MODULE 2.4

NON-CLINICAL OVERVIEW

NAME OF PRODUCT:	**GLYCOPYRROLATE 1 MG AND 2 MG TABLETS**
ACTIVE SUBSTANCE:	**GLYCOPYRROLATE**
FORMULATION:	**1 MG AND 2 MG TABLETS**
DATE OF REPORT:	**AUGUST 2019**

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2.4 Non-Clinical Overview

Background

Sialorrhea, also known as hypersalivation, ptyalism or drooling, is excessive salivation associated with neurological disorders or localized anatomical abnormalities in the oral cavity. Pathologic sialorrhea may develop due to hypersalivation, together with various neurologic disorders including cerebral palsy, Parkinson's disease, and amyotrophic lateral sclerosis, or as an adverse effect of medications. Sialorrhea results in numerous problematic physical and psychosocial complications and has a significant negative impact on quality of life for both the patient and caregiver (Guvenc IA 2019).

Drooling can result in perioral chapping, irritation, and maceration, with secondary infection of the facial skin, dehydration due to chronic loss of fluids, and increased risk of silent saliva aspiration that can result in recurrent respiratory infections.

Treatment options explored to control drooling in children and adults include behavioral approaches, such as prompts to swallow or wipe or preventing individuals from putting their fingers or objects into their mouths; surgery to decrease salivary flow and anticholinergic agents such as glycopyrrolate.

(Zeller Robert S. et al 2012).

Introduction

The applicant Kinedexe Limited, U.K., intends to file an application for marketing authorisation of glycopyrronium bromide (glycopyrrolate) 1 mg and 2 mg Tablet for "use in drooling", in accordance with Article 10a of Directive 2001/83/EC, as amended.

This Nonclinical Overview is to evaluate the pharmacological and toxicological properties of glycopyrrolate, as reflected in the available literature. The data are presented in accordance with Article 10a of Directive 2001/83/EC, as amended. Thus, this application refers to published scientific literature presented in accordance with Article 10a stating that Annex 1 of Directive 2001/83/EC, as amended, shall apply by analogy.

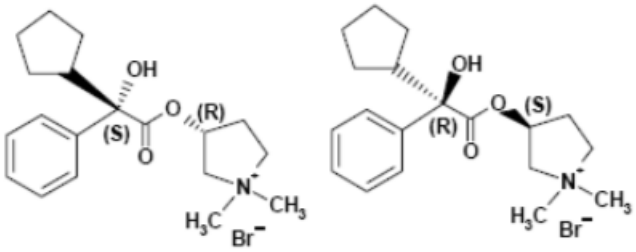
According to a recent UK Public Assessment Report (Kinedexe Ltd) glycopyrronium bromide tablets are a medicine with well-established use having been employed in the EU for at least 10 years with recognized efficacy and an acceptable level of safety. A presentation by the EMA (European Medicines Agency) indicates that for Art 10a applications test and trial results shall be replaced by appropriate scientific literature. Regulatory assessment reports are not considered acceptable for this purpose although bridging studies may be provided to support the relevance of literature information. A peer-reviewed article by Chabicovsky et al, 2019, recently published in *Toxicology and Applied Pharmacology* (Pharmacology, toxicology and clinical safety of glycopyrrolate) meets the EMA criterion of "scientific literature" although it contains multiple references to EMA assessment reports and FDA reviews. Since the acceptability of using information obtained from regulatory assessment

reports but published in a peer-reviewed article in a highly respected toxicology journal is unclear, such information, when cited, is depicted in *italics*.

2.4.1 Overview of the Non-Clinical Testing Strategy

Glycopyrrolate is an anticholinergic drug available as 1 mg and 2 mg tablets.
The chemical name for glycopyrrolate is pyrrolidinium, 3-[(cyclopentylhydroxyphenylacetyl)oxy]-1,1-dimethyl-, bromide.

Chemical structure is:



Empirical formula: $C_{19}H_{28}BrNO_3$
CAS number (of racemate): 596-51-0
Molecular weight: 398.33.
Log p -1.18 (Gujjar et al 2014)

Glycopyrrolate contains four chiral centres and is a 1:1 mixture of R,S and S,R diastereomers, both being pharmacologically active. The active moiety, glycopyrronium constituting 80% of the nominal tablet strength, is the free-base form of glycopyrronium bromide. [In this overview "glycopyrrolate" refers to glycopyrronium bromide.]

Indications:
Glycopyrrolate 1 mg and 2 mg tablets are indicated in symptomatic treatment of severe sialorrhoea (chronic pathological drooling) in children and adults with chronic neurological disorders. Each 1mg tablet contains 1 mg of glycopyrronium bromide which is equivalent to 0.8 mg of glycopyrronium.

The dosing schedule for Glycopyrronium bromide tablets is based on the weight of the child with the initial dosing of 0.02 mg/kg to be given orally three times daily and titrate in increments of 0.02 mg/kg every 5-7 days based on therapeutic response and adverse reactions. The maximum recommended dosage is 0.1 mg/kg three times daily not to exceed 1.5-3 mg per dose based upon weight. (CUVPOSA Oral Solution, FDA Label of Merz Pharmaceuticals, 2018)

Search Strategy:
A search of the published literature was carried out to locate original research articles and review papers employing internet search engines and PubMed abstracts.

Many of the published articles are pre-GLP but appear to be of an acceptable scientific standard. Where appropriate, data from experiments in human volunteers and from clinical trials have been cited.

2.4.2 Pharmacology

Glycopyrronium bromide is a quaternary ammonium antimuscarinic agent and like other anticholinergic agents, it inhibits the action of acetylcholine (Ach) on structures innervated by postganglionic cholinergic nerves and on smooth muscles that respond to acetylcholine but lack cholinergic innervation. ACh, which is synthesized from acetyl-coenzyme A and choline, acts at neuromuscular junctions, synapses in the ganglia of the visceral motor system, and at a variety of sites within the central nervous system (CNS). The effects of ACh (cholinergic effects) are mediated by binding to and subsequent activation of receptors located on the surface of cells.

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels, and are classified depending on their locations as presynaptic,axonal or postsynaptic nAChRs. Binding of ACh to nAChRs increases the permeability for cations resulting in a depolarization of the cell; the subsequent calcium influx leads to muscle contraction. Muscarinic acetylcholine receptors (mAChRs), are G protein-coupled receptors (GPCRs), being more sensitive to muscarine than to nicotine. mAChRs are located in postganglionic parasympathetic neurons and also in certain sweat glands (Haga T, 2013) Almost all organs are targeted by parasympathetic neurons.

Ach elicits excitatory or inhibitory effects depending on the mAChR receptor subtype. Five mAChR subtypes, M1 to M5, have been identified. Receptor subtypes M1, M3, and M5 are coupled to G proteins of type Gq, and binding of ACh to these subtypes activates phospholipase C, which in turn activates a signalling pathway that leads to increased intracellular calcium levels. In smooth muscles, this results in excitation and contraction. The receptor subtypes M2 and M4, on the other hand, are coupled to the Gi protein, thus activation of those receptors inhibits the adenylyl cyclase upon ACh binding. The resulting drop in cAMP levels is associated with further inhibitory effects depending on cell type.

The different mAChR subtypes are located at various sites in the body:

- Receptors of the M1 subtype are mainly present on the CNS, gastricparietal cells and salivary glands and activation results primarily in stimulation of the CNS.
- The M2 subtype is predominantly found on cells of the heart, and activation results primarily in cardiac inhibition.
- Receptors of the M3 subtype are located on exocrine glands and smooth muscle, and receptor activation leads to contraction of smooth muscle and secretion of exocrine glands (e.g. saliva and sweat).

- The M4 and M5 subtypes are mainly present on the CNS. Binding of ACh to M4 receptors inhibits acetylcholine release, i.e. M4 receptors are inhibitory autoreceptors for ACh.
- The M5 subtype is involved in the regulation of dopamine release in the brain and in rewarding brain stimulation

Various classes of anticholinergics drugs that block the action of the neurotransmitter ACh on ACh receptors have been developed. Anticholinergic drugs are classified either as nicotinic receptor antagonists or as muscarinic receptor antagonists, depending on AChR specific binding. Nicotinic receptor antagonists act either on the skeletal muscle, on autonomic ganglia and adrenal medulla or on the CNS Clinical use of nicotinic receptor antagonists is mainly restricted to anesthesiology, as neuromuscular blocking agents. Muscarinic receptor antagonists (antimuscarinics) include naturally occurring alkaloids such as atropine and scopolamine, as well as semisynthetic and synthetic alkaloid derivatives. As previously indicated glycopyrrolate is part of the antimuscarinic group of anticholinergics which find applications as bronchodilators, urinary or gastrointestinal antispasmodics, as well as mydriatic and antiparkinsonian drugs.

2.4.2.1 Primary Pharmacology

Glycopyrrolate is a competitive inhibitor of acetylcholine receptors of the M3 subtype that are located on peripheral tissues such as salivary glands. It blocks stimulation of these receptors thereby reducing the extent of salivation.

The effect of glycopyrrolate on salivary secretion was investigated in anesthetized mongrel dogs by Franko et al, 1962. Methacholine was used as the stimulating agent and salivary output resulting from each methacholine stimulation was measured before and after IV glycopyrrolate. The results indicated that an IV dose of 5µg/kg produced a marked reduction of salivary secretion, as shown below in Figure 1 (figure from Franko et al, 1962).

In 1974 Wyant and Kao reported the results of a similar experiment in human volunteers using a combination of carbamylcholine chloride and adrenaline to stimulate release of saliva. An IV dose of 0.2mg glycopyrrolate was found to be more or similarly effective as 0.4mg IV atropine in relation to the inhibition of salivary secretion, as shown in Figure 2 (taken from Wyant and Kao).

Mirakhur et al reported in 1978 the results of an investigation of salivary secretion carried out on six adult volunteers. Each volunteer had nine doses of glycopyrrolate by three routes given in a random order. These were 0.1, 0.2 and 0.4 mg intramuscularly (IM), 0.1, 0.14 and 0.2 mg IV and 2.0, 4.0 and 8.0 mg orally (PO). There was great individual variation in the salivary secretion before any drug was given. The effects of drug varied in a dose-related manner from slight with lower doses to a marked and persistent effect with higher doses. With 0.4 mg IM the depression in salivation was significant throughout the period of study and secretion was less than 15% of its original volume between 1 and 3 h. IV administration produced a more

rapid effect; 0.2 mg IV produced a maximum to about 25% of the control value at 1 h. When given orally 2.0 mg had little effect, 4.0 mg caused significant depression from 4 to 6 h (maximum to 50% of control value at 6 h), while with 8.0 mg significant reduction occurred after 3 h. Dose-response results are shown in Figure 3 from Mirakhur et al, 1978.

Mirakhur and Dundee (1980) compared the antisialogue effects of IM atropine (0.5, 1.0 and 2mg) and glycopyrrolate (0.1, 0.2 and 0.4mg) in six adult volunteer anaesthetists. The effects of both drugs were dose-related. Salivary secretion was reduced by 43, 72 and 85%with 0.5, 1.0 and 2.0 mg atropine respectively, the peak effect being achieved at one hour. The effects of glycopyrrolate were similar to those of atropine, but the depression in salivary secretion was more prolonged. The average maximal reduction with the three doses was 43, 74 and 94% respectively. The dose-response results are shown in Figure 4. Various physical parameters were also measured and glycopyrrolate produced a significant effect on sweat-gland activity, more pronounced and longer-lasting than the effect of atropine.

