CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20984

PHARMACOLOGY REVIEW(S)

PHARMACOLOGY CONSULT REVIEW FOR CHEMIST

Reviewer: Dou Huey (Lucy) Jean, Ph.D.

NDA 20-984 RAPLON (rapacuronium bromide) for Injection
Sponsor: Organon Inc.
TYPES, DATES AND REVIEW OF SUBMISSIONS: Original Amendment (AZ) of June 17, 1999
DATE OF review: July 22, 1999

COMMENTS:
The review is in response to Juanita Ross's request for consult review on the safety of impurities and degradation products as specified (attached JR's comments, received by LJ 7/20/99).

The Sponsor sets specification limits for ORG 9488 (impurity F) at % and total impurities at %. The chemist has requested the sponsor to lower these limits to % and %, respectively. The Sponsor, nonetheless, chose not to do so, instead presenting the results of two clinical studies using large quantities of ORG 9488 (2 to 10 times the amount that would be given to patients receiving Raplon for Injection) to support the reasonable safety of the new spec limit.

From pharmtox 's viewpoint, it can be concluded that the reasonable safety of ORG 9488 (%) and total impurities %) were shown in animal studies.

(1) The total impurity %):
The total impurity %) without ORG 9488 was studied in an acute toxicity study in dogs. Except for N-M blockade, no mortality, adverse effects or target organ toxicity was observed at 5.1 mg/kg, a dose probably 10-20X that of a patient can receive from Raplon containing % of the impurities.

(2) ORG 9488 %)
The major metabolite or degradation product, ORG 9488, was not specifically studied in animals. In both acute and multiple dose toxicity studies in cats and dogs, however, the extent of exposure to ORG 9488 was approximately % (ranging %) that of the parent compound. The results of the toxicity studies support the recommended human dose for rapacuronium. It is, therefore, reasonable to conclude that a specification limit of % for ORG 9488 is also safe based on these animal data.

From the discussions (1) and (2) above, it is concluded that the specification limits for both total impurities at % and ORG 9488 at % are reasonable safe based on the animal data.

Dou Huey (Lucy) Jean, Ph.D.
Pharmacologist Team Leader

cc
Original NDA 20-984
HFD-170/Div. File
HFD-170/DHJean
HFD-170/SSamanta
F/T by DHJean 7/22/99
N20984a

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
DIVISION OF ANESTHETIC, CRITICAL CARE, AND ADDICTION DRUG PRODUCTS (HFD-170)

Reviewer: Dou Huey (Lucy) Jean, Ph.D.

Original Summary

NDA 20-984 RAPLON (rapacuronium bromide) for Injection

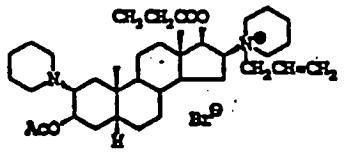
Sponsor:

Organon Inc.
375 Mt. Pleasant Avenue
West Orange, NJ 07052

TYPES, DATES AND RECEIPT OF SUBMISSIONS: Original of June 30, 1998
Receipt by: June 30, 1998 (CDER); June 30, 1998 (HFD-170) & July 1, 1998 (Reviewer)
Date Review completed: February 8, 1999

Drug:

Trade name: Raplon
Generic name: rapacuronium bromide
Code name: Org 9487 or ORG 9487
Chemical name: 1-[(2β,3α,5α,16β,17β)-3-(Acetyloxy)-17-(1-oxopropoxy)-2-(1-piperidinyl) androstan-16-yl]-1-(2-propenyl)-piperidinium bromide



$C_{37}H_{61}BrN_2O_4$

677.81

CAS# 156137-99-4

CATEGORY: Non-depolarizing neuromuscular blocking agent with rapid onset and short duration of action.

INDICATIONS: As an adjunct to general anesthesia to facilitate endotracheal intubation, and to provide skeletal muscle relaxation during surgical procedures.

RECOMMENDED DOSAGES:

Adults & geriatric:

Intubation: Initial (1.5 mg/kg) + maintenance [0.5 mg/kg (bolus) or 1.36 mg/kg or 2.72 mg/kg/hr for 30 min (infusion)].

Pediatric: intubation: 2-3 mg/kg.

DOSAGE FORM: Raplon, a 2% isotonic aqueous solution for parenteral administration, Is supplied as a sterile, lyophilized cake* packaged in 5 or 10 mL vials containing 114 & 227 mg of ORG 9487 (equivalent to 100 & 200 mg of active moiety) for reconstitution with 5 or 10 mL of Water for Injection. ***Refer to COMPOSITIONS for other ingredients and details**

COMPOSITIONS:

Ingredient	Amount/mL
ORG 9487,	
As Bromide	mg
Citric Acid, Anhydrous, USP	mg
Sodium Phosphate,	
Dibasic Anhydrous, USP	mg
Mannitol, USP	mg
Sodium Hodroxide, NF	Trace
And/or	
Phosphoric Acid, NF as 5% solution	Trace
To adjust pH 3.9 ± 0.1	
Water for Injection, USP q.s. to	mL

RELATED DRUG/INDs/NDAs/DMFs:

IND

IND

NDA 20-214: Zemuron (rocuronium bromide) Injection

NDA 18-776: Norcuron (vecuronium bromide) for Injection

DMFs

Note: Portions of the review were excerpted from the submissions. ORG 9487, Org 9487 and ORG were used interchangeably. Safety studies were conducted under GLP unless specified.

Redacted 6

pages of trade

secret and/or

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commercial

information

Unpublished Articles

I. PHARMACOLOGY:

(A) Efficacy: N-M blockade was shown in standard in vivo and in vitro studies.

(a) In vivo studies: Animals: anesthetized and ventilated cats, Beagle dogs, Rhesus monkeys and pigs; anesthetized and not ventilated rats (Vol. 1. 24).

Preparations: sciatic-nerve-stimulated twitch tension of the tibialis anterior or soleus (cat, dog & pig) and ulnar-nerve-stimulated adductor pollicis (monkeys).

Measurements: inhibition of twitch tension following intravenous administration of ORG 9487. Comparison drugs: vecuronium and/or succinylcholine.

Neuromuscular blocking profiles: potency, onset, duration, recovery rate, and/or reversibility.

The results are summarized as follows:

1. ORG 9487 and succinylcholine (SCC):

Species	ED_{90} µg/kg (iv)	Onset min	Duration min	Recovery min 25%-75%	
Cat	300(tibia.)	1.8	4.9	2.8	
	330(soleus)	2.3	7.3	2.6	
	55(tibia.)	*1.6*	*5.1*	*1.7*	*(SCC)*
	180(soleus)	*2.9*	*10.6*	*3.4*	*(SCC)*
Dog	270	1.4	4.7	1.8	
Monkeys (Rhesus)	192	1.5	9.8	5.0	(Org)
	2000	*1.5*	*9.7*	*2.9*	*(SCC)*
	570	1.2	23.2	7.5	($3XED_{90}$ Org)
	6000	*1.0*	*12.9*	*2.8*	*($3XED_{90}$ SCC)*
Pig	500(tibia.)	1.4	9.4	3.6	
	(soleus)	2.8	20.1	7.2	
Man	1500*	1.5	15	9	
Rat	2500	0.4**	5.4**	1.0**	

*Man ED_{90} estimated at 1.15 mg/kg iv

** At rat $2XED_{90}$

Note: for profiles following intramuscular injection of ORG & SCC, refer to page 20 of this review.

2. Vecuronium:

Species	ED_{90} µg/kg (iv)	Onset min	Duration min	Recovery min 25%-75%
Cat	50 (tibia.)	4.3	10.1	3.0
	38 (soleus)	5.0	17.4	5.2
Dog	25	3.0	8.3	2.9
Monkey (Rhesus)	9	6.7	33.2	10.1
Pig	150 (tibia.)	1.8	9.2	3.6
	(soleus)	3.2	18.6	5.9
Man	57*	3-5**	45-65**	25-40**

*ED90 **intubating dose

The *in vivo* studies showed ORG 9487 (ORG) is most potent in Rhesus monkeys and least potent in humans. In general, the time profiles are comparable among the animal species and humans. ORG is much less potent than vecuronium both in animals and in humans. The potency of ORG ranged from 1/3 (pig), 1/6 (cat), 1/10 (dog), to 1/20 (monkey), and 1/20 (human) that of vecuronium. But the N-M blockade profile compares more favorably for ORG than for vecuronium with shorter onset, duration and recovery rate: onset (1-3 min vs 2-7 min), duration (9-14 min vs 8-33 min), and the recovery rate from 25-75% control (3-5 vs 3-10 min). The blockade could be reversed by anti-ChE such as neostigmine, or pyridostigmine. At 3X ED_{90}, a clinically useful dose, the onset was shortened to approx. 1 min. Although the recovery and the duration were prolonged, the time profile still compared favorably for ORG in several animal species except in the pig, where ORG had a longer recovery rate and duration than vecuronium. In humans following a bolus of 1.5 mg/kg ORG (estimated ED_{90} 1.15 mg/kg), the onset was 1.5 min. The duration was 15 min and the recovery rate was 9.5 min. Intubation at 5-10 min was reported as good to excellent. The time profiles were longer than those obtained from 1 mg/kg succinylcholine.

3. Infusion data: obtained from pigs, refer to Report 070-1137-PH, on page 31. When a steady state of 60% block was maintained for 75 min, the required dose was

reduced from an initial dose of 0.039 to 0.029 mg/kg/min toward the end of infusion suggesting cumulative effects. The recovery rate (11 min) was also slower than that (5 min) following bolus administration.

(b) In vitro preparations:

Guinea pig diaphragm and chick biventer (potency study): standard experimentals.

Report 070-1082-PH, Vol.1. 24

Potency: ED_{50} values.

50% neuromuscular blocking concentrations of Org 9487, vecuronium and Suxamethonium in the isolated guinea-pig diaphragm and chick biventer nerve Muscle preparations

Compound	n	G P diaphragm Preparation (μM)	n	Chick biventer preparation (μM)
Org9487	12	3.4+/-0.3	10	0.39+/-0.02
Vecuronium	9	0.22+/-0.3	10	0.047+/-0.004
Succinylcholine	2	5.6+/-0.6*	7	1.1+/-0.1*

*concentration-effect dose response constructed from data obtained using single additions of different concentrations of drug, with washings between drug addition.

ORG was approximately 8-15X less potent than vecuronium; but ORG was approx. 2-3X more potent than succinylcholine. Chick preparation was more sensitive than the guinea pig preparation.

(c) Comparison with succinylcholine (SCC)

(1) In vivo:

ORG was shown to be approx. 1/5X (cat) to 10X (monkey) more potent than SCC in *in vivo* studies. At 1X ED_{90} dose, the time profile is similar for both compounds in both cats and monkeys. In monkeys following 3X ED_{90}, however, ORG showed longer duration and slower recovery rate than SCC.

1. Cats:

Neuromuscular Blocking Profiles of Org 9487, Vecuronium and Succinylcholine in Anesthetized Cats.

