day p.o.), ≤53 in female mice (100 mg/kg/day p.o.) and ≤79 in rats (≤0.45 mg/kg/day by inhalation); relative local exposure in the rat study was ≤194. Non neoplastic lesions were observed in the stomach of mice (epithelial hyperplasia, hyperkeratosis and mixed cell infiltration) and are consistent with local irritation following oral administration at high doses (AusPAR, Glycopyrronium bromide, 2013).

2.4.4.5 Reproductive and Developmental Toxicity

No teratogenicity was observed in rat treated with glycopyrrolate ([redacted]).

Glycopyrrolate was assessed for effects on fertility or general reproductive function in rats. Rats of both genders received glycopyrrolate at dosages up to 100 mg/kg/day *via* oral gavage (approximately 50 times the maximum recommended human dose, when comparing on the basis of body surface area estimates). No treatment-related effects on fertility or reproductive parameters of both genders were observed (Prescribing Information, Cuvposa (glycopyrrolate), 2013).

Reproductive studies in rats and rabbits revealed no teratogenic effects from glycopyrrolate. However, diminished rates of conception and of survival at weaning were observed in rats, in a dose-related manner. Studies in dogs suggest that this may be due to diminished seminal secretion which is evident at high doses of glycopyrrolate (Canada Product Monograph, Glycopyrrolate Injection, 2005).

No or very limited placental transfer was reported for glycopyrrolate in mice and dogs. In a rabbit study, no foetuses from dams treated with glycopyrrolate contained quantifiable levels of drug, although this was not measured until more than 24 h after the last dose. Excretion of glycopyrrolate and its metabolites in milk was shown in lactating rats after i.v. administration, with concentrations higher in milk than in plasma (approximately 2-times the C_{max} and 11-times the AUC for the unchanged drug) (AusPAR, Glycopyrronium bromide, 2013).

Male and female fertility were unaffected in rats treated with doses ≤1.5 mg/kg/day s.c. (relative exposure, ≤894 in males and ≤500 in females). There was evidence of slight inhibition of ovulation (decreased corpora lutea) and increased pre-implantation loss at the highest dose, though, together causing a reduction in viable litter size. The no observed effect level (NOEL) is 0.5 mg/kg/day (relative exposure, 162) (AusPAR, Glycopyrronium bromide, 2013).

No effects on male rat fertility parameters (including sperm counts and sperm motility) were noted at plasma exposure levels (AUC) up to 895-fold higher than is observed clinically at therapeutic doses. Decreases in the number of corpora lutea and implantation sites were observed females with a NOAEL of 0.5 mg/kg/day. This gives rise to an AUC based safety margin of approximately 160-fold. Overall, it is considered unlikely that treatment with glycopyrronium bromide will affect fertility in humans at therapeutic doses (EPAR, Glycopyrronium bromide, 2012).

Embryo-foetal development was unaffected in rats (≤3.05 mg/kg/day; relative exposure, ≤731) and rabbits (≤3.5 mg/kg/day; relative exposure, ≤254). Pup birth weight and postnatal body weight gain were significantly reduced in rats treated at 1.5 mg/kg s.c. (relative exposure, 500). No effects on other developmental parameters were observed. Relative exposure at the NOEL for pup development (0.5 mg/kg/day s.c. is 162 (AusPAR, Glycopyrronium bromide, 2013).

No effects on embryo-foetal development were observed in pregnant rats exposed to glycopyrronium *via* inhalation during gestation days 6 to 17 and in pregnant rabbits inhaling glycopyrronium during gestation days 7 through 19. The maximal plasma exposures (AUC) achieved in the pregnant rats and rabbits were around 670 and 250-fold higher than is observed in humans at the maximal recommended daily dose. Furthermore, it should be underlined that glycopyrronium is unlikely to have reached significant concentrations in the foetuses. Nevertheless, this also seems to be the case in humans (EPAR, Glycopyrronium bromide, 2012).

