

**Results:** ORG per se, not the drug product to be marketed, showed the following:

- (1) Blood sampling of 5 ml each represent 12-16% of total blood volume in the rat. The volume was replaced with normal saline, this caused hemodilution (lower Hgb conc.).
- (2) At 6 and 15 mg/kg, dose-related hypotension and bradycardia were observed; one rat (15 mg/kg) died of severe hypotension.
- (3) Red cell hemolysis: Significant increases of Hgb concentration occurred at 15 mg/kg.
- (4) Osmotic red cell stability: no significant effects.
- (5) Serum albumin and total protein concentration: Both parameters decreased with time in all 3 groups due to hemodilution; no significant dose-related effect.
- (6) Serum protein flocculation: no significant effect.

**2. Report on the effect of ORG9487 on hemolysis, osmotic red cell stability and precipitation of plasma proteins in human blood in vitro.** Report 070-1069-TX, Vol. 1.25

Performed by: not specified, undated

**Formulation:** ORG freshly dissolved in 0.1 mL 0.9% NaCl acidified with HCl to pH 4.0; compositions not identical to the marketed formulation.

**Experimental:** Blood samples were obtained from 4 human volunteers: 15 ml each for heparinized and non-heparinized tubes. Two (2) ml aliquots from each tube were added to 0.1 ml each of 0.9% NaCl, 2.4 mg/ml or 6.0 mg/ml ORG9487 (i.e., similar to plasma conc. after rapid iv of 5 X ED<sub>90</sub> and 13 X ED<sub>90</sub> in humans).

**Hemolysis:** Heparinized blood samples were incubated at 37C for 60 min, centrifuged and Hgb conc. of the plasma was determined in a spectrophotometer at 560 nm.

**Osmotic red cell fragility:** 50 µl of heparinized blood were added to 5 ml each of a series (0.9%-0.1%) of NaCl concentrations. After incubation at 37C for 30 min, Hgb concentrations of the supernatants were determined in a spectrophotometer.

**Albumin and total protein:** Determined as in the rat study.

**Turbidity:** Optical absorbency of sera containing 0, 120 µg/ml or 300 µg/ml ORG9487 were determined in a spectrophotometer at 850 nm. Standard curve was obtained from 2.5-10 µg/ml latex particles of 0.455 µm diameter

**Results:** ORG per se, not the drug product to be marketed showed the following:

- (1) Hemolysis: No effect on serum Hgb concentration at ORG conc. of 120-300 µg/ml.
- (2) Osmotic red cell fragility: No drug effect
- (3) Serum albumin and total protein concentrations: No effect
- (4) Flocculation: No effect.

**(B) Local tolerance**

**1. An intramuscular irritation study in rabbits with a neuromuscular blocking agent, ORG 9487.**

Report 070-1146-TX, Vol. 1.32

Performed by  
1996-September 24, 1996

July 8,

**Animals:** New Zealand white rabbits, (NZW) fBR, mean BW: 3 kg (M) & 3.3 kg (F).

approx 5 months old,

(3M + 3F)/group

**Formulation:** Lyophilized with phosphate buffer at 200 mg/vial, reconstituted with sterile water for injection to 20 mg/ml; Lot#: OA.03.95/B-3.

**Treatment route and dosage:** Commercial succinylcholine (SCC), 20 mg/mL, as a comparison drug.

Group	Dose (mg/kg)	vol (mL/kg)*	# sacrificed Day 8	Day 15
(1)	2; 4	0.1 (R); 0.2 (L)	1M + 2F	
(2)	6	0.3 (R)	0M + 1F	3M + 3F
(3)	4	0.2 ((R)	2M + 4F	4M + 4F
(4)SCC	2; 4	0.1 ((R); 0.2 (L)	3M + 3F	

\*Site of injection: right (R) and/or left (L) dorsal m. longissimus muscle. Parameters: signs, BW, food consumption; injection site: gross observation (daily), histopathology (day 8 & 15/16).

**Results:**

- (1) Mortality : Some rabbits receiving 0.3 ml/kg, either as 0.1 + 0.2 ml/kg (2M + 1F) or as a 0.3 ml/kg (3M + 1F) died. Cause of death: prolonged N-M blockade (4-8 hrs) and anesthesia (5-10 hrs). Signs before deaths: poor recovery from anesthesia, labored breathing and apparent pulmonary irritation. The group to receive 0.4 ml/kg, therefore, was canceled. Non-lethal dose: 0.2 ml/kg as one dose. One rabbit receiving 0.1 + 0.2 ml/kg succinylcholine died due to prolonged N-M blockade (twice the duration of other rabbits in this group).
- (2) Signs: Unremarkable except for N-M blockade.
- (3) BW/FC: No significant findings
- (4) Gross observation at the injection site: Only 2 animals showed effects. One (0.1 + 0.2 ml/kg) had subcutaneous red and multifocal discoloration on the dorsal thorax; the other (0.3 ml/kg): red and mild discoloration at the injection site.
- (5) Microscopic examinations:

On day 8: Similar findings (graded as mild-severe) were between ORG & succinylcholine groups: muscle injury (necrosis) associated with interstitial edema and hemorrhage. They were surrounded by a region of tissue reactions consisting of fibrous connective tissue, degenerative atrophy & leukocyte infiltration. On day 15/16, some of the above reactions were still present, but regenerative reactions were also observed.

**Conclusion:** Intramuscular injections of ORG product and commercial succinylcholine caused similar irritations in the muscle at the injection site. A deficiency of the study was the failure to include saline control sites to validate the applicant's claim that the irritations were due to injection trauma.

**2. A local irritation study in rabbits with two presentations of the finished product of Org9487 for injection via intravenous, intramuscular, intraarterial and perivascular routes of administration**

Report 070-1188-TX, Vol. 1.32

Performed by

May, 1997

**Animals:** 55 anesthetized and ventilated New Zealand white rabbits, (NZW) fBR, mean BW: 3.4 kg (M)

**Formulation:** Same as the study above. Drug products in two different vials with two different stoppers: (a) vial with rubber stopper and (b) vial with rubber stopper

**Treatment route and dosage:** A total of 8 groups (6M/gr): 4 received presentation (a) and 4 receiving presentation (b). Each group received a single bolus injection of 0.2 mL/kg into the dorsal m. longissimus muscle (im), marginal ear vein (iv), medial ear artery (ia) or parallel to the marginal ear vein (pv). Parameters: signs, BW; injection sites: gross observation (daily), histopathology (day 3); animals were sacrificed on day 3.

**Results:**

**Mortality:** 6 animals died due to complications from anesthesia and/or poor recovery from anesthesia, according to the report. Findings before deaths included labored breathing, pulmonary irritation and fluid in the lungs. These animals were replaced.

**Signs/BW:** N-M blockade 1-2.5 hrs (iv, ia, pv) & 3-4 hrs (im), no significant difference between the two presentations. Others were unremarkable, according to the report

**Injection sites:** Comparable incidence and severity for both presentations

**Gross:** mainly slight erythema in 1-6 rabbits of each group; but none in im groups.

**Microscopic:** Slight to moderate perivascular hemorrhage (iv, ia, pv) & muscle necrosis (im) in one to several animals. Applicant attributed to mechanical trauma of injection. Since a saline-control group was not included, these findings should also be considered as possibly product related.

**(C) Malignant hyperthermia induction in susceptible swine following exposure to Org 9487.**

Report O70-1187-TX, Vol. 1.32

Performed by

11/28/95-3/19/96

**Animals:** 9 Purebred Pietrain swine and 7 normal swine (controls), suppliers: (9MHS\* & 6 control animals) (3 control animals), approx 2-4 mo old, mean BW: MHS: 20 (10-44) & controls: 29 (16-49) kg Genetic testing indicated presence of the ryanodine receptor gene mutation. Known homozygous boars and sows were bred to produce homozygous offspring.

**Formulation:** Same as study above, Lot#OA.03.95/B-3

**Experimentals:** Animals were anesthetized and ventilated. Bolus doses of 1X, 3X or 6X ED90 were given iv via the jugular vein, each following complete recovery from the previous dose.

**Parameters studied:**

MH: End-tidal CO<sub>2</sub>, oral force (Intraoral force transducer was placed between the upper and lower molars to measure masseter spasm), hind limb rigidity, BP, HR, PAP, CO, core and muscle temperature, serum lactate & blood gases. N-M block and relative force of hindlimb were monitored.

In vitro: Muscle specimens (gracilis muscle) were dissected into small muscle bundles of fiber segments of 2-3 cm long & 2 mm in diameter. Contracture was measured after exposure to 3% halothane or 0.5-32 mM caffeine. Positive: contracture of 0.8 gm (halothane), or 0.2 gm (caffeine at 2 mM or lower).

**Results:**

- (1) N-M blockade showed a dose-related duration which was similar in MHS and control groups. The ED<sub>90</sub> was at 0.40-0.55 mg/kg (iv).
- (2) MH: Following 3X & 6X ED<sub>90</sub> (1.32 and 2.64 mg/kg), no consistent effects suggesting MH in the MHS swines were observed.
- (3) In both MHS and control groups, MAP decreased following Org; but HR was not affected.
- (4) One MH pig died of hemodynamic instability following 3X ED<sub>90</sub>.
- (5) Muscle high energy phosphates: phosphocreatine, AMP, ADP and ATP were comparable in both groups.
- (6) Verification of the animals susceptibility was satisfactory based on the results from:
  - (a) *in vivo*: challenge at end of study with succinylcholine and halothane.
  - (b) *in vitro*: muscle strip contraction in response to either halothane or caffeine.Two control pigs were excluded due to hypermetabolic response to halothane exposure (possible heterozygote for the MH trait)

**IV. REPRODUCTION STUDIES:**

**1. An embryotoxicity study (including teratogenicity) with ORG 9487 in the rat by t.i.d. intravenous injection** Report No. 070-1052-TX, Vol. 1.33

Performed by:

Nov. 14-Dec. 9, 1993, a GLP study.

**Animals:** Conscious non-ventilated Sprague-Dawley mated female rats (25/group), 11 wk old, 217-296 g.

**Drug:** lot no. 0A.04.93/A-3. Lyophilized cake to be reconstituted with Water for Injection to form a citrate/phosphate buffer pH4 aqueous solution at 10 mg/mL.

**Route and duration of administration:** Bolus iv injection into a caudal vein in 3 subdoses, at 3 hrs intervals, from day 6-17 post coitum.

**Dosage levels:** Refer to table under **Results**.

**Experimentals:** Mortality, clinical signs, BW/FC: daily and weekly interval; on day 21 post coitum, animals were sacrificed. Dams were examined for gross findings, corpus lutea, and implantation/resorption sites, live or dead fetuses; fetuses weighed, and examined for external, visceral and skeletal abnormalities according to standard procedures.

**Results** (table copied from the submission):**Reproduction and Fetal Data Segment 2 Study in Rats**

Group Dose (mg/kg)	Placebo 3 x 0	Low Dose 3 x 0.25	Interm. Dose 3 x 0.50	High Dose 3 x 0.75
No. of mated females	25	25	25	30
No. of pregnant females	24	24	24	29
No. of females with live fetuses	24	23	24	25
Post-Implantation Loss (%)	3.5	3.3	2.8	5.5
Embryonic resorptions (%)	3.2	3.3	2.1	2.9
Fetal resorptions (%)	0.3	0.0	0.8	2.6**
No of fetuses	362	350	375	364
% Males	44	47	45	46
% Females	56	53	55	54
Mean fetal weight (g)	5.4	5.4	5.3	5.2**
Malformed fetuses (%)				
External malformations	0	0	0	0
Visceral malformation	0	0	0	0
Skeletal malformations	0	0	0	0

\*/\*\* Fisher's Exact Test significant at 5% (\*) or 1% (\*\*) level

**Dam data:**

**Mortality:** 5F of high dose group died of apnea. Necropsy was unremarkable. These animals were replaced.

**Signs:** N-M blockade showed dose-related duration and depth. Other signs observed during drug administration and persisting between 10 sec and 4.5 min, were excitation, clonic spasms, breathing sounds, respiratory distress and apnea of short duration. Apnea was the cause of death in 5-high F.

