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APPLICATION NUMBER:

207947Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 207947
Submission date: 12/22/14
Drug: selexipag
Applicant: Actelion Pharmaceuticals

Indication: treatment of pulmonary arterial hypertension to delay disease progression

Reviewing Division: Division of Cardiovascular and Renal Products, Division of Hematology Products

Discussion:
The primary reviewer and supervisor found the nonclinical information adequate to support the approval of selexipag for the indication listed above.

The carcinogenicity of selexipag was assessed in 2-year rat and mouse studies. These studies were found to be acceptable by the executive carcinogenicity assessment committee and the committee concluded that there were no drug-related neoplasms in either species.

The applicant provided embryofetal studies in rats and rabbits with selexipag These studies showed few adverse effects such as decreased feta body weight in rats at a dose that produced an exposure estimated to be 47 times higher than that achieved in humans at the maximum recommended dose.

A pre/postnatal study has not been conducted with selexipag. Given the role of prostacyclin in pregnancy and parturition, some effects might be anticipated in late term pregnancy women.

An acceptable established pharmacologic class for selexipag could be "prostacyclin receptor agonist".

Conclusions: I agree that this NDA can be approved from a pharm/tox perspective for the indication listed above. I have provided comments on labeling separately.

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/s/

PAUL C BROWN
12/18/2015

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	207947
Supporting document/s:	001, 052, 053
Applicant's letter date:	12/22/2014
CDER stamp date:	12/22/2014
Product:	Uptravi®
Indication:	Uptravi is a selective IP prostacyclin receptor agonist indicated for the treatment of pulmonary arterial hypertension (PAH, WHO Group I) to delay disease progression. (b) (4) (b) (4) (b) (4)
Applicant:	Actelion Pharmaceuticals
Review Division:	Division of Cardiovascular and Renal Products
Reviewer:	James M. Willard, Ph.D.
Supervisor/Team Leader:	Albert De Felice, Ph.D.
Division Director:	Norman Stockbridge, M.D., Ph.D.
Project Manager:	Wayne Amchin

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Selexipag (Uptravi®), submitted as IND 104504 in 2009, is the first non-prostanoid prostacyclin agonist submitted to the FDA for the treatment of Pulmonary Arterial Hypertension. Intact selexipag is active at the prostacyclin receptor; but it is also rapidly converted to an active metabolite with a longer half-life. This should allow for oral b.i.d. dosing. Most of the selexipag data was submitted under the cognate IND in 2009. The data was reviewed at that time and can be found in Appendix I. Uptravi is intended to treat pulmonary arterial hypertension, (b) (4)

1.2 Brief Discussion of Nonclinical Findings

Selexipag is rapidly absorbed and metabolized to ACT-333679 that has a longer half-life. Both selexipag and its active metabolite are specific for the prostacyclin receptor. Pharmacokinetic behavior may be age-related. Juvenile and adult dogs had similar Cmax levels of the parent compound. However AUC values in the juvenile dogs were more than 10-fold higher than in the adults. Safety pharmacology studies showed no effects on hERG channels. Due to its pharmacodynamics activity of relaxing blood vessels, especially arterioles, It also lowered systemic blood pressure and raised heart rate. Flushing of the skin in particular was observed, and not unexpected. At high dosages, increased respiratory rates and tidal volumes, hunching, increased struggling when handled, tip-toe gait and impaired righting reflex were observed, as well as low body temperature, soft stools and impaired grasping. Lesions of the rat and/or dog GI tract included lethal intussusception, decreased peristalsis, increased gastric acid secretion and lethal gastric erosion and ulceration. Renal effects included decreased urinary output and decreased sodium and chloride transport, as expected of vasodilators. All effects were reversible after withdrawal of the drug. Similar results were found for the active metabolite, ACT-333679.

In toxicology studies, the GI tract and the skin were the primary target organs of toxicity. High dosages of Uptravi caused lethal intussusception in the adult and juvenile dog. In the 100-104 month mouse carcinogenicity studies, the high dosage caused lethal gastric erosion in 24/60 females beginning after approx. 72 weeks. The adverse gastrointestinal effects likely reflect the prominence of prostacyclin receptors in the gastrointestinal tract. Cutaneous effects were expressed as flushing, scaling, piloerection, alopecia, and hair clumping. Adrenal glands showed hypertrophy. Bones exhibited increased ossification of the periosteum and trabeculae. In mice, Uptravi upregulated CYP-450 enzymes in the liver and increased T3 and T4 levels, but did not affect the pituitary gland or TSH levels. In rats, reduced presence of Leydig cell

was noted in the carcinogenicity assay, but no effects on testosterone levels were seen that could be related to Uptravi exposure.

The pharmacology and toxicology profiles of selexipag are similar to those of eicosanoid prostacyclin agonists. Most of the adverse effects are believed to be extensions of, or related to, the pharmacology of the drug — namely severe gastrointestinal effects at high dosages (i.e. gastric erosion and intussusception), and some level of skin flushing at all dosage levels. Other cutaneous effects included scaling, alopecia and piloerection, as well as hair clumping, at the highest dosages.

While some antithrombotic activity is to be expected, bleeding time may also be prolonged as slight increases in PT and APT were observed.

1.3 Recommendations

1.3.1 Approvability

Although Uptravi is a non-eicosanoid prostacyclin agonist, it is expected to have toxicity, especially GI, similar to that of other prostacyclin agonists though head-to-head comparisons were not made in the animal toxicity studies. Oral bioavailability, and twice a day dosing, should promote compliance. Uptravi is approvable from a non-clinical pharmacology-toxicology perspective.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Preliminary Labeling

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis: In the 2-year carcinogenicity studies, chronic dietary administration of selexipag revealed no evidence of carcinogenic potential in rats or mice at up to 100 and 500 mg/kg/day, respectively. The mid dosage in the mouse study afforded AUC exposures of parent and metabolite that were 800 and 100 times, respectively, the values in patients at a dose of 1.8 mg. In the rat, the high dosage afforded multiples of clinical exposures of approximately 170 for the parent compound and >300 for the active metabolite, and, at the mid-dosage, multiples of approx. 50 and 90, respectively.

Mutagenesis: selexipag and ACT-333679 were negative in a battery of *in vitro* and *in vivo* genotoxicity tests for mutagenicity and clastogenicity that included Ames, Chinese Hamster Lung and rodent micronucleus tests.

Impairment of Fertility: Fertility and early embryonic development in the rat were not affected at dosages up to 60 mg/Kg. Selexipag was not teratogenic in rats or rabbits at up to 20 and 30 mg/Kg, respectively.

2. Drug Information

2.1 Drug:

CAS Registry Number (Optional): 475086-01-2

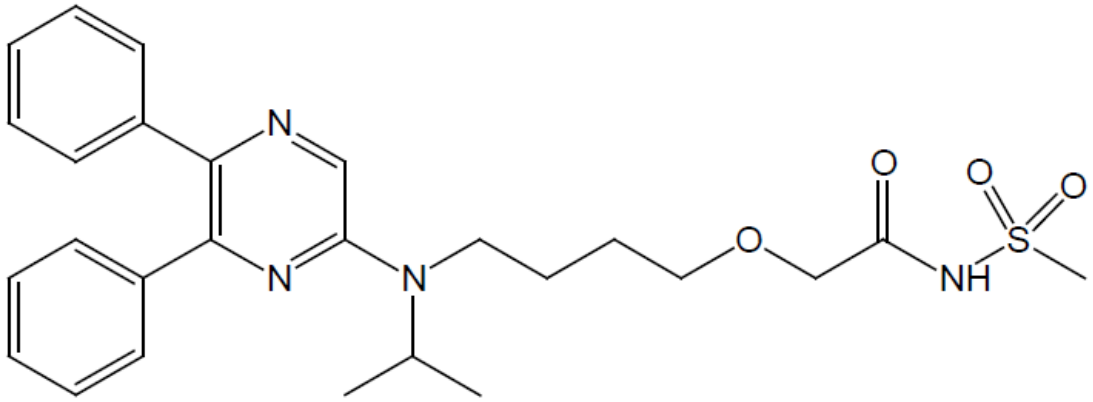
Generic Name

Code Name

Chemical Name 2-{4-[(5, 6-diphenylpyrazin-2-yl) (isopropyl) amino] butoxy}-N-(methylsulfonyl) acetamide

Molecular Formula/Molecular Weight $C_{26}H_{32}N_4O_4S$ MW 496.62

Structure or Biochemical Description



Pharmacologic Class: Platelet aggregation inhibitors excl. heparin; prostacyclin receptor agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs:

IND 104504

2.3 Drug Formulation:

Table 1. Tablet composition (200-1600 mcg strength).

Table 1 Composition of selexipag film-coated tablets (200–800 µg)

Ingredients	Selexipag film-coated tablet			
	200 µg	400 µg	600 µg	800 µg
Selexipag	0.2 mg	0.4 mg	0.6 mg	0.8 mg
D-mannitol	(b) (4)			
Corn starch				
Low substituted hydroxypropylcellulose				
Hydroxypropylcellulose				
Magnesium stearate				
(b) (4)				
Hypromellose				
Propylenglycol				
Titanium dioxide				
Iron oxide red				
Iron oxide black				
Iron oxide yellow				
Carnauba wax				
Coating weight				
Total weight of film-coated tablet		(b) (4)		
(b) (4)				

Table 2 Composition of selexipag film-coated tablets (1000–1600 µg)

Ingredients	Selexipag film-coated tablet			
	1000 µg	1200 µg	1400 µg	1600 µg
Selexipag	1.0 mg	1.2 mg	1.4 mg	1.6 mg
D-mannitol	(b) (4)			
Corn starch				
Low substituted hydroxypropylcellulose				
Hydroxypropylcellulose				
Magnesium stearate				
(b) (4)				
Hypromellose				
Propylenglycol				
Titanium dioxide				
Iron oxide red				
Iron oxide black				
Iron oxide yellow				
Carnauba wax				
Coating weight				
Total weight of film-coated tablet		(b) (4)		
(b) (4)				

2.4 Comments on any novel excipients:

There are no novel excipients.

Table 2. composition film coat

Table 5 Color and composition of the film-coat (commercial production)

	Selexipag dose strength			
	200 µg	**400 µg**	**600 µg**	**800 µg**
Color	Light yellow	Red	Light violet	Green
Hypromellose	(b) (4)			
Propylenglycol				
Titanium dioxide				
Iron oxide red				
Iron oxide black				
Iron oxide yellow				

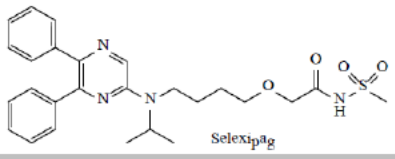
	Selexipag dose strength			
	1000 µg	**1200 µg**	**1400 µg**	**1600 µg**
Color	Orange	Dark violet	Dark yellow	Brown
Hypromellose	(b) (4)			
Propylenglycol				
Titanium dioxide				
Iron oxide red				
Iron oxide black				
Iron oxide yellow				

Company proposes varying color (b) (4) (b) (4) (b) (4) (b) (4)

2.5 Comments on any impurities/degradants of concern

Figure 1. degradation pathways, below

Figure 3 Degradation pathway of selexipag in the film-coated tablets



(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4). Stability is not a concern.

2.6 Proposed Clinical Population and Dosing Regimen

Uptravi is proposed for treating patients with pulmonary arterial hypertension at. 200, 400, 600, 800, 1000, 1200, 1400 or 1600 mcg bid.

2.7 Regulatory Background

IND 104504 (selexipag) was submitted on September 29, 2009. Uptravi was submitted as NDA 207947 on December 22, 2014.

3. Studies Submitted

3.1 Studies reviewed herein:

- Mouse Carcinogenicity study, Report # T-10.648
- Rat Carcinogenicity study, Report # T-10.649
- Juvenile Toxicology study
- Genetic toxicology (multiple metabolites, degradants and contaminants)
- Mechanistic study: Mouse liver and thyroid hormones
- Mechanistic study: testosterone-production in rat Leydig cell cultures (Selexipag and ACT-333679)
- Mechanistic study: Rat pituitary-testicular hormones

3.2 Studies Not Reviewed

3.3 Studies previously reviewed under IND 104504 (see Appendix I).

Safety pharm:
CNS, Cardiovascular, Pulmonary, Renal, GI, Repro (uterine contraction)

Pharmacology:
Prostanoid receptor selectivity
Smooth muscle effects
Platelet aggregation Model of PAH

Pharmacokinetics:
ADME Drug interactions (P450)

Toxicology:
Single dose: mice, rats, dogs

Repeat dose:
Mouse: 4 and 13 weeks
Rat: 4 weeks+ 4 weeks recovery; 26 weeks + 4 weeks recovery.
Dog: 2, 4 and 39 weeks.

Genotoxicity:
Ames
Chinese Hamster Lung
Rat Micronucleus.

Reproductive Toxicity:
Fertility & early development: Rat
Embryo-fetal development (teratogenicity): Rabbit, Rat.

Phototoxicity

4. Pharmacology:

The primary, secondary and safety pharmacology studies, previously reviewed under IND 104504, are included in Appendix I

5. Pharmacokinetics/ADME/Toxicokinetics

PK, ADME, and toxicokinetic studies, previously reviewed in IND 104504, are included in the Appendix 1.

6. General Toxicology

Single and repeat dose toxicity, previously reviewed in IND 104504, is included in the appendix.

7. Genetic Toxicology

Tests for mutagenicity and clastogenicity, previously reviewed in IND 104504, are included in the appendix.

Other Genetic Toxicity Studies:

Several other metabolites were screened under IND 104504. Compounds identified as (b) (4) were all assessed in the Ames assay. None of the metabolites or

degradants were mutagenic under the conditions tested, i.e., presence and absence of rat mitochondrial S9 metabolic enzyme fraction.

8. Carcinogenicity

A. Mouse

Study title: Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice

Study no.:	(b) (4) 5939 (T-10.648)
Study report location:	Nippon Shinyaku Co., Ltd. 14, Nishinosho-Monguchi-cho, Kisshoin, Minami-Ku, Kyoto 601-8550, Japan
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 28, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NS-304, lots 26 & 33, 100% purity
CAC concurrence:	No

Key Study Findings:

There were no positive carcinogenicity findings per both Sponsor and FDA survival-adjusted statistical analyses. A slight numerical excess of thyroid tumors was not statistically significant, and, moreover was within historical control incidence

Adequacy of Carcinogenicity Study:

Dosages were not sanctioned by the CDER execCAC. The dosages used exceeded the MTD for females at 500 mg/kg, a dosage that was tolerated by the males. With the exception of the high dose female group, the study lasted 105 weeks, an acceptable duration. Females in the high dosage group were terminated at week 100 since only 27% of animals were alive. Severely reduced survival was evident by approximately week 72 with severe gastric erosion being the adjudicated cause of death.

Appropriateness of Test Models:

Test model was of a standard design, and utilized dosages that provided at least 25 times the clinical AUC exposure, satisfying a criterion for an adequate exposure margin.

Methods (from sponsor)

Doses:	125, 250 and 500 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg body weight
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% methylcellulose
Basis of dose selection:	13 week study
Species/Strain:	B6C3F1/Crlj SPF mice
Number/Sex/Group:	55/M or F

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Age:	Beginning at 5 weeks of age
Animal housing:	The animals were housed in an animal room at 23±3°C; relative humidity at 50±20% ;air ventilation at 12 to 17 times per hour; and 12-hour illumination. The animals were housed individually, and on a pellet diet Drinking water was provided *ad libitum*.
Paradigm for dietary restriction:	Food consumption was measured twice in the first week of administration: on Day 1 (one days consumption from the day before) and Day 7 (6 days cumulative consumption) of administration for each animal. Thereafter, 7 days cumulative consumption was recorded weekly at 7-day intervals until Week 14 of administration and thereafter every 2 weeks at 14-day intervals, and one days food consumption was calculated from the cumulative consumption (note 1). Food consumption was also recorded in all surviving animals of both sexes on the day before premature necropsy (Day 697) (note 2).

Dual control : No

Interim sacrifice: No
Satellite groups: No

Cohorts (per sponsor):

Group composition

Test group	Dose (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg/day)	Sex	Main group		Satellite group	
					No. of animals	Animal No.	No. of animals	Animal No.
Control[a)]	0	0	10	M	55	1001-1055	9	1201-1209
				F	55	1101-1155	9	1301-1309
Low	125	12.5	10	M	55	2001-2055	24	2201-2224
				F	55	2101-2155	24	2301-2324
Middle	250	25	10	M	55	3001-3055	24	3201-3224
				F	55	3101-3155	24	3301-3324
High	500	50	10	M	55	4001-4055	29	4201-4229
				F	55	4101-4155	29	4301-4329

a): 0.5 w/v% methylcellulose solution, M: Male, F: Female

Mortality:

Figure 2 : Male mortality

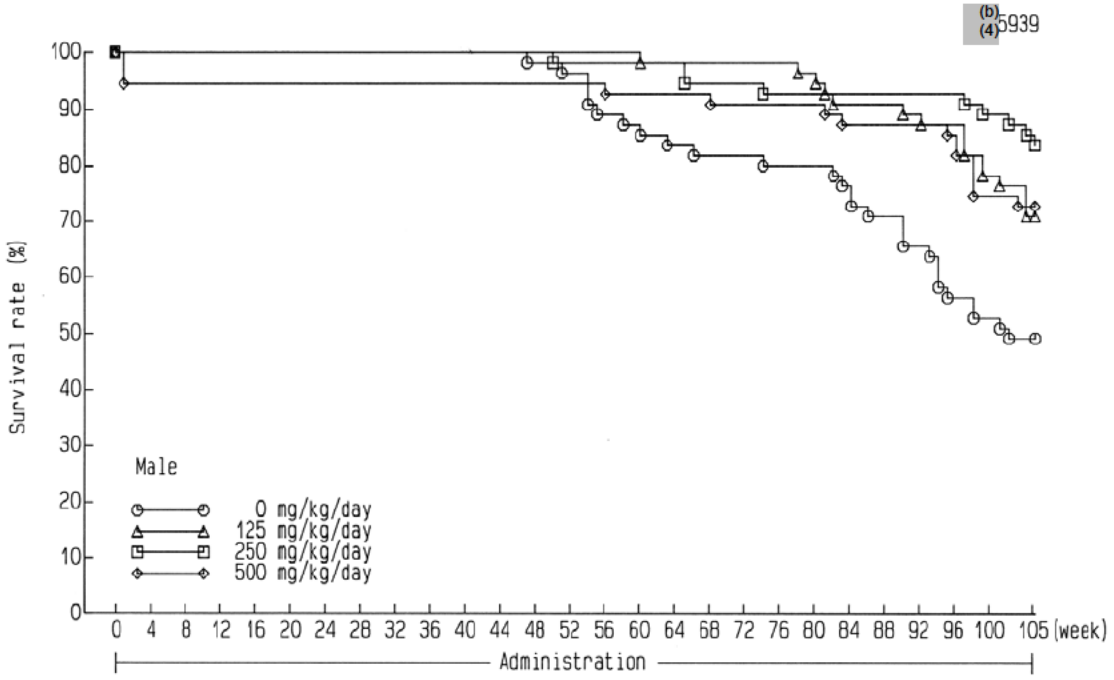


Fig.1 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice

——— Survival rate ———

Survival in treated males was not reduced, rather numerically exceeded that of controls at study end.

Figure 3. Female mortality

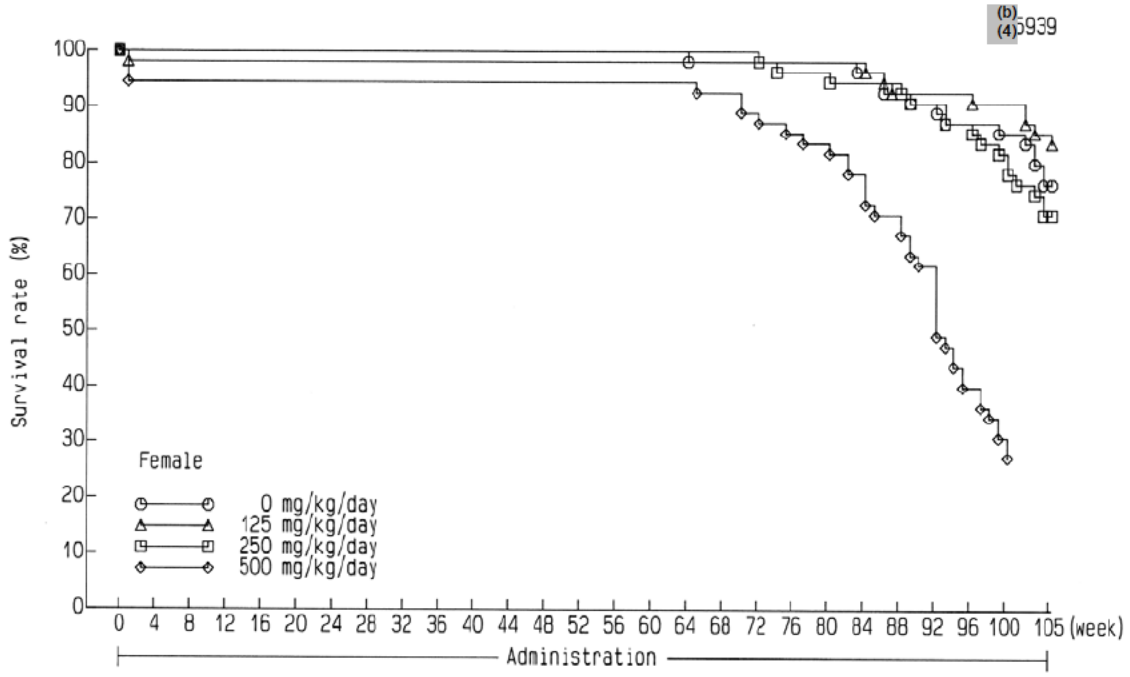


Fig.2 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice

——— Survival rate ———

For females in the high dosage cohort, morality was persistently highest starting at 68 weeks. The high dosage female group was sacrificed at 100 weeks, rather than 105 weeks, when survival had been reduced to approximately 27%. Lethal gastric erosion was the primary pathology reported.

Table 3. Cause of death

Text Table 2. Presumptive cause of demise

Sex	Male				Female			
Dose (mg/kg/day)	0	125	250	500	0	125	250	500[a)]
No. of animals used	55	55	55	55	55	55	55	55
No. of deaths	28	16	9	15	13	9	16	40
Tumor lesion								
Adrenomedullary tumor	0	0	0	0	0	1	0	0
Bone tumor	0	0	1	0	0	0	1	0
Histiocytic tumor	2	0	0	1	2	0	3	0
Liver tumor	5	4	2	4	0	0	1	0
Lung tumor	1	0	0	0	2	0	0	0
Malignant lymphoma	2	2	1	1	6	1	3	0
Mesenchymal tumor	0	0	0	0	0	0	1	0
Muscular tumor	0	0	0	1	0	0	0	0
Subcutaneous tumor	0	0	0	0	1	2	1	1
Vascular tumor	0	1	1	0	1	0	0	0
Ovarian tumor	NA	NA	NA	NA	0	1	0	0
Non-tumor lesion								
Intoxication	0	0	0	3[b)]	0	0	0	3[b)]
Gastrointestinal lesion	0	0	1	1	0	0	6	24
Kidney lesion	1	0	0	0	0	0	0	0
Fat necrosis	0	0	0	0	0	1	0	0
Skin lesion	1	0	0	0	0	0	0	0
Urogenital tract lesion	14	8	3	2	0	0	0	0
Unclear	2	1	0	2	1	2	0	12
Gavage error	0	0	0	0	0	1	0	0

a): All surviving females in the 500 mg/kg group were sacrificed in Week 100 after administration for 99 weeks.
b): These animals died on Days 3 and 4 of administration.
Values in the table indicate the number of animals that died or were sacrificed as moribund.
NA: Not applicable

According to the Sponsors adjudication, there was no excess cancer death in this study in either gender. Primary causes of premature death were gastrointestinal lesions in high dose females, and, in the male control group, urogenital lesions. The numerical incidence of cancer was appreciably less in the treated cohorts (Table 6 below)

Clinical Signs

Table 4. Treatment related clinical signs

Text Table 3. Incidence summary of treatment-related clinical signs

Sex	Male				Female			
Dose (mg/kg/day)	0	125	250	500	0	125	250	500[a)]
No. of animals used	55	55	55	55	55	55	55	55
No. of deaths	28	16	9	15	13	9	16	40
Creeping position	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1)
Flaccidity	0(0)	0(0)	19(2)	34(9)	0(0)	0(0)	15(3)	46(34)
Eye discharge	0(0)	0(0)	33(7)	48(13)	0(0)	0(0)	48(14)	54(39)
Lacrimation	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	13(6)	30(21)
Salivation	0(0)	0(0)	2(0)	14(4)	0(0)	0(0)	10(5)	32(26)
Flush (limbs)	0(0)	3(0)	55(9)	55(15)	0(0)	9(4)	55(16)	55(40)

a): All surviving females in the 500 mg/kg group were sacrificed in Week 100 after administration for 99 weeks.
Number in the table indicates the total number of animals and number in parentheses indicates the number of animals that died or were sacrificed as moribund.

Flaccidity, flushing and "cholinergic" effects (lacrimation, salivation) predominated.

Body Weight:

Figure 4. Male Body Weights

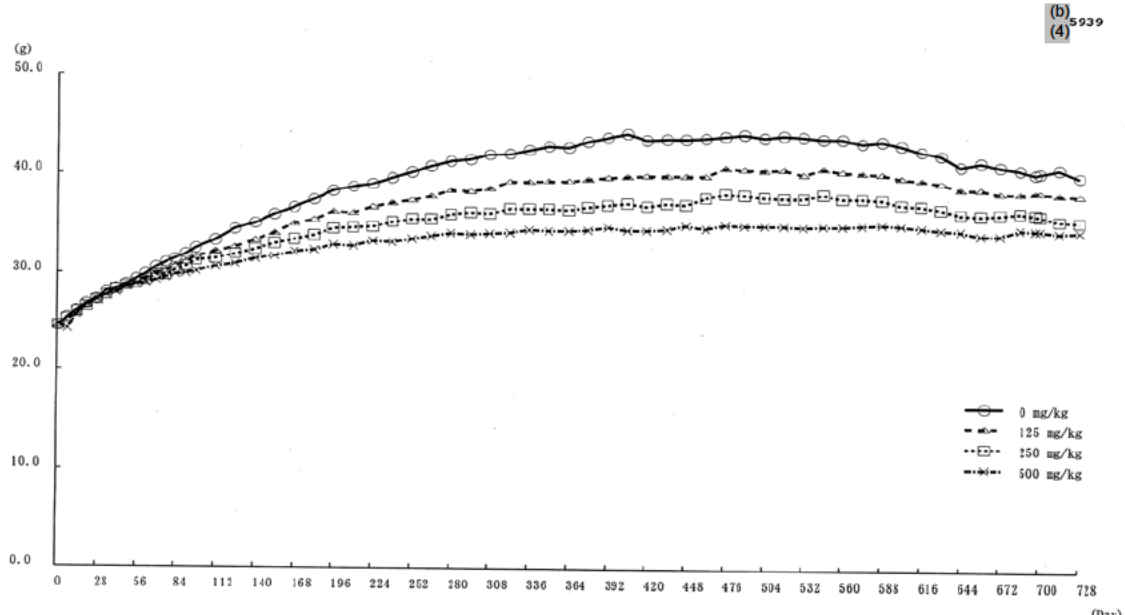
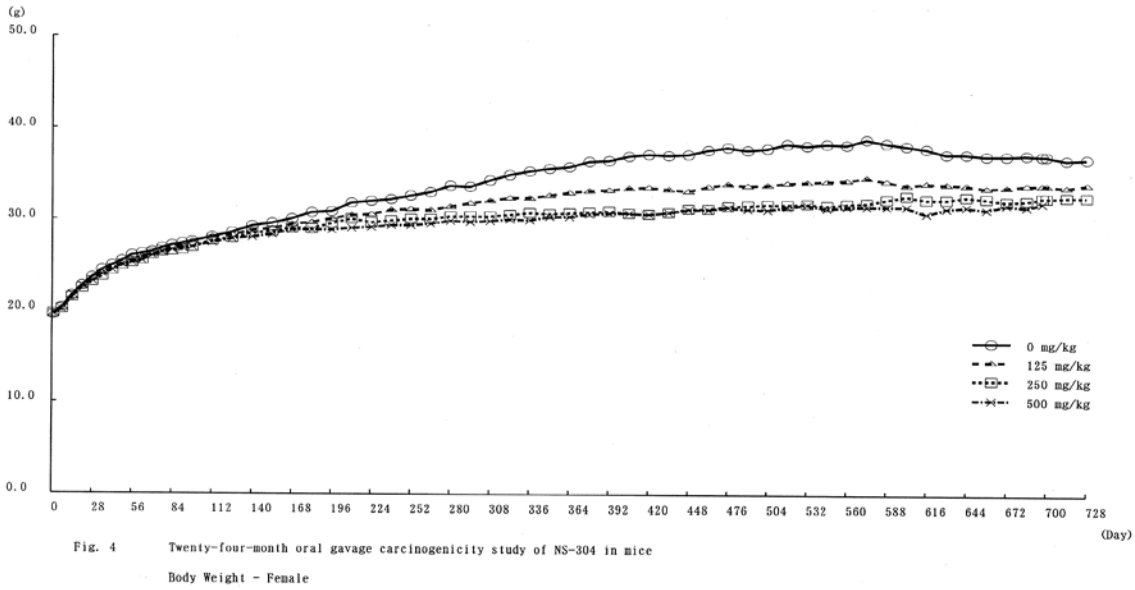


Fig. 3 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice

Body Weight - Male

Rate of gain in body weight was depressed in a dose dependent manner. Numerical mean body weight of the high dose cohort was about 85% of that of control.

Figure 5. Female Body Weights



Rate of gain in female body weight was reduced by selexipag evident by week 30, although final body weight was not appreciably depressed

Food Consumption

Figure 6. Male Food Consumption

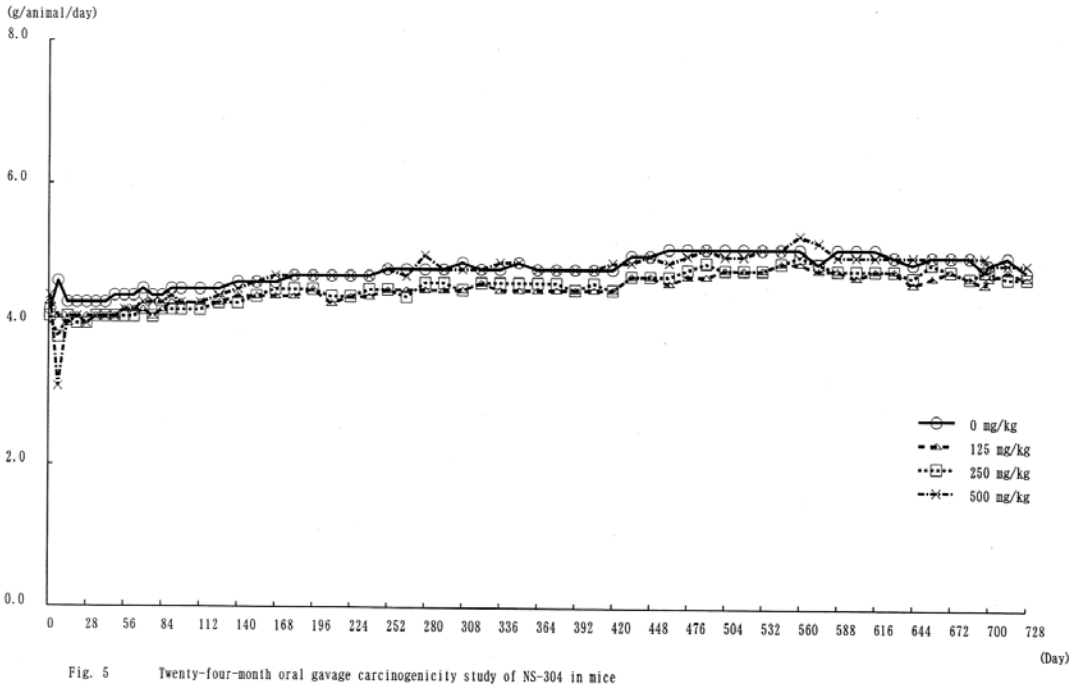


Fig. 5 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice

Food Consumption - Male

Except for transient depression in the high dosage group, food consumption was not different between the groups, Accordingly, enhanced survival in treated males noted above (Figure 2) was associated with dose-dependent reduced body weight of up to approx.15-20% by study end), but not with decreased food intake.

Figure 7. Female food consumption

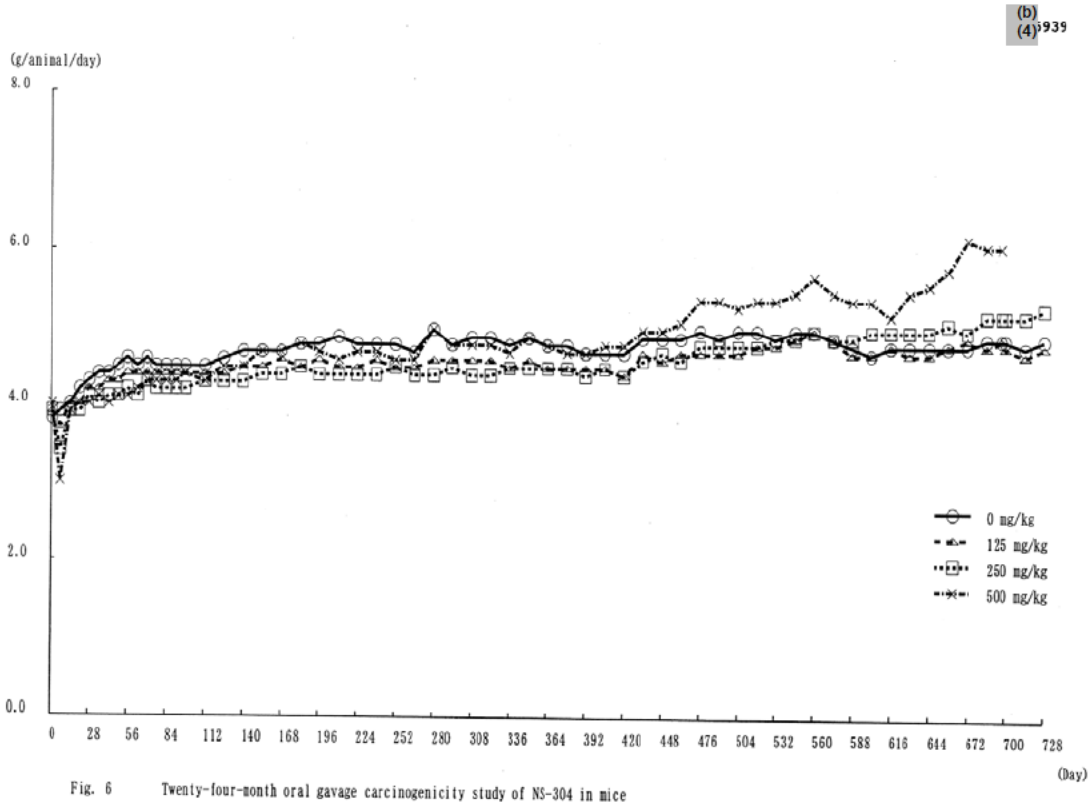


Fig. 6 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice

Food Consumption - Female

As with the males, food consumption was similar for all groups, except the high dosage female group, which had lower consumption during the first few weeks, then a return to normal consumption, followed by higher consumption after day 440.

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Gross Pathology:

Table 5. Treatment related gross lesions with sponsor annotation

Text Table 8. Incidence summary of treatment-related gross lesions (all animals)

Sex	Male				Female			
Dose (mg/kg/day)	0	125	250	500	0	125	250	500[a)]
No. of animals used	55	55	55	55	55	55	55	55
Stomach								
Focus, dark red, glandular stomach	5	3	5	5	1	3	2	26
Perforation, glandular stomach	0	0	0	1	0	0	4	4
Adhesion	0	0	0	0	0	0	4	4
Focus, depressed, glandular stomach	0	0	0	0	0	0	1	2
Thickening, wall, glandular stomach	0	0	0	0	0	0	0	2
Intestine, duodenum								
Nodule	0	0	0	0	0	1	2	4
Focus, white	0	0	0	0	0	0	0	3
Perforation	0	0	0	1	0	0	0	1
Spleen								
Small	1	0	0	3	0	0	0	11

a): All surviving females in the 500 mg/kg group were sacrificed prematurely in Week 100 after administration for 99 weeks.
Number in the table indicates the number of animals with respective lesions.

Stomach: Increased incidence of dark red foci in the glandular stomach was observed in females in the 500 mg/kg group. Perforation in the glandular stomach was observed in males in the 500 mg/kg group and in females in the 250 and 500 mg/kg groups. Adhesion with surrounding tissues and depressed foci in the glandular stomach were observed in females in the 250 and 500 mg/kg groups. Thickening of the glandular stomach wall was observed in females in the 500 mg/kg group.

Duodenum: Nodule that corresponds to erosion/ulcer in histopathology was observed in females in the 250 and 500 mg/kg groups. White foci were observed in females in the 500 mg/kg group. Perforation was observed in both sexes in the 500 mg/kg group.

Spleen: Smallness was observed in females in the 500 mg/kg group.

The approximately 50% mortality in the females in the high dosage group was attributed to severe gastric erosion and perforation. Otherwise, gross pathology was not remarkable.

Histopathology per Peer review:

PEER REVIEW STATEMENT

A microscopic peer review was performed as follows for this study:

1. Reexamination of all tissues from 10% of the control group (Group 1) male and female mice and 10% from the high dose (Group 4) male and female mice selected randomly:

Group 1M	1019, 1023, 1028, 1033, 1036, 1044
Group 4M	4002, 4028, 4032, 4034, 4042, 4047
Group 1F	1103, 1124, 1135, 1140, 1143, 1147
Group 4F	4102, 4118, 4121, 4136, 4152, 4155

2. Reexamination of all diagnoses from the adrenal; bone+bone marrow, femoral; bone+bone marrow, sternum; intestine, duodenum; kidney; liver; pancreas; salivary gland, sublingual; spleen; stomach; thymus; thyroid; from all male and female animals in all groups and mammary gland; ovary; uterus; and vagina from all female animals in all groups.

3. Reexamination of all neoplasms and hyperplasias.

Following the review of the microscopic findings reported by the study pathologist, the results were discussed and appropriate terminology and diagnoses mutually agreed on. Differences of opinion between the study and reviewing pathologists were resolved with agreement on the diagnoses.

Evaluation of Tumor Findings:

Statistical analysis revealed no increase in any tumor type. Sponsor noted a small and insignificant trend in mouse thyroid tumors. Moreover, incidences of thyroid tumors at the high dosages were near the mean values for historical controls.

Neoplastic findings:

Table 6. Tumors and tumor bearing animals

Text Table 9. Number of tumors and tumor bearers

Sex	Male				Female			
Dose (mg/kg/day)	0	125	250	500	0	125	250	500[a)]
No. of animals used	55	55	55	55	55	55	55	55
Total No. of tumors	62	46	44	35	89	81	66	30
No. of benign tumors	37	24	23	21	45	40	27	21
No. of malignant tumors	25	22	21	14	44	41	39	9
Total No. of tumor bearing animals	38	31	26	27	45	41	42	19
No. of benign tumor bearers	24	16	16	16	31	26	23	15
No. of malignant tumor bearers	21	18	17	13	32	30	32	8
No. of multiple tumor bearers	16	13	10	6	26	23	19	9

a): All surviving females in the 500 mg/kg group were sacrificed prematurely in Week 100 after administration for 99 weeks.

Figure 8. Thyroid tumors and hyperplasia (sponsor's Text Table 10).

Text Table 10. Incidence summary of tumors and hyperplasia in the thyroid

Sex	Male				Female			
Dose (mg/kg/day)	0	125	250	500	0	125	250	500[a)]
No. of animals used	55	55	55	55	55	55	55	55
Thyroid								
Adenoma, follicular cell	0	0	1	2	1	1	1	3
Carcinoma, follicular cell	0	0	1	0	0	0	0	0
Adenoma + Carcinoma, follicular cell	0	0	2	2	1	1	1	3
Hyperplasia/hypertrophy, follicular cell (total)	1	2	17	36	2	51	52	52
(±)	1	2	17	33	2	41	20	4
(+/++)	0	0	0	3	0	10	32	48

a): All surviving females in the 500 mg/kg group were sacrificed prematurely in Week 100 after administration for 99 weeks.
Numbers in the table indicate the number of animals with lesions.
±; Minimal, +; Mild, ++; Moderate

Thyroid: Slightly higher incidence of follicular cell tumors (adenoma + carcinoma) were observed in 2 males each in the 250 and 500 mg/kg groups and in 3 females in the 500 mg/kg group, although there were no statistically significant differences in either trend analysis or pairwise comparison between the control and any dose group. At the same time, increased incidence and severity of hyperplasia/hypertrophy of the follicular cells were observed in the above groups. Follicular cell adenoma was also observed in 1 female each in the 125 and 250 mg/kg groups, respectively, and the possibility of involvement of treatment could not be completely excluded, since increased incidence and severity of hyperplasia/hypertrophy of the follicular cells were also observed in these groups. However, it was hardly judged to be treatment-related since follicular cell adenoma was also observed in 1 control female in the present study.

Overall, the incidence of tumors, both benign and malignant, was not enhanced; rather, tumor burden was numerically reduced in both mice genders. Thyroid tumor burden was lower than that encountered historically in controls, and the sponsor determined that the numerical slight excess was not statistically significant. In that context, the significance of the dosage -dependent hyperplasia/hypertrophy of the follicular cells is moot.

Non neoplastic lesions:

Table 7. Non-tumor lesions

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Text Table 11. Incidence summary of non-tumor lesions

Sex	Male				Female			
Dose (mg/kg/day)	0	125	250	500	0	125	250	500[a)]
No. of animals used	55	55	55	55	55	55	55	55
Stomach					n=54		n=54	n=54
Erosion/ulcer, glandular stomach (total)	6	3	3	7	0	3	18	37
(±)	5	2	2	3	0	2	2	9
(+ to +++)	1	1	1	4	0	1	16	28
Adhesion (total, ±/+)	0	0	0	0	0	0	2	4
Duodenum	n=52	n=51	n=51	n=47	n=52		n=50	n=52
Erosion/ulcer (total)	0	0	0	1	1	0	12	27
(±)	0	0	0	0	1	0	4	7
(+ to +++)	0	0	0	1	0	0	8	20
Regeneration, mucosal (total, ±/+)	0	0	0	0	0	0	4	1
Adhesion (total, +)	0	0	0	0	0	0	1	0
Liver								
Hypertrophy, hepatocytic, central (total, ± to ++)	0	20	43	51	0	0	6	26
Necrosis, hepatocytic, central (total, ±/+)	0	1	0	9	1	0	0	1
Multinucleated hepatocyte (total, ±)	0	0	17	21	0	0	0	0
Pancreas							n=54	
Decreased zymogen granule (total, ++/+++)	3	3	0	3	1	2	3	30
Hyperplasia, acinar cell, focal (total, ±/+)	0	1	5	4	0	6	22	25
Salivary gland, sublingual								
Atrophy, acinar cell (total, + to +++)	1	3	2	3	0	0	1	13
Hypertrophy, acinar cell (total, ±/+)	1	2	22	26	5	7	28	9
Adrenal								
Hypertrophy, cortical, diffuse (total, ±/+)	17	6	3	11	8	19	30	49
Kidney							n=54	
Dilatation, tubular (total, ± to +++)	4	1	0	3	0	5	24	42
Hypertrophy, tubular, papilla (total, ±)	0	0	0	9	0	30	30	20
Basophilia, tubular, cortical (total, ± to ++)	0	0	0	0	0	1	3	9
Eosinophilic droplet, papillary epithelium (total, ±/+)	0	0	0	3	0	43	42	13
Hypertrophy, tubular, outer medulla (total, +)	0	0	0	0	0	0	0	5
Hyperplasia, papillary epithelium (total, ±)	0	0	0	1	0	0	0	1
Regeneration, tubular (total, ± to ++)	28	47	49	45	13	10	18	10
Urinary cast, hyaline (total, ±/+)	20	28	52	48	48	34	37	50
Vacuolation, tubular cell (total, ±/+)	50	55	0	0	0	0	0	4
Spleen								
Increased pigment, red pulp (total, +/++)	0	0	0	0	0	37	34	34
Atrophy (total, +/++)	1	0	0	1	1	0	0	22

a): All surviving females in the 500 mg/kg group were sacrificed prematurely in Week 100 after administration for 99 weeks.
Number in the table indicates the number of animals with respective lesions.
±; Minimal, +; Mild, ++; Moderate, +++; Severe

(Continued to the next page)

Text Table 11. (Continued)

Sex	Male				Female			
Dose (mg/kg/day)	0	125	250	500	0	125	250	500[a)]
No. of animals used	55	55	55	55	55	55	55	55
Thymus	n=54		n=54	n=53			n=54	n=53
Atrophy (total, + to +++)	19	23	24	26	8	9	18	41
Femur (bone and bone marrow)								
Granulopoiesis (total, +/++)	14	7	3	3	1	3	10	12
Fibro-osseous lesion (total, ±/+)	6	8	9	31	34	47	29	36
Sternum (bone marrow)								
Granulopoiesis (total, +/++)	14	8	3	3	1	3	10	12
Ovary								
Hypertrophy, corpora lutea (total, ± to ++)	NA	NA	NA	NA	6	22	28	33
Vagina								
Mucification (total, ±/+)	NA	NA	NA	NA	4	12	17	17
Mammary gland								
Hyperplasia, lobular (total, ± to ++)	NA	NA	NA	NA	1	22	30	18

a): All surviving females in the 500 mg/kg group were sacrificed prematurely in Week 100 after administration for 99 weeks.
Number in the table indicates the number of animals with respective lesions.
NA: Not applicable
±; Minimal, +; Mild, ++; Moderate, +++; Severe

Stomach: Increased incidence and severity of erosion/ulcer in the glandular stomach were observed in males in the 500 mg/kg group and in females in the 250 and 500 mg/kg groups. Adhesion was observed in females in the 250 and 500 mg/kg groups, and this was considered to be associated with erosion/ulcer.

Duodenum: Increased incidence and severity of erosion/ulcer were observed in males in the 500 mg/kg group and in females in the 250 and 500 mg/kg groups. Mucosal regeneration was observed in females in the 250 and 500 mg/kg groups. Adhesion was observed in females in the 250 mg/kg group. Mucosal regeneration and adhesion were considered to be associated with erosion/ulcer.

Liver: Hypertrophy of the centrilobular hepatocytes was observed in males in the 125 mg/kg and higher groups and in females in the 250 and 500 mg/kg groups. Increased incidence of necrosis of the centrilobular hepatocytes was observed in males in the 500 mg/kg group. Multinucleated hepatocytes were observed in males in the 250 and 500 mg/kg groups.

Pancreas: Increased incidence of decreased zymogen granules was observed in females in the 500 mg/kg group. Increased incidence of hyperplasia of the acinar cells was observed in females in the 250 and 500 mg/kg groups.

Salivary gland, sublingual:

Increased incidence of atrophy of the acinar cells was observed in females in the 500 mg/kg group. Increased incidence of hypertrophy of the acinar cells was observed in males in the 250 and 500 mg/kg groups and in females in the 250 mg/kg group.

Adrenal: Increased incidence of diffuse hypertrophy of the cortical cells was observed in females in all dose groups.

Kidney: Increased incidence of tubular dilatation was observed in females in the 250 and 500 mg/kg groups. Tubular hypertrophy in the papilla and eosinophilic droplet of the papillary epithelium in males in the 500 mg/kg group and in females in the 125 mg/kg and higher groups, tubular basophilia in cortex in females in the 125 mg/kg and higher groups, tubular hypertrophy in the outer medulla in females in the 500 mg/kg group and hyperplasia of the papillary epithelium in both sexes in the 500 mg/kg group were observed. In males only, increased incidences in tubular regeneration in the 125 mg/kg and higher groups and hyaline casts in the 250 and 500 mg/kg groups were observed.

Spleen: Increased pigments in the red pulp were observed in females in the 125 mg/kg and higher groups. Increased incidence of atrophy was observed in females in the 500 mg/kg group.

Thymus: Increased incidence of atrophy was observed in females in the 250 and 500 mg/kg groups.

Femur and sternum (bone marrow):

Increased incidence of granulopoiesis was observed in females in the 250 and 500 mg/kg groups.

Femur (bone): Increased incidence of fibro-osseous lesion was observed in males in the 500 mg/kg group.

Ovary: Increased incidence of hypertrophy of the corpora lutea was observed in females in the 125 mg/kg and higher groups.

Vagina: Increased incidence of mucification was observed in females in the 125 mg/kg and higher groups.

Mammary gland: Increased incidence of lobular hyperplasia was observed in females in the 125 mg/kg and higher groups.

Hyperplasia/hypertrophy was found in multiple organs, but without any associated neoplasia. Female reproductive organs were affected, which is perhaps not surprising since prostacyclin is involved in pregnancy and may have roles beyond affecting implantation and uterine contractility. Thymic and splenic atrophy suggest a capacity for immune suppression although this was a high dosage phenomenon, and confined to one gender. Kidney tubular dilatation may be an extension of the pharmacodynamics activity of prostacyclin as one of the primary actions of the eicosanoid is to increase renal blood flow and urine output in electrolyte homeostasis. The increased incidence of centrilobular hepatocytes was observed only at the highest dosage, and not in both genders. As noted below, even at the mid-dosage, the exposure of parent and metabolite exceed that in patients by several orders of magnitude.

Toxicokinetics in the mouse cancer trial

Table 8. Toxicokinetics

Text Table 12. Summary of TK parameters

Sex	Male (n=3)			Female (n=3)		
Dose (mg/kg/day)	125	250	500	125	250	500
NS-304						
T_{max} (hr)						
Day 1	0.5	0.5	0.5	0.5	0.5	0.5
Week 26	0.5	0.5	2	0.5	0.5	1
C_{max} (ng/mL)						
Day 1	16900	27000	24900	18500	39800	43900
Week 26	12600	22600	24700	16600	20200	38900
C_{2h} (ng/mL)						
Day 1	1460	5440	10800	2540	11000	15600
Week 13	554	1930	9900	1430	2860	20000
Week 26	902	4050	24700	1150	4590	16400
$AUC_{0\text{-}24h}$ (ng·hr/mL)						
Day 1	18500	60800	247000	24900	67500	199000
Week 26	11600	36800	84400	18600	36700	91700
MRE-269						
T_{max} (hr)						
Day 1	0.5	0.5	1	0.5	0.5	0.5
Week 26	0.5	0.5	2	0.5	0.5	1
C_{max} (ng/mL)						
Day 1	12500	18500	18400	11800	18900	23000
Week 26	11800	12600	18000	12000	10300	17500
C_{2h} (ng/mL)						
Day 1	3070	8650	11700	3860	8840	11900
Week 13	841	3070	9240	1750	2690	18300
Week 26	1430	3840	18000	1120	4020	13300
$AUC_{0\text{-}24h}$ (ng·hr/mL)						
Day 1	25700	77300	215000	24800	58800	153000
Week 26	13900	31700	67500	16000	30800	65500

In healthy human subjects, the AUC for an 1,800 mcg dose was 44.8 ng-hr/mL for the parent compound (ACT-293987 or NS-304 or MRE-304) and 276 ng-hr/mL for the active metabolite (ACT-333679 or MRE-269). In the mouse carcinogenicity trial, the AUC for both parent and metabolite at the well tolerated dosage of 250 mg/Kg, was in the range of about 31,000 to 37,000ng hr/ml. This provides very reassuring safety margins of exposure of approximately 800x for the parent compound and 100X for the active metabolite.

Sponsor Tables 10-1 to 10-11 of tumor incidences in the mouse carcinogenicity study are in Appendix IV.

B. Rat:

Study title: Twenty-four month oral gavage carcinogenicity study of NS-304 in rats

Study no.:	(b) (4) 5940
Study report location:	Nippon Shinyaku co. Ltd. 14, Nishinosho-Monguchi-cho, Kisshoin, Minami-Ku, Kyoto 601-8550, Japan
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 29, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NS-304 (or MRE-304), lot # 26 and 33, 100% purity
CAC concurrence:	Yes

Key Study Findings: No statistically significant increase in tumor burden was seen. Non-tumor related findings were primarily gastric ulceration in the high dose groups, mild hypertrophy of the liver and thymus, and pancreatic acinar cell atrophy.

Adequacy of Carcinogenicity Study: The design, dosages and species were approved by the CDER executive committee.

Appropriateness of Test Models: Sprague-Dawley rats are commonly used in carcinogenicity studies.