Compound	Muscle	Dose (mg/kg)	Onset (min)	25-75% Recovery (min)	Duration 90 (min)
Org 9487	Tibialis	0.31±0.027	1.9±0.1	1.5±0.2	5.0±0.2
	Soleus	0.3±0.021	2.3±0.1	2.6±0.3	7.3±0.4
Vecuronium[c]	Tibialis	0.04±0.006	4.3±0.3[a]	3.0±0.2[a]	10.1±0.7[a]
	Soleus	0.038±0.007	5.0±0.2[a]	5.2±0.4[a]	17.4±1.5[a]
Succinylcholine	Tibialis	0.055±0.007	1.6±0.1[b]	1.7±0.1[b]	5.1±0.2[b]
	Soleus	0.18±0.03	2.9±0.5[a,b]	3.4±0.5[b]	10.6±0.9[b]

3X ED_{90} was injected 60 min after the recovery from the first ED_{90} determination to obtain the time profile. Again, 60 min after full recovery from the $3XED_{90}$ study, a second $3XED_{90}$ was injected. One minute after complete block, a mixture of atropine (30 µg/kg) and neostigmine (50 µg/kg) was injected i.v. and the recovery of N-M function was recorded.

The time profiles are copied as follows:

Table: Comparison of the time course of neuromuscular block of Org 9487 and vecuronium (3 x 90%Blocking doses) on the tibialis anterior muscle of the anesthetized cat in the absence and Presence of neostigmine.

	Dose (ug/kg-1)	Onset (min)	Recovery	Recovery 90 (min)	100% Block (min)
Org 9487(b) (Control) n=5	1190±129	0.7±0	2.2±0.2	11.5±1.5	7.6±1.1
Org 9487(b) (neostigmine)	1190±129	0.8±0	1.5±0.1**	6.1±0.4**	3.5±0.2*
Vecuronium (Control) n=5	113±9	1.4±0.1	4.6±0.6	24.7±1.6	15.2±0.7
Vecuronium (neostigmine)	113±9	1.4±0.1	3.6±0.4	14.0±1.1**	6.6±0.6**

* $p<0.05$; ** $p<0.001$. Data are mean ± SEM

Recovery 90% - time difference from one minute after the onset of profound (100%) block (ie when neostigmine is administered in the test groups) to 90% recovery of single twitch tension.

(1) AntiChEs were studied 1 min after complete block by Org. Neostigmine (0.05 mg/kg) reduced the duration of 90%- and 100%-block, 25-75% recovery time and 90% recovery time. Increasing neostigmine to 0.1 mg/kg did not significantly improve the reversal time profile.

(2) Pyridostigmine (0.2 mg/kg) showed similar effects, but to a lesser extent than neostigmine.

(3) Edrophonium (0.2-0.4 mg/kg iv) did not show significant reversal effects.

(4) At higher stimulation frequencies, recovery of the T_4/T_1 ratio appeared to be more rapid following neostigmine than pyridostigmine during the early restoration of neuromuscular function.

(b) *in vitro* data:

Studies with Org 9487, vecuronium and suxamethonium in isolated muscle preparations: determination of neuromuscular blocking potency, fade and reversibility with anticholinesterase agents. Report 070-1082-PH, Vol.1. 24

Preparations:

Guinea pig diaphragm and chick biventer (potency study)

Chick semi-spinalis muscle (affinity constants study)

Guinea pig diaphragm (effects at different stimulation frequencies)
Chick biventer (reversibility study)

Results:

(1) Potency: Refer to (A) Efficacy (b), in vitro preparations, on page 11.

(2) Affinity constants and PA_2: for the affinity for carbachol-receptor of avian muscle, ORG was approx 17X less than that of vecuronium.

(3) Effects on TOF fade and on tetanic block at 50% block of the single twitch responses; (4) Effects on T_4/T_1 ratio; (5) Effects of stimulation at different frequencies; and (6) Reversal of the block; discussed under **mechansim and site of action** section.

(C) Cumulative effects:

The twitch tension of soleus and its time course were studied in anesthetized cats following 7 consecutive doses (250 µg/kg each). The injections were made immediately after 90% twitch recovery from the previous injection.

Results: The blockade and time profile at Injection 1, 2 & 7 are copied from the submission:

Effects of Repeat Dosing on Depth and Time Course of Org 9487-Induced Paralysis in Anesthetized Cats (Mean±SEM)

Dose (mcg/kg)	Injection No.	% Block	Onset (min)	Recovery (min)	Duration (min)
274±10	1	46±2	2.0±0.1	-	5.0±0.3
	2	86±3	2.0±0.1	2.5±0.3	7.6±0.8
	7	89±2	2.3±0.4	2.8±0.4	8.9±0.6

Greater blockade and duration were observed between the first and second dose. From the third to seventh dose no additional effects on the block, onset, recovery and duration were observed.

(D) Mechanism and site of action:

Classified as a non-depolarizing type agent acting at the neuromuscular junction: As evidenced by the reversal by AntiChE, differential effects on muscle contraction following muscle and nerve stimulation, and effects on TOF and tetanic fade.

1. *In vitro* guinea pig nerve-muscle preparation (Report 070-1082-PH, page 13 of this review): to study prejunctional role. At 20% block of single twitch responses, increased stimulation rate caused decreases in frequency-dependent contraction of twitches or tetani. The decreases were significantly greater for vecuronium than ORG.

Effects on T_4/T_1 ratio: Contractions at different stimulation frequencies are shown:

Effect of Org 9487 on the Force of Contraction at Different Stimulation Frequencies in the Guinea-Pig Hemidiaphragm Preparation

Compound	Conc (μM)	% Change in contraction force at the following stimulation frequencies (Hz)						Reduction in 50 Hz Tetanus (%)	Tetanic Fade (%)
		0.1	1	2	3	5	10		
Vehicle		-	+6.8	+7.5	+2.3	-3.5	-14.0	-	-
Org 9487[a]	1.3	-19.6	-29.2	-41.9 *	-50.8 **	-63.4 **	-73.1 **	-52.6	-33.2 **
Vecuronium[a]	0.13	-24.3	-42.1	-60.6	-73.3	-82.0	-90.9	-52.9	-60.9

data presented as the mean of 10-22 observations

*p<0.01 and **p<0.001 significant difference compared to vehicle and vecuronium using the unpaired Student t-test

[a] sufficient drug added to the bath to produce ~20% 0.1 Hz twitch block before the stimulation frequency was increased

Conc = Concentation

Effects on TOF fade and on tetanic block in *in vitro* preparations: TOF fade is significantly more pronounced with vecuronium than ORG in the guinea pig preparation:

Effect of Org 9487 and Vecuronium on TOF Fade and Tetanic Block in Isolated Muscle Preparations

In vitro preparation	Compound	TOF fade	% Tetanic block
Guinea-pig diaphragm	Org 9487	0.22±0.05*	78.0±1.4
	Vecuronium	0.56±0.06	83.6±4.1
Chick biventer	Org 9487	0.19±0.02	62.2±1.2
	Vecuronium	0.23±0.03	65.3±3.5

data presented as mean±SEM

*p<0.01 significant difference compared to vecuronium using the unpaired Student t-test

Effects measured at ~50% block of single twitch responses

Both ORG and vecuronium induced train-of-four and tetanic fade and decreased diaphragm contraction force as the frequency of stimulation was increased. Fade produced by ORG was less marked than that by vecuronium, suggesting a less pronounced prejunctional blocking action or a lower affinity for these receptors.

2. Reversal of the block: Refer to page 12 of this review for *In vivo* data.

In *in vitro* preparations, neostigmine and pyridostigmine reversed only to the extent of 40-66% both ORG- and vecuronium-induced block; edrophonium was a poor reversal agent for ORG (27%). Incomplete reversal in the *in vitro* preparations may have been due to different drug/receptor kinetics and inhibition of antiChE by some N-M blockers.

3. The effect of Org 9487 on the in vitro rat sciatic nerve preparation Report 070-1076-PH, Vol. 1.25

Preparation: *in vitro* sciatic nerve from Sprague-Dawley rats. Compound action potentials (CAPs) were recorded during the 30-min exposure to 1 mM Org 9487 or 1 mM procaine. There were 60 min of control and washout periods between drug testings.

Results:

(1) Org 9487 up to 1 mM showed no effects on the height or duration of nerve CAPs. Procaine (1 mM) produced an 85% reduction in amplitude and a 300% increase in duration of CAPs.

(2)The data showed that Na^+ channels (either in nerve or muscle) did not play role in ORG's blocking effect.

4. Neuromuscular blocking profile of the vecuronium analogue, Org 9487, in the in vitro rat hemidiaphragm preparation. Report 070-1092-PH, Vol. 1.24

Preparation: Standard *in vitro* rat hemidiaphragm preparation

Experimentals: Miniature endplate currents (mepcs) and endplate currents (epcs) were measured from muscle fiber endplates using a conventional two microelectrode voltage-clamp technique.

To study the relative pre- and post junctional effects of Org 9487. The mepcs and epcs (elicited at 50 Hz for 2 sec) were recorded at room temperature and at a single holding potential of -50 mV in the presence and absence of a single concentration of Org 9487 (3 μM producing 30% reduction in the amplitude of the mepcs)

To assess the endplate ion channel blocking activity of Org 9487 in each fiber studied, 10 and 30 epcs were recorded at 32°C at -30 to -70 mV in the absence and presence of 10 μM of Org 9487. The ion channel blocking experiments were performed at 32°C to minimize the effects of repetitive binding of ACh to its postjunctional receptors on the decay phase of epcs. ORG was superinfused (5-15 ml/min) for 5 min. Quantal transmitter release was calculated.

The effect on the AChE from rat brain was determined in the presence of Org 9487.

Results:

(1) Org 9487 (5-100 μM) decreased amplitude of singly evoked twitches (0.1 Hz) and TOF stimulation at 2 Hz, indicating a prejunctional component of action.

(2) ORG inhibited AChE from the rat brain with an approx. IC_{50} of 10^{-4}M.

(3) AntiChEs (neostigmine, edrophonium) only partially reversed the effect of ORG on twitch responses. Possible reason: ORG possesses antiChE activity (shown in rat brain preparation).

(4) ORG (3 μM) increased the rundown of endplate current amplitudes during a 2-sec train of 50 Hz nerve stimulation. This was because ORG increased the quantal content of the first endplate current in the train without affecting ACh release towards the latter part of the train.

(5) ORG (10 μM) produced a voltage-dependent decrease in the time constant of decay of endplate currents at 32°C and 0.5 Hz, indicative of a block of endplate ion channels. The blocking rate constant increased with membrane hyperpolarization.

5. Effect in anesthetized pigs of Org 9487 on skeletal muscle contraction induced by direct stimulation. Report 070-1095-PH, Vol. 1.25
Performed by the sponsor in Feb-Mar., 1995
Animals: cross strain pigs (5/M + F), wt. 10.3-11.9 kg. Anesthetized (azaperone-a tranquilizer; 3-4% halothane for induction, chloralose for maintenance) and mechanically ventilated with room air via a tracheal cannula (28 b/m @ 12-12 ml/kg). BP/HR were monitored.
Experimentals: The anterior tibialis muscles of both hind limbs and their small branches of sciatic nerves were prepared. After full recovery from Org 9487 induced-blockade, vecuronium Br was administered to maintain 100% block. At this point, indirect stimulation of the right tibialis muscle via the motor nerve was discontinued and the muscle was directly stimulated. Stimulation of the left tibialis muscle via the motor nerve was continued to confirm complete curarization of the muscle to indirect stimulation by vecuronium during dosing with Org 9487. The effects of 2X ED_{90} of Org 9487 on the directly-stimulated twitch was measured. Five min later, vecuronium was discontinued and indirect stimulation of the right tibialis anterior muscle was re-established. Recovery of twitches in both tibialis muscles was recorded.
Results:

	Effects on the block (mean±sem)			
N	indirect muscle stimulation		direct muscle stimulation	
	ED_{90} dose (μg/kg)	% block	ED_{90} dose (μg/kg)	% block
6	542±28	91.1±2.0	1084±57	-0.7%±1.7

In pigs, 90% blockade was obtained at 542 μg/kg, whereas in the presence of 1084 μg/kg or 2X ED_{90}, a direct muscle stimulation showed no blockade. The results showed that the site of action is at the neuromuscular junction.