**BW/FC:** Slight reduction in the high-dose F.

**Litter data:**

**Post-implantation losses** (due to fetal death and subsequent resorption): Increased in the high-dose group (Refer to the table above). Although the total embryonic and fetal deaths were 13, 12, 11 and 21 for C, L, M and H dose groups, respectively, the significant differences were due to the fetal resorptions (i.e., 1, 0, 3 and 10 for C, L, M and H dose groups, respectively).

**Fetal wt (mean):** Slightly reduced in the high dose group

**External, visceral:** No abnormal findings.

**Skeletal examination:** Incomplete, none, or abnormal ossifications of various bones were observed in some fetuses of all groups. No apparent drug related effect.

**2. Org 9487-Dose Range Finding Study by Continuous Intravenous Infusion in the Pregnant Rabbit.** Report 070-1150-TX, Vol. 1.33

Performed by:

Oct. 29-Nov. 21, 1996, a GLP study.

**Animals:** Mated New Zealand white rabbits (6/group); Supplier

**Route and duration of administration:** Continuous iv infusion using a catheter implanted

**Results:** (table copied from the submission):**Reproduction and Fetal Data Segment 2 Study in Rabbits**

Group Dose (mg/kg/24 hr)	Placebo 0	Low Dose 0.75	Interm. Dose 1.5	High Dose 3.0
No. of mated females	20	20	20	10
No. of mortalities (P/NP)	0/0	0/1	1/0	3/1
No. of pregnant females	17	18	18	7
No. of females that aborted	0	0	1	0
No. of females with live fetuses	17	18	16	4
Post-Implantation Loss (%)	5.4	11.2	14.6	13.6
Embryonic resorptions per dam	0.5	1.2	1.4	1.0
Fetal resorptions per dam	0.0	0.0	0.1	0.0
No. of fetuses per dam	7.9	9.3	8.3	7.8
Total no. of fetuses	134	167	133	31
% Males	49	46	44	46
% Females	51	54	56	54
Mean fetal weight (g)	40.2	38.0	38.5	35.0
External malformations	1/134	1/167	0/133	1/31
Visceral variations	1/62	1/79	1/61	1/14
Skeletal malformations	2/134	2/167	1/133	1/31

P = pregnant NP = nonpregnant

**Dam data:**

**Mortality:** 4/10F of the 3 mg/kg group died due to treatment; signs included body tremors, subdued behavior and rapid breathing; the rest of the group was canceled (10 total instead of 20). Necropsy was unremarkable. Two deaths, one each from the low and mid group were not considered as drug-related by the applicant.

**Signs:** Only seen at high dose as noted above under Mortality

**BW/FC:** reduced BW gain only at 3 mg/kg during GD 6-9. FC correlated with the BW changes.

**Litter data:**

**Post-implantation losses (%):** increased in the dosed groups without apparent dose-related effects (C: 5.4%, vs dosed: 11.2%, 14.6% and 13.6%, but H had only 4 litters). The total resorptions (early + late)/litter were 8/17, 21/18, 22/16 and 4/4 for C, L, M and H dose groups, respectively. Note that clinical signs of N-M blockade were not observed in the low- and mid-dose groups.

**Fetal wt (mean):** No significant effect.

**Sex ratio:** Slightly decreased in the dosed group (C: 50/50 vs dosed: 45/55)

**External, visceral:** No significant findings.

**Skeletal examination:** Variations such as incomplete ossification of phalanges, and incomplete and unossified sternbrae were increased in the dosed groups.

### V. Mutagenicity studies:

Note: All *in vitro* studies were tested in the presence of the parent compound and substantial amounts of hydrolytic products, most of which are likely the same as the major metabolites. The stability study conducted concurrently with the Ames test showed that at room temperature, the parent compound was reduced by 50% in pH 7.4 buffer in 3 hrs or in water in 1 hr. In the pH 7.4 buffer, complete decomposition was observed in 24 hrs. ORG in DMSO was stable for at least 1 hr at room temperature. All *in vitro* assays were conducted at pH 7.4.

#### 1. Bacterial mutation assay (S. typhimurium and E. coli) on ORG 9487.

Report 070-1004-TX. Vol. 1.34

Performed by

Aug. 2-17, 1990

**Experimentals:** Ames standard protocol was used to evaluate the ability of inducing gene mutations in histidine-dependent *S. typhimurium* strains and a tryptophan-dependent *E. coli* as measured by reversion of autotrophy to prototrophy. Tester strains: TA 1535, 1537, 100, 98 and WP2 *uvrA* *pkM101*. Cytotoxicity was performed with and without S9 metabolic activation. Two Ames tests were performed: the second assay adjusted the highest concentration based on the results from the first assay. Solvent: DMSO.

Assay 1: ORG 200-3200\*/6400 µg/plate (- S9-mix); 313/625-10,000 µg/plate (+ S9).

Assay 2: ORG 200/313-3200\*/10,000 µg/plate (- S9-mix); 313-10,000 µg/plate (+ S9-mix). \*TA100 only.

**Results:**

- (1) Cytotoxicity: Cytotoxicity as evident by the thinning of the background lawn, reduction in revertant numbers or microcolony formation was observed at 10,000 µg/plate (- S9-mix), not observed in the presence of S9-mix.
- (2) Revertant colonies: None of the strains showed a doubling of the number of revertant colonies over the control in either of the two assays.
- (3) Precipitation was observed at higher concentrations of some tester strains.

#### 2. A gene mutation test in mouse lymphoma L5178Y TK +/- cells in vitro with ORG 9487.

Report 070-1109-TX, Vol. 1.34

Performed by

May 22-July 7, 1995.

**Experimentals:** (Note: Hydrolysis occurs at pH 7.4, thus the study cells were exposed to both parent and metabolites). Drug: Batch 395/0016

- (1) **Cytotoxicity:** conducted in the absence and presence of metabolic activation system (S-9). Osmolarity was decreased slightly and pH increased slightly at end of incubation, but mutation frequency was not affected.
- (2) **Standard protocol:** (Batch 395/0024). S-9 was prepared from Sprague Dawley rats, induced with Aroclor 1254. Mutational colonies: Large (mutation) & small (clastogenicity). Negative control: DMSO diluted with treatment medium



chromosomal aberrations. Three conc. examined at 20-hr and one conc. examined at 44 hr.

**Results:**

(1) **Cytotoxicity:** Observed at 1200 µg/mL in the absence of S9-mix (20 hr exposure/harvest) and at 2450 µg/mL in the presence of S9-mix (3 hr exposure/20 hr harvest). Precipitate observed at at 5000 µg/mL.

(2) Table copied from the submission:

**Cytotoxicity and Chromosome Aberrations (Initial Study)**

Exposure time (hr)	Harvest time (hr)	Conc. (µg/mL)	RMI (%)	No. aberrant cells per 200 cells examined
in the absence of S9-mix				
20	20	0	100	1
		1400	100	6*
		1600	56	7*
		1800	39	8*
		4NQO	-	12 <sup>2)***</sup>
20	20	0	100	1
		1500	60	11**
		1600	49	4
		1700	51	5
		4NQO	-	21 <sup>1)***</sup>
44	44	0	100	2
		1800	78	0
in the presence of S9-mix				
3	20	0	100	4
		2200	97	2
		2800	56	3
		3400	48	6
		CP	-	14 <sup>1)***</sup>
3	44	0	100	4
		3400	68	3

<sup>1)</sup> 50 cells examined

<sup>2)</sup> 75 cells examined

\* significantly different from control at p < 0.05 (Fisher's exact test)

\*\* significantly different from control at p < 0.01 (Fisher's exact test)

\*\*\* significantly different from control at p < 0.001 (Fisher's exact test)

Note: (1) Where the number of cells with aberrations increased, the total number of aberrations (both with and without gaps) also increased. (2) Most increases (both numbers of cells and aberrations) were outside historical normal ranges, although increases were not always present in both duplicate samples.

**4. A chromosome aberration test in peripheral human lymphocytes in vitro with Org 9487. Report 0070-1148-TX, Vol. 1.34**

Performed by  
13, 1996-Jan 22, 1997

Nov.

**Experimentals:**

Vehicle: DMSO; positive controls: mitomycin C (-S9) and cyclophosphamide (+ S9); precipitate at 1200 µg/mL (note: the first study above precipitated at 5000 µg/mL)

The test concentrations are:

(-S9): 1400-5000 µg/mL: 10 levels total (3-hr-exposure/24-hr harvest);

1200-2400 µg/mL: 10 levels total (24 & 48 hr exposure/harvest)

(+S9): 2200-4000 µg/mL: 10 levels total (3 hrs exposure/24 & 48 hrs harvest)

Colchicine was added during the last 3 hrs of incubation to arrest dividing cells in metaphase. Two hundred (100 per culture) cells in metaphase per test conc. were examined for chromosome aberrations. Based on the mitotic index, three concs. were selected for scoring at the 24-hr harvest time in the presence and absence of S9-mix; one conc. of the 48 hr harvest time was selected for scoring in the presence and absence of S9-mix. Refer to the table under Results for details:

Note: Where the number of cells with aberrations increased, the total number of aberrations (both with and without gaps) also increased. RMI (relative mitotic index) as an index of cell viability.

### Results:

#### (1) Stability (copied from the submission):

Precipitate: Contrary to the experience at \_\_\_\_\_ where concentrations of Org 9487 of up to 5000 µg/mL were prepared in serum-holding culture medium, it appeared at Notox that concentrations higher than 1200 µg/mL precipitated. Therefore, Org 9487 dissolved in DMSO, was added by force to pre-heated (37 °C) serum-free culture medium. Serum was added after 30 min incubation at 37 °C. Although precipitation was not reported, it cannot be excluded that crystal-clear precipitates, not visible to the naked eye, were present.

#### (2) Cytotoxicity and Chromosome Aberrations (Repeat Study)

Exposure time (hr)	Harvest time (hr)	Conc. (µg/mL)	RMI (%)	No. aberrant cells per 200 cells examined
in the absence of S9-mix				
3	24	0	100	0
		1400	82	3
		1800	64	5
		2600	40	16***
		MMC	59	64 <sup>1)***</sup>
24	24	0	100	4
		1200	86	2
		1700	64	3
		2200	47	2
		MMC	48	95***
48	48	0	100	1
		2200	79	6
		MMC	75	76 <sup>1)***</sup>
in the presence of S9-mix				
3	24	0	100	4
		2200	84	3
		2800	64	4
		3600	48	5
		CP	62	69 <sup>1)***</sup>
3	48	0	100	2
		3600	102	10*

" 150 cells examined

\* significantly different from control at  $p < 0.05$  (Chi-square test)

\*\*\*significantly different from control at  $p < 0.001$  (Chi-square test)

Note: (1) Where number of cells with aberrations increased, the total number of aberrations (both with and without gaps) also increased. (2) Most increases (both numbers of cells and aberrations) were outside historical normal ranges, though increases were not always present in both duplicate samples.

**5. A micronucleus test in rats with ORG 9487, Report 070-1049-TX, Vol. 1:34**

Performed by the sponsor in The Netherlands, Feb 21-May 30, 1994.

**Animals:** SD Wistar rats, Wt. 184-270 g (M), 149-201 g (F)

**Drugs:** ORG 9487 in lyophilized form, reconstituted in Water for Injection, and diluted with vehicle. Placebo vehicle: Citrate/phosphate buffer system pH 4, Citric acid anhydrous 9.1 mg/ml, sodium phosphate dibasic anhydrous 5.2 mg/ml, Mannitol 20 mg/ml, NaOH/phosphoric acid to pH 4 in Water for Injection.