Methods (from sponsor):

Dosages:

Group composition

Test group	Dose (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg/day)	Sex	Main group		Satellite group	
					No. of animals	Animal No.	No. of animals	Animal No.
Control[a)]	0	0	5	M	60	1001-1060	5	1201-1205
				F	60	1101-1160	5	1301-1305
Low	10	2	5	M	60	2001-2060	8	2201-2208
				F	60	2101-2160	8	2301-2308
Middle	30	6	5	M	60	3001-3060	8	3201-3208
				F	60	3101-3160	8	3301-3308
High	100	20	5	M	60	4001-4060	8	4201-4208
				F	60	4101-4160	8	4301-4308

a): 0.5 w/v% methylcellulose solution, M: Male, F: Female

Frequency of dosing:
Dose volume: 5 mL/kg of body weight
Route of administration: Oral gavage
Formulation/Vehicle: 0.5% (w/v) methyl cellulose solution
Basis of dose selection: 26 week toxicity study
Species/Strain: Sprague-Dawley
Number/Sex/Group: 60/M or F/4
Age: 6 weeks old
Animal housing:

Paradigm for dietary restriction: NA
Dual control employed: No
Interim sacrifice: Not planned
Satellite groups: 4
Deviation from study protocol:

Observations and Results

Mortality

Figure 9. Survival, male

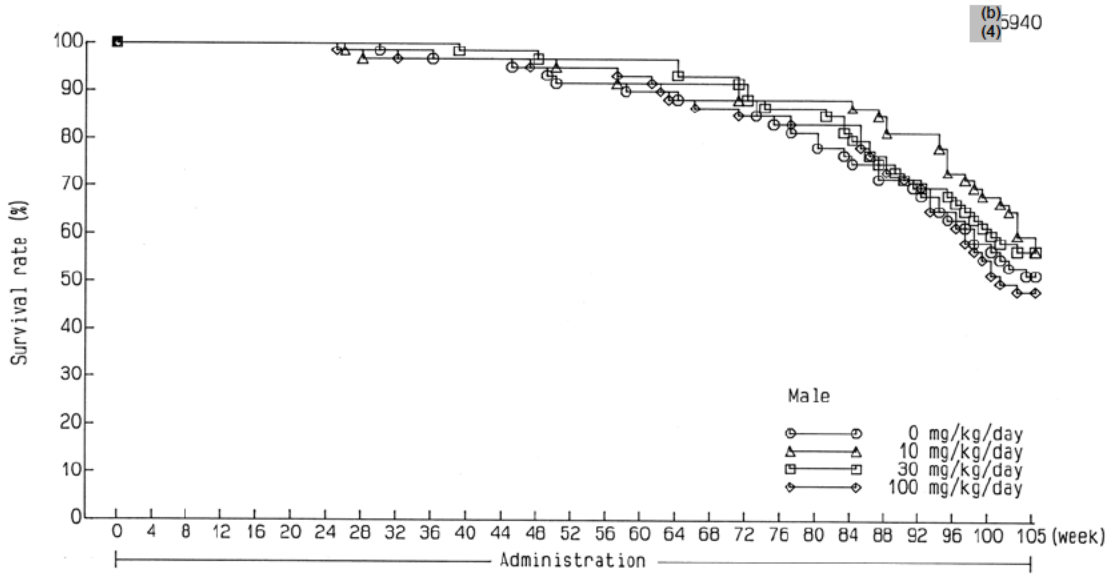


Fig.1 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats

——— Survival rate ———

Selexipag did not significantly affect male survival

Figure 10. Survival, female

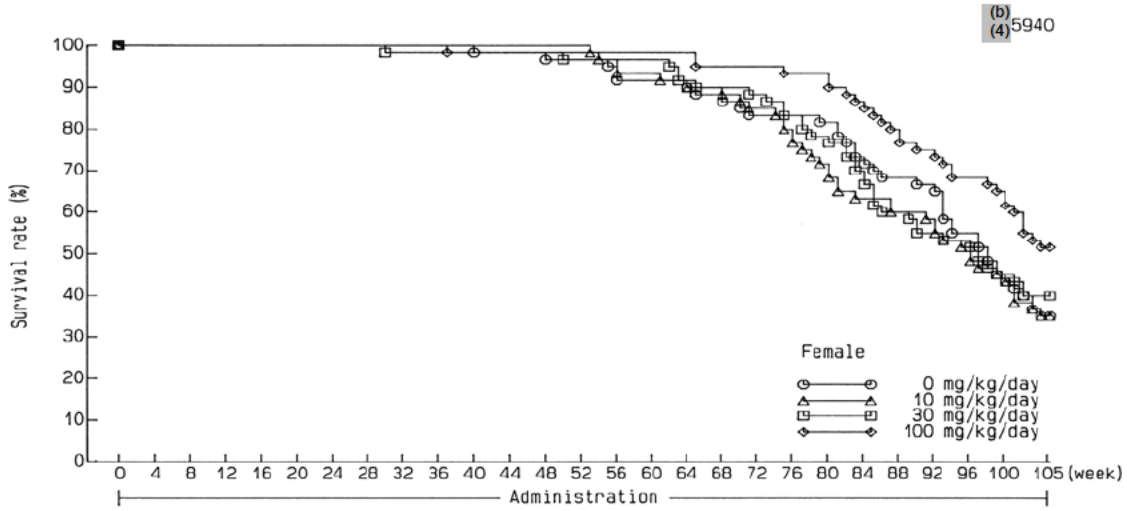


Fig.2 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats

——— Survival rate ———

The only effect of selexipag on survival of females *prima facie* seems to be prolongation at the high- dosage.

Clinical Signs

Table 9. Treatment related clinical signs

Text Table 3. Incidence summary of treatment-related clinical signs

Sex	Male				Female			
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of animals used	60	60	60	60	60	60	60	60
No. of deaths	29	26	26	31	39	39	36	29
Soft feces	0(0)	0(0)	0(0)	6(4)	0(0)	0(0)	0(0)	0(0)
Lacrimation	0(0)	0(0)	9(5)	41(21)	0(0)	0(0)	37(22)	51(27)
Salivation	0(0)	0(0)	18(12)	49(23)	0(0)	0(0)	8(4)	42(21)
Alopecia	0(0)	0(0)	0(0)	1(0)	0(0)	0(0)	5(2)	12(4)
Flush (pinna, limbs)	0(0)	60(26)	60(26)	60(31)	0(0)	60(39)	60(36)	60(29)
Flush (whole body)	0(0)	0(0)	0(0)	16(7)	0(0)	0(0)	0(0)	14(6)
Flaccidity	0(0)	0(0)	0(0)	24(11)	0(0)	0(0)	0(0)	10(5)
Creeping position	0(0)	0(0)	0(0)	1(0)	0(0)	0(0)	0(0)	0(0)

Number in the table indicates the total number of animals and number in parentheses indicates the number of animals that died or were sacrificed as moribund.

Flushing was seen at all dosages, and, at the high dosage, included the whole body. Other symptoms, i.e. flaccidity, lacrimation, salivation and alopecia, were high-dosage phenomena. .

Body Weight

Figure 11. Male Body Weights

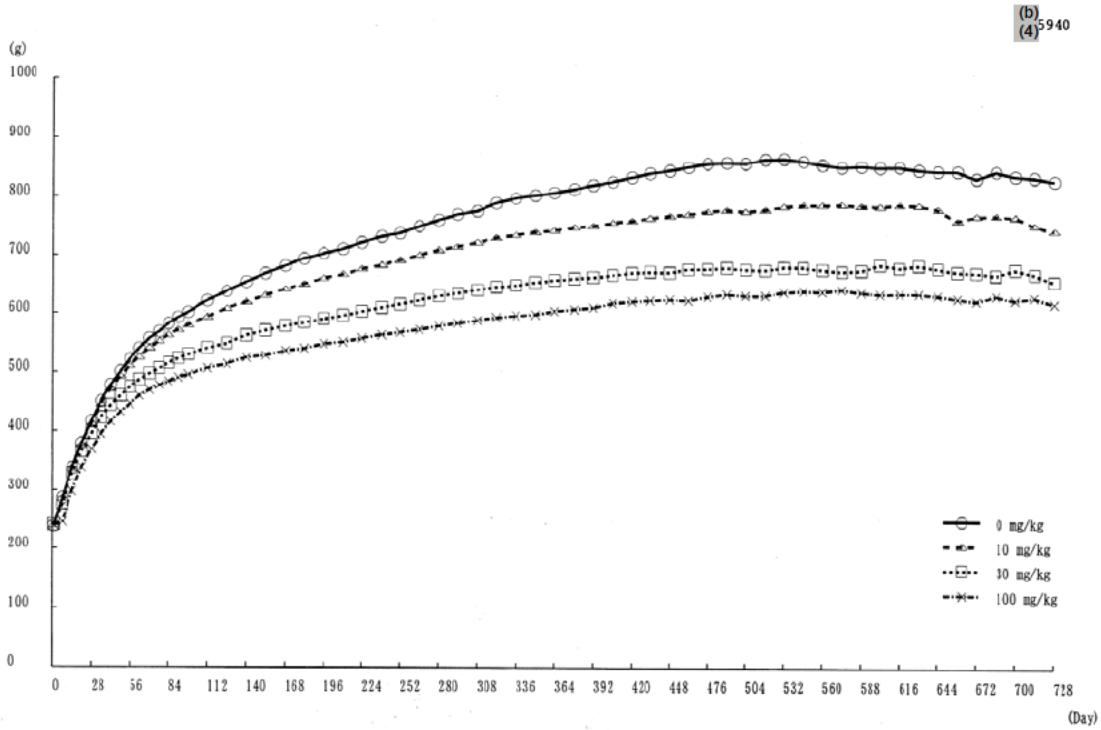
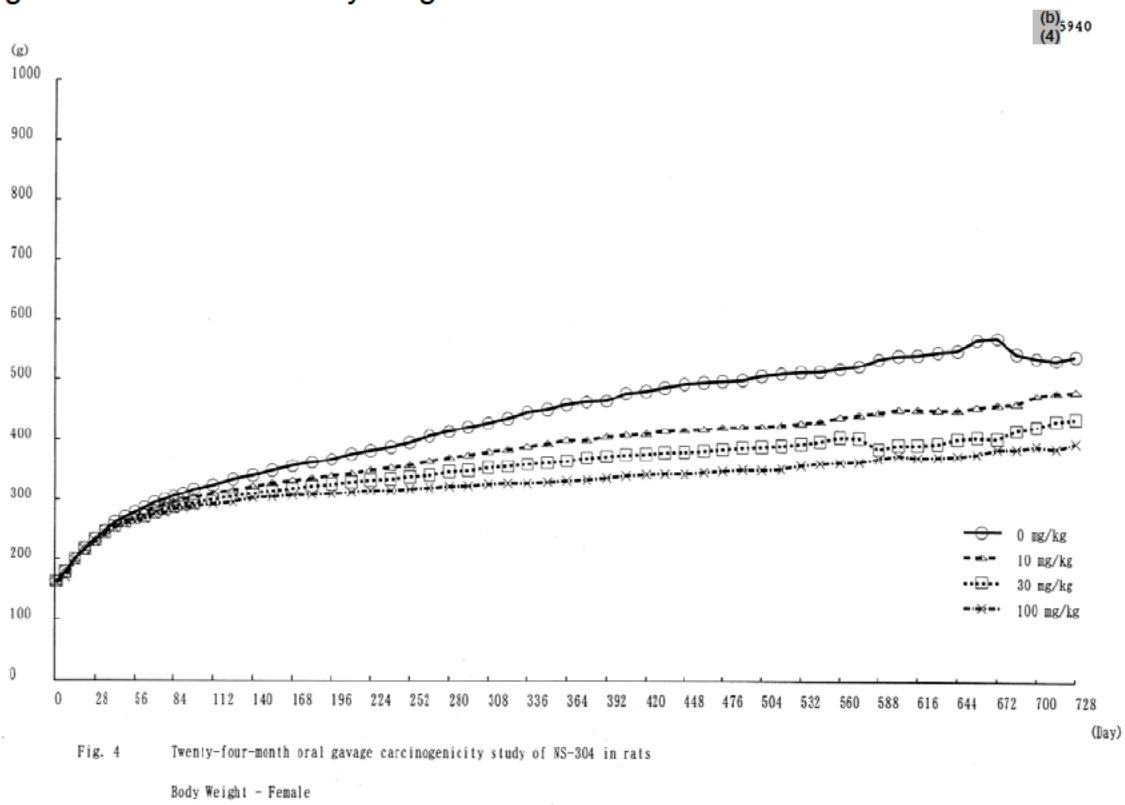


Fig. 3 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats

Body Weight - Male

There was an appreciable and dosage-related reduction in body weight of males of up to approximately 50%.

Figure 12. Female rat body weights



Body weights in both genders decreased dose-dependently up to approximately 20% (male) to 30% (female). However, as can be seen in the food consumption section, there was no apparent effect of dose on food consumption, therefore dietary restriction per se cannot be invoked to explain some of the results of this study e.g., the suggestion of reduced tumor burden., though the relatively reduced body weight may be a factor.

Food Consumption

Figure 13. Male Food consumption

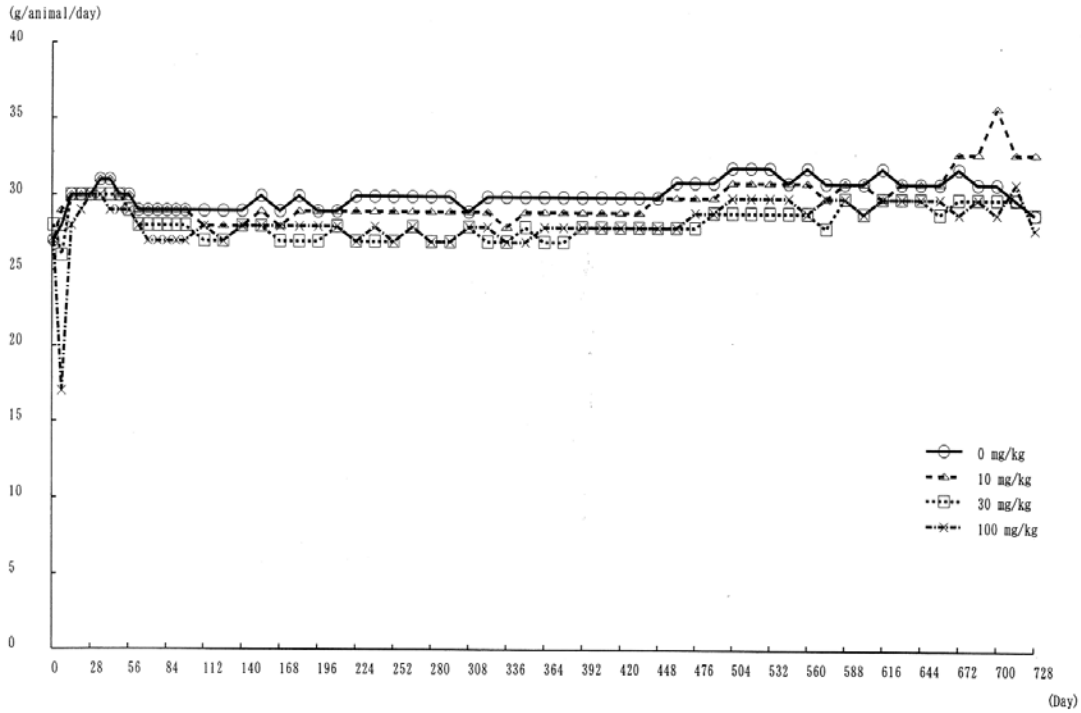


Fig. 5 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats

Food Consumption - Male

Figure 14. Female Food consumption

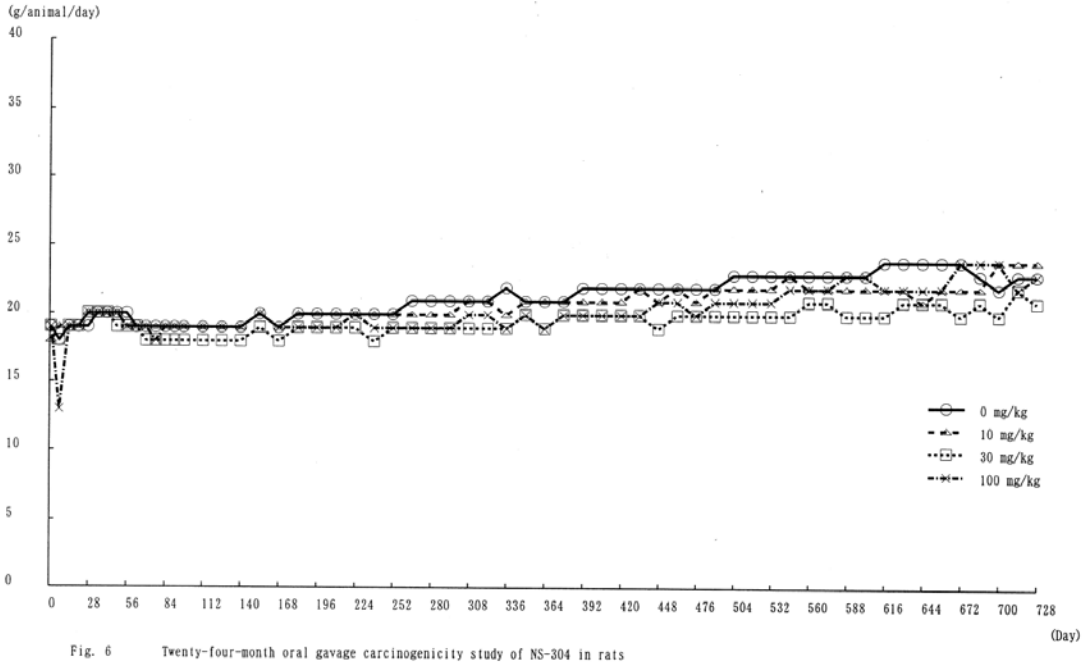


Fig. 6 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats

Food Consumption - Female

With the exception of a transient early reduction in both genders, food consumption was not affected by treatment. The previously noted dose-related reduced body weight in both genders cannot be attributed to reduced food intake

Gross Pathology

Table 10. Treatment related lesions

Text Table 10. Incidence summary of treatment-related gross lesions (all animals)

Sex	Male				Female			
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of animals used	60	60	60	60	60	60	60	60
Skin								
Alopecia	0	0	0	0	0	0	5	7
Stomach								
Focus, dark red, glandular stomach	9	15	9	19	4	7	9	15

Number in the table indicates the number of animals with respective lesions.

Skin: Alopecia was observed in females in the 30 and 100 mg/kg groups.

Stomach: Increased incidence of dark red foci in the glandular stomach was observed in both sexes in the 100 mg/kg group.

At necropsy, the only treatment-associated effect was, besides alopecia at higher dosages, a relative increase in incidence of dark red foci in the stomach indicative of local gastric damage and extravasation of RBCs.

Peer-reviewed histopathology:

(b) (4) 5940
Attached Data 5

(b) (4)

(b) (4)

STUDY NUMBER (b) (4) 5940
(b) (4) PROJECT NO.: 908-001

TWENTY-FOUR MONTH ORAL GAVAGE
CARCINOGENICITY STUDY OF
NS-304 IN RATS

PEER REVIEW STATEMENT

A microscopic peer review was performed as follows for this study:

1. Reexamination of all tissues from 10% of the control group (Group 1) male and female rats and 10% from the high dose (Group 4) male and female rats selected randomly:

Group 1M	1006, 1018, 1034, 1052, 1057, 1059
Group 4M	4011, 4013, 4029, 4038, 4052, 4058
Group 1F	1112, 1121, 1129, 1130, 1131, 1146
Group 4F	4103, 4104, 4123, 4132, 4136, 4141

2. Reexamination of all diagnoses from the stomach, pancreas, thymus, sternum and femur from all animals from all dose groups.

3. Reexamination of all neoplasms and hyperplasias.

Following the review of the microscopic findings reported by the study pathologist, the results were discussed and appropriate terminology and diagnoses mutually agreed on. Differences of opinion between the study and reviewing pathologists were resolved with agreement on the diagnoses.

(b) (4)

Study Pathologist — Reviewing Pathologist

(b) (4)

August 28, 2010 — August 28, 2010
DATE — DATE

Table 11. Tumors and tumor-bearing rats

Text Table 11. Number of tumors and tumor bearers

Sex	Male				Female			
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of animals used	60	60	60	60	60	60	60	60
Total No. of tumors	121	124	91	84	159	153	128	125
No. of benign tumors	102	107	70	69	104	94	95	79
No. of malignant tumors	19	17	21	15	55	59	33	46
Total No. of tumor bearing animals	52	55	47	43	56	57	59	52
No. of benign tumor bearers	46	52	37	38	52	52	57	46
No. of malignant tumor bearers	18	17	18	13	29	28	23	27
No. of multiple tumor bearers	31	34	23	20	41	37	39	36

As in mice, there may be a slight reduction in tumor burden *prima facie,* more evident in the male, except for Leydig cell neoplasia and focal hyperplasia, although those numbers were similar to historical control levels. Sponsor did not specify whether tumors in any cohort were benign or malignant. The sponsor's interpretation follows:

Tumor that showed an increased incidence in dose groups as compared to the control group were observed in the testis and pituitary and incidences of testicular tumor and focal hyperplasia and pituitary tumor are summarized in the following Text Table 12 *(cf* Tables 11-1 to 11-3, 11-8 and 11-9, Table 12-15).

Table 12. major tumors and hyperplasia

Text Table 12. Incidence summary of major tumors and hyperplasias

Sex	Male				Female			
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No.of animals used	60	60	60	60	60	60	60	60
Testis								
Leycligcell tumor	2$')	0	2	5	NA	NA	NA	NA
Hyperplasia, Leycligcell, focal (total, ±J+)	0	2	2	6	NA	NA	NA	NA
Pituitary								
Adenoma		20	40**	23				

Numbers in the table indicate the number of animals with lesions.
$:p<0.025 (statistically significant positive trend, rare tumor, Petois test)
**: p<Opole (statistically significant clifference from the control group, conmmon tumor, Petois test)
a): TIere was no statistically significant positive trend in the control, 10 and 30 mg/kg groups.
±: Minimal, +: Mild, NA: Not applicable

Testis: Marginally increased incidence of Leydig cell tumor was observed in the

100 mg/kg group, and statistically significant positive trend was noted (rare tumor, p<0.025); however, there was no statistical significance in pairwise comparison between the control and 100 mg/kg groups. Also, slightly higher incidence in focal hyperplasia of Leydig cells was observed in this group, although the difference in incidence between the two groups was very small. The tumor incidence of the 100 mg/kg group (5/60 animals, 8%) was marginally higher than that in our historical data (0 to 4% in the incidence). In addition, there was no statistically significant positive trend in incidence of Leydig cell tumor in the control, 10 and 30 mg/kg groups.

Table 13. Non-tumor lesions

Text Table 13. Incidence summary of non-tumor lesions

Sex	Male				Female			
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of animals used	60	60	60	60	60	60	60	60
Stomach								
Erosion/ulcer, glandular stomach (total)	10	18	10	20	4	11	7	17
(±)	6	8	4	4	1	5	3	8
(+)	4	9	6	14	2	4	4	5
(++)	0	1	0	2	1	2	0	4
Liver								
Hypertrophy, centrilobular (total, ±)	0	0	4	19	0	0	0	11
Pancreas								
Atrophy, acinar (total)	15	26	25	39	8	19	35	37
(±)	11	14	12	19	7	11	25	22
(+)	4	10	12	19	1	8	6	13
(++/+++)	0	2	1	1	0	0	4	2
Hyperplasia, acinar cell (total, ± to ++)	5	11	10	19	0	1	3	10
Thymus			(n=58)	(n=59)				
Hyperplasia, epithelial (total)	1	3	6	3	37	44	44	53
(±)	0	2	5	3	32	39	35	34
(+/++)	1	1	1	0	5	5	9	19
Femur								
Enostosis (total)	2	0	3	1	9	14	22	26
(±)	1	0	3	0	9	13	20	19
(+)	1	0	0	1	0	1	2	7
Sternum								
Enostosis (total)	1	0	1	1	4	12	20	29
(±)	0	0	1	0	4	11	19	23
(+)	1	0	0	1	0	1	1	6

Number in the table indicates the number of animals with respective lesions.
±: Minimal, +: Mild, ++: Moderate, +++: Severe

Stomach: Increased incidence and severity of erosion/ulcer in the glandular stomach were observed in both sexes in the 100 mg/kg group. A high incidence of erosion/ulcer in the glandular stomach was also found in males in the 10 mg/kg group; however, it was judged to be incidental, since it was not dose-related.

Liver: Hypertrophy in the centrilobular hepatocytes was observed in males in the 30 and 100 mg/kg groups and in females in the 100 mg/kg group.

Pancreas: Increased incidence and severity of acinar atrophy was observed in both sexes in the 10 mg/kg and higher groups. Increased incidence of hyperplasia of the acinar cells was observed in males in all dose groups and in females in the 100 mg/kg group.

Thymus: Increased incidence and severity of epithelial hyperplasia were observed in females in the 100 mg/kg group.

Femur and sternum:

Increased incidence and/or severity of enostosis were observed in the sternum in females in the 10 mg/kg and higher groups and in the femur in females in the 30 and 100 mg/kg groups. Enostosis in the dose groups except for the 100 mg/kg group was observed more frequently in the dead and moribund sacrificed animals than the scheduled sacrificed animals.

As the sponsor notes, there was an increase in non-lethal stomach ulcers in the rats. Effects of selexipag on the stomach and pancreatic acinar cells may forecast an effect on the digestive system. One minor effect that was only noted in the 2 year rat carcinogenicity study was retinal tortuosity. It was only seen in the mid- and high dose groups, which provides a significant safety margin, and was thought to be related to dilataion of the retinal blood vessels due to selexipag treatment. There were no histopathological findings associated with retinal tortuosity and the finding is thought to be of little toxicological significance. Retinal tortuosity is only known to cause a slight increase in risk of retinal hemorrhages and is not linked to any vision or visual problems.

Toxicokinetics in the rat cancer trial: (Sponsor's Text table 14 below).

Text Table 14. Summary of TK parameters

Sex	Male (n=3)			Female (n=3)		
Dose (mg/kg/day)	10	30	100	10	30	100
NS-304						
T_{max} (hr)						
Day 1	0.5	0.5	0.5	0.5	0.5	0.5
Week 26	0.5	0.5	0.5	0.5	0.5	0.5
C_{max} (µg/mL)						
Day 1	0.451	1.16	3.80	0.209	1.22	3.72
Week 26	0.419	1.28	6.41	0.888	3.35	9.83
$C_{0.5h}$ (µg/mL)						
Day 1	0.451	1.16	3.80	0.209	1.22	3.72
Week 13	0.185	0.653	3.34	0.494	1.67	4.84
Week 26	0.419	1.28	6.41	0.888	3.35	9.83
$AUC_{0\text{-}24h}$ (µg·hr/mL)						
Day 1	0.395	1.84	11.1	0.206	1.53	11.7
Week 26	0.342	1.69	7.78	0.630	2.18	13.3
MRE-269						
T_{max} (hr)						
Day 1	1	1	1	1	1	1
Week 26	1	1	0.5	1	0.5	1
C_{max} (µg/mL)						
Day 1	2.30	7.55	14.9	1.56	8.38	15.6
Week 26	2.29	7.64	15.4	2.95	9.70	27.0
$C_{0.5h}$ (µg/mL)						
Day 1	1.13	3.21	8.85	1.03	3.90	10.3
Week 13	0.930	2.47	9.43	1.83	6.31	13.0
Week 26	1.22	4.12	15.4	2.63	9.70	19.1
$AUC_{0\text{-}24h}$ (µg·hr/mL)						
Day 1	12.9	46.2	212	7.16	35.5	219
Week 26	7.62	25.4	90.7	11.4	26.9	162

Table 15. Human PK data

Appendix 21 Study AC-065-101: pharmacokinetic parameters of ACT-293987 and ACT-333679 on each 3rd day following multiple oral dose administrations of ACT-293987 to healthy male subjects (fed)

	Dose (µg)	N	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0\text{-}12h}$ (ng·h/mL)	$AUC_{0\text{-}\infty}$ (ng·h/mL)	$t_{½}$ (h)
ACT-293987	400	12	2.94 ± 1.17	2.00 (2.00-4.00)	9.78 ± 4.60	–	–
	600	11	3.57 ± 1.43	2.00 (2.00-4.00)	12.2 ± 6.72	–	–
	800	10	6.92 ± 3.47	2.00 (2.00-2.00)	20.5 ± 10.7	–	–
	1,000	9	8.76 ± 4.72	2.00 (2.00-2.00)	26.3 ± 14.9	–	–
	1,200	9	10.4 ± 5.63	2.00 (2.00-2.00)	30.4 ± 15.1	–	–
	1,400	9	11.2 ± 4.15	2.00 (2.00-2.00)	31.7 ± 12.2	–	–
	1,600	9	11.0 ± 5.16	2.00 (2.00-2.00)	34.8 ± 14.9	–	–
	1,800	8	13.8 ± 6.71	2.00 (2.00-2.00)	44.6 ± 17.4	44.8 ± 17.7	1.47 ± 0.3
ACT-333679	400	12	5.59 ± 2.20	4.00 (2.00-6.00)	41.4 ± 17.1	–	–
	600	11	7.73 ± 2.81	4.00 (2.00-6.00)	54.7 ± 22.0	–	–
	800	10	11.1 ± 5.14	4.00 (2.00-4.00)	74.1 ± 29.6	–	–
	1,000	9	13.7 ± 5.79	4.00 (2.00-4.00)	98.8 ± 43.6	–	–
	1,200	9	16.0 ± 7.55	4.00 (2.00-6.00)	111 ± 48.8	–	–
	1,400	9	16.3 ± 6.23	4.00 (2.00-4.00)	117 ± 45.6	–	–
	1,600	9	17.9 ± 8.17	4.00 (2.00-6.00)	137 ± 58.8	–	–
	1,800	8	22.9 ± 9.55	4.00 (2.00-4.00)	166 ± 77.1	276 ± 114	8.74 ± 1.25

Name:	NS-304 (MRE-304)
Lot number:	33
Assay:	99.8%

Organs

In healthy human subjects, the AUC for the 1,800 mcg dose was 44.8 ng-hr/mL for the parent compound (ACT-293987 or NS-304 or MRE-304) and 276 ng-hr/mL for the active metabolite (ACT-333679 or MRE-269). In the rats, the AUC was 7780 ng-hr/mL for the parent compound, and 90700 ng-hr/mL for the active metabolite. This provides margins of exposure of approximately 170x for the parent compound and >300x for the active metabolite at the high dosage, and approx. 50X and 90X, respectively, at the mid-dosage.

Tables of tumor incidences in the rat study (Sponsor tables 11- 1 to7, for male; 11- 8 to13, for female) are found in Appendix V.

Prenatal and postnatal development:

Study title: STUDY FOR EFFECTS ON PRE- AND POSTNATAL DEVELOPMENT INCLUDING MATERNAL FUNCTION IN RATS TREATED ORALLY WITH NS-304"

Key study findings: Animals received 0, 2, 6, or 20 mg/kg of selexipag between the period of time of implantation of the embryos to weaning. The high dose dams did have reduced body weights and food consumption, however, the NOAEL for the pups was 20 mg/kg, since there were no abnormalities in any of the pups.

Study no.: R-1048
Volume #, and page #: eCTD
Conducting laboratory and location:
(b) (4)
Date of study initiation: December 10, 2009
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Methods

Doses: see chart below

Species/strain: Sprague-Dawley rats
Number/sex/group:

Test group	Dose level (mg/kg)	Dose concentration (mg/mL)	Dose volume (mL/kg)	Main group		Satellite group	
				Number of copulated animals*	Animal number	Number of copulated animals*	Animal number
Control	0	0	5	20 (18)	1101-1120	4 (4)	1121-1124
Low dose	2	0.4	5	20 (20)	2101-2120	8 (8)	2121-2128
Middle dose	6	1.2	5	20 (19)	3101-3120	8 (8)	3121-3128
High dose	20	4.0	5	20 (20)	4101-4120	8 (7)	4121-4128

*: Numbers between parentheses indicate the number of pregnant females.

Route, formulation, volume, and infusion rate: oral gavage
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality (dams): no mortalities occurred in the dams in the study

Clinical signs (dams): The primary clinical signs were flushing of the ears beginning approximately one hour post-dosing. There was also some minor muscle flaccidity on the first day of treatment in 3 dams in the high dose group, day 7 of gestation is when implantation occurs.

Table 1 Study for effects on pre- and postnatal development including maternal function in rats treated orally with NS-304

Clinical signs in dams during the gestation period

Dose mg/kg	Signs	Administration																	
		0-6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23a)
0	No. of dams	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	2	0
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	No. of dams	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	3	0
	No. of dams with abnormal findings	0	6	7	8	6	7	10	4	6	6	6	3	5	4	5	0	2	
	Flush (pinna) b)	0	6	7	8	6	7	10	4	6	6	6	3	5	4	5	0	2	
6	No. of dams	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	1	0
	No. of dams with abnormal findings	0	19	19	18	19	19	16	15	15	13	18	9	11	10	8	5	0	
	Flush (pinna) b)	0	16	11	13	11	12	10	12	6	8	13	9	9	9	7	4	0	
	Flush (pinna and limbs) b)	0	3	8	5	8	7	6	3	9	5	5	0	2	1	1	1	0	
20	No. of dams	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	19	3	0
	No. of dams with abnormal findings	0	20	20	20	20	20	20	20	20	20	19	16	9	12	9	10	0	
	Flush (pinna) b)	0	0	0	0	1	3	5	9	11	14	11	11	7	9	8	6	0	
	Flush (pinna and limbs) b)	0	20	20	20	19	17	15	11	9	6	8	5	2	3	1	4	0	
	Flaccidity b)	0	3	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	

a): Gestation day
b): Clinical signs were observed at approximately 1 hour after dosing.

Body weight (dams):

Slight decreases in body weight occurred in the high dose group of dams and persisted through lactation.

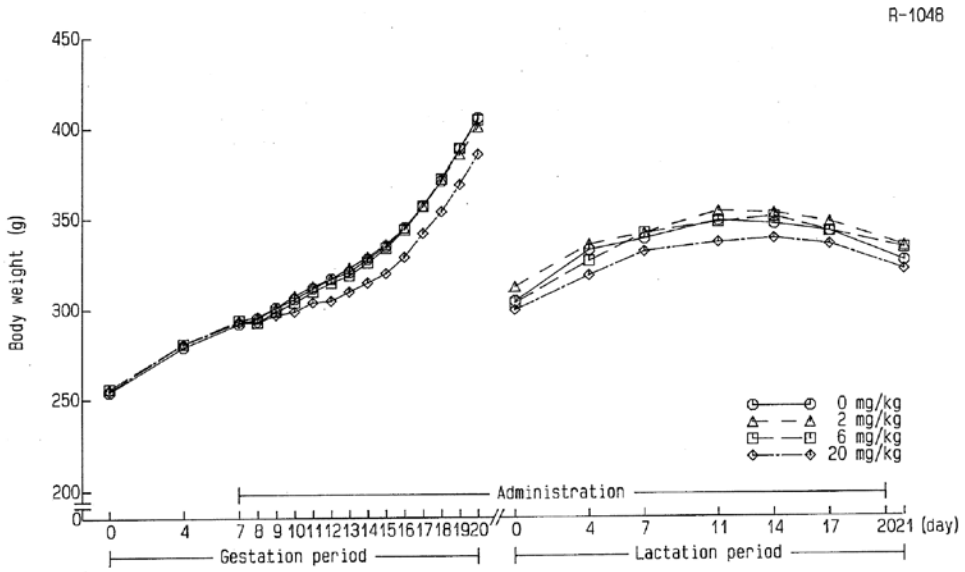


Fig.1 Study for effects on pre- and postnatal development including maternal function in rats treated orally with NS-304

Body weight of dams

Food consumption (dams): Food consumption declined in the high dose dams immediately after dosing with selexipag began. However, differences were not large.

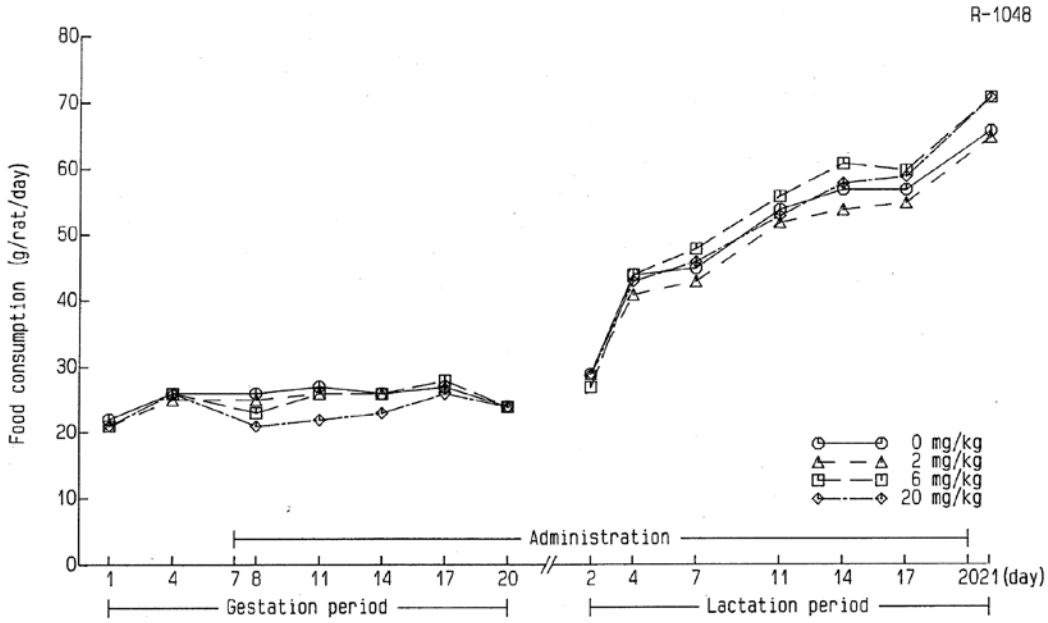


Fig.2 Study for effects on pre- and postnatal development including maternal function in rats treated orally with NS-304

Food consumption of dams

.

Toxicokinetics:
Selexipag never reached detectable levels in the 2 mg/kg group, and by one hour

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Table 28-2 Study for effects on pre- and postnatal development including maternal function in rats treated orally with NS-304
Plasma concentration of NS-304 (final administration)

Dose (mg/kg)		Plasma Concentration (μg/mL)																
		Animal No.	Pre	Animal No.	0.5h	Animal No.	1h	Animal No.	2h	Animal No.	4h	Animal No.	8h	Animal No.	24h	Tmax (h)	Cmax (μg/mL)	AUC 0-24h (μg·h/mL)
0						1121	N.D.											
						1122	N.D.											
						1123	N.D.											
	Mean						0											
	S.D.						0											
2		2125	N.D.	2121	N.D.	2125	N.D.	2121	N.D.	2125	N.D.	2121	N.D.	2125	N.D.			
		2126	N.D.	2122	N.D.	2126	N.D.	2122	N.D.	2126	N.D.	2122	N.D.	2126	N.D.			
		2127	N.D.	2123	N.D.	2127	N.D.	2123	N.D.	2127	N.D.	2123	N.D.	2127	N.D.			
	Mean		0		0		0		0		0		0		0	N.C.	N.C.	N.C.
	S.D.		0		0		0		0		0		0		0			
6		3125	N.D.	3121	0.316	3125	0.0699	3121	0.0683	3125	N.D.	3121	N.D.	3125	N.D.			
		3126	N.D.	3122	0.153	3126	N.D.	3122	N.D.	3126	N.D.	3122	N.D.	3126	N.D.			
		3127	N.D.	3123	0.379	3127	N.D.	3123	N.D.	3127	N.D.	3123	N.D.	3127	N.D.			
	Mean		0		0.283		0.0233		0.0228		0		0		0	0.5	0.283	0.193
	S.D.		0		0.117		0.0404		0.0394		0		0		0			
20		4125	N.D.	4121	2.18	4125	0.0770	4121	N.D.	4125	N.D.	4121	N.D.	4125	N.D.			
		4126	N.D.	4123	0.171	4126	0.0942	4123	N.D.	4126	N.D.	4123	0.0928	4126	N.D.			
		4127	N.D.	4124	0.250	4127	0.183	4124	0.126	4127	0.0615	4124	0.0648	4127	N.D.			
	Mean		0		0.867		0.118		0.0420		0.0205		0.0525		0	0.5	0.867	1.17
	S.D.		0		1.138		0.057		0.0727		0.0355		0.0476		0			

S.D. : Standard Deviation
N.D. : Not detected(<0.0500 μg/mL)
N.D. was calculated as zero(0) for Mean and S.D.
N.C. : Not calculated

in the other groups was not detectable, while approximately one hour was Tmax for the active metabolite.

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Table 29-1 Study for effects on pre- and postnatal development including maternal function in rats treated orally with NS-304
Plasma concentration of MRE-269 (first administration)

Dose (mg/kg)		Plasma Concentration (μg/mL)															
		Animal No.	0.5h	Animal No.	1h	Animal No.	2h	Animal No.	4h	Animal No.	8h	Animal No.	24h	Tmax (h)	Cmax (μg/mL)	AUC 0-24h (μg·h/mL)
0				1121	N.D.											
				1122	N.D.											
				1123	N.D.											
	Mean				0											
	S.D.				0											
2		2121	0.145	2125	0.216	2121	0.160	2125	0.0535	2121	0.0513	2125	N.D.			
		2122	0.122	2126	0.229	2122	0.115	2126	0.0743	2122	N.D.	2126	N.D.			
		2123	0.140	2127	0.319	2123	0.0790	2127	0.138	2123	N.D.	2127	N.D.			
	Mean		0.136		0.255		0.118		0.0886		0.0171		0	1.0	0.255	0.873
	S.D.		0.012		0.056		0.041		0.0440		0.0296		0			
6		3121	0.805	3125	1.58	3121	0.735	3125	0.166	3121	0.0742	3125	N.D.			
		3122	0.526	3126	1.14	3122	0.488	3126	0.172	3122	0.0549	3126	N.D.			
		3123	0.904	3127	1.74	3123	0.873	3127	0.372	3123	0.101	3127	N.D.			
	Mean		0.745		1.49		0.699		0.237		0.0767		0	1.0	1.49	4.02
	S.D.		0.196		0.31		0.195		0.117		0.0232		0			
20		4121	5.59	4125	8.35	4121	6.17	4125	1.44	4121	1.97	4125	N.D.			
		4123	6.23	4126	11.4	4123	2.69	4126	2.07	4123	0.647	4126	N.D.			
		4124	2.12	4127	9.33	4124	2.48	4127	3.30	4124	0.893	4127	N.D.			
	Mean		4.65		9.69		3.78		2.27		1.17		0	1.0	9.69	33.8
	S.D.		2.21		1.56		2.07		0.95		0.70		0			

S.D. : Standard Deviation
N.D. : Not detected(<0.0500 μg/mL)
N.D. was calculated as zero(0) for Mean and S.D.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Table 8 Study for effects on pre- and postnatal development including maternal function in rats treated orally with NS-304

Gross pathological findings in dams

Dose (mg/kg)	0	2	6	20
No. of dams examined	18	20	19	20
No. of dams with abnormal findings	0	0	0	0

As can be seen by the above table, no abnormal findings occurred in any of the dams from the study.

Offspring (malformations, variations, etc.):

No differences were seen between the control and treated animals, no malformations or irregularities. Neurological and learning tests also showed no differences between the treated and controls into the F1 generation.

Table 9 Study for effects on pre- and postnatal development including maternal function in rats treated orally with NS-304

Sex ratio, body weight and external finding of liveborns at birth

Dose mg/kg	No. of dams		No. of males	No. of females	Sex ratio a)	Body weight(g) Male	Body weight(g) Female	External b) abnormalities(%)c)
0	18	Total	137	124	0.52			0
		Mean	7.6	6.9		6.4	6.0	(0.0)
		S.D.	1.5	1.6		0.4	0.3	(0.0)
2	20	Total	125	126	0.50			0
		Mean	6.3	6.3		6.5	6.1	(0.0)
		S.D.	2.3	2.7		0.8	0.7	(0.0)
6	19	Total	151	121	0.56			0
		Mean	7.9	6.4		6.4	6.0	(0.0)
		S.D.	2.4	2.3		0.5	0.4	(0.0)
20	20	Total	131	139	0.49			0
		Mean	6.6	7.0		6.6	6.2	(0.0)
		S.D.	2.3	2.3		0.4	0.4	(0.0)

a): No. of males / No. of liveborns
b): No. of liveborns with external abnormalities
c): (No. of liveborns with external abnormalities / No. of liveborns) X 100
No significant difference in any treated groups from control group

Table 16 Study for effects on pre- and postnatal development including maternal function in rats treated orally with NS-304

Gross pathological findings in pups at weaning

Dose (mg/kg)	0	2	6	20
Male				
No. of pups examined	36	36	41	39
No. of pups with abnormal findings	0	0	0	1
Thymic remnant in neck	0	0	0	1
Female				
No. of pups examined	36	34	36	40
No. of pups with abnormal findings	0	0	0	0

Table 26 Study for effects on pre- and postnatal development including maternal function in rats treated orally with NS-304

Terminal examination at the middle stage of gestation in F_1 dams

Dose mg/kg	No. of F_1 dams		No. of corpora lutea	No. of implantations	Implantation index % a)	No. of dead embryos (%)b)	No. of live embryos
0	16	Total	225	213		16	197
		Mean	14.1	13.3	94.3	(7.6)	12.3
		S.D.	1.9	2.5	10.8	(9.5)	2.6
2	19	Total	288	279		24	255
		Mean	15.2	14.7	96.9	(8.8)	13.4
		S.D.	1.8	1.8	4.0	(7.0)	2.1
6	19	Total	296	284		9	275
		Mean	15.6*	14.9*	96.2	(3.2)	14.5**
		S.D.	2.0D	1.9D	5.7	(4.7)	2.1D
20	19	Total	296	289		18	271
		Mean	15.6*	15.2*	97.9	(6.3)	14.3*
		S.D.	1.4D	1.4D	6.5	(6.6)	1.7D

a): (No. of implantations / No. of corpora lutea) X 100
b): (No. of dead embryos / No. of implantations) X 100
*: $p<0.05$; **: $p<0.01$ (Significant difference from control group)
D: Dunnett's test

Table 27 Study for effects on pre- and postnatal development including maternal function in rats treated orally with NS-304

Gross pathological findings in F_1 animals used for reproductive performance test

Dose (mg/kg)	0	2	6	20
Male				
No. of F_1 animals examined	18	20	19	20
No. of F_1 animals with abnormal findings	0	0	0	0
Female				
No. of F_1 animals examined	18	20	19	20
No. of F_1 animals with abnormal findings	0	0	0	0

Summary and conclusions: Selexipagtreatment had essentially no effect on the progeny including the F1 generations. The only difference was selexipag treatment led to higher implantation rates and corpora lutea. Otherwise, there were no treatment related differences. Study was well planned and executed.

2.6.6.7 Local tolerance

ACT-293987:
Local Tolerability Study in Rabbits – Intravenous and Paravenous Application

Key Findings: Uptravi was dosed to animals at rates of 0.5 mL/min from stock solutions of 8 or 40 mcg/mL for 20 or 25 minutes. No signs of irritation at the infusion or injection sites were reported.

Young adult New Zealand White rabbits were utilized in the study allocated in the following fashion:

Allocation and Dose Concentrations µg/mL	**Group 1 i.v. 8**	**Group 2 i.v. 40**	**Group 3 p.v. 8**	**Group 4 p.v. 40**
Males	1	2	3	4
Females	5 - 6	7 - 8	9 - 10	11 - 12

APPEARS THIS WAY ON ORIGINAL

2.1 Test Item

Information as provided by the Sponsor (see Appendix I on p. 61)

Identification:	ACT-293987
Description:	[redacted] (b) (4)
Batch Number:	F496-02-001p092
Purity of Selexipag:	99.8% (Assay by HPLC)
Correction for Purity:	No
Expiry Date (Retest Date):	[redacted] (b) (4)
Storage Conditions:	[redacted] (b) (4) [redacted] (b) (4) [redacted] (b) (4) [redacted] (b) (4) as handled by [redacted] (b) (4))
Safety Precautions:	Routine hygienic procedures (gloves, goggles, face mask).

2.1.1 Vehicle and Control Item

Identification:	Physiological saline (0.9% NaCl)
Source:	[redacted] (b) (4) [redacted]
Description:	Colorless liquid
Batch Number:	0427H51
Stability of the Vehicle:	Stable under storage conditions.
Expiry Date:	[redacted] (b) (4) (handled at [redacted] (b) (4) [redacted] ;)
Storage Conditions:	Ambient (20 ± 5 °C)
Safety Precautions:	Routine hygienic procedures (gloves, goggles, face mask).

3.6 Treatment

Route of Application:	Groups 1 and 2: Intravenously, by infusion, into the marginal ear vein, without congestion at the edge of the vein during the injection Groups 3 and 4: Paravenously, slow bolus
Rationale:	Intravenous infusion is the intended application method for Phase I. Paravenous injection may accidentally occur during clinical use.
Frequency of Application:	One single administration per ear
Dose Volume:	Intravenous injection: 10 mL / ear Paravenous injection: 0.5 mL / ear
Injection Rate:	Intravenous injection: 0.5 mL / min (10 mL in 20 min) Paravenous injection: 0.1 mL / 5 sec (0.5 mL in 25 sec)
Dose Formulation Concentration:	Groups 1 and 3: 8 µg/mL Groups 2 and 4: 40 µg/mL The test item formulations were injected into the right ear; vehicle was injected into the left ear, which serves as reference. The paravenous injections were performed one after the other, starting with the vehicle. The intravenous infusion was performed simultaneously in both ears.
Rationale for Dose Level Selection:	ACT-293987 is an orally available long-acting non prostanoid agonist of the prostacyclin receptor (IP). Enzymatic hydrolysis of ACT-293987 by carboxilyesterase 1 yields ACT-333679, the active metabolite of ACT-293987. The concentration of 8 µg/mL should cover the intended intravenous administration of ACT-293987 in man. In humans, conversion of ACT-293987 into its active metabolite ACT-333679 after iv injection is unknown. An additional concentration of 40 µg/mL should provide preclinical cover to any significantly different conversion of ACT-293987 into the active metabolite in humans.
Duration of Acclimatization Period:	7 days
Duration of Treatment and Observation Period:	8 days

3.2 Test System

Animals:	Young Adult New Zealand White Rabbit, SPF
Rationale:	Commonly used in studies of this type.
Breeder:	(b) (4) [redacted]
Number of Animals per Group:	One male and two females (nulliparous and non-pregnant) per group
Total Number of Animals:	4 males and 8 females
Age (at Delivery):	12 to 15 weeks
Body Weight Range (at Acclimatization Start):	2539 - 3303 g
Identification:	By unique cage number and corresponding marking with indelible pen on the ear.
Randomization:	Randomly allocated to groups by hand upon delivery.
Acclimatization:	Under test conditions after health examination. Only animals without any visible signs of illness were used for the study.

Results:

No mortalities occurred in the study.

No clinical signs were observed during the study with the exception of one female rabbit with slight erythema on days 2 and 3 post-infusion. Erythema subsequently resolved.

Body weights were not affected by the study.

No gross pathology was noted.

No histopathology was seen related to the infusion.

Conclusions and summary: The study was sufficient, but not optimal. Rabbits received in one ear the test substance via intravenous infusion and paravenously, while the contralateral ear received vehicle both intravenously and paravenously. No signs of irritation were apparent visually or microscopically. Animals were observed for 7 days post-treatment before sacrifice. Perhaps a better protocol would have specified a repeat dose after 7 days with sacrifice afterwards. However, the study is acceptable as performed.

Juvenile Toxicology study:

ACT-293987 – Juvenile up to 39-week toxicity study in the beagle

dog by the oral (gavage) route.
Study #AB09680.

Actelion Pharmaceuticals Ltd
Gewerbestrasse 16
CH 4123 Allschwil
Switzerland.

Methods (from sponsor):

On the first day of dosing (day 0), for all arms of the study, dogs were 27 to 32 days old. Animals were assigned to the following groups:

Group	Dose level (mg/kg/day)	Dose volume (mL/kg/day)	Dose concentration (mg/mL)	Number of animals – Sacrifice in					
				Week 13		Week 26		Week 39	
				M	F	M	F	M	F
1. Control	0	5	0	4	4	4	4	6	6
2. Low dose	1	5	0.2	4	4	4	4	4	4
3. Intermediate dose	3	5	0.6	4	4	4	4	4	4
4. High dose	6/4[1]	5	1.2/0.8[1]	4	4	0[2]	0[2]	4	4

M: males
F: females
[1]: high dose was reduced from 6 mg/kg/day to 4 mg/kg/day from day 42 of the 39-week arm of the study. High dose animals from the 13-week arm of the study were treated at a dose level of 4 mg/kg/day from day 0 onwards.
[2]: no high dose group was included in the 26-week arm of the study as not enough animals were available from the supplier.

Control animals received the vehicle (0.5 % (w/v) methylcellulose 400 centipoises in water for injection).

The following was assessed: morbidity/mortality, clinical signs, skin examinations, growth parameters, post-weaning development, limb function, ophthalmology, body weights, food consumption, cardiovascular parameters, clinical pathology, bone biomarkers, toxicokinetics, organ weights and gross and microscopic pathology.

Results:

Toxicokinetics.

Cmax parameters between adults and juvenile dogs were similar; however, AUC parameters were significantly higher in the latter. This indicates reduced rates of excretion or reduced first pass liver metabolism or greater absolute bioavailability in the juveniles. . In any case, the relative AUCs are evidence of significantly higher systemic exposures in juveniles. Although sponsor felt that 1 mg/kg was a

NOAEL dose at 13 and 26 weeks, it was not a NOAEL dose at 39 weeks, i.e., there appeared to be delayed toxicity.

Table 16. Juvenile dog.

Table 4 **Toxicokinetic parameters and dose-dependence of ACT-333679 exposure after oral administration of ACT-293987 to dogs**

Dose (mg/kg/day)	Week	Sex	Tmax (h)	Cmax (ng/mL)	AUClast (h*ng/mL)	Tlast (h)	Cmax/D (ng/mL)/(mg/kg)	AUClast/D (h*ng/mL)/(mg/kg)
1	1	f	4	1260	15800	24	1300	16000
1	1	m	4	982	13300	24	980	13000
1	13	f	2	884	6470	24	880	6500
1	13	m	1	773	6770	24	770	6800
1	26	f	2	1900	14300	24	1900	14000
1	26	m	2	2110	20900	24	2100	21000
1	39	f	2	1900	13000	24	1900	13000
1	39	m	2	1940	16400	24	1900	16000
3	1	f	4	4810	63800	24	1600	21000
3	1	m	4	5360	70700	24	1800	24000
3	13	f	2	3510	25200	24	1200	8400
3	13	m	1	3510	25000	24	1200	8300
3	26	f	2	5290	52800	24	1800	18000
3	26	m	2	5230	55200	24	1700	18000
3	39	f	2	5330	45400	24	1800	15000
3	39	m	2	6510	44700	24	2200	15000
4	1	f	8	5570	79900	24	1400	20000
4	1	m	4	5730	71200	24	1400	18000
4	13	f	1	4320	36300	24	1100	9100
4	13	m	2	5350	34700	24	1300	8700
4	26	f	2	5600	48700	24	1400	12000
4	26	m	4	6200	57000	12[#]	1600	14000
4	39	f	2	6470	50300	24	1600	13000
4	39	m	1	5600	48800	12[#]	1400	12000
6	1	f	8	5920	83000	24	990	14000
6	1	m	8	5590	89800	24	930	15000

[#] Concentrations available at 1, 4 and 12 h post-dose from two dogs only

Mortality

Table 17(sponsor Text table 1 below)

Text Table 1 Summary of mortality

Group	Animal number	Gender	Day of death	Age at death	Status	Cause of death or moribundity
1	5	M	9	PND37	Premature euthanasia	Bronchopneumonia
2	14	M	30	PND60	Premature euthanasia	Lung inflammation
4	31	M	9	PND37	Found dead	Intestinal intussusception
4	32	M	34	PND62	Premature euthanasia	Protozoal enteritis
4	35	F	41	PND69	Premature euthanasia	Intestinal intussusception

Two animals in the high dose group died of intestinal intussusception, a lethal lesion in the high dose group in the toxicity study in adults as well. Intussusception is rare, but has also occurred in patients receiving prostacyclin therapy .The other animals died incidentally of non-treatment related causes.