Figure 1: Inhibitory effect of glycopyrrolate on methacholine-stimulated salivary secretion in the dog

INHIBITORY EFFECT OF GLYCOPYRROLATE ON METHACHOLINE-STIMULATED SALIVARY SECRETION IN THE DOG

Compound	I. v. dose	Time of dose	Volume of salivary secretion per 10 min.
	μg./kg.	*min.*	*ml.*
	11	−30	0.60
Methacholine chloride	11	−20	0.60
	11	−10	0.60
Glycopyrrolate (Br)	5	0	
	11	+10	0.28
	11	+20	0.37
Methacholine chloride	11	+30	0.48
	11	+40	0.19
	11	+50	0.20

Figure 2: Inhibition of salivary secretion by IV glycopyrrolate in 12 human volunteers

VOLUME OF SALIVA (ml) SECRETED FROM BEGINNING OF ADMINISTRATION OF CARBACHOL/EPINEPHRINE TO FIVE MINUTES AFTER COMPLETION OF ADMINISTRATION

Subject	Saline (1 ml)	Atropine (0.4 mg)	Glycopyrrolate (0.1 mg)	Glycopyrrolate (0.2 mg)
1	4.4	0.2	0.9	0.1
2	4.5	0	4.3	0.05
3	5.2	0.5	2.7	0.3
4	2.05	0	0.25	0
5	11.15	1.0	0.60	0.6
6	5.1	0.5	0.8	0
7	5.7	0	1.2	0
8	6.9	0.65	1.05	0.3
9	8.0	0.3	1.8	0
10	5.1	0.05	3.2	0.08
11	2.7	0.0	0.3	0.03
12	16.6	0.3	3.4	0.2

Figure 3: Human volunteer dose-response data from Mirakhur et al, 1978

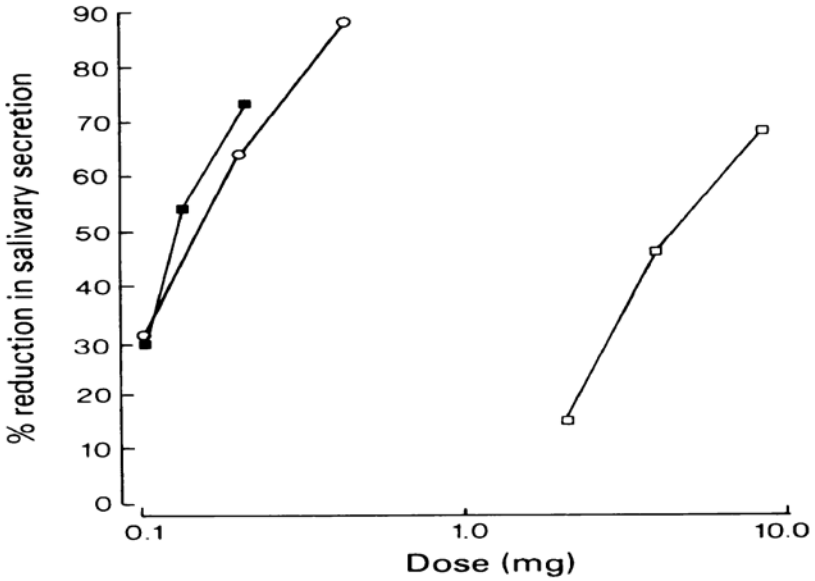
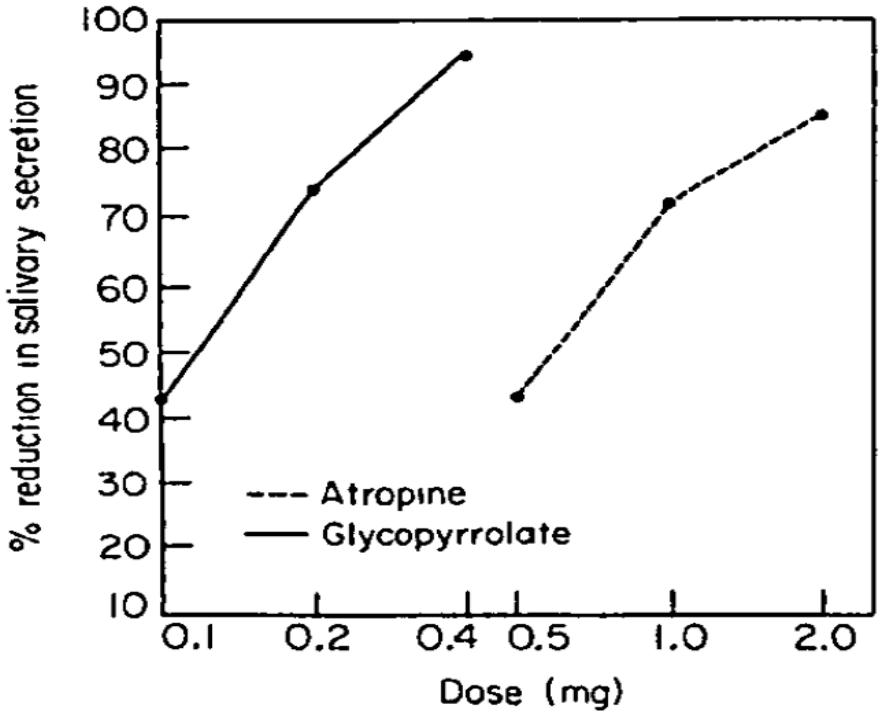


Figure 3 Dose response after glycopyrronium administration (○ intramuscular; ■ intravenous; □ oral) on salivary secretion. The oral dosage to achieve a 50% reduction in salivary secretion was about 35 times the parenteral dose.

Figure 4: Dose-response of IM atropine and glycopyrrolate on salivary secretion in human volunteers (Mirakhur and Dundee, 1980)



In a review article targeted at anaesthetic practice, Mirakhur and Dundee (1983) summarised the differences in pharmacological actions of glycopyrrolate and atropine as shown in Table 1, based on both animal and human data.

Table 1: Summary of the principal effects of atropine and glycopyrrolate in volunteers

	Atropine	**Glycopyrrolate**
Salivation	Marked inhibition	Marked and prolonged inhibition
Sweat glands	Marked inhibition	Marked and prolonged inhibition
Heart rate	Increase	Minimal Change
Pupil size	Increase	No change
Near point of vision	Increase	No change

In the context of veterinary practice, both atropine sulfate and glycopyrrolate are effective when administered to anesthetized dogs and cats in inhibiting sialorrhea and intestinal peristalsis as well as controlling bradycardia. In these species, glycopyrrolate has been

shown to be more potent and have more long-lasting effects than atropine (Short and Miller, 1978; Beleslin, 1984).

2.4.2.2 Secondary Pharmacology

In-vitro investigations and in-vivo studies in numerous small- and large-animal species have shown various effects typical of muscarinic receptor antagonists, thus confirming the mode of action of glycopyrrolate.

An investigation by Lau et al examined the antagonism by glycopyrrolate of the negative inotropic effects due to acetylcholine and carbachol in guinea-pig atrium. Glycopyrrolate had biphasic effects on the contraction of guinea-pig atrium. At concentrations between 0.4 and 20 μM the drug induced a small but consistent increase in the contraction force. This negative ionotropic effect was opposed by a Ca+2 channel agonist. Glycopyrrolate also antagonized potently the depressant effects of carbachol and acetylcholine in guinea-pig atrium. Radioligand binding studies showed that g1ycopyrrolate displaced pirenzepine (PZ) and AF-DX 384 from their binding to the M1 and M2 muscarinic receptors in guinea-pig brain membranes respectively. Study results confirm the antimuscarinic properties of g1ycopyrrolate. Author suggests that the therapeutic value of g1ycopyrrolate in the treatment of organophosphate poisoning is associated with its potency against prolonged muscarinic receptor activation by cholinergic agonists. (Lau W. M. et al 1992)

Effects of glycopyrronium, indacaterol and their combination on electrically-induced ACh-release and contractile response were studied in isolated bovine trachealis. Glycopyrronium 10^{-8} M decreased contractile response by 19 ± 6% without altering ACh-release whereas 10^{-7} M and 10^{-6} M almost abolished contractile responses even if the ACh-release was increased by 27 ± 19% and 20 ± 8%, respectively. Indacaterol 10^{-7} M had no significant effects on contractile response and ACh-release, whereas indacaterol 10^{-6} M reduced contractile response by 24 ± 12% without altering ACh-release. IND 10^{-5} M decreased contractile response by 51 ± 17% and ACh-release by 22 ± 11%. Co-incubation with glycopyrronium 10^{-8} M and indacaterol 10^{-7} M did not alter ACh-release but inhibited contractile response by 41 ± 8%. Results conclude that glycopyrronium had a significantly larger inhibitory effect on electrically- induced contraction of airway smooth muscle than indacaterol, even if it caused an increase in ACh-release. Adding Glycopyrronium to indacaterol had a greater effect than increasing the concentration of indacaterol alone. (Baroffio Michele et al 2017)

Posey E. L. et al compared the effects of atropine sulfate, propantheline bromide and glycopyrrolate on the ultrastructure of canine parietal cells and determining the effects of glycopyrrolate on the parietal cells of dogs previously subjected to vagus section. Atropine sulfate and propantheline bromide induced alterations in the nucleus, mitochondria, cytoplasmic vesicles and vacuoles, and intracellular canaliculi of parietal cells. Vagotomy failed to induce parietal cell alterations. Glycopyrrolate induced profound cytoplasmic vacuolization, in addition to producing alterations of the parietal cell nucleus, mitochondria and intracellular canaliculus. Atropine was accompanied by the most profound mitochondrial

derangements. Glycopyrrolate administration was marked by the most extensive cytoplasmic vacuolization. This study provided confirmation of the development of glycopyrrolate induced parietal cell changes, as well as the absence of alterations induced by vagotomy. (Posey E. L. et al 1970)

A blinded, placebo-controlled study was designed to evaluate oesophageal and gastric pH in anesthetized dogs. Preanesthetic administration of atropine and glycopyrrolate had no effect on oesophageal, gastric, or tracheal pH before, during, or after surgery but did result in increased heart rate. (Roush James K. et al 1990)

Test Meal Progression in Rats

The charcoal meal progression test in rats indicated that glycopyrrolate affected gastrointestinal propulsive activity. The effect of glycopyrrolate in this test was comparable to that of isopropamide iodide and significantly less than that of atropine sulfate. At an oral dose of 2.5 mg/kg., atropine sulfate limited progression of the test meal to 23% (mean value for the group) of the intestinal length, while with glycopyrrolate and isopropamide iodide at 25 mg/kg (10 times the atropine dose) the respective average values were 39% and 56%. (Franko B. V. et al 1962)

Unanesthetized Thiry-Vella Loop Dogs

A method using unanesthetized Thiry-Vella loop dogs was used to determine the inhibitory effect of glycopyrrolate on intestinal activity. Glycopyrrolate was administered in intravenous doses of 5 to 50 pg/kg or in oral doses of 1 to 5 mg/kg. A marked effect of glycopyrrolate on intestinal activity in a typical experiment with one of these trained, unanesthetized animals. There was a prolonged lowering of intestinal tone and a decrease in amplitude of intestinal contractions of relatively short duration. In another experiment it was found that the tone remained lowered for at least ***3*** hours after the intravenous administration of 25 µg/kg. (Franko B. V. et al 1962)

Effect on basal gastric secretion in chronic fistula rats

Intragastric glycopyrrolate, 1 mg/kg. as the base, was very effective in reducing volume and acidity of basal secretion in rats. A highly significant decrease in free hydrochloric acid output was produced in each of the animals, and the depression in volume response was significant in those animals that had a relatively high basal secretion. (Franko B. V. et al 1962)

Cardiovascular Effects

Glycopyrrolate effect on heart rate has been studied in horses following a 2.5-, 5-, and 10-µg/kg intravenous bolus doses. Mean heart rate (n = 5) after the 2.5-µg/kg dose did not demonstrate a significant difference from the control group for measurements taken up to 120 min. However, the 5- and 10- µg/kg doses both produced elevated mean heart rates compared with the control group beginning 5 min after and ending 60 min after treatment in conscious horses. In conclusion, 5 µg/kg glycopyrrolate appears to be a useful dose in conscious,

unsedated horses to elevate heart rate within reasonable limits. (Rumpler M. J. et al 2013 and Singh S. et al 1997)

Another study by Dyson et al determined effect of glycopyrrolate on heart rate (HR) and blood pressure (BP) in anesthetized horses with low HR (< 30 beats/min). The horses were randomly treated with glycopyrrolate (2.5 µg/kg body weight) or saline, intravenously (n = 17). Heart rate increased by > 5 beats/min in 3 out of 9 horses following the initial glycopyrrolate treatment. Overall changes in HR and mean BP were not significantly different, while systolic and diastolic BP increased significantly. On the 2nd treatment, 3 out of 7 horses given 2.5 µg/kg glycopyrrolate, and 4 out of 5 horses given 5.0 µg/kg showed an increase in heart rate of > 5 beats/min, which was significant. A significant increase in BP was produced following treatment with 2.5 µg/kg, but not following 5.0 µg/kg. A final increase in HR, of > 5 beats/min, was associated with a significant rise in BP. In conclusion, an increase in HR can occur with 2.5 to 5.0 µg/kg of intravenous glycopyrrolate and results in improvement in BP in anesthetized horses. Glycopyrrolate appears to be an effective treatment for low HR in the anesthetized horse at the recommended dose range of 2.5 to 5.0 µg/kg. [Dyson Doris H. et al 1999(a)]

Effects of intravenously administered atropine (0.2 mg/kg) and glycopyrrolate (0.01 mg/kg) on heart rate were studied in 10 conscious mature goats. In a drug cross-over fashion, either atropine, glycopyrrolate or 0.9% saline solution was administered. Heart rate increased significantly after administration of atropine and glycopyrrolate but not after 0.9% sodium chloride solution. The magnitude of the changes after atropine and glycopyrrolate were similar, up to 14 minutes after drug administration. Thereafter, glycopyrrolate maintained a significantly greater change in heart rate, up to 29 minutes after drug administration. Within the atropine group, the mean percentage changes in heart rate became significantly lower compared with the initial increase (1 minute) starting at 11 minutes. For the glycopyrrolate group, the mean percent changes became significantly lower starting at 27 minutes. Glycopyrrolate and atropine had a mean percentage change in heart rate of greater than 1%, up to 31 and 22 minutes, respectively. At the doses used, glycopyrrolate had longer duration of action than atropine but the magnitude of increase was similar. (Pablo Luisito S. et al 1995)

Cardiopulmonary consequences of intravenous administered glycopyrrolate followed by butorphanol and xylazine were evaluated in 06 dogs, with and without nasal administration of oxygen. For either treatment (with or without oxygen), glycopyrrolate caused significant increases in heart rate and cardiac index and significant decreases in stroke index. There were no other significant changes after glycopyrrolate administration. (Jacobson John D. et al 1994)

Lemke K. A. et al compared the hemodynamic changes caused by the administration of intravenous atropine or glycopyrrolate after intravenous xylazine in isoflurane-anesthetized dogs. Heart rate, cardiac index, and rate pressure product increased after both drug administration. Significant differences between atropine and glycopyrrolate were not

observed in any of the hemodynamic parameters. The authors conclude that atropine and glycopyrrolate have equivalent hemodynamic actions during the increased pressure phase after intravenous xylazine in isoflurane-anesthetized dogs. (Lemke K. A. et al 1993)

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 [pCO2] 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 at 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 glycopyrrolate significantly increased ADE but was associated with higher rate pressure products and myocardial work (Lemke K. A. et al 1994).