**Positive control:** methylmethanesulfonate, 50 mg/kg ip (single dose)

**Route and duration of administration:** iv, 3 sub-doses per day at 3-hr apart, for 3 consecutive days. Vol: 1 mL/kg.

**Dosage level:** (6M + 6F)/group  
3X 0.25, 3X 0.50 and 3X 0.75 mg/kg

**Experimentals:** Daily observation on signs, BW at pre-dose and on day of autopsy; FC similarly monitored. Animals were killed by cervical dislocation and under CO<sub>2</sub>; left femur of hind limb was removed at 24 hrs after the last dose. Bone marrow cells were prepared and scored according to the standard procedure. Examination/scoring of slides, however, were made only on placebo, high-dose and positive control groups. If required, the mid- and low-dose groups were scored. A total of 1000 PCEs (polychromatic erythrocytes) was examined for the presence of micronuclei. The ratio of PCE/NCE was determined in a total of 500 erythrocytes.

**Results:**

**Mortality:** None. **BW/FC:** no significant effect.

**Clinical signs:** Respiratory disturbances were severe in the high dose group such that after the 1st dose, the regimen for this group was readjusted to two 0.5 mL/kg 10 min apart to reach the target dose of 1 mL/kg or 0.75 mg/kg. Low- & mid-dose groups did not show the effect. Muscle relaxation: Mid- and high-dose groups showed dose-related depth and duration. Bulging eyes: The 3 dose groups showed dose-related effects.

**Micronuclei:** No increase in frequency in the high-dose group; positive control showed increases. **PCE/NCE ratio:** No increase in the high-dose group comparing with the control.

**VI. PK/ADME/TK:****1. Determination of Org 9487 and its metabolites, org 9488 and org 9504 in plasma and urine (cat).** Report 70-1089-MT, Vol. 1.35

A modification of the method used for Org 9426 was used. Inter-day variation was large, others such as sensitivity, accuracy were acceptable.

**2. In vitro binding of Org 9487 to dog, cat and human plasma proteins (pilot study).** Report #070-1050-MT, Vol. 1.35

Performed by the sponsor, 1994

**Experimentals:** Pooled plasma samples were obtained from male Beagle dogs, male cats and male human volunteers.

Drug concentrations tested: Dog, 95-15625 ng/ml; cat, 136-18,801 ng/ml; and human, 148-21,951 ng/ml (+ 395-547 ng/ml Org 9488 for the 3 highest concentrations).

Equilibrium dialysis was done at 37C for 2 hrs and for drug analysis.

**Results:**

Extent of plasma protein binding (approximate values from this pilot study):

Dog: 20-75%

Cat: 40-85%

Human: 50-90%

**3. In vitro metabolism of Org 9487 by rat, dog and human postmitochondrial liver fractions.**

Study Report 070-1132-MT Vol 1.35

**Experimentals:**

Rat, dog and human postmitochondrial liver fractions were incubated with <sup>14</sup>C-Org 9487 equiv to 5 nmol/ml at 37C for 1-30 min. The metabolites were determined with Incubation without NADP and with <sup>14</sup>C-testosterone served as controls for non-NADPH-dependent reactions and enzymatic activity, respectively.

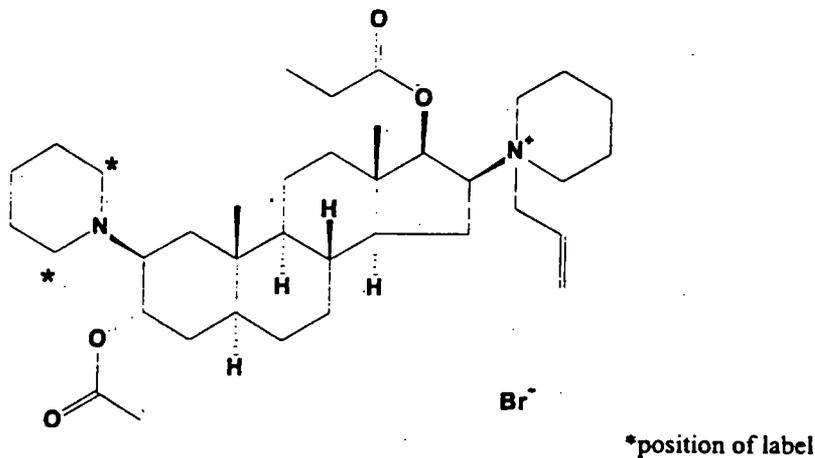
**Results:**

(1) After 30 min of incubation approx. 83% (rat), 69% (dog) and 88% (human) of radioactivity were recovered as parent compound. Org was metabolized to greater extent in dogs (30%) than in rats (17%) and humans (12%).

(2) The major metabolite, Org 9488 (3-OH), accounted for 7-8% (rat and human) and over 10% (dog). Unidentified M3 was present only in rats and dogs, but not in humans.

**4. Rat liver perfusion with [<sup>14</sup>C]-Org 9487.** Study Report 070-1135-MT, Vol 1.35**Compound labeled with <sup>14</sup>C as shown:**

(formulated with citric acid, Na<sub>2</sub>HPO<sub>4</sub> and mannitol at 9.1, 5.2 and 19 mg/mL, respectively, pH 4)

**Experimentals:**

Male rat liver for 2-hr in situ perfusion: [ $^{14}\text{C}$ ]-Org 9487 at 4.9 ug/ml. Collection time: 0 and 1-120 min. Org 9487 and metabolites were analyzed in perfusate, liver homogenate and bile.

**Results:**

- (1) After 2-hr infusion, approx. 3%, 30% and 68% of the added radioactivity were recovered in the perfusion buffer, bile and the liver homogenate, respectively.
- (2) Org 9487 (70-80% of the recovered radioactivity) and Org 9488 (10-16%) and M3 (4-6%) were present in the bile and liver supernatant.

**5. An entero-hepatic circulation study after a single intravenous administration of [ $^{14}\text{C}$ ]-Org 9487 to male and female Wistar rats.** Study Report 070-1100-MT, Vol. 1.35

Performed by the sponsor in 1994

**Animals:** Wistar rats, 8 M & 8 F, wt. 208-287 g (M); 197-200 g (F)

**Experimentals:**

Donor rats: 4M & 4F were cannulated in the bile duct towards the liver and in the duodenum. The two cannulas were led subcutaneously to the back of the head and connected; bile flowed overnight into the duodenum. The next day, the cannulas were disconnected and the 3M & 3F with the highest bile flow (observed for 5 min pre-dose) were selected for study. Each rat received  $^{14}\text{C}$  compound iv (tail vein) at 0.2 g (radioactivity at 1.42 & 1.23 Mbq for M & F respectively, or 0.2 mg/mL) in 1 ml/kg. The vehicle was citric acid/disodium phosphate/mannitol buffer pH 4. The bile was collected at 0-4, 4-24 and 24-48 hr fractions. The fractions from 0-4 and 4-24 hrs were combined to obtain approx. 35 ml as donor bile.

Acceptor rats: same set-up as donor rats. The next day, each of 3M & 3F were dosed intraduodenally (dosing time approx. 1.5 hrs) with approx. 9 ml pooled donor bile (from the same sex). The bile was collected as in the donor rats. The radioactivity and metabolites were determined.

**Results:** copied from the submission.**Biliary excretion of male and female donor rats.**

Male rats:		
25-3F78	41 %	MEAN ( $\pm$ st. dev.) 47 ( $\pm$ 10)
24-4DB9	39 %	
8-C7FB	40 %	
Female rats:		
24-53AB	64 %	MEAN ( $\pm$ st. dev.) 55 ( $\pm$ 8)
6-1F39	53 %	
24-4B29	48 %	

**Biliary excretion of male and female acceptor rats.**

Male rats:		
8-E2B5	0.46 %	MEAN ( $\pm$ st. dev.) 0.29 ( $\pm$ 0.15)
9-BD0D	0.23 %	
9-C8EC	0.18 %	
Female rats:		
25-2D3A	2.60 %	MEAN ( $\pm$ st. dev.) 1.09 ( $\pm$ 1.31)
D-F0B1	0.28 %	
25-4129	0.39 %	

Total biliary excretions in 48 hrs were approx. 47% (M) and 55% (F) of the administered radioactivity. Approx. 80% of the radioactivity in the bile was the parent drug. Most of the biliary excretion occurred during the first 4 hrs: 28% (M) and 44% (F).

In the acceptor rats the total biliary excretion in 48 hrs was 0.3% (M) and 1% (F) of the dose.

Bile was the major route and the parent compound was the major component.

Enterohepatic recirculation was not significant. Female rats appeared to have slightly greater bile excretion.

**6. Hepatic distribution and biological effects of ORG 9487.** Report 070-1153-TX, Vol. 1.32

Performed at:

Mitochondrial study:

Batch 5117, lot 7,  $^{14}$ C-Org 9487, sp act 103 mCi/mmol

Outline of the study:

- (1) Subcellular distribution of Org in the liver: Intravenous injection of an ED<sub>90</sub> of 2.5 mg/kg (2.2 $\mu$ Ci) to rats. At 5 & 120 min, animals (3 dosed + 2 control)/time point were sacrificed and liver homogenate prepared and fractionated. The radioactivity of each subfraction (nuclei, mitochondria, microsome, lysosome and cytosol) was determined.
- (2) Biological effects of Org in isolated rat hepatocytes: Incubation with Org to study the effect on viability, leakage of LDH, cell content of ATP and energy charge of hepatocytes
- (3) Biological effects of Org in isolated rat liver mitochondria: effects on membrane potential and respiration.

**Results:**

- (1) Mitochondria and lysosomes took up relative high radioactivity by 120 min
- (2) No significant effects on LDH leakage, ATP content of rat hepatocyte up to 200  $\mu$ g/mL (human plasma level: 40  $\mu$ g/mL @ 1.5 mg/kg).
- (3) No significant effect on membrane potential and respiration of mitochondria at 136  $\mu$ g/mL (200 $\mu$ M). The coupling of oxidative phosphorylation was improved at 20-30  $\mu$ M.

**Conclusion:** ORG was taken up by liver mitochondria and lysosomes and had a relatively long residence time. ORG had no effect on mitochondrial functions, no uncoupling effect, no LDH leakage or dissipation of ATP in hepatocytes.

**7. [<sup>14</sup>C]-Org 9487: Whole body autoradiography study in the pregnant rat.** Report 070-1093-MT, Vol. 1.35

Performed by [redacted] 11/17/94-2/15/95.

**Animals:** Day-14 pregnant (278-306 gm) and non-pregnant (223-249 gm) pigmented rats, 2 each at 5 min, 2, 5, 24 and 96 hrs. Dosage: single iv dose of 0.35 mg/kg (1 mL/kg). Placenta, amniotic fluid (liquid scintillation counting); and 3 fetuses each from half of the pregnant rats for whole body radioautography.

**Results:**

- (1) Fetuses, placenta and amniotic fluid had very low or no radioactivity (radiogram and scintillation counting).
- (2) Whole body radioautography showed similar and wide distributions in both pregnant and non-pregnant rats; the CNS had low level of radioactivity.
- (3) At 5 min, the highest levels were in the kidney and liver. The salivary, Harderian and pituitary glands, cartilaginous material, eye, heart, tongue and adrenals also showed substantial levels. Blood had low level.
- (4) At 24 and 96 hrs, the radioactivity decreased with time to almost undetectable levels; but the kidney, eye and to lesser extent, the heart, some glandular tissues and muscle still had some low levels.

**8. Tissue distribution and excretion balance after a single intravenous dose of [<sup>14</sup>C]-Org 9487 to male Wistar rats.** Report #070-1120-MT, Vol. 1.35

Performed by the sponsor in The Netherlands.

**Animals:** Male Wistar rats, 245-293 gm. **Dosage:** A single iv dose of  $3.4 \pm 0.9$  Mbq/kg [<sup>14</sup>C]-Org 9487 (equivalent to approx.  $530 \pm 136$  ug/kg).