(E) Cardiovascular (CV) effects: Refer to page 3-5 for list of studies

(a) In vivo studies: The CV effects were studied in pithed rats, anesthetized dogs, cats, monkeys and pigs following bolus injections. The vehicle was saline at pH 3. The results are summarized as follows:

1. Pithed rats, ORG 9487 (0.1-3.0 mg/kg) did not modify electrically induced tachycardia. At 10 mg/kg the induced tachycardia was reduced by 9 beats/min. Pancuronium (0.3-3.0 mg/kg) and cocaine (0.5 mg/kg) increased both the magnitude and duration of the induced tachycardia. ORG did not affect on chronotropy, but pancuronium showed positive chronotropic effects. The results suggest that ORG is not likely to cause CV effects by inhibiting reuptake of norepinephrine into sympathetic nerve terminals, which were observed with pancuronium and cocaine.

The potential metabolites, ORG 9488, 9502 and 9504, did not have effects on electrically-induced tachycardia at low doses (0.1-3.0 mg/kg). At the highest dose (10 mg/kg), the induced tachycardia was reduced by 17 beats/min (ORG 9504) and 8-14 beats/min (ORG 9488 and 9502).

2. Anesthetized dogs: Transient effects occurred within the first 5 min and recovered by 10 min. Org 9487 at ED_{90} decreased MAP by 10% and pulmonary artery pressure by 5%. At 3 X ED_{90} , MAP decreased by 20% within 2 min, but returned to control level within 10 min. No other significant effects were observed. The results are copied as follows:

Effect of Org 9487 (3 x 90% Blocking Dose) on Hemodynamics in Anesthetized Beagle Dogs

Time (min)	0	1	2	5	10	20	30
MAP	100	80±2	85±2	91±2	99±2	100±1	101±1
RAP	100	96±7	100±2	100	100	100	100
MPAP	100	94±2	98±1	100	100	100	100
HR	100	93±3	97±2	108±2	110±3	105±1	103±1
PCWP	100	-	91±8	94±5	100	100	100
CO	100	-	110±5	110±6	105±2	106±2	101±4

data presented as mean±SEM for n=5 dogs

MAP - mean arterial pressure, RAP - right atrial pressure, MPAP - mean pulmonary artery pressure, HR - heart rate, PCWP - pulmonary capillary wedge pressure and CO - cardiac output. Values expressed as % of pre-drug control values (=100).

3. Anesthetized cats: Transient effects occurred in 1-5 min and recovered in 8-10 min. BP first increased (14-15%), then decreased (12-19%).

4. Anesthetized pigs:

BP: Within 5-10 min of drug administration, there were decreases of 10 & 20% of control level at ED_{90} and 3X ED_{90}, respectively. HR: no effect.

5. Anesthetized Rhesus monkeys: Monkeys were anesthetized with ketamine HCl and pentobarbital and ventilated with N_2O/O_2. Twitch tension was obtained from adductor pollicis muscle with supramaximal stimulation (0.1 Hz and 0.2 msec duration) of the ulnar nerve via needle electrodes positioned subcutaneously. BP (measured from the forearm, leg or tail using a non-invasive method -Finapres) and HR (from ECG signal) were studied.

Results: No significant changes of BP & HR following ED_{90} and 3X ED_{90} administration.

6. The acute effects on QT-interval of the ECG in anesthetized pigs of intravenous administered high dose Org 9487 and 3-OH Org 9487 (Org 9488). Report 070-1086-PH, Vol. 1. 25

Animals: Young (12-15 wks old) large white strain domestic pigs, anesthetized with halothane and α-chloralose and mechanically ventilated with oxygen via tracheal cannula.

Experimentals: BP, HR and ECG (Lead I, II and III) were measured; fast ECG recordings were made before dosing, at 0.5-10 min after each sub-dose, and at 15-360 min after the third sub-dose. Since QT-interval is influenced by the heart rate, corrected QT (QTc) intervals were computed.

Compounds, dissolved in pH4 phosphate buffer, were given in bolus at 0.5-1.5 mL/kg, at 10X ED_{90}, three times at 10-min intervals to two groups; a third group received vehicle.

Results:

(1) QTc-intervals showed no significant compound-related changes following 3 subdoses of 10X ED_{90} of either Org 9487 or 9488.

(2) Heart rates were slightly increased following the first sub-dose of either Org 9487 (from 110 to 124 b/m) or Org 9488 (115 to 128 b/m), attributed to affinity for the cardiac muscarinic receptors and low vagal tone. Control group showed no changes of HR.

(3) Both QTc-interval and heart rate showed similar decreases following the third sub-dose in all 3 groups.

(b) In vitro studies:

Effects of Org 9487 and Org 9488 on the fast action potential of rabbit papillary muscle. Report 070-1056-PH, Vol. 1. 25. Performed in May, 1994

Experimetals: Right papillary muscles from male fawn rabbits, 1.5-2.5 kg, 5-6 preparations, were superfused and electrically stimulated at 0.5 Hz. Compounds were dissolved in phosphate/citrate buffer and diluted with saline. Infusion: 70 min.

Measurements: Resting membrane potential (RP), action potential (APA), overshoot (Ovs), the maximum rate of depolarization (Vmax) and action potential duration (APD) were measured at 30, 50, 90 and 95% of repolarization, respectively. Decreases in Vmax indicate sodium channel block and lengthening of the action potential (APD) suggests a possible propensity to block repolarizing currents.

Results:

(1) Phosphate/citrate buffer did not cause significant changes in all parameters studied.

(2) Org 9487 decreased Vmax in a concentration-dependent manner (from 3 μM). APD at 50, 90 and 95% repolarization were increased from 0.3-100 μM (concentration-dependent); at 30%, APD was increased at concentration ≥ 1μM.

(3) Org 9488 increased Vmax significantly at 1-30 μM; APD at 30-95% repolarization were

significantly increased at 0.3-100 μM

(4) RP and Ovs were not affected by either agent at any concentration.

(F) Effects on autonomic nervous system:

1. Anesthetized cats, iv administration (data from Report 070-1101-PH).
For Org 9487 the separation ratio of vagolytic vs N-M blocking activity is 2.6 (ED_{50} vagus/tibialis); that of ganglionic blocking vs N-M blocking is > 20. The ED_{50} blocking doses were 0.22 (tibialis), 0.57 (vagus) and >4.49 (nictitating membrane) mg/kg.
For details refer to page 29.

2. Anesthetized cats, im administration: Neuromuscular blocking potency, time course profile and autonomic blocking effects of intramuscularly administered Org 9487 in anesthetized cats; a comparison with succinylcholine. Report 070-1133-PH, Vol. 1. 24
Performed by the sponsor in Scotland, Jan.-Mar., 1996.
Animals: Chloralose/pentobarbital-anesthetized female cats were artificially ventilated with room air; BW: 2-3 kg
Experimentals: Standard set-up
N-M block: sciatic nerve-tibialis anterior muscle preparation; CV: BP/HR
Vagus-nerve induced bradycardia and contractions of the nictitating membrane induced by cervical sympathetic nerve stimulation were recorded.
Dosages and route: After establishing the iv ED_{90}, doses of 2X, 4X and 8X iv ED_{90} were then given im.
Results: iv ED_{90}: 237 μg/kg
(1) N-M profiles of Org and succinylcholine were copied from the submission as follows:

Pharmacodynamic Profiles of Org 9487 and Succinylcholine Administered by the i.m. Route in Anesthetized cats

Dosing/potency/profile data	Org 9487	Succinylcholine
Multiple of the i.v. dose	6x	5x
i.m. Dose (μg/kg)	1714±52*	428±83
% Block	100±0	100±0
Onset Time (min)	3.7±0.3	3.2±0.5
100% Block Duration (min)	3.8±1.5	8.0±1.3
25-75% Recovery Time (min)	4.8±0.8	2.4±0.3
Duration 90 (min)	18.9±1.0	18.3±1.7

data are presented as mean±SEM for n=4 per group; summarized data shown only for cats in which the Org 9487 and succinylcholine produced complete neuromuscular block
potency and time course data on the sciatic nerve/tibialis anterior muscle preparation
*p<0.001 significant difference compared to succinylcholine using the unpaired Student t-test

(2) It required higher doses of i.m. ORG to produce complete block; also ORG more unpredictable or variable because 2/6 cats had less than 20% block. SCC produced

100% (4 cats) and greater than 90% (2 cats) blockade. Rapid onset and short duration were comparable between Org and SCC; but the recovery rate was slower with Org.

(3) Selectivity (im vagal ED_{50}/NM ED_{50}) ratio: 3.4 (Org 9487);

(4) Ganglionic blocking activity was absent at 8X iv ED_{90} (Org 9487)

Succinylcholine increased baseline tension of the nictitating membrane, probably a direct depolarizing action on the smooth muscle of the nictitating membrane.

(5) BP: decreased 6-12% at 2-8X iv ED_{90}. HR: no significant changes

(G) Interactions:

Interactions of ORG 9487 and standard volatile and intravenous anesthetic agents, premedicating agents and streptomycin were studied in cats. Report 2 includes the results from those of the interactions of ORG with 90 min (chronic) inhalation studies of volatile anesthetics in addition to reports of the 10-20 min (acute) interactions study from Report 1

1. & 2. Interactions in cats between the neuromuscular blocking agent Org 9487 and some drugs used in clinical anesthesia. Reports 070-1030-PH, Vol. 1.25 & 070-1087-PH, Vol. 1.26

Animals: Chloralose and pentobarbital anesthetized and ventilated cats.

Experimentals: Twitch tension, TOF, 95%, 75-25% recovery rate of sciatic nerve-anterior tibialis preparation were measured. Voltage at twice that required to produce maximum twitches was used. Two patterns of stimulation were employed: single pulses at 10-sec intervals (0.1 Hz, left leg), and train-of-four (TOF) at 12-sec intervals (four pulses delivered in 2 sec, right leg). Infusion (at 0.35 mg/mL) to a steady state of 50% block as control. Following recovery, a second infusion to 50% block was again maintained and the interaction studies* were conducted as follows (copied from the submission). BP & HR were measured in some cases.

*In Study 2, volatile anesthetics were administered for 90 min to simulate some clinical application. The infusion doses required for maintaining 50% block were determined before and after anesthetic administration.