Figure 15. Juvenile male body weights

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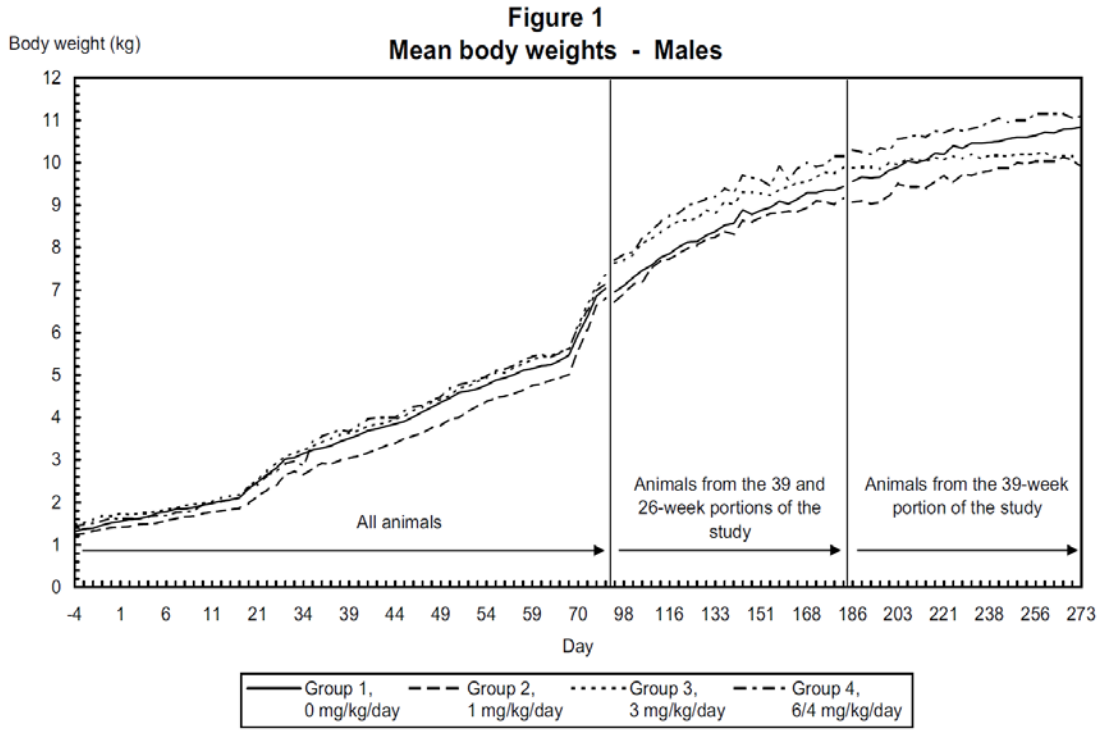

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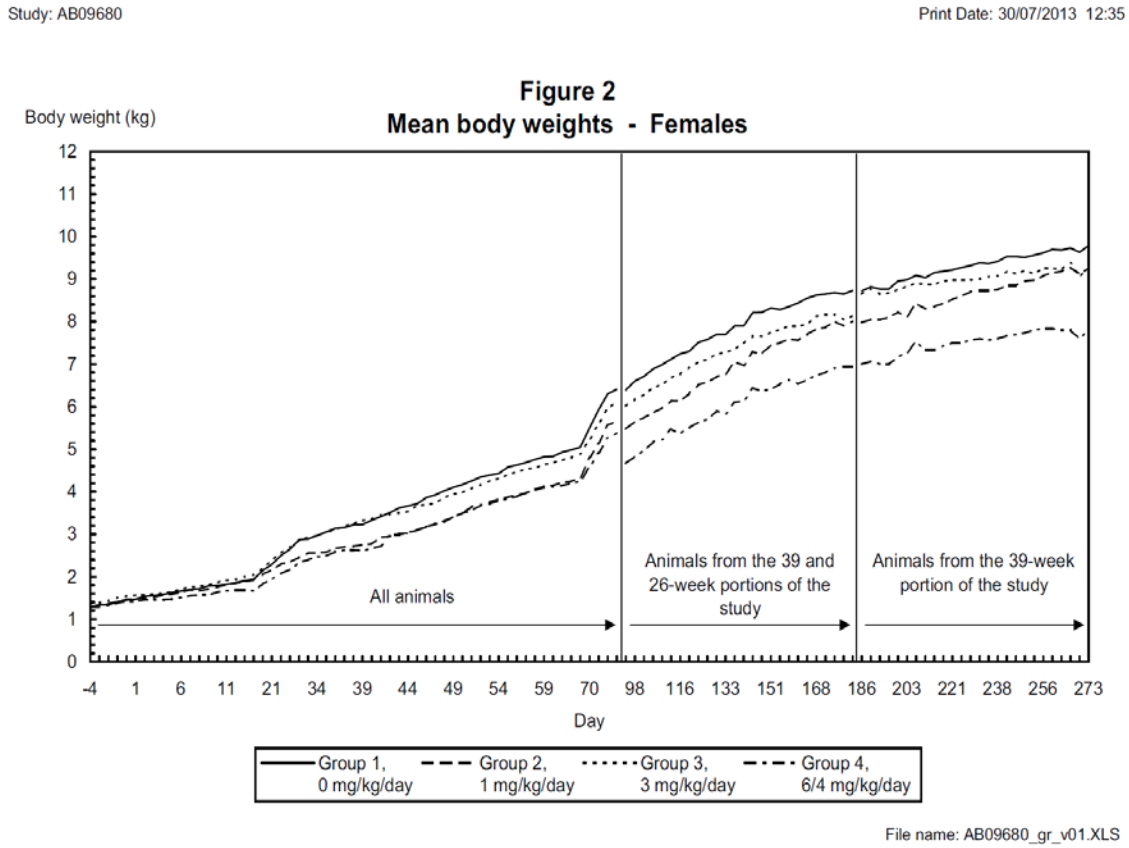
Figure 16. Juvenile female body weights



Male high dosage animals had the greatest weight gain, while the high dosage females had the lowest weight gain. The changes were not significant in juvenile males, but body weight was significantly depressed in the high dose juvenile female group.

Clinical Signs: the primary sign was flushing due to the vasodilator actions of prostacyclin, along with alopecia, especially in the first 13 weeks. This was more severe than in the adults, probably due to the higher exposures received by the juvenile animals. No other effects were considered toxicological.

No treatment-related effects were observed on growth, limb function, ophthalmology, heart rate, or on cardiac conduction, rhythm or intervals.

No effects were noted on hematology, or coagulation; clinical chemistry, or urine analysis parameters, or on markers of bone turnover.

Gross Pathology:
At necropsy, dermatitis, scaly skin, hair clumping and alopecia were the primary gross findings in the treated animals.

Both relative and absolute weights of the thymus, adrenal glands, prostate uterus and ovaries were reduced. The ovaries were also still immature by the end of the study in the intermediate and high dose animals. Although the study ended while development was ongoing, it is possible that the treatment was potentially slowing sexual development in dogs.

Bone: By 39 weeks of treatment, 2 of 3 low dose, and all the intermediate and high dose males had increased ossification of the femoral shaft. Tibial and femoral growth plates remained open. However, as they close between 8 and 10 months of age (approximately between 35 and 41 weeks), open plates would not be unexpected in a 39 week study.

Ovaries: Intermediate and high dose females had delayed ovarian maturation, characterized by pre-antral follicles rather than mature corpora lutea as seen in the control and low dose animals. Sponsor considered that the delay in sexual maturation may have been due to the lower weights (20% decrease, on average) and food consumption in the high dose females. In any case, sexual maturity seems to have been delayed in both genders.

Skin: Dose-related incidence and severity of dermatitis was a major issue. Per sponsor's description:
"This change was characterized by varying severities of orthokeratotic hyperkeratosis, parakeratosis, epidermal hyperplasia, spongiosis with irregular rete ridges, lymphocytic exocytosis of the epidermis, intraepidermal neutrophilic or eosinophilic pustules, dermal mixed perivascular or diffuse inflammatory cell infiltration with lymphocytes, plasma cells, histiocytes and often neutrophils, eosinophils and mast cells, as well as hyperplasia of sebaceous glands and decreased numbers of hair follicles."
The skin reactions were far more common and severe in juveniles versus adult dogs. The increased flushing seems to have led to further infiltration by cells of the immune system, potentially a factor in the increase in dermatitis in the juvenile dogs.

Summary: Juvenile animals have increased drug exposure levels vs. adults. Decreased food consumption and body weights were associated with early death for the high dose adult females, and delays in ovarian maturation. Toxicological dermatitis, accelerated bone ossification, and lethal intestinal intussusception are prominent in this population.

The 10-fold increase in AUC in juveniles is noteworthy and a consideration in the design and monitoring of any pediatric trials.

9 Special Toxicology Studies:

The effect of ACT-293987 (selexipag) and its metabolite ACT-333679 on testosterone production in rat Leydig cells *in vitro*

Primary cultures of rat Leydig cells were isolated and cultured to examine the effects of Uptravi on testosterone production. Cells were treated with vehicle, or exposed to concentrations of Uptravi and the active metabolite at 3, 10, 30, 50, and 100 mcM. .

Table 17. Selexipag cytotoxicity in rat Leydig cells

Table 4 Cytotoxicity in cultured rat Leydig cells treated for 20 h

Low LH (0.1 ng/mL)			Cell viability (%)	
Treatment	**(µM)**	n	Mean	% of control
Control	0	2	83.0	100
ACT-293987	3	2	82.7	100
	10	2	82.0	99
	30	2	78.5	95
	50	1	69.2	83
	100	2	53.7	65
ACT-333679	3	2	81.0	98
	10	2	81.3	98
	30	2	73.8	89
	50	1	71.4	86
	100	2	65.8	79
Ketoconazole	1	2	80.6	97
Aminoglutethimide	100	2	86.6	104

Values are means of single incubations from 2 independent experiments.

Sponsor Table 4 shows that at 30-50 mcM and above Uptravi and the active metabolite exhibited cytotoxicity. This is also apparent in the assays of testosterone production where the 100 mcM group usually showed a severe decline though lower concentrations promoted testosterone synthesis. The decline is probably related to the cytotoxicity shown in this table.

Table 18. Basal testosterone production in Leydig cells

Table 1 Testosterone production in isolated rat Leydig cells treated for 3 h under basal conditions

Unstimulated cells (basal)			Testosterone (ng/10^6 cells/3h)			
Treatment	**(µM)**	n	Mean		SD	% of control
Control	0	4	3.16	±	0.71	100
ACT-293987	3	4	3.69	±	0.26	117
	10	4	3.98	±	0.21	126
	30	4	4.66*	±	0.52	147
	50	2	8.39***	±	0.75	266
	100	4	1.50***	±	0.49	47
ACT-333679	3	4	3.05	±	0.26	96
	10	4	3.58	±	0.36	113
	30	4	4.30*	±	0.46	136
	50	2	7.21***	±	0.01	228
	100	4	1.77*	±	0.76	56
Ketoconazole	1	2	0.57*	±	0.48	18
Aminoglutethimide	100	2	0.72*	±	0.52	23

Values are means ± standard deviations of n incubations from 2 independent experiments. Asterisks indicate results significantly different (Student's t-test) from the control: *:$p<0.05$, **:$p<0.01$, ***:$p<0.001$.

The effect of selexipag and its active metabolite on basal testosterone production was identified in the absence of luteinizing hormone (LH). Basal production under these conditions was low as anticipated. Uptravi treatment effect was biphasic, i.e., steroid production was enhanced by up to 2.6-fold at 50 mcM but at 100 mcM, synthesis was reduced to 50% of basal production.

Table 19. Testosterone production in Leydig cells stimulated by luteinizing hormone

Table 2 Testosterone production in isolated rat Leydig cells treated for 3 h under LH-stimulated conditions

LH-stimulated cells (100 ng/mL)			Testosterone (ng/10^6 cells/3h)			
Treatment	**(µM)**	n	Mean		SD	% of control
Control	0	4	39.31	±	1.65	100
ACT-293987	3	4	36.93	±	1.80	94
	10	4	33.51	±	5.74	85
	30	4	44.76	±	8.01	114
	50	2	49.01*	±	5.88	125
	100	4	3.11***	±	0.88	8
ACT-333679	3	4	34.93	±	1.68	89
	10	4	36.62	±	0.89	93
	30	4	39.24	±	5.71	100
	50	2	29.09**	±	2.66	74
	100	4	3.79***	±	1.07	10
Ketoconazole	1	2	0.78***	±	0.54	2
Aminoglutethimide	100	2	1.73***	±	1.83	4

Values are means ± standard deviations of n incubations from 2 independent experiments. Asterisks indicate results significantly different (Student's t-test) from the control: *:$p<0.05$, **:$p<0.01$, ***:$p<0.001$.

Effect of selexipag was biphasic whereas the active metabolite did not stimulate synthesis of this steroid. At 100µM, both the parent and the active metabolite significantly depressed synthesis of testosterone. The positive controls Ketoconazole and aminoglutethimide behaved as expected.

Table 20. Leydig cell testosterone production in 20 hr. treatment

Table 3 Testosterone production in cultured rat Leydig cells treated for 20 h

Low LH (0.1 ng/mL)			Testosterone (ng/10^6 cells/20h)			
Treatment	**(µM)**	n	Mean		SD	% of control
Control	0	6	37.05	±	2.01	100
ACT-293987	3	4	39.96	±	2.06	108
	10	4	38.39	±	3.11	104
	30	4	58.73***	±	9.69	159
	50	2	105.30***	±	6.69	284
	100	4	2.92***	±	0.86	8
ACT-333679	3	4	35.01	±	1.29	94
	10	4	38.31	±	2.21	103
	30	4	68.53***	±	8.55	185
	50	2	124.78***	±	2.74	337
	100	3	6.21***	±	2.22	17
Ketoconazole	1	3	1.18***	±	1.97	3
Aminoglutethimide	100	3	2.18***	±	3.83	6

Values are means ± standard deviations of n incubations from 2 independent experiments. Asterisks indicate results significantly different (Student's t-test) from the control: *:p<0.05, **:p<0.01, ***:p<0.001.

Sponsor's Table 3 shows the effects of Uptravi and the active metabolite on testosterone production after 20 hrs. of exposure to 100 ng/mL of LH. Both significantly increased testosterone synthesis at 30 and 50 mcM, and both decreased production at 100 mcM due to cytotoxicity. The positive controls behaved as expected in this assay.

Since clinical serum levels will be closer to 3 mcM, selexipag and the active metabolite are expected to have minimal, if any, effect on testosterone levels.

The effect of ACT-293987 (selexipag) on the pituitary-testicular endocrine axis in the rat: Determination of hormone levels

Sprague-Dawley rats were treated with 100 or 150 mg/kg/day of selexipag to examine its effects on the pituitary-testosterone axis per luteinizing hormone (LH) and testosterone levels. Ketoconazole, which inhibits testosterone production, was the positive control, and vehicle was the negative control.

Methods (from sponsor):

Treatments (dose levels) Allocation	**Group 1** Control# vehicle	**Group 2** **Selexipag** 100 mg/kg/day	**Group 3** **Selexipag** 150 mg/kg/day	**Group 4** **Ketoconazole** 50 mg/kg/day
A	1-10	11-20	21-30	31-35
B	36-38	39-41	42-44	-

#Control animals were treated with the vehicle only.

A: main study animals.

B: animals for PK.

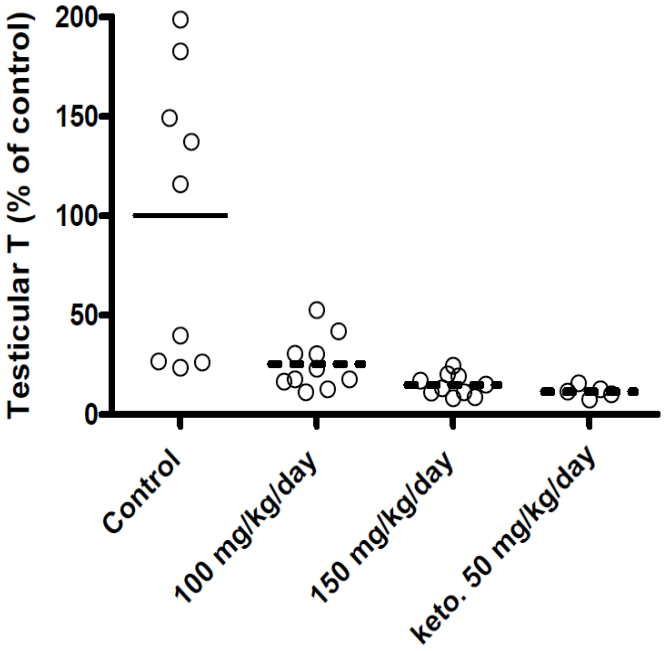
6.8 Route and duration of administration

6.8.1 Main study (allocation A)

The animals were treated orally with the test or reference compound daily for 28 days. On Day 29, the animals were killed 2 h after last dosing. A constant dose volume of 5 mL/kg was used. Intergroup variability was minimized by 4 days stratification of treatments and sacrifices across the dose cohorts. Rats in Allocation B were used to assay for blood levels.

Figure 17. Testosterone levels

Figure 15 Individual and mean testicular testosterone concentrations

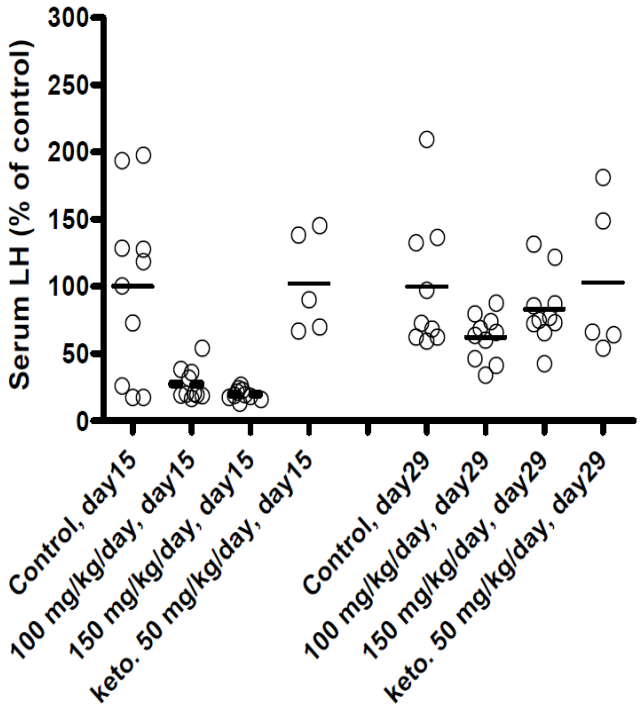


The effect of selexipag and ketoconazole (keto.) on testicular testosterone content after 29 days of treatment. For each group, the horizontal line represents group mean of the presented individual values (dashed line when statistically different from control). Values are expressed in % of the mean value observed with the control group (100%). Absolute values are presented in Table 10 and Table 11.

Sponsor's Figure 15 shows that selexipag has reduced testosterone production after 29 continuous days of oral treatment. The next figure provides some mechanistic insight by showing that selexipag inhibits luteinizing hormone, which is required for testosterone production by the Leydig cells.

Figure 18. Luteinizing hormone concentrations

Figure 12 Individual and mean serum luteinising hormone concentrations



The effect of selexipag and ketoconazole (keto.) on serum luteinising hormone content after 15 and 29 days of treatment. For each group, the horizontal line represents group mean of the presented individual values (dashed line when statistically different from control). Values are expressed in % of the mean value observed with the respective control groups (100%). Absolute values are presented in Table 8, Table 9, Table 10, and Table 11.

LH was reduced at day 15, although there was some tolerance to this effect noted by day 29. Selexipag reduced Prolactin slightly but not FSH or, dihydrotestosterone levels.

Other selexipag-associated findings were: decreased body weights, weight gain, and increased water consumption; significantly lowered absolute, but not relative, prostate weights; significantly lowered absolute and relative seminal vesicle weights; and increased adrenal gland weights. Testis weight and pituitary gland size were unaffected.

In conclusion, selexipag significantly decreases testosterone production and luteinizing hormone levels in 29 day trials in rats. However, there was no effect on pituitary gland, and prolactin levels were not significantly depressed.

Effects of NS-304 (MRE-304) on Liver Drug Metabolizing Enzymes and Thyroid Hormones in Mice

This study was designed to follow up on thyroid hyperplasia expressed in the 2-year carcinogenicity study of selexipag in mice. The study examined CYP450 activity as well as thyroid hormones T4, TSH, and T3. The selexipag treatment groups were configured as follows (from sponsor):

6.7 Group composition

6.7.1 Main study group

Group	Test substance	Dose (mg/kg)	Number of animals (animal number)	
			Male	Female
1	Control [*1]	0	10 (10101 – 10110)	10 (50101 – 50110)
2	NS-304	62.5	10 (10201 – 10210)	10 (50201 – 50210)
3	NS-304	125	10 (10301 – 10310)	10 (50301 – 50310)
4	NS-304	250	10 (10401 – 10410)	10 (50401 – 50410)
5	NS-304	400	10 (10501 – 10510)	10 (50501 – 50510)

[*1] Vehicle (0.5 w/v% MC)

6.7.2 TK satellite group

Group	Test substance	Dose (mg/kg)	Number of animals (animal number)	
			Male	Female
1	Control [*1]	0	6 (20101 – 20106)	6 (60101 – 60106)
2	NS-304	62.5	39 (20201 – 20239)	39 (60201 – 60239)
3	NS-304	125	6 (20301 – 20306)	6 (60301 – 60306)
4	NS-304	250	6 (20401 – 20406)	6 (60401 – 60406)
5	NS-304	400	39 (20501 – 20539)	39 (60501 – 60539)

[*1] Vehicle (0.5 w/v% MC)

Animals were dosed once daily via oral gavage for 28 days.

Table 21. T3, T4 and TSH values in males

Table 1 Plasma T_3, T_4, and TSH concentrations in male mice treated with NS-304

Group No.	Test substance	Dose (mg/kg)		T_3 (ng/mL)		T_4 (μg/dL)		TSH (ng/mL)	
1	NS-304	0	Mean	0.50		2.74		1.68	
			S.D.	0.06		0.41		0.60	
			n	10		10		10	
2	NS-304	62.5	Mean	0.54	(108.0)	2.73	(99.6)	1.95	(116.1)
			S.D.	0.07		0.36		0.34	
			n	10		10		10	
3	NS-304	125	Mean	0.50	(100.0)	2.67	(97.4)	2.41	(143.5)
			S.D.	0.04		0.31		0.51	
			n	10		10		10	
4	NS-304	250	Mean	0.61	(122.0)	2.58	(94.2)	2.52 $	(150.0)
			S.D.	0.13		0.31		0.78	
			n	10		10		10	
5	NS-304	400	Mean	0.85 ##	(170.0)	2.92	(106.6)	2.72 $$	(161.9)
			S.D.	0.17		0.60		1.00	
			n	10		10		10	

##: P<0.01 (significantly different from the control group by the Steel test)

$: P<0.05, $$: P<0.01 (significantly different from the control group by the Dunnett test)

Figures in parenthesis indicate the percentage of concentration of NS-304 treatment group to the 0 mg/kg group.

Table 22. T3, T4, and TSH values in females

Table 2 Plasma T_3, T_4, and TSH concentrations in female mice treated with NS-304

Group No.	Test substance	Dose (mg/kg)		T_3 (ng/mL)		T_4 (μg/dL)		TSH (ng/mL)	
1	NS-304	0	Mean	0.50		2.54		1.02	
			S.D.	0.08		0.45		0.75	
			n	10		10		10	
2	NS-304	62.5	Mean	0.55	(110.0)	2.33	(91.7)	1.55	(152.0)
			S.D.	0.10		0.42		0.92	
			n	10		10		10	
3	NS-304	125	Mean	0.48	(96.0)	2.50	(98.4)	1.05	(102.9)
			S.D.	0.07		0.52		0.82	
			n	10		10		10	
4	NS-304	250	Mean	0.67 $$	(134.0)	2.57	(101.2)	1.31	(128.4)
			S.D.	0.10		0.59		0.89	
			n	10		10		10	
5	NS-304	400	Mean	0.68 $$	(136.0)	2.37	(93.3)	1.50	(147.1)
			S.D.	0.12		0.32		0.93	
			n	10		10		10	

$$: P<0.01 (significantly different from the control group by the Dunnett test)

Figures in parenthesis indicate the percentage of concentration of NS-304 treatment group to the 0 mg/kg group.

The results of thyroid hormone assays are not readily interpretable. Hepatic CYP450 enzymes are responsible for the breakdown of thyroid hormones T4 and T3. Since liver enzyme activity is apparently increased in treated mice(see table 23 immediately below), the sponsor proposes that the reason T4 and T3 levels remain essentially constant except at the highest doses, is that the increased TSH production is able to increase T3 and T4 levels above the rate of destruction due to the increase in liver enzyme activity..

Table 23. Liver microsomal protein, male

Table 7 Microsomal protein contents in liver Male

Test Substance Dose	Animal Number	Microsomal protein concentration mg protein/mL		Microsomal protein content in liver mg protein/g liver	
NS-304 0 mg/kg	Mean	13.6	(100.0)	28.5	(100.0)
	S.D.	1.1		2.5	
	n	10		10	
NS-304 62.5 mg/kg	Mean	12.9	(94.9)	27.5	(96.5)
	S.D.	0.8		1.6	
	n	10		10	
NS-304 125 mg/kg	Mean	14.3	(105.1)	30.3	(106.3)
	S.D.	1.0		2.0	
	n	10		10	
NS-304 250 mg/kg	Mean	14.3	(105.1)	30.7	(107.7)
	S.D.	1.3		2.9	
	n	10		10	
NS-304 400 mg/kg	Mean	15.9 **	(116.9)	33.7 **	(118.2)
	S.D.	0.9		1.6	
	n	10		10	

**: P<0.01 (significantly different from the NS-304 0 mg/kg by Dunnett test)
Figures in parenthesis indicate the percentage to NS-304 0 mg/kg group.

Table 24. Liver microsomal protein, female

Table 8 Microsomal protein contents in liver Female

Test Substance Dose	Animal Number	Microsomal protein concentration mg protein/mL		Microsomal protein content in liver mg protein/g liver	
NS-304 0 mg/kg	Mean	16.0	(100.0)	33.7	(100.0)
	S.D.	1.1		1.8	
	n	10		10	
NS-304 62.5 mg/kg	Mean	14.6 *	(91.3)	30.6 **	(90.8)
	S.D.	1.2		2.6	
	n	10		10	
NS-304 125 mg/kg	Mean	14.7 *	(91.9)	31.2 *	(92.6)
	S.D.	1.0		2.1	
	n	10		10	
NS-304 250 mg/kg	Mean	15.1	(94.4)	32.1	(95.3)
	S.D.	1.0		1.6	
	n	10		10	
NS-304 400 mg/kg	Mean	15.9	(99.4)	33.7	(100.0)
	S.D.	1.2		2.3	
	n	10		10	

*: P<0.05, **: P<0.01 (significantly different from the NS-304 0 mg/kg by Dunnett test)
Figures in parenthesis indicate the percentage to NS-304 0 mg/kg group.

Sponsor tables 23 and 24 reveal a 15% increase in liver microsomal proteins in males treated with 400 mg/Kg of selexipag.

Table 25. Liver cytochrome P450 levels, male mice

Table 9 Cytochrome P450 contents in liver Male

Test Substance		Cytochrome P450 content					
		nmol/mg protein		nmol/g liver		nmol/total liver	
Dose	Animal Number						
NS-304	Mean	0.759	(100.0)	21.5	(100.0)	30.7	(100.0)
0 mg/kg	S.D.	0.088		2.6		4.1	
	n	10		10		10	
NS-304	Mean	0.855	(112.6)	23.4	(108.8)	34.8	(113.4)
62.5 mg/kg	S.D.	0.071		1.5		2.8	
	n	10		10		10	
NS-304	Mean	0.935 **	(123.2)	28.4 ##	(132.1)	42.3 **	(137.8)
125 mg/kg	S.D.	0.080		3.5		5.9	
	n	10		10		10	
NS-304	Mean	1.10 **	(144.9)	33.8 ##	(157.2)	53.4 **	(173.9)
250 mg/kg	S.D.	0.12		4.8		7.3	
	n	10		10		10	
NS-304	Mean	1.25 **	(164.7)	42.0 ##	(195.3)	73.6 **	(239.7)
400 mg/kg	S.D.	0.10		2.0		4.4	
	n	10		10		10	

**: P<0.01 (significantly different from the NS-304 0 mg/kg by Dunnett test)
##: P<0.01 (significantly different from the NS-304 0 mg/kg by Steel test)
Figures in parenthesis indicate the percentage to NS-304 0 mg/kg group.

Table 26. Liver cytochrome P450 levels, female mice

Table 10 Cytochrome P450 contents in liver Female

Test Substance		Cytochrome P450 content					
		nmol/mg protein		nmol/g liver		nmol/total liver	
Dose	Animal Number						
NS-304	Mean	0.629	(100.0)	21.2	(100.0)	29.3	(100.0)
0 mg/kg	S.D.	0.044		1.3		2.1	
	n	10		10		10	
NS-304	Mean	0.719 **	(114.3)	22.0	(103.8)	31.0	(105.8)
62.5 mg/kg	S.D.	0.065		2.1		2.9	
	n	10		10		10	
NS-304	Mean	0.758 **	(120.5)	23.7	(111.8)	32.9 #	(112.3)
125 mg/kg	S.D.	0.057		2.8		2.8	
	n	10		10		10	
NS-304	Mean	0.914 **	(145.3)	29.3 **	(138.2)	42.2 ##	(144.0)
250 mg/kg	S.D.	0.036		1.3		2.5	
	n	10		10		10	
NS-304	Mean	1.23 **	(195.5)	41.3 **	(194.8)	63.9 ##	(218.1)
400 mg/kg	S.D.	0.07		3.4		7.6	
	n	10		10		10	

**: P<0.01 (significantly different from the NS-304 0 mg/kg by Dunnett test)
#: P<0.05, ##: P<0.01 (significantly different from the NS-304 0 mg/kg by Steel test)
Figures in parenthesis indicate the percentage to NS-304 0 mg/kg group.

Selexipag doubles the CYP450 activity in males and females over a dose range of 62-400 mg/Kg. To support this, the sponsor tables 11-18 (not shown) show that all the basic substrates for CYP 450 activity showed increased activity to go along with the increase in CYP450 protein. The study was GLP-compliant, and toxicokinetics and clinical signs were similar to those reported in the other toxicology studies of selexipag.

11.0 Integrated Summary and Safety Evaluation

Selexipag (Uptravi®) is the first non-eicosanoid prostacyclin agonist submitted to the FDA for the treatment of Pulmonary Arterial Hypertension. While the intact molecule is active at the prostacyclin receptor, it is rapidly converted to a metabolite with a longer half-life. This allows for orally-active selexipag to be given bid, unlike Ventavis that requires approximately 9 administrations per day or some of the other prostanoids that require continuous infusion due to short half-life.

Most of the selexipag data was submitted with ND in 2009. The data was reviewed at that time and can be found in Appendix I.

Uptravi is intended to treat pulmonary arterial hypertension, (b) (4) (b) (4).

Selexipag, and its active metabolite, are specific for the prostacyclin receptor. Safety pharmacology studies show no effects on hERG channels. However, due to its pharmacological activity of relaxing arterioles it can lower blood pressure and raise heart rate. Flushing of the skin in particular was noted. High doses also increased respiratory rates and tidal volumes. Hunching, increased struggling when handled, tip-toe gaits, righting reflex problems, low body temperature, soft stools and impaired grasping were noted at higher dosages. The GI effects (decreased peristalsis, decreased gastric acid secretion) are not unexpected. Decreased urinary output with decreased sodium and chloride transport were also expected consequences of selexipag treatment. All effects were reversible after withdrawal of the drug. Similar activity was expressed for the active metabolite, ACT-333679.

Selexipag is the parent compound is rapidly absorbed and metabolized to ACT-333679 that has a much longer half-life. This allows for bid dosing in humans. Pharmacokinetic behavior may be age-dependent as juvenile and adult dogs had similar Cmax levels, but the former had AUC values for the metabolite that were over 10-fold that of adults.

The toxicity of selexipag in safety studies was overtly dose-related i.e., no idiosyncratic reactions were observed. In dogs, lethal intussusception occurred in the highest dose groups in both the adult and juvenile studies. In the mouse carcinogenicity studies, severe lethal gastric erosion occurred in 24/60 females in the high dosage group. Adverse GI effects may reflect the prominence of prostacyclin receptors in that tract. The skin was also a major target of toxicity. With higher dosing, flushing, scaling, piloerection, alopecia, and hair clumping were observed. Dermatological effects were significant by the end of the carcinogenicity study, especially in the high dosage group. Adrenal glands developed hypertrophy, while bones had increased ossification of the periosteum and trabeculae. In mice, selexipag treatment upregulates CYP 450 enzymes in the liver and leads to an increase in T3 and T4 levels. In rats, there was a concern over testosterone production since Leydig cell neoplasia and hyperplasia were detected in the carcinogenicity assay. Selexipag had a biphasic effect on testosterone production *in vitro*, and depressed secretion in vitro and in vivo only at concentrations that will not be achieved clinically.

Non-clinical findings indicate that selexipag is pharmacodynamically and toxicologically similar to other prostacyclin agonists. Most of the adverse effects are considered to be extensions of the pharmacology of the drug. This prominently includes gastric erosion, intussusception, and some level of skin flushing at all dosage levels

Toxicokinetic data (Sponsor tables 27 and 28 below) show that for the adverse events, there is a reassuring safety margin for human usage. Juvenile dogs,

evidently less capable than adults at metabolizing selexipag, however provide data that would forecast a smaller safety margin for pediatric use absent a dose adjustment.

Although selexipag is a nonprostanoid prostacyclin agonist, it is forecast to carry the risk of gastro-intestinal side effects, including diarrhea and nausea, vaso-dilation and some increased bleeding that other prostacyclin agonists do. The primary improvement would be oral bioavailability and b.i.d. dosing. This should afford better compliance. The non-clinical toxicological and pharmacokinetic data are adequate to identify both safety issues and safety margins (table 27) of selexipag to support its approval.

Table 27. Exposure multiples based on AUC at NOAELs vs. clinical:

Table 21 Exposure ratios based on systemic exposures of ACT-333679 in animals at NOEL/NOAEL/LOEL and in healthy subjects at 1600 µg b.i.d.

ACT-333679 - exposure ratio based on AUC			
	Animal data		Exposure ratio[1]
Study	**Dose (mg/kg/day)**	**AUC_{0-24} (ng·h/mL) Mean (M+F)**	**Human exposure:** AUC_{0-24}: 276 ng·h/mL
26 week rat	NOAEL: 6	5780	20.9
39 week dog	LOEL: 1	29400	107
39 week dog (intussusception)	NOEL: 2	49700	180
[Corrected for potency]			*[2.20]*
104 week rat (neoplastic findings)	NOEL: 30	25400[2]	92.0
104 week mouse (neoplastic findings)	NOEL: 125	14950	54.2

ACT-333679 - exposure ratio based on C_{max}			
	Animal data		Exposure ratio[1]
Study	**Dose (mg/kg/day)**	**C_{max} (ng/mL) Mean (M+F)**	**Human exposure:** C_{max}: 27 ng/mL
26 week rat	NOAEL: 6	1520	56.3
39 week dog	LOEL: 1	2930	109
39 week dog (intussusception)	NOEL: 2	5580	207
[Corrected for potency]			*[2.52]*
104 week rat (neoplastic findings)	NOEL: 30	7640[2]	283
104 week mouse (neoplastic findings)	NOEL: 125	11900	441

[1] Human AUC_{0-12h} (geometric mean of male and female mean AUC on Day 23) is doubled to account for b.i.d. dosing to obtain AUC_{0-24h} [D-13.117, Table 23].
[2] End of treatment mean values from male animals due to testes findings.
AUC: area under the plasma concentration vs time curve; b.i.d.: twice daily; C_{max}: maximum observed plasma concentration; IP: prostacyclin; LOEL: lowest-observed-effect level; NOAEL: observed-adverse-effect level; NOEL: no-observed-effect level.

Table 28. Exposure ratio comparison to animals and humans

Table 22 Exposure ratios based on systemic exposures of selexipag in animals at NOEL/NOAEL/LOEL and in healthy subjects at 1600 µg b.i.d.

	Selexipag - exposure ratio based on AUC		
	Animal data		Exposure ratio[1]
Study	**Dose (mg/kg/day)**	**AUC_{0-24} (ng·h/mL) Mean (M+F)**	**Human exposure:** AUC_{0-24}: 276 ng·h/mL
26 week rat	NOAEL: 6	260	2.95
39 week dog	LOEL: 1	2715	30.9
39 week dog (intussusception)	NOEL: 2	7345	83.5
104 week rat (neoplastic findings)	NOEL: 30	1690[2]	19.2
104 week mouse (neoplastic findings)	NOEL: 125	15100	172

	Selexipag - exposure ratio based on C_{max}		
	Animal data		Exposure ratio[1]
Study	**Dose (mg/kg/day)**	**C_{max} Mean (M+F) (ng/mL)**	**Human exposure:** C_{max}: 27 ng/mL
26 week rat	NOAEL: 6	1782	99.0
39 week dog	LOEL: 1	1400	77.8
39 week dog (intussusception)	NOEL: 2	2445	136
104 week rat (neoplastic findings)	NOEL: 30	1280[2]	71.1
104 week mouse (neoplastic findings)	NOEL: 125	14600	811

[1] Human AUC_{0-12h} (geometric mean of male and female mean AUC on Day 23) is doubled to account for b.i.d. dosing to obtain AUC_{0-24h} [D-13.117, Table 22].
[2] End of treatment mean values from male animals due to testes findings.
AUC: area under the plasma concentration vs time curve; b.i.d.: twice daily; C_{max}: maximum observed plasma concentration; IP: prostacyclin; LOEL: lowest-observed-effect level; NOAEL: observed-adverse-effect level; NOEL: no-observed-effect level.

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12 Appendix/Attachments:

Appendix I. IND 104504 review.

Appendix II. Statistical review of carcinogenicity data

Appendix III. CAC data sheets for rat and mouse carcinogenicity studies.

Appendix IV. Sponsor tables of mouse carcinogenicity study tumor incidence.

Appendix V. Sponsor table of rat carcinogenicity study tumor incidence.

Appendix VI. Executive CAC minutes for Selexipag

APPENDIX I: IND 104504 review (J. Willard,6/28/2010) :

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW – Appendix I

2.6.1INTRODUCTION AND DRUG HISTORY

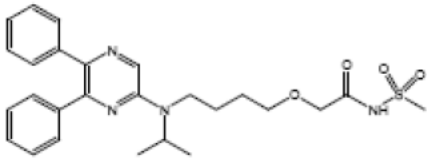
IND number: 104504
Review number: 1
Sequence number/date/type of submission:
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: Actelion Pharmaceuticals, Ltd.
Manufacturer for drug substance: (b) (4)

Reviewer name: James M. Willard, Ph.D.
Division name: **Division of Cardiovascular and Renal Products**
HFD #: **110**
Review completion date: 6/28/2010

Drug:

Trade name:
Generic name:
Code name: ACT-293987 (also NS-304)
Chemical name: 2-{4-[(5, 6-diphenylpyrazin-2-yl) (isopropyl) amino] butoxy}-N-
(methyl sulfonyl) acetamide
CAS registry number:
Molecular formula/molecular weight: /$C_{26}H_{32}N_4O_4S$/496.62

Structure:



Relevant INDs/NDAs/DMFs:

Drug class: Prostacyclin (IP) receptor agonist

Intended clinical population: Patients with Pulmonary Arterial Hypertension (PAH)

Clinical formulation:

Table 1: Quantitative compositions of ACT-293987 Tablet

Ingredient	Specification	Function	ACT-293987 Tablet 200 µg	ACT-293987 Tablet (Placebo)
ACT-293987	In house	API	0.2 mg	-
D-Mannitol	USP/NF			(b) (4)
Corn starch	USP/NF			
Low substituted Hydroxypropylcellulose	USP/NF			
Hydroxypropylcellulose	USP/NF			
Magnesium stearate	USP/NF			
(b) (4)				
Hypromellose	USP/NF			
Propylene glycol	USP/NF			
Titanium oxide	USP/NF			
Yellow ferric oxide	USP/NF			
Carnauba wax	USP/NF			
Coating weight				
Total weight of film-coated tablet				(b) (4)

Route of administration: oral

Proposed clinical protocol:
From the sponsor:

> "The sponsor intends to start two identical multicenter, double-blind, randomized, placebo-controlled, parallel group, event-driven clinical studies in adult patients with symptomatic PAH. Each trial will have 272 patients to be randomized into 2 groups (n = 136/group), placebo: active (1:1). The primary objective for each single study will be to demonstrate the effect of ACT-293987 on 6 minute walk distance (6MWD) from baseline to Week 16. The co-primary objective for the pool of the two studies will be to demonstrate the effect of ACT-293987 on time to clinical worsening up to the end of treatment (EOT) (expected maximum duration 4.1 years). An open-label extension (AC-065A303) of these two studies will be aimed at collecting long-term safety and tolerability in patients who will have experienced a clinical worsening of PAH or who will have completed the double-blind phase of the two studies."

Previous clinical experience: Six clinical studies have been done with ACT-293987, originally developed by Nippon Shinyaku Co. Ltd., Kyoto, Japan. Five Phase I studies were done, fulfilling a first in man study, and looking at pharmacokinetics and tolerability. One Phase 2a study has been carried out of 17 weeks duration, with an open-label extension still ongoing. Approximately 184 patients have been exposed to ACT-293987 with the following side effects seen: headache, myalgia, arthralgia, flushing, jaw pain, nausea, vomiting and diarrhea.

Disclaimer: Tabular and graphical depictions of information are constructed by the reviewer unless cited otherwise.
Note: For IND reviews, unused headings may be deleted.

Studies reviewed within this submission:
Studies not reviewed within this submission:

TABLE OF CONTENTS

2.6.2PHARMACOLOGY

2.6.2.1 Brief summary

Prostacyclin receptors are primary targets in Pulmonary Arterial Hypertension, however, this development area has had issues with most of the compounds not being orally available and having very short half-lives requiring frequent administration. ACT-293987 is a pro-drug of ACT-333679, which is produced by hydrolysis of the parent compound in the liver. ACT-333679 is a non-prostanoid compound that acts as an agonist at the IP receptor with high affinity. Studies show ACT-333679 is highly selective for IP receptors, with some small reactivity with EP_2 receptors. In animal models of PAH, ACT-333679 improved hemodynamic parameters indicative that it has a potential to help clinical cases of PAH. One important issue is that all the non-prostanoid prostacyclin agonists have more the properties of a partial agonist and are not fully able to activate all the functions of both IP1 and IP2 receptors that endogenous prostacyclin does. (Seller, et al., 1997, Prostaglandins 53:21-35)

2.6.2.2 Primary pharmacodynamics

Mechanism of action: ACT-293987 is a pro-drug that is hydrolyzed into ACT-333679, which is a high affinity agonist at IP receptors, and intended for relief from PAH.

Drug activity related to proposed indication: ACT-293987 is a pro-drug that is hydrolyzed into ACT-333679, which is a high affinity agonist at IP receptors intended to relieve PAH.

2.6.2.3 Secondary pharmacodynamics. No studies have been conducted beyond the safety pharmacology studies noted below which are observational rather than mechanistic, and typically explore high dosages.

2.6.2.4 Safety pharmacology

Neurological effects:

Central nervous system						
Irwin screen (b) (4) 08.267]	Y	Oral	Single dose	Sprague-Dawley rats	0, 10, 30, 100 mg/kg	6 m / group
Body temperature, sleep & pain response [T-08.383]	Y	Oral	Single dose	Sprague-Dawley rats	0, 10, 30, 100 mg/kg	6 m / group

The modified Irwin Screen performed by the sponsor was designed to look for signs of behavioral or physiologic changes indicative of effects on the central or peripheral nervous systems. The sponsor used high doses of the compound, 10, 30, and 100 mg/kg in rats. The low and mid dose had no noticeable effects on the rats, however, the high dose group exhibited hunchback position, reddened skin, struggle response to handling, tip-toe gait, problems with righting reflex, prolonged standing on the hind paws, low body temperature, deep respiration, soft stools, and difficulty with the wire maneuver. All signs disappeared within 24 hrs. of drug administration.

The second study on body temperature, sleep and pain responsiveness used a control group, and doses of 10, 30, and 100 mg/kg. The high dose group saw all three parameters impacted, with a body temperature lowered7%, sleep prolonged by 77%, and pain response reduced by 26%. The mid dose group only saw a reduction in body temperature.

Summary: The neurological safety pharmacology studies indicate at high doses notable effects occur in the modified Irwin screening, along with results of an examination of CNS effects (body temperature, sleep, and pain response).

Cardiovascular effects:

Cardiovascular system						
hERG channel K^+ current (ACT-293987) (b)(4)-08.268]	Y	*In vitro*	N/A	CHO-K1 cell line expressing hERG K^+ channel	0, 3, 10, 30, 100 µM	6 replicates
hERG channel K^+ current (ACT-333679) (b)(4)-08.270]	Y	*In vitro*	N/A	CHO-K1 cell line expressing hERG K^+ channel	0, 3, 10, 30 µM	6 replicates
Contractile force & heart rate (right atria) (ACT-293987) (b)(4)-08.271]	Y	*In vitro*	N/A	Isolated right atrium from Hartley guinea pig	0, 3, 10, 30, 100 µM	6 m
Contractile force & heart rate (right atria) (ACT-333679) (b)(4)-08.382]	Y	*In vitro*	N/A	Isolated right atrium from Hartley guinea pig	0, 3, 10, 30, 100 µM	6 m
Cardiac electrophysiology (ACT-293987) (b)(4)-08.269]	Y	*In vitro*	N/A	Isolated papillary muscle from Hartley guinea pig	0, 3, 10, 30, 100 µM	6 m
Hemodynamics & cardiac electrophysiology (telemetry study) (b)(4)-08.265]	Y	Oral	Single dose × 4 (crossover design)	Conscious Beagle dogs	0, 1, 3, 10 mg/kg	4 m
Blood coagulation [T-08.387]	Y	Oral	Single dose	Sprague-Dawley rats	0, 10, 30 or 100 mg/kg	6 m / group

Several studies were done to explore potential effects of ACT-293987 on the cardiovascular system. Prostacyclin agonists are known to be vasodilators, the basis for expecting efficacy in Pulmonary Arterial Hypertension (PAH). Therefore it is important to assess any impact on the cardiovascular system.

ACT-293987 at concentrations up to 30 mcM had no effect on hERG channels, and only had effects on guinea pig atria and papillary muscle in vitro at the highest dose tested, 100 mcM, indicating it probably affects Na or Ca channels. ACT-293987 had no effect on blood coagulation in vivo in rats. However, the compound reduced blood pressure at its lowest dose, and at the mid dose it also increased heart rate and respiration rates. There were no effects on ECG parameters or hemoglobin oxygenation.

Pulmonary effects:

Respiratory system						
Whole body plethysmography (b)(4)-08.266]	Y	Oral	Single dose	Conscious, unrestrained Sprague-Dawley rats	0, 10, 30, 100 mg/kg	6 m / group

Rats were treated with control, 10, 30, 100 mg/kg of ACT 293987. Animals in the mid and high dose groups showed higher respiration rates, tidal volumes and minute volumes.

Renal effects:

Urinary system						
Water & electrolyte excretion (b) (4)08.272]	Y	Oral	Single dose	Sprague-Dawley rats	0, 10, 30, and 100 mg/kg	6 m / group

Results indicate that ACT-293987 at all tested doses decreased chloride and sodium/potassium ratio in rats.

Gastrointestinal effects:

Gastrointestinal system						
Intestinal transport [T-08.384]	Y	Oral	Single dose	Sprague-Dawley rats	0, 10, 30, 100 mg/kg	6 m / group
Gastric secretion [T-08.384]	Y	Intra-duodenal	Single dose	Sprague-Dawley rats	0, 10, 30, 100 mg/kg	6 m / group

Prostacyclin receptors are common in the gastrointestinal tract. All doses of ACT-293987 tested inhibited GI transit of the carbon powder and decreased acid output in the stomach.

Abuse liability: n/a

Other:

Reproductive system						
Amplitude & frequency of uterine contraction (ACT-333679) [T-08.385]	Y	*In vitro*	N/A	Sprague-Dawley rats	0, 10, 30, 100 µM	6 f / group (4 f for 100 µM)

Spontaneous contractions of the uterine muscle were not affected by the low dose of ACT-293987, while the mid and high doses significantly reduced uterine muscle contractions.

2.6.2.5 Pharmacodynamic drug interactions - No studies done, but see below for anticipated interactions based on pharmacology of prostacyclin.

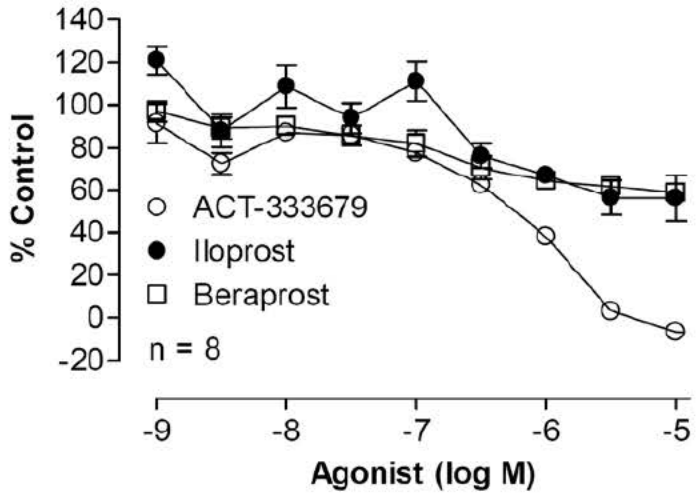
2.6.3 PHARMACOLOGY TABULATED SUMMARY:

Table 1 **Affinities of ACT-293987, ACT-333679 and beraprost for human prostanoid receptors**

	Binding Affinity (K_i, μM)							
	IP	EP_1	EP_2	EP_3	EP_4	DP	FP	TP
ACT-293987	0.26	> 10	> 10	> 10	> 10	> 10	> 10	> 10
ACT-333679	0.02	> 10	5.8	> 10	4.9	2.6	> 10	> 10
Beraprost	0.039	> 10	> 10	0.68	7.2	> 10	> 10	> 10

IP = PGI_2 receptor; EP_{1-4} = PGE_2 receptor; DP = PGD_2 receptor; FP = $PGF_{2\alpha}$ receptor; TP = TXA_2 receptor

Figure 1 **Effect of ACT-333679 on proliferation of human pulmonary arterial smooth muscle cells**



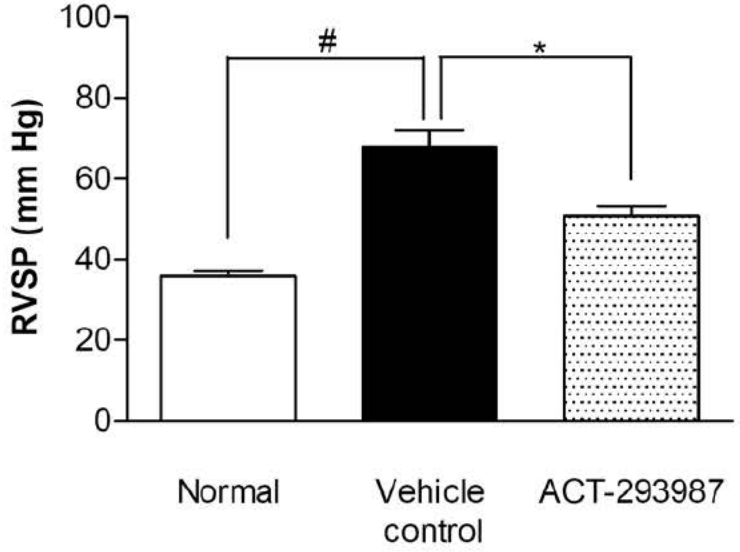
Cells were stimulated with PDGF (10 ng/mL) either in the absence or presence of test compounds for 24 h, and cellular incorporation of radioactivity was measured. Data is presented as mean ± S.E.M. (n = 8).

Table 2 **Inhibition of platelet aggregation by ACT-293987 and ACT-333679 in platelet-rich plasma from different species**

	Platelet aggregation IC_{50} (μM)			
Drug	Human	Monkey	Dog	Rat
ACT-293987	5.5 ± 0.8	3.4 ± 1.1	456 ± 23	–
ACT-333679	0.21 ± 0.04	0.21 ± 0.02	25 ± 1.5	10 ± 0.4

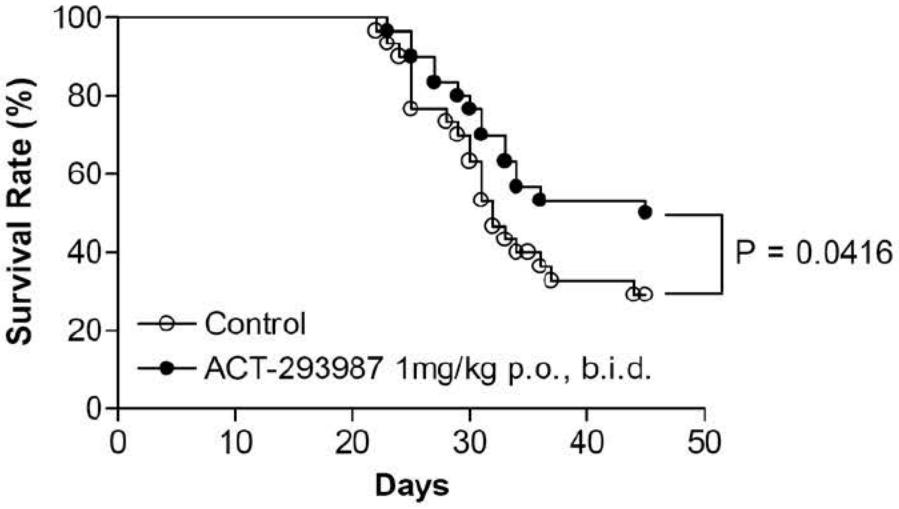
– = not determined; data is presented as mean ± S.E.M. (n = 4).

Figure 4 **Effect of ACT-293987 on RVSP in rats with MCT-induced PAH**



Data is presented as mean ± S.E.M. #, P < 0.01 vs normal rats, *, P < 0.01 vs vehicle control rats. (n = 12).

Figure 8 **Effect of ACT-293987 on survival of rats with MCT-induced PAH**



Survival data is presented using the Kaplan-Meier method and compared by the log-rank test. Day 0 indicates the time of injection of MCT and the beginning of administration of ACT-293987. (n = 30).