**Experimentals:** At 2, 24, 96 and 168 hrs after dosing, 2-3 rats were sacrificed, blood and various tissues/organs were obtained. At 96 and 168 hr, 24-hr urine and feces samples were also collected.

**Results:**

- (1) Within 96-168 hrs, the total excretion was approx. 73% (64% in feces and 9% in urine). The parent compound accounted for approx. 50% of radioactivity in both feces and urine and the major metabolite, 3-OH metabolite, accounted for 33%. Urine had M1, which was absent in the feces. The other two metabolites, M2 and M3, were present in urine and feces. Most excretion (approx. 63%) occurred within 48-hr.
- (2) The drug eliminated from the blood/plasma rapidly such that, at 2-hr, very low concentration of radioactivity was detected. At 2-hr, the highest drug concentrations were in the small intestine, kidneys, and liver. Brain/other CNS, muscle, fat and skin had low levels except hypophysis, where low level remained during the entire study period. In most other organs the radioactivity was low by 96-168 hrs.
- (3) The mean binding radioactivity to erythrocytes was 44% (at 2-hr) and 79% (at 24-hr).

**9. Recovery of radioactivity after a single intravenous dose of [<sup>14</sup>C]-Org 9487 to male Wistar rats.**  
Report #070-1195-MT, Vol. 1.35

Performed by the sponsor, Sept 1-Oct. 1, 1997

**Animals and experimentals:** Similar to the above study, but n = 2. Collection times: 24, 168 and 336 hrs. Dosage: 2.7 MBq/kg or 474 ug/kg iv (approx ED<sub>90</sub>: 2.5 mg/kg iv)

**Results:**

- (1) Total excretion was 83% (71% in feces and 12% in urine) in 7 days, and 91% (78% feces and 13% urine) in 14 days. The majority (65-75%) was excreted within 48 hrs feces. Initially, both parent compound and 3-OH metabolite (Org 9488) were the major compounds in feces, urine and tissues; but from day 7 on, only the 3-OH metabolite was present in the tissues.
- (2) On day 14, the tissue radioactivity (% of the dose) was mainly present in hind and foreleg muscles (approx. 3%) and kidneys (approx. 1%).
- (3) At all time points, the highest concentration of radioactivity (% dose/g) was in the kidneys and heart. The concentration in plasma was negligible at all time points.

**10. An ADME study after a single intravenous dose of [<sup>14</sup>C]-Org 9487 to male and female Beagle dogs.** Report #070-1147-MT, Vol. 1.35

Performed by the sponsor, Sept 16-30, 1996

**Animals and experimentals:** M & F Beagle dogs (n = 3/sex). Collection times: up to 168 hrs at 24-hr fractions. Dosage: 2 kBq/kg or approx 20 ug/kg iv. Analysis:

**Results:**

In 7 days, total excretion was approx. 65% of the dose (56% in feces and 9% in urine) in M, and 67% (55% feces + 13% urine) in F. The majority was excreted in feces within 48 hrs. Parent compound and Org 9488 were the major compounds in feces and urine. In female dogs, 60% of the radioactivity excreted in the urine during the first 24-hr was as the 3-OH metabolite, which was twice as high as in urine from the male dogs. Five other minor metabolites were also detected, two of which were not detected in rats.

**11. The roles of liver and kidneys on N-M blockade, time profiles and/or PK were studied in anesthetized cats.** The data were discussed under **I. PHARMACOLOGY**

**TOXICOKINETICS:**

**1. An acute toxicokinetic study in dogs with a neuromuscular blocking agent Org 9487 via intravenous injection in five sub-doses.** Report 070-1054-MT, Vol. 1.35

This is part of the acute toxicity study in dogs. Blood samples were taken before (- 2 min) and after (2 min) each subdose and, in addition, some extra samples were taken after the fifth subdose. Blood samples were from 1M + 1F from the low, 2M + 2F from the mid and 1M + 1F from the high dose groups. Analysis: detection limit, 80 ng/mL.

**Results** (copied from the submission):

Org 9487				
Dose (mg/kg)	Sex	AUC <sub>t</sub> (pg-h/ml)	t <sub>1/2</sub> (min)	V <sub>central</sub> (ml/kg)
5 x 0.6	Male	1.6	-	63
	Female	0.6	-	64
5 x 1.9	Male	3.9	47.7	85
	Female	4.3	66.6	63
5 x 6.0	Male	15.5	71.1	76
	Female	19.8	66.0	56

Org 9488			
Dose (mg/kg)	Sex	AUC <sub>t</sub> (pg-h/ml)	t <sub>1/2</sub> (min)
5 x 0.6	Male	-	-
	Female	-	-
5 x 1.9	Male	0.3	-
	Female	0.5	39.8
5 x 6.0	Male	2.1	47.4
	Female	2.9	85.3

- = could not be calculated

Dose-related increases of exposure (AUC) to the parent compound and its metabolite were observed. T<sub>1/2</sub> was 48-71 min; V<sub>c</sub> was 60-70 mL/kg. Extent of exposure to Org 9488 was approx. 10% of the parent compound; T<sub>1/2</sub> of Org 9488 was 40-85 min. No significant gender difference.

**2. An acute toxicokinetic study in cats with a neuromuscular blocking agent Org 9487 via intravenous injections in five sub-doses.** Report 070-1088-MT, Vol. 1.35

This is part of the acute toxicity study in cats. Blood samplings and assays were identical to the acute dog study above.

**Results** (copied from the submission):

Org 9487				
Dose (mg·kg <sup>-1</sup> )	Sex (n)	AUC <sub>t</sub> (µg·h·ml <sup>-1</sup> )	t <sub>1/2</sub> (elim.) (h)	V <sub>central</sub> (ml·kg <sup>-1</sup> )
5x0.6	Male (1)	1.4	-	36
	Female (1)	0.9	-	33
5x1.9	Male (2)	5.1	1.5	20
	Female (2)	4.6	1.4	44
5x6.0	Male (1)	30.6	1.4	36
	Female (1)	27.5	1.4	34

Org 9488			
Dose (mg·kg <sup>-1</sup> )	Sex (n)	AUC <sub>t</sub> (µg·h·ml <sup>-1</sup> )	t <sub>1/2</sub> (elim.) (h)
5x0.6	Male (1)	0.08	-
	Female (1)	0.01	-
5x1.9	Male (2)	0.11	-
	Female (2)	0.33	-
5x6.0	Male (1)	3.23	3.3
	Female (2)	-	1.3

Dose-related increases of exposure (AUC) to both the parent and the metabolite were observed. T<sub>1/2</sub> was approx. 1.4 hrs, and V<sub>c</sub> was 20-44 mL/kg for ORG9487. Exposure to Org 9488 was 5-10% that of the parent compound; T<sub>1/2</sub> of Org 9488 was 1.3-3.3 hrs. No gender differences were observed.

**3. A 4-week toxicokinetic study in Beagle dogs with a neuromuscular blocking agent, Org 9487 via intravenous injections, 2 days a week.** Report 070-1099-MT, Vol. 1.35

This is part of the subacute toxicity study in dogs. After the first and the last dose, the blood samples were obtained from 3M & 3F of each dose group in the series. The parent drug and metabolite Org 9488 were determined using an ion-pair liquid chromatographic assay with post-column extraction and detection by fluorescence.

**Results** (copied from the submission): sd: single dose; md: multiple doses.

Table 24: Pharmacokinetic Parameters for Org 9487 in Dogs

Dose (mg/kg)	Sex	Org 9487				V <sub>d</sub> (mL/kg)	
		AUC <sub>0-24h</sub> (µg·h/mL)		t <sub>1/2</sub> (h)			
		s.d.	m.d.	s.d.	m.d.		
3 x 1.0	Male	1.2	2.0	0.2	0.4	103	60
	Female	2.0	1.4	0.2	0.2	75	122
3 x 2.45	Male	6.0	10.0	0.3	0.3	76	17
	Female	4.1	5.3	0.2	0.2	113	142
3 x 6.0	Male	12.3	13.5	0.2	0.3	87	76
	Female	11.4	7.8	0.2	0.3	87	137

s.d. = single dose      m.d. = multiple dose

Table 25: Pharmacokinetic Parameters for Org 9488 in Dogs

Dose (mg/kg)	Sex	Org 9488			
		AUC <sub>0-24h</sub> (µg·h/mL)		t <sub>1/2</sub> (h)	
		s.d.	m.d.	s.d.	m.d.
3 x 1.0	Male	0.1	0.1	-	1.2
	Female	0.1	0.1	-	-
3 x 2.45	Male	0.3	0.4	0.9	1.0
	Female	0.3	0.3	0.5	0.8
3 x 6.0	Male	1.0	1.1	0.9	0.9
	Female	0.8	0.8	1.0	0.7

- could not be calculated because of lack of data  
s.d. = single dose      m.d. = multiple dose

Dose-related increases of exposure (AUC) were observed following single and multiple dosing. Male dogs showed slightly higher exposure following 8 doses than after 1 dose, whereas female dogs showed slightly less exposure following 8 doses. Male dogs also showed slightly higher extent of exposure (AUC) and lower central volume of distribution than females. These slight differences probably are not statistically significant due to variations among the small number of animals. T<sub>1/2</sub> were approx. 0.2 hr for ORG 9487 and 0.9 hr for Org 9488. Exposure to the metabolite Org 9488 was approx. 8-10% that of the parent compound.

**4. A 4-week toxicokinetic study in cats with a neuromuscular blocking agent, Org 9487 via intravenous injections.** Report 070-1085-MT, Vol.1.35

This is part of the subacute toxicity study in cats. After 3 iv doses on Day 1 and Day 25, a series of blood samples was obtained from 2-3M & 1-4F in each dose group (refer to the table under Results). Drug and metabolite Org 9488 determination: same as the dog study above. Performed by the sponsor in October, 1994.

**Results** (copied from the submission):

A summary of the results is given in the following tables:

Org 9487				
Dose (mg·kg <sup>-1</sup> )	Sex (n)	AUC <sub>0-24h</sub> (µg·h·mL <sup>-1</sup> )	t <sub>1/2</sub> (elim.) (h)	V <sub>d</sub> (mL·kg <sup>-1</sup> )
3 x 1.0	Male (2)	2.5	0.3	51
	Female (2)	1.0	0.3	138
3 x 2.45	Male (3)	3.7	0.3	104
	Female (2)	2.7	0.2	105
3 x 6.0	Male (3)	9.8	0.3	143
	Female (4)	10.0	0.2	99
3 x 1.0	Male (3)	2.5	0.2	72
	Female (2)	1.5	0.2	90
3 x 2.45	Male (3)	3.7	0.2	99
	Female (3)	4.9	0.2	150
3 x 6.0	Male (3)	13.3	0.2	104
	Female (1)	14.0	0.2	126

Org 9488			
Dose (mg·kg <sup>-1</sup> )	Sex (n)	AUC <sub>0-24h</sub> (µg·h·mL <sup>-1</sup> )	t <sub>1/2</sub> (elim.) (h)
3 x 1.0	Male (1)	0.2	0.3
	Female (1)	0.2	0.7
3 x 2.45	Male (2)	0.3	0.3
	Female (2)	0.2	0.3
3 x 6.0	Male (3)	1.1	0.4
	Female (4)	1.3	0.6
3 x 1.0	Male (3)	0.2	0.5
	Female (0)	-	-
3 x 2.45	Male (3)	0.4	0.6
	Female (3)	0.5	0.6
3 x 6.0	Male (3)	3.6	1.1
	Female (1)	1.8	0.6

-- Calculation not possible

Dose-related increases of exposure (AUC) to ORG 9487 and ORG 9488 were observed following single or multiple dosings. Cumulation was not apparent. The metabolite Org 9488 was present at approx. 10% of the parent compound. The T<sub>1/2</sub> (elim) is approx. 12 min for ORG 9487 and 36 min for ORG9488. The V<sub>c</sub> for ORG 9487 was approx. 100 mL/kg. No gender difference was apparent.