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 bradyarrhythmia's, 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 (Clutton R. E. et al 1992).

Kneip C. F. et al 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. (Kneip C. F. et al 2010)

Central Nervous Effects

The effects of atropine and glycopyrrolate on neuromuscular transmission and on muscle contraction were studied in the rat diaphragm preparation. Atropine increased the indirectly-elicited twitch tension by 22 ± 2.1%, tetanus by 15 ± 1.1%, post-tetanic twitch response by 33 ± 3.1% and post-tetanic twitch potentiation value by 36 ± 1.9%. 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. 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%), post-tetanic twitch response (by 17± 1.2%) and post-tetanic twitch potentiation values (by 24 ± 3.1%). Results conclude 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. (Wali F. A. et al 1988)

Ocular Effects

Varssano David et al assessed the topical mydriatic effect of glycopyrronium bromide solution in the eyes of albino rabbits in comparison with atropine. Glycopyrrolate 0.5% and atropine 1.0% were instilled separately in the eyes of albino rabbits. Mydriasis was noted within 5 min of glycopyrrolate instillation, reached near-maximal level at 15 rain and persisted for 1 week. Glycopyrrolate 0.5% showed a faster, stronger and more peristent 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. Glycopyrrolate showed an ability to penetrate the eye when applied topically, paralyzing the pupillary sphincter swiftly. The drug is evidently more potent than atropine even when applied at half the percentage concentration. Glycopyrrolate solution has the potential to deliver an ocular anticholinergic effect without causing associated central anticholinergic hazards. Given its lack of central nervous system penetration and its topical effect on the eye, glycopyrrolate may be an important tool in the ophthalmologist’s arsenal. (Varssano David et al 1996)

Ji F. et al evaluated the pharmacological effect (mydriatic activity) of soft drug analog (SG-1) of glycopyrrolate and compared to that of glycopyrrolate in rabbits. At the pharmacodynamically equivalent doses (the lowest dose that induces the maximum response) of SG-1 (1%) and glycopyrrolate (0.1%), the mydriatic activities lasted for 5 and 100 h, respectively. Compared to glycopyrrolate, the intrinsic pupil dilation potency of SG-1 was lower (1/10th) but the duration of action was much shorter (<1/20th) as SG-l is susceptible to facile enzymatic hydrolysis/deactivation in the rabbit eyes. (Ji. F. et al 2002)

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 unanesthetized 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 (Frischmeyer K. J. et al 1993).

2.4.4.3 Safety Pharmacology

There appear to be no scientific publications on conventional animal safety pharmacology studies examining for example CNS, cardiovascular and respiratory effects. However, studies in rats, dogs and rabbits (see below) have shown no marked effects of glycopyrrolate on these endpoints.

Olson et al evaluated the influence of atropine and glycopyrrolate on heart rate, systolic blood pressure, respiration rate and core body temperature as well as the efficacy of these anticholinergic agents in preventing bradycardia in animals anesthetized with an injectable anesthetic combination. In rats, atropine sulfate (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 sulfate 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 anesthetized 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. Glycopyrrolate also prevented a decrease in heart rate in rabbits anesthetized 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 anesthetic. Study results suggest that glycopyrrolate is an effective anticholinergic agent in rabbits and rodents and more useful as a preanesthetic agent than atropine sulfate in these animals. (Olson M. E. et al 1993)

Cardiovascular effects of intravenous administration of glycopyrrolate were determined in adult dogs. Glycopyrrolate did not significantly affect heart rate, but induced a significant decrease in systemic vascular resistance, rate-pressure product, and mean arterial pressure, and significantly increased cardiac index. Study results suggest that glycopyrrolate is safe and efficacious anesthetic regimen in adult dogs. (Benson G. J. et al 1987)

Franko et al, 1962 found no evidence for glycopyrrolate-related CNS effects in general pharmacodynamics studies in anaesthetised dogs, EEG recordings in cats and acute toxicity studies in mice, rats, rabbits, dogs and cats. The authors provide the following explanation for the absence of CNS stimulation:

Franko et al, 1962 found no evidence for glycopyrrolate-related CNS effects in general pharmacodynamics studies in anaesthetised dogs, EEG recordings in cats and acute toxicity studies in mice, rats, rabbits, dogs and cats. The authors provide the following explanation for the absence of CNS stimulation: The lack of CNS-stimulating action by Glycopyrrolate was not an unexpected finding, since quaternary ammonium compounds in general do not exhibit this property. The non-quaternized analog of glycopyrrolate is known to be a potent anticholinergic agent. It also causes excitation in unanaesthetised animals, blocks tremorine induced tremors in mice and readily causes the appearance of an atropine like effect on the EEG of Cats.it seems therefore that the quaternary ammonium character of glycopyrrolate eliminate CNS activity. Since Glycopyrrolate in doses below the lethal range did not produce symptoms of CNS stimulation in the dog, cat or rat the non-quaternized analog was apparently not readily formed in these species.

In relation to potential effects on QT prolongation no publications could be found describing in-vitro Ikr assays evaluating the effects on the ionic current through a native or expressed Ikr channel protein, such as that encoded by hERG. On the other hand, extensive human cardiovascular safety data are available in the literature from inhaled glycopyrrolate (62µg once daily) employed to treat COPD. A prolongation in the QT interval was noted in 4% of the patients receiving glycopyrronium versus only 1.1% in the placebo group, in the GLOW1 clinical trial. However, none of the patients had a prolongation of more than 60 ms or a QT interval of more than 500 ms. (D'Urzo et al, 2011).

	NVA237 50 µg (n = 550)	Placebo (n = 267)
Electrocardiographic abnormalities, n (%)		
Notable QTcF[†]	22 (4.0)	3 (1.1)
QTcF > 500 ms	0	0
Increase from baseline of 30-60 ms	59 (10.7)	21 (7.9)
Increase from baseline of > 60 ms	6 (1.1)	1 (0.4)

In another similar study using 16.6µg glycopyrrolate bd no differences in QT interval prolongation were observed among the study groups. The proportion of patients with newly occurring or worsening clinically notable Fridericia's corrected QT interval (QTcF) values was similar between the treatment groups. One patient in the glycopyrrolate group had a QTcF value >480 msec. No meaningful differences between treatment groups were seen for laboratory evaluations and clinically notable vital signs. Adverse events were reported for 111 (51.4%) patients in the glycopyrrolate group versus 91 (42.5%) patients in the placebo group. (Kerwin et al, 2016). Thus, across these two studies reassuring safety data on QT interval prolongation are available from a total of 661 patients.

Comment on safety pharmacology by Chabicovsky et al, 2019.

The long history of marketed glycopyrronium -containing products for a range of therapeutic uses provides ample information on glycopyrrolate-related adverse effects. Initial reports on the potential impact of glycopyrrolate on vital functions in animals (mice, guinea pigs, cats and dogs) date back to the 1960s (Franko et al., 1962). These early reports on the effects of glycopyrrolate on the cardiovascular system did not suggest any effects of glycopyrrolate on heart rate, carotid arterial blood pressure, or respiration in anesthetized dogs receiving i.v. (5–10 µg/kg) or oral (0.5–5 mg/kg) doses of glycopyrrolate (Franko et al., 1962).

*More recently, more standardized safety pharmacology endpoints including effects on the cardiovascular system, CNS and respiratory system were specifically evaluated in vitro, in animals and in humans. Inhibition of the hERG current was only observed at concentrations significantly higher than the maximum human exposure (Cmax) at the recommended clinical dose. ®®Following i.v. administration of 0.01 mg/kg glycopyrrolate in beagle dogs, transient effects on heart rate and blood pressure were seen. A transient increases in heart rate, a transient decreases in heart rate-corrected QT-intervals as well as effects on PR and P widths were observed following an inhaled dose of 0.149 mg/kg. Moreover, tachycardia was a frequent finding in repeat-dose toxicity studies in dogs at doses ≥0.077 mg/kg/day (AUC 0–6.5h 15.6 to 20.8 ng*h/mL, Cmax 7.8 to 11.8 ng/mL) (EMA, 2012).*

The risk of adverse effects of glycopyrrolate on the CNS are considered unlikely due to the limited ability of the quaternary structure to penetrate the blood-brain barrier (Proakis and Harris, 1978). Similarly, in safety pharmacology studies, no treatment-related effects were seen on the central nervous system (except for slight and transient pupil dilation).

Of note, safety pharmacology studies revealed no treatment-related effects on the respiratory system in rats at an inhaled dose of 0.168 mg/kg glycopyrrolate (EMA, 2012; EMA, 2016).

After the 7-day dermal treatment of minipigs with doses of up to 4% glycopyrrolate in cream or gel no clinical signs or evidence for behavioural changes were noted. (Dr. Wolff, data on file). Similar results were seen in a 39 week study where formulations containing glycopyrronium tosylate at concentrations of 0%, 2%, 6% or 20% w/w were topically applied to minipigs once daily. No apparent effects of treatment were seen (no effect on survival, clinical signs, mean body weight, clinical pathology or histopathology) (FDA, 2018a).

The reported side effects result mainly from exaggerated pharmacological effects (i.e. anticholinergic effects) such as increased pupil size, inhibition of accommodation (blurred vision), increased heart rate, and impaired urination and gastrointestinal motility.

The results of these studies collectively show that no major safety signals are seen in animal trials when glycopyrrolate is applied i.v., orally or topically and exposures levels expected with topical use.

2.4.2.4 Pharmacodynamic Drug Interactions

A number of studies involving glycopyrrolate drug combinations have been undertaken in animals and humans. Franko et al (1971) evaluated the pharmacological and toxicological profile of glycopyrrolate in combination with a variety of other drugs including: phenobarbital, meprobamate, tybamate, mephenoxalone and butaperazine. Several animal species were employed namely mouse, rat and dog, Overall, there was no evidence for any significant pharmacological interaction. In terms of inhalation therapy, combinations of glycopyrrolate with formoterol and indacaterol have been authorised on the basis of showing improved efficacy (compared to monotherapy) in COPD. (Tashkin & Gross, 2018) In both cases the enhanced efficacy appears to relate to the different but complementary modes of action of the two active ingredients.

Additive effects along with potential toxicity are possible (at least in theory) from co-administration of glycopyrrolate with other drugs possessing anticholinergic properties (Hoeft D 2014).

2.4.3 Pharmacokinetics

A brief summary of the status of the ADME profile of glycopyrrolate based on published data up to the mid-80s is provided by Mirakhur & Dundee (1983)**Error! Bookmark not defined.**.

This (ADME) has been studied with ^{14}C-labelled glycopyrrolate in the mouse. Following intravenous administration, peak radioactivity was found in all organs at 5-10 minutes except brain: liver, kidney and intestines showed traces of activity at 24 hours. Following oral administration, stomach and small intestine showed the maximum amount of radioactivity and absorption from the gastrointestinal tract was poor. Minimal amounts of glycopyrrolate cross the blood-brain barricr. Both animal and human studies show that placental transfer is limited. 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. A study using intravenous ^{3}H-glycopyrrolate in humans showed the disappearance of more than 90% from the serum in *5* minutes and almost 100% in 30 minutes. Urinary radioactivity was highest in the first 3 hours and 85% was excreted in the urine within 48 hours. Paper chromatography showed 80% of the radioactivity in bile and urine corresponding to unchanged glycopyrrolate. Following oral administration to mice, 7.6% was excreted in the urine and about 79%,in the faeces.

Metabolic fate of glycopyrrolate in mouse and rat was investigated by a radiochemical method, thin-layer chromatography, and gas chromatography-mass spectrometry. When drug was intravenously and orally administered, six metabolites were detected in urine. Major metabolites were considered to be 1,1-dimethyl-3-hydroxypyrrolidinium bromide a-(2- or 3-hydroxy-cyclopentyl) mandelate and 1,1-dimethyl-3-hydroxypyrrolidinium bromide benzoyl formate. 1,1-Dimethyl-3-hydroxypyrrolidinium bromide was identified as minor metabolite. According to these results, major metabolic pathways of glycopyrrolate were assumed to be hydroxylation of cyclopentyl ring and oxidation of hydroxyl group in mandelic acid moiety, then simultaneous removal of cyclopentyl ring. (Takada Shoichi et al 1973)

Data from more formal studies are reported by Chabicovsky et al, 2019 and are shown in sections 2.4.3.2-2.4.3.5.