2.6.4PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary: ACT-293987 is rapidly absorbed from the Gastro-intestinal tract with a Tmax of approximately 1 hr. and a bioavailability of 80 to 90%. Radiolabeled ACT-293987 accumulates first in the liver after oral dosing, followed by accumulation in the stomach. This is probably due to the high level of activity and receptors present in the stomach, where prostacyclin agonists apparently have high activity. Most adverse events for prostacyclin agonists are related to their effects on the gastrointestinal system. ACT-293987 is primarily excreted through the biliary system, with only 2% of its excretion due to renal pathways. ACT-293987 is a prodrug, although it also has activity at prostacyclin receptors, and is hydrolyzed to ACT-333697, the primary prostacyclin agonist. ACT-333697 is glucuronidated primarily and excreted through the biliary system, although *in toto* there are up to 15 potential metabolites of ACT-293987**.**

2.6.4.2 Methods of Analysis

HPLC [redacted] (b) (4) nm or mass spectrophotometric methods was used to quantify the amounts of ACT-293987 and/or ACT-333697 in serum. Both methods provided a wide and useful range

2.6.4.3 Absorption

	Rat	Dog
Bioavailability	72%	84%
Dose Proportionality	Linear	Linear
Food Effects	Decreased Cmax AUC unchanged Increased Tmax	
Gender Differences	No effect	Slight increases in Females vs. Males
Accumulation	None	None
Tmax	1 hr.	1 hr

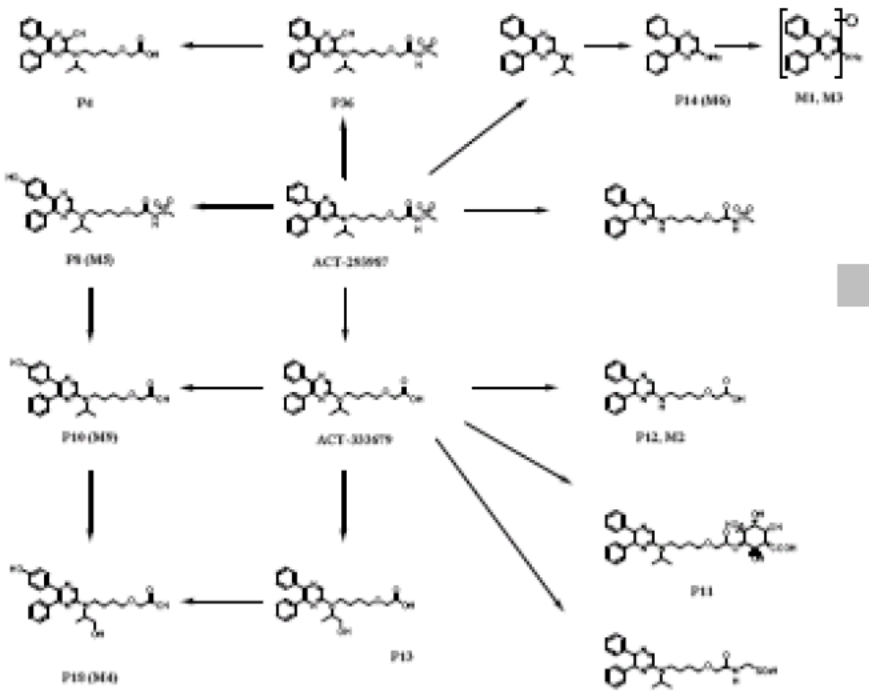
2.6.4.4 Distribution

Upon initial absorption of ACT-293987, the liver is the primary site of accumulation, presumably part of the normal first pass effect. It is also desirable with ACT-293987 since it is a prodrug and is metabolized to the active product, ACT-33679, primarily in the liver. After that, the stomach becomes the primary organ of accumulation of ACT-293987 and its active metabolite, ACT-333679. Although the sponsor does not comment on this (and seems to ignore it), presumably this, among other possibilities, is due to the importance of prostacyclins in the gastric environment and may reflect a receptor mediated accumulation of the drug products. Since gastro-intestinal effects are the primary drivers of the adverse events seen in the dog and human studies, the issue is worth taking note of. Prostacyclins are thought to increase blood flow to the gastric mucosa and also to inhibit gastric acid secretion.

2.6.4.5 Metabolism

ACT-293987 is a pro-drug that has modest activity by itself as a prostacyclin agonist, and is hydrolyzed to ACT-333679, an active metabolite at prostacyclin receptors. As shown in the metabolic pathways figure below, ACT-333679 is not the only metabolite of ACT-293987, with a total of 15 possible metabolites.

Figure 5 Metabolic pathways of ACT-293987



2.6.4.6 Excretion In rats:

Biliary excretion accounted for 95% of the excretion of ACT-293987 and its metabolites, while in dogs, 80-88% was recovered in the bile. 85% of the radiolabeled compound was eliminated within the first 24 hrs.

2.6.4.7 Pharmacokinetic drug interactions

ACT-293987 and ACT-333697 are poor substrates for most of the CYP-450 enzyme systems, having only weak activity at CYP2C8 and CYP 2C9, which are more minor metabolic enzymes. Both substrates are also negligible at the multi-drug resistance transporters. These results would predict minimal interactions with most drugs.

2.6.4.8 **Other Pharmacokinetic Studies** none

2.6.4.9 Discussion and Conclusions

The oral pro-drug, ACT-293987, is hydrolyzed by carboxyesterases to ACT-333697, a prostacyclin agonist. ACT-293987 is highly bioavailable, with approximately 80-90% absorbed from the gastro-intestinal tract. Hydrolysis to ACT-333697 is variable by species, rats having high serum levels of carboxyesterases, while dogs, monkeys, and man have high levels primarily in the liver. Although the half-life is approximately 14 hrs, distribution of oral doses are first primarily to the liver followed by accumulation in the stomach, an area with a significant level of prostacyclin receptors and activity. Of note is that most of the adverse reactions involving prostacyclin agonists are related to their activity in the gastro-intestinal system. ACT-293987 is primarily metabolized to ACT-333697 which is glucuronidated and excreted primarily through the biliary system. Only 2% of radiolabel from ACT-293987 is excreted through the kidneys. ACT-293987 and its metabolites are only minor substrates for 2 minor CYP enzymes, 2C8 and 2C9, and are not substrates for the multi-drug transporters, thereby minimizing the potential for major interactions with other drugs. Possible additive or synergistic effects with other vasodilators (e.g. nitrates), H2 blockers or other acid blockers, and anti-platelet drugs is possible in view of the vasodilating activity of ACT-293987 and ACT-333697 and inhibition of gastric acid secretion and platelet aggregation. Reduction of acid in the stomach is known to affect the uptake of vitamin B12 in man, and it is also known to affect absorption of some drugs, for example clopidogrel. So, although drug-drug interactions at the metabolic or drug transport level are not expected to occur, there is still potential for interactions based on vasodilating, gastro-intestinal, and/or platelet-modulating activities of ACT-293987 and ACT-333697.

2.6.4.10 Tables and figures to include comparative TK summary

Table 2 **Summary of ACT-293987 and ACT-333679 exposures in the 4- and 13-week toxicity studies with ACT-293987 in mice**

ACT-293987 (mg/kg)	Time		C_{max} (µg/mL)		AUC_{0-24} (µg·h/mL)	
			Male	Female	Male	Female
4-week toxicity study in mice [T-08.291]: NOAEL at 100 mg/kg/day						
30	Day 1	ACT-293987	1.49	1.50	1.33	1.41
		ACT-333679	2.14	1.91	2.86	2.45
100	Day 1	ACT-293987	7.77	10.1	11.3	13.9
		ACT-333679	6.53	6.93	16.2	17.0
300	Day 1	ACT-293987	14.5	22.4	64.2	70.0
		ACT-333679	10.7	13.2	65.9	62.2
30	Week 4	ACT-293987	1.45	1.54	1.07	1.41
		ACT-333679	2.16	1.76	2.25	1.99
100	Week 4	ACT-293987	8.32	11.3	9.36	10.6
		ACT-333679	6.01	6.9	11.9	10.5
300	Week 4	ACT-293987	8.33	15.1	24.8	23.2
		ACT-333679	8.03	8.02	28.7	24.3
13-week toxicity study in mice [T-08.292]: NOAEL at 100 mg/kg/day						
100	Day 1	ACT-293987	9.66	12.2	15.0	19.5
		ACT-333679	10.0	8.23	23.6	21.4
300	Day 1	ACT-293987	26.5	35.9	88.4	115
		ACT-333679	19.5	19.1	99.6	98.8
500	Day 1	ACT-293987	21.1	45.6	295	310
		ACT-333679	17.5	17.0	250	223
100	Week 13	ACT-293987	16.9	10.8	12.2	10.1
		ACT-333679	11.4	7.15	12.4	9.99
300	Week 13	ACT-293987	23.2	24.9	52.1	44.4
		ACT-333679	12.4	13.5	51.5	39.8
500	Week 13	ACT-293987	27.3	18.8	90.0	74.0
		ACT-333679	20.3	12.4	71.5	58.0

Values are means of 3 mice/sex/dose/sampling time.

Table 3 **Summary of ACT-293987 and ACT-333679 exposures in the 4- and 26-week toxicity studies in rats**

ACT-293987 (mg/kg)	Time		C_{max} (µg/mL)		AUC_{0-24} (µg·h/mL)	
			Male	Female	Male	Female
4-week toxicity study in rats [T-08.275]						
20	Day 1	ACT-293987	0.28	0.63	0.29	0.46
		ACT-333679	2.76	2.8	13.3	9.53
60	Day 1	ACT-293987	1.05	2.1	2.41	3.64
		ACT-333679	7.15	9.3	64.5	55.4
180	Day 1	ACT-293987	2.5	5.3	8.22	13.7
		ACT-333679	14.7	16.68	210	226
20	Week 4	ACT-293987	0.27	0.53	0.32	0.51
		ACT-333679	3.04	3.25	12.2	9.54
60	Week 4	ACT-293987	1.2	2.3	2.14	2.92
		ACT-333679	8.99	11.9	38.8	36.3
180	Week 4	ACT-293987	2.5	6.01	8.77	24.8
		ACT-333679	14.5	27.2	137	304
4-week toxicity study in rats [T-08.276]: NOAEL at 6 mg/kg/day						
2	Day 1	ACT-293987	n.d.	n.d.	n.d.	n.d.
		ACT-333679	0.16	0.10	1.23	0.34
6	Day 1	ACT-293987	0.10	0.13	0.09	0.09
		ACT-333679	0.86	0.63	4.38	2.89
60	Day 1	ACT-293987	1.29	3.66	2.51	5.92
		ACT-333679	8.69	13.2	61.4	75.0

2	Week 4	ACT-293987	n.d.	n.d.	n.d.	n.d.
		ACT-333679	0.11	0.11	0.21	0.12
6	Week 4	ACT-293987	0.02	0.08	0.01	0.04
		ACT-333679	0.53	0.46	1.40	0.95
60	Week 4	ACT-293987	1.21	2.30	2.10	4.09
		ACT-333679	9.63	13.6	50.9	63.6
26-week toxicity study in rats [T-08.285]: NOAEL at 6 mg/kg/day						
6	Day 1	ACT-293987	0.20	0.18	0.14	0.13
		ACT-333679	1.12	0.90	6.43	3.10
25	Day 1	ACT-293987	1.27	2.08	1.73	1.72
		ACT-333679	7.06	6.85	38.5	33.2
100	Day 1	ACT-293987	4.89	5.51	13.1	12.4
		ACT-333679	22.3	20.0	192	190
6	Week 26	ACT-293987	0.4	0.40	0.23	0.29
		ACT-333679	1.26	1.78	4.77	6.79
25	Week 26	ACT-293987	0.50	2.77	1.43	2.81
		ACT-333679	4.46	11.9	22.7	45.7
100	Week 26	ACT-293987	2.07	10.1	5.33	18.8
		ACT-333679	12.7	43.7	76.3	202

Values are means of 3 rat/sex/dose/sampling time. n.d. = not determined.

Table 5 **Summary of ACT-293987 and ACT-333679 exposures in the 2-, 4-, and 39-week toxicity studies in dogs**

ACT-293987 (mg/kg/day)	Time		C_{max} (µg/mL)		AUC_{0-24} (µg·h/mL)	
			Male	Female	Male	Female
2-week toxicity study in dogs [T-08.277]: NOAEL at 2 mg/kg/day						
2	Day 1	ACT-293987	3.17	2.36	7.42	6.18
		ACT-333679	5.16	5.59	59.5	62.4
6	Day 1	ACT-293987	9.59	5.48	25.7	22.2
		ACT-333679	15.2	12.1	186	181
20	Day 1	ACT-293987	10.1	18.2	45.5	59.9
		ACT-333679	18.8	27.4	281	403
2	Week 2	ACT-293987	3.19	2.90	7.50	6.72
		ACT-333679	5.99	6.34	50.8	67.8
6	Week 2	ACT-293987	10.1	8.50	24.3	25.1
		ACT-333679	14.8	13.5	154	155
20	Week 2	ACT-293987	22.0*	23.3**	77.2*	103**
		ACT-333679	44.6*	35.9**	576*	532**
4-week toxicity study in dogs [T-08.290]: NOAEL at 1.5 mg/kg/day						
1.5	Day 1	ACT-293987	2.23	3.39	5.47	7.95
		ACT-333679	2.80	5.22	31.7	50.2
3	Day 1	ACT-293987	5.25	5.08	14.4	14.8
		ACT-333679	7.13	8.20	91.0	105
6	Day 1	ACT-293987	9.38	10.6	23.8	31.5
		ACT-333679	12.6	15.2	168	211

1.5	Week 4	ACT-293987	1.98	3.09	5.39	7.29
		ACT-333679	3.72	4.71	39.7	54.6
3	Week 4	ACT-293987	4.25	4.99	13.1	14.4
		ACT-333679	6.82	8.33	82.9	85.6
6	Week 4	ACT-293987	6.77	13.2	29.2	35.4
		ACT-333679	12.4	15.9	159	201
39-week toxicity study in dogs [T-08.286]						
1	Day 1	ACT-293987	0.98	1.53	2.55	3.36
		ACT-333679	2.50	3.05	24.6	33.2
2	Day 1	ACT-293987	2.54	2.87	8.44	7.30
		ACT-333679	5.27	5.56	49.9	54.0
4	Day 1	ACT-293987	5.77	5.21	17.4	17.6
		ACT-333679	9.17	8.62	94.4	104
1	Week 26	ACT-293987	1.83	0.97	3.49	2.85
		ACT-333679	3.29	2.52	28.8	24.6
2	Week 26	ACT-293987	4.50	3.26	10.2	9.15
		ACT-333679	6.40	6.13	55.8	52.2
4	Week 26	ACT-293987	5.66	5.03	18.1	16.2
		ACT-333679	6.89	10.4	58.4	90.8
1	Week 39	ACT-293987	1.03	1.36	2.55	2.88
		ACT-333679	2.60	3.26	23.1	35.7
2	Week 39	ACT-293987	3.10	1.79	8.15	6.54
		ACT-333679	5.29	5.87	40.5	58.9
4	Week 39	ACT-293987	8.62	6.88	21.3	18
		ACT-333679	9.54	12.5	109	120

Values are means of 3 dog/sex/dose/sampling time. *: 1 dog/sex/dose; **: 2 dog/sex/dose.

2.6.5PHARMACOKINETICS TABULATED SUMMARY

2.6.6TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

From the mouse studies:

Study Length	Doses	Results	NOAEL
4 weeks	0, 30, 100, 300 mg/kg/day	No mortality, @300 mg/kg/day flaccidity and flushing	100 mg/kg/day

13 weeks	0, 100, 300, 500 mg/kg/day	500 mg/kg/day one mortality, CK & ALT increase; 300 mg/kg/day decrease in food consumption, flushing, flaccidity, BUN decreased, kidney tubular vacuolation	100 mg/kg/day

From the rat studies:

Study Length	Doses	Results	NOAEL
4 weeks	0, 20, 60, 180 mg/kg/day	20 mg/kg/day alveolar hemorrhage and decreased platelets, loss or black discoloration of tail tip;	Not determined
4 weeks + 4 weeks recovery	2, 6, 60 mg/kg/day	60 mg/kg/day flushing, decreased movement, piloerection, reduced body wt and food consumption. Reversible	6 mg/kg/day
26 weeks + 4 week recovery	0, 6, 25, 100 mg/kg/day	>25, 100 mg/kg/day liver and adrenal hypertrophy, hyperplasia mammary gland, salivary gland, follicular cells in thyroid; all treated animals, flushing, decreased movement, reversible; one animal died in high dose group of malignant lymphoma	6 mg/kg/day

From the dog studies:

Study length	Doses	Results	NOAEL	
2 weeks	2, 6, 20 mg/kg/day	20 mg/kg/day led to mortalities, Intussuseception, QTc prolongation; 6 mg/kg/day increased ossification, bone marrow fibrosis; 2 mg/kg/day decreased platelet, wbc, & neutrophils.	2 mg/kg/day	
4 weeks	1.5, 3, 6 mg/kg/day	6 mg/kg/day intussusception; 3 mg/kg/day intussusception, bone marrow fibrosis, ossification; 1.5 mg/kg/day vomiting, diarrhea, jelly feces	1.5 mg/kg/day	
39 weeks	1, 2, 4 mg/kg/day	4 mg/kg/day 2 mortalities, intussusception; 1mg/kg/day bone marrow fibrosis and ossification	Not determined	

Genetic toxicology: ACT-293987 was negative in most of the genotoxicity testing. The exception was a small signal of clastogenicity in the Chinese Hamster Lung cell in vitro assay. This was not present in the in vivo mouse micronucleus study.

Carcinogenicity: not done

Reproductive toxicology: For the rat studies, the NOAEL was the same as for the standard toxicity testing, 6 mg/kg/day. In the rat studies, the primary issue appeared to be low birth weight, which in humans is correlated with developmental difficulties.
In the rabbit study, the NOAEL was 10 mg/kg/day, with one animal dying at 30 mg/kg/day, reproductive function and fetal development were not affected.

Special toxicology: **Study title**: *in vitro* phototoxicity study

Key study findings: both ACT-293987 and ACT-333697 were positive for phototoxicity with UVA light, suggesting absorption in the UV spectrum.

2.6.6.2 Single-dose toxicity

Table 1 Overview of completed toxicity studies with ACT-293987

Study type [Reference] *Batch number**	GLP	Route	Species / test system	Treatment duration	Dose (mg/kg/day) / concentration	Animals / sex/ groups
Single dose						
Acute [T-08.284] *Lot 20*	Y	i.v.	Slc:ddy mice (m & f)	Single dose	0, 10, 20, and 40	5/sex/group
Acute [T-08.274] *Lot 20*	Y	Oral	SD rats (m & f)	Single dose	0, 125, 250, 500, and 1,000	5/sex/group
Acute [T-08.283] *Lot 20*	Y	i.v.	SD rats (m & f)	Single dose	0, 10, 20, and 40	5/sex/group
Acute [T-08.273] *Lot 20*	Y	Oral	Beagle dogs (m)	Single dose	20, 200, and 2,000	2/group

Single intravenous doses of up to 40 mg/kg were given to mice and rats. There were no mortalities, in mice, there was flushing at 10 mg/kg, decreased movement at 20 mg/kg, and prone position at 40 mg/kg, in rats, prone position, decreased movement, flaccidity and flush were observed at all doses above 10 mg/kg, with severity and reversibility dependent on the dose.

Single oral doses were given to rats and dogs. In rats the oral lethal dose was 500 mg/kg, in dogs 2,000 mg/kg. Reasons for mortality were different, with the cause of death evidently being severe hypotension due to peripheral vasodilation in rats, while in

dogs the cause of death was intussusception. In general, the dogs showed more adverse clinical signs related to gastrointestinal effects, with some of the animals flushing as a sign of vasodilatation., In the rats, signs were similar to those seen after 40 mg/Kg iv , but with increased severity resulting in death at 500mg/Kg po.

No serum levels were reported, making it difficult to compare the toxicities of the oral vs. intravenous routes of administration as a function of blood levels.

2.6.6.3 Repeat-dose toxicity

Study title: Twenty-six-week oral gavage toxicity study of NS-304 in rats with 4-week recovery period

Key study findings: One male died in the high dose group, however, upon necropsy it was determined the animal had malignant lymphoma. This was thought to be not drug related, but it will not be known until the carcinogenicity study is done. Doses were 6, 25, and 100 mg/kg/day, with 6 mg/kg/day being the NOAEL dose. Flushing was the primary observation, with alopecia and reduced body weights and food consumption observed in the high dose groups. . Many of the changes seen were reversible in the 4 week recovery period.

Study no.: T-08.285
Volume # and page #: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: July 7, 2006
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity:
Test Article
The test article, NS-304, was supplied by Nippon Shinyaku Co Ltd. The lot number, appearance and other information on the test article used in the present study were as follows (Attached Data 1).
Name: NS-304 (another name; MRE-304)
Lot number: 20
Content: 100.3%
Appearance: Pale yellow crystalline powder
Stability: Characterization analysis was conducted at (b) (4) after animal experiment, and the stability during the administration period was deduced (Study No. A-1942, Attached Data 2-1).
Handling precautions: Mask and gloves were worn as the test article is a skin irritant.
Storage conditions: (b) (4)

(b) (4)

Storage place: (b) (4) (b) (4) (b) (4)

Methods

Doses:

Text Table 1. Group composition

Test group	Dose (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg/day)	Sex	Main group		Recovery group	
					No. of animals	Animal No.	No. of animals	Animal No.
Control	0	0	5	M	12	1001-1012	6	1013-1018
				F	12	1101-1112	6	1113-1118
Low	6	1.2	5	M	12	2001-2012	–	–
				F	12	2101-2112	–	–
Middle	25	5	5	M	12	3001-3012	6	3013-3018
				F	12	3101-3112	6	3113-3118
High	100	20	5	M	12	4001-4012	6	4013-4018
				F	12	4101-4112	6	4113-4118

M: Male, F: Female

(continued)

Test group	Dose (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg/day)	Sex	Satellite group	
					No. of animals	Animal No.
Control	0	0	5	M	8	1201-1208
				F	8	1301-1308
Low	6	1.2	5	M	8	2201-2208
				F	8	2301-2308
Middle	25	5	5	M	8	3201-3208
				F	8	3301-3308
High	100	20	5	M	8	4201-4208
				F	8	4301-4308

M: Male, F: Female

Species/strain: Crl:CD(SD) SPF rats ((b) (4))

Route, formulation, volume, and infusion rate: oral gavage, 0.5% w/v% methyl cellulose, 5 mL/kg body weight,

Age: 4 weeks old

Weight: 195-234 g in males, 137-177 g in females

Sampling times: see observation and times

Unique study design or methodology (if any):

Observation and Times:

6.11.1 Clinical Observation

All animals were observed for clinical signs including external appearance, nutritional condition, posture, behavior and excretions 3 times a day (before, immediately after and approximately 2 hours after dosing; however twice on Saturdays, Sundays and holidays: before and immediately after dosing) during the administration period.

During the recovery period, all animals were observed once a day in the morning.

6.11.2 Measurement of Body Weight

During the administration period, all animals were weighed before administration twice in week 1 (days 1 and 7) of administration, and once a week every 7 days thereafter. During the recovery period, all animals were weighed twice in week 1 (days 1 and 7) of recovery and once a week at 7-day intervals thereafter. Measurement was done between 08:30 and 11:32. Body weight gains for the entire period of administration (26 weeks) or recovery period (4 weeks) were also calculated. In order to calculate the relative organ weight of the animals sacrificed as scheduled, the body weight was also recorded on the day of necropsy after depriving the animals of food overnight (approximately 16 hours). The animal that died was weighed before carrying the animal out of the animal room for necropsy; however, this data was omitted from the table and appendix.

6.11.3 Measurement of Food Consumption

During the administration period, food consumption was measured for each animal twice in week 1 (days 1 and 7) of administration, and once a week every 7 days thereafter. During the recovery period, food consumption was measured for each animal once a week. Measurement was done between 08:57 and 12:09. On day 1 of administration, one day's food consumption was measured from the day before the start of administration and that on day 7 was calculated from 6-day's cumulative food consumption. Thereafter, 7-day's cumulative consumption was measured and one day's food consumption was calculated. On day 7 of recovery, one day's food consumption was measured from 6-day's cumulative consumption and thereafter, 7-day's cumulative consumption was measured and one day's food consumption was calculated.

6.11.4 Ophthalmology

Before the start of administration (during the quarantine/acclimatization period, 11 and 12 days before administration), all animals were examined, and the animals with pre-existing ophthalmological abnormalities that might affect the toxicity evaluation were excluded from animal grouping for the main and recovery groups (note).

During the administration period, examination was done in month 3 (week 12, days 80 and 81) and month 6 (week 25, days 171 and 172) of administration. All survivors of each sex in control and high dose group were examined after dosing on the day of examination. Examination was not done in middle and low dose groups, since no treatment-related changes were observed in the high dose group. During the recovery period, examination was done in week 4 (day 22) of recovery. All survivors of each sex in each test group were examined. The procedure for examination was as follows.

First, the light reflex was tested using an ophthalmoscope (BXα-13 type: NEIZ Inc.). Then mydriatic agent (Mydrin® P: Santen Pharmaceutical Co., Ltd., Lot Nos. MP0872, MP0931 and MP0947) was applied to dilate the pupil and the anterior portion, transparent body (optic media) and fundus oculi were examined using a hand slit lamp (SL-14 type: Kowa, Inc.) and an ophthalmoscope (Omega 200: HEINE OPTOTECNIK GmbH & Co. KG, Germany).

note: Four males and 3 females with severe ophthalmological abnormalities such as aqueous flare, hemorrhage of iris, retinal hemorrhage and impossibility of observation in transparent body and fundus were excluded from the present study. Twelve males and 19 females with ophthalmological abnormalities such as focal corneal vascularization, residue in anterior chamber, synechia, focal opacity in lens, adhesion posterior lens capsule, persistent tunica vasculosa lentis, persistent hyaloids artery, vitreous hemorrhage,

persistent hyperplastic primary vitreous and retinal hold were excluded as candidates for the main and recovery groups since it was judged that the above abnormalities might affect the toxicity evaluation. However, they were included as candidates for the satellite group.

6.11.5 Urinalysis (including water intake)

Examination was done in all survivors of each sex in months 3 (week 13, days 85 to 87) and 6 (week 25, days 169 to 171) (note) of administration and in week 4 (days 24 to 25) of recovery.

During the administration period, after the dosing, all survivors of each sex in main and recovery groups were accommodated in cages with trays attached for urine collection and 4-hour urine samples were collected under fasting conditions but withfree access to water. Then the following 20-hour urine samples were collected under free access to food and water.

In week 4 of recovery, the survivors were accommodated in cages with trays attached for urine collection, and 4-hour urine samples were collected under fasting but free access to water. The following 20-hour urine samples were collected under free access to food and water. The parameters listed in the following were examined. The 4-hour urine samples were used for examination of pH, protein, ketone body, glucose, occult blood, bilirubin, urobilinogen, color, urinary sediments and urine volume. The urinary sediments obtained by centrifugation (set at 1,500 rpm, approximately 440×g for 5 minutes) were subjected to microscopic examination without fixation or staining.

Urine volume (24h) was calculated by totaling the volume of 4-hour and 20-hour urine.

One day's output of electrolytes was calculated by their concentration and 24-hour urine volume.

One day's water intake was measured using water bottles on the day of urinalysis for each animal.

Text Table 2. Items, Methods and Equipment for Urinalysis Examinations

Item	Method	Unit
pH	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)a)	
protein	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)a)	
ketone body	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)a)	
glucose	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)a)	
occult blood	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)a)	
bilirubin	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)a)	
urobilinogen	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)a)	
color	macroscopic observation	
urinary sediments	microscopic examination	
urine volume (24h)	volumetry	mL/24h
osmotic pressure	freezing point methodb)	mOsm/kg
sodium (Na)	ion selective electrode methodc)	mmol/24h
potassium (K)	ion selective electrode methodc)	mmol/24h
chloride (Cl)	coulometric titration methodc)	mmol/24h

Equipment used

a): AUTION MINITM AM-4290 (ARKREY, Inc.)

b): Osmotic Pressure AUTO & STAT OM-6030 (ARKREY, Inc.)

c): Automatic Electrolyte Analyzer PVA-α II (Analytical Instrument Inc.)

note: At the examination in month 6, 2 males was subjected to re-collection of urine sample or re-examination was conducted (days 176 and 177).

In No. 2001, re-collection of the urine sample was conducted to examine osmotic pressure and output of electrolytes, since an initial urine sample was dropped on the floor and could not be collected. The data from re-collected urine for urine volume was adopted.

In No. 4007, re-examination of water intake was conducted, since a relatively high value compared to other animals was recorded, although there was no apparent abnormality in any other parameter. The data from re-examination were adopted, since it was judged that the re-examined value was normal for these weeks of age.

6.11.6 Hematology

At the time of necropsy conducted on the day following the end of the administration period or the end of the recovery period, the survivors were deprived of food overnight (approximately 16 to 20 hours) prior to blood collection. Blood samples were collected from the abdominal aorta under ether anesthesia into blood collection tubes (approximately 1 mL, SB-41: Sysmex Corp.) containing an anticoagulant (EDTA-2K) and the following items were determined. May-Gruenwald-Giemsa staining smears from all animals were prepared as reserve in the case of microscopic examination, although examination was not actually conducted. However, for determining PT, APTT and fibrinogen, plasma obtained by centrifuging (set at 3100 rpm, approximately 1690 × g for 12 minutes) blood samples (approximately 0.9 mL) treated with 3.8 w/v% sodium citrate (1 vol sodium citrate solution/9 vol blood) was used.

Text Table 3. Items, Methods and Equipment for Hematological Examinations

Item Method Unit
red blood cell count (RBC) dual angle laser flow-cytometric measurement[a)] $10^4/\mu L$
hemoglobin (HGB) modified cyanmethemoglobin method[a)] g/dL
hematocrit (HCT) calculated from mean corpuscular volume and red blood
cell count[a)]
%
mean corpuscular volume (MCV) dual angle laser flow-cytometric measurement[a)] fL
mean corpuscular hemoglobin (MCH) calculated from red blood cell count and hemoglobin[a)] pg
mean corpuscular hemoglobin concentration
(MCHC)
calculated from hematocrit and hemoglobin[a)]
g/dL
reticulocyte ratio (Reticul.) laser flow-cytometric measurement with RNA stain[a)] %
platelet count (PLT) dual angle laser flow-cytometric measurement[a)] $10^4/\mu L$
white blood cell count (WBC)
dual angle laser flow-cytometric measurement[a)]
$10^2/\mu L$
differential leukocyte count (note) peroxidase flow-cytometric measurement and dual angle
laser flow-cytometric measurement[a)]
%
prothrombin time (PT) clot method[b)] s
activated partial thromboplastin time
(APTT)
clot method[b)] s
Fibrinogen (FIB) thromboplastin method[b)] mg/dL
Equipment used
a): ADVIA®120 Hematology System (Bayer Corporation, New York, USA)
b): Coagulometer ACL 100 (Instrumentation Laboratory)
note: Lymphocytes (LYM), neutrophils (NE), eosinophils (EOSINO), basophils (BASO), monocytes (MONO) and large unstained cells (LUC)

6.11.7 Blood chemistry

At the same time as hematology, blood samples were collected from the abdominal aorta into blood collection tubes (approximately 5 or 6 mL, Venoject II-Autosep: Terumo Corporation) containing serum separator, and the serum was obtained by centrifugation (set at 3,100 rpm, approximately 1,690×g for 12 minutes). The following items were determined on the serum. However, for determining AST, ALT and CK, the plasma obtained by centrifuging (set at 3,100 rpm, approximately 1,690×g for 12 minutes) blood samples collected into test tubes (approximately 2 mL) containing anti-coagulant heparin sodium salt (approximately 20 units/mL blood) was used. The following parameters were determined.

Text Table 4. Items, Methods and Equipment for Blood chemistry Examinations

Item Method Unit
AST UV-rate method[a)] IU/L
ALT UV-rate method[a)] IU/L

AlP (ALP) Bessey-Lowry method[a)] IU/L
CK UV-rate method[a)] IU/L
total cholesterol (T-CHO) CEH-COD-POD method[a)] mg/dL
triglyceride (TG) LPL-GK-GPO-POD method[a)] mg/dL
total bilirubin (T-BIL) bilirubin oxidase method[a)] mg/dL
glucose (GLU) glucose dehydrogenase method[a)] mg/dL
blood urea nitrogen (BUN) urease-LEDH method[a)] mg/dL
creatinine (CRNN) creatininase-creatinase-sarcosine oxidase-POD method[a)] mg/dL
sodium (Na) ion selective electrode method[a)] mmol/L
potassium (K) ion selective electrode method[a)] mmol/L
chloride (Cl) ion selective electrode method[a)] mmol/L
calcium (Ca) OCPC method[a)] mg/dL
inorganic phosphorus (P) molybdic acid method[a)] mg/dL
total protein (TP) Biuret method[a)] g/dL
A/G ratio (A/G) calculated from protein fractions
protein fractions cellulose acetate membrane electrophoresis[b)] %
Equipment used
a): Toshiba Biochemical Analyzer Model TBA-120FR (Toshiba Corp.)
b): CLINISCAN SA-V (Helena Co. Ltd.)

6.11.8 Pathology

6.11.8.1 Necropsy

After collecting blood samples, all survivors were sacrificed by exsanguination from the abdominal aorta under ether anesthesia. External appearance and all the organs/tissues in the cranial, thoracic and abdominal cavities were carefully examined and the results were recorded. The animal that was found dead was necropsied as soon as it was discovered.

6.11.8.2 Organ weights

After necropsy, the organs listed below of all survivors were weighed (absolute weight) and organ weight per 100 g body weight (relative weight) was calculated based on the fasted animal's body weight and absolute organ weight. The paired organs indicated by asterisks (*) were weighed separately; however, evaluation was done on the total value of the right and left organs. Organ weights were not measured on the animals that were found dead.

brain, pituitary, thyroids (including parathyroids)*, adrenals*, thymus, spleen, heart, lungs (including bronchus), salivary glands (submandibular + sublingual glands)*, liver, kidneys*, testes*, ovaries*, epididymides*, uterus, prostate and seminal vesicles

6.11.8.3 Histopathology

All the organs/tissues listed below of all animals were fixed and preserved in phosphate buffered 10 vol% formalin. However, the eyeballs and optic nerves were fixed with a mixture containing 3 w/v% glutaraldehyde and 2.5 vol% formalin, and the testes and epididymides of all survivors were fixed with Bouin's solution, and then preserved in phosphate buffered 10 vol% formalin. All organs/tissues of all animals were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H.E). Of these, all organs/tissues from the control and the high dose groups were examined histopathologically in the main group (26-week sacrificed group). In addition, sections of the adrenal and liver in both sexes and mammary gland, submandibular gland and thyroid in females from all other groups were subjected to histopathological examination, since treatment-related lesions were suspected in these organs. The submandibular gland in males was also suspected to be a target organ and it was also examined; however, it was ultimately judged that there were no treatment-related changes. In the recovery groups, the above organs/tissues suspected to be the target organ from all animals were examined. The paired organs indicated by asterisks (*) were examined bilaterally; however, the organs marked with # were examined

unilaterally:
cerebrum, cerebellum, spinal cord (cervical, thoracic and lumbar), sciatic nerves#, eyeballs*, optic nerves*, Harderian glands*, pituitary, thyroids*, parathyroids*, adrenals*, thymus, spleen, submandibular lymph node, mesenteric lymph node, heart, thoracic aorta, trachea, lungs (including bronchus), tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, submandibular glands*, sublingual glands*, parotid glands*, liver, pancreas, kidneys*, urinary bladder, testes*, epididymides*, prostate, seminal vesicles*, ovaries*, uterus*, vagina, mammary glands (inguinal region, both sides)#, sternum (including bone marrow), femurs (including bone marrow)#, femoral skeletal muscles#, skin (inguinal region, both sides)#, preputial glands*, clitoral glands* and gross lesions
Besides the organs/tissues listed above the oviducts, extraorbital lacrimal glands, Zymbal's glands, larynx, nasal cavity and the site of animal identification (ear auricle) were preserved.

6.11.8.4 Electron microscopy

At the time of necropsy conducted on the day following the end of the administration period or the end of the recovery period, liver and kidney (cortex and medulla) from 2 animals in each sex in the control and 100 mg/kg group (note) were fixed with phosphate buffered 0.5 w/v% glutaraldehyde and 1.5 w/v% paraformaldehyde, post fixed with 1 w/v% tetroxide osmium and embedded in epoxy resin.
note: animal Nos. 1001, 1003, 4002, 4004, 1101, 1103, 4101 and 4103 at week 26 of administration, animal Nos. 1013, 1015, 4013, 4015, 1113, 1115, 4113 and 4115 at week 4 of recovery

Adequate

Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )

Results:

Mortality: Below is the sponsor table on the progression for the one animal that died in the study in the high dose group.

Table 1-1 Twenty-six-week oral gavage toxicity study of NS-304 in rats with 4-week recovery period
Clinical signs (Administration period, dead animals)

Sex	Dose mg/kg	Animal number	Day of death	Week of administration 1	2-14	15	16	17	18-26
Male	100	4001	113	AB	A	AJK	AKL	+	

A : Flush(pinna, limbs)
B : Flush(whole body)
J : Decrease, spontaneous movement
K : Deep breathing
L : Unkempt fur
+ : Dead

Clinical signs:

Body weights:

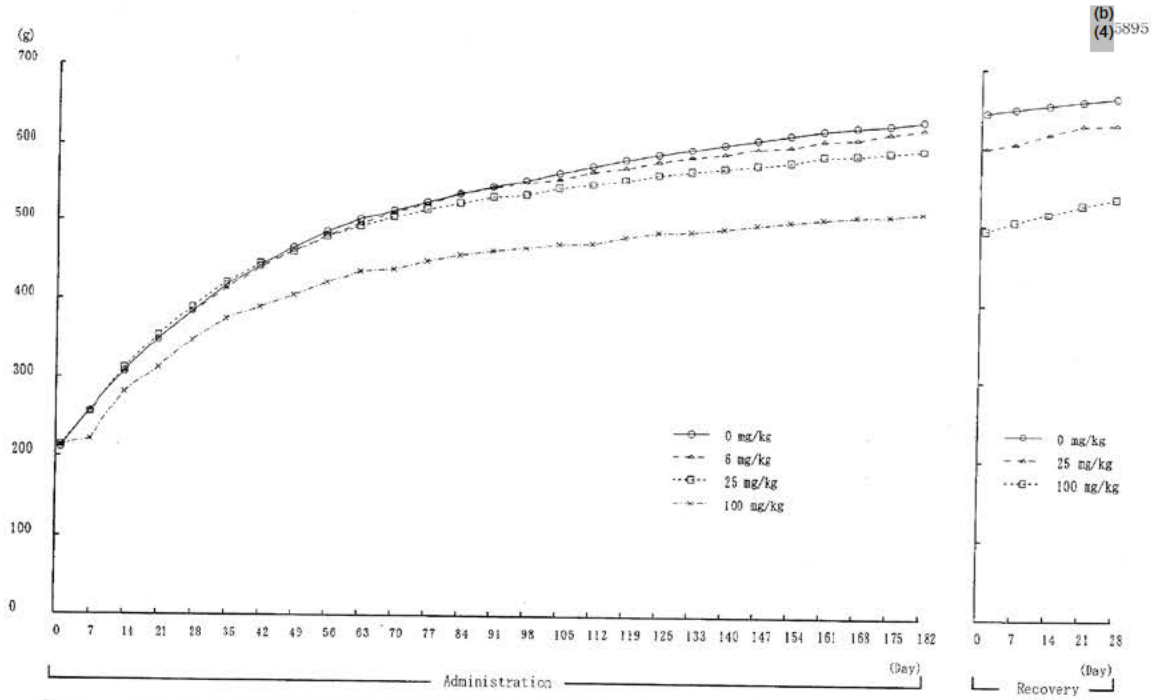


Fig. 1 Twenty-six-week oral gavage toxicity study of NS-304 in rats with 4-week recovery period
Body Weight - Male

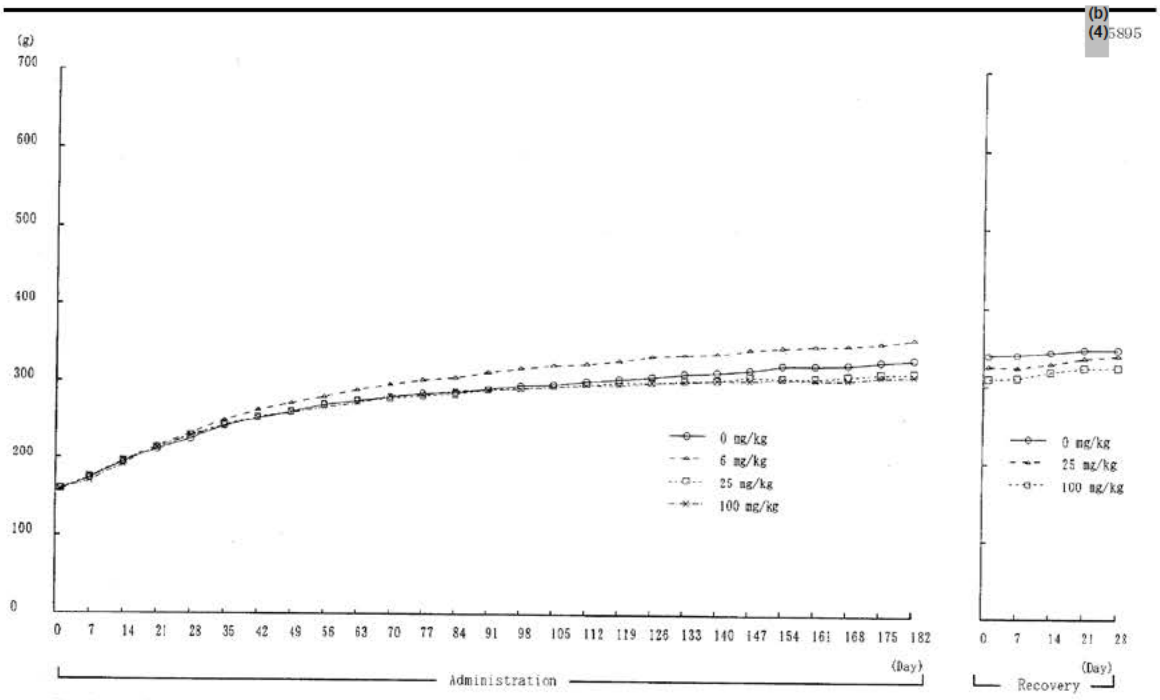


Fig. 2 Twenty-six-week oral gavage toxicity study of NS-304 in rats with 4-week recovery period
Body Weight - Female

<u>Food consumption</u>:

(b) (4)5895

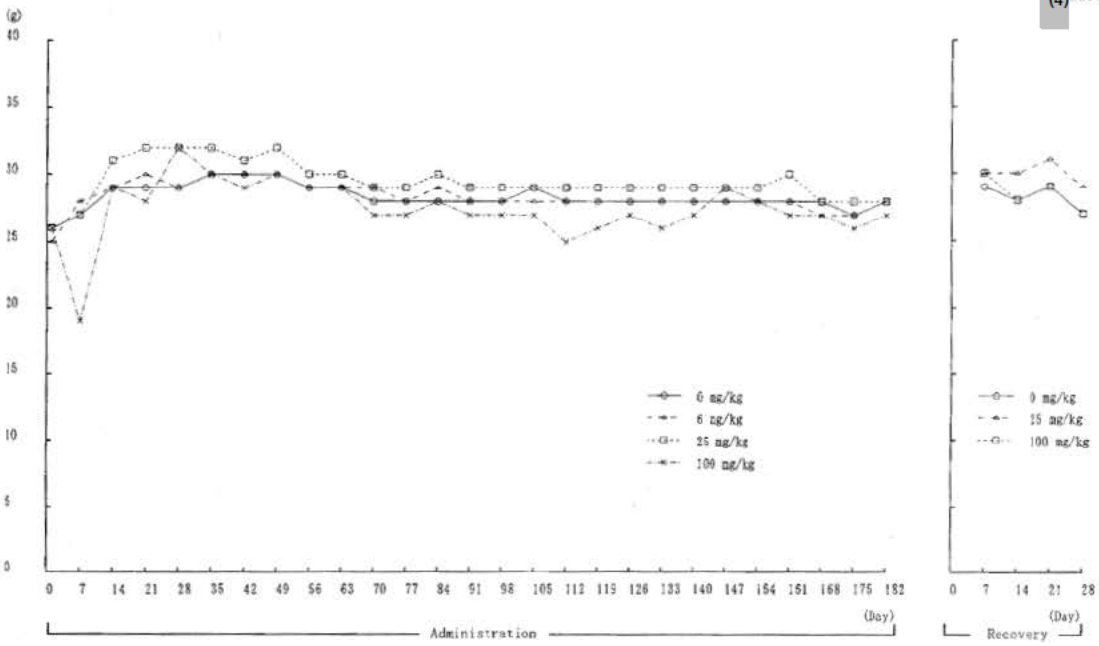


Fig. 3 Twenty-six-week oral gavage toxicity study of NS-304 in rats with 4-week recovery period
Food Consumption - Male

(b) (4)5895

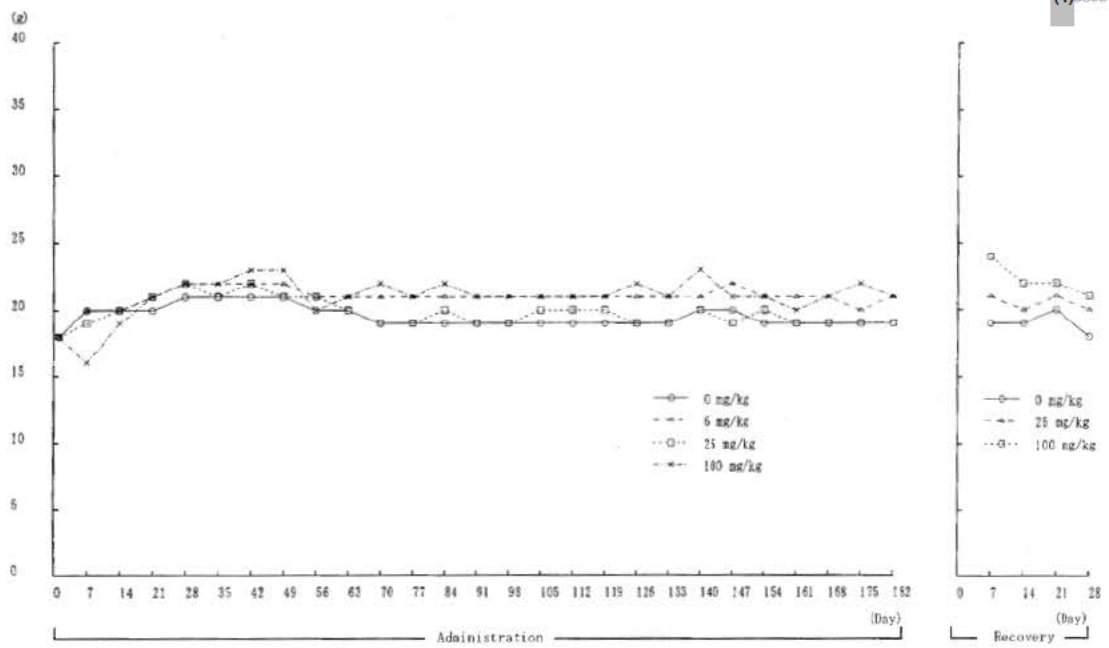


Fig. 4 Twenty-six-week oral gavage toxicity study of NS-304 in rats with 4-week recovery period
Food Consumption - Female

Ophthalmoscopy: no treatment related abnormalities were reported

EKG: not done

Hematology:

Text Table 3-1. Summary of hematology –End of the administration period–

Sex	Male			Female		
Dose (mg/kg/day)	6	25	100	6	25	100
No. of animals	12	12	11	12	12	12
HGB	N	N	N	N	N	-4%**
HCT	N	N	N	N	N	-4%*
MCV	N	N	N	N	+4%*	N
WBC	-31%**	N	-23%*	N	N	N
Differential leukocyte ratio						
Lymphocyte ratio	N	+23%**	+16%**	N	N	N
Neutrophil ratio	N	-41%**	-27%*	N	N	N
Monocyte ratio	N	N	-34%*	N	N	-35%*
Platelet	-15%**	-16%**	-32%**	N	N	-17%**
Fibrinogen	N	N	N	N	+23%*	+46%**

Values in the table indicate percentage of change against the control mean (-: decrease, +: increase).
N: No remarkable changes
* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)

Text Table 3-2. Summary of WBC and differential leukocyte count[a)]

–End of the administration period–

Sex	Male				Female			
Dose (mg/kg/day)	0	6	25	100	0	6	25	100
No. of animals	12	12	12	11	12	12	12	12
WBC ($10^2/\mu L$)	107.2	74.3**	84.2	82.1*	60.9	59.2	54.3	71.9
Lymphocyte ratio	62.6%	N	76.8%**	72.8%**	71.0%	N	N	N
actual number	67.1	NC	64.7	59.8	NC	NC	NC	NC
Neutrophil ratio	30.8%	N	18.2%**	22.6%*	22.8%	N	N	N
actual number	33.0	NC	15.3	18.6	NC	NC	NC	NC
Monocyte ratio	3.8%	N	N	2.5%*	3.4%	N	N	2.2%*
actual number	4.1	NC	NC	2.1	2.1	NC	NC	1.6

Values are group mean.
a): Actual number of each cell type was calculated from WBC and differential leukocyte ratio (unit: $10^2/\mu L$).
N: No remarkable changes
* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)
NC: Not calculated, since there were no statistical differences from the control group in the ratio

Text Table 3-3. Summary of hematology –End of the recovery period–

Sex	Male		Female	
Dose (mg/kg/day)	25	100	25	100
No. of animals	6	6	6	6
Differential leukocyte ratio				
Basophil ratio	-25%*	-50%**	N	N
PT	N	-4%*	N	N
APTT	-10%*	-10%*	N	N

Values in the table indicate percentage of change against the control mean (-: decrease).
N: No remarkable changes
* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)

Clinical chemistry:

Text Table 4-1. Summary of blood chemistry –End of the administration period–

Sex	Male			Female		
Dose (mg/kg/day)	6	25	100	6	25	100
No. of animals	12	12	11	12	12	12
AST	N	N	N	N	-49%**	-56%**
ALT	-51%*	N	N	N	-48%**	-52%**
CK	N	N	N	N	-14%*	-18%**
AlP	N	N	N	N	N	+90%**
T-CHO	N	N	N	N	N	+29%*
TG	N	N	-51%**	N	N	-62%*
Glucose	N	N	-18%**	N	N	N
BUN	N	N	N	N	N	-20%*
K	N	N	-8%**	N	N	N
Protein fractions						
Albumin ratio	N	+6%*	N	N	N	N
α_2-globulin ratio	N	N	N	N	N	+20%**
β-globulin ratio	-6%*	-7%**	N	N	N	+10%*
γ-globulin ratio	N	N	+17%*	N	N	N
A/G ratio	N	+14%*	N	N	N	-11%*

Values in the table indicate percentage of change against the control mean (-: decrease, +: increase).
N: No remarkable changes
* (**): p≤0.05 (0.01) (significantly different from the control group)

Text Table 4-2. Summary of blood chemistry –End of the recovery period–

Sex	Male		Female	
Dose (mg/kg/day)	25	100	25	100
No. of animals	6	6	6	6
CK	-21%*	-22%*	N	N
BUN	N	+17%**	N	N
P	+14%*	N	N	N
Protein fractions				
β-globulin ratio	N	-10%*	N	N
γ-globulin ratio	N	N	N	+28%*

Values in the table indicate percentage of change against the control mean (-: decrease, +: increase).
N: No remarkable changes
* (**): p≤0.05 (0.01) (significantly different from the control group)

Urinalysis:

Text Table 2-1. Summary of urinalysis –Month 3 of administration–

Sex	Male			Female		
Dose (mg/kg/day)	6	25	100	6	25	100
No. of animals	12	18	18	12	18	18
Urine volume	N	+54%*	+36%	N	+166%**	+305%**
Water intake	N	+30%	+24%	N	+24%	+82%**
Osmolality	N	-23%	-28%*	N	-46%**	-60%**
Na	N	N	N	N	+44%**	+33%*
K	N	N	N	N	+53%**	+35%*
Cl	N	N	N	N	+55%**	+36%*

Values in the table indicate percentage of change against the control mean (-: decrease, +: increase).
N: No remarkable changes
* (**): p≤0.05 (0.01) (significantly different from the control group)

Gross pathology:

1) Dead animal (one male in the 100 mg/kg group, Animal No. 4001)

Nodule in the thymus, enlargement of the submandibular, mesenteric and other lymph node, excess fluid in abdominal and thoracic cavities, enlargement of the spleen and liver, dark red focus in the femur and unkempt fur were observed.

2) End of the administration period

(1) Males

There were no treatment-related changes in any animal.

(2) Females

Hair loss (alopecia) was observed in 2/12 females in the 100 mg/kg group.

3) End of the recovery period

There were no treatment-related changes in any animal in either sex.