The data showed that dogs and cats had similar extents of exposure and other PK profiles

under similar experimental conditions. The extent of exposure showed dose-related effects. In both species, the acute and 4-wk studies showed different  $T_{1/2}$  (elim) and  $V_c$  both for the parent and the metabolite. Specifically, shorter  $T_{1/2}$  (elim) and larger  $V_c$  were observed in the subacute than in the acute toxicity study. The reasons are not known, but the dosages in the acute and subacute studies were different.

### VII. PACKAGE INSERT

In conformance with 21CFR 201.57, amendments of various Precautions subsections are indicated.

### VIII. OVERALL SUMMARY AND EVALUATION:

RAPLON (rapacuronium bromide) or code named ORG 9487 (ORG) is a non-depolarizing neuromuscular (N-M) blocking agent with rapid onset and short duration of action. As an aminosteroid compound, it is structurally related to pancuronium, pipecuronium, vecuronium and rocuronium which are marketed by the same sponsor (Organon). The chemical name is designated as 1-(17 $\beta$ -acetoxy-3 $\alpha$ -hydroxy-2 $\beta$ -morpholino-5 $\alpha$ -androstan-16 $\beta$ -yl)-1-allylpyrrolidinium bromide. It will be marketed as RAPLON (rapacuronium bromide) for Injection and supplied as a sterile, lyophilized cake packaged in vials for reconstitution to 2% (Refer to COMPOSITIONS for detailed ingredients). The drug product is for intravenous administration. The recommended human dose is an initial bolus dose of 1.5 mg/kg, followed by maintenance dose of either a bolus dose of 0.5 mg/kg or a 30-min infusion at 2.72 mg/kg/hr (1.36 mg/kg). It is indicated as an adjunct to general anesthesia to facilitate tracheal intubation, and to provide skeletal muscle relaxation during surgical procedures. Many preclinical studies were conducted using vecuronium, another shorter acting aminosteroid N-M blocker, and in a few studies, succinylcholine as comparison drugs.

#### EFFICACY:

The standard *in vivo* N-M blocking studies in cats, dogs, pigs and monkeys showed ORG 9487 is an N-M blocker with low potency. Comparing the  $ED_{90}$  values in different species, it is most potent in Rhesus monkeys and least potent in humans, pigs and rats. In pigs ( $ED_{90}$  = 500  $\mu$ g/kg) the compound is twice as potent as in humans ( $ED_{90}$  = 1.15 mg/kg). In general, the time profiles are comparable among the animal species and humans. ORG 9487 is much less potent than vecuronium both in animals and in humans. The potency of ORG 9487 ranged from 1/3 (pig), 1/6 (cat), 1/10 (dog), to 1/20 (monkey), and 1/20 (human) that of vecuronium. The N-M blockade time profile following 1-3X  $ED_{90}$  compares more favorably for ORG 9487 than for vecuronium with shorter onset, duration and recovery rate. For example, at  $ED_{90}$ , onset of ORG vs vecuronium was 1-3 min vs 2-7 min, duration was 9-14 min vs 8-33 min, and the recovery rate from 25-75% control was 3-5 min vs 3-10 min. In pigs, however, the duration was similar (at  $ED_{90}$ ) or longer (at

3X ED<sub>90</sub>) than vecuronium depending on the dose. In cats, dogs, monkeys and pigs by increasing dose from ED<sub>90</sub> to 2-3X ED<sub>90</sub>, a clinically useful dosage, the onset was shortened. Although the duration and recovery rate were prolonged, the time profile again compares more favorably for ORG than for vecuronium. The efficacy following infusion was shown in pigs. Infusion showed cumulative effects and prolonged the recovery rate (discussed under Activity of metabolites). In humans the ED<sub>50</sub> is approximately 0.3 mg/kg for adults, elderly, and neonates; children of 2-12 yrs old have an ED<sub>50</sub> of 0.4 mg/kg. Following an intubation dose of 1.5 mg/kg, the onset is 1.5 min, the duration is 15 min and the recovery index is 9 min. Intubation at 50 seconds was rated as good to excellent.

In comparison with succinylcholine; however, the compound does not possess the exact same profile. Although ORG has been shown to have similarly rapid onset, short duration and fast recovery rate following an intravenous ED<sub>90</sub> dose in cats, at 3X ED<sub>90</sub> (a clinical useful dose) a fast onset was followed by a longer duration and a slower recovery rate for ORG. In addition, in cats, intramuscular (i.m.) injection showed less potency than i.v. injection, requiring a higher dose of ORG (1714 µg/kg or 6X iv ED<sub>90</sub>) to produce effects comparable to i.m. succinylcholine (428 µg/kg or 5X iv ED<sub>90</sub>). At these doses, the onset (3-4 min) and duration (18-19 min) were similar in both groups; but a slower recovery rate was observed in the ORG group. It should be noted that i.m. ORG produced more variable and unpredictable block than i.m. succinylcholine: 2/6 cats failed to show N-M blockade following ORG, whereas succinylcholine produced complete block in all 6 cats. Similar to that following i.v. administration, i.m. injection of ORG (2-8X iv ED<sub>90</sub>) produced slight hypotension (6-12% decreases of mean arterial blood pressure) but no effect on heart rate. The separation ratio of vagolytic/N-M blocking ED<sub>50</sub> dose was approx. 3, and no ganglionic blocking effects were observed up to the highest i.m. doses tested, as also observed following i.v. administration. In *in vivo* studies, the potency of ORG is 0.2 (cat) and 10 (monkey) times that of succinylcholine. The *in vitro* studies of nerve-muscle preparations from guinea pigs, chicks and rats showed that ORG was more potent than succinylcholine, but less potent than vecuronium. The data indicate species and preparation differences in sensitivity to the compounds.

The mechanism of action was shown to be non-depolarizing or competitive at the neuromuscular junction, mainly at the postjunctional AChR site: AntiChE reversed and shortened the blockade in *in vivo* and *in vitro* studies. It lacked direct effects on nerve conduction, and muscle contraction could be elicited by direct stimulation of the muscle in pigs. The prejunctional site of action to inhibit ACh release was less pronounced than vecuronium: There was less TOF fade and a less marked frequency-dependent decrease in the force of contraction in the partially paralyzed guinea pig preparation. In the rat sciatic nerve preparation, electrophysiological measurements showed ORG had no effects on the amplitude or duration of nerve compound action potentials, whereas procaine decreased the amplitude and increased the duration (local anesthetic effect). The data showed that Na<sup>+</sup> channels (in either nerve or muscle) did not play a role in the blocking effect on N-M transmission.

**Reversibility by antiChE:**

The blockade can be partially reversed by anti-ChE, such as neostigmine. Pyridostigmine and edrophonium were less potent reversal agents. In anesthetized cats, the ED<sub>50</sub> for neostigmine was estimated at 36 µg/kg. At 80 µg/kg, 80% reversal was obtained. The recovery index and duration were shortened by neostigmine. Early reversal by neostigmine and physostigmine, but not edrophonium, can shorten the blockade. In addition, reversal by neostigmine is not affected by pH or acid-base imbalance.

**Activity of the metabolites:**

The potential metabolites, Org 8433 (3-OH rapcuronium), Org 9502 (17-OH rapcuronium) and Org 9504 (3,17 di-OH rapcuronium) were evaluated for N-M blocking, vagolytic and ganglionic blocking activities in cats and pigs. The potency was: in cats, Org 8433 2/3X, Org 9502 1/2X, and Org 9504 1/4X; and in pigs, Org 8433 was 2X and Org 9504 was 2/5X as potent as the parent compound. The onset was similarly rapid among the parent, 17-OH and 3,17 di-OH metabolites, whereas 3-OH had a slower onset. The duration and recovery were longer with 3-OH and 3,17-di-OH metabolites than with the parent compound. The 3-OH metabolite can have a significant role in the N-M profile because of its substantial N-M blocking activity and longer duration of action. For all 3 potential metabolites, the separation of N-M blocking and vagolytic activities was poor (ED<sub>50</sub> ratio of less than 1). Thus, the possibility of the metabolites contributing to tachycardia should be considered whenever substantial amounts of the metabolites are formed, e.g., following prolonged infusion or repeated high dose. Ganglionic blocking activity was observed at very high doses for all compounds. The recovery rate following infusion of either the parent compound or the metabolites was longer than that following bolus administration. Following a 75-min infusion of ORG 9487 in pigs, the 50%-90% recovery time was 11 min compared to 5 min following bolus injection. The corresponding times for 3-OH and 3,17 di-OH were 15 and 14 min after infusion, and 9 min and 6 min following bolus injection, respectively. Steady state infusion doses of Org 9487 and 3-OH metabolite were approx. 25% and 36% lower towards the end of the infusion compared to the start dose suggesting cumulative effects. The infusion dose of 3,17 di-OH remained constant throughout the infusion. The infusion study again showed that the metabolites, especially the 3-OH, can have significant roles because of its potency, long duration and recovery.

**Cumulative effects:**

In cats, Org 9487 did not show significant cumulative effects except during the first two bolus doses. In pigs, however, potential cumulative effects were shown following a 75-min infusion (discussed above).

**Acidosis and alkalosis:**

In pigs, respiratory acidosis and metabolic alkalosis potentiated the blockade and increased the time profiles, whereas metabolic acidosis potentiated only the blockade. Respiratory alkalosis decreased the blockade and time profiles. *In vitro* preparations of guinea pig phrenic nerve hemidiaphragm also showed similar effects of acidosis and alkalosis on the depth of blockade, except

that both metabolic and respiratory alkalosis antagonized the blockade.

**Role of liver in N-M blockade:**

The role of the liver was studied in cats following both liver shunt model (portacaval shunt) and intraportal injection. The duration and recovery were slightly prolonged following liver bypass, but intraportal injection did not show significant effects. The data suggest minor roles for liver in the pharmacodynamics of the compound. Because the liver is probably the site of metabolism and the major route of excretion is in the bile/feces, the role of the liver should be considered important under certain circumstances, e.g., liver insufficiency or cirrhosis.

**Role of kidney in N-M blockade:**

Renal pedicle ligation in anesthetized cats showed only minor effects on the recovery and dose requirement following either bolus or infusion. The PK data were not significantly altered either. The data indicate a minor role of the kidney in the ORG-induced N-M blockade. Under certain circumstances, such as renal insufficiency or failure, the role of the kidney may play a significant role in the N-M blockade, because urinary excretion accounted for approximately 10% of the dose in rats, cats and dogs.

**Interactions:**

Interaction studies in anesthetized cats showed that volatile anesthetics (halothane, isoflurane and especially enflurane) potentiated and prolonged recovery from the N-M blockade of ORG. Intravenous anesthetics (thiopental, propofol, midazolam and droperidol) did not show significant interaction. Premedication agents such as diazepam and chlorpromazine were without significant interaction; morphine and fentanyl slightly potentiated the block. Streptomycin and phenytoin potentiated the blockade and slowed the recovery. Interactions with other N-M blockers have not been studied in animals despite the agency's recommendation to do this during drug development. The available clinical data probably can address this issue.

**Inhibition of acetylcholinesterase:**

In *in vitro* human plasma ChE and RBC AChE assays, ORG inhibited acetylcholinesterase to a greater extent than vecuronium or pancuronium. ORG also inhibited non-specific plasma ChE. Thus, with ORG's low potency, it compares less favorably than vecuronium or pancuronium in certain clinical applications where metabolism by AChE is considered important.