2.4.3.1 Methods of Analysis

Many of the early studies employed radiolabelled glycopyrrolate in order to determine PK parameters, Proakis and Harris (1978) administered ^{14}C-glycopyrrolate by IV injection to anaesthetised mongrel dogs in order to assess the potential for transfer across the blood-brain and placental barriers. Kaltiala et al (1974) administered ^{3}H-glcopyrrrolate in their studies in order to assess its fate in man. More recent reports have employed more sophisticated analytical methods including a radioreceptor assay (Kaila et al, 1990) and ultra-high-performance liquid chromatography (UHPLC) and tandem mass spectrometry (MS/MS) (Rumpler et al., 2011).

2.4.3.2 Absorption

The principal sources of data on the absorption of ^{14}C-glycopyrrolate are publications by Kagiwada et al, 1973 and Proakis and Harris, 1978.

Following oral administration ^{14}C-glycopyrrolate at 4.83 μCi/mouse, minimal amounts of radioactivity were present in the blood by 0.5 hours and remained detectable for up to 6 hours at concentrations of 1.1-1.6 μμCi/mg. Oral absorption was low most likely due to the permanently ionised nature of the drug, 1.9% of the dose being found in the stomach and 6.4% in the small intestine at 3 h post-dose. After IV dosing of 0.966 μCi/mouse, peak radioactivity in blood was observed at 5 minutes after administration (Kagiwada et al, 1973).

The levels of ^{14}C-glycopyrrolate appearing in cerebrospinal fluid (CSF) and serum (S) following a single intravenous dose were determined in anaesthetized mongrel dogs and compared with levels reached in dogs treated with similar doses of ^{3}H-atropine. As early as 10 min after injection, 23% of atropine levels were detected as compared to 2% of glycopyrrolate levels. Peak CSF/S concentration ratios for ^{3}H-atropine averaged 0.87, vs. a mean ratio of 0.1 for ^{14}C-glycopyrrolate within the four-hour observation period. In pregnant dogs peak mean fetal serum (FS) levels of.13 ± 1.5 ng/ml occurred 10 min after a single intravenous dose of 0.1 mg/kg ^{3}H-atropine administered to the mother and represented 30% of the corresponding maternal serum (MS) concentration. The maximum FS/MS concentration ratio observed within the four-hour post-drug period for ^{3}H-atropine was 1.0 vs. 0.04 for ^{14}C-glycopyrrolate. The evidence cited in this study indicates that glycopyrrolate is significantly more resistant than atropine to penetration through the blood-brain and placental barriers. (Proakis and Harris 1978).

In children aged 7–14 years, the mean absolute bioavailability of oral glycopyrrolate (50 µg/kg) was reported to be low and variable (median 3.3%; range 1.3 to 13.3%)) (Rautakorpi et al., 1998). The figure shown below is taken from this publication.

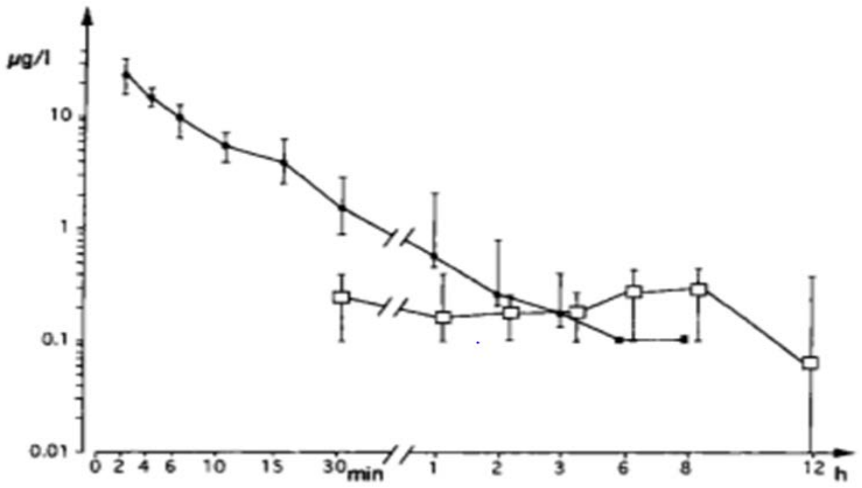


Fig. 1. Plasma concentrations of glycopyrrolate (median (range)) after a single intravenous (●) (5 µg/kg) and oral (□) (50 µg/kg) dose of the drug.

*In a 13-week repeated dose study in mice, glycopyrrolate was administered orally at doses of 30, 100 or 300 mg/kg/day once daily. Reported values for the peak plasma concentration (Cmax) and the total drug expose over time (AUC) of glycopyrronium varied widely and lack reliability, but was not unexpected given the low oral bioavailability. In general, exposure was reported to increase with increasing dose, and Cmax and AUClast (AUC from time 0 to the last measurable concentration) values were in the range of 1–100 ng/mL and 10–100 h*ng/mL, respectively. However, no Cmax and AUC values were reported for the highest applied dose of 300 mg glycopyrrolate/kg/day. In another 13-week repeated dose study in rats, doses of 40, 120 or 360 mg glycopyrrolate/kg/day were administered orally once daily*

*over a period of 84 days. In that study, Cmax and AUC values ranged from 3 ng/mL and 9 h*ng/mL at the lowest to 1153 ng/mL and 2014 h*ng/mL glycopyrronium at the highest dose administered, with a clear dose dependent increase observed (FDA, 2010c).*

*Absorption of glycopyrronium tosylate was investigated in male Göttingen Minipigs after a single i.v. dose (0.25 mg/kg of glycopyrrolate). The values for mean C2min and AUC0-∞ of 458.5 ng/mL and 76.83 ng*h/mL, respectively were reported (FDA, 2018a). In humans, the bioavailability of glycopyrrolate oral solution was compared to glycopyrrolate tablets in an open-label, randomized, single-dose study. In the same study, bioavailability of glycopyrrolate oral solution was also investigated under different dietary conditions in healthy subjects (FDA, 2010b; FDA, 2016). Both solution and tablet contained 2 mg of the compound and were given to healthy adults under fasting conditions.*

*Glycopyrronium absorption from the oral solution was compared between the fasting state and following a high-fat meal. Cmax and AUC24h were reduced by 74% (to 0.084 ng/mL) and 78% (to 0.38 h*ng/mL), respectively, when glycopyrrolate was given after a high fat meal. Hence, a high fat meal markedly reduces the bioavailability of orally administered glycopyrronium (FDA, 2016).*

Administration of glycopyrrolate oral solution 2 mg in fasting healthy adults resulted in mean C_{max} of 0.318 ng/ml after a median time (t_{max}) of 2.53 h (mean t_{max} of 3.10 h). The mean $AUC_{0-\infty}$ was 1.81 ng × h/ml. Compared with glycopyrrolate tablets, glycopyrrolate oral solution had a 23% lower C_{max} and a 28% lower $AUC_{0-\infty}$ suggesting that absorption of glycopyrrolate from the tablet formulation is superior to that from the oral solution (Garnock-Jones KP, 2012).

2.4.3.3 Distribution

After IV administration of ^{14}C-glycopyrrolate to mice at 0.966 μCi/mouse, total radiolabelled material was rapidly distributed throughout the body. High levels of radioactivity were observed mainly in the liver (26.1% reducing to 2.1% of dose at 12h) and also in the kidney, but not in the brain, only a trace of radioactivity being detected at 5 minutes and none thereafter. After 6h a total of 13.8% of dose was present in the small intestine, caecum and large intestine, suggesting a modest extent of biliary excretion.

Radioactivity after oral administration was distributed initially to the stomach and at later time-points to the small intestine, caecum and small intestine. No radioactivity was detected in the brain. Liver radioactivity was highest after 6h at 1.3% of dose.

Whole-body autoradiograms showed no radioactivity in the foetus following administration to pregnant animals. After multiple oral administration of radiolabelled glycopyrronium at 3.86 μCi/mouse/day over 1 week, the radioactivity disappeared entirely from organs at 72 hours after the last dose, and accumulation was not observed (Kagiwada et al, 1973).

Several tissues in mice (eye, brown fat, Harderian gland, kidney and liver) displayed very slow elimination following IV route of administration. Distribution was generally comparable between pigmented and non-pigmented mice, except for higher levels of radioactivity in the eye and skin of pigmented mice, with values that were above those observed in blood. This suggests that the uptake, which is at least partly reversible, occurs most likely into melanin-containing structures (EMA, 2012).

The distribution of glycopyrronium following either IV, intratracheal or oral administration appears to not differ between these routes of administration. Qualitatively higher values were only recorded in gastrointestinal tissue after oral administration (EMA, 2012).

Plasma protein binding of glycopyrronium was tested in different species (i.e., mice, rats, rabbits, dogs, and humans). Overall, plasma protein binding was weak without relevant differences between species. In these species, protein binding values ranged from 23 to 44% (EMA, 2012). For Göttingen Minipigs and humans, protein binding values ranging from 24% to 45% for glycopyrronium concentrations of 0.0799 ng/mL, 7.99 ng/mL and 799 ng/mL (0.1, 10 and 1000 ng/mL GPB) were determined (Dr. Wolff, data on file). It should be noted that values for rabbits and humans tend to be higher than for the other tested species (FDA, 2015). Accordingly, the values for the free drug concentrations can be expected to be higher in toxicological studies conducted, for example, in mice and rats than in humans (FDA, 2015).

The blood/plasma concentration ratios were comparable between species (0.48–0.67) with no apparent concentration dependency (FDA, 2015). The volume of distribution of glycopyrronium has been studied after IV administration and after inhalation. The steady-state volume of distribution of glycopyrronium was 83 L after IV administration. The volume of distribution in the terminal phase was 376 L. The apparent volume of distribution in the terminal phase following inhalation was considerably higher (7310 L), reflecting the much slower elimination after inhalation (EMA, 2012).

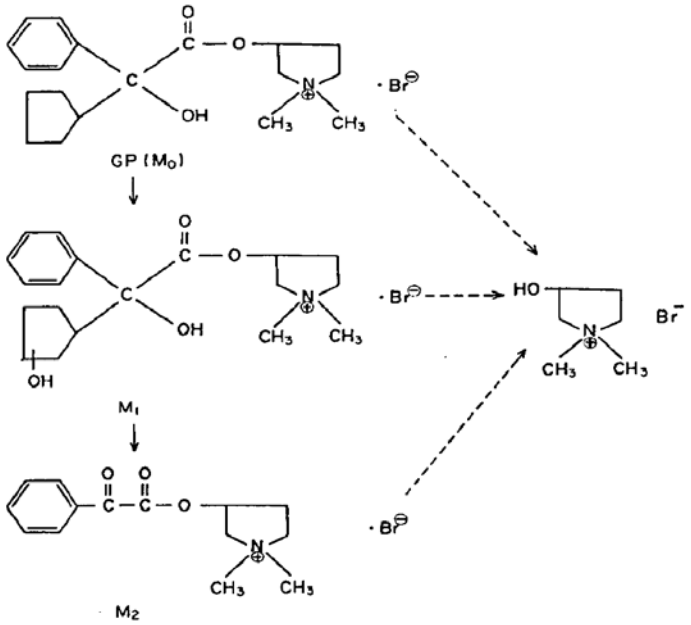
2.4.3.4 Metabolism

The metabolic fate of glycopyrrolate in mouse and rat was investigated by a radiochemical method, thin-layer chromatography, and gas chromatography-mass spectrometry. When drug was intravenously and orally administered, six metabolites were detected in urine. Major metabolites were considered to be 1,1-dimethyl-3-hydroxypyrrolidinium bromide a-(2- or 3-hydroxy-cyclopentyl) mandelate (M_1) and 1,1-dimethyl-3-hydroxypyrrolidinium bromide benzoyl formate (M_2). 1,1-Dimethyl-3-hydroxypyrrolidinium bromide (M_3) was identified as minor metabolite. According to these results, major metabolic pathways of glycopyrrolate were assumed to be hydroxylation of cyclopentyl ring and oxidation of hydroxyl group in mandelic acid moiety, then simultaneous removal of cyclopentyl ring. (Takada Shoichi et al 1973). The metabolic pathways proposed in this publication are shown in Figure 5.

Kagiwada et al, 1973 identified three metabolites in the mouse that were present mainly in the liver and kidney after oral administration of glycopyrrolate. The two main metabolites were 1,1-dimethyl-3-hydroxypyrrolidinium bromide a-(2- or 3-hydroxy-cyclopentyl) mandelate and 1,1-dimethyl-3-hydroxypyrrolidinium bromide benzoyl formate. No drug substance was detected in urine. The pattern of the intestinal metabolites at different time-points provided evidence for enterohepatic circulation.

The work of Kaila et al (1990) involving the use of a sensitive radioreceptor assay for glycopyrrolate effectively shows that the metabolites of glycopyrrolate lack pharmacological activity.