Organ weights (specify organs weighed if not in histopath table):

Text Table 5-1. Summary of organ weights –End of the administration period–

Sex	Male			Female		
Dose (mg/kg/day)	6	25	100	6	25	100
No. of animals	12	12	11	12	12	12
Body weight at necropsy	N	-5%	-17%**	N	N	N
Brain						
absolute	N	N	N	N	N	N
relative	N	N	+22%**	N	N	N
Thyroid						
absolute	N	N	N	N	N	N
relative	N	N	N	N	+26%*	+31%**
Salivary gland						
absolute	N	N	N	N	N	+21%**
relative	N	N	+23%**	N	N	+26%**
Heart						
absolute	N	N	N	N	N	+23%**
relative	N	+12%*	+23%**	N	+16%**	+29%**
Lung						
absolute	N	N	N	N	N	+15%**
relative	N	+15%**	+31%**	N	+14%*	+22%**
Liver						
absolute	N	N	N	N	N	+25%**
relative	N	N	+21%**	N	+13%**	+30%**
Kidney						
absolute	N	N	-11%*	N	N	N
relative	N	N	N	N	N	+8%*
Adrenal						
absolute	N	+15%*	+22%**	N	+23%**	+42%**
relative	N	+22%**	+56%**	N	+29%**	+48%**
Testis						
absolute	N	N	N	NA	NA	NA
relative	N	N	+21%**	NA	NA	NA
Prostate						
absolute	N	N	-19%**	NA	NA	NA
relative	N	N	N	NA	NA	NA
Epididymis						
absolute	N	N	-10%*	NA	NA	NA
relative	N	N	N	NA	NA	NA
Seminal vesicle						
absolute	N	N	-19%**	NA	NA	NA
relative	N	N	N	NA	NA	NA

Values in the table indicate percentage of change against the control mean (-: decrease, +: increase).
N: No remarkable changes
NA: Not applicable
* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)

Text Table 5-2. Summary of organ weights –End of the recovery period–

Sex	Male		Female	
Dose (mg/kg/day)	25	100	25	100
No. of animals	6	6	6	6
Body weight at necropsy	-5%	-20%**	N	N
Brain				
absolute	N	N	N	N
relative	N	+22%**	N	N
Pituitary				
absolute	-18%**	-24%**	N	N
relative	-12%**	N	N	N
Thyroid				
absolute	N	N	+41%*	N
relative	N	+49%*	+45%*	N
Salivary gland				
absolute	N	-16%**	N	N
relative	N	N	N	N
Thymus				
absolute	N	N	N	+71%*
relative	N	N	N	+88%*
Heart				
absolute	N	N	N	N
relative	+8%*	+12%**	N	+29%*
Lung				
absolute	+10%*	N	N	+14%*
relative	+15%*	+19%**	N	+19%**
Liver				
absolute	N	N	N	N
relative	N	N	N	+17%*
Spleen				
absolute	N	N	N	N
relative	+14%*	N	N	N
Kidney				
absolute	N	-18%**	N	N
relative	N	N	N	N
Adrenal				
absolute	N	-21%*	N	N
relative	N	N	N	+42%*

Values in the table indicate percentage of change against the control mean (-: decrease, +: increase).
N: No remarkable changes
* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )

Text Table 6. Incidence summary of histopathological lesions –End of the administration period–

Sex	Male				Female			
Dose (mg/kg/day)	0	6	25	100	0	6	25	100
No. of animals	12	12	12	11	12	12	12	12
Adrenal								
Hypertrophy, zona glomerulosa (±, +)	2	1	4	11	1	3	10	12
Liver								
Hypertrophy, hepatocytic, central (±)	0	0	0	5	0	0	0	9
Mammary gland								
Hyperplasia, acinar cell, diffuse (total)	0	NE	NE	0	8	6	9	11
(±)	0	NE	NE	0	7	5	9	1
(+)	0	NE	NE	0	1	1	0	10
Submandibular gland								
Hypertrophy, acinar cell (±)	0	1	1	1	0	0	7	10
Thyroid								
Hyperplasia, follicular cell, diffuse (±)	0	NE	NE	0	0	0	1	4

Values in the table indicate the number of animals with lesions.
±: Minimal, +: Mild
NE: Not examined

Toxicokinetics:

Text Table 7. Summary of TK parameters

Sex	Male (n=3)			Female (n=3)		
Dose (mg/kg/day)	6	25	100	6	25	100
NS-304						
T_{max} (h)						
Day 1	0.5	0.5	1.0	0.5	0.5	0.5
Week 26	0.5	0.5	1.0	0.5	0.5	0.5
C_{max} (µg/mL)						
Day 1	0.204	1.27	4.89	0.181	2.08	5.51
Week 26	0.398	0.498	2.07	0.405	2.77	10.1
C_{1h} (µg/mL)						
Day 1	0.0462	0.787	4.89	0.0476	0.642	3.60
Week 13	0.0626	0.415	1.37	0.0609	0.368	3.59
$AUC_{0\text{-}24h}$ (µg·h/mL)						
Day 1	0.137	1.73	13.1	0.126	1.72	12.4
Week 26	0.229	1.43	5.33	0.286	2.81	18.8
MRE-269						
T_{max} (h)						
Day 1	1.0	1.0	1.0	1.0	1.0	1.0
Week 26	1.0	1.0	1.0	1.0	1.0	1.0
C_{max} (µg/mL)						
Day 1	1.12	7.06	22.3	0.904	6.85	20.0
Week 26	1.26	4.46	12.7	1.78	11.9	43.7
C_{1h} (µg/mL)						
Day 1	1.12	7.06	22.3	0.904	6.85	20.0
Week 13	1.31	5.07	8.13	1.71	6.01	24.8
$AUC_{0\text{-}24h}$ (µg·h/mL)						
Day 1	6.43	38.5	192	3.10	33.2	190
Week 26	4.77	22.7	76.3	6.79	45.7	202

Value in the table (C_{max} and $AUC_{0\text{-}24h}$) indicates the mean value of 3 animals.

Other:

Study title: 39-week oral (capsule) toxicity study in the Beagle dog

Key study findings: dogs were placed in 4 groups of 6 males and 6 females receiving 0, 1, 2 or 4 mg/kg/day. 2 Females in the high dose group were sacrificed moribund on day 24 and 124, respectively.

Study no.: T-08.286
Volume #, and page #: eCTD 4.2.3.2
Conducting laboratory and location: (b) (4)

Date of study initiation: July 25, 2006
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: NS-304 (MRE-304)/ Lot # 20/ 100.3% purity

Methods

Doses:

Text Table 1. Group Composition

Test group	Dose level (mg/kg)	Sex	26-week necropsy group		39-week necropsy group	
			No. of animals	Animal number	No. of animals	Animal number
Control group	0	Male	3	1001-1003	3	1004-1006
		Female	3	1101-1103	3	1104-1106
Low dose group	1	Male	3	2001-2003	3	2004-2006
		Female	3	2101-2103	3	2104-2106
Middle dose group	2	Male	3	3001-3003	3	3004-3006
		Female	3	3101-3103	3	3104-3106
High dose group	4	Male	3	4001-4003	3	4004-4006
		Female	3	4101-4103	3	4104-4106

Species/strain: Beagle dogs

Route, formulation, volume, and infusion rate: Oral/gelatin capsules packed with drug compound.

Satellite groups used for toxicokinetics or recovery: none

Age: 6 months

Weight: 6.6 to 9.4 kg in males and 6.1 to 8.3 kg in females

Sampling times:

Text Table 2. Items and Schedule for Observation and Examination (In-life)

Item	Schedule / Frequency
Observation of general condition	Week –1 of administration: once daily (morning) Administration period: before and 1 hour after dosing (every day) and 3 hours after dosing (once a week)
Measurement of body weight	Once in Week –1 of administration (morning) Until Week 13 of administration: On Day 1 of administration and once a week thereafter (before dosing of the day) From Week 14 of administration: Once every 2 weeks (morning) and before necropsy on the day of necropsy
Measurement of food consumption	Every day from Week –1 of administration until the end of the administration period
Measurement of water intake	Once in Week –2 of administration Administration period: Once each in Weeks 4, 13, 26 and 39 of administration
Ophthalmological examination	Week –2 of administration: Once Administration period: Once each in Weeks 12, 25 and 38 of administration (1 to 4 hours after dosing)
Electrocardiography	Week –2 of administration: Once (morning) Administration period: Once each in Weeks 13, 26 and 39 of administration (before dosing and 1 to 4 hours after dosing)
Urinalysis	Week –2 of administration: Once Administration period: Once each in Weeks 4, 13, 26 and 39 of administration
Hematological examination	Week –1 of administration: Once Administration period: Once each in Weeks 13, 26 and 39 of administration (before dosing)
Blood chemistry examination	Week –1 of administration: Once Administration period: Once each in Weeks 13, 26 and 39 of administration (before dosing)

Unique study design or methodology (if any):

Observation and Times:

6.13.1 Clinical Observation

All animals were observed for clinical abnormalities such as the condition of visual mucosa and appearance of excreta and abnormal behaviors and abnormalities observed were recorded.

6.13.2 Measurement of Body Weight

All animals were weighed between 08:12 and 9:50 on the days of measurement (before dosing of the day during the administration period). On the day of necropsy, animals were weighed after fasting for at least 16 hours from the previous day. Moribund animals were weighed before necropsy.

6.13.3 Measurement of Food Consumption

For all animals, feed was supplied and removed as described in 6.5, one-day food consumption was calculated from the residual amount, and mean daily food consumption was calculated from the cumulative food consumption for 1 week.

6.13.4 Measurement of Water Intake

For the animals that were alive at the time of urinalysis, 1000 mL of water was put into a 1000-mL polyethylene container for each animal, and the amount of water that remained was measured (by weight and recorded by volume) at 08:40-09:44 on the following day to calculate daily intake. During the measurement of water intake, the automatic water supply system was stopped. For the animal (No. 3001) for which contamination of the urine collected in Week 13 of administration with drinking

water was suspected and for the animal (No. 2001) which took all the water given and thus correct water intake could not be measured, the amount of water intake was measured again and urinalysis was done again using the urine re-collected.

6.13.5 Ophthalmological Examination

For all animals, using the equipments listed in Text Table 3, each item was examined macroscopically, or using a slit lamp, binocular indirect ophthalmoscope or fundus camera. Light reflex test was done first, and the anterior portion and fundus of the eye were examined after application of Mydrin® P (Lot Nos. MP0932, MP0940, MP0935, Santen Pharmaceutical Co., Ltd.), a mydriatic agent, to the eyes. Fundus oculi was photographed for all animals before the start of administration and only for one animal (No. 2002) in Week 25 of administration. For this animal, fundus oculi was also examined and photographed in Week 26 of administration to examine progress of the changes.

Text Table 3. Ophthalmological Examinations

Examination	Equipment	Items
Macroscopic observation	Pen light	Macroscopic examination of external appearance of the eyes
Examination of anterior portion, optic media and fundus oculi	Binocular indirect ophthalmoscope [a)] and slit lamp [b)]	Observation of cornea, conjunctiva, lens, iris, vitreous body and fundus oculi
Photography of fundus oculi	Fundus camera [c)]	Photography of fundus oculi
Instruments used a): Omega 200, HEINE OPTOTECHNIK, Germany b): Slitlamp (SL-14, Kowa Co., Ltd.) c): Fundus camera (Kowa GENESIS, Kowa Co., Ltd.)		

6.13.6 Electrocardiography

For all animals, items listed in Text Table 4 were recorded and calculated. During the administration period, the examination after dosing was performed in such a way that the examination time was approximately the same time for each group.

Text Table 4. Electrocardiography

Recording conditions	Position of animals etc.: Unanesthetized, right recumbent position, standard limb lead Equipment used: Electrocardiograph (LABO-SYSTEM ZM-5012; Fukuda M.E. Co., Ltd.)
Items calculated	Heart rate, P-R, Q-T and QRS intervals, QTc: Q-T interval (s) / $\sqrt{\text{R-R interval (s)}}$ × 1,000

6.13.7 Urinalysis

For all animals, fresh urine and cumulative urine were collected and items listed in Text Table 5 were examined by the method described in the same table. A urine collector was placed under each cage and fresh urine (comparatively fresh urine after urination) was collected between 08:50 and 13:20 before dosing during the administration period under deprivation of feed and drinking water. Cumulative urine was collected thereafter for approximately 20 hours from 13:00 (after dosing during the administration period) to 09:20 next morning with free access to feed and drinking water. At the same time as for cumulative urine collection, water intake was measured.

Text Table 5. Items, Methods and Equipment in Urinalysis

1) Examination on fresh urine		2) Examination on cumulative urine	
Item	Method	Item	Method
pH protein glucose ketones occult blood urobilinogen bilirubin	Multistix Test Paper (Bayer Medical Ltd., Lot Nos. 5K11C, 6B10C, 6C15D, 6H19C, 6L09D)	urine volume	measuring cylinder (Unit: mL)
sediment	microscopic examination	sodium potassium chloride	ion selective electrode method [b)] (Unit: mmol/20h)
specific gravity	refractometry [a)]		
color	macroscopic examination		
Equipment used a): Clinical Refractometer (Erma Inc.) b): Clinical Laboratory System TBA-120 FR (Toshiba Corporation)			

For the animal (No. 2001) which took all the water given and thus accurate water intake could not be measured and for the animal (No. 3001) for which contamination of the urine collected with drinking water was suspected in Week 13 of administration, the amount of water intake was measured again and urinalysis was done again using the re-collected urine. The data of re-urinalysis were adopted since they were all within the range of the background data of the testing facility. For the animals that showed occult blood (± to ++) or protein (++) in the urinalysis of fresh urine in Week –2 of administration and Week 13 of administration, re-examination was done (2 to 4 times) for confirmation. However, since the values obtained in the re-examination were similar to the initial values and the initial data were adopted, the data obtained in the re-examinations were regarded as supporting data.

6.13.8 Hematological Examination

After deprivation of feed for at least 16 hours from the previous day, blood samples (approximately 2 mL) were collected via the cephalic vein of all animals. Blood samples (approximately 1 mL) that were put into blood collecting tubes containing EDTA-2K (SB-41: Sysmex Corporation, Lot Nos. G5090, G6002, G6040) and items listed in Text Table 6-1) were measured by the method specified in the same table. Blood smear specimens were prepared by the May-Gruenwald-Giemsa staining method for all animals. In addition, blood samples (approximately 1 mL) that were collected into blood collecting tubes containing 3.8 w/v% sodium citrate solution (volume ratio of blood to citrate solution = 9:1) were centrifuged (approximately 1,600 × g, 10 minutes) and plasma samples were examined for items listed in Text Table 6-2) by the method specified in the same table. For moribund animals (Nos. 4102, 4106), blood was collected in the same manner and examined, but these animals were not fasted before blood collection.

Text Table 6. Items, Methods and Equipment for Hematological Examinations

1) Examination on EDTA-2K treated blood samples		
Item	Method	Unit
red blood cell count (RBC)	dual-laser flow cytometry [a)]	$10^4/\mu L$
hemoglobin (Hb)	modified cyanmethemoglobin method [a)]	g/dL
hematocrit (Ht)	calculated from red blood cell count and mean corpuscular volume [a)]	%
mean corpuscular volume (MCV)	dual-laser flow cytometry [a)]	fL
mean corpuscular hemoglobin (MCH)	calculated from red blood cell count and hemoglobin [a)]	pg
mean corpuscular hemoglobin concentration (MCHC)	calculated from hematocrit and hemoglobin [a)]	g/dL
reticulocyte percentage (Reticulocyte) and count	laser flow cytometry by RNA staining [a)]	%, $10^4/\mu L$
platelet count (Platelet)	dual-laser flow cytometry [a)]	$10^4/\mu L$
white blood cell count (WBC)	dual-laser flow cytometry [a)]	$10^2/\mu L$
differential white blood cell percentage and differential white blood cell count	flow cytometry by peroxidase staining + dual-laser flow cytometry [a)]	% $10^2/\mu L$
2) Examination on plasma samples separated from sodium citrate-treated blood samples		
Item	Method	Unit
prothrombin time (PT)	clot method [b)]	s
activated partial thromboplastin time (APTT)	clot method [b)]	s
fibrinogen	thromboplastin method [b)]	mg/dL
Equipment used a): Advia 120 Hematology System (Bayer Corporation, New York, USA) b): Coagulometer ACL 100 (Instrumentation Laboratory)		

White blood cells were classified as monocytes, neutrophils, eosinophils, basophils, lymphocytes and large unstained cells.

6.13.9 Blood Chemistry Examination

Portions of blood samples (approximately 4 mL) that were collected for hematology were put into test tubes, and allowed to stand at room temperature. The serum samples obtained by centrifugation (approximately 1,600 × g, 10 minutes) were examined for items listed in Text Table 7-1) by the method specified in the same table. Blood samples (approximately 2 mL) that were collected into blood collecting tubes containing heparin (approximately 20 units of heparin per 1 mL blood, Heparin sodium for injection "Simizu", Ajinomoto Pharma Co., Ltd., Lot No. 40231) were centrifuged (approximately 1,600 × g, 10 minutes) and the plasma samples obtained were examined for items listed in Text Table 7-2) by the method specified in the same table. For moribund animals, examination was done as far as possible. Fractions for protein electrophoresis were evaluated as _ ($\__1 + \__2 + \__3$), _ ($\__1 + \__2$), and _. The serum samples remaining after examination were sent to the Sponsor in a freezing condition for their use.

Text Table 7. Items, Methods and Equipment for Blood Chemistry Examinations

1) Examination on sera separated after standing at room temperature		
Item	Method	Unit
ALP	Bessey-Lowry method [a)]	IU/L
total cholesterol (CHO)	CEH-COD-POD method [a)]	mg/dL
triglyceride (TG)	LPL-GK-GPO-POD method [a)]	mg/dL
total bilirubin (BIL)	bilirubin oxidase method [a)]	mg/dL
glucose (GLU)	glucose dehydrogenase method [a)]	mg/dL
blood urea nitrogen (BUN)	urease-LEDH method [a)]	mg/dL
creatinine (CRE)	creatininase-creatinase-sarcosine-oxidase-POD method [a)]	mg/dL
sodium (Na)	ion selective electrode method [a)]	mmol/L
potassium (K)	ion selective electrode method [a)]	mmol/L
chloride (Cl)	ion selective electrode method [a)]	mmol/L
calcium (Ca)	OCPC method [a)]	mg/dL
inorganic phosphorus (IP)	molybdic acid method [a)]	mg/dL
total protein (TP)	biuret method [a)]	g/dL
albumin (ALB)	BCG method [a)]	g/dL
A/G ratio	calculated from total protein and albumin	
protein fractions	electrophoresis using cellulose acetate membrane [b)]	%
2) Examination of plasma samples from heparin treated blood sample		
Item	Method	Unit
AST (GOT)	UV-rate method [a)]	IU/L
ALT (GPT)	UV-rate method [a)]	IU/L
LDH	UV-rate method [a)]	IU/L
CPK	UV-rate method [a)]	IU/L
Equipment used a): Clinical Laboratory System TBA-120FR (Toshiba Corporation) b): Cliniscan 2 (K.K. Helena Kenkyujyo)		

6.13.10 Pathological Examinations

1) Necropsy

All animals subjected to necropsy were fasted for at least 16 hours from the previous day. They were sacrificed humanely by exsanguination via the cervical artery under anesthesia by intravenous injection of pentobarbital sodium (30 mg/kg, Tokyo Kasei Kogyo Co., Ltd., Lot No. GM01). They were subjected to necropsy as soon as possible after sacrifice. The organs/tissues weighed and collected for histopathological examination are listed in Text Table 8.

2) Measurement of Organ Weight

For the organs listed in Text Table 8, absolute weight was measured and the weight per body weight (relative weight) was calculated from the animal's terminal body weight and the absolute organ weight. For the paired organs, organ weight was measured separately but evaluation was done on the total weight.

3) Histopathological Examination

The subject organs/tissues are shown in Text Table 8. For all animals, the organs and tissues were removed and fixed in phosphate buffered 10 vol% formalin. However, the eyeballs and optic nerves were fixed in phosphate buffered 3 vol% glutaraldehyde/2.5 vol% formalin and the testes were fixed in Bouin's solution and these organs/tissues were preserved in phosphate buffered 10 vol% formalin. All the organs/tissues of all animals were embedded in paraffin, and sections were prepared, stained with hematoxylin/eosin (H&E) and subjected to microscopy.

Text Table 8. List of Organs/tissues for Pathological Examination

Organ/tissue	Histopathology H&E	Organ weight
cerebrum	√	√ (together)
cerebellum	√	
medulla oblongata	√	
spinal cord	√	
optic nerve	√	
sciatic nerve	√	
eyeball	√	
lacrimal gland	√	
pituitary	√	√
thyroid	√	√
parathyroid	√	
adrenal	√	√
thymus	√	√
spleen	√	√
submandibular lymph node	√	
mesenteric lymph node	√	
heart	√	√
aorta (aortic arch)	√	
larynx	√	
trachea	√	
lung (including bronchus)	√	√
tongue	√	
esophagus	√	
stomach	√	
duodenum	√	
jejunum	√	
ileum	√	
cecum	√	
colon	√	
rectum	√	
submandibular gland	√	√
sublingual gland	√	
parotid gland	√	
liver	√	√ (together)
gallbladder	√	
pancreas	√	√
kidney	√	√
ureter	√	
urinary bladder	√	
testis/ovary	√ / √	√ / √
epididymis/uterus	√ / √	√ / √
prostate/vagina	√ / √	√ /
mammary gland	√	
sternum (including bone marrow)	√	
femur (including bone marrow)	√	
femoral skeletal muscle	√	
skin (abdominal region)	√	
part for individual identification (ear auricle)	(preservation only)	

Adequate Battery: yes (x), no ( )—explain

Peer review: yes (), no (x)

Results:

Mortality:

Clinical signs:

Body weights:

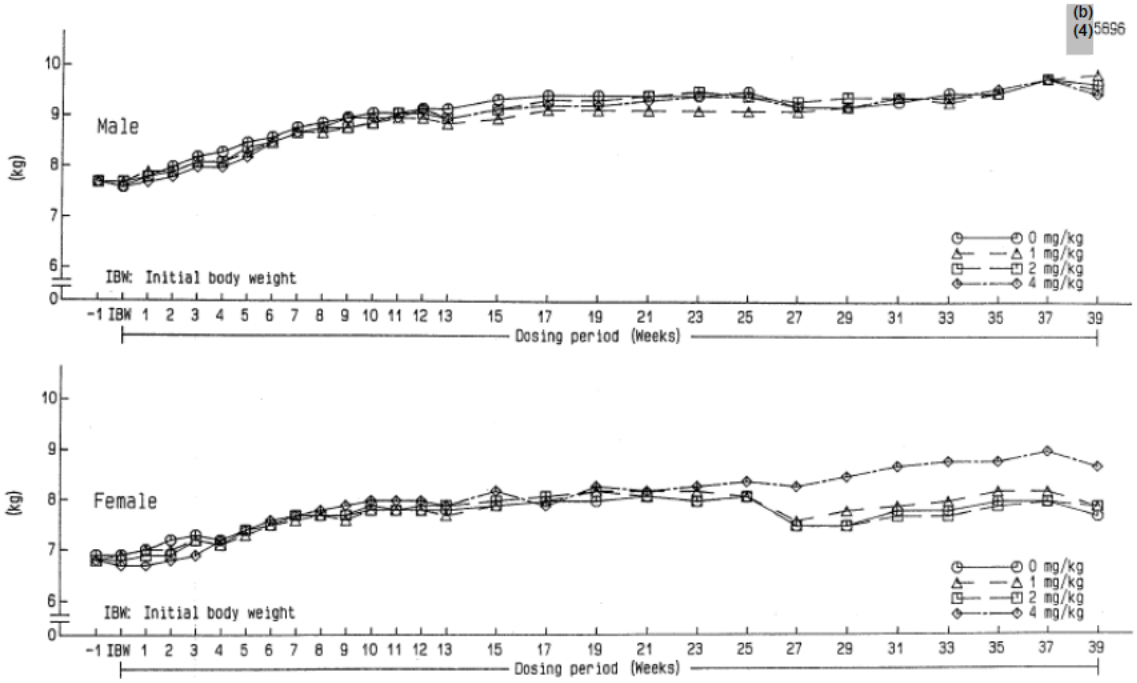


Fig.1 39-week repeated dose oral toxicity study of NS-304 in dogs
Body weight changes

Food consumption:

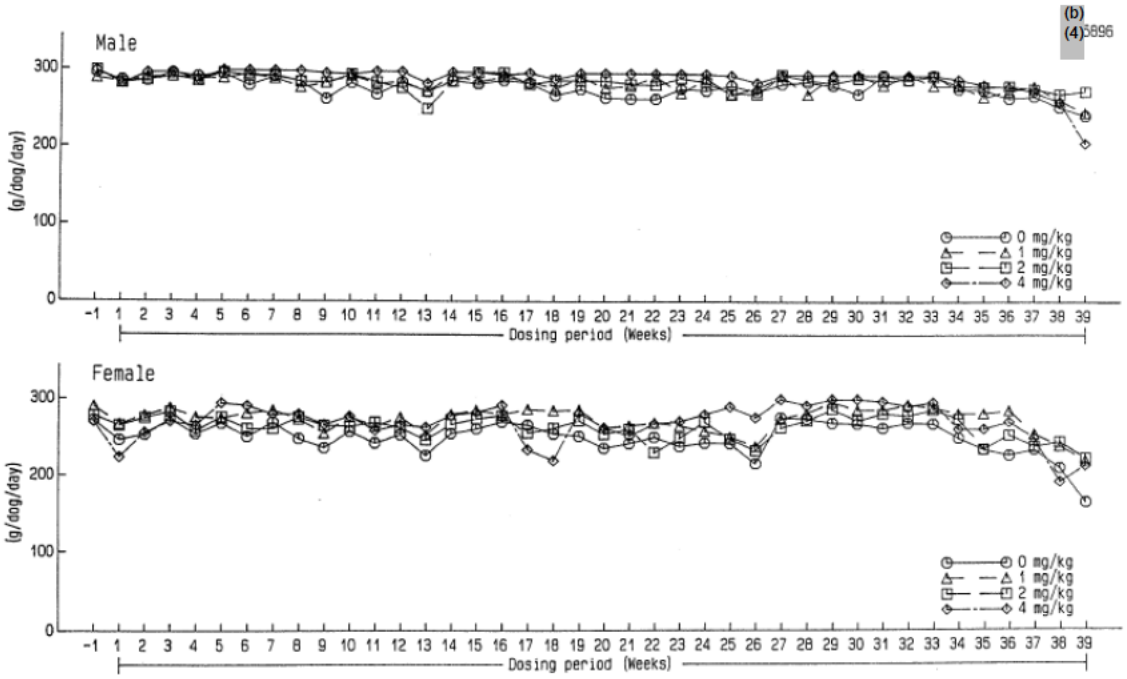


Fig.2 39-week repeated dose oral toxicity study of NS-304 in dogs
Food consumption changes

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )

Text Table 9-1 TK Parameters of NS-304 in Plasma (Mean Values)

Dose (mg/kg)	Week		Male/ Female		
			C_{max} (μg/mL)	T_{max} (h)	AUC_{0-24h} (μg·h/mL)
1	Day 1		0.977 / 1.53	1.1 / 1.3	2.55 / 3.36
	13W	4h	0.163 / 0.228		
		24h	0 / 0		
	26W		1.83 / 0.974	1.2 / 1.8	3.49 / 2.85
	39W		1.03 / 1.36	1.2 / 1.3	2.55 / 2.88
2	Day 1		2.54 / 2.87	2.3 / 1.6	8.44 / 7.30
	13W	4h	0.946 / 0.726		
		24h	0 / 0		
	26W		4.50 / 3.26	1.8 / 1.5	10.2 / 9.15
	39W		3.10 / 1.79	1.7 / 2.7	8.15 / 6.54
4	Day 1		5.77 / 5.21	1.5 / 1.4	17.4 / 17.6
	13W	4h	1.33 / 1.38		
		24h	0 / 0		
	26W		5.66 / 5.03	1.7 / 2.0	18.1 / 16.2
	39W		8.62 / 6.88	1.0 / 2.0	21.3 / 18.0

Text Table 9-2 TK Parameters of MRE-269 in Plasma (Mean Values)

Dose (mg/kg)	Week		Male/ Female		
			C_{max} (μg/mL)	T_{max} (h)	AUC_{0-24h} (μg·h/mL)
1	Day 1		2.50 / 3.05	3.7 / 2.7	24.6 / 33.2
	13W	4h	2.25 / 2.06		
		24h	0.154 / 0.213		
	26W		3.29 / 2.52	2.7 / 4.3	28.8 / 24.6
	39W		2.60 / 3.26	2.7 / 3.3	23.1 / 35.7
2	Day 1		5.27 / 5.56	4.0 / 3.7	49.9 / 54.0
	13W	4h	4.94 / 3.45		
		24h	0.239 / 0.228		
	26W		6.40 / 6.13	3.3 / 3.3	55.8 / 52.2
	39W		5.29 / 5.87	3.3 / 4.0	40.5 / 58.9
4	Day 1		9.17 / 8.62	3.2 / 4.0	94.4 / 104
	13W	4h	6.60 / 3.87		
		24h	0.380 / 0.548		
	26W		6.89 / 10.4	3.0 / 3.5	58.4 / 90.8
	39W		9.54 / 12.5	2.7 / 4.0	109 / 120

Toxicokinetics:

Other:

Study title: Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice

Key study findings: Study was primarily to determine doses for the carcinogenicity study. Doses of 0, 100, 300, and 500 mg/kg/day were administered. The top dose of 500 mg/kg/day had one animal die, and liver and kidney effects after only 13 weeks. That may be too high a dose for a 104 week carcinogenicity study, leading to loss of the high dose group.

Study no.: T-08.292
Volume #, and page #: eCTD 4.2.3.2
Conducting laboratory and location: (b) (4)

Date of study initiation: Oct. 24, 2006
GLP compliance: Yes
QA report: yes (x) no ( )

Drug, lot #, and % purity: NS-304, lot # 20, 100.3% pure

Methods

Species/strain: B6C3F1/Crlj SPF mice
Route, formulation, volume, and infusion rate: Oral Gavage
Age: 5 weeks of age
Weight:

group, respectively, to evaluate toxicity and systemic exposure. Individual body weight on the starting day of administration ranged from 21.1 to 24.8 g in males and 17.2 to 20.3 g in females for the main group, and that for the satellite group ranged from 21.2 to 25.4 g in males and 17.2 to 21.3 g in females.

Sampling times: see observation & times

Text Table 1. Group composition

Test group	Dose (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg/day)	Sex	Main group		Satellite group	
					No. of animals	Animal No.	No. of animals	Animal No.
Control	0	0	10	M	10	1001-1010	20	1201-1220
				F	10	1101-1110	20	1301-1320
Low	100	10	10	M	10	2001-2010	42	2201-2242
				F	10	2101-2110	42	2301-2342
Middle	300	30	10	M	10	3001-3010	42	3201-3242
				F	10	3101-3110	42	3301-3342
High	500	50	10	M	10	4001-4010	42	4201-4242
				F	10	4101-4110	42	4301-4342

M: Male, F: Female

Observation and Times: Clinical signs:

All animals were observed for clinical signs including external appearance, nutritional condition, posture, behavior and excretions 3 times a day (before, immediately after and approximately 2 hours after dosing; however twice on Saturdays, Sundays and holidays: before and immediately after dosing) during the administration period. In addition, the observation of 2 hours after dosing was done at days 3 (Saturday) and 4 (Sunday).

Body weights:

All animals were weighed twice in week 1 (days 1 and 7) of administration, and once a week every 7 days thereafter. Measurement was done before administration between 08:37 and 10:23. In order to calculate the relative organ weight, the body weight was also recorded on the day of necropsy (not fasted).

Food consumption:

Food consumption was measured for each animal twice in week 1 (days 1 and 7) of administration, and once a week every 7 days thereafter. Measurement was done between 08:50 and 11:12. On day 1 of administration, one day's food consumption was measured from the day before the start of administration and that on day 7 was calculated from 6-day's cumulative food consumption. Thereafter, 7-day's cumulative consumption was measured and one day's food consumption was calculated.

Ophthalmoscopy:

Before the start of administration (during the quarantine/acclimatization period, 3 days before administration), all candidate animals for main group were examined, and the animals with ophthalmological abnormalities that might affect the toxicity evaluation were excluded from animal grouping for the main group (note).

In month 3 of administration (week 13, day 85), all animals of each sex in the control and 500 mg/kg groups were examined after dosing on the day of examination. The examination in the 100 and 300 mg/kg groups were not done, since no treatment-related changes were observed in the 500 mg/kg group. The procedure for examination was as follows.

The mydriatic agent (Mydrin® P: Santen Pharmaceutical Co., Ltd., Lot No. MP0947) was applied to dilate the pupil and the anterior portion, transparent body (optic media) and fundus oculi were examined an ophthalmoscope (Omega 200: HEINE OPTOTECNIK GmbH & Co. KG, Germany).

note: Two males and one female with severe ophthalmological abnormalities such as focal opacity in lens, persistent tunica vasculosa lentis and myelinated nerve fiber were excluded from the present study.

EKG: not done

Hematology:

At the time of necropsy on the day following the end of the administration period, blood samples were collected from the vena cava inferior of all animals (not fasted) using syringes treated with heparin sodium under ether anesthesia into blood collection tubes (approximately 0.35 mL) containing an anticoagulant (EDTA-2K, Microtainer® Tube: Japan Becton Dickinson Inc.). The following parameters were determined. May-Gruenwald-Giemsa staining smears from all animals were prepared as reserve in the case of microscopic examination. (Ultimately, the microscopic examination was not conducted.)

Text Table 2. Items, Methods and Equipment for Hematological Examinations

Item	Method	Unit
red blood cell count (RBC)	dual angle laser flow-cytometric measurement[a)]	$10^4/\mu L$
hemoglobin (Hb)	modified cyanmethemoglobin method[a)]	g/dL
hematocrit (HCT)	calculated from mean corpuscular volume and red blood cell count[a)]	%
mean corpuscular volume (MCV)	dual angle laser flow-cytometric measurement[a)]	fL
mean corpuscular hemoglobin (MCH)	calculated from red blood cell count and hemoglobin[a)]	pg
mean corpuscular hemoglobin concentration (MCHC)	calculated from hematocrit and hemoglobin[a)]	g/dL
reticulocyte ratio (Reticul.)	laser flow-cytometric measurement with RNA stain[a)]	%
platelet count (PLT)	dual angle laser flow-cytometric measurement[a)]	$10^4/\mu L$
white blood cell count (WBC)	dual angle laser flow-cytometric measurement[a)]	$10^2/\mu L$
differential leukocyte count (note)	peroxidase flow-cytometric measurement and dual angle laser flow-cytometric measurement[a)]	%

Equipment used

a): ADVIA®120 Hematology System (Bayer Corporation, New York, USA)

note: Lymphocytes (LYM), neutrophils (NE), eosinophils (EOSINO), basophils (BASO), monocytes (MONO) and large unstained cells (LUC)

Clinical chemistry:

At the same time as hematology, blood samples were collected from the vena cava inferior into blood collection tubes (approximately 0.45 or 0.55 mL) containing anticoagulant heparin lithium (Capiject®: Capillary blood collection tubes, Terumo Corporation). The plasma samples were obtained by centrifugation (set at 3,100 rpm, approximately 1,690×g for 12 minutes) and the following items were determined.

Text Table 3. Items, Methods and Equipment for Blood chemistry Examinations

Item	Method	Unit
AST	UV-rate method[a)]	IU/L
ALT	UV-rate method[a)]	IU/L
AlP	Bessey-Lowry method[a)]	IU/L
CK	UV-rate method[a)]	IU/L
total cholesterol (T-CHO)	CEH-COD-POD method[a)]	mg/dL
triglyceride (TG)	LPL-GK-GPO-POD method[a)]	mg/dL
total bilirubin (T-BIL)	bilirubin oxidase method[a)]	mg/dL
glucose (GLU)	glucose dehydrogenase method[a)]	mg/dL
blood urea nitrogen (BUN)	urease-LEDH method[a)]	mg/dL
creatinine (CRNN)	creatininase-creatinase-sarcosine oxidase-POD method[a)]	mg/dL
sodium (Na)	ion selective electrode method[a)]	mmol/L
potassium (K)	ion selective electrode method[a)]	mmol/L
chloride (Cl)	ion selective electrode method[a)]	mmol/L
calcium (Ca)	OCPC method[a)]	mg/dL
inorganic phosphorus (P)	molybdic acid method[a)]	mg/dL
total protein (TP)	Biuret method[a)]	g/dL
albumin (ALB)	BCG method[a)]	g/dL

Equipment used

a): Toshiba Biochemical Analyzer Model TBA-120FR (Toshiba Corp.)

Urinalysis: not done

Gross pathology:

After collecting blood samples, all animals were sacrificed by exsanguination from the abdominal aorta under ether anesthesia. External appearance and all the organs/tissues in the cranial, thoracic and abdominal cavities were carefully examined and the results were recorded.

Organ weights (specify organs weighed if not in histopath table):

After necropsy, the organs listed below of all animals were weighed (absolute weight) and organ weight per 100 g body weight (relative weight) was calculated based on the animal's body weight (not fasted) and absolute organ weight. The paired organs indicated by asterisks (*) were weighed separately; however, evaluation was done on the total value of the right and left organs.

brain, spleen, heart, lungs (including bronchus), salivary glands (submandibular + sublingual glands)*, liver (including gall bladder), kidneys*, testes* and uterus

Histopathology:

All the organs/tissues listed below of all animals were fixed and preserved in phosphate buffered 10 vol% formalin. However, the eyeballs and optic nerves were fixed with a

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mixture containing 3 w/v% glutaraldehyde and 2.5 vol% formalin, and the testes and epididymides were fixed with Bouin's solution, and then preserved in phosphate buffered 10 vol% formalin. The organs/tissues marked with # listed below of all animals were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H.E). Of these, all organs/tissues from the control and the high dose groups were examined histopathologically. In addition, sections of the liver in both sexes and kidney in males from all other groups were subjected to histopathological examination, since treatment-related lesions were suspected in these organs. In addition, the mammary gland in females from all groups was observed, since treatment-related changes were observed in a 26-week oral gavage toxicity study of NS-304 in rats with 4-week recovery period ((b) (4) Study No. (b) (4) 5895)[3]. Ultimately, no treatment-related changes were observed in mammary gland in the present study. Other than the above, the animal that was found dead on day 3 in the 500 mg/kg group (No. 4328, satellite group) was necropsied and all the organs/tissues listed below were fixed, preserved and examined histopathologically to presume the cause of death, since no treatment-related deaths were observed in the main or satellite groups during the administration period except for this animal. The paired organs indicated by asterisks (*) were examined unilaterally; however, these were preserved bilaterally.

cerebrum, cerebellum, spinal cord (cervical, thoracic and lumbar), sciatic nerves*, eyeballs#*, optic nerves#*, Harderian glands*, pituitary#, thyroids#*, parathyroids*, adrenals#*, thymus#, spleen#, submandibular lymph node, mesenteric lymph node, heart#, thoracic aorta, trachea, lungs (including bronchus)#, tongue, esophagus, stomach#, duodenum#, jejunum, ileum, cecum#, colon, rectum, submandibular glands#*, sublingual glands#*, parotid glands*, liver#, gallbladder#, pancreas, kidneys#*, urinary bladder#, testes#*, epididymides*, prostate, seminal vesicles*, ovaries#*, uterus#*, vagina, mammary glands (inguinal region, females only)#*, sternum (including bone marrow), femurs (including bone marrow)#*, femoral muscles*, skin (inguinal region)*, oviducts*, extraorbital lacrimal glands*, Zymbal's glands*, larynx, nasal cavity, preputial glands*, clitoral glands* and gross lesions (note)

Besides the organs/tissues listed above, the site of animal identification (ear auricle) was preserved.

note: Gross lesions were observed only in the animal that was found dead in the satellite group.

Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )

Results:

Mortality: one female in the high dose group died of treatment related causes on treatment day 3, but this individual was in the toxicokinetics group. One male died in the low dose group, but it was not thought to be due to treatment.

Clinical signs:

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Table 1 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Clinical signs

Sex	Dose mg/kg/day	Findings	Week of administration												
			1	2	3	4	5	6	7	8	9	10	11	12	13
	0	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	10	10	10	10	10	10	10	10	10	10	10	10	10
Male	100	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	10	10	10	10	10	10	10	10	10	10	10	10	10
	300	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	0	9	8	6	7	5	5	5	6	4	5	6	4
		Flaccidity	7	0	0	0	0	0	0	0	0	0	0	0	0
		Flush(limbs)	10	1	2	4	3	5	5	5	4	6	5	4	6
	500	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	0	2	1	2	1	1	1	0	2	1	1	0	0
		Flaccidity	10	0	0	0	0	0	0	0	0	0	0	0	0
		Flush(limbs)	10	8	9	8	9	9	9	10	8	9	9	10	10
		Creeping position	1	0	0	0	0	0	0	0	0	0	0	0	0
	0	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	10	10	10	10	10	10	10	10	10	10	10	10	10
Female	100	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	10	10	10	10	10	10	10	10	10	10	10	10	10
	300	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	2	6	6	2	3	2	2	4	4	1	3	3	4
		Flaccidity	6	0	0	0	0	0	0	0	0	0	0	0	0
		Flush(limbs)	6	4	4	8	7	8	8	6	6	9	7	7	6
	500	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	0	1	1	2	1	1	0	1	1	0	1	1	3
		Flaccidity	10	0	0	0	0	0	0	0	0	0	0	0	0
		Flush(limbs)	10	9	9	8	9	9	10	9	9	10	9	9	7
		Creeping position	2	0	0	0	0	0	0	0	0	0	0	0	0
		Eye discharge	2	0	0	0	0	0	0	0	0	0	0	0	0

Body weights:

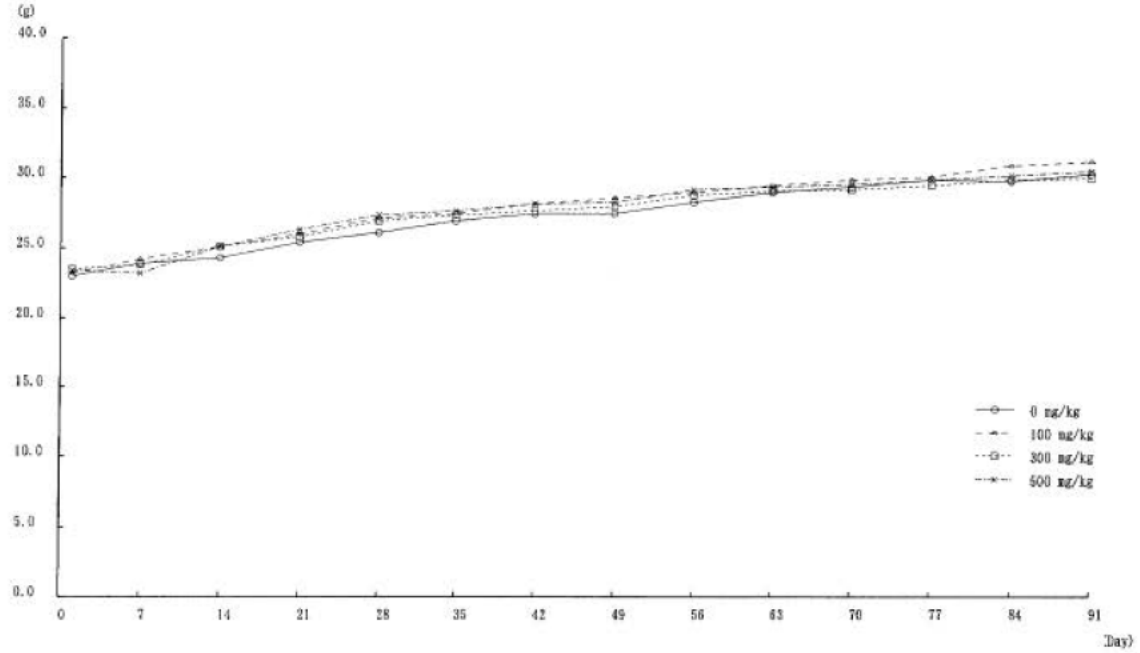

Fig. 1 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Body Weight - Male

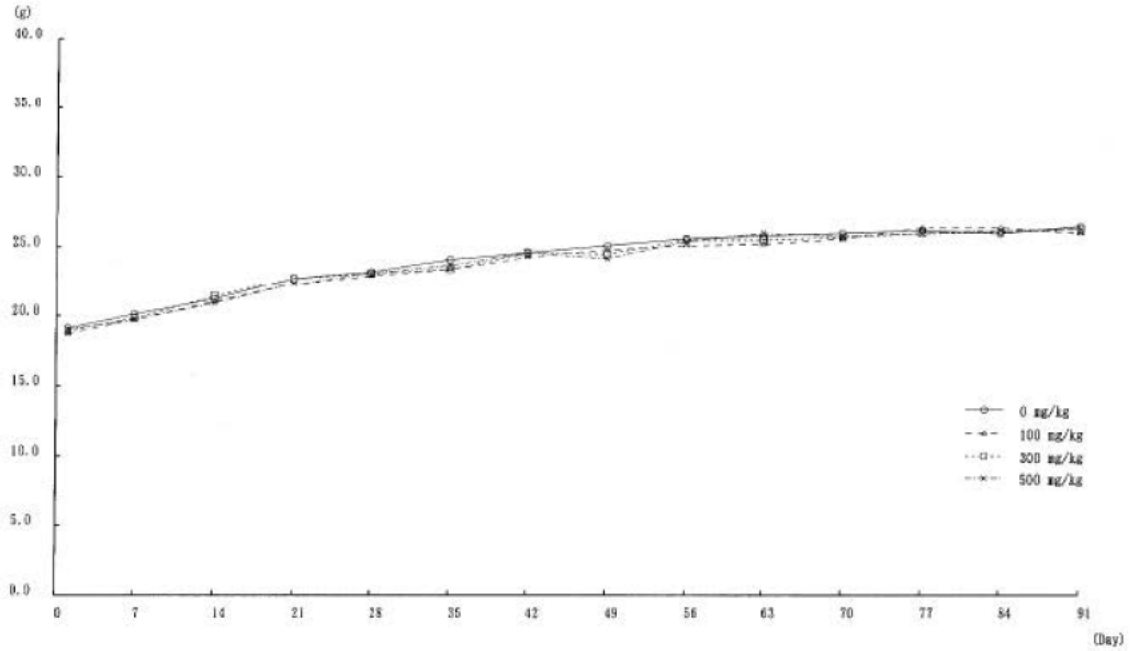

Fig. 2 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Body Weight - Female

Food consumption:

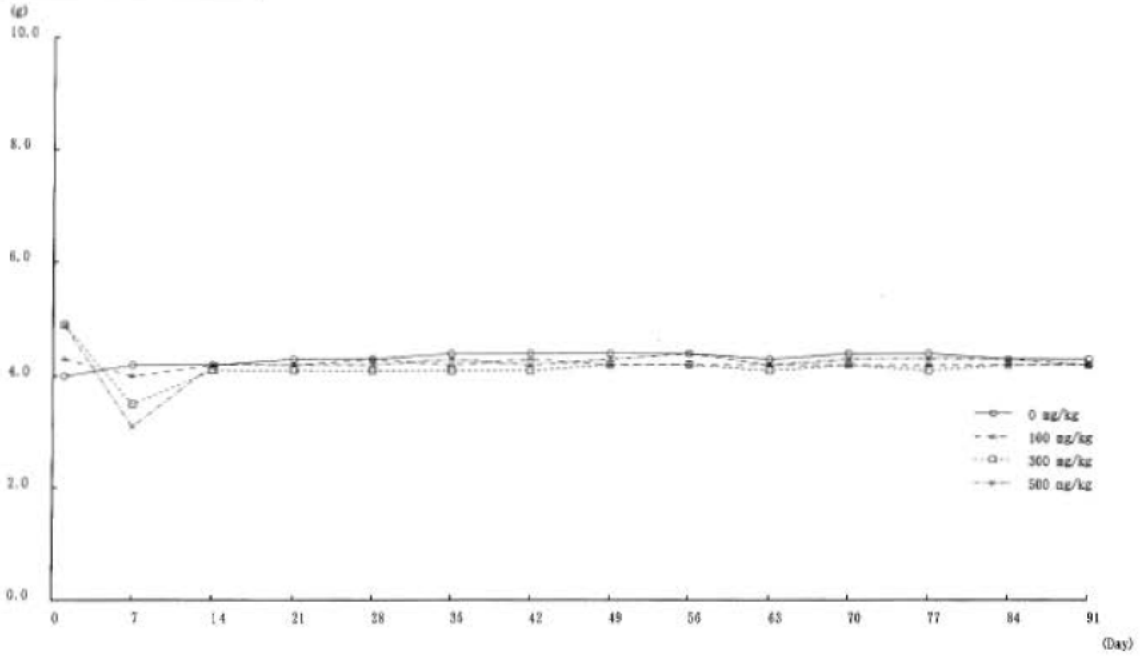

Fig. 3 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Food Consumption - Male

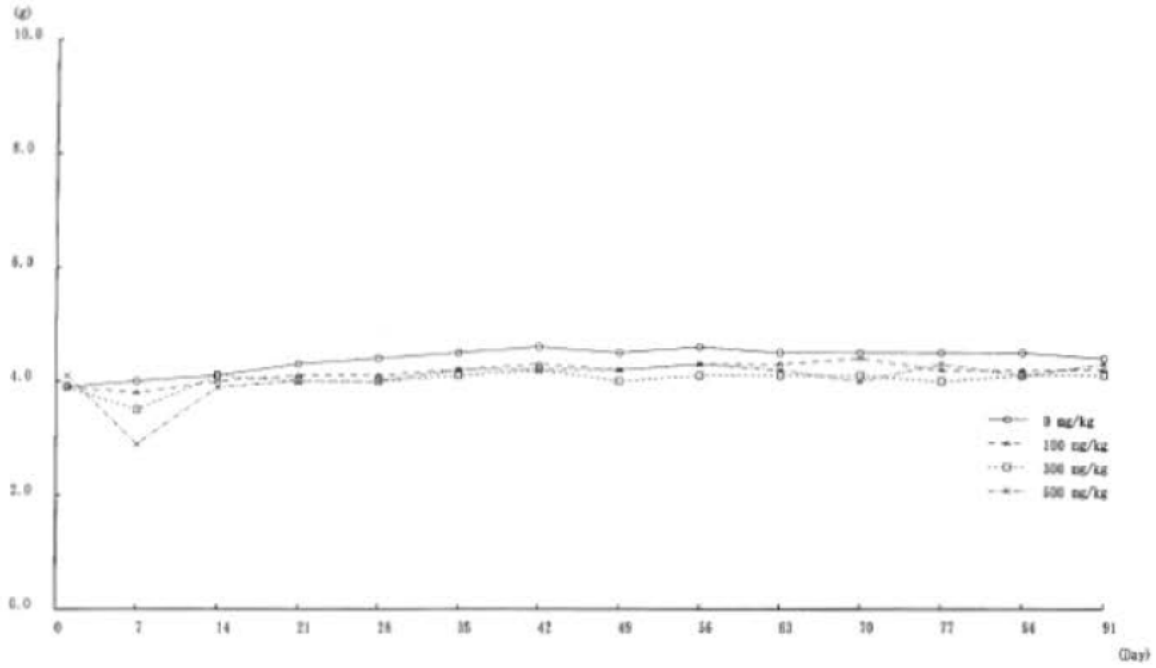

Fig. 4 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Food Consumption - Female

Ophthalmoscopy: no abnormalities reported

EKG: not done

Hematology:

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Table 5 - 1 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Hematology Stage : Week 13
Sex : Male

Test Article Dose		RBC 10E4/uL	Hb g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL
NS-304 0 mg/kg	Mean	979	15.7	44.2	45.1	16.0	35.5
	S.D.	27	0.5	1.4	0.3	0.1	0.4
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	997	16.2	45.8	45.9D2**	16.2D2**	35.4
	S.D.	43	0.6	1.8	0.5	0.2	0.3
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	1013	16.6D2**	46.8D2**	46.2D2**	16.4D2**	35.5
	S.D.	48	0.8	2.3	0.6	0.1	0.4
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	1009	16.5D2*	46.7D2*	46.3D2**	16.4D2**	35.4
	S.D.	35	0.6	1.8	0.5	0.2	0.2
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side

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Table 5 - 4 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Hematology Stage : Week 13
Sex : Female

Test Article Dose		RBC 10E4/uL	Hb g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL
NS-304 0 mg/kg	Mean	990	16.1	45.4	45.8	16.2	35.4
	S.D.	25	0.4	1.0	0.5	0.2	0.5
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	1018	16.9D2**	48.0D2**	47.1D2**	16.6D2**	35.3
	S.D.	42	0.7	1.8	0.5	0.1	0.2
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	1040D2**	17.1D2**	48.8D2**	46.9D2**	16.5D2*	35.1
	S.D.	36	0.6	1.6	0.5	0.1	0.4
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	1049D2**	17.3D2**	49.4D2**	47.0D2**	16.5D2*	35.0
	S.D.	34	0.5	1.4	0.4	0.2	0.5
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side

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Table 5 - 5 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Hematology Stage : Week 13
Sex : Female

Test Article Dose		Reticulocyte %	PLT 10E4/uL	WBC 10E2/uL	LYM %	NE %	EOSINO %
NS-304 0 mg/kg	Mean	2.5	108.8	23.0	82.0	15.5	1.2
	S.D.	0.5	5.0	9.3	4.3	4.6	0.7
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	2.4	110.4	18.4	84.8	12.2	1.3
	S.D.	0.3	3.5	8.4	3.8	4.1	1.0
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	2.6	118.0D2**	22.5	82.2	14.9	1.3
	S.D.	0.5	5.8	7.4	5.1	5.6	0.9
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	3.0D2*	116.3D2**	29.5	85.1	12.8	0.8
	S.D.	0.3	5.8	16.1	1.9	2.3	0.3
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side

Clinical chemistry:

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Table 6 - 1 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Blood Chemistry Stage : Week 13
Sex : Male

Test Article Dose		AST IU/L	ALT IU/L	CK IU/L	ALP IU/L	T-CHO mg/dL	TG mg/dL
NS-304 0 mg/kg	Mean	41	25	37	258	102	60
	S.D.	3	3	15	17	9	13
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	42	24	66	262	113D2*	64
	S.D.	4	2	41	20	10	23
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	44	24	93	266	121D2**	55
	S.D.	6	2	78	21	10	18
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	42	25	63S2*	274	133D2**	67
	S.D.	5	5	29	23	6	15
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side ,S2:Steel Test Two-Side

(b)
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Table 6 - 2 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Blood Chemistry Stage : Week 13
Sex : Male

Test Article Dose		T-BIL mg/dL	GLU mg/dL	BUN mg/dL	CRNN mg/dL	Na mmol/L	K mmol/L
NS-304 0 mg/kg	Mean	0.1	202	29	0.07	154	4.6
	S.D.	0.0	20	5	0.01	1	0.4
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	0.1	196	27	0.08	155	4.8
	S.D.	0.0	16	6	0.01	1	0.4
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	0.1	198	25	0.09	155	4.5
	S.D.	0.0	19	5	0.01	1	0.3
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	0.1	186	20D2**	0.08	155	4.4
	S.D.	0.0	17	3	0.01	2	0.2
	n	10	10	10	10	10	10

Significantly different from control : ** P≦0.01
D2:Dunnett Test Two-Side

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Table 6 - 4 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Blood Chemistry Stage : Week 13
Sex : Female

Test Article Dose		AST IU/L	ALT IU/L	CK IU/L	AlP IU/L	T-CHO mg/dL	TG mg/dL
NS-304 0 mg/kg	Mean	48	23	46	441	96	44
	S.D.	9	2	10	48	11	6
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	47	25	66	428	103	56
	S.D.	7	3	31	34	9	20
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	49	26	53	434	119D2**	49
	S.D.	11	9	17	45	11	10
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	58	41S2*	51	440	126D2**	58
	S.D.	24	23	22	39	9	17
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side ,S2:Steel Test Two-Side

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Table 6 - 5 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Blood Chemistry Stage : Week 13
Sex : Female

Test Article Dose		T-BIL mg/dL	GLU mg/dL	BUN mg/dL	CRNN mg/dL	Na mmol/L	K mmol/L
NS-304 0 mg/kg	Mean	0.1	192	18	0.09	154	4.1
	S.D.	0.0	16	3	0.02	1	0.6
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	0.1	202	16	0.11	155	4.1
	S.D.	0.0	11	2	0.01	1	0.4
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	0.1	210	15D2**	0.11	154	4.6
	S.D.	0.0	15	2	0.01	3	1.1
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	0.1	198	15D2**	0.11	154	4.0
	S.D.	0.0	25	1	0.02	2	0.3
	n	10	10	10	10	10	10