**CV/autonomic nervous system:**

The cardiovascular activities were studied in anesthetized and ventilated dogs, cats, monkeys, and pigs. Although biphasic or triphasic changes were possible, in most instances, transient, dose-related decrease in BP and increase in HR were observed at 5 min, and by 10 min, these effects had returned to normal. For example, following 3X ED<sub>90</sub>, ORG produced transient hypotension in dogs (-20%) and pigs (-10%), hypertension (+15%) in cats but no effects on BP in monkeys. The hypotension could be, in part, due to a direct relaxant effect on vascular smooth muscle through inhibition of voltage-activated, L-type Ca<sup>++</sup> channels, as observed with other structurally related

N-M blockers. Heart rates were not significantly affected in cats, monkeys and pigs, but increased 10% in dogs. The absence of apparent HR effect in some species may be due to low vagal tone in cats and pigs, and administration of atropine in the monkey studies. In dogs, where detailed measurements were made, 3X ED<sub>90</sub> decreased right atrial pressure, pulmonary artery pressure, pulmonary capillary wedge pressure, caused an initial decrease followed by an increase in heart rate, and cardiac output, but the changes were not greater than 10%. In cats, ORG had similar CV effects under both normal and abnormal acid-base conditions. In pithed rats, no norepinephrine reuptake blocking activity was observed. Propensity for histamine release has not been studied in animals, despite a request from the agency to study this during drug development. The available clinical data showing histamine release in some patients has been included in the label.

Adverse ECG changes were dose- and duration-related. For example, following a bolus dose of 27 mg/kg in dogs, prolonged QT interval, sinus arrhythmia with prolonged PR, P widening, some P and T complexes, and AV dissociation with accrochage were observed. In cats, right bundle branch block pattern and prolonged PR intervals were observed following a bolus dose of 26 mg/kg. These doses are approximately 23X the human ED<sub>90</sub>. At lower doses, several days of treatment was needed in order to see some of the ECG effects. For example, in subacute toxicity studies following seven days of treatment at 3 subdoses of 6 mg/kg at 30-min intervals on each treatment day, prolonged QT interval was observed only in dogs, but not in cats. The total daily dose (18 mg/kg) is approximately 16X the human ED<sub>90</sub>. When cats and dogs were treated for only one day with 5 subdoses of 6 mg/kg at 30-min intervals, there were no ECG changes in either species. Although this dose is approximately 26X the human ED<sub>90</sub>, the dose was divided into five subdoses, each approximately 5X the human ED<sub>90</sub>. (Refer to Acute and Subacute toxicity studies). In a specific study in pigs, no ECG changes were observed following 3 subdoses of 10X the pig ED<sub>90</sub>, given at 10-min intervals. In terms of the actual dosage, 3X 5 mg/kg (pig ED<sub>90</sub> = 500 µg/kg), this "no-effect level" is lower than that employed in the one-day studies in cats and dogs, i.e., 5X 6 mg/kg. Thus, in various animal species the NOAELs for ECG effects are 15-30 mg/kg when given for a one-day treatment or 7.5 mg/kg for multiple-day treatment, in protocols where the doses were given in divided subdoses. Extrapolation of these results to humans would suggest a safe dose at 7.5 mg/kg given in 3 subdoses which is approximately equivalent to the recommended human dose based on a mg/m<sup>2</sup>. The exact mechanism of this QT prolongation is unknown, but the available data suggest inhibition of cardiac K<sup>+</sup> channels. In isolated rabbit papillary muscles, lengthening of repolarization (inhibition of the rectifying repolarizing K<sup>+</sup> current) suggests inhibition of cardiac K<sup>+</sup> channels. An *in vitro* receptor binding study showed significant inhibition of the apamin-sensitive K<sup>+</sup> channel (controlling low conductance potassium channels) prepared from rat forebrain; but it is not known whether the apamin-sensitive K<sup>+</sup> channel is also present in cardiac tissues. The exact mechanism remains to be elucidated. The animal data suggest potential dose- and duration-related adverse ECG effects in patients receiving a single high bolus dose, such as 23X ED<sub>90</sub>, or following repeated treatment or prolonged infusion at a total dose of 16X ED<sub>90</sub>. It is noted that 5X the human ED<sub>90</sub> can be given 3-5 times in one day without causing ECG effects, whereas multiple dosings of this same dose over several days can have adverse ECG effects. The potential for adverse ECG effects is recommended to be included in the

package insert.

Vagolytic effect may contribute to tachycardia. Inhibition of autonomic nervous system was studied in pentobarbital/chloralose-anesthetized cats. Separation ratio ( $ED_{50}$  values) of vagolytic/N-M blocking activities was 3 (similar to pancuronium), and that of ganglionic/N-M blocking activities was 24 (poorer than most other N-M blockers). The data suggest that following clinical relevant doses of 3X  $ED_{90}$ , some hypotension and tachycardia can be observed in patients. In pithed rats, ORG did not appear to block catecholamine reuptake in sympathetic nerve terminals which contributes, in addition to a vagolytic effect, to pancuronium's tachycardic effect. The potential metabolites of ORG produced phasic effects on BP (moderate hypertension followed by hypotension and then hypertension); but there were no effects on heart rate in cats. The major metabolite is the 3-OH compound.

#### Receptors:

A wide variety of receptors was included in the *in vitro* binding study. At  $10^{-5}M$  both Org 9487 and the 3-OH metabolite were without significant effects on most receptors except for a 50% inhibition at the apamin receptor (controlling low conductance potassium channels). The role of this inhibition in the QT prolongation is discussed under CV effects.

#### Hormonal effects:

In the immature rat model, ORG (1.22-2.45 mg/kg/day sc for 7 days) did not show significant dose related effects on various organ weights. According to the applicant, the data suggest no significant effects on estrogenic, androgenic, anabolic, gonad-inhibiting, or glucocorticoid-like effects. But this is a crude screening, the exact hormonal effects remain unknown.

#### Toxicity:

Acute intravenous toxicity studies were carried out in anesthetized and ventilated cats and dogs, first in dose range finding and then in 5-subdose studies. The results show no mortalities at 13.5 mg/kg (single) or 39-41 mg/kg (two doses at ½ hr apart: 13-14 + 26-27 mg/kg) or 5 X 6 mg/kg (at ½ hr apart) in dogs or cats, except for one cat that died of severe hypotension after 13 mg/kg. Drug-related ECG changes were observed in the cat at 26 mg/kg and the dog at 27 mg/kg (most likely due to the second dose of 27 mg/kg). The ECG changes included prolonged QT interval, sinus arrhythmia with prolonged PR interval, P-wave widening and some P & T complexes, AT dissociation with accrochage in the dog, and right bundle branch block, prolonged PR interval and QRS and ST segment deviation in the cat. The ECG abnormalities were reversible during a 2-week recovery period. It should be noted that in pigs, no QT effect was observed following 3 subdoses of 10X the pig  $ED_{90}$ . The potential for ECG effects in humans should be considered probable following high bolus dose or prolonged infusion (Refer to page 67 for detailed discussion). Hypotention that was dose-related was observed in both dogs and cats at 6.5-27 mg/kg, but was significant only at 10X the human  $ED_{90}$ . The BP effect was reversible on the same day of the study. No target organ toxicity was established.

Subacute toxicity studies (3 subdoses/day at ½ hr intervals, 2 treatments/wk for 4 weeks) were carried out in anesthetized and ventilated Beagle dogs and cats. The iv dosages were 3X 1.0, 3X 2.45, and 3X 6.0 mg/kg. In dogs, QT prolongation was observed in the ECG following 3 X 6 mg/kg on the 7th dosing days (total dosing days was 8). In cats, one high-dose cat died of severe hypotension. Increased BUN was observed at the high dose in both dogs and cats, but only cats showed renal interstitial inflammation, mineral deposits in the medulla and protein in the urine. The data strongly suggest the kidney is the target organ. No other significant drug-related effects on body weight, food consumption, clinical pathology, gross or histopathology were observed. The injection site showed subcutaneous hemorrhages, inflammation, and fibrosis in many of the animals, including the controls. The adverse effects (prolonged QT interval, hypotension, and target organ toxicity in the kidney) were observed at the high dose of 18 mg/kg/day (LOAEL). This high dose is equivalent to approximately 3X the recommended human dose on a mg/m<sup>2</sup> basis. The NOAEL is at 7.5 mg/kg in both species, or approximately 1.5X the recommended human dose on a mg/m<sup>2</sup> basis. The corresponding AUC's are approximately 11-14 (LOAEL) and 4-8 (NOAEL) µg·hr/ml. The plasma C<sub>max</sub>'s are not known, because the data were not submitted. The subacute toxicity data support the reasonable safety for the recommended dose in humans.

The extent of exposure was dose-related and similar in both species. For example, in subacute toxicity studies following 3X 1 to 3X 6 mg/kg, the AUCs were approximately 1.7-11 µg·h/ml in dogs, and 2-14 µg·h/ml in cats. No apparent accumulation or gender difference was observed. The T<sub>1/2</sub> (elim) and V<sub>c</sub> were different following acute and subacute toxicity studies, the reasons are not known. The dosages were different in the acute and subacute studies.

#### Toxicity of Impurities and degradation products:

The acute toxicity of seven impurities and degradation products at a total concentration of 5.1 mg/mL was studied in dogs. Refer to page 38 for details of the components. Dosages in the range of 0.1-1.5 mg/kg (or approx. 12X the recommended human dose of 3 mg/kg) were given in 3 subdoses at 30-min intervals. The animals were observed for 14 days before necropsy. No significant toxicity or treatment-related adverse effects, including ECG changes, were observed. As expected from these active impurities and degradation products, the N-M blockade following the last subdose had a duration of 2-4 hrs.

#### Special studies:

##### Compatibility with blood components:

The drug, but not the final product, was studied. ORG adjusted to pH 4 caused slight hemolysis at 6 and 15 mg/kg in the rat *in vivo*; but not at 120-300 µg/mL in the human blood *in vitro*. The peak human plasma concentration was approx. 40 µg/mL following an intubation dose of 1.5 mg/kg. Therefore, no hemolytic potential is anticipated following the recommended dose, but at high bolus doses of 5-12X the human ED<sub>90</sub>, hemolytic potential is present based on the *in vivo* rat data. ORG did not cause significant osmotic red cell fragility or protein flocculation in both *in vivo* and *in vitro* studies. No plasma protein flocculation was observed at 300 µg/mL, equivalent to human plasma concentration following 13X the human ED<sub>90</sub>. The data do not indicate compatibility

problem for the proposed market product, but the available clinical data using the final formulation should provide confirmation on this aspect of safety.

**Local Tolerance:**

Local tolerance was studied in two separate studies in rabbits. Intramuscular injection of 2% formulation up to 0.3 mL/kg produced mild irritations of incidental hemorrhage, inflammation or necrosis that were similar to those observed following im injection of a commercial succinylcholine (2%) at 0.2 mL/kg. Following iv, im, intraarterial, or paravenous site injection of 0.2 mL/kg of 2% solution stored in either a Wheaton vial with rubber stopper 4416/50 or a Schott vial with rubber stopper 9310/50, mild irritations were observed.

**Malignant hyperthermia:**

ORG did not trigger malignant hyperthermia in MH-susceptible swine which indicates a lack of this potential in humans. At doses up to 6X ED<sub>90</sub> (2.64 mg/kg), no signs or parameters consistent with malignant hyperthermia were observed. The susceptibility of the animals was verified by both *in vivo* challenge with halothane and succinylcholine and *in vitro* muscle strip contraction in the presence of halothane and caffeine.

**Effects on subcellular uptake and functions of rat hepatocytes:**

Similar to that of pancuronium and rocuronium, accumulation of ORG in the mitochondrial and lysosomal fractions of rat hepatocytes was observed at 120 min. The recovery of radioactivity was 13% (5 min) and 2.5% (120 min) of dose. Mitochondrial respiratory function was not affected. There was no effect on viability, LDH leakage, ATP content and energy charge of hepatocytes at 40 µg/mL, the peak human plasma concentration following 1.5 mg/kg. At concentrations greater than 200-500 µg/mL, however, effects on some of these parameters were observed.