Results (copied from the submissions):

Effect of Various Drugs on the Depth of Stable Neuromuscular Block Induced by Infusing Org 9487 in Anesthetized cats

Agent	Conc (%)[a] or Total dose (mg/kg)	Placebo		Interaction	
		% Block[b] pre-	% Change	% Block[b] pre-	Δ % Change
Halothane	2[a]	58	1	56	14*
Isoflurane	2[a]	55	-2	53	21*
Enflurane	2[a]	57	2	55	31**
Nitrous Oxide	60[a]	54	-1	56	3*
Thiopentone	10	52	0	51	-4
Midazolam	0.5	57	1	58	3

Ketamine	5	58	1	58	5*
Droperidol	0.35	54	1	58	2
Etomidate	0.6	61	1	57	3
Propofol	5	57	1	61	2
Fentanyl	0.01	51	1	46	7*
Morphine	2	58	1	62	8*
Diazepam	2	55	3	53	5
Chlorpromazine	5	59	2	58	2
Streptomycin	5	54	1	55	15**
Vancomycin	10	60	3	59	3*
Cefuroxime	20	56	4*	58	5*
Phenytoin	10	55	2	60	8***

[a]data presented as mean % block and mean % change for 5-6 cats per group

*p<0.05, **p<0.005 ***p<0.001 significant difference compared to % block values prior to administration of placebo or interacting substance using the Student paired t-test

[b]% block determined in the sciatic nerve/tibialis anterior muscle preparation

Table 23 **Effect of Various Drugs on 50-90% Recovery Time Following the Infusion of Org 9487 in Anesthetized Cats**

Interacting agent	n	50-90% recovery time (min)[a]			
		Placebo	Interaction	Change	Δ% Change
Halothane	5	2.3	2.0	-0.3	-11
Isoflurane	4	1.5	1.8	0.3	19
Enflurane	4	1.8	3.4*	1.5	101
Nitrous Oxide	5	1.7	1.6	-0.1	+0.6
Thiopentone	1	2.8	1.5	-1.3	-47
Midazolam	4	1.8	2.3	0.5	14
Ketamine	6	1.5	1.8	0.3	+41
Droperidol	4	1.0	1.0	0	-6
Etomidate	5	1.4	1.3	-0.1	-1
Propofol	4	1.8	2.2	0.4	+16
Fentanyl	4	2.0	1.9	-0.1	-9
Morphine	4	1.8	2.0	0.2	14
Diazepam	3	0.9	1.2	0.3	20
Chlorpromazine	4	1.4	1.5	0.1	+7
Streptomycin	4	1.1	2.5	1.4	87
Vancomycin	5	1.1	1.0	-0.1	-2
Cefuroxime	5	1.4	1.6	0.2	+4
Phenytoin	5	0.9	1.1	0.2	+15

50-90% recovery times determined in the sciatic nerve/tibialis anterior muscle preparation

*p<0.05 significant difference compared to placebo 50-90% recovery time using the Student paired t-test

[a]data presented as mean of 3-6 observations, with the exception of thiopentone (n=1)

Infusion Doses of Org 9487 to Maintain Stable Neuromuscular Block, Before, During and After Prolonged Exposure to Volatile Anesthetics

Agent	Infusion Dose (mg/kg/hr)		
	Pre-interaction[a]	Interaction[b]	Post-interaction[c]
Halothane	2.21±0.35	1.26±0.17*	2.11±0.43
Enflurane	2.41±0.41	1.01±0.14**	2.09±0.32
Isoflurane	1.82±0.23	1.00±0.15*	1.83±0.27

data presented as mean±SEM from n=4-5 experiments

the mean exposure times to the 3 anesthetics were, halothane 100±4 min, enflurane 101±14 min and isoflurane 101±3 min

*p<0.01, **p<0.001 significant difference compared to pre-interaction infusion rate using the Student paired t-test

[a] before, [b] during and [c] after prolonged exposure to halothane, enflurane and isoflurane

The mean infusion dose of Org 9487 was 2.12 (1.15-3.79) mg/kg/hr; the mean steady-state blocks were 58% (stimulated at 0.1 Hz) and 78% (TOF).

(1) Volatile anesthetics: Acute (10 min) exposure potentiated the block and prolonged recovery; enflurane was most potent. Exposure (90 min) to volatile inhalation anesthetic, halothane, enflurane and isoflurane, 2% each, reduced the steady state infusion doses of Org 9487 by 43%, 58% and 46%, respectively. Enflurane increased the recovery time. BP and HR were decreased.

(2) N_2O/O_2 (60%/40%) administered for 10 min increased blockade (3%), but the rate of recovery was not affected.

(3) Ketamine (5 mg/kg) administered over 2 min increased both the blockade and recovery rate. Morphine slightly potentiated the block

(4) Streptomycin potentiated the block by 10-15% and slowed the recovery. Vancomycin and cefuroxime administered slightly increased the blockade.

(5) Phenytoin increased blockade (8%) and the recovery rate.

(6) Droperidol, etomidate, propofol, thiopental, midazolam, chlorpromazine and diazepam had no significant effects on the block and recovery.

(H) Effects of Acidosis and Alkalosis on N-M blockade:

(a) *In vivo* studies:

1. The effects of respiratory and metabolic acid-base changes on the neuromuscular and cardiovascular effects of Org 9487: studies in anesthetized pigs and in the isolated guinea-pig diaphragm. Report 070-1033-PH Vol. 1.25

Animals: Anesthetized large white strain domestic pigs (11.5-16.3 kg, M & F) were used with the standard set-up.

Results:

Depth of Neuromuscular Block and Pharmacodynamic Profile of Org 9487 in the Pig, Before (pre) and During Experimentally-Induced Respiratory and Metabolic Acidosis and Alkalosis.

Acid-base Status		Block (%)	Onset (min)	Duration 90 (min)	50-75% rec (min)	50-90% rec (min)
Respiratory Acidosis	pre	79.3	1.4±0.1	9.3±0.9	2.5±0.3	5.5±0.5
	during	84.5	1.9±0.1 **	15.4±0.8 ***	3.9±0.2 **	9.4±0.9 **
Respiratory Alkalosis	pre	76.9	1.6±0.1	9.8±2.5	2.7±0.1	6.0±0.4
	during	70.4	1.4±0.1 **	6.9±0.3 **	1.8±0.1 ***	4.3±0.2 **
Metabolic Acidosis	pre	73.0	1.4±0.1	8.8±0.7	2.4±0.3	5.7±0.5
	during	80.9	1.6±0.1	14.1±2.1 *	4.0±0.6 *	9.1±1.6
Metabolic Alkalosis	pre	75.5	1.3±0.1	8.9±1.1	2.2±0.3	5.6±0.9
	during	83.9	1.2±0.1	11.9±1.8	2.8±0.4	8.2±1.6

data presented as mean±SEM for n=6 pigs per group

neuromuscular profile data obtained in the sciatic nerve/tibialis anterior muscle preparation using ~75-80% blocking doses of Org 9487

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ significant difference compared to pre-respiratory and metabolic acidosis and alkalosis values using the paired Student t-test

(1) Respiratory acidosis: Plasma PCO_2 inc. from 43 mm Hg to 84 mm Hg, dec pH by 0.27 unit. These were accompanied by small statistically significant decreases in PO_2, bicarbonate and base excess. N-M block was increased by 5.3% and the onset, recovery and duration were all increased significantly.

(2) Metabolic acidosis: dec. plasma bicarbonate and base excess; pH and PCO_2 changes were less marked than respiratory acidosis. The block was increased by 8%; the recovery and duration were prolonged but there was no effect on onset.

(3) Respiratory alkalosis: inc. plasma pH by 0.24 unit and PCO_2 from 45 to 22 mm Hg. No changes occurred in plasma bicarbonate values. N-M block was decreased by 6%; and the onset, recovery and duration were shortened.

(4) Metabolic alkalosis: increased plasma pH by 0.21 unit, bicarbonate (12 mM) and base excess (11.3 mM). These were accompanied by small decreases in PCO_2 and PO_2. The block was increased by 8% ; recovery and duration were slightly increased, but the onset was not affected.

When the effects were re-evaluated 60-75 min after resuming normal ventilation and termination of infusion, partial or full recovery of time course was found in respiratory acidosis and alkalosis and metabolic acidosis. The effects of metabolic alkalosis were less readily reversed.

2. Changes in the time course profile of Org 9487 during experimentally-induced abnormal

pH and acid-base balance in anesthetized pigs following early administration of the reversal agent, neostigmine. Report 070-1168-PH, Vol. 1.25

Performed by the sponsor

Animals: In domestic pigs 9.6-15.5 kg, sciatic nerve-tibialis anterior muscle preparation was studied under experimentally-induced abnormal acid-base conditions.

Results:

(1) Respiratory and metabolic acidosis slowed N-M recovery and prolonged the blocking action of ORG, whereas respiratory alkalosis hastened recovery and shortened N-M block. Metabolic alkalosis did not have significant effect.

(2) Neostigmine, immediately preceded by atropine, shortened the recovery time under respiratory and metabolic acidosis.

(3) Respiratory alkalosis also shortened the recovery time, but metabolic alkalosis had no effect. Early administration of neostigmine further shortened the recovery.

(b) *In vitro* studies:

The potency of ORG 9487 was tested in standard phrenic nerve-hemidiaphragm preparations from guinea pigs using a cumulative dose-response method. Acidosis (pH 7.2) and alkalosis (pH 7.7) were induced by adding an appropriate volume of HCl or N_aHCO_3 to the bathing fluid. Alternatively, the pH was altered by increasing (with 7.5% CO_2/O_2) or decreasing (with 2.5% CO_2/O_2) the CO_2 tension of the bathing fluid.

Results

(1) The IC_{50} values are 3.4 X 10^{-6} M (ORG9487) and 2.2 X 10^{-7} M (vecuronium).

(2) In the absence of a N-M blocker, acidosis (HCl or 7.5%CO_2) decreased the twitch tension slightly, whereas alkalosis (2.5% CO_2) potentiated contractions. Bicarbonate did not have significant effects.

(3) Acidosis (respiratory and metabolic) significantly potentiated the block as evidenced by a reduction of the twitch tension in the presence of either ORG (19-20%) or vecuronium (12%). Conversely, both respiratory and metabolic alkalosis antagonized the block by increasing the twitch tension: ORG (13-17%) and vecuronium (9-15%).

(l) Inhibition of Cholinesterases:

Inhibition of human plasma butyrylcholinesterase and erythrocyte acetylcholinesterase by new muscle relaxants Org 9453, Org 9487 and Org 9489 in vitro. Report 070-1039, Vol. 1.25

In vitro assays used human plasma for butyrylcholinesterase (substrate: benzoylcholine) and human RBC for AChE activity. The activity of the enzyme was measured spectrophotometrically by the decrease of benzolycholine.

Results:

Inhibitory IC_{50} values were

pseudoChE: 8.1 X 10^{-7}M (ORG), 6.0 X 10^{-7}M (vecuronium); 2.2 X 10^{-7} M (pancuronium);

AchE : 5.4 X 10^{-6}M (ORG), 2.0 X 10^{-5}M (vecuronium); 5.2 X 10^{-5}M (pancuronium).

Both ORG and vecuronium are potent pseudoChE inhibitors (ORG 3-4X more potent than pancuronium). ORG also inhibited AChE (ORG 5-10X more potent than vecuronium and pancuronium) but was less potent against AChE than pseudoChE.