Significantly different from control : ** P≦0.01
D2:Dunnett Test Two-Side

Urinalysis: not done

Gross pathology:

(b) (4)

Table 8-1 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Gross pathological findings (Dead animal, Satellite group)

Organs / Findings	Sex:	F
	Dose (mg/kg/day):	500
	Number:	1
Stomach		
Focus, dark red, glandular stomach		1
Tongue		
Focus, dark red		1

(b) (4)5938

Table 8-2 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Gross pathological findings (Survivors, Main group)

Organs / Findings	Sex:	M	M	M	M	F	F	F	F
	Dose (mg/kg/day):	0	100	300	500	0	100	300	500
	Number:	10	10	10	10	10	10	10	10
All tissues									
Not remarkable		10	10	10	10	10	10	10	10

Organ weights (specify organs weighed if not in histopath table):

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Table 7 - 1 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Absolute Organ Weight Stage : Week 13
Sex : Male

Test Article Dose		Body weight g	Brain mg	Salivary gland-RL mg	Heart mg	Lung mg	Liver g
NS-304 0 mg/kg	Mean	30.3	505	201.6	148	152	1.37
	S.D.	1.5	14	16.0	12	10	0.12
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	31.1	513	224.6D2**	141	151	1.39
	S.D.	1.4	11	11.8	10	7	0.14
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	30.1	497	223.1D2**	141	154	1.53D2**
	S.D.	1.2	7	9.5	16	9	0.11
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	30.5	496	225.3D2**	129D2**	155	1.88D2**
	S.D.	0.8	7	11.0	9	10	0.05
	n	10	10	10	10	10	10

Significantly different from control : ** P≦0.01
D2:Dunnett Test Two-Side

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Table 7 - 2 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Absolute Organ Weight Stage : Week 13
Sex : Male

Test Article Dose		Spleen mg	Kidney-RL mg	Testis-RL mg
NS-304 0 mg/kg	Mean	62	477	187
	S.D.	7	31	19
	n	10	10	10
NS-304 100 mg/kg	Mean	58	461	194
	S.D.	5	38	15
	n	10	10	10
NS-304 300 mg/kg	Mean	57	419D2**	189
	S.D.	3	24	10
	n	10	10	10
NS-304 500 mg/kg	Mean	56D2*	407D2**	187
	S.D.	5	15	17
	n	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side

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Table 7 - 3 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Absolute Organ Weight Stage : Week 13
Sex : Female

Test Article Dose		Body weight g	Brain mg	Salivary gland-RL mg	Heart mg	Lung mg	Liver g
NS-304 0 mg/kg	Mean	26.0	521	121.6	127	143	1.25
	S.D.	0.8	7	8.8	8	8	0.07
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	26.2	518	135.7D2**	124	142	1.26
	S.D.	1.2	13	7.1	7	9	0.10
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	26.0	508D2*	137.8D2**	121	143	1.38D2**
	S.D.	0.6	13	10.0	6	8	0.06
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	26.2	506D2*	140.4D2**	114D2**	147	1.70D2**
	S.D.	0.9	13	8.6	5	10	0.11
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side

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Table 7 - 4 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Absolute Organ Weight Stage : Week 13
Sex : Female

Test Article Dose		Spleen mg	Kidney-RL mg	Uterus mg
NS-304 0 mg/kg	Mean	90	330	154
	S.D.	11	12	36
	n	10	10	10
NS-304 100 mg/kg	Mean	75D2**	321	148
	S.D.	8	19	29
	n	10	10	10
NS-304 300 mg/kg	Mean	76D2**	306D2**	137
	S.D.	6	13	32
	n	10	10	10
NS-304 500 mg/kg	Mean	73D2**	306D2**	131
	S.D.	6	14	36
	n	10	10	10

Significantly different from control : ** P≦0.01
D2:Dunnett Test Two-Side

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Table 7 - 5 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Relative Organ Weight Stage : Week 13
Sex : Male

Test Article Dose		Body weight g	Brain mg/100g	Salivary gland-RL mg/100g	Heart mg/100g	Lung mg/100g	Liver g/100g
NS-304 0 mg/kg	Mean	30.3	1673	666.1	488	502	4.53
	S.D.	1.5	107	38.9	37	30	0.35
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	31.1	1651	724.0D2*	454	486	4.46
	S.D.	1.4	61	49.4	35	25	0.35
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	30.1	1653	742.9D2**	469	512	5.08D2**
	S.D.	1.2	69	42.0	52	28	0.34
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	30.5	1626	738.5D2**	424D2**	509	6.17D2**
	S.D.	0.8	50	37.0	32	26	0.21
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side

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Table 7 - 6 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Relative Organ Weight Stage : Week 13
Sex : Male

Test Article Dose		Spleen mg/100g	Kidney-RL mg/100g	Testis-RL mg/100g
NS-304 0 mg/kg	Mean	204	1577	621
	S.D.	22	68	72
	n	10	10	10
NS-304 100 mg/kg	Mean	187	1482D2*	625
	S.D.	13	106	55
	n	10	10	10
NS-304 300 mg/kg	Mean	188	1395D2**	630
	S.D.	11	66	40
	n	10	10	10
NS-304 500 mg/kg	Mean	183D2*	1335D2**	613
	S.D.	16	37	56
	n	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side

(b)
(4)5938

Table 7 - 7 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Relative Organ Weight Stage : Week 13
Sex : Female

Test Article Dose		Body weight g	Brain mg/100g	Salivary gland-RL mg/100g	Heart mg/100g	Lung mg/100g	Liver g/100g
NS-304 0 mg/kg	Mean	26.0	2006	467.4	490	548	4.80
	S.D.	0.8	74	31.8	38	22	0.26
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	26.2	1981	518.4D2**	475	544	4.80
	S.D.	1.2	75	23.5	23	22	0.23
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	26.0	1953	529.7D2**	466	551	5.32D2**
	S.D.	0.6	69	41.5	21	36	0.23
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	26.2	1934	535.7D2**	437D2**	561	6.48D2**
	S.D.	0.9	51	25.8	28	31	0.31
	n	10	10	10	10	10	10

Significantly different from control : ** P≦0.01
D2:Dunnett Test Two-Side

(b)
(4)5938

Table 7 - 8 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Relative Organ Weight Stage : Week 13
Sex : Female

Test Article Dose		Spleen mg/100g	Kidney-RL mg/100g	Uterus mg/100g
NS-304 0 mg/kg	Mean	346	1268	593
	S.D.	46	48	147
	n	10	10	10
NS-304 100 mg/kg	Mean	287D2**	1224	567
	S.D.	30	54	110
	n	10	10	10
NS-304 300 mg/kg	Mean	292D2**	1176D2**	525
	S.D.	20	44	128
	n	10	10	10
NS-304 500 mg/kg	Mean	279D2**	1166D2**	500
	S.D.	25	27	137
	n	10	10	10

Significantly different from control : ** P≦0.01
D2:Dunnett Test Two-Side

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

(b) (4)

Table 9-2 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Histopathological findings (Survivors, Main group)

Organs / Findings	Sex:	M	M	M	M	F	F	F	F
	Dose(mg/kg/day):	0	100	300	500	0	100	300	500
	Number:	10	10	10	10	10	10	10	10
Adrenal									
Number examined		10	0	0	10	10	0	0	10
Hyperplasia, subcapsular cell		1	0	0	1	10	0	0	9
minimal		1	0	0	1	10	0	0	9
Eye									
Number examined		10	0	0	10	10	0	0	10
Dysplasia, retinal		0	0	0	0	1	0	0	0
minimal		0	0	0	0	1	0	0	0
Kidney									
Number examined		10	10	10	10	10	0	0	10
Vacuolation, tubular cell		8	5	0	0	0	0	0	0
minimal		5	5	0	0	0	0	0	0
mild		3	0	0	0	0	0	0	0
Regeneration, tubular		0	0	0	0	2	0	0	4
minimal		0	0	0	0	2	0	0	4
Urinary cast, hyaline		1	1	0	0	2	0	0	3
minimal		1	1	0	0	2	0	0	3
Cell infiltration		1	0	0	0	2	0	0	0
minimal		1	0	0	0	2	0	0	0
Liver									
Number examined		10	10	10	10	10	10	10	10
Necrosis, focal		0	0	0	0	1	0	0	0
minimal		0	0	0	0	1	0	0	0
Microgranuloma		1	3	0	1	4	3	5	5
minimal		1	3	0	1	4	3	5	5
Hypertrophy, hepatocyte, centrilobular		0	0	6	10	0	0	6	10
minimal		0	0	6	2	0	0	6	2
mild		0	0	0	8	0	0	0	8
Lung(bronchus)									
Number examined		10	0	0	10	10	0	0	10
Cell infiltration		0	0	0	0	1	0	0	0
minimal		0	0	0	0	1	0	0	0
Thickening, arterial wall		0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	1
Pituitary									
Number examined		10	0	0	10	10	0	0	10
Cyst		1	0	0	0	0	0	0	0
minimal		1	0	0	0	0	0	0	0
Spleen									
Number examined		10	0	0	10	10	0	0	10
Hematopoiesis, extramedullary		0	0	0	0	3	0	0	3
minimal		0	0	0	0	3	0	0	3
Stomach									
Number examined		10	0	0	10	10	0	0	10
Ectopic tissue		0	0	0	0	0	0	0	1
minimal		0	0	0	0	0	0	0	1
Thymus									
Number examined		10	0	0	10	10	0	0	10
Cyst		0	0	0	1	0	0	0	0
minimal		0	0	0	1	0	0	0	0
Thyroid									
Number examined		10	0	0	10	10	0	0	10
Dilatation, follicular		0	0	0	0	1	0	0	0
mild		0	0	0	0	1	0	0	0
Hyperplasia, C cell, focal		0	0	0	0	1	0	0	0
minimal		0	0	0	0	1	0	0	0
Urinary bladder									
Number examined		10	0	0	10	10	0	0	10
Cell infiltration		2	0	0	0	1	0	0	1
minimal		2	0	0	0	1	0	0	1

Toxicokinetics:

Text Table 5. Summary of TK parameters

Sex	Male (n=3)			Female (n=3)		
Dose (mg/kg/day)	100	300	500	100	300	500
NS-304						
T_{max} (h)						
Day 1	0.5	0.5	8	0.5	0.5	0.5
Week 13	0.5	0.5	2	0.5	0.5	0.5
C_{max} (ng/mL)						
Day 1	9660	26500	21100	12200	35900	45600
Week 13	16900	23200	27300	10800	24900	18800
AUC_{0-24h} (ng·h/mL)						
Day 1	15000	88400	295000	19500	115000	310000
Week 13	12200	52100	90000	10100	44400	74000
MRE-269						
T_{max} (h)						
Day 1	1	1	8	0.5	1	0.5
Week 13	0.5	0.5	2	0.5	0.5	2
C_{max} (ng/mL)						
Day 1	10000	19500	17500	8230	19100	17000
Week 13	11400	12400	20300	7150	13500	12400
AUC_{0-24h} (ng·h/mL)						
Day 1	23600	99200	250000	21400	98800	223000
Week 13	12400	51500	71500	9990	39800	58000

Value in the table (C_{max} and AUC_{0-24h}) indicates the mean value of 3 animals.
T_{max} indicates the time shown C_{max}.

Other:

Histopathology inventory

Study				
Species				
Adrenals				
Aorta				
Bone Marrow smear				
Bone (femur)				
Brain				
Cecum				
Cervix				
Colon				
Duodenum				
Epididymis				
Esophagus				
Eye				
Fallopian tube				
Gall bladder				
Gross lesions				
Harderian gland				

Heart				
Ileum				
Injection site				
Jejunum				
Kidneys				
Lachrymal gland				
Larynx				
Liver				
Lungs				
Lymph nodes, cervical				
Lymph nodes mandibular				
Lymph nodes, mesenteric				
Mammary Gland				
Nasal cavity				
Optic nerves				
Ovaries				
Pancreas				
Parathyroid				
Peripheral nerve				
Pharynx				
Pituitary				
Prostate				
Rectum				
Salivary gland				
Sciatic nerve				
Seminal vesicles				
Skeletal muscle				
Skin				
Spinal cord				
Spleen				
Sternum				
Stomach				
Testes				
Thymus				
Thyroid				
Tongue				
Trachea				
Urinary bladder				
Uterus				
Vagina				
Zymbal gland				

2.6.6.4 Genetic toxicology

Study title: Reverse mutation test of NS-304

Key findings: ACT-293987 and ACT-333679 were non-mutagenic under the conditions used in this Ames bacterial assay

Study no.: T-08.279, T-08.278

Volume #, and page #: eCTD
Conducting laboratory and location: Name of facility where the test was conducted: Toxicology Department, Research and Development Division, Nippon Shinyaku Co., Ltd.
Address:
14 Kisshoin-Nishinosho-Monguchi-cho, Minami-ku, Kyoto
Date of study initiation: Study initiation date: 24-Aug-2004
GLP compliance: yes
QA reports: yes ( ) no ( )
Drug, lot #, and % purity: 1.1 Compound code: MRE-269
1.2 Lot number: 9
1.3 Date received: 31-Aug-2004
1.4 Source and responsible person: H. Yamashita, Test Article Management Unit
1.5 Quantity received (b) (4) g
1.6 Characteristics[1)]
1.6.1 Description: A pale yellow crystalline powder, meeting the specified requirements
1.6.2 Identification (ultraviolet spectrophotometry): MRE-269 proved to meet the specified requirements.
1.6.3 Purity (liquid chromatography): The total area of the peaks (b) (4) other than the peak of MRE-269 was (b) (4) %, thus meeting the specified requirements (not more than (b) (4) %).
Study No.: TX-1298
1.6.4 Assay: The assay value was (b) (4) %, hence meeting the specified requirements (98.0 to
101.0%).

Methods

Strains/species/cell line:

Strain designation	Genetic characteristics
TA100	His⁻, uvrB⁻, rfa, R
TA1535	His⁻, uvrB⁻, rfa
TA98	His⁻, uvrB⁻, rfa, R
TA1537	His⁻, uvrB⁻, rfa
WP2*uvr*A	Trp⁻, uvrA⁻

His⁻: Histidine-requirement
Trp⁻: Tryptophan- requirement
uvrB⁻: Defective of DNA repair system (sensitive to ultraviolet rays)
uvrA⁻: Defective of DNA repair system (sensitive to ultraviolet rays)
rfa: Membrane mutation (sensitive to crystal violet)
R: Ampicillin-resistant

Doses used in definitive study: see tables below

Basis of dose selection: dosing study done

Negative controls: see tables below

Positive controls: see tables below

Incubation and sampling times: from the sponsor:

13 Test Procedure

13.1 Test method

The test was performed by the preincubation method with and without a metabolic activation system (S9 liver microsomal fraction from arochlor-treated rats).

13.2 Number of assay plates

In both the dose-finding assay and the main assay, the test was conducted with a negative control (DMSO), the test article and each positive control by treatment (incubation) with and without metabolic activation using duplicate assay plates per dose except the negative control for which triplicate plates were used.

13.3 Identification of plates

Each plate was marked with the study number, name of tester strain, presence or absence of S9, name of test article or control article, concentration (treatment concentration and stock solution number) of test article or control article, and intra-treatment identification number.

13.4 Treatment with test article or control article, and incubation

To each tube, 0.1 mL of the test article solution or control article solution, 0.5 mL of S9 mix, and 0.1 mL of the bacterial suspension were added, mixed, and agitated for 20 minutes at 37º_C. Two mL of the top agar was then added to the mixture and poured evenly over a minimum glucose agar plate, and the plate was observed for test article

precipitation. After the top agar hardened, the plates were incubated in a T.H. Type Digital Incubator Model HD-12-b (Hirasawa Works Co., Ltd.) at 37_C for about 48 hours.

13.5 Observation of revertant colonies

Plates were observed for inhibition of bacterial growth of the tester strain under the inverted microscope to check for antibacterial (cytotoxic) effect of the test article. They were then examined for the number of colonies formed by prototrophic revertants that emerged, and observed for test article precipitation. Colonies were counted with an automated colony counter (Bio-Multiscanner BMS-400; Toyo Sokki Co., Ltd.). Colony enumeration data were tallied using the Genotoxicity Study System MUTAPACK, of which protocol registration was made by the study director.

13.6 Sterility test

Top agar was added, 2 mL each, to 0.1 mL of the test article solution and to 0.5 mL of S9 mix, and these were poured evenly over nutrient agar plates. After the top agar layer hardened, the plates were incubated in the same manner as indicated in Section 13.4, and examined for bacterial contamination.

13.7 Interpretation of results

Plates were subjected to interpretation of results for mutagenic activity of the test article providing that the sterility test showed no bacterial contamination and that the numbers of revertant colonies on plates treated with the negative control and those on plates treated with positive control articles were within the limits (mean_3SD) of background data[10)].

The test was interpreted as positive when the number of revertant colonies (mean per plate) on plates treated with the test article was twice or more than that on negative control plates and when the increase was dose-dependent. No statistical processing of the data was performed.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): studies followed guidelines, had sufficient replicates, used typical criteria, and positive and negative controls behaved as expected.

Study outcome: Findings were interpreted as negative

Table 2 Reverse mutation test of MKE-269
(Main assay)

Compound	S9mix	Revertant colonies/plate				
		Base exchange type			Frameshift type	
(μg/plate)		TA100	TA1535	WP2 *uvrA*	TA98	TA1537
		79	6	29	18	8
		95	7	17	14	5
0	−	87 (87)	7 (7)	16 (21)	16 (16)	5 (6)
		91	15	17	11	6
78.13	−	89 (90)	7 (11)	17 (17)	16 (14)	5 (6)
		84	8	18	11	6
156.25	−	86 (85)	5 (7)	19 (19)	11 (11)	2 (4)
		94	5	25	17	7
312.5	−	95 (95)	5 (5)	17 (21)	17 (17)	2 (5)
		99	8	17	13	4
625	−	85 (92)	11 (10)	17 (17)	17 (15)	7 (6)
		83	8	11	6	2
1250	−	83 (83)	6 (7)	13 (12)	12 (9)	2 (2)
		71	4	24	11	3
2500	−	66 (69)	5 (5)	19 (22)	10 (11)	2 (3)
		29	11	17	10	2
5000	−	29 (29)*	10 (11)	19 (18)	6 (8)	4 (3)
		497	358	81	334	805
PC	−	568 (533)	331 (345)	86 (84)	335 (335)	1087 (946)
		111	8	22	33	14
		112	10	23	28	9
0	+	97 (107)	10 (9)	13 (19)	22 (28)	11 (11)
		99	6	21	25	5
78.13	+	98 (99)	8 (7)	24 (23)	26 (26)	4 (5)
		86	6	25	21	8
156.25	+	108 (97)	7 (7)	27 (26)	24 (23)	13 (11)
		104	6	31	21	16
312.5	+	107 (106)	12 (9)	31 (31)	20 (21)	10 (13)
		100	11	30	16	2
625	+	95 (98)	11 (11)	20 (25)	15 (16)	7 (5)
		80	6	21	17	7
1250	+	88 (84)	6 (6)	20 (21)	28 (23)	10 (9)
		91	6	12	11	7
2500	+	104 (98)	5 (6)	16 (14)	12 (12)	5 (6)
		41	5	11	15	6
5000 †	+	33 (37)*	11 (8)	19 (15)	13 (14)	6 (6)
		1140	265	611	305	290
PC	+	1226 (1183)	278 (272)	445 (528)	324 (315)	331 (311)

Vehicle control : Dimethyl sulfoxide
PC(positive controls) (μg/plate) :

	Without S9mix	With S9mix
TA100	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (0.01)	2-aminoanthracene (1)
TA1535	Sodium azide (0.5)	2-aminoanthracene (2)
WP2 *uvrA*	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (0.01)	2-aminoanthracene (10)
TA98	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (0.1)	2-aminoanthracene (0.5)
TA1537	9-aminoacridine (80)	2-aminoanthracene (2)

* : Toxic effect was observed.
† : Precipitates of test chemical were observed.
The number in parentheses means the average of plates.

Table 2 Reverse mutation test of NS-304
(Main assay)

Compound (μg/plate)	S9mix	Revertant colonies/plate: Base exchange type TA100	TA1535	WP2 uvrA	Frameshift type TA98	TA1537
0	−	96 83 112 (97)	11 10 9 (10)	30 30 30 (30)	20 19 19 (19)	8 10 11 (10)
78.13	−	93 82 (88)	11 11 (11)	28 24 (26)	13 10 (12)	8 10 (9)
156.25	−	78 91 (85)	8 7 (8)	23 23 (23)	11 10 (11)	10 10 (10)
312.5	−	91 106 (99)	7 6 (7)	28 33 (31)	12 12 (12)	6 7 (7)
625	−	100 104 (102)	6 6 (6)	30 25 (28)	18 20 (19)	5 7 (6)
1250	−	87 93 (90)	6 4 (5)	32 33 (33)	18 17 (18)	7 10 (9)
2500	−	78 88 (83)	6 7 (7)	25 27 (26)	17 22 (20)	9 10 (10)
5000	−	40 40 (40)*	7 7 (7)	27 27 (27)	20 20 (20)	9 8 (9)
PC	−	544 560 (552)	298 300 (299)	95 96 (96)	307 345 (326)	941 1020 (981)
0	+	108 122 110 (113)	11 8 8 (9)	29 30 28 (29)	25 27 26 (26)	12 14 14 (13)
78.13	+	122 131 (127)	12 12 (12)	30 28 (29)	16 25 (21)	13 13 (13)
156.25	+	123 116 (120)	11 11 (11)	34 32 (33)	29 23 (26)	12 11 (12)
312.5	+	112 120 (116)	13 7 (10)	31 33 (32)	23 18 (21)	18 19 (19)
625	+	105 114 (110)	8 8 (8)	32 31 (32)	13 12 (13)	12 11 (12)
1250	+	91 80 (86)	10 11 (11)	32 30 (31)	13 16 (15)	11 11 (11)
2500	+	80 86 (83)	10 10 (10)	32 34 (33)	14 16 (15)	8 11 (10)
5000	+	96 85 (91)	10 11 (11)	28 28 (28)	16 16 (16)	12 11 (12)
PC	+	1271 1101 (1186)	250 275 (263)	582 505 (544)	327 341 (334)	291 250 (271)

Vehicle control : Dimethyl sulfoxide
PC(positive controls) (μg/plate) :

	Without S9mix	With S9mix
TA100	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (0.01)	2-aminoanthracene (1)
TA1535	Sodium azide (0.5)	2-aminoanthracene (2)
WP2 uvrA	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (0.01)	2-aminoanthracene (10)
TA98	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (0.1)	2-aminoanthracene (0.5)
TA1537	9-aminoacridine (80)	2-aminoanthracene (2)

* : Toxic effect was observed.
The number in parentheses means the average of plates.

Study title: Chromosome Aberration test for MRE-269 and MRE-304

Key findings: ACT-269987 in the absence of S9 was clastogenic at a high dose of 250 mcg/mL, but negative in the presence of S9, ACT-333697 was negative for chromosomal aberrations in Chinese hamster lung cells.

Study no.: T-08.280 and T-08.281

Volume #, and page #: eCTD
Conducting laboratory and location:
Name of facility where the test was conducted:
Toxicology Department, Research and Development Division, Nippon Shinyaku Co., Ltd.
Address:
14 Kisshoin-Nishinosho-Monguchi-cho, Minami-ku, Kyoto
Date of study initiation: Study initiation date: 16-Sep-2004
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:
1.1 Compound code: MRE-269
1.2 Lot number: 9
1.3 Date received: 30-Sep-2004
1.4 Quantity received: 8.0 g
1.5 Source and responsible person: H. Yamashita, Test Article Management Unit
1.6 Characteristics[1)]
1.6.1 Description: A pale yellow crystalline powder, meeting the specified requirements
1.6.2 Identification by ultraviolet spectrophotometry: MRE-269 proved to meet the specified requirements.
1.6.3 Purity by liquid chromatography: The total area of the peaks (of related substances) other than the peak of MRE-269 was (b) (4) %, thus meeting the specified requirements (not more than (b) (4) %).
1.6.4 Assay: The assay value was (b) (4) %, hence meeting the specified requirements (98.0 to 101.0%)

Methods

Strains/species/cell line: A cell line derived from newborn Chinese hamster lung (CHL/IU) was used.

Doses used in definitive study:

Basis of dose selection: cytotoxicity and solubility

Negative controls: DMSO

Positive controls: Mitomycin C (-S9), Benzo(a)pyrene (+S9)

Incubation and sampling times: see below tables

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): study used typical criteria for positive and negative results, counting methods and a typical number of replicates.

Study outcome:

Table 1 Chromosome aberration test of NS-304 : Short Time Treatment Method

Substance	Dose (μg/mL)	Treatment time (hr)	-	Recovery time (hr)	S9 mix	Number of analyzed cells	Number of structural aberrant cells							Number of polyploid cells (%)	Cell proliferation rate (%)
							gap	ctb	cte	csb	cse	frg	Total (%) -gap		
DMSO	-	6	-	18	-	200	0	2	0	0	0	0	2 (1.0)	0 (0.0)	100.0
NS-304	31.25	6	-	18	-	200	0	1	1	0	0	0	2 (1.0)	0 (0.0)	100.8
	62.5	6	-	18	-	200	0	1	1	0	0	0	2 (1.0)	0 (0.0)	91.7
	125	6	-	18	-	200	0	0	0	0	0	0	0 (0.0)	1 (0.5)	72.0
	250	6	-	18	-	131	1	19	45	0	0	0	53 (40.5) **	0 (0.0)	54.7
	500	6	-	18	-	0	-	-	-	-	-	-	- (-)	- (-)	8.1
	1000 P	6	-	18	-	0	-	-	-	-	-	-	- (-)	- (-)	2.8
MMC	0.1	6	-	18	-	200	1	58	106	0	0	0	123 (61.5) **	0 (0.0)	94.7
DMSO	-	6	-	18	+	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	100.0
NS-304	25	6	-	18	+	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	84.8
	50	6	-	18	+	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	80.8
	100	6	-	18	+	200	0	0	2	0	0	0	2 (1.0)	0 (0.0)	60.2
	200	6	-	18	+	200	0	1	3	0	0	0	3 (1.5)	0 (0.0)	48.3
	400	6	-	18	+	0	-	-	-	-	-	-	- (-)	- (-)	8.0
	800 P	6	-	18	+	0	-	-	-	-	-	-	- (-)	- (-)	2.0
B(a)P	20	6	-	18	+	200	0	30	106	0	1	0	116 (58.0) **	0 (0.0)	62.4

ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange; frg, fragmentation

MMC, mitomycinC; B(a)P, Benzo(a)pyrene

* Significantly different from negative control ($p<0.05$)

** Significantly different from negative control ($p<0.01$)

P, Precipitation

Table 2 Chromosome aberration test of NS-304 : Long Time Treatment Method

Substance	Dose (μg/mL)	Treatment time (hr)	-	Recovery time (hr)	Number of analyzed cells	Number of structural aberrant cells							Number of polyploid cells (%)	Cell proliferation rate (%)
						gap	ctb	cte	csb	cse	frg	Total (%) -gap		
DMSO	-	24	-	0	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	100.0
NS-304	15	24	-	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	103.4
	30	24	-	0	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	105.3
	60	24	-	0	200	0	1	1	0	0	0	2 (1.0)	0 (0.0)	88.4
	120	24	-	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	64.1
	240	24	-	0	0	-	-	-	-	-	-	- (-)	- (-)	39.1
	480	24	-	0	0	-	-	-	-	-	-	- (-)	- (-)	31.2
	960 P	24	-	0	0	-	-	-	-	-	-	- (-)	- (-)	0.9
MMC	0.05	24	-	0	200	0	33	62	0	0	0	83 (41.5) **	0 (0.0)	83.6
DMSO	-	48	-	0	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	100.0
NS-304	6.25	48	-	0	200	0	1	1	0	0	0	2 (1.0)	0 (0.0)	100.9
	12.5	48	-	0	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	99.7
	25	48	-	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	101.2
	50	48	-	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	83.7
	100	48	-	0	200	0	2	4	0	0	0	6 (3.0)	0 (0.0)	39.1
	200	48	-	0	0	-	-	-	-	-	-	- (-)	- (-)	20.0
	400	48	-	0	0	-	-	-	-	-	-	- (-)	- (-)	18.1
MMC	0.05	48	-	0	200	0	45	111	0	0	0	123 (61.5) **	0 (0.0)	74.5

ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange; frg, fragmentation

MMC, mitomycinC

* Significantly different from negative control ($p<0.05$)

** Significantly different from negative control ($p<0.01$)

P, Precipitation

Table 1 Chromosome aberration test of MRE-269 : Short Time Treatment Method

Substance	Dose (μg/mL)	Treatment time (hr)	–	Recovery time (hr)	S9 mix	Number of analyzed cells	Number of structural aberrant cells							Number of polyploid cells (%)	Cell proliferation rate (%)
							gap	ctb	cte	csb	cse	frg	Total (%) –gap		
DMSO	–	6	–	18	–	200	0	3	1	0	0	0	4 (2.0)	0 (0.0)	100.0
MRE-269	36.25	6	–	18	–	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	98.8
	72.5	6	–	18	–	200	0	2	1	0	0	0	3 (1.5)	0 (0.0)	109.0
	145	6	–	18	–	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	94.9
	290 P	6	–	18	–	200	0	4	4	0	0	0	7 (3.5)	0 (0.0)	47.9
	580 P	6	–	18	–	0	–	–	–	–	–	–	– (–)	– (–)	6.5
	1160 P	6	–	18	–	0	–	–	–	–	–	–	– (–)	– (–)	7.6
MMC	0.1	6	–	18	–	200	1	48	96	0	0	0	112 (56.0) **	0 (0.0)	88.9
DMSO	–	6	–	18	+	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	100.0
MRE-269	17.5	6	–	18	+	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	101.2
	35	6	–	18	+	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	92.5
	70	6	–	18	+	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	86.7
	140	6	–	18	+	200	0	1	1	0	0	0	2 (1.0)	0 (0.0)	80.6
	280	6	–	18	+	22	–	–	–	–	–	–	– (–)	– (–)	24.2
	560 P	6	–	18	+	0	–	–	–	–	–	–	– (–)	– (–)	7.5
B(a)P	20	6	–	18	+	200	0	21	69	0	1	0	75 (37.5) **	0 (0.0)	73.5

ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange; frg, fragmentation
MMC, mitomycinC; B(a)P, Benzo(a)pyrene
* Significantly different from negative control ($p<0.05$)
** Significantly different from negative control ($p<0.01$)
P, Precipitation

Table 2 Chromosome aberration test of MRE-269 : Long Time Treatment Method

Substance	Dose (μg/mL)	Treatment time (hr)	–	Recovery time (hr)	Number of analyzed cells	Number of structural aberrant cells							Number of polyploid cells (%)	Cell proliferation rate (%)
						gap	ctb	cte	csb	cse	frg	Total (%) –gap		
DMSO	–	24	–	0	200	0	0	0	0	0	0	0 (0.0)	1 (0.5)	100.0
MRE-269	22.5	24	–	0	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	90.3
	45	24	–	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	93.3
	90	24	–	0	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	74.6
	180	24	–	0	200	0	2	0	0	0	0	2 (1.0)	0 (0.0)	54.2
	360 P	24	–	0	0	–	–	–	–	–	–	– (–)	– (–)	32.4
	720 P	24	–	0	0	–	–	–	–	–	–	– (–)	– (–)	11.7
MMC	0.05	24	–	0	200	0	32	90	0	0	0	104 (52.0) **	0 (0.0)	77.1
DMSO	–	48	–	0	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	100.0
MRE-269	13.75	48	–	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	104.4
	27.5	48	–	0	200	0	2	0	0	0	0	2 (1.0)	0 (0.0)	98.7
	55	48	–	0	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	93.3
	110	48	–	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	48.0
	220	48	–	0	0	–	–	–	–	–	–	– (–)	– (–)	21.2
	440 P	48	–	0	0	–	–	–	–	–	–	– (–)	– (–)	15.3
MMC	0.05	48	–	0	200	1	59	140	0	0	0	148 (74.0) **	0 (0.0)	67.7

ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange; frg, fragmentation
MMC, mitomycinC
* Significantly different from negative control ($p<0.05$)
** Significantly different from negative control ($p<0.01$)
P, Precipitation

Study title: Micronucleus test of NS-304 (MRE-304) with mouse bone marrow cells

Key findings: No indications of *in vivo* clastogenic activity by ACT-293987 or ACT-333679 were seen in the assay.

Study no.: T-08.282

Volume #, and page #: eCTD

Conducting laboratory and location:
Date of study initiation:
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Methods

Strains/species/cell line: male S1c:ddY mice

Doses used in definitive study: 125, 250, and 500 mg/kg/day

Basis of dose selection: previous dose and toxicity studies

Negative controls: 0.5% methylcellulose

Positive controls: mitomycin C at 2 mg/kg

Incubation and sampling times: from the sponsor:

5. Preparation and Administration of the Test Article and Negative Control
5.1 Route of Administration and Rationale
5.1.1 Route of Administration: Oral
5.1.2 Rationale for Selection: Oral route of administration was selected according to the envisaged administration route for humans and the Guideline for Genotoxicity Studies.
5.2 Dosing Volume: 10 mL/kg. The animals were weighed immediately before administration on the first day of administrating using an LP2200S electronic balance (Sartorius K.K.) and a computer system for genotoxicity studies MUTAPACK. Based on the body weight measured, the dosing volume for each animal was determined at 10
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mL/kg of body weight. The dosing volume was calculated to 2 decimal places by rounding up the third decimal place.
5.3 Method of Administration and Rationale
5.3.1 Method of Administration: The test solution was administered to mice by oral gavage using a sterile disposable syringe (Terumo Corporation) attached with a metallic oral sonde.
5.3.2 Rationale for Selection: The method was selected because it is generally used for oral administration of drugs to mice, and, as documented by Sponsor, it is orally absorbed, and metabolized to active drug in the mouse .
5.4 Number of Administration and Rationale
5.4.1 Number of Administration: Once daily for two days.
5.4.2 Rationale for Selection: The frequency and number of administration was decided according to the Guideline for Genotoxicity Studies.
5.5 Time of Administration

5.5.1 Dose Finding Study: 9:30 to 9:55 a.m. on January 25, 2005 and 9:36 to 9:56 a.m. on January 26, 2005
5.5.2 Main Study: 9:43 to 10:23 a.m. on February 15, 2005 and 9:44 to 10:25 a.m. on February 16, 2005
5.5.3 TK Measurement Study: 10:00 a.m. to 4:20 p.m. on March 1, 2005 and 10:01 a.m. to 4:20 p.m. on March 2, 2005
5.6 Doses and Rationale for Selection
5.6.1 Dose Finding Study
Doses: 62.5, 125, 250, 500, 1000 and 2000 mg/kg
The highest dose was set at 2000 mg/kg according to the Guideline for Genotoxicity Studies. Totally 6 doses were set using a common ratio of 1/2.
5.6.2 Main Study
Doses: 62.5, 125, 250, 500 and 1000 mg/kg
More than half the animals died at 1000 mg/kg in the dose finding study (See Results).
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Results

Study validity: Study followed OECD approved methods, criteria, and replicates.

Study outcome: Study was considered to be negative for clastogenicity.

Table 4 Micronucleus test of NS-304 [male mice] Study No. TX-1321

Compound	Dose (mg/kg)	Number of animals	Frequency of MNPCE [%] (Mean ± S.D.)	Range of MNPCE/2000PCE (Min - Max)	Ratio of PCE [%] (Mean ± S.D.)
0.5%MC	0	6	0.26 ± 0.36	4 - 7	52.3 ± 1.9
NS-304	125	6	0.24 ± 0.37	3 - 7	51.8 ± 2.2
	250	6	0.25 ± 0.37	3 - 7	51.2 ± 1.9
	500	5	0.19 ± 0.39	1 - 6	48.6 ± 4.9
MMC	2.0	6	5.27 ± 2.35 **	56 - 155	51.4 ± 1.4

Kasten-Baum Significant difference from control *:p<0.05, **:p<0.01
Student t-test Significant difference from control #:p<0.05, ##:p<0.01

2.6.6.5 Carcinogenicity not done

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: study of fertility and early embryonic development to implantation in rats treated orally with ns-304.

Key study findings: The high dose group females experienced a brief weight drop on day 4, but then recovered and gained weight at the same rate as the other groups.

The high dose group (60 mg/kg) was delayed in time to copulation, and all the treated animals had reduced litter size, however, the reduction was not statistically significant.and there was no effect on number of corpora lutea, or pre or post-implantation loss.
Study no.: R-950
Volume #, and page #: eCTD
Conducting laboratory and location:
(b) (4)

Date of study initiation: October 3, 2006
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Name:	NS-304 (MRE-304)
Lot number:	20
Assay:	100.3%

Methods

Test group	Dose level (mg/kg)	Dose concentration (mg/mL)	Dose volume (mL/kg)	Sex	Main group		Satellite group	
					Number of animals	Animal number	Number of animals	Animal number
Control	0	0	5	Male Female	20 20	1001-1020 1101-1120	4 4	1021-1024 1121-1124
Low dose	6	1.2	5	Male Female	20 20	2001-2020 2101-2120	8 8	2021-2028 2121-2128
Middle dose	20	4	5	Male Female	20 20	3001-3020 3101-3120	8 8	3021-3028 3121-3128
High dose	60	12	5	Male Female	20 20	4001-4020 4101-4120	8 8	4021-4028 4121-4128

Doses:
Species/strain: Sprague-Dawley rats
Number/sex/group:
Route, formulation, volume, and infusion rate: oral gavage,
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality: none

Clinical signs: At all doses, flushing occurred in the ears and extremities.

Body weight:

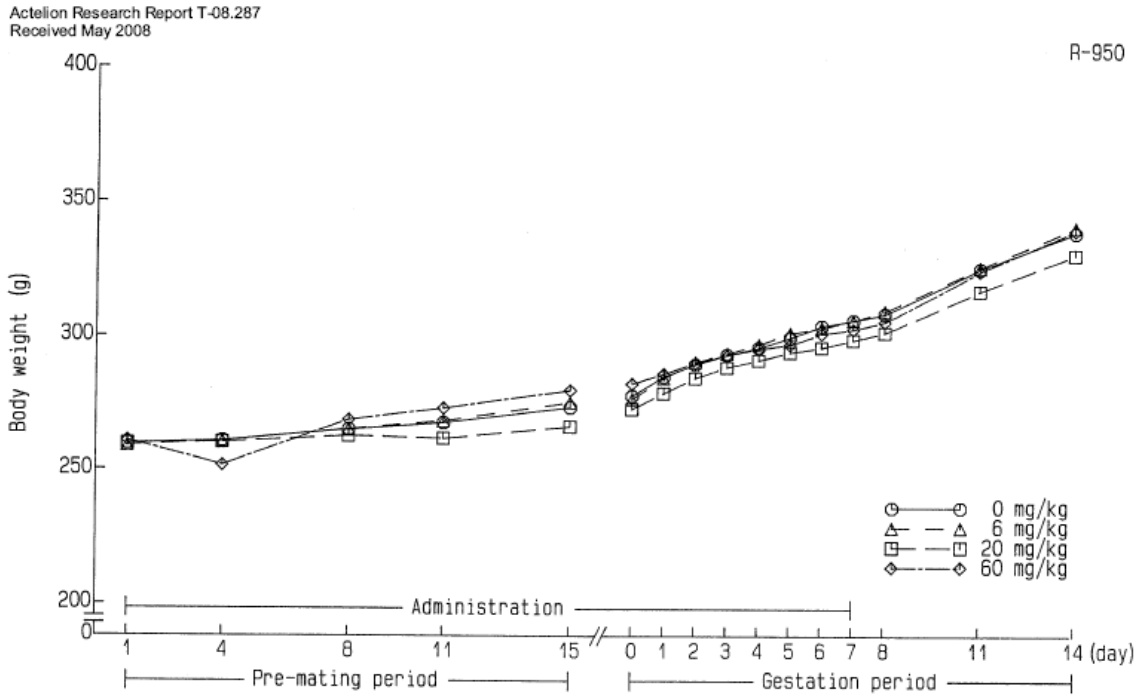

Fig.3 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Body weight of female rats

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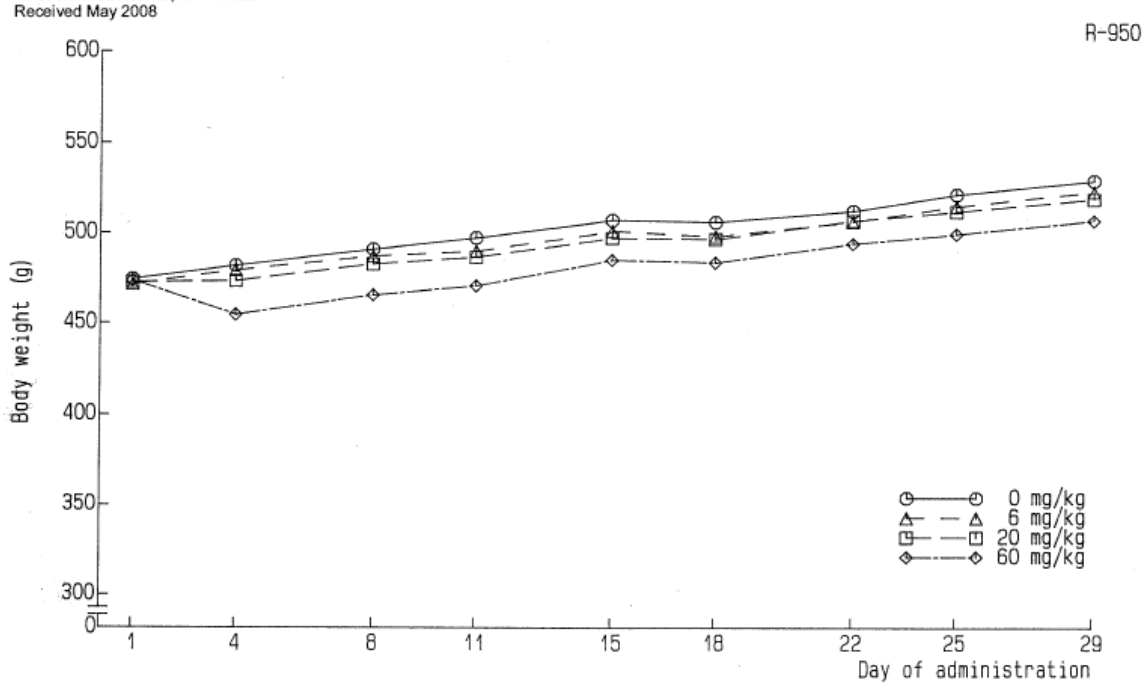

Fig.1 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Body weight of male rats

Food consumption:

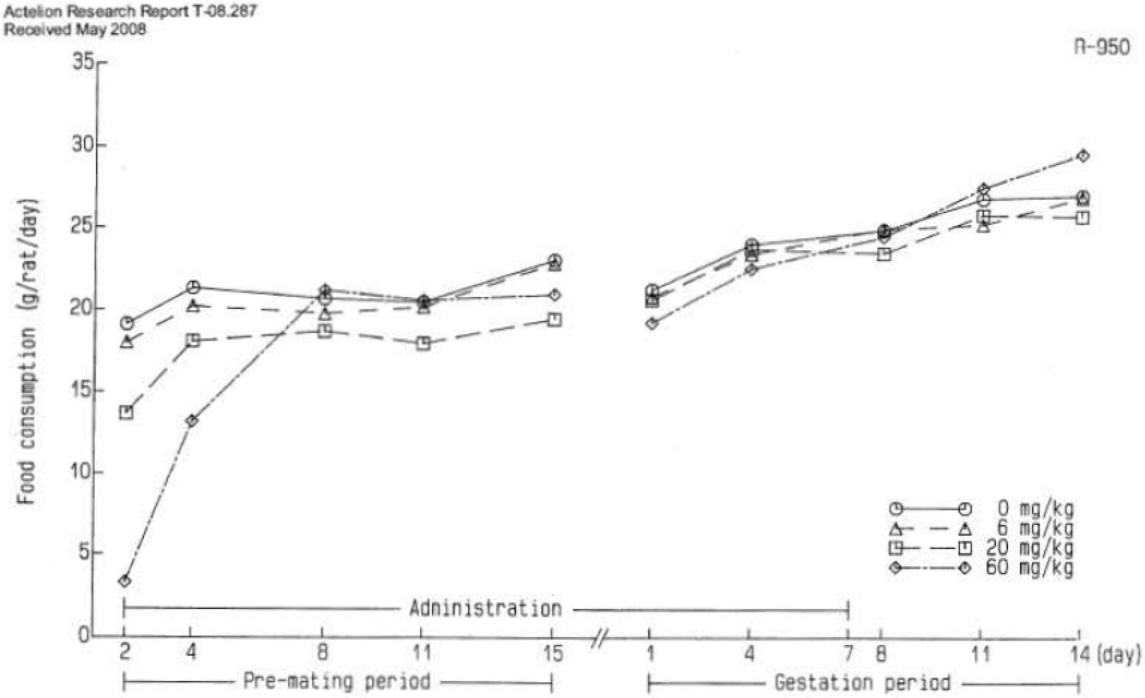

Fig.4 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Food consumption of female rats

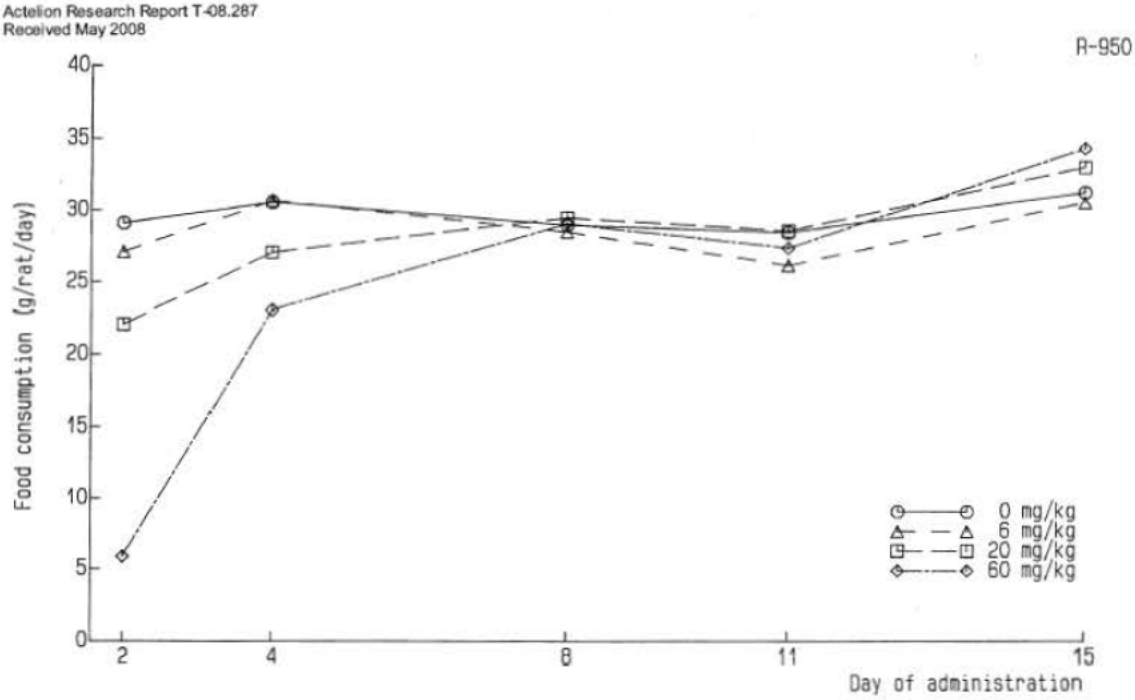

Fig.2 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Food consumption of male rats

Toxicokinetics:

Table 15-1 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Plasma concentration of NS-304 (Day 1)

Male

Dose mg/kg	Plasma concentration (μg/mL) Animal number	0.5h	Animal number	1h	Animal number	2h	Animal number	4h	Animal number	8h	Animal number	24h	Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
0			1021	N.D.											
			1022	N.D.											
			1023	N.D.											
	Mean			0											
	S.D.														
6	2021	N.D.	2024	N.D.	2021	N.D.	2024	N.D.	2021	N.D.	2024	N.D.			
	2022	0.118	2025	0.0605	2022	N.D.	2025	N.D.	2022	N.D.	2025	N.D.			
	2023	0.111	2026	0.0976	2023	N.D.	2026	N.D.	2023	N.D.	2026	N.D.			
	Mean	0.0763		0.0527		0		0		0		0	0.0763	0.5	0.0777
	S.D.	0.0662		0.0493											
20	3021	0.123	3024	0.0739	3021	N.D.	3024	N.D.	3021	N.D	3024	N.D.			
	3022	0.177	3025	0.129	3022	N.D.	3025	N.D.	3022	N.D	3025	N.D.			
	3023	0.223	3026	0.234	3023	N.D.	3026	N.D.	3023	N.D	3026	N.D.			
	Mean	0.174		0.146		0		0		0		0	0.174	0.5	0.197
	S.D.	0.050		0.081											
60	4021	0.520	4024	1.70	4021	N.D.	4024	0.704	4021	N.D	4024	0.110			
	4022	0.940	4025	1.50	4022	0.0817	4025	0.490	4022	N.D	4025	N.D.			
	4023	4.41	4026	1.96	4023	0.329	4026	1.16	4023	N.D	4026	N.D.			
	Mean	1.96		1.72		0.137		0.785		0		0.0367	1.96	0.5	5.12
	S.D.	2.14		0.23		0.171		0.342				0.0635			

N.D. : Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean± S.D.

Table 15-2 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Plasma concentration of NS-304 (Day 15)

Male

Dose mg/kg	Plasma concentration (μg/mL) Animal number	Pre	Animal number	0.5h	Animal number	1h	Animal number	2h	Animal number	4h	Animal number	8h	Animal number	24h	Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
0					1021	N.D.											
					1022	N.D.											
					1023	N.D.											
	Mean					0											
	S.D.																
6	2024	N.D.	2021	0.243	2024	0.0573	2021	N.D.	2024	N.D.	2021	N.D.	2024	N.D.			
	2025	N.D.	2022	0.176	2025	0.0867	2022	N.D.	2025	N.D.	2022	N.D.	2025	N.D.			
	2026	N.D.	2023	0.206	2026	0.0596	2023	N.D.	2026	N.D.	2023	N.D.	2026	N.D.			
	Mean	0		0.208		0.0745		0		0		0		0	0.208	0.5	0.160
	S.D.			0.034		0.0106											
20	3024	N.D.	3021	0.423	3024	0.191	3021	N.D.	3024	0.0555	3021	N.D.	3024	N.D.			
	3025	N.D.	3022	0.530	3025	0.172	3022	N.D.	3025	N.D.	3022	0.0728	3025	N.D.			
	3026	N.D.	3023	0.676	3026	0.191	3023	N.D.	3026	N.D.	3023	N.D.	3026	N.D.			
	Mean	0		0.543		0.185		0		0.0185		0.0243		0	0.543	0.5	0.709
	S.D.			0.127		0.011				0.0320		0.0420					
60	4024	N.D.	4021	2.31	4024	1.19	4021	0.0862	4024	0.0704	4021	0.108	4024	N.D.			
	4025	N.D.	4022	2.19	4025	1.74	4022	0.216	4025	0.279	4022	0.0985	4025	N.D.			
	4026	N.D.	4023	1.41	4026	1.62	4023	0.459	4026	0.121	4023	0.190	4026	N.D.			
	Mean	0		1.97		1.52		0.254		0.157		0.132		0	1.97	0.5	4.30
	S.D.			0.49		0.29		0.189		0.109		0.050					

N.D. : Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean± S.D.

Table 15-3 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Plasma concentration of NS-304 (Day 1)

Female

Dose mg/kg	Plasma concentration (μg/mL)												Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
	Animal number	0.5h	Animal number	1h	Animal number	2h	Animal number	4h	Animal number	8h	Animal number	24h			
0			1121 1122 1123	N.D. N.D. N.D.											
	Mean S.D.			0											
6	2121 2122 2123	0.221 0.139 0.204	2124 2125 2126	N.D. N.D. N.D.	2121 2122 2123	N.D. N.D. N.D.	2124 2125 2126	N.D. N.D. N.D.	2121 2122 2123	N.D N.D N.D	2124 2125 2126	N.D. N.D. N.D.			
	Mean S.D.	0.188 0.043		0		0		0		0		0	0.188	0.5	0.0940
20	3121 3122 3123	0.236 0.226 0.290	3124 3125 3126	0.584 0.262 0.759	3121 3122 3123	0.0647 N.D. N.D.	3124 3125 3126	0.0617 N.D. N.D.	3121 3122 3123	N.D. N.D. N.D.	3124 3125 3126	N.D. N.D. N.D.			
	Mean S.D.	0.251 0.034		0.535 0.252		0.0216 0.0374		0.0206 0.0356		0		0	0.535	1.0	0.621
60	4121 4122 4123	6.24 5.25 7.66	4124 4125 4126	2.93 4.55 9.92	4121 4122 4123	2.20 1.52 1.31	4124 4125 4126	1.10 1.66 2.51	4121 4122 4123	0.225 0.12[illegible] 0.194	4124 4125 4126	0.0717 N.D. 0.0921			
	Mean S.D.	6.38 1.21		5.80 3.66		1.68 0.47		1.76 0.71		0.180 0.053		0.0546 0.0484	6.38	0.5	17.6

N.D. : Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean ± S.D.

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Table 15-4 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Plasma concentration of NS-304 (Day 15)

Female

Dose mg/kg	Plasma concentration (μg/mL)														Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
	Animal number	Pre	Animal number	0.5h	Animal number	1h	Animal number	2h	Animal number	4h	Animal number	8h	Animal number	24h			
0					1121 1122 1123	N.D. N.D. N.D.											
	Mean S.D.					0											
6	2124 2125 2126	N.D. N.D. N.D.	2121 2122 2123	0.252 0.241 0.148	2124 2125 2126	N.D. N.D. N.D.	2121 2122 2123	N.D. N.D. N.D.	2124 2125 2126	N.D. N.D. N.D.	2121 2122 2123	N.D. N.D. N.D.	2124 2125 2126	N.D. N.D. N.D.			
	Mean S.D.	0		0.214 0.057		0		0		0		0		0	0.214	0.5	0.107
20	3124 3125 3126	N.D. N.D. N.D.	3121 3122 3123	0.124 0.953 0.645	3124 3125 3126	0.321 0.0888 0.267	3121 3122 3123	0.0902 N.D. N.D.	3124 3125 3126	N.D. N.D. N.D.	3121 3122 3123	N.D. N.D. N.D.	3124 3125 3126	N.D. N.D. N.D.			
	Mean S.D.	0		0.574 0.419		0.226 0.122		0.0301 0.0521		0		0		0	0.574	0.5	0.502
60	4124 4125 4126	N.D. N.D. 0.124	4121 4122 4123	0.543 1.20 2.21	4124 4125 4126	3.04 0.652 0.396	4121 4122 4123	0.136 0.0524 0.534	4124 4125 4126	N.D. 0.156 0.235	4121 4122 4123	0.0731 N.D. 0.0592	4124 4125 4126	N.D. N.D. N.D.			
	Mean S.D.	0.0413 0.0716		1.32 0.84		1.36 1.46		0.241 0.257		0.130 0.120		0.0441 0.0388		0	1.36	1.0	2.88

N.D. : Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean ± S.D.