Figure 5: Glycopyrrolate: Proposed Metabolic Pathways in Mouse and Rat (from Takada Shoichi et al, 1973)



The in vitro metabolism of glycopyrronium has been investigated in hepatocytes of various species (mouse, rat, rabbit, dog, human) and also in liver and lung microsomes obtained from rat, dog and human. Quantitative, but no qualitative differences were observed between species, and no unique human metabolites were identified in vitro (EMA, 2012). Glycopyrrolate (1 and 10 µM) was poorly metabolized by human liver microsomes (only 20% after 150 min), while it was more readily metabolized in mouse, rat and minipigs liver microsomes with clearance values of 121, 593 and 346 µL/min/mg protein respectively (FDA, 2018a). Furthermore, in vitro metabolism studies suggest that glycopyrronium undergoes hydroxylation resulting in a variety of mono-and bishydroxylated metabolites and direct hydrolysis resulting in the formation of a carboxylic acid derivative termed 2(RS)-2-cyclopentyl-2-hydroxy-2-phenylacetic acid (M9). Multiple cytochrome P450 isoenzymes appear to contribute to the oxidative biotransformation of glycopyrronium (FDA, 2015).

In vivo investigations of the glycopyrronium metabolism in mice and rats were conducted after i.v., oral, or intratracheal administration, the latter only in rats. Glycopyrronium was

extensively metabolized in both, mice and rats. Glycopyrronium and the M9 metabolite constituted the majority of the plasma exposure. Mean human systemic exposure to M9 was similar in magnitude as the exposure to parent drug after inhalation. In contrast, M9 exposure was only a minor component after i.v. administration. Moreover, only minimal amounts of M9 were found in the urine after both routes of administration (EMA, 2012). After inhalation of glycopyrrolate, M9 is formed from the oral dose fraction (FDA, 2015). A single enantiomer of M9 metabolite appears to lack pharmacological activity as was demonstrated in an assay for muscarinic (M1-M5) and off-target activity of M9 using a panel of 65 Gprotein coupled receptors, transporters, ion channels and enzymes (EMA, 2012).

2.4.3.5 Elimination

Kagiwada et al, 1973, have reported that following oral administration of radiolabelled glycopyrrolate in mice 7.6% of radioactivity was excreted in urine and approximately 78% in faeces. No data were presented on urinary excretion after IV administration.

A study using ^{3}H-glycopyrrolate in humans showed that 85% of an IV dose was excreted in urine within 48h and 80% of the activity in bile and urine corresponded to unchanged drug (Kaltiala et al, 1974). It thus appears that glycopyrrolate reaching the systemic circulation is not significantly metabolised in humans. Hydrolysis with Glusulasc increased glycopyrrolate concentrations in urine only by 15% in 3h indicating the minor contribution of glucuronide and sulfate conjugation reactions in the metabolism of glycopyrrolate (Kentala et al, 1990).

Non-clinical investigations of glycopyrrolate excretion have been performed in mice and rats after oral and IV administration and in rats after intratracheal administration. After intratracheal and oral administration, glycopyrronium was mainly excreted via feces. In contrast, glycopyrronium was found in higher amounts in the urine than in the feces or bile after IV administration. Hence, it is likely that the majority of orally administered glycopyrrolate is not absorbed, which is consistent with the low bioavailability observed in rats and humans after oral administration. This hypothesis is further supported by the fact that in rodents, the majority of the radioactivity in feces was attributed to unchanged drug (> 60% of the detected radioactivity) following oral administration (EMA, 2012). In humans, glycopyrrolate excretion has been investigated after i.v. administration and after inhalation. Glycopyrronium is predominantly excreted via urine. Reports vary between 60% and 85% of the dose (EMA, 2012; FDA, 2016) Non-renal clearance is mainly due to metabolism as biliary clearance is limited. In adult human subjects receiving IV radiolabeled glycopyrronium,>80% of the excreted drug in urine and bile was attributed to unchanged drug (FDA, 2018b), in line with the results described in rodents.

Glycopyrronium plasma concentrations in humans decline in a multi-phasic manner. The mean terminal elimination half-life depends on the route of administration: 2.8 h after oral administration, 6.2 h after IV. administration, and 33 to 53 h after inhalation (FDA, 2015) Clearance values for glycopyrronium after IV. administration differ between adults and

pediatric patients, with higher clearance values observed in pediatric patients (1.01–1.41 L/kg/h vs 0.54 L/kg/h) (FDA, 2016).

In renally-imparied patients, the elimination of IV administered glycopyrronium (4 μg/kg) is severely impaired. Compared to control patients, a significantly smaller plasma clearance, longer elimination half-life and larger AUC were reported. While the 24 h renal excretion was 65% in control patients, it was only 7% in uremic patients (Kirvela et al., 1993).

2.4.3.6 Pharmacokinetic Drug Interactions

Although nebulized glycopyrrolate is metabolized by various enzymes, including the cytochrome P (CYP) and cholinesterase families, via first-pass metabolism, it has no in vitro effects on the activity of a wide range of CYP family members; efflux transporters, including MDR1; and uptake transporters such as OATP1 (Pleasants RA 2018)

2.4.4 Toxicology

The published literature on the toxicology of Glycopyrrolate is summarised and reviewed to ascertain whether the studies conducted since the original marketing authorisation for the first Glycopyrrolate containing product indicate any significant change in safety assessment for the non-clinical (animal) information. The detail description of various studies undertaken to investigate toxicity of Glycopyrrolate in various animal species such as a rats, mice, rabbits, cats and dogs etc is provided as below.

2.4.4.1 Single-Dose Toxicity

Single-dose toxicity studies in mice, rats, rabbits, cats and dogs using several routes of administrations revealed clinical signs that included mydriasis, tachycardia, prostration, anorexia and diarrhoea consistent with exaggerated pharmacological effects and, at very high doses, drug-induced deaths. There are three main relevant publications: Franko et al, 1962, Franko et al, 1970 and Saito et al, 1973.

The following table summarised results obtained by Franko et al, 1962

ACUTE (SINGLE DOSE) TOXICITY OF GLYCOPYRROLATE

Species	Sex	Route	LD_{50}	95% confidence limits	Slope
			mg./kg.		
Mouse	M	I.v.	14.7	11.9–18.4	1.2
	M	I.p.	112	93–134	1.2
	M	Oral	550	430–704	1.4
Rat	F	I.v.	14.6	13.9–15.3	1.1
	F	Oral	1280	1180–1389	1.2
Rabbit	Either	I.v.	25*		
Dog	Either	I.v.	15–30*		
Cat	Either	I.v.	15–30*		

* Approximations.

Higher doses by all routes in mice and rats caused mydriasis, 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 hours. No outstanding gross pathological changes attributable to glycopyrrolate were found in the survivors or the animals that died. There was an unusually large difference between the oral and intravenous LD_{50}s in the mouse and rat suggesting low oral bioavailability of glycopyrrolate in these species.

Franko et al, 1970 evaluated the acute toxicity of glycopyrrolate in mice and rats. Following intraperitoneal (IP) administration, the LD_{50} was estimated to be 107 mg/kg in mice and 196 mg/kg in rats. Following oral dosing, the LD_{50} was estimated to be 1150 mg/kg in rats. More details are shown below in Table 5. The principal clinical effects observed included: mydriasis, decreased motor activity, hyperflexia, laboured respiration, tonic and clonic convulsions (only at elevated doses). However, there were no gross autopsy findings, no organ-weight changes or adverse histopathology findings.

Table 05: Acute Toxicity of Glycopyrrolate (Franko et al, 1970)

Species	**Sex**	**Route**	**LD_{50} mg/kg**	**LD_{50} 95% confidence limits**
Mouse	F	IP	107	100-115
Rat	F	IP	196	177-217
Rat	M	PO	1150	915-1440

Results reported by Saito et al, 1973, are shown below.

TABLE 1 LD_{50} values of GP in mice, rats and rabbits by single administration

Species	Route	LD50(mg/kg) Male	LD50(mg/kg) Female	Slope	Method
Mouse	iv	19.6(* 18.9~ 20.3)	16.4(* 15.7~ 17.0)	1.2	
	ip	117.8(113.3~ 122.4)	122.1(117.2~ 127.3)	1.2	Van der Wärden
	sc	588.8(556.9~ 622.5)	645.0(619.8~ 671.2)	1.2	
	po	971.9(971.9~1,037.4)	903.6(863.0~ 946.0)	1.2	
rat	ip	282.4(275.6~ 289.3)	244.8(238.7~ 251.0)	1.1	
	sc	833.0(796.9~ 870.7)	963.9(919.2~1,010.8)	1.2	Van der Wärden
	po	1,825.0(1,712.2~1,945.2)	1,606.0(1,507.0~1,711.0)	1.2	
Rabbit	iv	29.1		1.05	Up and Down
	po	2,360.0		1.1	

Note; * Standard deviation

Clinical signs observed in acute toxicity tests were depression of spontaneous movement and mydriasis for all injection routes; symptoms of anorexia and diarrhoea were observed in higher dose groups. Injection-site necrosis and scarring were observed following SC administration. The results again show the markedly reduced toxicity following oral administration, most likely reflecting lower systemic exposure using this route.

2.4.4.2 Repeat-Dose Toxicity

Franko et al, 1970 reported results from subchronic and chronic toxicity studies of glycopyrrolate in rats and dogs using intravenous and oral (diet and gavage) routes of administration (Table 7). No adverse findings were noted in relation to autopsy, histology, organ weights, haematology and clinical biochemistry. There were no drug-related deaths. Clinical signs in dogs included: mydriasis, cycloplegia, xerostomia, emesis, occasional lacrimation, injection of sclera and rhinorrhoea (only at 64 mg/kg/day). Thus a NOAEL of 16 mg/kg/day can be determined. No clinical signs were noted in rat subacute and chronic toxicity studies. Decreased body weight gain (up to 24%) at the mid and high doses was noted in the 30-week rat study for which a NOAEL (in terms of body weight gain) of 20 mg/kg/day can be determined.

Table 7: Repeat-Dose Toxicity Studies Reported by Franko et al, 1970

Species; Strain	Animal numbers	Route; Test material	Duration (weeks)	Doses (mg/kg/day or ppm)
Dog; Beagle	2 M/F	IV; saline solution	4	0.4, 2.0
Dog; Beagle	1 M/F	PO; capsule	5	3,9,27
Rat; SD	2 M/F	Diet	6-7	250, 500, 1000, 2000*
Dog; Beagle	2 M/F	PO; capsule	27	4,16,64
Rat; SD	15 M/F	Diet	30	400,1300, 4000**

*Equivalent to 12.5, 25, 50 and 100 mg/kg/day (Food Additives Tox Guideline 2000)
Equivalent to 20, 65 and 200 mg/kg/day Error! Bookmark not defined.**

Saito et al, 1973, reported results from one-month and six-month toxicity studies in Wistar rats (Table 8). The only clinical effect observed was mydriasis. There were no drug-related deaths in either study. Slight anaemia was noted at the highest doses in both studies. Urinalysis parameters were normal in both studies. Clinical biochemistry evaluations revealed a slight reduction of A/G ratio and slight increases in serum GOT and GPT only at the highest dose in males in the one-month subacute study. Autopsies revealed no abnormalities except one high-dose rat with cystitis in the one-month study. In general only very minor histological changes were noted namely dose-related organ congestion and hyperaemia. Some effects were noted in the high-dose groups including reticular cellular proliferation in the lymph nodes and spleen, simple atrophic changes and flattening of gastric mucosa, and swelling of tubular epithelium and proteinaceous casts in the tubuli. Vacuolation and cloudy swelling of hepatocytes were noted at 220mg/kg/day in the one-month study. The NOAEL for this subacute study is considered to be 100 mg/kg/day. After a 3-week recovery period histological changes in the 6-month study were reversed. A worst-case NOAEL of 46 mg/kg/day glycopyrrolate for rat oral chronic toxicity can be determined.

Table 8: Repeat-Dose Toxicity Studies Reported by Saito et al, 1973

Species; Strain	Animal numbers	Route; Test material	Duration (months)	Doses (mg/kg/day)
Rat; Wistar	5 M/F	PO gavage; distilled water	1	22, 46, 100, 220
Rat; Wistar	10 M/F*	PO gavage; distilled water	6	10, 22, 46, 100

*8 M/F for 6 months; 2/M/F for 3-week recovery period

The repeat-dose toxicity studies described above were undertaken in a pre-GLP era although the technical standards appear to be more than adequate particularly for the Saito et al studies for which extremely detailed results are presented.

2.4.4.3 Genotoxicity

No genotoxicity data on glycopyrrolate could be found in scientific publications (except in the Chabicovsky et al, 2019 publication as shown below). However, Leadscope predictions have been generated as shown in Annex 1.All endpoints were predicted as negative. The reference compound pyridostigmine bromide (CAS no 101-26-8) tested negative in the Ames assay at up to 10mg/plate and showed no evidence for clastogenic potential in an in-vivo rat micronucleus assay (Pyridostigmine bromide 508).