(J) Roles of kidneys (renal pedicle ligation) and liver (hepatic vessels excluded and intraportal injection):

1. The effect in the cat of bilateral renal pedicle ligation on the pharmacokinetics and pharmacodynamics of the neuromuscular blocking drug, Org 9487. Report 070-1136-PH, Vol. 1.24

Animals: Cats, 2-4 kg, were anesthetized (α-chloralose and pentobarbital) and ventilated with oxygen via tracheotomy. BP/HR, twitches from both legs [left:single pulses at 12s intervals, 0.1 Hz, and right: train of four (4 pulses delivered in 2 s) applied at 12 s intervals] were monitored

Experimentals:

Protocol A: ED_{90} was determined in each of the 10 cats (5/gr); 3 hrs later ligation or sham ligation (urine was collected from urinary bladder) of the renal pedicles was carried out. Blood samples for drug and metabolite levels were collected before and after drug administration (3 X ED_{90}). Urine from sham ligation control was collected every half hr for 4 hrs.

Protocol B: ED_{90} determined as under Protocol A. The cats received ED_{90} as a bolus followed by an infusion at a rate maintaining T1 twitch of the TOF at 10%-15% of control height. Blood samples were obtained from each of 3 ligated and 3 sham ligated cats before, and at 10 min intervals during the steady state infusion. When the steady state block was maintained for 30 min, the renal vessels were ligated in 5 cats and sham-ligated in the other 5 cats. The infusion rate was adjusted to maintain the depth of block at the pre-ligation level. Blood samples were again obtained at 10-min intervals for 30 min and following termination of the infusion.

Results: copied from the submission.

Protocol A. Pharmacodynamic time course profile of Org 9487 administered at 3 x the 90% blocking dose in control (sham ligated) and renal pedicle ligated chloralose-anaesthetized cats. The tibialis anterior muscle was stimulated indirectly with a TOF burst every 12 s.

Experimental conditions	Dose ($\mu g.\ kg^{-1}$)	Onset time (min)	Duration of 100% block (min)	Recovery$_{25\text{-}75\%}$ time (min)	Duration$_{25\%}$ time (min)	Duration$_{90\%}$ time (min)
Control (sham ligated)	1092±106	0.60±0	5.96±0.47	3.12±0.31	8.00±0.56	12.92±0.91
Renal pedicle ligated	1132±141	0.55±0.04	6.75±0.25	2.85±0.30	8.06±0.49	13.3±0.43

Values expressed as mean±s.e.m. n=5 per group. No statistically significant differences between sham ligated controls and renal pedicle ligated cats.

Summary of pharmacokinetic variables for Org 9487 (3 x the 90% blocking dose) administered by bolus injection in sham ligated (Controls) and renal pedicle ligated (Ligation) cats. (n=6 per group).

		CONTROLS mean	sd	cv (%)
C1	µg/l	13747	1484	11
L1	/min	0.413	0.069	17
C2	µg/l	1060	517	49
L2	/min	0.0660	0.0063	13
t½β	min	10.5	1.4	14
CL	l/min/kg	0.0222	0.0033	15
V1	l/kg	0.074	0.011	15
Vss	l/kg	0.140	0.019	13
MRT	min	6.4	1.6	24

	CONTROLS mean	sd	cv (%)
keo	0.664	0.078	14
EC_{50}	2311	397	17
Gamma (Ce)	5.64	1.47	26
ED/EC	0.186	0.016	10
ED_{90}	577	112	19

		LIGATION mean	sd	cv (%)
C1	µg/l	16787	2496	15
L1	/min	0.334	0.071	21
C2	µg/l	1101	431	39
L2	/min	0.0498	0.0085	17
t½β	min	14.3	2.8	20
CL	l/min/kg	0.0153	0.0023	15
V1	l/kg	0.063	0.012	19
Vss	l/kg	0.125	0.022	18
MRT	min	8.3	1.8	22

	LIGATION mean	sd	cv (%)
keo	0.544	0.201	37
EC_{50}	2701	748	28
Gamma (Ce)	5.26	0.97	18
ED/EC	0.139	0.029	21
ED_{90}	562	131	23

(1) Following a bolus of 3X ED_{90}, no significant changes of blockade, time profiles of the renal-ligated and non-ligated cats occurred. Plasma Cl of Org 9487 decreased 30% and slight increase of recovery in the ligated animals. PK/PD analysis, however, revealed no significant differences in keq, and EC_{50} in 2 groups. In non-ligated cats, 6-9% of the administered dose was found in the urine.

(2) During infusion, renal ligation reduced the dose needed to maintain steady state train of four N-M block, but did not affect the rate of recovery. No differences in PKs were observed between the 2 groups.

(3) Renal exclusion did not have significant effects on the PD and PK of Org 9487.

2. Org 9487 in the portacaval shunt mode in the cat. **Report 070-1044-PH, Vol. 1.24**

Animals: Male cats (6) with portacaval shunt (bypassing the liver circulation) and intraportal injection were used. N-M blockade and time profiles were determined following ED_{90} and 1.5XED_{90}. Each dose was administered at least 45 min after recovery from the preceding dose. Performed by the sponsor.

Protocol A (n = 3):

(1) ED_{90} i.v. normal circulation (shunt closed)

(2) ED_{90} i.v with liver bypass (shunt open) normal circulation restored after 10 min

(3) ED_{90} intraportally with normal circulation

(4) ED_{90} identical to (1)

Protocol B (n = 3)

(1) 1.5X ED_{90} i.v. with normal circulation

(2) 1.5X ED_{90} i.v. with liver bypass, normal circulation after 10 min

(3) 1.5X ED_{90} identical to (1)

Results:

Table 10: Hepatic Effects on Neuromuscular Blocking Activity of Org 9487 in Anesthetized Cats

	Dose Number (ED_{90})			
Parameter	1 control i.v.	2 bypass i.v.	3 control intraportal	4 control i.v.
Maximum block (%)	90	98[a]	86	91
Onset time (min)	1.43	1.57	1.67[a]	1.52
Duration 25% (min)	2.27	3.33[a]	2.29	2.52
Duration 90% (min)	4.10	5.71[ab]	4.14	4.43
Recovery index (min)	1.16	1.52[a]	1.27	1.31

[a] Significantly different from dose 1 ($p<0.05$)
[b] Significantly different from dose 4 ($p<0.05$)

Liver bypass or intraportal administration affected the block and time profile. Protocol B: Similar results as A above. The report stated that the plasma levels at onset and recovery were higher in the liver bypass condition, but Vc and Cl were not significantly affected.

(K) Receptors activities:

Screening of the nondepolarizing neuromuscular blocking agents vecuronium bromide, Org 9487 and Org 9488 in selected receptor binding assays. Report 070-1080-PH, Vol. 1.25 performed by [redacted] June-July, 1994

Animals/receptors/tissues and concentrations: mostly from rat cortex, forebrain, cerebellum, spinal cord, and liver; one from bovine cerebellum. Receptors included adenosine,histamine, serotonin, nicotinic, NMDA, AMPA, glutamate (non selective), glycine, benzodiazepine (central), PCP, opiate, TRH, insulin, calcium channel typ N (w-conotoxin), nitrendipine (Ca channel type T & L), potassium, apamin (K^+ channel, low conductance-Ca^{2+}), sodium site 2, GABA uptake and norepinephrine uptake. Concentrations at 10^{-9}, 10^{-7} & 10^{-5} M were tested

Results:

(1) Org 9487 and Org 9488 did not inhibit specific ligand binding in various receptors/tissues except at the apamin receptor controlling low conductance potassium channels (70% inhibition at 10^{-5}M).

(2) Vecuronium had no significant effects on any of these receptors.

(L) Hormonal activities:

Effects of ORG 9487 in the Screening Test for Hormonal Activities in Immature Male and Female Rats. Report 070-1041-PH, Vol. 1. 25

Animals: Immature male and female rats, ORGA, 22-24 days old, BW 62-82 g,

Experimentals: Animals were treated with daily sc dose of 87.5 or 175 µg/rat (about 1.22 and 2.45 mg/kg) for 7 days; placebo group received vehicle. On day 8 animals were killed with CO_2 gas and various organs were weighed. The N-M blocking ED_{50} and ED_{90} in rats are approximately 2 mg/kg and 3.86 mg/kg iv.

Results:

Males: statistical decreases of adrenal wt at high dose and increases of pituitary gland and seminal vesicles only at the low dose. Females: statistical wt. increases of thyroid (low and high doses) and spleen (high dose). Based on the results of this screen, the sponsor concluded that ORG did not appear to show significant estrogenic, androgenic, anabolic, gonad-inhibiting, or glucocorticoid-like activities.

(M) Smooth muscle relaxant property:

In vitro relaxant activity of Org 9487 on the human internal mammary artery preparation: a comparison with vecuronium, pancuronium and the calcium channel blocking agent verapamil. Report 070-1198-PH, Vol 1. 26

Performed by _ Report dated May 25, 1998

Preparation: isolated human internal mammary artery (IMA)

EC_{50}: concentration producing 50% of maximum relaxation

Emax: maximal relaxation values

Results:

Verapamil and ORG relaxed both thromboxane-A2-induced and KCl-induced contraction whereas vecuronium and pancuronium only relaxed the former. The data suggest that at high concentrations ORG can inhibit both receptor and voltage activated Ca^{2+} channels to produce relaxation. Vecuronium and pancuronium blocked only the receptor activated Ca^{2+} channels. The effective concentrations were 6-14X higher than human peak plasma concentration of 8-20 µg/mL

(N) Potential metabolites of Org 9487; N-M blocking, CVS and autonomic nervous effects:

(a) In vivo; studied in cats and pigs: Three hydrolytic products are potential metabolites.

1. Anesthetized cats:

Neuromuscular and autonomic blocking activity and cardiovascular effects of the known impurities and degradation products of Org 9487 in anesthetized cats. Report 070-1101-PH, Vol. 1.24

Experimentals: Standard set-up (Refer to cat autonomic nervous system study for details).

Potential metabolites tested: ORG 9488 (3-hydroxy, 17-propionate derivative), ORG 9502 (3-acetoxy, 17-hydroxy derivative), and ORG 9504 (3, 17-dihydroxy derivative).

Animals: Anesthetized (chloralose and pentobarbital) cats (M & F) were ventilated with room air. BP & HR were monitored.

Results: Effects on the block, vagal and ganglion blocking activities and time profiles are shown as follows:

Neuromuscular, Vagal and Ganglion Blocking Potencies of Org 9487 and its Potential Metabolites in Anesthetized Cats

Compound	50% Blocking Doses (mg/kg)				Ratio
	Tibialis	Soleus	Vagus	Nic Mem[a]	Vagus/Tibialis[b]
Org 9487	0.22±0.024	0.20±0.017	0.57±0.003	>4.49	2.6
3-OH Org 9487	0.35±0.034	0.27±0.053	0.18±0.035	>2.34	0.5
17-OH Org 9487	0.47±0.16	0.54±0.17	0.40±0.13	>1.67	0.9
3, 17 -diOH Org 9487	0.93±0.061	1.12±0.14	0.35±0.15	>3.70	0.4

[a] dose producing 50% block of nictitating membrane contractions

[b] 50% vagal blocking dose/50% tibialis anterior blocking dose ratio

Pharmacodynamic Blocking Profiles of Org 9487 and its Potential Metabolites in Anesthetized Cats

Compound	Muscle	n	90% Blocking Dose (mg/kg)	Onset (min)	25-75% Recovery (min)	Duration 90 (min)
Org 9487	Tibialis	4	0.29±0.025	1.8±0.1	1.5±0.2	4.9±0.3
	Soleus		0.32±0.032	2.3±0.1	2.2±0.3	6.8±0.4
3-OH Org 9487	Tibialis	5	0.44±0.024	2.3±0.1	3.1±0.4	7.9±1.0
	Soleus		0.38±0.023	2.9±0.1	4.9±0.3	12.9±1.1
17-OH Org 9487	Tibialis	3	0.72±0.25	1.4±0.2	1.3±0.2	4.7±0.6
	Soleus		0.82±0.19	2.1±0.2	3.0±0.3	7.2±0.3
3,17- diOH Org 9487	Tibialis	3	1.59±0.17	1.6±0.2	2.6±0.3	7.3±1.0
	Soleus		1.98±0.29	2.4±0.7	5.6±1.5	15.3±3.4

data are presented as mean±SEM for n=3-5 cats per group

The 3-OH, 17-OH and 3,17-di-OH metabolites were approx. 2/3, ½ and 1/4 as potent as the parent compound, respectively. The time profiles varied with metabolites: increased duration and recovery in all, except for 17-OH, which had similar profiles as the parent compound. Separation ratio of vagolytic/N-M blockade ED_{50} was less than 1 for all metabolites whereas the parent compound had a ratio of approximately 3.