Necropsy:

Table 12 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Gross pathological findings in female rats

Dose (mg/kg)	0	6	20	60
No. of animals examined	20	20	20	20
No. of animals with abnormal findings	0	0	0	0

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Table 13 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Mating and fertility of animals

Dose mg/kg	No. of males	No. of females	Days until copulation Mean±S.D.	Copulation index (%) a)	Fertility index (%) b)
0	20	20	2.3±1.1	20/20(100.0)	20/20(100.0)
6	20	20	2.0±0.9	20/20(100.0)	19/20(95.0)
20	20	20	2.7±1.5	20/20(100.0)	19/20(95.0)
60	20	20	3.3±1.4*D	20/20(100.0)	20/20(100.0)

a): (No. of copulated animals / No. of mated animals) X 100
b): (No. of pregnant animals / No. of copulated animals) X 100
*: p<0.05 (Significant difference from control group)
D: Dunnett's test

Table 14 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Findings at examination at the middle of gestation in dams

Dose mg/kg	No. of dams		No. of corpora lutea	No. of implan-tations	Implan-tation index % a)	No. of dead embryos (%)b)	No. of live embryos
0	20	Total	326	300		15	285
		Mean	16.3	15.0	93.0	(5.4)	14.3
		S.D.	2.0	2.1	13.3	(8.0)	2.6
6	19	Total	304	287		23	264
		Mean	16.0	15.1	94.0	(7.5)	13.9
		S.D.	3.3	3.6	11.1	(7.9)	3.4
20	19	Total	315	290		21	269
		Mean	16.6	15.3	93.2	(7.4)	14.2
		S.D.	3.2	1.9	8.7	(7.1)	2.2
60	20	Total	329	290		23	267
		Mean	16.5	14.5	88.2	(7.9)	13.4
		S.D.	2.4	3.6	19.2	(9.1)	3.7

a): (No. of implantations / No. of corpora lutea) X 100
b): (No. of dead embryos / No. of implantations) X 100
No significant difference in any treated groups from control group.

Embryofetal development

Study title: Study for effects on embryo-fetal development in rats treated orally with NS-304

Key study findings: The high dose group (20 mg/kg) experienced slightly, but statistically significantly, decreased birth weights of the fetuses.
Study no.: R-951
Volume #, and page #: eCTD
Conducting laboratory and location:
(b) (4)

Date of study initiation: November 14, 2006
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Name:	NS-304 (MRE-304)
Lot number:	20
Assay:	100.3%

Methods

Doses:
Species/strain: Sprague-Dawley rats
Number/sex/group:

Test group	Dose level (mg/kg)	Dose concentration (mg/mL)	Dose volume (mL/kg)	Main group		Satellite group	
				Number of copulated animals*	Animal number	Number of copulated animals*	Animal number
Control	0	0	5	20 (20)	1101-1120	4 (4)	1121-1124
Low dose	2	0.4	5	20 (20)	2101-2120	8 (8)	2121-2128
Middle dose	6	1.2	5	20 (20)	3101-3120	8 (8)	3121-3128
High dose	20	4	5	20 (20)	4101-4120	8 (8)	4121-4128

*: The number between the parentheses indicates the number of pregnant animals.

Route, formulation, volume, and infusion rate: oral gavage
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality (dams): none

Clinical signs (dams): flushing of ears and extremities, sporadic flaccidity in the high dose group (20 mg/kg)

Body weight (dams): minor reductions in body weight in the high dose dams (20 mg/kg)

Food consumption (dams): food consumption reduced for one day in the 6 mg/kg group, for approximately 4 days in the 20 mg/kg group.

Toxicokinetics:

Table 9-1 Study for effects on embryo-fetal development in rats treated orally with NS-304
Plasma concentration of NS-304 (Day 1)

Dose mg/kg	Plasma concentration (μg/mL)												Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
	Animal number	0.5h	Animal number	1h	Animal number	2h	Animal number	4h	Animal number	8h	Animal number	24h			
0			1121	N.D.											
			1122	N.D.											
			1123	N.D.											
	Mean			0											
	S.D.														
2	2121	N.D.	2125	N.D.	2121	N.D.	2125	N.D.	2121	N.D.	2125	N.D.			
	2122	N.D.	2126	N.D.	2122	N.D.	2126	N.D.	2122	N.D.	2126	N.D.			
	2123	0.0527	2127	N.D.	2123	N.D.	2127	N.D.	2123	N.D.	2127	N.D.			
	Mean	0.0176		0		0		0		0		0	0.0176	0.5	0.00880
	S.D.	0.0304													
6	3121	0.238	3125	0.0517	3121	N.D.	3125	N.D.	3121	N.D.	3125	N.D.			
	3122	0.191	3126	N.D.	3122	N.D.	3126	N.D.	3123	N.D.	3126	N.D.			
	3123	0.143	3127	N.D.	3123	N.D.	3127	N.D.	3124	N.D.	3127	N.D.			
	Mean	0.191		0.0172		0		0		0		0	0.191	0.5	0.108
	S.D.	0.048		0.0298											
20	4121	1.19	4125	0.458	4121	0.0576	4125	0.0738	4121	N.D.	4125	N.D.			
	4122	1.22	4126	0.418	4122	0.0527	4126	N.D.	4122	N.D.	4126	N.D.			
	4123	1.68	4127	0.587	4123	0.0762	4127	0.0520	4123	N.D.	4127	N.D.			
	Mean	1.36		0.488		0.0622		0.0419		0		0	1.36	0.5	1.27
	S.D.	0.27		0.088		0.0124		0.0378							

N.D. : Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean±S.D.

Table 9-2 Study for effects on embryo-fetal development in rats treated orally with NS-304
Plasma concentration of NS-304 (Day 11)

Dose mg/kg	Plasma concentration (μg/mL)														Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
	Animal number	Pre	Animal number	0.5h	Animal number	1h	Animal number	2h	Animal number	4h	Animal number	8h	Animal number	24h			
0					1121	N.D.											
					1122	N.D.											
					1123	N.D.											
	Mean					0											
	S.D.																
2	2125	N.D.	2121	0.0914	2125	N.D.	2121	N.D.	2125	N.D.	2121	N.D.	2125	N.D.			
	2126	N.D.	2122	0.0707	2126	N.D.	2122	N.D.	2126	N.D.	2122	N.D.	2126	N.D.			
	2127	N.D.	2123	0.0814	2127	N.D.	2123	N.D.	2127	N.D.	2123	N.D.	2127	N.D.			
	Mean	0		0.0812		0		0		0		0		0	0.0812	0.5	0.0406
	S.D.			0.0104													
6	3125	N.D.	3121	0.193	3125	0.0812	3121	N.D.	3125	N.D.	3121	N.D.	3125	N.D.			
	3126	N.D.	3122	0.209	3126	0.0693	3122	N.D.	3126	N.D.	3122	N.D.	3126	N.D.			
	3127	N.D.	3123	0.219	3127	0.0603	3123	N.D.	3127	N.D.	3123	N.D.	3127	N.D.			
	Mean	0		0.207		0.0703		0		0		0		0	0.207	0.5	0.156
	S.D.			0.013		0.0105											
20	4125	N.D.	4121	0.684	4125	0.202	4121	0.0767	4125	N.D.	4121	0.0909	4125	N.D.			
	4126	N.D.	4122	0.799	4126	0.286	4122	N.D.	4126	0.0565	4122	N.D.	4126	N.D.			
	4127	N.D.	4123	0.598	4127	0.0969	4123	N.D.	4127	N.D.	4123	0.0806	4127	N.D.			
	Mean	0		0.694		0.195		0.0256		0.0188		0.0572		0	0.694	0.5	1.16
	S.D.			0.101		0.095		0.0443		0.0323		0.0498					

N.D. : Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean±S.D.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Table 4 Study for effects on embryo-fetal development in rats treated orally with NS-304

Gross pathological findings in dams

Dose (mg/kg)	0	2	6	20
No. of dams examined	20	20	20	20
No. of dams with abnormal findings	0	0	0	0

Table 5 Study for effects on embryo-fetal development in rats treated orally with NS-304

Cesarean section data

Dose mg/kg	No. of dams		No. of corpora lutea	No. of implantations	Implantation index % a)	No. of embryo-fetal deaths: Total (%)b)	Implantation trace	Resorbed embryo	Placental remnant	Early macerated fetus	Late macerated fetus	Dead fetus	No. of live fetuses
0	20	Total	320	306		11	0	10	1	0	0	0	295
		Mean	16.0	15.3	95.8	(3.5)							14.8
		S.D.	1.6	1.5	5.5	(3.6)							1.4
2	20	Total	322	309		19	0	16	2	1	0	0	290
		Mean	16.1	15.5	96.4	(6.1)							14.5
		S.D.	2.0	1.5	5.5	(6.1)							1.6
6	20	Total	307	298		19	0	17	2	0	0	0	279
		Mean	15.4	14.9	97.3	(6.2)							14.0
		S.D.	1.6	1.3	4.5	(6.3)							1.3
20	20	Total	303	289		15	0	15	0	0	0	0	274
		Mean	15.2	14.5	93.1	(4.8)							13.7
		S.D.	2.6	3.7	19.2	(6.2)							3.5

a): (No. of implantations / No. of corpora lutea) X 100
b): (No. of embryo-fetal deaths / No. of implantations) X 100
No significant difference in any treated groups from control group.

Table 6 Study for effects on embryo-fetal development in rats treated orally with NS-304

Examination of live fetuses

Dose mg/kg	No. of dams		No. of males	No. of females	Sex ratio a)	Fetal body weight(g): Male	Female	No. of fetuses with external abnormalities (%)b)	Gross evaluation of placenta
0	20	Total	156	139	0.53			0	
		Mean	7.8	7.0		4.12	3.90	(0.0)	No abnormal findings
		S.D.	2.4	2.3		0.30	0.27	(0.0)	
2	20	Total	148	142	0.51			0	
		Mean	7.4	7.1		3.96	3.80	(0.0)	No abnormal findings
		S.D.	2.2	1.8		0.21	0.17	(0.0)	
6	20	Total	140	139	0.50			0	
		Mean	7.0	7.0		4.08	3.86	(0.0)	No abnormal findings
		S.D.	1.7	1.4		0.24	0.23	(0.0)	
20	20	Total	136	138	0.50			0	
		Mean	6.8	6.9		3.82**	3.63**	(0.0)	No abnormal findings
		S.D.	2.2	2.5		0.280	0.360	(0.0)	

a): No. of males / No. of live fetuses
b): (No. of live fetuses with external abnormalities / No. of live fetuses) X 100
**: p<0.01 (Significant difference from control group)
D: Dunnett's test

Offspring (malformations, variations, etc.):

Table 7 Study for effects on embryo-fetal development in rats treated orally with NS-304

Visceral examination of live fetuses

Dose (mg/kg)	0	20
No. of fetuses examined	141	134
No. of fetuses with abnormality (%)	4(2.9± 7.5)	10(7.2±11.0)
Situs inversus totalis	0(0.0± 0.0)	1(0.7± 2.9)
Ventricular septal defect	2(1.4± 4.4)	4(3.1± 7.5)
Abnormal origin of left pulmonary artery	1(0.7± 3.2)	3(2.0± 6.3)
Abnormal lobation of liver	1(0.7± 3.2)	1(0.9± 3.8)
Dilatation of renal pelvis and ureter	0(0.0± 0.0)	1(0.7± 2.9)
No. of fetuses with variation (%)	11(8.2± 9.1)	3(2.5± 6.0)*J
Thymic remnant in neck	7(5.1± 7.1)	1(0.7± 2.9)*J
Convoluted ureter	2(1.7± 5.4)	1(1.1± 4.6)
Left umbilical artery	2(1.4± 4.4)	1(0.8± 3.3)

*: p<0.05 (Significant difference from control group)
J: Wilcoxon's rank sum test

Table 8 Study for effects on embryo-fetal development in rats treated orally with NS-304

Skeletal examination of live fetuses

Dose (mg/kg)	0	2	6	20
No. of fetuses examined	154	150	145	140
No. of fetuses with abnormality (%)	0(0.0± 0.0)	0(0.0± 0.0)	0(0.0± 0.0)	0(0.0± 0.0)
No. of fetuses with variation (%)	15(9.8±15.1)	14(9.9±13.3)	26(18.1±18.5)	14(9.9±15.1)
Wavy ribs	0(0.0± 0.0)	1(0.8± 3.7)	1(0.6± 2.8)	0(0.0± 0.0)
14th rib	15(9.8±15.1)	13(9.1±13.4)	25(17.5±17.6)	12(8.6±15.3)
Splitting of thoracic vertebral body	1(0.7± 3.2)	0(0.0± 0.0)	0(0.0± 0.0)	2(1.3± 4.1)
Lumbarization of sacral vertebra	1(0.6± 2.8)	0(0.0± 0.0)	0(0.0± 0.0)	0(0.0± 0.0)
Progress of ossification				
No. of ossified sternebrae (%)				
1st	154(100.0± 0.0)	150(100.0± 0.0)	145(100.0± 0.0)	140(100.0± 0.0)
2nd	154(100.0± 0.0)	149(99.2± 3.7)	145(100.0± 0.0)	140(100.0± 0.0)
3rd	154(100.0± 0.0)	150(100.0± 0.0)	145(100.0± 0.0)	140(100.0± 0.0)
4th	154(100.0± 0.0)	149(99.2± 3.7)	145(100.0± 0.0)	140(100.0± 0.0)
5th	143(93.1±13.1)	140(93.0±10.9)	132(91.3±17.1)	120(85.6±19.3)
6th	154(100.0± 0.0)	149(99.2± 3.7)	144(99.2± 3.7)	139(99.4± 2.8)
No. of ossified metacarpi [Mean±S.D.]				
Right	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Left	4.00±0.00	3.99±0.04	4.00±0.00	4.00±0.00
No. of ossified metatarsi [Mean±S.D.]				
Right	4.93±0.10	4.84±0.22	4.91±0.12	4.79±0.21
Left	4.96±0.06	4.87±0.21	4.88±0.16	4.83±0.27
No. of ossified sacral and caudal vertebrae [Mean±S.D.]	8.45±0.31	8.32±0.47	8.42±0.43	8.27±0.39

No significant difference in any treated groups from control group.

Study title: Study for effects on embryo-fetal development in rabbits treated orally with NS-304

Key study findings: Fetuses from the high dose group (30 mg/kg) experienced increased incidence of right retrocaval ureter. No other statistically significant excess soft tissue or skeletal anomalies were detected. Fertility parameters were not affected.

Study no.: R-952
Volume #, and page #: eCTD
Conducting laboratory and location:

(b) (4)

Date of study initiation: December 14, 2006
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Name:	NS-304 (MRE-304)
Lot number:	20
Assay:	100.3%

Methods

Doses:
Species/strain: New Zealand White Rabbits
Number/sex/group:

Text Table 1. Group Composition Table

Test Group	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Copulated Females	Animal Number
Control	0	0	5	22	1101 to 1122
Low dose	3	0.6	5	22	2101 to 2122
Middle dose	10	2	5	22	3101 to 3122
High dose	30	6	5	22	4101 to 4122

Route, formulation, volume, and infusion rate: oral gavage
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality (dams): one dam in the high dose group died one hour post-dose, probably test article related.

Clinical signs (dams):

Table 1 Study for effects on embryo-fetal development in rabbits treated orally with NS-304

Clinical signs in dams

Dose mg/kg	Signs	Administration 0-5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28 a)
0	No. of dams	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0
	Decrease in the amount of feces	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0
3	No. of dams	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
	No. of dams with abnormal findings	0	0	1	0	0	0	0	0	1	1	2	2	2	1	1	1	0	0	0	0	0	0	0	0
	Decrease in the amount of feces	0	0	1	0	0	0	0	0	1	1	2	2	2	1	1	1	0	0	0	0	0	0	0	0
10	No. of dams	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
	No. of dams with abnormal findings	0	20	21	19	19	16	16	16	10	12	12	10	7	5	0	0	0	0	0	0	0	0	0	0
	Flush(auricle)	0	19	19	16	15	14	8	6	4	6	5	4	3	1	0	0	0	0	0	0	0	0	0	0
	Flush(around upper and lower lip, around eyelid)	0	16	18	17	18	14	14	15	8	10	11	8	5	3	0	0	0	0	0	0	0	0	0	0
	Decrease in the amount of feces	0	0	3	0	0	0	1	0	0	1	1	2	2	2	0	0	0	0	0	0	0	0	0	0
30	No. of dams	21	21	21	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	No. of dams with abnormal findings	0	21	21	20	20	20	20	20	20	20	20	20	20	20	1	1	0	0	0	0	0	0	0	0
	Flush(auricle)	0	21	20	20	20	20	20	20	20	20	20	20	19	20	0	0	0	0	0	0	0	0	0	0
	Flush(around upper and lower lip, around eyelid)	0	21	20	20	20	20	20	20	20	20	20	20	20	20	0	0	0	0	0	0	0	0	0	0
	Hyperpnea	0	21	18	12	14	14	10	9	5	6	6	6	7	7	0	0	0	0	0	0	0	0	0	0
	Decrease in the amount of feces	0	0	17	7	4	1	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
	Staining around the anus region	0	3	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Decrease in locomotion activity	0	0	1	1	1	1	3	1	1	0	1	2	4	2	0	0	0	0	0	0	0	0	0	0
	Soft feces	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Death	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

a): Gestational day

Body weight (dams):

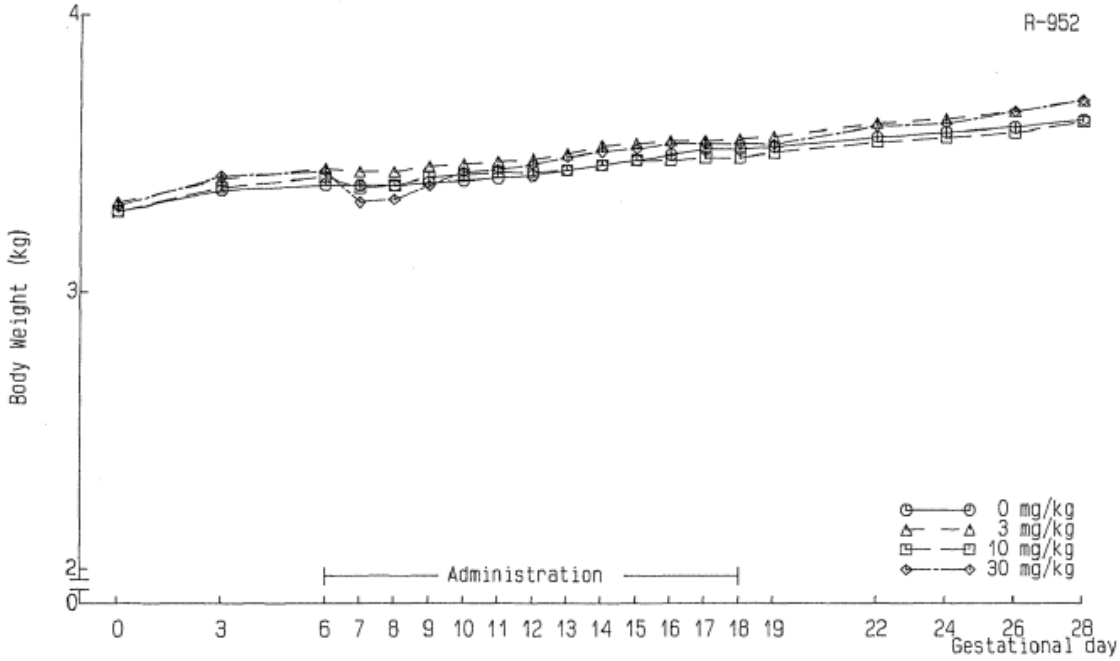

Fig. 1 Study for effects on embryo-fetal development in rabbits treated orally with NS-304

Body weight changes of dams

Food consumption (dams):

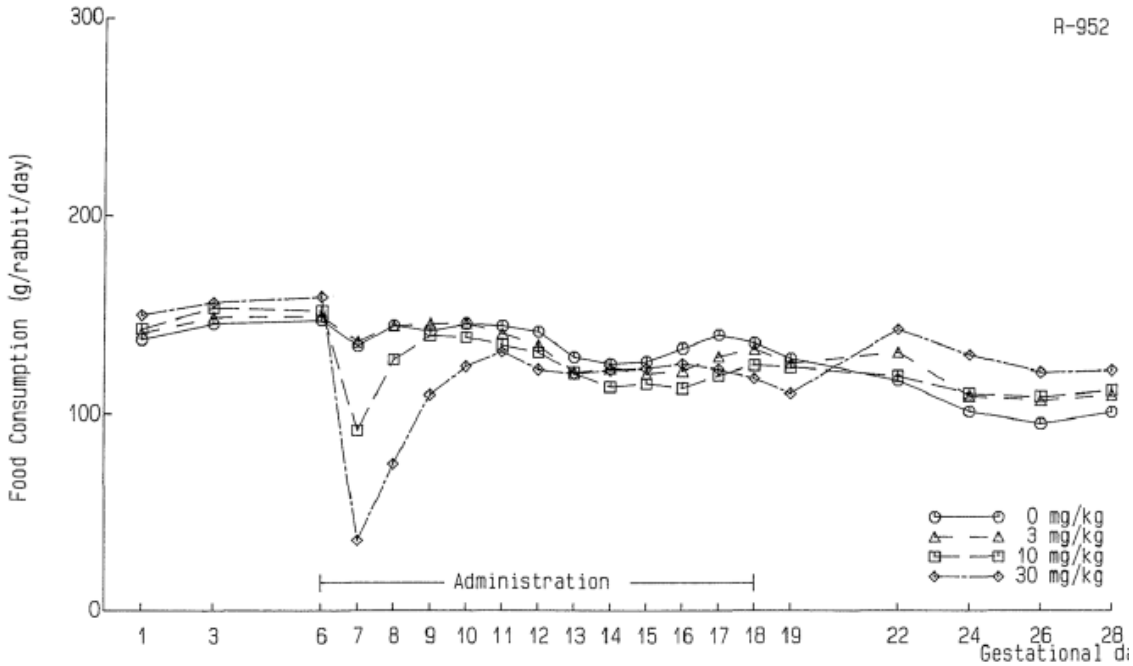

Fig. 2 Study for effects on embryo-fetal development in rabbits treated orally with NS-304
Food consumption of dams

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Toxicokinetics:

Table 10-1 Study for effects on embryo-fetal development in rabbits treated orally with NS-304
Plasma concentration of NS-304 (Gestational day 18)

Dose (mg/kg)	Animal number	Plasma concentration (μg/mL) Pre	0.5h	1h	2h	4h	8h	24h	Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
0	1118	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	-
	1119	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	-
	1120	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	-
	Mean	0	0	0	0	0	0	0			
	S.D.										
3	2118	N.D.	0.137	N.D.	N.D.	N.D.	N.D.	N.D.	0.137	0.5	0.0685
	2119	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	-	0
	2120	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	-	0
	Mean	0	0.0457	0	0	0	0	0	0.0457	0.5	0.0228
	S.D.		0.0791						0.0791		0.0395
10	3118	N.D.	0.0665	N.D.	N.D.	N.D.	N.D.	N.D.	0.0665	0.5	0.0333
	3119	N.D.	0.335	0.0784	N.D.	N.D.	N.D.	N.D.	0.335	0.5	0.226
	3120	N.D.	0.223	0.0820	N.D.	N.D.	N.D.	N.D.	0.223	0.5	0.173
	Mean	0	0.208	0.0535	0	0	0	0	0.208	0.5	0.144
	S.D.		0.135	0.0463					0.135	0.0	0.100
30	4119	N.D.	2.35	1.31	0.394	N.D.	N.D.	N.D.	2.35	0.5	2.75
	4121	N.D.	0.921	0.492	0.0961	N.D.	N.D.	N.D.	0.921	0.5	0.974
	4122	N.D.	0.740	0.554	0.120	N.D.	N.D.	N.D.	0.740	0.5	0.966
	Mean	0	1.34	0.785	0.203	0	0	0	1.34	0.5	1.56
	S.D.		0.88	0.455	0.166				0.88	0.0	1.03

N.D.: Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean±S.D. and AUC.

Table 10-2 Study for effects on embryo-fetal development in rabbits treated orally with NS-304
Plasma concentration of MRE-269 (Gestational day 18)

Dose (mg/kg)	Animal number	Plasma concentration (μg/mL) Pre	0.5h	1h	2h	4h	8h	24h	Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
0	1118	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	-
	1119	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	-
	1120	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	-
	Mean	0	0	0	0	0	0	0			
	S.D.										
3	2118	N.D.	0.420	0.500	0.204	0.102	N.D.	N.D.	0.500	1.0	1.20
	2119	N.D.	0.443	0.386	0.148	0.0703	N.D.	N.D.	0.443	0.5	0.944
	2120	N.D.	0.176	0.221	0.106	0.0713	0.0674	N.D.	0.221	1.0	1.30
	Mean	0	0.346	0.369	0.153	0.0812	0.0225	0	0.388	0.8	1.15
	S.D.		0.148	0.140	0.049	0.0180	0.0389		0.147	0.3	0.18
10	3118	N.D.	0.406	0.552	0.512	0.332	0.348	N.D.	0.552	1.0	5.86
	3119	N.D.	0.932	1.03	0.172	0.140	0.109	N.D.	1.03	1.0	3.01
	3120	N.D.	0.895	1.07	0.365	0.161	0.112	N.D.	1.07	1.0	3.40
	Mean	0	0.744	0.884	0.350	0.211	0.190	0	0.884	1.0	4.09
	S.D.		0.294	0.288	0.171	0.105	0.137		0.288	0.0	1.55
30	4119	N.D.	3.26	5.17	3.77	0.732	0.304	N.D.	5.17	1.0	16.4
	4121	N.D.	2.11	3.41	1.27	0.506	0.609	N.D.	3.41	1.0	13.1
	4122	N.D.	1.32	2.39	1.49	0.657	0.506	N.D.	2.39	1.0	11.7
	Mean	0	2.23	3.66	2.18	0.632	0.473	0	3.66	1.0	13.7
	S.D.		0.98	1.41	1.38	0.115	0.155		1.41	0.0	2.4

N.D.: Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean±S.D. and AUC.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

K-952

Table 4 Study for effects on embryo-fetal development in rabbits treated orally with NS-304
Gross pathological findings in dams

Dose mg/kg	0	3	10	30
No. of dams examined	22	21	22	21
No. of dams with abnormal findings	0	0	0	1 a)
Thymus : Discoloration, dark red	0	0	0	1
Lung : Discoloration, dark red	0	0	0	1
Stomach : Discoloration, dark red, mucosal	0	0	0	1
Subcutaneus : Hemorrhage, around the mammary gland region, many	0	0	0	1
: Hemorrhage, around the neck	0	0	0	1

a): Finding in dead dam

R-952

Table 5 Study for effects on embryo-fetal development in rabbits treated orally with NS-304

Cesarean section data on dams

Dose mg/kg	No. of dams		No. of corpora lutea	No. of implantations	Implantation index % a)	No. of resorbed or dead fetuses Total(%) b)	Early c)	Late d)	No. of live fetuses	Placental weight(g) male	female	Gross evaluation of placentae
0	22	Total	226	181		14	11	3	167			
		Mean	10.3	8.2	80.7	(8.2)			7.6	3.24	3.00	No abnormal findings
		S.D.	1.9	2.8	23.5	(10.1)			2.8	0.78	0.53	
3	21	Total	205	168		9	5	4	159			
		Mean	9.8	8.0	82.0	(5.4)			7.6	3.40	3.12	No abnormal findings
		S.D.	1.6	2.7	22.4	(8.5)			2.7	0.54	0.53	
10	22	Total	196	165		15	8	7	150			
		Mean	8.9	7.5	84.0	(12.0)			6.8	3.58	3.37	No abnormal findings
		S.D.	2.5	2.9	19.9	(21.4)			2.7	0.55	0.63	
30	20	Total	200	176		17	13	4	159			
		Mean	10.0	8.8	87.6	(10.4)			8.0	3.39	3.33	No abnormal findings
		S.D.	2.2	2.4	14.9	(16.4)			2.7	0.47	0.44	

a): (No. of implantations / No. of corpora lutea) X 100
b): (No. of resorbed or dead fetuses / No. of implantations) X 100
c): Implantation trace, resorbed embryo and placental remnant
d): Early macerated fetus, late macerated fetus and dead fetus
No significant difference from control group in any treated group

Offspring (malformations, variations, etc.):

R-952

Table 6 Study for effects on embryo-fetal development in rabbits treated orally with NS-304

External examination of live fetuses

Dose mg/kg	No. of dams		No. of males	No. of females	Sex ratio a)	Fetal body weight(g) Male	Female	No. of fetuses with external abnormalities (%) b)
0	22	Total	75	92				0
		Mean	3.4	4.2	0.45	34.80	33.68	(0.0)
		S.D.	1.7	2.0	0.15	5.90	4.32	(0.0)
3	21	Total	64	94				c) 1
		Mean	3.0	4.5	0.40	35.98	34.41	(2.4)
		S.D.	1.7	2.0	0.19	3.32	4.38	(10.9)
10	21	Total	75	72				d) 3
		Mean	3.6	3.4	0.51	36.31	34.89	(1.8)
		S.D.	1.8	1.6	0.24	3.54	4.31	(4.6)
30	20	Total	84	75				0
		Mean	4.2	3.8	0.57	33.80	33.77	(0.0)
		S.D.	1.7	2.2	0.21	3.43	3.48	(0.0)

a): No. of males / No. of live fetuses
b): (No. of live fetuses with external abnormalities / No. of live fetuses) X 100
c): Brachyury
d): Hypoplasia in cavity, Amelia in forelimbs, Spina bifida, Paw hyperflexion in forelimb (left), Gastroschisis
No significant difference from control group in any treated group

Table 7 Study for effects on embryo-fetal development in rabbits treated orally with NS-304

Visceral examination of live fetuses

Dose (mg/kg)	0	30
No. of fetuses examined	167	159
No. of fetuses with abnormalities (%:Mean±S.D.)	0(0.0± 0.0)	2(1.1± 5.0)
Absent gallbladder	0(0.0± 0.0)	2(1.1± 5.0)
No. of fetuses with variation (%:Mean±S.D.)	8(6.1±12.4)	10(5.1±10.6)
Abnormal lung lobation (Absent accessory lobe)	3(2.8±10.8)	3(1.5± 4.9)
Abnormal origin of right subclavian artery	5(3.3± 7.5)	1(0.5± 2.2)
Retrocaval ureter (right)	0(0.0± 0.0)	6(3.1± 7.5)*J

*: p<0.05 (Significant difference from control group)
J: Wilcoxon's rank sum test

R-952

Table 8-1 Study for effects on embryo-fetal development in rabbits treated orally with NS-304

Skeletal examination of live fetuses

Dose (mg/kg)	0	30
No. of fetuses examined	167	159
No. of fetuses with abnormalities (%:Mean±S.D.)	8(5.8±10.0)	3(1.5± 4.9)
Fused sternebra	5(3.7± 9.0)	2(1.0± 3.1)
Branched rib	1(1.1± 5.3)	1(0.5± 2.2)
Fused rib	1(1.1± 5.3)	0(0.0± 0.0)
Absent thoracic vertebral arch	0(0.0± 0.0)	1(0.5± 2.2)
Absent lumbar arch	1(0.5± 2.1)	0(0.0± 0.0)
Lumbar hemivertebra	1(0.5± 2.1)	0(0.0± 0.0)
Malpositioned caudal vertebra	1(0.5± 2.1)	1(0.5± 2.2)
No. of fetuses with variation (%:Mean±S.D.)	7(4.3± 9.4)	10(5.5± 8.1)
Cervical rib	1(0.9± 4.3)	3(1.8± 4.6)
Asymmetric sternebra	1(1.1± 5.3)	1(0.5± 2.2)
Splitting of sternebra	1(0.4± 1.9)	2(1.0± 3.1)
Unossified talus	4(1.8± 6.0)	4(2.2± 4.6)
No. of fetuses with 13th rib (%:Mean±S.D.)	128(76.6±27.7)	117(74.4±23.7)

No significant difference from control group in any treated group

Prenatal and postnatal development: Not done

2.6.6.7 Local tolerance not done

2.6.6.8 Special toxicology studies

Study title: *in vitro* phototoxicity study

Volume #, and page #: eCTD 2.6.6.8.1 or 4.2.3.7.7
Conducting laboratory and location: not provided
Date of study initiation: not provided
GLP compliance: no

QA reports: yes ( ) no (x)
Drug, lot #, and % purity: ACT-333679 (code: MRE-269; batch number: ELB0066-1217.00) and ACT-293987 (code: NS-304; batch number: ELB0066-1216.00)
Formulation/vehicle: 10 mM stock solution in DMSO, final dilution in phosphate buffered saline.

Methods

Doses:
ACT-333679; +UVA: 100.0, 33.3, 11.1, 3.6, 1.2, 0.4, 0.14, 0.05 µM
ACT-333679; -UVA: 100.0, 33.3, 11.1, 3.6, 1.2, 0.4, 0.14, 0.05 µM
ACT-333679; +UVA: 1,000.0, 333.3, 111.1, 37, 12.3, 4.1, 1.4, 0.5 nM
ACT-333679; -UVA: 1,000.0, 333.3, 111.1, 37, 12.3, 4.1, 1.4, 0.5 nM

ACT-293987; +UVA: 100.0, 33.3, 11.1, 3.6, 1.2, 0.4, 0.14, 0.05 µM
ACT-293987; -UVA: 100.0, 33.3, 11.1, 3.6, 1.2, 0.4, 0.14, 0.05 µM
ACT-293987; +UVA: 1,000.0, 333.3, 111.1, 37, 12.3, 4.1, 1.4, 0.5 nM
ACT-293987; -UVA: 1,000.0, 333.3, 111.1, 37, 12.3, 4.1, 1.4, 0.5 nM

Study design:

Results:

Table 1 Survival data

ACT-293987				ACT-333679			
ACT-293987 [uM]	ACT-293987 [nM]	Presence of UV (% Survival)	Absence of UV (% Survival)	ACT-333679 [uM]	ACT-333679 [nM]	Presence of UV (% Survival)	Absence of UV (% Survival)
100.00		25.96	21.05	100.00		25.24	24.14
33.33		26.35	21.39	33.33		26.19	24.38
11.11		25.45	22.96	11.11		24.88	25.09
3.60		26.99	55.32	3.60		25.48	76.45
1.20		26.09	97.65	1.20		25.48	98.22
0.40		26.22	93.39	0.40		25.71	96.33
0.14		26.99	95.07	0.14		24.40	97.87
0.05		29.95	100.00	0.05		23.57	100.00
	1000.00	24.31	92.55		1000.00	23.73	87.58
	333.30	32.67	94.38		333.30	25.90	87.20
	111.10	72.94	95.99		111.10	73.49	92.42
	37.00	92.85	98.05		37.00	96.75	94.41
	12.30	95.60	93.46		12.30	98.92	97.89
	4.10	97.14	96.22		4.10	96.39	92.17
	1.40	96.15	98.51		1.40	100.00	93.42
	0.50	95.05	99.08		0.50	96.27	98.39

% Survival was calculated with respect to mean maximal value of hexaplicates obtained across all treated and non-treated samples.

2.6.6.9 Discussion and Conclusions: Although both substances, at concentrations of approx 100nM and higher, were positive for phototoxicity enabled by UVA, the drug

does not accumulate in the skin or pigmented cells near the surface, so risk of such toxicity may be low.

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

APPEARS THIS WAY ON ORIGINAL

Table 8 Incidence of intussusception in the dog

Study	LOEL	Mortality (Found dead or humanely killed)	Day	Gender
Single-Dose (plus 15 days observation) [T-08.273]	200 mg/kg	N = 1 out of 6	7	♂ (FD)
2-week study [T-08.277]	20 mg/kg/day	N = 3 out of 6	6 10 13	♀ (FD) ♂ (UN) ♂ (FD)
4-week study + 4 week recovery [T-08.290]	6 mg/kg/day	N = 1 out of 10 Intussusception observed during routine necropsy	29	♂
39-week study [T-08.286]	4 mg/kg/day	N = 2 out of 12	24 124	♀ (UN) ♀ (UN)

FD = found dead; UN = unscheduled necropsy

Table 9 Safety margins for intussusception based on AUC data

Drug	Dog study	NOEL (mg/kg/day/day)	Mean AUC_{0-24} (ng.h/mL)	Safety* margins	Corrected safety margins**
ACT-333679	2-week study	6	154,000	562	5
ACT-293987	[T-08.277]		24,675	355	4
ACT-333679	4-week study	3	84,255	308	3
ACT-293987	recovery period (4-week) [T-08.290]		13,720	197	2
ACT-333679	39-week study	2	49,700	181	2
ACT-293987	[T-08.286]		7,345	106	1

*Achieved plasma exposure after administration of 1,600 µg ACT-293987 b.i.d. at steady state in humans subjects: ACT-293987 AUC_{0-24h} of 69.6 ng·h/mL; ACT-333679 (active metabolite) AUC_{0-24h} of 274 ng·h/mL

**Taking into account the species difference between dogs and humans of *in vitro* inhibition of ADP-induced platelet aggregation by ACT-333679 [(b)(4)08.253]. The exposure at the respective NOAEL is corrected for the species difference of *in vitro* IC_{50} values on platelet aggregation inhibition. The ratio $IC_{50\ dog}$/ $IC_{50\ human}$ is 119 with ACT-333679 and 83 with ACT-293987. The safety margin is corrected by this factor.

Table 10 Safety margins for intussusception based on C_{max} data

Drug	Dog Study	NOEL (mg/kg/day/day)	Mean C_{max} (ng/mL)	Safety* margins	Corrected Safety margins**
ACT-333679	2-week study	6	14,135	790	7
ACT-293987	[T-08.277]		9,290	845	7
ACT-333679	4-week study	3	7,575	423	4
ACT-293987	recovery period (4-week) [T-08.290]		4,620	387	3
ACT-333679	39-week study	2	5,580	312	3
ACT-293987	[T-08.286]		2,445	222	2

* Achieved plasma exposure after administration of 1,600 µg ACT-293987 b.i.d. at steady state in human subjects: ACT-333679 (active metabolite): C_{max} 17.9 ng/mL; ACT-293987 (drug): C_{max} 11 ng/mL

** Taking into account the species difference between dogs and humans of *in vitro* inhibition of ADP-induced platelet aggregation by ACT-333679 [(b)(4)08.253]. The ratio of inhibition of ADP induced platelet aggregation by ACT-333679 between dogs and humans is 119. The safety margin is corrected by this factor.

Table 11 Inhibition of platelet aggregation in platelet-rich plasma [(b)(4)-08.253]

	IC_{50} of platelet aggregation (µM)		
Drug	**Human**	**Dog**	**Rat**
ACT-333679	0.21 (88.1 ng/mL)	25 (10,488 ng/mL)	10 (4,195 ng/mL)
ACT-293987	5.5 (2,731 ng/mL)	456 (226,459 ng/mL)	–

– = not tested

ACT-333679 (active metabolite): C_{max} 17.9 ng/mL; ACT-293987 (drug): C_{max} 11 ng/mL

Table 12 Safety margins for drug-related bleeding potential based on AUC data

Compound	Human[1]		Dog[2]		Rat[3]		
	AUC (ng.h/mL)	Mean AUC (ng.h/mL)	Safety margin	Corrected safety margin[4]	Mean AUC (ng.h/mL)	Safety margin	Corrected safety margin[4]
ACT-333679	274	554,000	2,022	17	139,000	507	11
ACT-293987	69.6	90,000	1,293	16	12,000	172	na

1 Achieved plasma exposure after administration of 1,600 µg ACT-293987 b.i.d. at steady state in human subjects: ACT-293987 AUC_{0-24h} of 69.6 ng·h/mL; ACT-333679 (active metabolite) AUC_{0-24h} of 275 ng·h/mL.

2 Dog NOAEL 20 mg/kg/day/day, 2-week oral toxicity study [T-08.277]

3 Rat NOAEL 100 mg/kg/day/day, 26-week oral toxicity study [T-08.285]

4 The exposure at the respective NOAEL is corrected for the species difference of *in vitro* IC_{50} values on platelet aggregation inhibition. The ratio $IC_{50\ dog}$/ $IC_{50\ human}$ is 119 with ACT-333679 and 83 with ACT-293987. The ratio $IC_{50\ rat}$/ $IC_{50\ human}$ is 48 with ACT-333679 [Table 11].

Table 13 Safety margins for bleeding based on C_{max} data

Compound	Human[1]		Dog[2]		Rat[3]		
	C_{max} (ng/mL)	Mean C_{max} (ng/mL)	Safety margin	Corrected safety margin[4]	Mean C_{max} (ng/mL)	Safety margin	Corrected safety margin[4]
ACT-333679	17.9	40,000	2,235	19	28,175	1,574	33
ACT-293987	11	22,630	2,057	25	6,070	552	na

1 Achieved plasma exposure after administration of 1600 µg ACT-293987 b.i.d. at steady state in human subjects: ACT-333679 (active metabolite): C_{max} 17.9 ng/mL; ACT-293987 (drug): C_{max} 11 ng/mL.

2 Dog NOAEL 20 mg/kg/day/day, 2-week oral toxicity study [T-08.277]

3 Rat NOAEL 100 mg/kg/day/day, 26-week oral toxicity study [T-08.285]

4 The exposure at the respective NOAEL is corrected for the species difference of IC_{50} values on platelet aggregation inhibition. The ratio $IC_{50\ dog}$/ $IC_{50\ human}$ is 119 with ACT-333679 and 83 with ACT-293987.
The ratio $IC_{50\ rat}$/ $IC_{50\ human}$ is 48 with ACT-333679 [Table 11].

Table 14 Safety margin for increased ossification in dogs based on AUC data

Study	Bone finding in dogs	NOEL (mg/kg/day)	Mean AUC_{0-24} (ng·h/mL)	Safety margins for ACT-333679*	Corrected safety margins**
2-week study [T-08.277]	Bone (femur) increased ossification in the trabeculae (males and females) and/or increased ossification of the periosteum (females)	2	59,290	216	2
4-week study [T-08.290]	Bone (femur) increased ossification in the trabeculae (males and females) and/or increased ossification of the periosteum (males)	1.5	47,110	172	1
39-week study [T-08.286]	Bone (femur) increased ossification in the trabeculae (males and females)	**LOEL** 1	29,400	107	1
39-week study interim evaluation (26-week) [T-08.286]	Bone (femur) increased ossification in the trabeculae (females)	2	49,700***	181	2

* Achieved plasma exposure after administration of 1,600 µg ACT-293987 b.i.d. at steady state in humans subjects: ACT-333679 (active metabolite) AUC_{0-24h} of 274 ng·h/mL

** Taking into account the species difference between dogs and humans of *in vitro* inhibition of ADP-induced platelet aggregation by ACT-333679 (b)(4) [08.253]. The ratio of inhibition of ADP-induced platelet aggregation by ACT-333679 between dogs and humans is 119. The safety margin is corrected by this factor.

*** Exposures at the end of the study (39-week).

LOEL = lowest observed effect level

Table 15 Binding affinity for human receptors

Drug	Binding affinity (Ki, μM) for human receptor			
	IP	EP_2	EP_4	Fold differences for EP vs IP receptor affinities (worst case scenario)
ACT-333679 (MW 419)	0.02 (8.4 ng/mL)	5.8 (2,436 ng/mL)	4.9 (2,058 ng/mL)	245
ACT-293987 (MW 496)	0.26 (129 ng/mL)	>10 (4,960 ng/mL)	>10 (4,960 ng/mL)	38

References: [(b)(4)–08.217] to [(b)(4)08.222] and [(b)(4)08.255]
MW = molecular weight

Table 16 Human safety margin for EP_2/EP_4 subtype receptor binding

Drug	EP_2 (Ki)	Margins EP_2 vs human achieved** C_{max} at 1600μg b.i.d.	EP_4 (*Ki*)	Margins EP_4 vs human achieved** C_{max} at 1,600 μg b.i.d.
ACT-333679**	5.8 μM (2,436 ng/mL)	136	4.9 μM (2,058 ng/mL)	115
ACT-293987**	>10 μM (4,960 ng/mL)	451	>10 μM (4,960 ng/mL)	451

**Achieved concentration at 1,600 μg b.i.d: C_{max} 17.9 ng/mL (ACT-333679) or C_{max} 11 ng/mL (ACT-293987)

Table 17 Incidence of bone marrow fibrosis and associated findings in dogs

Study	Dose (mg/kg/day)	Incidence: Bone marrow fibrosis	Incidence: White/red cell counts decrease	Incidence: Extramedullary hematopoiesis
2-week study [T-08.277]	6	5 out of 6	√	X
	20	3 out of 3	√	X
4-week study [T-08.290]	3	4 out of 6	X	X
	6	6 out of 6	√	3 out of 6
4-week recovery period (4-week study) [T-08.290]	3	0 out of 4	X	X
	6	0 out of 4	X	X
39-week study [T-08.286]	4	3 out of 5	X	X

√ = sign present
X = not observed

Table 18 Safety margin for bone marrow fibrosis

Study	Bone marrow finding in dogs	NOEL (mg/kg/day)	Mean AUC_{0-24} (ng·h/mL)	Safety margins* ACT-333679	Corrected Safety margins** ACT-333679
2-week study [T-08.277]	Bone marrow fibrosis	2	59,290	216	2
4-week study [T-08.290]	Bone marrow fibrosis	1.5	47,110	172	1
4-week study + 4-week recovery period [T-08.290]	Bone marrow fibrosis	6	180,250	658	6
39-week study [T-08.286]	Bone marrow fibrosis	2	49,700	181	2

*Achieved plasma exposure after administration of 1,600 µg ACT-293987 b.i.d. at steady state in human subjects: ACT-333679 (active metabolite) AUC_{0-24h} of 274 ng·h/mL.
** Taking into account the species difference between dogs and humans of *in vitro* inhibition of ADP-induced platelet aggregation by ACT-333679 (b)(4)08.253]. The ratio of inhibition of ADP-induced platelet aggregation by ACT-333679 between dogs and humans is 119. The safety margin is corrected by this factor.
NOEL = No observed effect level

OVERALL conclusions and recommendations

Summary: ACT-293987 is a prostacyclin agonist that has an ester group conjugated to it to improve oral bioavailability .It is rapidly metabolized to ACT-333679, the primary active compound. For this submission, the indication is for treatment of pulmonary arterial hypertension (PAH).

The primary pharmacology studies project efficacy in PAH based on appreciable relief in models of PAH and an ability to reduce right ventricular hypertrophy (RVH)in such. The receptor binding profile suggests that the basis for this efficacy is selective binding to the IP receptor..

Some of the positive findings in the safety pharmacology studies reflect prostacyclin pharmacology. The cardiovascular safety studies indicated flushing, hypotension, and increased bleeding times - issues to be aware of, although platelet inhibiton and vasodilatation are expected of prostacyclin agonists.r There were also positive CNS findings i.e., in the modified Irwin screening, and manifest as changes in body temperature, sleep, pain response, and respiration rates. . ACT-293987 also decreased urinary sodium and potassium excretion.. Prostacyclin receptors mediating smooth muscle tone are common in the gastrointestinal tract, and intussusception was observed at necropsy, especially in the dog. Most adverse events for prostacyclin agonists are related to their effects on the gastrointestinal system.

ACT-293987 is rapidly and extensively absorbed from the gastro-intestinal tract with a Tmax of approximately 1 hr and a bioavailability of 80 to 90%. Radiolabeled ACT-293987 accumulates first in the liver after oral dosing, followed by accumulation in the stomach. This is probably due to the high concentration of receptors present in the stomach, where prostacyclin agonists apparently have high activity. ACT-293987 is primarily.,excreted through the biliary system (i.e., enterohepatic) with only 2% being excreted by the kidneys. ACT-293987 is a pro-drug, although it also has activity at prostacyclin receptors, and is hydrolyzed to ACT-333697, the primary prostacyclin agonist. ACT-333697, one of up to 15 potential metabolites of ACT-293987, is glucuronidated primarily and excreted through the biliary system..

General toxicology:

From the mouse studies:

Study Length	Doses	Results	NOAEL
4 weeks	0, 30, 100, 300 mg/kg/day	No mortality, @300 mg/kg/day flaccidity and flushing	100 mg/kg/day
13 weeks	0, 100, 300, 500 mg/kg/day	500 mg/kg/day one mortality, CK & ALT increase; 300 mg/kg/day decrease in food consumption, flushing, flaccidity, BUN decreased, kidney tubular vacuolation	100 mg/kg/day

From the rat studies:

Study Length	Doses	Results	NOAEL

4 weeks	0, 20, 60, 180 mg/kg/day	20 mg/kg/day alveolar hemorrhage and decreased platelets, loss or black discoloration of tail tip;	Not determined
4 weeks + 4 weeks recovery	2, 6, 60 mg/kg/day	60 mg/kg/day flushing, decreased movement, piloerection, reduced body wt and food consumption. Reversible	6 mg/kg/day
26 weeks + 4 week recovery	0, 6, 25, 100 mg/kg/day	>25, 100 mg/kg/day liver and adrenal hypertrophy, hyperplasia mammary gland, salivary gland, follicular cells in thyroid; all treated animals, flushing, decreased movement, reversible; one animal died in high dose group of malignant lymphoma	6 mg/kg/day

From the dog studies:

Study length	Doses	Results	NOAEL
2 weeks	2, 6, 20 mg/kg/day	20 mg/kg/day led to mortalities, Intussuseception, QTc prolongation; 6 mg/kg/day increased ossification, bone marrow fibrosis; 2 mg/kg/day decreased platelet, wbc, & neutrophils.	2 mg/kg/day
4 weeks	1.5, 3, 6 mg/kg/day	6 mg/kg/day intussusception; 3 mg/kg/day intussusception, bone marrow fibrosis, ossification; 1.5 mg/kg/day vomiting, diarrhea, jelly feces	1.5 mg/kg/day
39 weeks	1, 2, 4 mg/kg/day	4 mg/kg/day 2 mortalities, intussusception; 1mg/kg/day bone marrow fibrosis and ossification	Not determined

Genetic toxicology: ACT-293987 was negative in most testing except for a small signal of clastogenicity in the Chinese Hamster Lung cell in vitro assay. However, the in vivo mouse micronucleus assay for clastogenicity was negative..

Carcinogenicity: not done

Reproductive toxicology: For the rat studies, the NOAEL was the same as for the standard toxicity testing, 6 mg/kg/day. In this species, but not the rabbit, the primary issue appeared to be low birth weight, which in humans has been correlated with developmental difficulties.
In the rabbit study, the NOAEL was 10 mg/kg/day,and one animal died at the highest dose tested i.e., 30 mg/kg/day, Reproductive function and fetal development were not affected.

Special toxicology: In an *in vitro* phototoxicity study both ACT-293987 and ACT-333697 were positive for phototoxicity with UVA light. Phototoxic concentrations were approx 100 nm (0.1uM) and above, relatively high exposures unlikely to be achieved at therapeutic dosages since no accumulation was observed in skin or eye.

Based on the animal safety studies, ACT-293987 and its active metabolite ACT-333697 primarily carry, risk of intussusception, and bleeding due to platelet effects. and. However, PAH is a high risk disease state, and with the appropriate safety measures in place , it should be reasonably safe to begin clinical trials at the proposed starting dose . Also, the prospect of an orally available prostacyclin agonist may be attractive for the treatment of these patients considering the other prostacyclin agonists are only available in intravenous or nebulizer formulations.

Internal comments:

External comments (to sponsor):

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes _x__ No ___

Appendix/attachments

Appendix II. FDA Statistical Review of Carcinogenicity studies.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CARCINOGENICITY STUDY

NDA:	207947
Drug Name:	UPTRAVI® (Selexipag)
Indication:	Treatment of Pulmonary arterial hypertension.
Sponsor:	Actelion Pharmaceiticals Ltd. c/o Actelion Clinical Research Inc. 1820 Chapel Avenue West Cherry Hill, New Jersey 08002
CRO:	(b) (4) [redacted]
Date:	Data Submitted: 3 March 2015 Assigned to Reviewer: 8 January 2015
Review Priority:	Standard
Biometrics Division:	Division 6
Statistical Reviewer:	Steve Thomson
Concurring Reviewer:	Team Leader: Karl Lin, Ph. D.
Medical Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Toxicologist:	Reviewer: Matthew Whittaker, Ph.D. Team Leader: Marcie Wood, Ph.D.
Project Manager:	Michelle Jordan Garner, , MS, OTR/L CDR, U.S. Public Health Service
Keywords:	Carcinogenicity, Cox regression, Kaplan-Meier product limit, survival analysis, Trend test

Contents

1. EX ECUTIVE SUMMARY

Both the report for rats and the report for mice included the statement that: “The purpose of the present study is to evaluate carcinogenicity of NS-304 [(i.e., Uptravi®)] in rats by a 2-year oral gavage administration. In addition, systemic exposure to NS-304

was evaluated." (page 7 of both reports). Both studies were conducted at the (in English, rather unfortunately named) [redacted] (b) (4).

1.1. Conclusions and Recommendations

The Sponsor summarizes study design in the rat report as follows: "Carcinogenicity of NS-304 (prostaglandin I2 agonist) was evaluated in Crl:CD(SD) rats. NS-304 was administered orally by gavage to male and female rats (60/sex/group for a main group, 6 weeks of age at the start of treatment) for 104 weeks. The dosage levels of NS-304 were set at 0 (0.5 w/v% methylcellulose solution), 10, 30 and 100 mg/kg/day." (page 14 of rat report)

Alternatively, gross aspects of the study design for the rat study are summarized in Table 1 be1ow:

Table 1. Design of Rat Study (dose volume 5 mL/kg/day)

Treatment Group	Animals / Gender	Nominal Dose (mg/kg/day)	Concen-tration (mg/mL)
1. Vehicle[1]	60 (5)	0	0
2. Low	60 (8)	10	2
3. Medium	60 (8)	30	6
4. High	60 (8)	100	20

[1]0.5 w/v% methylcellulose solution (0.5 w/v% MC solution)

General aspects of the study design for the rather similar mice study are summarized in Table 2 be1ow. The Sponsor's report described the conduct of the mouse study as follows: "Carcinogenicity of NS-304 (prostaglandin I2 agonist) was evaluated in B6C3F1/Crlj mice. NS-304 was administered orally by gavage to male and female mice (55/sex/group for a main group, 6 weeks of age at the start of treatment) for 2 years. The dosage levels of NS-304 were set at 0 (0.5 w/v% methylcellulose solution), 125, 250 and 500 mg/kg/day. Since the survival rate in females in the 500 mg/kg group decreased with the progression of the administration period, all surviving females in this group were prematurely sacrificed in Week 100 after administration for 99 weeks. Further, systemic exposure to NS-304 was evaluated by determining plasma NS-304 and its metabolite, MRE-269 concentrations on the satellite animals." (page 14 of mice report)

Table 2. Design of Mice Study (dose volume 10 mL/kg)

Treatment Group[1]	# Main study animals (# toxicology study animals)/gender	Dose (mg/kg/day)	Concentration (mg/mL)

1. Vehicle[1]	55 (9)	0	0
2. Low	55 (24)	125	12.5
3. Medium	55 (24)	250	25
4. High	55 (29)	500	50

[1] 0.5% w/v% methylcellulose solution

.