Pre and postnatal development was not affected in a study applying s.c. glycopyrronium dosing of pregnant rats. Toxicokinetic evaluations were not performed however based on the

data from the rat s.c. fertility study; it is likely that the plasma exposure was at least 100-fold higher than is observed in humans at the recommended daily dose (EPAR, Glycopyrronium bromide, 2012).

2.4.4.6 Local Tolerance

Glycopyrrolate applied topically to rabbit skin produced only minimal signs of toxicity even though systemic absorption occurred as indicated by eye changes. Slight erythema of about 24 h duration was seen with the lowest dose, and higher doses caused slight oedema and more persistent erythema. Mild to moderate irritation with apparent extravasation of blood was noted at sites of i.m. injection of glycopyrrolate in rabbits. These areas were haemorrhagic and/or pocketed in some cases at the higher drug concentrations. The low concentration of glycopyrrolate had virtually no effect at sites of s.c. injection. Areas of extravasation of blood were found with the high concentration ([redacted] .

2.4.4.7 Other Toxicity Studies

Immunotoxicity

Standard toxicology studies did not reveal any adverse effects on the immune organs. Immune function assessments during a 4-week inhalation toxicity study in rats confirmed that there were no changes in leukocyte distribution or on the primary immune response to sheep erythrocytes. The lack of immunetoxicity testing was considered acceptable (EPAR, Glycopyrronium bromide, 2012).

Excipients

All excipients contained in Glycopyrronum bromide tablets are commonly used in the manufacture of oral preparation. The quality of all excipients complies with the requirements of Ph.Eur. Therefore, no safety concerns arise regarding the vehicle for oral preparation of glycopyrronim in Glycopyrronium bromide tablets.

Acute toxicity of glycopyrrolate has been studied in mice rat, and r abbit. The LD_{50} value by oral administration was found to be 550 mg/kg in mice, 1150 mg/kg in rats, and 2360 m g/kg in rabbits. Following intravenous administration, the LD_{50} value was found to be 15 mg/kg in mice and r ats, and approximately 25 m g/kg i n r abbits. F ollowing intraperitoneal administration, the LD_{50} value w as e stimated t o be 107 m g/kg and 196 m g/kg i n m ice and rats, respectively. The LD_{50} value was found to be 122 m g/kg in mice and 833 m g/kg in rats by subcutaneous administration. Repeated dose-toxicity conducted in mice, rats and dogs, has reported that glycopyrrolate produced mydriasis, cycloplegia, xerostomia, emesis, occasional lacrimation, injection of sclera and rhinorrhoea. There were no changes in organ weight and histopathology, showed no drug-related changes.

Glycopyrrolate did not induce gene mutations in vitro *and chromosomal aberrations* in vivo *studies. No carcinogenic potential was observed with glycopyrrolate.*

No teratogenic potential w as r eported with g lycopyrrolate in r ats and do gs. But, diminished rates of c onception and of s urvival at w eaning w ere obs erved i n r ats, i n a dos e-related manner. Studies in dogs suggest that this may be due to diminished seminal secretion which is evident at high doses of glycopyrrolate. Glycopyrrolate causes minor skin irritation at site of intramuscular injection in rabbits.

Thus, preclinical data of glycopyrrolate reveal no special hazard for humans based on conventional studies of acute dose toxicity, repeated dose toxicity, fertility, embryo-foetal development, genotoxicity and carcinogenic potential. The excipients used in the formulation of glycopyrronum bromide are well characterised and safe. It is concluded that glycopyrronium bromide can be safely used in humans according to conditions specified in the SmPC.

2.4.5 Integrated Overview and Conclusions

The applicant Kinedexe limited, UK, intends to file an application for marketing authorisation of glycopyrronium bromide (glycopyrrolate) 1 and 2 mg tablets for "**use in adults as add-on therapy i n t he t reatment o f peptic u lcer**", in accordance with Article 10a of Directive 2001/83/EC, as amended.