2. Anesthetized pigs:

Infusion and bolus data: Pharmacodynamics in anesthetized pigs of Org 9487 and its metabolites following single bolus dosing and I.V. infusion. Report 070-1137-PH, Vol. 1.24

Animals: Domestic pigs (M & F), 12-15 wks old, BW 12-14 kg, anesthetized and ventilated with room air.

Experimentals:

N-M block: Sciatic nerve-tibialis anterior muscle were studied in both legs (left: single pulses at 10 s intervals, 0.1 Hz; right: train-of-four, 4 pulses delivered in 2 s, applied every 12 s). BP/HR monitoring: standard set-up

Compounds and administration: Org 9487, 3-OH Org 9487, 3, 17-diOH Org9487, all batch b, at 5 mg/ml. Infusion rate was adjusted to maintain a constant block of approx 55% for 75 min. The recovery time profile (50-90%) was studied after termination of infusion. Bolus data were also obtained.

Results (copied from the submission):

Infusion data:

Rates of Infusion, Total Administered Doses and 50-90% Recovery Time of Org 9487 and its Potential Metabolites Following 75 Minute Infusions in Anesthetized Pigs

Compound	Block (%)	25-75% Recovery (min)	Infusion dose (mg/kg/min) 1	2	Total infusion dose (mg/kg)
Org 9487	60±3	11.0±0.8 (5)	0.039±0.004	0.029±0.003	3123±196
3-OH Org 9487	67±7	15.2±0.9 (3)	0.015±0.003	0.010±0.001	1289±226
3,17-diOH Org 9487	59±5	14.3±0.4 (2)	0.055±0.002	0.055±0.004	4294±192

1. Infusion dose in the early part of the infusion after achieving steady-state block.
2. Infusion dose immediately prior to stopping the infusion.
( ) number of experiments.

Potency data (in vivo):

50% neuromuscular blocking doses of Org 9487 and its 3-OH and 3,17-diOH metabolites in the sciatic nerve/tibialis anterior muscle preparation in the anaesthetised pig.

Compound	0.1 Hz	TOF
Org 9487	198±5	134±6
3-OH Org 9487	89±19	47±6
3,17 diOH Org 9487	482±31	330±8

Doses µg/kg

Results expressed as the mean±sem

Bolus data:

Potency and neuromuscular blocking time course profile of Org 9487 and its -OH and 3,17 diOH-metabolites in the chloralose-anaesthetized pig.

Compound	n	90% mean Blocking dose (µg.kg)	%block	onset time (min)	recovery time (min)	recovery time (min)	dura time (min
Org 9487	7	382±8	89±2	1.6±0.1	3.6±0.1	4.7±0.4	9.5±0
3-OH Org 9487	5	166±24	87±1	2.1±0.2	6.5±0.9	8.8±1.8	15.9±
3,17-diOH Org 9487	3	866±77	87±3	1.8±0.3	4.9±0.6	6.0±0.9	11.6±

The mean infusion dose required to maintain a steady depth of block was less toward the end of the infusion compared to the start, as follows: by approx. 25% (the parent compound), 36% (3-OH); and no change (3,17-diOH) suggesting a possible cumulative effect.

The 3-OH and 3,17-di-OH metabolites were approx. 2X and 1/2X as potent as the parent compound. The parent and 3,17-diOH had rapid onset (1.6-1.8 min) and short duration (9.5-11.6 min); 3-OH had slower onset (2.1 min) and longer duration (15.9 min).

In anesthetized pigs, the 3-OH metabolite was approx. 2X as potent as the parent compound following either bolus or infusion administration; its recovery rate and duration were also longer. The *in vitro* data (ED_{50}), however, showed approx equal potency. The study suggested PK may contribute to the difference in potency *in vivo*. By contrast, 3,17 di-OH is approx. half as potent as the parent compound. Following infusion of Org 9487, the 50-90% recovery time was 11 min compared to approx. 5 min following bolus injection. Both metabolites also showed longer recovery time following infusion (approx. 15 min vs 6-9 min).

(b) In vitro (guinea pig nerve muscle preparation):

In vitro guinea pig diaphragm preparation:

Potency: as EC_{50} & EC_{90} µM (n = 4-6)

Compound	EC_{50} µM	EC_{90} µM
Org 9487	1.81±0.05	3.73±0.18
3-OH	2.00±0.14	3.46±0.61
3,17-di-OH	13.2±0.06	19.4±0.05

(c) Cardiovascular and autonomic blocking effects in cats:

Effect of Org 9487 and its Potential Metabolites on Arterial Pressure in Anesthetized Cats

Compound	Mean arterial pressure (mmHg)		
	Pre-drug	Post-drug	% Change
Org 9487	73±2	84±4/64±4	+14±5/-12±4
3-OH Org 9487 (F)	67±3	81±3/45±3/90±6	+21±3/-33±6/+29±1
17-OH Org 9487 (E)	83±17	109±22/75±18	+32±4/-11±4
3, 17-diOH Org 9487 (G)	85± 21	126±31/56±14	+48±13/-16±0

data presented as mean±SEM for n=3-5 cats per group

Mean arterial blood pressure was increased 20-40% by metabolites and 14% by the parent compound.

Impurities: N-M and autonomic blocking effects in cats (copied from the submission):

Neuromuscular and Autonomic Blocking Effects of Impurities A, B, C, D and H in Anesthetized Cats

Impurity	n	50% blocking dose (mg/kg)			Time course profile (min)		
		NM (tibialis anterior)	Vagus	Ganglion	Onset	Recovery	Duration 90
A (Org 21616)	1	>10	>10	>10	-	-	-
B (Org 21621)	2	0.059	0.14	2.4	3.5	3.8	12.2
C (Org ND 24)	2	0.040	1.1	>3.0	4.1	2.6	10.1
D (Org 9486)	3	0.30	1.7	>5.9	1.7	1.9	5.4
H (Org 21607)	2	0.25	2.0	>2.8	1.4	1.1	4.1
Org 9487	5	0.22	0.57	>4.6	1.8	1.5	4.9

data are presented as mean for n=2-5 cats per group, except for impurity A (n=1)
potency and profile data in the sciatic nerve/tibialis anterior muscle preparation

II. TOXICOLOGY:

(A) Acute toxicity:

1. An acute intravenous toxicity study in the dog with a neuromuscular blocking agent, ORG 9487, Report 070-1022-TX, Vol. 1.26

Performed by July 10-24, 1990 (staggering dosings).

Animals: Beagle dogs, mean BW: 10.3 kg (M), 9.4 kg (F),

Treatment and duration: One day of dosing (1-3 subdoses at 30-min intervals), followed by a 2-wk recovery.

Dosage levels: (1.35-2.7 ml/kg, 1 dose; 0.68 ml/kg, 3 doses).

1M: one dose at 13.5 mg/kg

1F: two sub-doses 13.5 & 27.0 mg/kg

1M/1F: three sub-doses at 6.75 mg/kg

Experimentals: Halothane-anesthetized and ventilated dogs

Parameters studied: Ophthalmology, indirect BP and urinalyses: pretest and at termination Clinical sings, ECG, BW, FC, hematology & clinical chemistry: pretest, during the treatment and recovery period. Direct BP: on the day of dosing via a catheter placed in the femoral artery. At the end of a 2-wk recovery: organ weight (ratios with body weight and brain weight), gross and histopathology on selected tissues.

Results:

Mortality: None

Clinical signs: N-M blockade of 166-202 min (13.5/27 mg/kg-1 dose) and 79-99 min (3 X 6.75 mg/kg). No other significant signs.

BW/FC: No significant effects

ECG: Measured at 10-min intervals following dosing and at 30-min intervals during the relaxation period, at the end of 2-wk recovery and termination. Drug related ECG changes were seen in only the one F receiving a total dose of 40.5 mg/kg. Prolonged QT interval, sinus arrhythmia with prolonged PR, P widening and some P and T complexes were observed at 150-210 min following the second sub-dose. AV dissociation with acrochage was observed at 240-270 min. The sponsor attributed these changes to marked and persistent hypotension induced by the dose. At end of the 2-wk recovery, the ECG returned to normal but heart rate was increased (200B/min).

BP: Measurement schedules were the same for the ECG. Halothane caused hypotension in all animals. Dose-related decreases of BP were also observed. In the first M receiving a dose of 13.5 mg/kg, the BP dropped to 30/5 mmHg; no further injection was given to this animal. In the first F given 2 subdoses of 20.25 mg/kg at 30-min interval (total of 40.5 mg/kg), BP was decreased markedly following each injection and remained low for 270 min, but gradually recovered to pre-dose level. In animals receiving 3 subdoses of 6.75 mg/kg (total of 20.25 mg/kg), decreased BP was observed following each injection. BP returned to pretest levels in 30 min (M) and 60 min (F) following the last injection.

Ophthalmology and hematology: Unremarkable.

Serum Chemistry: The following changes were observed on the day following dosing and

returned to normal at the end of 2-wk recovery (exception: In the F receiving the highest dose of 40.5 mg/kg, SGPT remained elevated at the termination).

SGOT and SGPT: Slight increases in 2 M and 1 F (40.5 mg/kg). The F's SGPT remained elevated at the termination of study.

Creatinine phosphokinase (CPK): Increases were observed in all treated M and the F receiving the highest dose (40.5 mg/kg). Increased CPK of muscle origin has been observed for other N-M blockers.

Alkaline phosphatase: increased above pre-dosing level in all treated M & F and controls.

Urinalysis and organ weight: Unremarkable.

Pathology; gross and histopathology.

Liver (vacuolation of hepatocytes) appeared in most animals, which was attributed to halothane by the sponsor; however, drug-related effects can not be ruled out (↑SGPT & SGOT in dosed animals).

Lungs: Acute or chronic interstitial/granulomatous inflammation in most treated dogs.

Injection sites: Fibrosis, inflammation, squamous hyperplasia or hemorrhage appeared in most dogs; these reactions appeared to be enhanced by the dosage.

Based on this study, the maximum tolerated dose was 3X 6.5 mg/kg/day in dogs.

2. An acute toxicity study in dogs with ORG 9487 via intravenous injection in five sub-doses. Report 070-1003TX, Vol.1. 27

*Satellite groups were treated identically for a PK study (see under ADME/PK).

Performed by [redacted] May 6-June 29, 1993 (staggering dosings).