**Reproduction:**

Segment II reproduction studies were conducted in unanesthetized and unventilated Sprague-Dawley rats (bolus) and rabbits (continuous infusion). The pregnant rats (25/group) were treated intravenously with vehicle, 3X0.25, 3X0.5 and 3X0.75 mg/kg on days 6-17 post coitum. Three subdoses were given at ½ hr intervals. Fetuses were delivered by C-section on Day 21 and studied according to the standard protocol. There were 5 deaths from apnea at the high dose. Dose-related signs in all treated groups included excitation, clonic spasms, breathing sounds, respiratory distress and apnea of short duration. Signs lasted 10 sec to 4.5 min. Body weight and food consumption were reduced only at the high dose. No teratogenic effects were observed. Post-implantation losses due to fetal deaths with subsequent resorption were slightly increased at the high dose. Mean fetal weight was slightly reduced in the high dose group. Ossification variations, observed in all 4 groups, were within control ranges. The adverse fetotoxic effects occurred at the high dose of 3X 0.75 mg/kg, equivalent to approx. 2X the human ED<sub>90</sub>. The NOAEL of 3X0.5 mg/kg approximates the human ED<sub>90</sub>.

The Segment II study in non-ventilated rabbits was preceded by a dose-range finding study in pregnant rabbits. The main study included 20F/group, each treated at 0, 0.75, 1.5 or 3 mg/kg/d by continuous 24-hr infusion (@ 4.8 mL/kg/day) during days 6-18 of gestation. Because of 4 deaths at 3 mg/kg, only 10 animals were enrolled in this high-dose group. Signs preceding death were body tremors, subdued behavior and rapid breathing. These signs were also observed in the surviving high-dose F. There were no teratogenic effects, judging from the external, visceral and skeletal examinations. Increased post-implantation losses, due to early and late resorption, were observed in all dosed groups, but there was no obvious dose-related effect. However, the high dose group had a smaller number of animals (10 instead of 20 enrolled due to deaths), and the evaluable dams was reduced to 6 because of 4 deaths during the study. It is noted that clinical signs of N-M blockade were not observed in the low- and mid-dose groups, raising the question whether hypoxia contributed to the fetal deaths. The NOAEL is not established.

Because of embryo- and fetodeaths observed in rabbits and rats, the drug product should be placed under Pregnancy Category C in the label (Refer to pages 75 and 77 for details). Although hypoxia caused by the N-M blockade may contribute to this fetotoxicity, the low- and mid-dose groups of rabbits that showed increased post-implantation losses, did not exhibit signs of N-M blockade or hypoxia. The rabbit is known to be sensitive to non-depolarizing N-M blocking agents.

#### Mutagenicity:

Five tests were conducted. *In vitro* gene mutation assays in the Ames Test (*Salmonella typhimurium* and *E. coli*), and mouse lymphoma L5178Y TK +/- cells were negative, both in the presence and absence of S9 metabolic activation. The *in vivo* micronucleus assay in rats was also negative. *In vitro* human lymphocyte chromosomal aberration assays, however, showed significant increases of structural aberrations in the absence of metabolic activation. The sponsor felt that the findings were of no biological significance because the increases often were not present in both duplicates, not apparently dose-related, confounded by precipitate formation, and a repeat study in another contracting lab failed to substantiate the positive findings. This reviewer, however, disagrees with the sponsor's conclusion. The increases were outside the historical control values and attained statistical significance. Furthermore, in both assays where the number of cells with aberrations increased, the total number of aberrations (both with and without gaps) also increased. To resolve the discrepancy between this reviewer's and the sponsor's conclusion, on December 16, 1998, a consulting review on these two tests were requested of Dr. Anita Bigger, Cochair of the CDER Genetic Toxicology Committee. Her report of January 18, 1999 is attached. Her conclusion is that the first test is inconclusive and the second test is positive. Her more conservative recommendation is to have the second laboratory (NOTOX) repeat the test, and her less conservative recommendation is to include the results of both tests in the label. This reviewer opts to adopt her less conservative recommendation by including both tests in the label.

The Ames test was negative at concentrations up to 10,000 µg/plate (+ S-9) and 3200 µg/plate (- S-9) in 4 *S. typhimurium* strains and *E. coli*. The study was preceded by cytotoxicity tests. A

gene mutation test in mouse lymphoma cells was negative at concentrations up to 1500 µg/ml (+ S-9) and 1000 µg/ml (- S-9). The *in vivo* micronucleus test in rats (treated i.v. for 3 days at 0, 3X0.25, 3X0.50 and 3X0.75 mg/kg/day, three subdoses at 3 hrs apart), did not show significant micronucleated cells in polychromatic erythrocytes from the bone marrow of the femur. The ratio of polychromatic to normochromatic erythrocytes was unremarkable. The high dose is equivalent to approximately 2X the human ED<sub>90</sub>.

Although a clastogenic activity of ORG is shown in the *in vitro* human lymphocyte chromosomal aberration assays, three other mutagenicity tests of the standard battery were negative. In the absence of mechanistic data, and because of occasional clinical use of neuromuscular blocking agents, a genotoxic risk is not readily apparent. The results from all tests, including the positive finding, should be included in the package insert.

**Absorption, Distribution, Metabolism and Elimination/Pharmacokinetics/Toxicokinetics:**

The available PK/ADME data are similar in rats, cats and dogs. The available human data showed similarly rapid initial clearance as in cats; but humans had a larger V<sub>c</sub> and a slower terminal T<sub>1/2</sub> than cats. No PK data from the pig and monkey are available for comparison.

**Absorption:** Following intravenous administration, the compound showed rapid distribution and elimination from the plasma; terminal half-life was 12-18 min in cats and dogs, and 34 min in rats; and in humans T<sub>1/2</sub> was 5 and 28 min (rapid and slow distribution) and 141 min (terminal elimination). Preliminary data indicated that the extent of plasma protein binding was highly variable in each species, ranging 20-70% in dogs and 40-80% in cats and humans.

**Distribution:** Two tissue distribution studies were conducted in rats with radioactive Org 9487. High radioactivity levels were observed in small intestine, kidney and heart initially. By days 1, 4 or 7 days, most tissues had low levels. The level in the hypophysis, however, remained relatively constant. At all time points, the blood, plasma, brain and fat had low levels. On day 14, in terms of the % of the dose, the radioactivity was highest in the hindleg and foreleg muscles accounting for approx. 3% of the dose and less in the kidneys (1%), suggesting residual accumulation in these organs. In liver, kidneys, heart and muscles, both the parent compound and 3-OH metabolite were present; but by 7 days; these tissues contained only the metabolite. In rats, extents of enterohepatic circulation and placental transfer were not significant. The V<sub>d</sub> at the steady state is estimated at 292 mL/kg in humans.

**Metabolism:** ORG9487 was not metabolized extensively, as the parent compound was the major product excreted by rats, dogs and humans. Hydrolysis appears to be the major metabolic pathway. The liver is probably the major site of metabolism, based on the results from the *in vivo* and *in vitro* (liver perfusion and postmitochondrial incubation) studies. The liver-shunt model or intraportal injection in cats, however, showed only small extent of increases in the depth and time profile. The major 3-OH metabolite was detected in the plasma, feces/bile and urine. The extent of

exposure to this major metabolite was approximately 10% of the parent compound in the acute and subacute toxicity studies in cats and dogs. Other metabolites include 17-OH, and 3, 17-di-OH metabolites, plus several other minor metabolites. The metabolites and impurities show varying degrees of N-M blocking activity, as well as prolonged time profile and vagolytic effects. Therefore, their contributions to prolonged blockade and tachycardia should be considered following repeated high doses or prolonged infusion.

**Elimination:** The initial excretion is rapid. In rats and cats, total excretion was 40-50% of the dose in 2-6 hrs; 65-75% of the dose in rats and 51% of the dose in dogs by 48 hrs. The major elimination route was the bile/feces, accounting for approximately 40% of the dose (25% as parent compound + 5-10% as 3-OH metabolite + others). Urine was a minor route, accounting for 5-8% of the dose (mainly as the parent compound). Following initial rapid excretion, a slower elimination phase in rats and dogs was observed. In rats, total excretion in 7 days was 73-83% (54-71% in feces and 9-12% in urine) of the dose. This is similar to the findings in dogs following single intravenous administration: 66% in 7 days (55% in feces and 11% in urine). Again, the drug profile (major components as the parent compound and 3-OH metabolite) excreted by the dog was similar to those in rats and cats. In the dog, no significant gender effect was observed. In humans, the urinary excretion profile during 48 hrs [13% of the dose (8% parent compound + 5% 3-OH metabolite)] was qualitatively similar to that of rat, cat or dog. In humans, the mean Cl is 6.6 mL/kg/min.

**Roles of liver and kidney in the PD/PK:** Renal ligation in anesthetized cats showed only slight effects on PD/PK parameters following either bolus or infusion, indicating minor role of kidneys in the PD/PK of the compound. The liver-shunt model or intraportal injection in cats showed only small increases in the depth and time profile. PK/ADME data from other species, such as monkeys and pigs used in pharmacodynamic studies, are not available. The PK/PD relationship data is not available in animal species.

**TK data are discussed under Toxicity. The N-M blocking and adverse CV activities of the potential metabolites and impurities/degradation products are discussed under Potential Metabolites**

**Package Insert :** Deficiencies are noted. Before the NDA can be approved, amendment is needed in the Precautions subsections.

## **IX. CONCLUSION:**

Based on the available nonclinical data generated from both in vivo and in vitro studies, the following pharmacological and toxicological profiles can be made:

- (1) Org 9487, an aminosteroid compound, was shown to be a non-depolarizing neuromuscular blocking agent with rapid onset, short duration of action and rapid recovery rate. The site of action is mainly at the postsynaptic junctional ACh receptors. The potency is low: at approximately 1/3 (pig) to 1/20 (monkey) that of vecuronium. Org 9487 is least potent in man; the potency in animal species is 2-5X greater than in man. Org 9487 can be reversed by anti-ChE such as neostigmine. The time profile of Org 9487 is more similar to that of succinylcholine (SCC) than vecuronium, but this is dose-dependent. At 3X ED<sub>90</sub>, the onset is shortened, but ORG has longer duration and slower recovery rate than SCC. Intramuscular administration of ORG requires high doses to produce a rapid onset and short acting profile as that of intramuscular SCC, but the recovery rate is slower and the blockade is variable and unpredictable with ORG.
- (2) In cats, Org 9487 did not show significant cumulative effects except during the first two doses. In pigs cumulative effect was shown following infusion. In cats, separation of neuromuscular blocking activity and inhibition of the autonomic nervous system was not particularly marked, but generally free of vagolytic and ganglionic blocking activities at 3X ED<sub>90</sub> doses.
- (3) Org 9487 generally produced transient hypotension (10-20% decreases) following 3X ED<sub>90</sub> in cats, dogs, and pigs; hypertension preceding hypotension was observed in cats. Heart rate was not significantly affected or slightly increased at this dose. Effect on histamine release was not studied in animals. The available clinical data showing histamine release in patients has been included in the label. ECG changes were observed following high doses [Refer to (7) and (8)].
- (4) Interaction studies in cats showed that volatile anesthetics, especially enflurane, potentiated the depth and prolonged the recovery from the N-M blockade of Org 9487 whereas i.v. anesthetics (thiopental, propofol, midazolam and ketamine) did not show significant interactions. Streptomycin and phenytoin also potentiated the blockade. Opioids and benzodiazepines did not show significant interactions. Interactions with other N-M blockers have not been studied in animals and are probably similar to those of other non-depolarizing agents. The available clinical data probably can address this issue.
- (5) In pigs, respiratory acidosis and metabolic alkalosis increased the blockade and increased the time profiles, whereas metabolic acidosis only increased the block. Respiratory alkalosis decreased the blockade and time profiles.
- (6) *In vitro* receptor binding studies suggested ORG had high specificity for postjunctional Ach receptors at the N-M junction; the compound had low affinities for serotonin, histamine, opiate and various other receptors except the K<sup>+</sup> sensitive apamin receptor. Org 9487 inhibited acetylcholinesterase to greater extent than did vecuronium or pancuronium.
- (7) Acute intravenous toxicity studies did not show mortalities at 13.5 mg/kg (single) or 39-41 mg/kg (two doses at ½ hr apart: 13-14 + 26-27 mg/kg) or 5 X 6 mg/kg (at ½ hr apart) in dogs or

cats, with one exception where one cat died of severe hypotension after 13 mg/kg. Drug-related ECG changes were observed in cats and dogs following a total dose of 40 mg/kg (most likely due to the second dose of 26 mg/kg). The ECG changes included prolonged QT interval, sinus arrhythmia with prolonged PR interval, P widening and some P & T complexes, AT dissociation with accrochage in the dog, and right bundle branch block, prolonged PR interval and QRS and ST segment deviation in the cat. The ECG abnormalities were reversible following a 2-week recovery period. Thus, in cats and dogs, the LOELs for ECG effects are 27 mg/kg, and the NOAELs are 5 subdoses of 6 mg/kg for a one-day treatment. The ECG changes following multiple dosing is discussed under (8). Dose-related extent and duration of hypotension were observed in both dogs and cats from 6.5-27 mg/kg, with significance only at 10X the human ED<sub>90</sub>. The BP effect was reversible on the same day of the study.