Glycopyrrolate is a synthetic quaternary ammonium compound, has anti-muscarinic effects. It inhibits the action of acetylcholine on structures innervated by postganglionic cholinergic nerves and on smooth muscles that respond to acetylcholine but lack cholinergic innervation. Long-acting muscarinic antagonists showed high affinity and potency toward the human muscarinic M_3 receptor (tiotropium, pA_2 = 10.4; aclidinium, pA_2 = 9.6; and glycopyrrolate, pA_2 = 9.7). However, dissociation half-lives of the long-acting muscarinic antagonists from the human muscarinic M_3 receptor differed significantly (tiotropium, $t_{1/2}$ = 27 h; aclidinium, $t_{1/2}$ = 10.7 h; and glycopyrrolate, $t_{1/2}$ = 6.1 h). Glycopyrrolate reduces gastric acid secretions and antral activity. It is far superior to atropine and scopolamine. Glycopyrrolate has potential to depress neuromuscular transmission. Glycopyrrolate alters cardiac (increased heart rate, conduction, and force of contraction) and respiratory function. Glycopyrrolate has more persistent mydriatric effect than atropine.

Glycopyrrolate controls salivary and bronchial secretion during surgery and recovery. Glycopyrrolate did not show cardiac or respiratory abnormalities. Heart rates and respiratory rates remained within normal limits. Thus, it is a safe and effective pre-anaesthetic agent. Glycopyrrolate did not change pupil diameter or intraocular pressure.

Glycopyrrolate is poorly absorbed from the gastrointestinal tract. No significant systemic accumulation has been reported with repeat daily dosing in animals. Glycopyrrolate binds weakly to plasma proteins (range 23-44%). Minimal amounts of glycopyrrolate cross the blood brain barrier. Limited placenta transfer in pregnant mice, rabbits, dogs and humans have been reported. Glycopyrrolate and its metabolites distributed well into milk from lactating rats and generally reached higher concentrations in milk when compared with those observed in plasma (up to 11.3-times). Its significance to humans is unknown.

Glycopyrrolate is mainly metabolised by hydroxylation of the cyclopentyl ring and oxidation of the hydroxyl group in the mandelic acid residue. These metabolites have been mainly detected in the liver and kidney. Glycopyrrolate is mainly excreted *via* faeces (79%) and to lesser extent *via* urine (7.6%).

Acute toxicity of glycopyrrolate has been studied in mice rat, and rabbit. The LD_{50} value by oral administration was found to be 550 mg/kg in mice, 1150 mg/kg in rats, and 2360 mg/kg in rabbits. Following intravenous administration, the LD_{50} value was found to be 15 mg/kg in mice and rats, and approximately 25 mg/kg in rabbits. Following intraperitoneal administration, the LD_{50} value was estimated to be 107 mg/kg and 196 mg/kg in mice and rats, respectively. The LD_{50} value was found to be 122 mg/kg in mice and 833 mg/kg in rats by subcutaneous administration. Repeated dose-toxicity conducted in mice, rats and dogs, has reported that glycopyrronium bromide produced mydriasis, cycloplegia, xerostomia, emesis, occasional lacrimation, injection of sclera and rhinorrhoea. There were no changes in organ weight and histopathology, showed no drug-related changes.

Glycopyrrolate did not induce gene mutations *in vitro* and chromosomal aberrations *in vivo* studies. No carcinogenic potential was observed with glycopyrrolate.

No teratogenic potential was reported with glycopyrrolate in rats and dogs. But, diminished rates of conception and of survival at weaning were observed in rats, in a dose-related

manner. Studies in dogs suggest that this may be due to diminished seminal secretion which is evident at high doses of glycopyrrolate. Glycopyrrolate causes minor skin irritation at site of intramuscular injection in rabbits.

It is thus concluded that preclinical data of glycopyrrolate reveal no special hazard for humans based on conventional studies of acute dose toxicity, repeated dose toxicity, fertility, embryo-foetal development, genotoxicity and carcinogenic potential. The excipients used in the formulation of glycopyrronium bromide are well characterised and safe. Therefore, glycopyrronium bromide can be safely used in humans according to conditions specified in the SmPC.

2.4.6 Literature References

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