Animals: Beagle dogs, mean BW: 10.6 kg (M), 10.0 kg (F), 6-7 months old, [redacted] **Treatment route and duration:** iv bolus via femoral vein; one day of dosing (5 subdoses at 30-min intervals), followed by a 2-wk recovery.

Dosage levels: 5X 0.0 (placebo); 5X 0.6; 5X 1.9 and 5X 6.0 mg/kg : halothane/oxygen-nitrous oxide (1:1) anesthetized and ventilated dogs; control animals were anesthetized and restrained as that of the high dose group. Set-up similar to Study 1 above. **Parameters studied:** same as Study 1 above. Histopathology included wider range of tissues & organs. Anesthesia was monitored; N-M function assessed by twitch response to stimulation and spontaneous respiration.

Results:

Mortality: None

Clinical signs:

N-M blockade: dose-related duration, i.e., 1-20 min (low), 40-50 min (mid), 90-200 min (high). Spontaneous breathing, also dose-related, could be maintained approximately 1-4 hrs after the fifth dose. No other significant signs.

BW/FC: No significant effects

ECG: measurements were the same as study 1 above. No significant findings.

BP: Measurements were the same as Study 1 above. Dose-related transient hypotension

was observed: mid dose (dec. of 15-26 mmHG systolic & 10-20 mmHG diastolic), high dose (dec. of 20-40 mmHg systolic & 17-28 mmHG diastolic). Mid dose: BP recovered to control level before each sub-dose, whereas high dose recovered by the end of the relaxation period.

Hematology, serum chemistry, ophthalmology, urinalysis and organ weight: no significant treatment effects.

Pathology:

Gross: Thickening, mass and discoloration in the incision site for femoral artery in control and dosed dogs-were thought due to mechanic trauma.

Histopathology:

Injection site: chronic active inflammation, fibrin, necrosis, edema, granulation tissue granulomatous inflammation (around suture material) in control and dosed animals. No other drug-related effects were apparent.

3. An acute intravenous toxicity study in the cat with a neuromuscular blocking agent, ORG 9487

Report 070-1021-TX, Vol. 1. 28

Performed by _____ July 12-25, 1990 (staggering dosings) to establish the highest tolerated dose in cats for main study.

Animals: domestic short-hair cats, mean BW: 4.3 kg (M), 3.7 kg (F), Halothane-anesthetized and ventilated cats.

Treatment and duration: One day of dosing (1-3 subdoses at 30-min intervals), followed by a 2-wk recovery.

Dosage levels: (1.3-2.6 ml/kg: 1 dose; 0.65 ml/kg: 3 doses),

1M: two sub-doses at 13.0 and 26 mg/kg

1F: one dose at 13.0 mg/kg

1M/1F: three sub-doses at 6.5 mg/kg

Experimentals and Parameters studied: same as Study 1 in dogs.

Results:

Mortality: 1F died 1 hr after the first dose (total dose of 13 mg/kg) following precipitous fall in BP. The dosings were adjusted following this death.

Clinical signs: Only N-M blockade with dose-related increases of duration: 52-196 min.

BW/FC: No significant effects

ECG: Same as Study 1 in dogs. Drug-related ECG changes were seen in one M (total dose of 39 mg/kg). Right bundle branch block pattern and prolonged PR intervals after the second sub-dose and remained until the end of the recording, but normal at the end of 2-week recovery. One F (single dose of 13 mg/kg) had a precipitous fall in BP after the injection leading to death in 30 min. In this animal, 10 min after injection right bundle branch block with QRS and PR interval lengthening and ST segment deviation occurred. A hypoxic type ECG developed with AV dissociation, marked QRS widening and an injury type potential in lead v_2. In the two animals (1M & 1F) dosed with 3 sub-doses of 6.5 mg/kg, no ECG changes occurred, except those seen with halothane anesthesia alone.

BP: Measurement schedules same as ECG.

Control animals showed no significant changes of BP & HR. One F died after one dose of 13 mg/kg with precipitous fall of BP. The M (total dose 39 mg/kg) showed marked drop in BP after each injection; returned to control level only at 240 min. In animals receiving 3 sub-doses of 6.5 mg/kg each (total dose 19.5 mg/kg), a marked fall of BP was observed after each injection. The M's BP returned to control level in 30 min after the last injection, whereas it took 20 min for F to recover.

Hematology/Ophthalmology/Urinalysis/Organ weight: Unremarkable

Serum chemistry: Creatinine phosphokinase (CPK) was Increased in all treated M and the F on the day after dosing.

Pathology: gross and histopathology

Liver (vacuolation of hepatocytes/congestion/perivascular/periductal lymphoid cells) appeared in all animals.

Lungs: Subacute/chronic interstitial inflammation/perivascular/peribronchiolar lymphoid cells/alveolar/intraalveolar macrophages in most cats.

Nose/turbinates: eosinophilic material/free erythrocytes in some of the cats

Injection sites: subacute/chronic inflammation/fibrosis/granulation tissue/squamous cell hyperplasia/hyperkeratosis/ulcer: in some cats of all groups, no apparent drug-effects.

Based on this study, the maximum tolerated dose was 3X 6.5 mg/kg in cats.

4. An acute toxicity study in cats with ORG 9487 via intravenous injection in five sub-doses.

Report 70-1053-TX, Vol. 1.28-29. Satellite groups of 1M + 1F were for toxicokinetic study.

Performed by( ) May 25-July 6, 1993

Animals: Domestic short-hair cats, mean BW: 3.5 kg (M), 2.6 kg (F), 5-14 months old; Supplier:( )

Formulation: Org 9487, lyophilized cake, was mixed with 1 ml of the vehicle, sterile water for injection to yield 10 mg/ml.

Treatment route and duration: iv bolus via femoral vein; one day of dosing (5 subdoses at 30-min intervals), followed by a 2-wk recovery (staggering dosings).

Dosage levels: 5X 0.0 (placebo); 5X 0.6; 5X 1.9 and 5X 6.0 mg/kg

Experimentals: Same as Study 2 in dogs, conducted by the same contractor.

Results:

Mortality: None

Clinical signs:

N-M blockade: duration was dose related, i.e.,10-20 min (low), 30-40 min (mid), 90-130 min (high). The blockade was recovered before each sub-dose in the low, but not in the high groups. Spontaneous breathing, also dose-related, could be maintained approximately 1-4 hrs after the fifth dose. No other significant signs except swollen hind legs in some cats attributing to incision/surgical procedure.

BW/FC: No significant effects

ECG: No significant drug-related findings. A-V dissociation was observed in one or more cats of all groups on the day of dosing. This is a known effect of halothane anesthesia.

BP: Dose-related transient hypotension was observed: mid dose (from 75/53 to 50/35 mmHg), high dose (from 115/85 to 58/28 mmHg). Mid dose: BP recovered to control level before each sub-dose whereas high dose recovered by the end of relaxation period.

Hematology: no other significant effects.

Serum chemistry: On Day 2 relative to the control group, increases of mean AST and ALT in high M & F, and increases of CP*, LDH and BUN in high M, which returned to normal at the termination. *1 control F had unusually high CP on Day 2; but increase in high F was still apparent.

Ophthalmology, urinalysis and organ weight: unremarkable

Pathology: Gross: unremarkable

Histopathology:

Liver/kidneys: One high M had elevated AST, ALT and BUN, and had moderate mineralization and subacute/chronic inflammation of the kidneys, but only minimal granulomatous inflammation of the liver.

Injection site: Incidental granulomatous inflammation (around suture material), granulation tissue, fibrin, edema and acute/subacute inflammation occurred in control and dosed animals. These changes often obliterated the vascular structures.

5. An Acute Intravenous Toxicity Study in the Dog with the Impurities and Degradation Products of a Neuromuscular Blocking Agent, ORG9487 for Injection. Report 070-1189-TX, Vol. 1.32

Performed by August 21-September 12, 1997

Animals: Beagle dogs, mean BW: 8.2 kg (M) & 6.6 kg (F), 5-6 months old

Treatment route and duration: iv bolus, three times at ½-hr intervals, vol : 1mL/kg

Formulation (copied from the submission):

Summary of Impurities found in Org 9487

Code Letter	Org Code No.	Structural difference compared to Org 9487	Specification [c] (at release)
A[b]	Org 21616	16N-despropenyl	%
B[a]	Org 21621	2N-propenyl	%
C[a]	Org ND 24	17-acetyloxy	%
D[a]	Org 9486	3-(1-oxypropenyl)	%
E[b]	Org 9502	17-hydroxy	%
F[b]	Org 9488	3-hydroxy	%
G[b]	Org 9504	3,17-dihydroxy	%
H[a]	Org 21607	16α-isomer	%

[a] Process related impurity [b] Degradant

Dosage levels: 3X 0.0 (Placebo) and 3 X 1.7 mg/kg, iv at ½-hr intervals

Experimentals (staggering design): Dogs were anesthetized with propofol/halothane and mechanically ventilated. The study parameters were standard. The animals were necropsied following a 2-wk recovery period. Parameters studied: Physical observations, BW, food consumption, hematology and clinical chemistry, ophthalmology, organ wt, gross & histopathology. ECG (9 leads), HR, direct & indirect BP, N-M blockade: at pretest, and/or during anesthesia, and at 10-min intervals following the dosing.

Results:

Mortality: None

Clinical signs: N-M blockade lasted 2-3.5 hrs following the last dose.

BW & FC, ECG/BP/HR, Hematology, Serum chemistry & Ophthalmology: unremarkable

Organ weight: Both absolute and relative wt of the ovaries were decreased in the dosed F.

Pathology:

Gross:

Liver: Nodule/mass was observed in 1F of the dosed group.

Injection site: Unremarkable.

Histopathology:

Liver: Necrosis and mineral deposit were observed in only 1F of the dosed group

Injection site: perivascular hemorrhage or acute/subacute inflammation in 1 M & 1 F of the placebo group.

Testes (immature germinal epithelium) & epididymides (oligospermia): in all M dogs.

6. Determination of the maximum tolerated dose by continuous intravenous infusion in the non-pregnant rabbit. Report 070-1149-TX, Vol. 1. 26

Performed by [redacted] July 17, 1996-Sept. 24, 1996

Animals: NZ white rabbits, 3/gr except 4/gr for 6 mg/kg; non-ventilated.

Dosage levels: 0, 1, 2, 3, 6, 12 and 24 mg/kg/d, infusion @ 4.8 mL/kg/d

Parameters: Morbidity/mortality, BW/FC and necropsy on all dead, sacrificed and scheduled terminated animals.

Results:

Mortality: dose-related: 3 mg/kg/d (1/3 on day 2); 6 mg/kg/d (3/4 on day 8); 12 mg/kg/d (3/3 on day 2) and 24 mg/kg (3/3 within 4 hrs of first day). Convulsion and/or labored respiration were observed before some of the deaths, and necropsies showed dark, black or red lungs in some of the dead rabbits.

BW/FC: Unremarkable

The results show a maximum tolerated dose of 2 mg/kg/day given by continuous infusion in rabbits. The sponsor selected 1.5, 3 and 6 mg/kg for a subsequent D-R study in pregnant rabbits.