Glycopyrrolate contains no structural alerts for mutagenicity. It is a carboxylic acid ester of α-cyclopentylmandelic acid (CAS no 427-49-6) and 1,1-dimethyl-3-hydroxypyrrolidinium bromide (CAS no 51052-74-5). Mandelic acid itself (2-hydroxy-2-phenylacetic acid; CAS 90-

64-2) is reported to test negative in a bacterial reverse mutation assay using S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and E. coli WP2 ±S9 (Mandelic Acid ECHA Europa 2016). The inclusion of the non-alerting α-cyclopentyl substituent (to form CAS 427-49-6) will not change the non-mutagenic status of mandelic acid. As for the charged part of the molecule no mutagenicity data could be found on 1,1-dimethyl-3-hydroxypyrrolidinium bromide but 1-ethyl-1-methylpyrrolidinium bromide is reported to be Ames-negative (1-ethyl-1-methylpyrrolidinium bromide ECHA Europa 1992).

2.4.4.3.1 In-Vitro Genotoxicity

In vitro genotoxicity testing of glycopyrronium included the Ames mutagenicity assay and human (peripheral) lymphocyte chromosome aberration assay has been performed. All tests gave negative results

- *Three independent Ames assays using S. typyhimurium strains TA98, 100, 1535, 1537 and 102, ±S9*
- *Chromosome aberrations in cultured human peripheral lymphocytes*
- *Mouse lymphoma assay*

2.4.4.3.2 In-Vivo Genotoxicity

The in vivo bone marrow micronucleus assay was conducted in rats (and mice). No genotoxic effect was observed in any these tests (EMA, 2012) (FDA, 2018a). Glycopyrronium tosylate was negative in an Ames assay, with and without metabolic activation (FDA, 2018a)

2.4.4.4 Carcinogenicity

Glycopyrrolate is considered highly unlikely to possess carcinogenic potential for a variety of reasons, including:

- Its simple carboxylic acid ester structure with no structural alerts
- Absence of pre-neoplastic lesions in 36-week and 6-month repeat-dose toxicity studies (see section 2.3.5.2)
- Long history of safe therapeutic use (in both human and veterinary medicines) with no signals for carcinogenic potential found in extensive literature searching.
- Negative Leadscope predictions for carcinogenicity (Annex 1).

Chabicovsky et al, 2019 make the following comments on the results of formal carcinogenicity testing of glycopyrrolate in animal models.

*The carcinogenic potential of glycopyrronium was tested in a 26-week oral study in CByB6F1-Tg (HRAS)2Jic transgenic mice and a 104-week inhalation study in rats. In a 26-week oral carcinogenicity study, glycopyrronium treatment did not increase the incidence of neoplastic findings in CByB6F1-Tg (HRAS)2Jic transgenic (Tg) and wild-type (WT) mice at the highest doses (75 and 100 mg/kg/day for males and females, resp.; correlated with AUC values for males: 33.1 and 75.6 ng*h/mL for Tg or WT; females: 24.7 and 15.3 ng*h/mL for Tg or WT) (EMA, 2012) (Table 7). In this 2-year carcinogenicity study, the neoplastic*

*incidence rate including but not limited to endometrial stromal polyps and the combination of endometrial stromal polyps plus endometrial stromal sarcoma monitored in Wistar rats treated at dose of 0.06, 0.17 and 0.45 mg/kg glycopyrrolate per day did not exceed the spontaneous incidence rate observed in the control animals (twice air or once vehicle); at week 52, the mean plasma AUC0–24 values were 8.2, 22.2 and 36.5 ng*h/mL in the low, mid and high-dose group, respectively (EMA, 2012).*

Furthermore, glycopyrronium tosylate was not carcinogenic when topically applied to rats daily for up to 24 months in a solution at concentrations of 1%, 2%, and 4% (w/w; 300 µL application volume). The vehicle consisted of water, ethanol, and citrate (FDA, 2018b).

2.4.4.5 Reproductive and Developmental Toxicity

Adverse effects on the fetus seem highly unlikely given the findings reported by Proakis and Harris, 1978, to the effect that there is minimal transplacental passage of radioactivity in the pregnant dog.

2.4.4.5.1 Fertility and Early Embryonic Development

A three-generation rat study using 20 animals/sex/group using of 0.13% glycopyrrolate (1300ppm) in the diet (equivalent to an average dose of 130 mg/kg/day in young rats and 65mg/kg/day in more mature rats**Error! Bookmark not defined.**) was performed in which control males were administered plain diet for 56 days before mating and through 2 matings (28 weeks) and 0.035% (equivalent to 35 mg/kg/day in young rats) thereafter until termination of the study (FDA, Summary Basis for Approval of Robinul). The information cited by FDA appears to be the same or similar to that reported by Franko et al, 1970, although this publication indicates a pre-mating exposure period of 3 weeks and 5 weeks per subsequent mating cycle. Data are reported by Franko et al on three matings, as shown in Table 9. The results revealed a treatment-related decrease in the rate of conception and survival at weaning. [Because no information on food consumption and bodyweight are reported, the possibility of inanition as a causative factor owing to poor diet palatability cannot be ruled out.] Careful examination of offspring born to drug-treated animals showed no abnormalities attributable to drug administration. The pharmacological action of glycopyrrolate could well have caused diminished seminal secretion. No abnormalities were seen in the offspring of treated animals.

Table 9: Summary of reproductive performance of rats given glycopyrrolate (Franko BV et al., 1970).

Parameter	**Control**			**Glycopyrrolate**		
	First litters	**Second litters**	**Third litters**	**First litters**	**Second litters**	**Third litters**
No. litters/group	19/20	18/20	18/20	14/20	13/18	13/18
No. stillbirths	1	0	7	8	1	9
Mean No. live young at birth	11.2	10.7	12.1	7.7	8.8	8.0

Lactation index*	93	93	92	68	69	96
Mean weight (g)						
Birth	6.0	6.2	6.0	6.1	6.0	6.2
Weaning	33.6	37.6	41.3	34.0	34.8	41.3

2.4.4.5.2 Embryofetal Development

An embryotoxicity study (Segment II) was undertaken in the New Zealand White rabbit using glycopyrrolate at two IM doses of 0.05 and 0.5 mg/kg. Although maternal bodyweight was depressed at both dose levels during the treatment period no teratogenic effects were observed (Robinul, Summary Basis of Approval**Error! Bookmark not defined.**).

Rabbit - Segment II

Reference Firm: 4

Robinul Injectable was administered I.M. to 15 mated female New Zealand White rabbits from Day 7 - Day 19. Dose levels were 0.05 and 0.5 mg/kg/day. Controls received vehicle alone. Rabbits were sacrificed on Day 30 and examined. All fetuses were examined for external and visceral malformations and then cleared and their skeletons stained with Alizarin Red for visualization of skeletal anomalies

Results:

	Control Vehicle Group I	Robinul Injection mg/kg/day 0.05 Group II	0.5 Group III
No. pregnant dams/pregnancy rate %	14/93.3	10/67.7	11/73.3
Maternal deaths	0	0	0
Mean no. corpora lutea	10.1	10.2	9.7
Mean no. resorptions	1.4	0.6	1.4
Mean no. implants	8.9	9.4	8.4
Implant efficiency %	88.1	92.2	86.6
Resorptions/No. implants (%)	19/125(15.2)	5/85(17.0)	15/92(16.3)
Live fetuses/Dead fetuses	125/0	85/0	92/0
Mean no. live fetuses	7.6	8.9	7.0
Mean live fetal weight (gm)	42.43	39.64	41.66
Sex ratio (M/F)	0.83	0.87	0.79
Fetal malformations %	0.0	1.1*	0.0
Incidence of ossification variations %	40.6	48.4	41.6

*Slight hydronephrosis in one animal.

Maternal body weight gains were depressed during the dosing period (Days 7-19) in Group II (about 63%) and Group III (about 85%) compared to controls. A decreased body weight gain of approximately 59.5% observed overall (Days 7-30) was seen in Group III compared to the control group.

Some of the information on the reproductive toxicity of glycopyrrolate including the results of a study involving parenteral administration to pregnant rabbits is taken from an FDA review undertaken in 1974 and so the data-protection period expired many years ago. In due course generic products were licensed (in the USA) on the basis of the nonclinical data available for Robinul. Consequently, the information on reproductive toxicity contained in the FDA Summary Basis for Approval (SBA) is considered acceptable for citation in an application

made on the basis of well-established use. [Critical information on rat studies is reported in Franko et al, 1970 in a rather cryptic manner; the SBA data are helpful in terms of clarification.]

In addition, Kagiwada et al (1973) undertook conventional embryotoxicity studies using oral administration of glycopyrrolate:

- At 0, 4, 20 and 100 mg/kg/day with dosing from GD (gestation day) 7-18 in the mouse;
- At 0, 4, 25 and150 mg/kg/day with dosing from GD 9-21 in the rat.

The intrauterine mortality, fetal bodyweight, delivery rate, pub bodyweight, viability at weaning and incidence of gross and skeletal abnormalities showed no significant differences between control and treated animals in both rodent species.

The results obtained by Kagiwada et al are considered to be reliable since the numbers of pregnant dams employed and number of pups evaluated were more than adequate. In the teratology phase in mice there were 20 pregnant dams/group and 254-275 pups/group were evaluated for skeletal malformations. In rats the corresponding numbers were 15 dams/group and 195-200 pups/group.

2.4.4.5.3 Prenatal and Postnatal Development

Kagiwada et al also evaluated postnatal development (for up to three weeks postpartum) in mice and rats using the same doses as in the embryotoxicity studies. In the post-natal development phase in mice 7 pregnant dams/group were employed producing 87-93 pups/group that were evaluated for skeletal malformations. In rats the corresponding numbers were 5 dams/group and 53-58 pup/group. No adverse effects were noted. [Kagiwada, K., Ishizaki, O., and Saito, G. (1973) Effects of glycopyrrolate on pre- and post-natal development of the offspring of pregnant mice and rats. *Oyo Yakuri 7*, 617-626.]

Ishizaki & Saito, 1978: Effects of Glycopyrrolate on Lactation in Mice. In this study single oral doses of 0, 1, 10 and 100 mg/kg were administered to dams (26-29 per group) whose offspring had been removed on the 13^{th} or 14^{th} day of lactation. Two separate evaluations were made of effects on lactation. In both cases statistically significant effects were noted at the highest dose.

2.4.4.5.4 Juvenile Animal Toxicity

No reports of dedicated juvenile animal toxicity studies could be found in the scientific literature. On the other hand adverse effects in such studies are considered highly unlikely for two main reasons:

- There are extensive reassuring data from pre- and postnatal reproduction studies (Franko et al, 1972; Kagiwada et al, 1973) – which is considered useful information in the EMA guidance (EMEA Guideline on the need for non-clinical testing in Juvenile animals of pharmaceuticals for paediatric conditions 2008).

- Multiple clinical trials have been undertaken in children with chronic severe drooling. Glycopyrrolate was reasonably well tolerated in all studies (Table 9) and there was no evidence for any adverse developmental effects.

Table 10: Glycopyrrolate: Clinical Studies in Children with Chronic Severe Drooling

Patient Population	Patient Number and Age Range	Main Adverse Events	Reference
Cerebral palsy or other neurologic conditions	38; Age 3-23	Dry mouth, constipation, vomiting	Zeller et al, 2012
Cerebral palsy or other neurologic conditions	137; Age 3-18	Constipation, vomiting, diarrhoea	Zeller et al, 2012
Disabled children with sialorrhea	39	20% of patients with adverse events	Mier et al, 2000

For many years parenteral glycopyrrolate was used as premedication in order to attenuate cardiovascular depression in infants undergoing halothane anaesthesia. Relevant articles include those by Mirakhur, 1982 (20 patients), Annila et al, 1998 (27 patients) and Cartabuke et al, 1991 (25 patients). Glycopyrrolate has been widely used as a preoperative medication to inhibit salivary gland and respiratory secretions (Gallanosa A 2019). Alimelkkila et al, 1993 have reviewed the use of anticholinergic drugs in obstetrics for example in patients undergoing Caesarian section. They conclude that "from the pharmacokinetic and -dynamic point of view, glycopyrrolate appears to be the best choice when the use of anticholinergic drugs is indicated in obstetrics".

In 2010 the FDA Department of Pharmacovigilance undertook a review of postmarketing experience with the use of glycopyrrolate in children (FDA medical review - NDA-22-571) (particularly in children with cerebral palsy, CP).

Because of the glycopyrrolate has been marketed since 1961 in a tablet form for the treatment of gastric ulcer, but widely used off label for treatment of drooling in children, the post marketing experience is a valuable tool for the safety evaluation of this new dosing form and indication. The oral tablets are indicated for gastric ulcers in adults, and the injectable glycopyrrolate is approved as preoperative and intraoperative antimuscarinic agent. The oral tablets are crushed and currently used for treating drooling in children with cerebral palsy; the injectable glycopyrrolate has not been reported as being used for this purpose. But the AERS data were examined for it as well. Towards that end the division of pharmacovigilance (DPV) was enlisted through a formal consult from DDDP to help examine AERS data on the already marketed glycopyrrolate containing drugs. After conducting a complete search of all AERS data to date, they did not identify any new significant safety concerns associated with the use of any formulation of glycopyrrolate in children 0-18 years old. In addition, they had no additional labelling recommendation at this time, other than the label reflect currently available safety information.

The conclusion of the review is as follows:

The paucity of adverse events data from the AERS and other postmarketing safety data, in combination with a yearly usage of approximately (b) (4) prescriptions in the age group of 3 – 16 in patients with a significant number diagnosed with CP provides strong additional support to the safe use of glycopyrrolate.