(8) Subacute toxicity studies (a total of 8 treatment days in 4 weeks; on each treatment day 3 subdoses of 1, 2.45, and 6 mg/kg each given at ½ hr intervals) were carried out in Beagle dogs and cats. Drug-related effects, observed at the high dose, included prolongation of QT in the ECG in dogs, hypotension in both dogs and cats, and the kidney as a probable target organ of toxicity in both species. The renal effects included increased serum BUN in both species with accompanied inflammation and mineral deposits in the medulla and protein in the urine in the cat. In both cats and dogs, TK data showed dose-related extent of exposure to the parent compound and the 3-OH metabolite which was present at 10% of the parent compound. No apparent drug accumulation following repeated doses over a 4-wk period, nor was significant gender difference in the PK observed in these species. The effects were observed at the high doses of 18 mg/kg/day. This LOAEL is equivalent to approximately 3X the recommended human dose on a mg/m<sup>2</sup> basis. The NOAEL was estimated at 7.5 mg/kg, or approximately 1.5X the recommended human dose on a mg/m<sup>2</sup> basis. The corresponding AUC's are approximately 11-14 (LOAEL) and 4-8 (NOAEL) µg•hr/ml. The data support the reasonable safety for the recommended dose in humans.

(9) Following single administration, the drug did not cause severe local irritation to the vein, artery, muscle or paravenous sites. Repeated injections caused irritations of hemorrhage, inflammation, edema or redness.

(10) The drug was compatible with the blood components such that no hemolysis or protein flocculation is anticipated. Extremely high doses of 5-12X the human ED<sub>90</sub> may cause hemolysis based on an *in vivo* rat study. The drug product with its excipients was not specifically tested. If available, the clinical data can be used to address this issue.

(11) No teratogenic effects were observed in rats and rabbits up to the highest doses tested (2.25-3 mg/kg, or approximately 0.1X-0.3X recommended human dose on a mg/m<sup>2</sup> basis). Embryocidal effects as evidenced by increased resorption were observed in rabbits at 0.75 mg/kg, and fetotoxicity as evidenced by increased fetal deaths and subsequent resorption was observed in rats at 2.25 mg/kg. These doses are equivalent to approximately 0.1 times the recommended human dose on a mg/m<sup>2</sup> bases.

(12) Org 9487 was not mutagenic in the Ames Test, mouse lymphoma gene mutation and rat micronucleus assays. Org 9487 possesses a clastogenic activity in the *in vitro* human lymphocyte chromosomal aberration assay in the absence of metabolic activation.

(13) Org 9487 did not trigger malignant hyperthermia in susceptible swine, suggesting a lack of this potential in humans.

(14) The pharmacokinetic/ADME data are similar in rats, cats, dogs and humans. The compound showed rapid distribution and elimination from the plasma in animal species. Humans showed similarly rapid initial distribution, but had a larger  $V_c$  and a slower terminal  $T_{1/2}$  than did animal species. The extent of plasma protein binding ranging from 20 to 80% was not high. It was variable in animals and humans. Org 9487 was not metabolized extensively (no more than 50%). Hydrolysis, probably in the liver, is the major biotransformation pathway. Although the major 3-OH metabolite was present in the plasma, bile/feces and urine, the main excretion product was the parent compound. The excretion was rapid initially but was slow for the residual dose. Bile/feces were the major, and urine the minor, route of excretion. The urinary excretion profile in humans is similar to that of animals. In rats, Org 9487 was distributed mainly to the small intestine, kidneys, and heart. Brain, muscle, fat and skin had low levels. The leg muscles of rats, however, contained 3% of the dose 14 days after drug administration. The drug was taken up by mitochondria and lysosomes, but it did not affect mitochondrial functions or cause cell death. In rats, no significant enterohepatic circulation or placental transfer occurred. No PK data from monkeys or pigs are available for comparison with other animal species or humans.

(15) The major 3-OH metabolite was shown in cats and pigs to have a potency 2/3-2X that of the parent compound and a longer elimination half-life. The extent of exposure to this metabolite can be at 10% of the parent compound following single or repeated administrations in both dogs and cats. The metabolite had relatively high vagolytic activity which may contribute to tachycardia. To lesser extent were present two other hydrolytic metabolites 17-OH and 3,17 di-OH, which were also shown to possess varying degrees of N-M blocking activity, as well as vagolytic effects. The role of the metabolites in prolonging blockade and tachycardia in humans, therefore, should be considered following repeated high bolus dose or prolonged infusion of Org 9487.

(16) Impurities and degradation products did not show toxicity in dogs following an acute intravenous administration of 5 mg/kg/day. Most of the impurities and degradation products possess significant N-M and autonomic blocking activity. Therefore, their contributions to the blockade and tachycardia should be considered when the product is used at high, repeated bolus dose or following prolonged infusion.

In animal species, Org 9487 (rapacuronium bromide) has been shown to possess low potency, but with rapid onset, short duration and fast recovery rate as succinylcholine following an intravenous  $ED_{90}$  dose. At 3X  $ED_{90}$ , a clinically useful dose, however, both the duration and recovery rate

were slower with Org 9487 indicating that Org 9487 does not have an identical profile to that of succinylcholine. In addition, the intramuscular injection that is used with succinylcholine clinically not only required high doses of Org 9487 but the N-M blockade was unpredictable, whereas intramuscular succinylcholine consistently produced complete and satisfactory blockade. Org 9487 showed dose-related hypotensive and tachycardia effects, but within the clinically useful doses, the effects were generally mild and transient. In dogs and cats, drug-related toxicities included QT prolongation in the ECG, and kidney effects (as evidenced by increased serum BUN some accompanied by inflammation and mineral deposits in the medulla of the kidney and protein in the urine). The effects were observed at high doses of 18 mg/kg/day. This high dose is equivalent to approximately 3X the recommended human dose on a mg/m<sup>2</sup> basis. The NOAEL was estimated at 7.5 mg/kg, or approximately 1.5X the recommended human dose on a mg/m<sup>2</sup> basis. Adverse reproductive effects of postimplantation losses in rats and rabbits were observed at 0.75 and 2.25 mg/kg, respectively, or approximately 0.1X the recommended human dose on a mg/m<sup>2</sup> basis.

Other pharmacological and toxicological profiles generated from nonclinical *in vivo* and *in vitro* studies have provided efficacy and reasonable safety for Org 9487, such that the drug product can be labeled (on Mutagenesis and Pregnancy subsections) for human use. The product is, therefore, approvable based on the pharmacology. Before the NDA can be approved, however, revision of the proposed package insert is needed.

#### X. RECOMMENDATIONS:

The pharmacological and toxicological profiles generated from nonclinical *in vivo* and *in vitro* studies have provided efficacy and reasonable safety for Org 9487. It can be labeled for human use. The product is, therefore, approvable based on the pharmacology. Before the NDA can be approved, however, revision of the proposed package insert is indicated.

#### X. PHARMACOLOGY PORTION OF LETTER TO APPLICANT:

✓

✓

/S/

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

cc

Original NDA 20-984

HFD-170/Div. File

HFD-170/DHJean

HFD-170/SSamanta

HFD-345

F/T by DHJean 2/8/99

N20984

D. file  
APR 21 1999

**ADDENDUM TO PHARMACOLOGY REVIEW OF February 8, 1999**  
Reviewer: Dou Huey (Lucy) Jean, Ph.D.

**NDA 20-984 RAPLON (rapacuronium bromide) for Injection**

**Sponsor:**

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

**TYPES, DATES AND REVIEW OF SUBMISSIONS:** Original of June 30, 1998,  
reviewed on February 8, 1999

**DATE OF THIS ADDENDUM:** April 21, 1999

**COMMENTS:**

The addendum is in response to Dr. Joseph DeGeorge's comments  
on the proposed Phase IV nonclinical study and package insert.  
Dr. DeGeorge is Associate Director for Pharmacology/Toxicology, ORM, CDER.

**(A) Phase IV study as proposed by Dr. Joseph DeGeorge**

Although technically challenging\*, the proposed study will be forwarded to the  
Applicant

\*Intravenous administration, the intended route of administration for Raplon in  
patients, would be extremely difficult for newborn rat pups. Dr. DeGeorge  
indicated (during our telephone conversation of 4/15/99) that intraperitoneal or  
intramuscular injection would suffice. Survival of the unventilated pups due to  
respiratory distress/apnea given Raplon can be problematic; Dr. DeGeorge  
recommended sub-therapeutic doses. The sub therapeutic doses can be  
exceedingly low for newborn rat pups. The extent and rate of tissue distribution  
and elimination can be different following the ip/im vs iv. The interpretation of  
the data and its relevance to human use can be confounded by the factors just  
indicated.

**(B) Package Insert:**

**(1) Carcinogenesis, Mutagenesis, Impairment of Fertility:**

This section has been rewritten according to Dr. DeGeorge's comment.

**(2) Pregnancy Category C:**

This section has been re-written according to Dr. DeGeorge's comment. On Dr. DeGeorge's Item d) provide the exposure multiples at which the fetotoxic and embryo toxic effects were observed for rats and rabbits, the exposure data in rats and rabbits are not available.

**(3) Clinical Pharmacology:** Dr. DeGeorge commented "From my experience (although not specifically with this class of drugs) there is a rather lengthy clinical pharmacology section with more detailed study report data than is usually seen. Is this a unique feature about this class of drugs?"

Since this section was reviewed by medical officers, response from the medical team deems appropriate.

**X. DRAFT PHARMACOLOGY PORTION OF LETTER TO APPLICANT**

Redacted

1

pages of trade

secret and/or

confidential

commercial

information

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

cc

Original NDA 20-984

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MAR 31 1999

**ADDENDUM TO PHARMACOLOGY REVIEW OF February 8, 1999**

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

**NDA 20-984 RAPLON (rapacuronium bromide) for Injection**

**Sponsor:**

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

**TYPES, DATES AND REVIEW OF SUBMISSIONS:** Original of June 30, 1998,  
reviewed on February 8, 1999

**DATE OF THIS ADDENDUM:** March 31, 1999

**COMMENTS:**

The amendment of **X. PHARMACOLOGY PORTION OF LETTER TO APPLICANT** is indicated. The following section should replace the one in the original review:

**X. PHARMACOLOGY PORTION OF LETTER TO APPLICANT**

**/S/**

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

cc

Original NDA 20-984

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F/T by DHJean 3/31/99

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