(B) Subacute Toxicity studies:

1. An intravenous dose range finding study in Wistar rats with a neuromuscular blocking agent ORG 9487 (t,i,d). Report 070-1055-TX, Vol. 1. 26

Performed by the sponsor in the Netherlands,10/5/93-10/13/93, a GLP study

Animals: Wistar rats, :Wu (spf-bred),

F: 120-140 g; M: 140-160 g

Formulation: A lyophilized cake formulated with a phosphate buffer system adjusted to pH 4 was supplied in vials containing 10 mg Org 9487 for reconstitution with 1 ml water for injection to obtain an isotonic preparation for injection with a strength of 10 mg/ml.

Route and duration of treatment: iv in 3 divided doses at 2 hrs apart per day for 1-3 consecutive days. Animals were observed for clinical signs and sacrificed.

Dosage levels: (3M + 3F)/ level, except Group 4 (3M +1F) only. Group 4 only dosed for 1 day. Group (2): 3X 1.0 mg/kg/d; (3) 3X 0.5 mg/kg/d; (4) 3X 0.75 mg/kg/d.

Vol: 2-4 mL/kg. Note: 1 mg Br salt = 0.882 mg quaternary ammonium ion.

Results:

Mortality: occurred within 1 min of respiratory arrest at 1.5-2 mg/kg.

Signs: Bulging eyes and respiratory disturbances were dose-related at 0.75-1 mg/kg; at 1 mg/kg within 10 seconds of dosings and of short duration (1-2 min). Subsequent dosings on the same day or consecutive days did not enhance the clinical signs. Within one day, the conscious, and non-ventilated rats tolerated 0.75 mg/kg given at 2-hr-interval for 3 times with clinical signs as noted above. Subdoses of 0.5 mg/kg (3X/day for 3 days) caused minimal signs and were considered as NOAEL.

2. A four week toxicity study in dogs with ORG 9487 via intravenous injection in three sub-doses two times per week. Report 070-1067-TX, Vol. 1. 29-30

Performed by August 23-October 16, 1993 (staggering dosings).

Animals: Beagle dogs, mean BW: 9.8 kg (M), 8.0 kg (F), 7-8 months old,

Formulation: Same as cat study below. Certificate of analysis enclosed.

Treatment route and duration: iv bolus via cephalic vein; twice per week for 4 wks; on the day of dosing (3 subdoses at 30-min intervals). The control animals were anesthetized and dosed with the vehicle at the same volume as the high dose group.

Dosage levels: (1) Control: vehicle control; (2) 3X 1.0 mg/kg; (3) 3X 2.45 mg/kg; (4) 3X 6.0 mg/kg. **Volume of injection at 0.6, 0.1, 0.245 and 0.6 ml/kg, respectively.**

Experimentals: Halothane/oxygen-nitrous oxide (1:1) anesthetized and mechanically ventilated dogs; control animals were anesthetized and restrained like the high dose group. Set-up same as dog acute toxicity study 2.

Parameters studied: Ophthalmology, hematology and clinical chemistry: pretest and at termination. In addition, hematology and clinical chemistry: on Day 2. Detailed physical examination and visual food consumption : pretest and weekly. BW: pretest, weekly and

termination. Urinalyses: termination only. ECG (all leads): pretest, following anesthesia, at 10-min intervals following each sub-dose and at the end of dosing period. Lead II was briefly recorded every 30 min during the relaxation period. ECG and Lead II were recorded on the 1st day of dosing in Week 1 and the last day of dosing in Week 4. Direct BP: on the day of dosing via a catheter placed in the femoral artery.

Results:

Mortality: None

Clinical signs: N-M blockade with dose-related duration: 10-20 min (low), 30-70 min (mid), 60-190 min (high). Spontaneous breathing, also dose-related, could be maintained approximately 1/2-6 hrs after the last subdose. No other significant signs.

BW: all groups lost wt. during the study, this was due to the procedures (frequent fasting and anesthesia); no significant drug effects. FC: no obvious effect.

ECG: No significant findings in any group on the first day of treatment. But on Day 25 before the final (8th) dosing, the high-dose dogs had pronounced (3/6) and slight to moderate (3/6) QT prolongation. Since slower heart rate may lengthen QT interval, it was decided to measure PR, QRS and QT intervals and calculate the Qtc interval. This was found to be significantly prolonged in the high-dose dogs. The onset of the ECG change was not clear because ECG was recorded on Day 1 and Day 25 in the 28-day study.

Hematology: significant increase in mean Hgb and Hct in F (high-dose).

Serum chemistry: mean BUN increased in the high-dose M & F after 8 doses.

Ophthalmology, urinalysis and organ weight: No significant changes.

Pathology:

Gross: Redness at the injection site in 1 animal in each of all groups including control.

Histopathology of injection site: The following changes were graded minimal to moderate in 1-3 animals of some or all groups: hemorrhage, subacute/chronic inflammation, and fibrosis were noted in the perivascular, subcutaneous and other soft tissues at the injection sites. In some of these animals, intimal "tag" and medial hypertrophy and/or inflammation of the blood vessels were observed. In the skin at the injection sites, subacute/chronic inflammation, accumulation of inflammatory cells and squamous cell hyperplasia were reported.

3. A four week toxicity study in cats with ORG 9487 via intravenous injection in three sub-doses two times per week. Report #070-1051-TX, Vol. 1.31; satellite groups (3/sex/gr) for TK study. Performed by [redacted] October 18-December 11, 1993 (staggering dosings); Necropsies: Nov. 15,1993-Feb. 3, 1994

Animals: Domestic shorthair cats, mean BW: 4.3 kg (M), 2.9 kg (F), 9-11 months old, [redacted]

Formulation: Lyophilized cake, reconstituted with Water for Injection to 10 mg/mL, pH4 containing citrate/phosphate buffer, Lot# OA. 04.93/A-3

Treatment route and duration: iv bolus via cephalic vein; twice per week for 4 wks. On the day of dosing: 3 subdoses at 30-min intervals. The control animals were anesthetized and dosed with the vehicle at the same volume as the high dose group.

Dosage levels: (1) Control: vehicle control, (2) 3X 1.0 mg/kg; (3) 3X 2.45 mg/kg; (4) 3X 6.0 mg/kg. Volume of injection at 0.6, 0.1, 0.245 and 0.6 ml/kg, respectively.

Experimentals: Halothane/oxygen-nitrous oxide (1:1) anesthetized and mechanically ventilated (via endotracheal tube) cats; control animals were anesthetized and restrained like the high dose group. Set-up same as the dog subacute toxicity study 2 above. The animals were sacrificed at the end of dosing for routine pathology study.

Parameters studied: Same as the dog subacute toxicity study above. ECG (9 leads): Same as the dog subacute toxicity study above. Indirect BP: high-dose only; on the day of dosing via a catheter placed in the femoral artery.

Results:

Mortality: One high F died probably of severe hypotension during the first day of dosing on Week 2 (or the 3rd of 8 scheduled doses) following the 2nd sub-dose. Three others (2 deaths:1 control & 1 low/Day 1 dosing; 1 sacrifice: 1 high/Wk 2), were related to anesthetics or intubation and were replaced.

Clinical signs: N-M blockade was duration dose related: 10-20 (low), 20-40 min (mid), 40-90 min (high). Spontaneous breathing, also dose-related, could be maintained approximately 1/2-6 hrs after the third subdose. At the end of Wk 4 dosing, one high dose M was lethargic, having decreased activity, irregular gait and dilated pupils, but had normal BP and temperature. This animals had increased AST, BUN, creatinine, lactate dehydrogenase and creatine kinase.

Indirect BP (Measured only in the high dose group): Marked but transient decreases.

BW: All groups lost wt. during the study, this was due to the procedures (frequent fasting and anesthesia); no significant drug effects. FC: correlated with BW.

ECG: No significant findings; one cat had a single extra ventricular extrasystole following the first subdose: considered incidental.

Hematology: Unremarkable

Serum chemistry: Marked increase of BUN in high M & F at Wk 4, this increase was not present after first day of dosing.

Creatinine kinase and lactate dehydrogenase increases in high M & F, were considered to be muscular activity and stress related effects.

AST: Increased in the high M only

Ophthalmology: One low-dose M cat had focal retinopathy of both eyes at Wk 4 and was retained for 11 wks for ophthalmological evaluation.

Urinalysis: Increased protein in all 3 dosed M, and low- and mid-dose F groups.

Organ weight: Both absolute and relative wt of the following organs were affected.

M: increased prostates, testes, and spleen (mid & high).

F: decreased lungs (low and high), pancreas (all 3 doses), thymus (mid & high), spleen (high), and increased thyroid/parathyroid (all 3 doses).

Pathology:

Gross exam: Injection site (redness in many animals of control and dosed groups).

Histopathology:

Injection site: The following changes were graded minimal to moderate in 1-3 animals of

all groups: hemorrhage, subacute/chronic inflammation, and fibrosis were noted in the perivascular, subcutaneous and other soft tissues at the injection sites. Intimal "tag", a thrombus or fibrosis in the vein used for injection, as well as acute/subacute/chronic inflammation of the soft tissue and eosinophilic material on the surface of the skin at the injection site were observed in a few cats; no apparent dose-related effects.

Thymus: Involution/atrophy showed drug-related effect in M & F.

Spleen: Congestion (2/3 high F vs 0/3 control F).

Adrenal glands: Vacuolation in all cats including controls.

Kidneys: Bilateral interstitial subacute/chronic inflammation (2/3 high F vs 0/3 each in control, low, and mid F) & M (1/3 each in control, mid and high M).

Mineral deposits in the medulla (in 3/3 F each all 3 dosed groups vs 1/3F control; in 2-3/3M each of all groups).

Liver: Hepatocellular cytoplasm vacuolation in all cats, incidence and severity suggest a probable halothane effect.

III. SPECIAL TOXICITY STUDIES:

(A) Compatability with blood components:

1. Report of the effect of ORG9487 on hemolysis, osmotic red cell stability and precipitation of serum proteins in live, anesthetized rats. Report 070-1068-TX, Vol. 1.25

Performed by: not provided, undated

Animals: Male Sprague-Dawley rats, wt 300-400 g, anesthetized and ventilated with cannulated carotid artery for blood samping and jugular vein for drug/saline administration. 4 rats/group

Formulation: The drug was dissolved in 0.2 ml 0.9% NaCl, acidified with HCl to pH 4

Experimentals: (I) Control: saline; (ii) Low dose: 6 mg/kg (5X human ED_{90} of 1.15 mg/kg) and (iii) High dose: 15 mg/kg (13 X human ED_{90})

At 0, 4 and 10 min, blood samples were withdrawn into tubes with, or without, heparin.

(1) Effects of ORG9487 on osmotic red cell stability: An aliquot of 50 µl to a series of test tubes containing 5 ml of 0.9%-0.1% NaCl, incubated at 37C water bath for 30 min. The hemoglobin (Hgb) conc. determined with a spectrophotometer at 560 nm.

(2) Effects of ORG 9487 on red cell hemolysis: The remainder of the heparinized blood samples not used for the above red cell fragility experiment was centrifuged and Hgb concentration of the plasma was determined as above.

(3) Effects of ORG 9487 on serum albumin and total protein concentrations. The blood samples without heparin were allowed to clot for 60 min at 37C. The sera were analyzed for albumin, total protein concentration and SMA-22 chemistry.

(4) Effects of ORG9487 on flocculation: Flocculation in the sera was determined by comparing the absorbency of the sera with that the standard absorbance regression line of a 2.5-10.0 µg/ml suspension of 0.455 um diameter latex particles in a spectrophotometer.