Although this review forms part of a regulatory assessment it does not make use of any confidential company data and so is considered acceptable evidence in the context of this application based on well-accepted use.

Taken together the existing nonclinical data from pre- and postnatal reproduction studies and clinical evidence of extensive use in children over many decades particularly in anaesthesia and obstetrics strongly suggest a benign safety profile. In these circumstances performing studies in juvenile animals would seem to be unnecessary and contrary to the spirit of EMA guidance on the 3Rs principles (EMA Guidance on Ethical use of animals in medicine testing).

2.4.5 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 intramuscular injection of glycopyrrolate in rabbits. These areas were hemorrhagic and/or pocketed in some cases at the higher drug concentrations. The low concentration of glycopyrrolate had virtually no effect at sites of subcutaneous injection. Areas of extravasation of blood were found with the high concentration. (Franko B. V. et al 1970)

2.4.6 Other Toxicity Studies

2.4.6.1 Antigenicity

Local tolerance of glycopyrrolate (0.2%, 2.4% and 24% (w/v)) was assessed using the murine Balb/c local lymph node assay, an standard in vivo test for skin sensitization (OECD no. 429), showing reddening of the application site (ear skin) from the second day of application of the high dose and an increase in draining lymph node weights in high dosed animals. Yet, there was no associated lymph node hypercellularity. Glycopyrrolate was not identified as a sensitizer in this study (EMA, 2012).

Chabikovsky et al also cite multiple clinical studies (many of which are published) involving repeated topical application of glycopyrrolate-containing formulations with no evidence of antigenic activity.

2.4.6.2 Immunotoxicity

Standard toxicity studies (eg Franko et al, Saito et al) did not reveal any adverse effects on the immune system. *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.

2.4.6.3 Mechanistic Studies

In a series of investigations Lau and Szilagyi, 1992, provided a detailed quantitative characterization of the muscarinic properties of glycopyrrolate. For example: effects on the contraction of guinea-pig atrium, Schild analysis to determine a pA2 values against carbachol and acetylcholine, radioligand binding studies and determination of binding constants. These results are considered complementary to primary pharmacology data confirming the mode of action of glycopyrrolate in relation to the sialorrhoea indication.

The report of Proakis and Harris, 1978, demonstrating that glycopyrrolate is resistant to penetration across the blood—brain and placental barriers. These findings greatly help to explain the essential absence of CNS effects and the lack of teratogenic potential.

2.4.6.4 Dependence

No specific studies on dependence potential were found in the literature, which is unsurprising since, as previously stated, CNS activity is minimal and in nearly 60 years of clinical use there are no reports of dependence. Moreover, self-administration of elevated doses of glycopyrrolate is effectively limited by the drug's side-effects.

2.4.6.5 Metabolites

Metabolite-related toxicity issues are considered highly unlikely following administration of oral glycopyrrolate for the following reasons:

- Oral bioavailability in humans is low and variable, probably around 5-10% on average
- In humans the majority (ca 80%) of the absorbed portion of is excreted in urine and bile as unchanged glycopyrrolate (see introductory information in Section 2.4.3). [No drug substance was detected in urine in the mouse after oral administration however.]
- In humans metabolism after oral absorption is estimated to be ≤5% of dose (see integrated overview section)
- The metabolites appear to lack pharmacological activity at the acetyl choline receptor.

2.4.6.6 Impurities

Glycopyrronium bromide is the subject of a European Pharmacopoeia monograph. The active substance employed is also covered by a Certificate of Suitability issued by EDQM (which addresses potential issues concerning residual solvents). The most likely trace impurities could be the hydrolysis products of glycopyrrolate – these are discussed in the section on mutagenicity. Both substances [α-cyclopentylmandelic acid no and 1,1-dimethyl-3-hydroxypyrrolidinium bromide] are integral to the drug substance and so any suspicion of mutagenic potential would be manifested in the structure of glycopyrrolate – which is predicted as Ames-negative using Leadscope software (Annex 1). In addition, residues of bromomethane, the reagent used for quaternizing the N-methylpyrrolidone portion of the

molecule, could be present. Bromomethane (CAS no 74-83-9) has been extensively evaluated in toxicological studies. It is an unusual example of a mutagenic non-carcinogen. Bercu et al, 2018, have determined an oral PDE (Permitted Daily Exposure) of 2.2mg. A gas-chromatography method has been developed for analysis of bromomethane in the drug substance for which the LOD & LOQ are 38 ppm & 75 ppm respectively (equivalent to 228 ng and 450 ng respectively at the maximum dose of 6 mg drug substance). Using this method of analysis bromomethane was not detected in several batches of the drug substance.

Five excipients are employed in the tablet formulation. These are:

- Calcium hydrogen phosphate dehydrate
- Lactose (anhydrous)
- Povidone
- Sodium starch glycolate
- Magnesium stearate.

All excipients are Ph.Eur-compliant.

A risk assessment based on the MDD of 6 mg glycopyrrolate has been performed by Rhea Sawant (21.05.18) on elemental impurity following ICH Q3D guidance. The risk assessment takes into account potential elemental impurities in: drug substance, excipients, container/closure systems, manufacturing equipment, manufacturing environment, water and compressed air used in manufacturing process. It is concluded that for all potential elemental impurities levels in the drug product are lower than the relevant ICH Q3D limits.

2.4.6.7 Photosafety

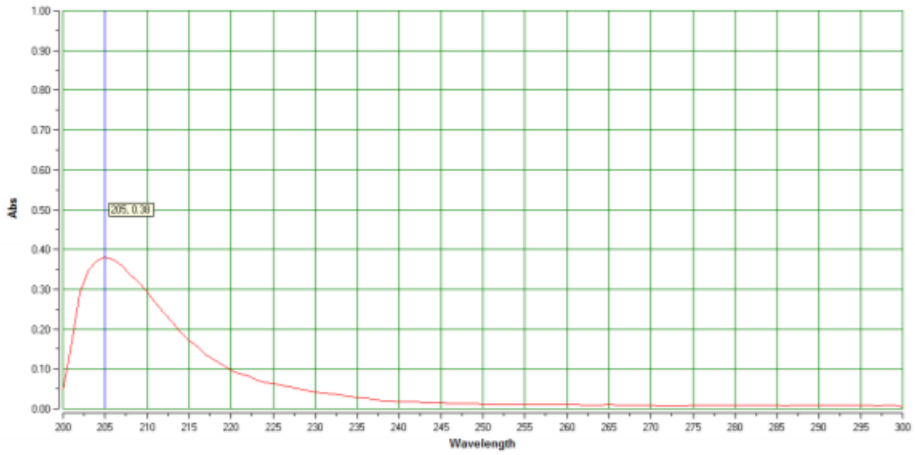
According to ICH S10 guidance on photosafety testing the following characteristics are considered critical:

- Absorbs light within the range of natural sunlight (290-700 nm);
- Generates a reactive species following absorption of UV-visible light;
- Distributes sufficiently to light-exposed tissues (e.g., skin, eye).

Concerning the first point the guidance contains the following statement: “A compound that does not have a Molar Extinction Coefficient (MEC) greater than 1000 L mol-1 cm-1 at any wavelength between 290 and 700 nm is not considered to be sufficiently photoreactive to result in direct phototoxicity”.

This criterion is considered to apply to glycopyrrolate based on an application of the Beer-Lambert law to literature data on glycopyrrolate’s UV spectrum shown in Figure 5 (Kapupara P 2018).

Figure 5: UV Absorption Spectrum of Glycopyrrolate (1 cm cells; 4µg/mL; methanol solvent)



MEC = ε = A/c.l, where A=absorbance; c= molar concentration and l=path length (in cm).
In this case at 290nm, A=<0.01; c=4µg/mL=4mg/L=0.01004millimolar (mol wt of glycopyrrolate being 398.335); l=1.0.
Hence MEC=<0.01x1000/0.01004=<996 $Lmol^{-1}cm^{-1}$

Thus the MEC at 290nm is lower than the threshold value triggering concern over photoreactivity. Although there is evidence for distribution of radioactivity to melanin-containing tissues in ADME studies the amount of drug reaching such tissues will be minimal following oral administration. Moreover there are no reports of photosensitivity following nearly 60 years of clinical use.

2.4.7 Integrated Overview and Conclusions

Glycopyrrolate is a permanently charged quaternary ammonium antimuscarinic agent which the antagonized action of acetylcholine on structures innervated by postganglionic cholinergic nerves and on smooth muscles that respond to acetylcholine but lack cholinergic innervation. It diminishes the volume and free acidity of gastric secretions and controls excessive pharyngeal, tracheal and bronchial secretions. Owing to its ionic nature passage across lipid membranes, such as the blood-brain barrier and placenta is greatly inhibited. Its mode of action is by competitive blockade of muscarinic receptors of various subtypes thus denying access by acetylcholine.

As early as 1962 a demonstration of the marked antisialogue effect of IV glycopyrrolate was achieved in anaesthetized mongrel dogs using methacholine as the saliva-stimulating agent. Qualitative information from the veterinary literature indicates that glycopyrrolate is effective against sialorrhea in dogs and cats. Several small-scale human volunteer studies undertaken in the period 1974-1980 confirmed the antisialogue effect of glycopyrrolate using IV, IM and PO routes of administration; effective doses were 0.2mg IV and IM and 8 mg PO (possibly suggesting that oral bioavailability is around 2.5%).

The database on secondary pharmacology is extremely extensive particularly in relation to anaesthetics, obstetrics and COPD/asthma. Although no scientific publications could be located on conventional animal safety pharmacology studies examining for example CNS, cardiovascular and respiratory effects, studies in rats, dogs and rabbits have shown no marked effects on these endpoints. In addition, various clinical evaluations have shown that glycopyrrolate exerts minimal effects in terms of QT prolongation.

Following oral administration of ^{14}C-glycopyrrolate in the mouse the rate of absorption was slow and bioavailability appeared low based on the small amounts of radioactivity detected in blood. After 6 hours around 8% of the dosed radioactivity was found in the stomach and small intestine. The absolute oral bioavailability was determined in children and found to be highly variable (1.3-13.3%) with a median value of 3.3%. Based on the results of experiments involving IV administration of radiolabelled glycopyrrolate to anaesthetized dogs it can be concluded that penetration of the drug through the blood-brain and placental barriers is minimal.Following oral and IV administration of ^{14}C-glycopyrrolate to mice, total radioactivity was distributed throughout the body, elevated levels of radioactivity being observed in highly perfused tissues (kidney, liver, small intestine and various glands) but not in the brain (consistent with the data in anaesthetized dogs). Whole-body autoradiograms showed no radioactivity in the foetus following administration to pregnant animals. The metabolic fate of glycopyrrolate has been investigated in mice and rats. The major metabolic pathways are considered 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. Following oral administration of radiolabelled glycopyrrolate in mice 7.6% of radioactivity was excreted in urine and approximately 79% in faeces. No drug substance was detected in mouse urine after oral administration. A study using intravenous ^{3}H-glycopyrrolate in humans showed the disappearance of more than 90% from the serum in *5* minutes and almost 100% in 30 minutes. Urinary radioactivity was highest in the first 3 hours and 85% was excreted in the urine within 48 hours. Paper chromatography showed that 80% of the radioactivity in bile and urine corresponded to unchanged glycopyrrolate.

Taken together the published animal and human data on ADME (absorption, distribution, metabolism and excretion) indicate that oral absorption is low and variable (probably in the region of 10-20%) with widespread distribution of the absorbed fraction. After oral administration the majority of the dose (approximately 80%) is excreted in faeces. Since 65-80% of an IV dose is excreted unchanged in urine it can be concluded that after an oral dose in humans the majority of the absorbed fraction will remain as unchanged drug substance with no more 5% of dose being converted to metabolites. [Absolute oral bioavailability appears to be markedly lower than oral absorption suggesting that a significant portion of the absorbed dose does not enter the systemic circulation possibly explained by biliary excretion and/or first-pass metabolism. Indirect evidence for biliary excretion arises from the fact that around 14% of an IV dose in mice is found in the large and small intestine and the metabolite pattern over time after oral administration in mice is suggestive of enterohepatic circulation.]

Attempts to integrate estimates of bioavailability with data on pharmacological effectiveness against sialorrhoea in human volunteers and the results of acute toxicity studies by different routes of administration were only partially successful owing to biological variability and the patchy nature of data available in the public domain.

Extensive data on acute toxicity are available from several publications. LD_{50} values are not entirely consistent across publication probably reflecting differences in experimental procedures, animal strains and genders. Reported oral LD_{50}s in mice ranged from 550-900 mg/kg and from 1150-1800 mg/kg in rats. In rats signs of toxicity were tremors, clonic and tonic convulsions and laboured breathing, A single experiment in rabbits produced an oral LD_{50} of 2360 mg/kg. Parenteral administration led to much lower LD_{50}s, strongly supporting the results of PK studies showing low bioavailability. Intravenous LD_{50}s were in the range 15-20 mg/kg in the mouse, 15-20 mg/kg in the rabbit and *ca* 15 mg/kg in the rat. On the other hand SC LD_{50}s in rodents were in the range 600-900 mg/kg possibly suggesting metabolic clearance by tissue-related carboxylesterases. Oral LD_{50}s in the rat are around twice those in the mouse, possibly suggesting higher bioavailability in the latter species. This is reflected in the data on reproductive toxicity in that the maximum feasible doses in pregnant mice were only two thirds of those in pregnant rats (100 vs 150 mg/kg/day) as discussed below. [The ADME data are suggestive of a greater extent of metabolism of glycopyrrolate in mice compared to humans.]

The results of seven repeat-dose toxicity studies (four in the rat and three in the dog) are reported in the literature as shown in Table 11. All of these studies date back to the early 1970s when the inclusion of toxicokinetic (TK) evaluations was not part of the testing paradigm. Indeed ICH guidance on TK studies (ICH S3 1995) did not become effective until mid-1995, over two decades later. The absence of TK data is not considered to impair the validity of these animal safety assessments since oral absorption of glycopyrrolate has been shown to be similar (and rather low) in both animals and humans, and moreover the lowest chronic NOAEL (16 mg/kg/day in the dog) is considerably higher than exposure of a 10kg child at the maximum dose of glycopyrrolate (ie 0.6 mg/kg).

Table 11: Repeat-Dose Toxicity Studies Reported by Franko et al, 1970 and Saito et al, 1973 [NOAELs are shown in **bold]**

Species; Strain	**Animal numbers**	**Route; Test material**	**Duration (weeks)**	**Doses (mg/kg/day or ppm)**
Dog; Beagle	2 M/F	IV; saline solution	4	0.4, 2.0
Dog; Beagle	1 M/F	PO; capsule	5	3,9,27
Rat; SD	2 M/F	Diet	6-7	250, 500, 1000, 2000*
Dog; Beagle	2 M/F	PO; capsule	27	4,**16**,64
Rat; SD	15 M/F	Diet	30	**400**,1300, 4000**
Rat; Wistar	5 M/F	PO gavage; distilled	4	22, 46, **100**, 220

		water		
Rat; Wistar	10 M/F***	PO gavage; distilled water	26	10, 22, **46**, 100

*Equivalent to 12.5, 25, 50 and 100 mg/kg/day (Food Additives Tox Guideline 2000)
Equivalent to **20, 65 and 200 mg/kg/day**Error! Bookmark not defined.**
***8 M/F for 6 months; 2/M/F for 3-week recovery period

No genotoxicity data on glycopyrrolate could be found in scientific publications in the public domain (except for the Chabicovsky et at, 2019, article). Yet there are a number of important pointers indicating a lack of genotoxic potential. Firstly, the molecule contains no structural alerts for mutagenicity; secondly, the reference substance pyridostigmine bromide is reported to be non-mutagenic, and thirdly Leadscope QSAR evaluation predicts all relevant genotoxicity endpoints to be negative. Similar considerations apply to carcinogenicity in that the only literature information on formal testing is located in Chabicovsky et al. On the other hand it should be stressed that glycopyrrolate can be reasonably assumed to be non-genotoxic; no pre-neoplastic lesions were detected in chronic toxicity studies in the rat and dog and no evidence of carcinogenic potential has emerged in nearly six decades of clinical use.

Conventional reproductive toxicity studies have been undertaken in mice and rats using oral administration and rabbits by the IM route. The results are summarised in Table 12. No adverse effects were noted at the highest doses used in mice and rats (up to 150 mg/kg/day) on embryofetal development or on pre- and postnatal development. No teratogenic effects were noted in the rabbit in spite of an overall 60% decrease in bodyweight gain in treated animals. A dietary multigeneration study in rats showed a treatment-related decrease in the rate of conception and survival at weaning although it is unclear whether factors such as diet palatability and glycopyrrolate-induced suppression of sperm secretion affected these outcomes. Although the animal data on reproductive toxicity are reassuring, since relevant human data are lacking use of glycopyrrolate in pregnancy and lactation is contraindicated.

Table 12: Reproductive Toxicity Studies on Glycopyrrolate [NOAELs are shown in **bold]**

Study Type	**Species; route**	**Doses (mg/kg/day)**	**Animal numbers**	**Reference**
Fertility and early embryonic development	Rat; diet	65, 130	20 M/F	Franko et al, 1970
Embryofetal development	Rabbit; IM	0.05, **0.5**	15 F	Robinul SBA
Embryofetal development	Rat; oral gavage	4, 25, **150**	15 F	Kagiwada et al, 1973
Embryofetal development	Mouse, oral gavage	4, 20, **100**	20 F	Kagiwada et al, 1973
Pre- and postnatal development	Rat; oral gavage	4, 25, **150**	5 F	Kagiwada et al, 1973

Pre- and postnatal development	Mouse, oral gavage	4, 20, **100**	7 F	Kagiwada et al, 1973
Lactation	Mouse; oral gavage; single dose	1, **10**, 100	26-29 F	Ishizaki & Saito, 1978

The available published data indicate that glycopyrrolate is neither antigenic nor immunotoxic and it is minimally irritant on skin contact. Impurity toxicity is not considered to be an issue since the drug substance complies with the relevant Ph.Eur monograph and residues of the quaternizing agent (bromomethane) are orders of magnitude lower than the PDE of 2.2 mg. In addition there should be no concerns regarding phototoxicity given the absence of a chromophore that absorbs in the UV region. The MEC at 290 nm is lower than the threshold value triggering concern over photoreactivity.

It is concluded that the cited public-domain nonclinical data strongly support the safety-in-use of glycopyrrolate in the intended indication (treatment of sialorrhoea). In the case of those endpoints where conventional study data are not available (for example genotoxicity and carcinogenicity) ancillary information indicates a lack of concern, but these omissions are noted in the SmPC.

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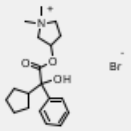
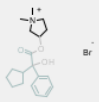
ANNEX 1

LEADSCOPE MODEL APPLIER 2.2.1: PREDICTIONS FOR GB IN RELATION TO MUTAGENICITY, GENOTOXICITY, CARCINOGENICITY AND REPRODUCTIVE TOXICITY

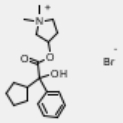
ICH M7 Suite – Mutagenicity

Structure	ICH M7 Consensus	Ames QSAR Consensus
 Glycopyrronium bromide	Negative	Negative

Statistical Models

E Coli - Sal 102 A-T Mut v1 Prediction	E Coli - Sal 102 A-T Mut v1 E Coli - Sal 102 A-T Mut structure	Salmonella Mut v3 Prediction	Salmonella Mut v3 Salmonella Mut structure
Negative	 Glycopyrronium bromide	Negative	 Glycopyrronium bromide

Expert Alerts

Bacterial Mutation v4 Prediction	Bacterial Mutation v4 Matched Alerts	Bacterial Mutation v4 Bacterial Mutation Bacterial Mutation structure
Negative	No Alerts	 Glycopyrronium bromide

Genotoxicity/Clastogenicity

	Statistical Models									
Structure	In Vitro Chrom Ab CHL v2	In Vitro Chrom Ab CHL v2 In Vitro Chrom Ab CHL structure	In Vitro Chrom Ab CHO v2	In Vitro Chrom Ab CHO v2 In Vitro Chrom Ab CHO structure	In Vitro SCE CHO v1	In Vitro SCE CHO v1 In Vitro SCE CHO structure	In Vitro SCE Comp v1	In Vitro SCE Comp v1 In Vitro SCE Comp structure		
 Glycopyrronium bromide_clastogenicity	Negative	 Glycopyrronium bromide_clastogenicity	Negative	 Glycopyrronium bromide_clastogenicity	Negative	 Glycopyrronium bromide_clastogenicity	Negative	 Glycopyrronium bromide_clastogenicity		

SCE in vitro other (A8I) v1	SCE in vitro other (A8I) v1 In Vitro SCE Other structure	chrom ab(A7P) v1 Prediction	chrom ab(A7P) v1 In Vivo Chrom Ab Comp structure	chrom ab other rodent(A7R	chrom ab other rodent(A7R) v1 In Vivo Chrom Ab Other structure	chrom ab rat (A7Q) v1	chrom ab rat (A7Q) v1 In Vivo Chrom Ab Rat structure
Not In Do...	 Glycopyrronium bromide_clastogenicity	Negative	 Glycopyrronium bromide_clastogenicity	Negative	 Glycopyrronium bromide_clastogenicity	Negative	 Glycopyrronium bromide_clastogenicity

In Vivo Micronuc Mouse v2	In Vivo Micronuc Mouse v2 In Vivo Micronuc Mouse structure	HGPRT Mut v1 Prediction	HGPRT Mut v1 HGPRT Mut structure	Mouse Lymphoma Act v2	Mouse Lymphoma Act v2 Mouse Lymphoma Act structure	Mouse Lymphoma Unact v2	Mouse Lymphoma Unact v2 Mouse Lymphoma Unact structure
Negative	 Glycopyrronium bromide_clastogenicity	Negative	 Glycopyrronium bromide_clastogenicity	Negative	 Glycopyrronium bromide_clastogenicity	Not In Do...	 Glycopyrronium bromide_clastogenicity

In Vivo Rodent DL Mut v1	In Vivo Rodent DL Mut v1 In Vivo Rodent DL Mut structure	In Vivo Rodent Mut v1	In Vivo Rodent Mut v1 In Vivo Rodent Mut structure	E Coli - Sal 102 A-T Mut v1	E Coli - Sal 102 A-T Mut v1 E Coli - Sal 102 A-T Mut structure	Salmonella Mut v3 Prediction	Salmonella Mut v3 Salmonella Mut structure
Not In Do...	 Glycopyrronium bromide_clastogenicity	Not In Do...	 Glycopyrronium bromide_clastogenicity	Negative	 Glycopyrronium bromide_clastogenicity	Negative	 Glycopyrronium bromide_clastogenicity

Genetox Statistical*

Effect	Model	Prediction	Positive Prediction Probability
Genetox Suite*			
Clastogenicity In Vitro	In Vitro Chrom Ab CHL v2	Negative	0.298
	In Vitro Chrom Ab CHO v2	Negative	0.211
	In Vitro SCE CHO v1	Negative	0.219
	In Vitro SCE Comp v1	Negative	0.008
	In Vitro SCE Other v1	Not in Domain	Not in Domain
Clastogenicity In Vivo	In Vivo Chrom Ab Comp v1	Negative	0.0
	In Vivo Chrom Ab Other v1	Negative	0.0207
	In Vivo Chrom Ab Rat v1	Negative	0.0075
	In Vivo Micronuc Mouse v2	Negative	0.219
Gene Mutation	HGPRT Mut v1	Negative	0.0737
	Mouse Lymphoma Act v2	Negative	0.0486
	Mouse Lymphoma Unact v2	Not in Domain	Not in Domain
	In Vivo Rodent DL Mut v1	Not in Domain	Not in Domain
	In Vivo Rodent Mut v1	Not in Domain	Not in Domain
	E Coli - Sal 102 A-T Mut v1	Negative	0.0197
	Salmonella Mut v3	Negative	0.0465

Carcinogenicity

	Statistical Models							
Structure	Cell Transform BALBc v1	Cell Transform BALBc v1 Cell Transform BALBc structure	Cell Transform C3H v1	Cell Transform C3H v1 Cell Transform C3H structure	cell transform (A8D) v1	cell transform (A8D) v1 Cell Transform Comp structure	SHE v1 Prediction	SHE v1 SHE structure
Glycopyrronium bromide_carcinogenicity	Negative	Glycopyrronium bromide_carcinogenicity	Positive	Glycopyrronium bromide_carcinogenicity	Negative	Glycopyrronium bromide_carcinogenicity	Negative	Glycopyrronium bromide_carcinogenicity

carc female mouse v3 Prediction	carc female mouse v3 carc female mouse structure	carc female rat v3 Prediction	carc female rat v3 carc female rat structure	carc male mouse v3 Prediction	carc male mouse v3 carc male mouse structure	carc male rat v3 Prediction	carc male rat v3 carc male rat structure
Negative	Glycopyrronium bromide_carcinogenicity	Negative	Glycopyrronium bromide_carcinogenicity	Negative	Glycopyrronium bromide_carcinogenicity	Negative	Glycopyrronium bromide_carcinogenicity

Rodent Carcinogenicity Statistical*

Effect	Model	Prediction	Positive Prediction Probability
Rodent Carcinogenicity Suite*			
Carcinogenicity In Vitro	Cell Transform BALBc v1	Negative	0.00822
	Cell Transform C3H v1	Positive	0.621
	Cell Transform Comp v1	Negative	0.123
	SHE v1	Negative	0.375
Carcinogenicity In Vivo	carc female mouse v3	Negative	0.2
	carc female rat v3	Negative	0.306
	carc male mouse v3	Negative	0.0633
	carc male rat v3	Negative	0.0308

Comments:

- Predictions based on in-vivo bioassays are negative
- Positive prediction for a cell-transformation (CT) endpoint considered unconvincing because:
    - CT assay results are highly unreliable and not used in a regulatory context
    - The positive prediction is based on a non-alerting structure (see below) but there appears to be no in-vivo data available on this compound.