Summary tables of survival in rats are presented in Tables 18 and 19, below, with corresponding tables in mice in tables 26 qnd 27. Kaplan-Meier survival curves for the both genders in both studies are presented in Appendix 1, Figures A.1.1 through A.1.4. The interpretation of these plots is supported by the tests of homogeneity and differences in survival over the treatment groups. The statistical significance levels (i.e., p-values) for rats are provided in Table 3, below. One might note that the log rank tests place greater weight on later events, while the Wilcoxon test tends to weight them more equally, and thus, it actually tends to place more weight on differences in earlier events than does the log rank test.

Table 3. Statistical Significances of Tests of Homogeneity and Trend in Survival in Rats

Hypotheses	Males		Females	
	Logrank	Wilcoxon	Logrank	Wilcoxon
Homogeneity over all four groups	0.5181	0.4880	0.1189	0.0656
No Trend over all four groups	0.3716	0.4084	0.0200	0.0095
No difference between high dose and vehicle	0.7931	0.8753	0.0444	0.0380

Figure A.1.1, in Appendix 1, the Kaplan-Meier estimated survival curves in male rats suggest that during the last third of the study the low dose group had slightly higher survival than the other three study groups, which in turn, were largely intertwined. However, none of the comparisons in male rats would be categorized as being statistically significant (i.e., all six $p \geq 0.3716$). In almost a reversal of fate, in Figure A.1.2, in female rats, the the high dose group seems to have higher survival than the other study groups, but again with the remaining dose groups largely intertwined. These differences are not sufficient to result in a statistically significant, though close to significance, test of overall homogeneity (Logrank p=0.1189, Wilcoxon p=0.0656). However, there is evidence of a statistically significant test of trend in dose (Logrank p=0.0200, Wilcoxon p=0.0095), and a somewhat weaker result in the test of differences between the high dose and vehicle control (Logrank p=0.0444, Wilcoxon p=0.0380).

Similar results in mice are presented below.

Table 4. Statistical Significances of Tests of Homogeneity and Trend in Survival in Mice

Hypotheses	Males		Females	
	Logrank	Wilcoxon	Logrank	Wilcoxon
Homogeneity over all four groups	0.0002	0.0001	<0.0001	<0.0001
No Trend over all four groups	0.0100	0.0104		<0.0001
No difference between high dose and	0.0080	0.0083		<0.0001

In particular, from the Kaplan-Meier plot in Figure A.1.3. in male mice the vehicle control has, by a considerable extent, the lowest survival, with eventually the medium group having the highest survival, and the low and high dose groups eventually largely intertwined between these two curves. Note these differences are sufficient to result in statistically significant differences in tests for homogeneity in both genders (both $p \leq 0.0002$), as is the test of trend in dose (both $p \leq 0.0104$), as wells as the test of no differences in the high and control low doise u. Similarly, the comparison between the high dose and vehicle test of was statistically significant (both $p \leq 0.0083$). In female mice the high dose groups have much higher mortality than the other study groups, resultin in highly statistically significant tests of homogeneity, trend, and sifference between high dose and control (all six $p < 0.0001$). Note that even if the most conservative adjustment for multiplicity were applied the results in female rats would still be highly significant.

Typically a large number of tumors are identified in the analysis of neoplasms, implying a large number of statistical tests. Following the frequentist paradigm, when interpreting significance levels (i.e., p-values), one can use the Haseman-Lin-Rahman (HLR) rules to adjust for the multiplicity of tests. Two approaches have been investigated, one for testing dose related trend and pairwise comparison between the high dose and control seperately and the other these hypotheses jointly (please see Section 1.3.1.5, below, for details). Usual statistical practice would be to test these hypotheses separately, but some scientists want to control Type I error only when simultaneously testing both the trend and pairwise hypotheses. That is, in the two year study, when testing for trend over dose and, separately, the difference between the highest dose group with a control group, to control the overall Type I error rate for the joint tests in a two species submission to roughly 10%, one compares the unadjusted significance level of the trend test to 0.005 for common tumors and 0.025 for rare tumors, and the pairwise test to 0.01 for common tumors and 0.05 for rare tumors. For the testing these hypothese jointly for common tumors one compares the unadjusted significance level of the trend test to 0.005 and the pairwise test to 0.05, and for rare tumors 0.025 for tests of trend and 0.10 the pairwise comparison. Using these adjustments for other tests, like testing the comparisons between the Low and Medium dose groups versus vehicle can be expected to increase the overall type I error rate to

some value above the nominal rough 10% level, possibly considerably higher than the nominal 10% rate. .

Table 5 and 6 below show the tumors in rats and mice that had at least one non-multiplicity adjusted test that was statistically significant at or close, to a 0.10 level (or contributed to a significant test). For each tumor-organ combination the tumor incidence over the four dose groups is listed first, followed by the significance levels of the overall test of trend over all four dose groups, and finally the comparison of the high, medium and low dose groups with vehicle.

Table 5. Potentially Statistically Significant Results for Organ-Tumor Combinations in Rats

Gender	Overall Results Tumor Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Male Rats								
Testis								
# Evaluated	60	60	60	60				
Adj. # at Risk	45.8	50.1	48.1	46.3				
LEYDIG CELL TUMOR	2	0	2	5	.0226	.2264	.7157	1
Thyroid								
# Evaluated	58	57	56	59				
Adj. # at Risk	44.9	47.4	46.4	45.6				
CARCINOMA,C-CELL	0	0	2	2	.0705	.2528	.2584	.
Female Rats								
Adrenal								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.4	42.4	49.5				
PHEOCHROMOCYTOMA	0	0	1	3	.0261	.1467	.4941	.
Adj. # at Risk	43.3	41.4	42.4	49.5				
Pheochromocytoma, Any	0	1	1	3	.0590	.1467	.4941	.4881
Thyroid								
# Evaluated	59	59	59	59				
Adj. # at Risk	42.5	40.9	42.9	49.5				
ADENOMA, C-CELL	1	5	5	7	.0999	.0478	.1008	.0899

Using the tumor incidence in the vehicle to determine whether a tumor should be classified as rare or common, only c-cell carcinoma and pheochromocytoma (both above) were classified as rare tumors, the remainder common. Although some of

these tumors exceed the 0.10 level, after adjusting for multiplicity using the Haseman-Lin-Rahman rules only the test of trend for Pheocromocytoma is close to statistical significance (p = 0.0261 ≈ 0.025). Complete tables of tumor incidence are given in Tables A.2.2 and A.2.3, in Appendix 2, below.

Similar results in mice are presented in Table 6, below.

Table 6. Potentially Statistically Significant Results for Organ-Tumor Combinations in Mice

organ/tumor	Overall Results Tumor Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Male Mice								
Testis								
# Evaluated	55	55	55	55				
Adj. # at Risk	42.4	51.5	52.0	49.0				
LEYDIG CELL TUMOR	0	0	1	2	.0644	.2816	.5484	.
Thyroid								
# Evaluated	55	55	55	55				
Adj. # at Risk	42.4	51.5	52.0	49.0				
ADENOMA,FOLLICULAR CELL	0	0	1	2	.0644	.2816	.5484	.
Adj. # at Risk	42.4	51.5	52.0	49.0				
Foll. Cell Adenoma/Carcinoma	0	0	2	2	.0749	.2816	.3034	.
Female Mice								
Thyroid								
# Evaluated	55	55	55	55				
Adj. # at Risk	52.4	52.8	52.2	41.1				
ADENOMA,FOLLICULAR CELL	1	1	1	3	.0889	.2245	.7524	.7524

Only the tests of trend above show significance levels below 0.10 (i.e. 10%). Using the tumor incidence in the vehicle to determine whether a tumor should be classified as rare or common, only follicular cell adenoma would be classified as common, the remaining tumors as rare. But then, after adjusting for multiplicity using the Haseman-Lin-Rahman rules, no tests would be categorized as statistical significant.

Complete tables of tumor incidence are given in Tables A.2.5 and A.2.6, in Appendix 2, below.

1.2. Brief Overview of the Studies

Two studies were submitted, conducted at (b) (4) :

Study No. (b) (4) 5940: Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats.

and,

Study No. (b) (4) 5939: Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice.

These studies were designed to assess the carcinogenic potential of Uptravi (Selexipag). In both studies, the actual dose groups were labeled in this report as the Low, Medium, and High dose groups, respectively, plus the Vehicle control group.

1.3. Statistical Issues and Findings

1.3.1. Statistical Issues

In this section, several issues, typical of statistical analyses of these studies, are considered. These issues include comments on the details of the survival analyses, tests on tumorigenicity, multiplicity of tests on neoplasms, and the validity of the designs.

1.3.1.1. Survival Analysis:

The survival analyses presented here are based on both the log rank test and the Wilcoxon test comparing survival curves. The Wilcoxon statistic provided by SAS® (technically the Gehan-Wilcoxon statistic) can be cast as a log rank test weighted by the number of subjects at risk, and thus is more sensitive to earlier differences (when more subjects are at risk). The logrank test is most powerful when the survival curves track each other, and thus the hazards, i.e., the conditional probability of the event in the next infinitesimal interval, would be roughly proportional. Note the logrank test seems to be the test usually recommended by statisticians, and is one of the tests used by the

Sponsor (in addition to the Tarone's test). Both the logrank and the Wilcoxon tests are used in the FDA analysis of mortality.

Appendix 1 reviews the specific FDA animal survival analyses in more detail. The results of the Sponsor's analysis are summarized in Sections 3.2.1.1 and 3.2.2.1.

1.3.1.2. Multiplicity of Tests on Survival:

Using both the logrank and Wilcoxon tests, for each gender in rats and, ignoring the positive control, mice there are six tests of survival differences in each gender in each species. Assuming tests were performed at the usual 0.05 level, and the tests were stochastically independent, but there were actually absolutely no differences in survival across groups (so one would hope no tests would be statistically significant), the probability of at least one statistically significant result in each gender in each species was about 0.2649 in rats and 0.708 in both genders in both species. These bounds assume the tests are stochastically independent, which they clearly are not, but these values can give some idea of the possible price paid for the multiplicity of hypothesis tests in the statistical frequentist paradigm. Further general comments on adjusting for the multiplicity of tests are presented in Section 1.3.1.4 below.

1.3.1.3. Tests on Neoplasms:

Sponsors are requested to provide data in either SEND (Standard for the Exchange of Nonclinical Data) format, part of the CDISC consortium, or in the older FDA Biometrics format. Data from both studies fit the latter format. The FDA Biometrics format data sets requested for the analysis of rodent carcinogenicity studies are supposed to include a record for each animal organ combination that was not evaluated. If a number of the animals are not examined, but the proportions of animals showing the tumor under study in each treatment group is roughly the same as in the subset of animals actually reported the calculated p-values will generally be too large, i.e., results will be less statistically significant than they should be, possibly much less

The Sponsor's analyses of tumorigenicity in both species are Peto tests, with incidental and fatal plus mortality independent tumors. Note that Peto methods require accurate determination of whether a tumor is fatal or incidental. In both species survival was generally consistent across study dose groups. .

The FDA analysis in both species is based on a modification of the Cochran-Armitage test of trend (please see Bailer & Portier, 1988, Bieler & Williams, 1993), adjusted for differential mortality. Inspecting a large number of studies, Bailer and Portier noted that survival time seemed to fit a Weibull distribution, generally with a shape parameter of between 1 and 5, with 3 a typical value. With t_{max} denoting the maximal time to terminal sacrifice and t_{obs} the time to detection of the tumor in the animal, they proposed weighting the animal by $(t_{obs}/t_{max})^k$, so that an animal that survives for say 52 weeks in 104 week study without the tumor being analyzed is counted as $(1/2)^k$ of an animal in the risk set for that tumor. For k = 3, that means that particular animal would count as 1/8 of an animal. Further, the k = 3 specification seems to represent tumor incidence where some animals are perhaps more sensitive

and respond earlier to the insult than the remining animals. Under this structure time to incidence would tend to follow a cubic expression. Thus an animal with the specific tumor being studied or who survives to terminal sacrifice without the tumor will be given a weight of 1 when counting the number of animals at risk. However, animals that die early without the tumor are down weighted when counting the number of animals in the risk set for that specific tumor. With differential mortality, as in male mice, this can mean a substantial reduction in the size of that risk set. Note this seems to be an appropriate adjustment for dose groups that are terminated early. The report of the Society of Toxicological Pathology "town hall" meeting in June 2001 recommeded the use of this poly-k modification of the so-called Cochran-Armitage tests of trend over the corresponding Peto tests used by the Sponsor.

The computed significance levels are based on small sample exact permutation tests of tumor incidence. In the tumor incidence tables the effective size of the risk set for each tumor is listed in the row labeled "Adjusted # at risk ", and seems to be a more appropriate denominator when comparing incidence rates than the simple unadjusted number evaluated.

1.3.1.4. Multiplicity of Tests on Survival and Neoplasms:

In each species and gender combination there were tests of homogeneity in survival or tumorigenicity over dose, tests of trend in survival, and comparisons of the high (and possibly other) dose to vehicle control. The individual p-values for hypothesis test are based on controlling the probability of rejecting a true null hypotheses in each seperate test. There are a number of ways of controlling the overall error of rejecting any true null hypothesis among a set of such hypotheses. The most conservative test is based on so called Bonferroni comparisons where the individual p-value is divided by the number of comparisons. While experimenting with other approaches, current FDA practice in testing tumorigenicity is usually based on the Haseman-Lin-Rahman multiplicity adjustments..

The Haseman-Lin-Rahman rules are based on the original multiplicity adjustment of Haseman (1983) and extended by Lin and Rahman on the basis of various simulations. Based on his extensive experience with such analyses, for pairwise tests in a two species study comparing control to the High dose group, Haseman (1983) claimed that for a roughly 0.10 (10%) overall false positive error rate, rare tumors should be tested at a 0.05 (5%) level, and common tumors (with a historical control incidence greater than 1%) at a 0.01 level. Lin & Rahman (1998) proposed a further p-value adjustment for tests of trend. That is, for a roughly 0.10 (10%) overall false positive error rate in tests of trend, rare tumors should be tested at a 0.025 (2.5%) level and common tumors at a 0.005 (0.5%) level. The general specifications are presented in the Table 4 below. This approach is intended to balance both Type I error and Type II error (i.e., the error of concluding there is no evidence of a relation to tumorgenicity when there actually is such a relation).

The proposed Haseman-Lin-Rahman bounds are taken from *Guidance for Industry Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals*, (HHS, 2013). The bounds on the right in table 7, below, are grouped so that the last four columns correspond to testing both trend and the pairwise comparison between the high dose and control jointly. The first four columns (columns 2-5), correspond to testing both overall trend and pairwise tests between the high dose and control seperately. Within each group there is a column giving the corresponding bounds for a two year, one species study, and another column for the alternate 6-month study. In this analysis we follow the usual practice of testing parameters separately, so the bounds in the leftmost columns are used. The observed tumor incidence in the vehicle group is used to decide if a tumor is classified rare or common.

Table 7. Recommended Multiplicity Adjusted Bounds on Significance Levels

Tests	Separate testing of trend and pairwise differences		Joint testing of trend and pairwise Differences	
	Trend	Pairwise	Trend	Pairwise
Common Tumor	0.005	0.01	0.005	0.05
Rare Tumor	0.025	0.05	0.025	0.10

In words, as noted in the FDA Guidance (2013) “For tests for positive trend alone, it is recommended that common and rare tumors are tested at 0.005 and 0.025 significance levels, respectively, in the two-year study …

“For [the] control-high pairwise comparison alone, it is recommended that common and rare tumors are tested at 0.01 and 0.05 significance levels, respectively, in the two-year study .. .

“For tests for positive trend and control-high pairwise comparison jointly, it is recommended that common and rare tumors are tested at 0.005 and 0.025 significance levels, respectively in trend test, and at 0.05 and 0.10 significance levels, respectively, in control-high pairwise comparison in the two-year study . . . ” (page 32 of 2013 Guidance)

The significance levels of the pairwise tests between the vehicle control with the Low and Medium dose groups are also provided in the tumor analysis tables below. Following the HLR rules, adding these comparisons can be expected to increase the overall type I error rate to some level above the usual rough 10% level, possibly considerably larger. Again, because of the possibility of genetic drift and for convenience, incidence in the vehicle group is used to determine if the tumor is classified as rare or common.

1.3.1.6. Validity of the Designs:

When determining the validity of designs there are two key points:
1) adequate drug exposure
2) tumor challenge to the tested animals.

1) is related to whether or not sufficient animals survived long enough to be at risk of forming late-developing tumors and 2) is related to the Maximum Tolerated Dose (MTD), designed to achieve the greatest likelihood of tumorigenicity.

Lin and Ali (2006), quoting work by Haseman, have suggested that in standard laboratory rodent species, a survival rate of about 25 animals, out of 50 or more animals (i.e. 50%), between weeks 80-90 of a two-year study may be considered a sufficient number of survivors as well as one measure of adequate exposure. From tables 14 and 15 in Section 3.2.1.2 and tables 14 and 15 in Section 3.2.1.2 below, as a percentage of the High dose group animals that survived to week 91, this criterion is considerably exceded in both genders (Male rats high dose: 71.7% and Female rats: 75.0%, Male mice high dose: 87.3% and Female mice: 61.8%). This may be interpreted as evidence that the MTD was not achieved in both genders in both species. However, such a determination requires the expertise of the toxicologist.

Tables 8 through 11 below indicate the weight changes in those animals that survived to near the end of each study. Chu, Ceuto, and Ward (1981), citing earlier work by Sontag et al. (1976) recommend that the MTD "is taken as 'the highest dose that causes no more than a 10% weight decrement as compared to the appropriate control groups, and does not produce mortality, clinical signs of toxicity, or pathologic lesions (other than those that may be related to a neoplastic response) that would be predicted to shorten the animal's natural life span' " From Tables 8 through 11 below, it seems that the weight criterion is exceded in all dose groups in both genders in both species. This also may be further evidence that the MTD was exceded in species, but such a determination requires the expertise of the toxicologist.

Table 8. Mean Weights and Changes (in g) in Male Rats

Dose Group	Dose mg/kg/day	N	Day		Change from Baseline	% change relative to vehicle
			0	700		
1. Vehicle	0	34	236.0	842.4	606.5	
2. Low	10	41	238.2	771.8	533.5	88.0%
3. Medium	30	36	238.0	681.3	443.3	73.1%
4. High	100	31	236.43	627.75	391.3	64.5%

Table 9. Mean Weights and Changes (in g) in Female Rats

Dose Group	Dose mg/kg/day	N	Day 0	Day 700	Change from Baseline	% change relative to vehicle
1. Vehicle	0	27	164.0	542.3	378.4	
2. Low	10	26	159.7	479.5	319.8	84.5%
3. Medium	30	27	161.7	427.0	265.3	70.1%
4. High	100	37	162.3	395.5	233.2	61.6%

The Sponsor's rat report notes the following:
"1) Male
In the 10 mg/kg group, statistically significant lower values for body weight were observed
from Week 18 (Day 126) to the end of the administration period and mean body weight at the
end of the administration period was 10% lower than that in the control group.
"In the 30 mg/kg group, lower values for body weight were observed from Week 1 (Day 7) to
the end of the administration period and statistically significant differences were recorded at
almost all measured points. Mean body weight at the end of the administration period was 21%
lower than that in the control group.
In the 100 mg/kg group, statistically significant lower values for body weight were observed
from Week 1 (Day 7) to the end of the administration period and mean body weight at the end
of the administration period was 25% lower than that in the control group.

"2) Female
In the 10 mg/kg group, lower values for body weight were observed from Week 14 (Day 98) to
the end of the administration period and statistically significant differences were recorded at
many measured points. Mean body weight at the end of the administration period was 11%
lower than that in the control group.
In the 30 mg/kg group, lower values for body weight were observed from Week 8 (Day 56) to
the end of the administration period and statistically significant differences were recorded at
almost all measured points. Mean body weight at the end of the administration period was 19%

lower than that in the control group.
In the 100 mg/kg group, statistically significant lower values for body weight were observed in
Week 1 (Day 7) and from Week 6 (Day 42) to the end of the administration period, and mean
body weight at the end of the administration period was 27% lower than that in the control
group. (pages 39-40)

At least one way to indicate results in mice is as follows:

Table 10. Mean Weights and Changes (in g) in Male Mice

Dose Group	Dose mg/kg/day	N	Day		Change from Baseline	% change relative to vehicle
			0	700		
1. Vehicle	0	29	24.7	40.6	15.9	
2. Low	125	43	24.6	38.6	14.0	88.1%
3. Medium	250	49	24.7	36.3	11.6	73.0%
4. High	500	41	24.6	34.8	10.2	64.2%

Table 11. Mean Weights and Changes (in g) in Female Mice

Dose Group	Dose mg/kg/day	N	Day		Change from Baseline	% change relative to vehicle
			0	630		
1. Vehicle	0	50	19.6	37.4	17.8	
2. Low	125	51	19.6	34.1	14.5	81.5%
3. Medium	250	50	19.6	32,4	12.8	71.9%
4. High	500	34	19.5	31.4	11.9	66.9%

The Sponsor's mice report notes the following:
"1) Male
In the 125 mg/kg group, statistically significant lower values for body weight were observed
from Week 10 (Day 70) to the end of the administration period and mean body weight at the
end of the administration period was 5% lower than that in the control group.
In the 250 mg/kg group, statistically significant lower values for body weight were observed
from Week 9 (Day 63) to the end of the administration period and mean body weight at the end
of the administration period was 11% lower than that in the control group.

In the 500 mg/kg group, statistically significant lower values for body weight were observed in
Week 1 (Day 7) and from Week 9 (Day 63) to the end of the administration period and mean
body weight at the end of the administration period was 14% lower than that in the control
group.

"2) Female
In the 125 mg/kg group, lower values for body weight were observed from around Week 26
(Day 182) to the end of the administration period and statistically significant differences were
recorded at almost all measured points. Mean body weight at the end of the administration
period was 8% lower than that in the control group. Although statistically significant
differences were observed in Weeks 5 (Day 35) and 8 (Day 56), they were judged to be
incidental, since they were temporary and minimal.
In the 250 mg/kg group, lower values for body weight were observed from around Week 4
(Day 28) to the end of the administration period and statistically significant differences were
recorded at many measured points. Mean body weight at the end of the administration period
was 11% lower than that in the control group.
In the 500 mg/kg group, lower values for body weight were observed from around Week 4
(Day 28) to the end of the administration period (Day 697) and statistically significant
differences were recorded at many measured points. Mean body weight at the end of
the administration period was 14% lower than that in the control group." (pages 39-40 of mouse report)

The Sponsor also report indicate that only the 10 mg/kg dose in rats was associated with food consumption comparable to the vehicle control, with lower consumption in the medium (30 mg/kg) and high (100 mg/kg) dose groups. In mice the higher dose groups were described as having lower food consumption during the first half or more of the study.

Again from 2) above, excess mortality not associated with any tumor or sacrifice in the higher dose groups might suggest that the MTD was exceeded. This suggests that a potentially useful way to assess whether or not the MTD was achieved is to measure early mortality not associated with any identified tumor. If this mortality is related to dose, it suggests that animals tend to die before having time to develop

tumors. From the table below it seems that in rats there is no particular evidence of heterogeneity across dose groups.

Table 12. Natural Death with No Identified Tumor in Rats (Male/Female)

	1.Vehicle	2. Low	3.Medium	4.High
Males Event	5	3	4	11
No event	55	57	56	49
Females Event	1	1	1	3
No event	59	59	59	57

The apparent lack of homogeneity in natural death without tumor is confirmed the results of Fisher exact tests of homogeneity (Males p = 0.0004 and Females p = 0.0297). This does seem to suggest that the high dose may have been above the MTD, however to be conclusive this observation requires the expertice of the toxicologist.

Table 13. Natural Death with No Identified Tumor in Mice (Male/Female)

	1.Vehicle	2. Low	3.Medium	4.High
Males Event	12	4	4	6
No event	43	51	51	49
Females Event	1	4	3	32
No event	54	51	52	23

There also seems to be an apparent lack of homogeneity in natural death without tumor in mice, although it is structurally different than the simple results in mice. In male mice the difference seems to be largely due to early deaths in the vehicle control, while in female mice has many more deaths without tumor in the high dose group. This is confirmed the results of Fisher exact tests of homogeneity (Males p = 0.0773 and Females p < 0.0001). This does seem to suggest that at least in female mice the high dose may have been above the MTD, however, again, such a conclusion requires the expertice of the toxicologist

1.3.2. Statistical Findings

Please see Section 1.1 above.

2. INTRODUCTION

2.1. Overview

The Sponsor's reports summarize results from two two-year studies, one in rats, and the other in mice, both with daily gavage, to assess the carcinogenic potential of Uptravi (Selexipag) in the Sponsor's reports.

2.2. Data Sources

SAS data sets for both species, following the requested FDA Biostatistcs format, both labeled tumor.sas7bdat, plus were translated from SAS transport files both labeled tumor.xpt. In addition both studies included SAS data sets labeled as food.sas7bdat and weights.sas7bdat as translated from the corresponding SAS transport files.

3. STATISTICAL EVALUATION

3.1. Evaluation of Efficacy

NA

3.2. Evaluation of Safety

More detailed results on the studies in rats and mice are presented below.

3.2.1. Study No. (b) (4) 5940: Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats.

CRO: (b) (4)
STUDY DURATION: 104 Weeks
DOSING STARTING DATE: December 13, 2007
STUDY COMPLETED (Terminal Necropsy): Males: December 11 and 14, 2009
Females: December 10, 2009. :
RAT STRAIN: Crl:CD(SD) Rats
ROUTE: Daily Oral gavage

The Sponsor's report describes the study as follows: "Two hundred and ninety-nine (299) male and 299 female Crl:CD(SD) SPF rats ((b) (4)), at 5 weeks of age, were obtained on December 3, 2007. . . . The animals were quarantined and acclimatized for 10 days, and clinical observations (once a day) and measurement of body weight (3 times) were conducted. In addition, ophthalmological examination was conducted once on all animals. Based on the above examinations, healthy animals showing normal body weight gains and no abnormal clinical signs were selected and used at 6 weeks of age. During the

quarantine/acclimatization period, 1 female showed excoriation and was removed from animal allocation. Except this, no observable abnormalities were found in clinical observations or body weight; however, the animals with ophthalmological abnormalities were removed from the present study . . . .

"After animal selection, the animals were ranked by individual body weight on the day of animal allocation (2 days before the start of administration). Then the animals were assigned to each group so as to ensure the homogeneity of group means for body weight. This procedure was done using a computer by block placement and randomization methods (the appropriate number of groups was composed by block placement method, and test groups and animal numbers in each test group were randomly assigned).

"Two hundred and forty (240) animals of each sex were provided for a main group to evaluate toxicity and 29 animals of each sex were provided for a satellite group to evaluate toxicokinetics. Individual body weight on the starting day of administration ranged from 212 to 269 g in males and 142 to 184 g in females for the main group, and that for the satellite group ranged from 220 to 268 g in males and 145 to 184 g in females.

"Of the animals remained after animal allocation, 12 animals of each sex (total of 5 planned animals and 7 reserve animals) were reserved as monitor animals for microbiological test. Other animals were excluded from the study and they were used for collection of TK matrix.
"note: The number of animals ordered according to the protocol was 290 of each sex, but 299 of each sex" (pages 21-22 of rat report)

Gross aspects of the study designs for the main study animals are summarized in Table 14 be1ow (a repeat of Table 1 above):

Table 14. Design of Rat Study (dose volume 5 mL/kg/day)

Treatment Group	Animals / Gender	Nominal Dose (mg/kg)	Concen-tration (mg/kg)
1. Vehicle[1]	60 (5)	0	0
2. Low	60 (8)	10	2
3. Medium	60 (8)	30	6
4. High	60 (8)	100	20

The Sponsor's report indicates that dose levels were based upon results: "In a 26-week repeated dose toxicity study of NS-304 in rats with 4-week recovery period ([redacted] (b) (4), Study No. [redacted] (b) (4) 5895, dosage levels: 0, 6, 25 and 100

mg/kg/day) …, suppression of body weight gain (male: -19%, female: -6%) and increased incidence or severity of diffuse acinar cell hyperplasia in the mammary gland was observed in females in the 100 mg/kg group. Therefore, the high dose level in the present study was set at 100 mg/kg/day and the middle and low dose levels were set at 30 and 10 mg/kg/day, respectively. There were 4 test groups including a control group for each sex. Sixty (60) animals of each sex were provided for each test group as the main group. In addition, in each group, another 5 (control group) or 8 (dose groups) animals of each sex served as a satellite group for determination of plasma drug concentrations." (pages 23-24 of rat report)

Animals were housed individually with water available ad libitum. The Sponsor states that all animals were observed for clinical signs three times a day.

3.2.1.1 Sponsor's Results and Conclusions

This section will present a summary of the Sponsor's analysis on survivability and tumorigencity in rats.

Survival analysis:

The Sponsor's conclusions on survival in rats are summarized as follows:
"1) Male
There were no effects of NS-304 on the survival rate.
"2) Female
A statistically significant trend of an increase in the survival rate with increasing dose levels was
observed, and the survival rate of the 100 mg/kg group was significantly higher than that of the control group." (page 36 of rat report)

Table 15: Sponsor's "Text Table 1. Summary of mortality and survival rate"

Sex	Male				Female			
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of animals used	60	60	60	60	60	60	60	60
Week of administration								
1-26	0	1	0	1	0	0	0	0
1-52	5	3	2	3	2	0	2	1
1-78	11	7	8	10	10	16	13	4
1-105[a)]	29	26	26	31	39	39	36	29
No. of survivors	31	34	34	29	21	21	24	31
Survival rate (%)	51.7	56.7	56.7	48.3	35.0$	35.0	40.0	51.7*

Values in the table indicate the cumulative number of animals that died or were sacrificed as moribund.
$: p<0.05 (statistically significant trend, Tarone.s test)
*: p<0.05 (statistically significant difference from control group, log-rank test)
a): All surviving animals were necropsied in Week 105 after administration for 104 weeks."
(page 36 of rat report)

Tumorigenicity analysis:

The Sponsor's report states that: "Tumors that occurred with high incidence (10 or more animals in total for each sex, >10) were evaluated using survival-adjusted Peto's test11) to assess

increasing trend of incidence to dose level for all groups and to compare the incidence between the control group and each dose group. Tumors that occurred with low incidence (less than 10 animals in total for each sex, <10) were evaluated using exact permutation trend test to assess increasing trend of incidence to dose level for all groups and to compare the incidence between the control group and each dose group. For incidental tumors, the analysis intervals were: weeks 0 through 52, tumors and 0.025 (one tailed-level) for rare tumors. Pairwise comparison was conducted at the significance levels of 0.01 (one tailed-level) for common tumors and 0.05 (one tailed-level) for rare tumors. Common tumors were defined as those with a historical incidence in controls (Crl:CD(SD) rats) at [redacted] (b) (4) exceeding 1% (>1%) and rare tumors as 1% or less (<1%)," (pages 34-35 of rat report)

The overall count of tumors is summarized in the following Table 16 (Text Table 11):

Table 16: Sponsor's "Text Table 11. Number of tumors and tumor bearers"

Sex	Male				Female			
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of animals used	60	60	60	60	60	60	60	60
Total No. of tumors	121	124	91	84	159	153	128	125
No. of benign tumors	102	107	70	69	104	94	95	79
No. of malignant tumors	19	17	21	15	55	59	33	46
Total No. of tumor bearing animals	52	55	47	43	56	57	59	52
No. of benign tumor bearers	46	52	37	38	52	52	57	46
No. of malignant tumor bearers	18	17	18	13	29	28	23	27
No. of multiple tumor bearers	31	34	23	20	41	37	39	36

"There was no treatment-related increase in either number of tumors or tumor bearing animals in
either sex.

"A decrease in the number of benign tumors was observed in males in the 30 and 100 mg/kg
groups. Decreases in the total number of tumors, number of malignant tumor bearing animals
and number of multiple tumor bearing animals were observed in males in the 100 mg/kg group.
However, they were not regarded as an adverse effect of treatment, as these changes were decreases." (page 45 of rat report)

"Tumor[s] that showed a increased incidence in dose groups as compared to the control group were
observed in the testis and pituitary and incidences of testicular tumor and focal hyperplasia and
pituitary tumor are summarized in the following . . . :"

Table 17.Sponsor's"Text Table 12. Incidence summary of major tumors and hyperplasias"

Sex	Male				Female			
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of animals used	60	60	60	60	60	60	60	60
Testis								
Leydig cell tumor	2$a)	0	2	5	NA	NA	NA	NA
Hyperplasia, Leydig cell, focal (total, ±/+)	0	2	2	6	NA	NA	NA	NA
Pituitary								
Adenoma, anterior	20	40**	23	22	49	44	44	38

Numbers in the table indicate the number of animals with lesions.
$: p<0.025 (statistically significant positive trend, rare tumor, Peto.s test)
**: p<0.01 (statistically significant difference from the control group, common tumor, Peto.s test)
a): There was no statistically significant positive trend in the control, 10 and 30 mg/kg groups.
±: Minimal, +: Mild, NA: Not applicable" (page 45 of rat report)

The Sponsor's report further summarizes results as:

"Testis:
"Marginally increased incidence of Leydig cell tumor was observed in the 100 mg/kg group, and statistically significant positive trend was noted (rare tumor, p<0.025); however, there was no statistical significance in pairwise comparison between the control and 100 mg/kg groups. Also, slightly higher incidence in focal hyperplasia of Leydig cells was observed in this
group, although the difference in incidence between the two groups was very small. The tumor incidence of the 100 mg/kg group (5/60 animals, 8%) was marginally higher than that in our historical data (0 to 4% in the incidence). In addition, there was no statistically significant positive trend in incidence of Leydig cell tumor in the control, 10 and 30 mg/kg groups.

"In the pituitary, increased incidence of anterior adenoma was observed in males in the 10 mg/kg
group with statistical significance (common tumor, p<0.01). However, it was not considered to
be treatment related, since it was not dose-related.

"The other tumors . . . were judged to be incidental since they were consistent with the spectrum of spontaneous tumors expected in aged rats of this strain." (pages 46-47 of rat report)

3.2.1.2 FDA Reviewer's Results

This section will present the current Agency findings on survival and tumorigenicity in male and female rats.

Survival analysis:

Kaplan-Meier plots comparing treatment groups in both studies are given in Appendix 1, along with more details of the analyis. The following tables (Table 18 for male rats, Table 19 for female rats) summarize the mortality results for the dose groups. The data were grouped for the specified time period, and present the number of deaths

during the time interval over the number at risk at the beginning of the interval. The percentage cited is the percent survived at the end of the interval.

Table 18. Summary of Male Rats Mortality (dose/kg/day)

Period	1.Vehicle	2.Low	3.Medium	4.High
0-50	5/60 91.7%	3/60 95.0%	2/60 96.7%	3/60 95.0%
51-70	2/55 88.3%	2/57 91.7%	2/58 93.3%	5/57 86.7%
71-90	10/53 71.7%	6/55 81.7%	13/56 71.7%	9/52 71.7%
91-105	12/43 51.7%	13/49 60.0%	9/43 56.7%	14/43 48.3%
terminal	31	36	34	29

[1] number deaths / number at risk
[2] per cent survival to end of period.

Table 19. Summary of Female Rats Mortality (dose/kg/day)

Period	1.Vehicle	2.Low	3.Medium	4.High
0-50	2/60 96.7%	0/60 100.0%	2/60 96.7%	1/60 98.3%
51-70	7/58 85.0%	8/60 86.7%	4/58 90.0%	2/59 95.0%
71-90	11/51 66.7%	16/52 60.0%	21/54 55.0%	12/57 75.0%
91-105	19/40 35.0%	15/36 35.0%	9/33 40.0%	14/45 51.7%
terminal	21	21	24	31

[1] number deaths / number at risk
[2] per cent survival to end of period.

Kaplan-Meier survival curves for the rat study are presented in Appendix 1. The results of statistical tests of differences in survival are given below (a repeat of Table 3):

Table 20. Statistical Significances of Tests of Homogeneity and Trend in Survival in Rats

Hypotheses	Males		Females	
	Logrank	Wilcoxon	Logrank	Wilcoxon
Homogeneity over all four groups	0.5181	0.4880	0.1189	0.0656
No Trend over all four groups	0.3716	0.4084	0.0200	0.0095
No difference between high dose and		0.8753		0.0380

vehicle	0.7931		0.0494	

From Figure A.1.1 in the appendix, in male rats the Kaplan-Meier estimated survival curves are largely intertwined, consistent with no tests of differences in survival being close to statistical significance. From Figure A.1.2 survival in female, the vehicle and low dose groups track each other closely with the higher survival than the high and medium dose groups. These differences were statistically significant (Logrank p= 0.0204, Wicoxon p= 0.0225). The high and medium dose groups track each other somewhat closely, but with some tendency for higher survival in the high dose group. No other tests or comparisons quite reached the usual 0.05 level of statistical significance.

Tumorigenicity analysis:

Table 21 below, a repeat of Table 6 above and Table A.2.1 below, shows the tumors in rats that had at least one non-multiplicity adjusted test that was statistically significant at a 0.10 or lesser level . For each tumor-organ combination the tumor incidence over the four dose groups is listed first, followed by the significance levels of the overall test of trend over all four dose groups, and finally the comparison of the high, medium and low dose groups with vehicle. .

Table 21. Potentially Statistically Significant Results for Organ-Tumor Combinations in Rats

Gender Organ/Tumor	Overall Results Tumor Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Male Rats								
Testis								
# Evaluated	60	60	60	60				
Adj. # at Risk	45.8	50.1	48.1	46.3				
LEYDIG CELL TUMOR	2	0	2	5	.0226	.2264	.7157	1
Thyroid								
# Evaluated	58	57	56	59				
Adj. # at Risk	44.9	47.4	46.4	45.6				
CARCINOMA,C-CELL	0	0	2	2	.0705	.2528	.2584	.
Female Rats								
Adrenal								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.4	42.4	49.5				
PHEOCHROMOCYTOMA	0	0	1	3	.0261	.1467	.4941	.
Adj. # at Risk	43.3	41.4	42.4	49.5				
Pheochromocytoma, Any	0	1	1	3	.0590	.1467	.4941	.4881
Thyroid								

```
        # Evaluated            59   59   59   59
        Adj. # at Risk         42.5 40.9 42.9 49.5
        ADENOMA, C-CELL         1    5    5    7   .0999  .0478
.1008  .0899
```

Using the tumor incidence in the vehicle to determine whether a tumor should be classified as rare or common, only c-cell carcinoma and pheochromocytoma (both above) were classified as rare tumors, the remainder common. Although some of these tumors exceed the 0.10 level, after adjusting for multiplicity using the Haseman-Lin-Rahman rules only the test of trend for Pheocromocytoma is close to statistical significance (p = 0.0261 ≈ 0.025).

Complete tables of tumor incidence are given in Tables A.2.2 and A.2.3, in Appendix 2, below.

3.2.2 Study No. (b) (4) 5939: Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice.

CRO: (b) (4)
STUDY DURATION: Up to 104 Weeks
DOSING STARTING DATE: December 28, 2007
STUDY COMPLETED: February 9. 2011
MOUSE STRAIN: SB6C3F1/Crlj SPF mice
ROUTE: Daily Oral gavage

The Sponsor's report indicates that in the mouse study: "Carcinogenicity of NS-304 (prostaglandin I2 agonist) was evaluated in B6C3F1/Crlj mice. NS-304 was administered orally by gavage to male and female mice (55/sex/group for a main group, 6 weeks of age at the start of treatment) for 2 years. The dosage levels of NS-304 were set at 0 (0.5 w/v% methylcellulose solution), 125, 250 and 500 mg/kg/day. Since the survival rate in females in the 500 mg/kg group decreased with the progression of the administration period, all surviving females in this group were prematurely sacrificed in Week 100 after administration for 99 weeks. Further, systemic exposure to NS-304 was evaluated by determining plasma NS-304 and its metabolite, MRE-269 concentrations on the satellite animals." (page 14 of mouse report)

General aspects of the study design for the mice study are also summarized in Table 18 be1ow (a repeat of Table 2 above):

Table 22. Design of Mice Study (Volume 10 mL/kg)

Treatment Group[1]	# Main study animals (# toxicology study animals)/gender	Dose (mg/kg/day)	Concentration (mg/mL)
1. Vehicle[1]	55 (9)	0	0

2. Low	55 (24)	125	12.5
3. Medium	55 (24)	250	25
4. High	55 (29)	500	50

[1] 0.5% w/v% methylcellulose solution

The Sponsor's report indicates that the:"oral route was selected since it was an intended route in clinical use. The length of the administration period was 24 months (104 weeks, until the day before necropsy) according to the toxicity guideline. In females in the 500 mg/kg group, since the mortality increased with the progression of the administration period, all surviving females in this group were prematurely sacrificed in Week 100 after administration for 99 weeks when survivors decreased to 15 (73% mortality). The frequency of administration was once daily (7 times per week), which is ordinary for repeated dose toxicity studies. The dose volume was set at 10 mL/kg body weight and dosing formulations were administered orally once daily (between 08:38 and 15:10) by gavage using a flexible stomach tube. Animals in the control group received the vehicle (0.5 w/v% MC solution) in the same manner. Individual dose volumes (unit: 0.01 mL) were calculated based on the most recently measured body weight." (page 24 of mouse report)

Animals were approximately six weeks old at first dosing. Animals were housed individually with food and water available ad libitum. The Sponsor states that animals were checked three times daily.

3.2.2.1 Sponsor's Results and Conclusions

This section will present a summary of the Sponsor's analysis on survivability and tumorigencity in mice.

Survival analysis:

The Sponsor's report notes that "The survival rate in females in the 500 mg/kg group was extremely low, where many deaths occurred in the late phase of the administration period. Contrary to females, in males, higher survival rate was observed in all dose groups." (page 14 of mouse report)

Specifically, mortality and survival rate are summarized in the following table:.

Table 23 Text Table 1. Summary of mortality and survival rate

Sex	Male				Female			
Dose (mg/kg/day)	0	125	250	500	0	125	250	500 [a)]
No. of animals used	55	55	55	55	55	55	55	55
Week of administration								
1-26	0	0	0	3	0	1	0	3
1-52	2	0	1	3	0	1	0	3
1-78	11	2	4	5	1	1	2	9
1-100	26	12	6	14	8	5	12	40
1-105[b)]	28	16	9	15	13	9	16	NA
No. of survivors	27	39	46	40	42	46	39	15
Survival rate (%)								
Week 100	52.7	78.2	89.1	74.5	85.5	90.9	78.2	27.3*
Week 105b)	49.1$	70.9*	83.6*	72.7*	76.4$	83.6	70.9	NA

a): All surviving females in the 500 mg/kg group were sacrificed in Week 100 after
administration for 99 weeks.
b): All surviving animals except for females in the 500 mg/kg group were necropsied
as scheduled in Week 105 after administration for 104 weeks.
Values in the table indicate cumulative number of animals that died or were sacrificed as
moribund.
$: p<0.05 (statistically significant trend, Tarone's test)
*: p<0.05 (statistically significant difference from the control group, log-rank test)
NA: Not applicable

"1) Male
A statistically significant trend of an increase in the survival rate with increasing dose levels was observed, and the survival rates in all dose groups were significantly higher than that in the control group.
"2) Female
A statistically significant trend of a decrease in the survival rate with increasing dose levels was observed, and the survival rate in the 500 mg/kg group was extremely low with statistical significance, where many deaths occurred in the late phase of the administration period." (page 36 of mouse report)

Tumorigenicity analysis:
The Sponsor's analysis of carcinogenicity is based on Peto survival-adjusted tests. Where results with low tumor incidence were computed using exact permutation tests. In particulat The Sponsor claims that the test drug did not increase any specific tumor deaths.

Table 24: Text Table 9. Number of tumors and tumor bearers

Sex	Male				Female			
Dose (mg/kg/day)	0	125	250	500	0	125	250	500[a)]
No. of animals used	55	55	55	55	55	55	55	55
Total No. of tumors	62	46	44	35	89	81	66	30
No. of benign tumors	37	24	23	21	45	40	27	21
No. of malignant tumors	25	22	21	14	44	41	39	9
Total No. of tumor bearing animals	38	31	26	27	45	41	42	19
No. of benign tumor bearers	24	16	16	16	31	26	23	15
No. of malignant tumor bearers	21	18	17	13	32	30	32	8
No. of multiple tumor bearers	16	13	10	6	6	23	19	9

a): All surviving females in the 500 mg/kg group were sacrificed prematurely in Week
100 after administration for 99 weeks.

"There was no treatment-related increase in either number of tumors or tumor bearing animals in either sex.

"Decreases in total number of tumors and number of benign tumors were observed in females in the 250 and 500 mg/kg groups. Decreases in number of malignant tumors, total number of tumor bearing animals, number of benign tumor bearing animals and number of malignant tumor bearing animals were observed in females in the 500 mg/kg group. A decrease in number of multiple tumor bearing animals was observed in both sexes in the 500 mg/kg group. However, they were not regarded as an adverse effect of treatment, as these changes were decreases." (page 45 of mouse report)

"Tumors that showed a tendency toward increase in the incidence in the dose groups as compared to the control group were observed in the thyroid and the incidence of tumors and related non-tumor findings are summarized in the following:

Table 25: Text Table 10. Incidence summary of tumors and hyperplasia in the thyroid

Sex	Male				Female			
Dose (mg/kg/day)	0	125	250	500	0	125	250	500[a)]
No. of animals used	55	55	55	55	55	55	55	55
Thyroid								
Adenoma, follicular cell	0	0	1	2	1	1	1	3
Carcinoma, follicular cell	0	0	1	0	0	0	0	0
Adenoma + Carcinoma, follicular cell	0	0	2	2	1	1	1	3
Hyperplasia/hypertrophy, follicular cell (total)	1	2	17	36	2	51	52	52
(±)	1	2	17	33	2	41	20	4
(+/++)	0	0	0	3	0	10	32	48

[a)]: All surviving females in the 500 mg/kg group were sacrificed prematurely in Week 100 after administration for 99 weeks.
Numbers in the table indicate the number of animals with lesions.
±; Minimal, +; Mild, ++; Moderate

"Thyroid:
"Slightly higher incidence of follicular cell tumors (adenoma + carcinoma) were observed in 2 males each in the 250 and 500 mg/kg groups and in 3 females in the 500 mg/kg group, although there were no statistically significant differences in either trend analysis or pairwise comparison between the control and any dose group. At the same time, increased
incidence and severity of hyperplasia/hypertrophy of the follicular cells were observed in the above groups. Follicular cell adenoma was also observed in 1 female each in the 125 and 250 mg/kg groups, respectively, and the possibility of involvement of treatment could not be completely excluded, since increased incidence and severity of hyperplasia/hypertrophy of the
follicular cells were also observed in these groups. However, it was hardly judged to be treatment-related since follicular cell adenoma was also observed in 1 control female in the present study.

"Decreased incidences of tumors were observed in the following; however, reduced incidences

of common spontaneous tumors are not regarded as adverse effects of treatment.
Liver: Hepatocellular adenoma in both sexes in the 250 and 500 mg/kg groups
Pituitary: Anterior adenoma in females in the 500 mg/kg group
Hemolymphoreticular: Malignant lymphoma in females in the 500 mg/kg group

"The other tumors described in the Tables and Appendices were judged to be incidental since they were consistent with the spectrum of spontaneous tumors expected in aged mice of this strain." (page 46 of mouse report)

3.2.1.2 FDA Reviewer's Results

This section will present the current Agency findings on survival and tumorigenicity in male and female mice.

Survival analysis:

Kaplan-Meier plots comparing treatment groups in are given in Appendix 1, along with more details of the analyis. The following tables (Table 26 for male mice and Table 27 for female mice) summarize the mortality results for the dose groups. As with rats, the data were grouped for the specified time period, and present the number of deaths during the time interval over the number at risk at the beginning of the interval. The percentage cited is the percent survived at the end of the interval.

Table 26. Summary of Male Mice Mortality

Period	1.Vehicle	2.Low	3.Medium	4.High
0-50	1/55 98.2%	0/55 100.0%	1/55 98.2%	3/55 94.5%
51-70	9/54 81.8%	1/55 98.2%	2/54 94.5%	2/52 90.9%
71-90	9/45 65.5%	5/54 89.1%	1/52 92.7%	2/50 87.3%
91-105	9/36 49.1%	10/49 70.9%	5/51 83.6%	8/48 72.7%
terminal	27	39	46	40

[1] number deaths / number at risk
[2] per cent survival to end of period.

Table 27. Summary of Female Mice Mortality

Period	1.Vehicle	2.Low	3.Medium	4.High
0-50	0/55 100.0%	1/55 98.2%	0/55 100.0%	3/55 94.5%
51-70	1/55 98.2%	0/54 98.2%	0/55 100.0%	3/52 89.1%
71-90	4/54 90.9%	3/54 92.7%	5/55 90.9%	15/49 61.8%
91-105	8/50 76.4%	5/51 83.6%	11/50 70.9%	19/34 27.3%
terminal	42	46	39	15 [i]

[1] number deaths / number at risk
[2] per cent survival to end of period.

Table 28. Statistical Significances of Tests of Homogeneity and Trend in Survival in Mice

Hypotheses	Males		Females	
	Logrank	Wilcoxon	Logrank	Wilcoxon
Homogeneity over all four groups	0.0002	0.0001	<0.0001	<0.0001
No Trend over all four groups	0.0100	0.0104		<0.0001
No difference between high dose and	0.0080	0.0083		<0.0001

In Appendix 1. from Figure A.1.3 in male mice the vehicle control has, by a considerable extent, the lowest survival, with eventually the medium group having the highest survival, and the low and high dose groups eventually largely intertwined between these two curves. Note these differences are sufficient to result in statistically significant differences in tests for homogeneity in both genders (both $p \leq 0.0002$), as is the test of trend in dose (both $p \leq 0.0104$), as wells as the test of no differences in the high and control low doses. Similarly, the comparison between the high dose and vehicle test of was statistically significant (both $p \leq 0.0083$). In female mice the high dose groups have much higher mortality than the other study groups, resultin in highly statistically significant tests of homogeneity, trend, and difference between high dose and control (all six $p < 0.0001$).

Again, Kaplan-Meier plots comparing treatment groups in both studies are given in Appendix 1, along with more details of the analysis.

Tumorigenicity analysis:

Table 29 below, a repeat of Table 7 above and Table A.2.2 below, shows the organ-tumor combinations associated with at least one non-multiplicity adjusted test that was statistically significant at a 0.10 level.

Table 29. Potentially Statistically Significant Results for Organ-Tumor Combinations in Mice

	Overall Results Tumor Incidence				Significance Levels		
organ/tumor	Veh	Low	Med	High	ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Male Mice								
Testis								
# Evaluated	55	55	55	55				

```
 Adj. # at Risk                42.4 51.5 52.0 49.0
 LEYDIG CELL TUMOR              0    0    1    2    .0644  .2816
.5484  .
Thyroid
 # Evaluated                   55   55   55   55
 Adj. # at Risk                42.4 51.5 52.0 49.0
 ADENOMA,FOLLICULAR CELL        0    0    1    2    .0644  .2816
.5484  .
 Adj. # at Risk                42.4 51.5 52.0 49.0
 Foll. Cell Adenoma/Carcinoma   0    0    2    2    .0749  .2816
.3034  .
```

Table 29. (cont.) Potentially Statistically Significant Results for Organ-Tumor Combinations in Mice

```
                                Overall Results
                                Tumor Incidence          Significance Levels
organ/tumor                   Veh  Low  Med  High   ptrend phigh
pmed   plow
                                                                vsVeh
vsVeh  vsVeh

Female Mice
Thyroid
 # Evaluated                   55   55   55   55
 Adj. # at Risk                52.4 52.8 52.2 41.1
ADENOMA,FOLLICULAR CELL         1    1    1    3    .0889  .2245
.7524  .7524
```

Using the tumor incidence in the vehicle to determine whether a tumor should be classified as rare or common, only follicular cell adenoma would be classified as common, the remainder rare. Again, several of the tests of trend fall below the 0.10 level, after adjusting for multiplicity using the Haseman-Lin-Rahman rules no tests would be categorized as statistical significant. Complete tables of tumor incidence are given in Tables A.2.5 and A.2.6, in Appendix 2, below.

4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

NA

5. SUMMARY AND CONCLUSIONS

5.1. Statistical Issues and Collective Evidence

Please see Section 1.3 above.

5.2. Conclusions and Recommendations

Please see section 1.1 above.

APPENDICES (for FDA statistical review of carcinogenicity studies)

Appendix 1. Survival Analyses

Simple summary life tables in mortality in rats are presented in the report (Tables 18 and 19, above). Kaplan-Meier estimated survival curves across study groups for each gender in rats are displayed below in Figures A.1.1 and A.1.2. The plots include 95% confidence intervals around each survival curve (colored area around each curve). These plots are also supported by tests of homogeneity in survival over the treatment groups. The statistical significance levels (i.e., p-values) are provided in Table A.1.1., below. One might note that the log rank tests place greater weight on later events, while the Wilcoxon test tends to weight them more equally, and thus, it actually tends to place more weight on differences in earlier events than does the log rank test.

Table A.1.1 Statistical Significances of Tests of Homogeneity and Trend in Survival in Rats

Hypotheses	Males		Females	
	Logrank	Wilcoxon	Logrank	Wilcoxon
Homogeneity over all four groups	0.5181	0.4880	0.1189	0.0656
No Trend over all four groups	0.3716	0.4084	0.0200	0.0095
No difference between high dose and vehicle	0.7931	0.8753	0.0444	0.0380

Kaplan-Meier survival curves for these studies are presented below. From Figure A.1.1, the Kaplan-Meier estimated survival curves in male rats suggest that during the last third of the study the low dose group had slightly higher survival than the other three study group, which in turn, were largely intertwined. However, none of the comparisons in male rats would be categorized as being statistically significant (i.e., all six $p \geq 0.3716$). In almost a reversal of fate, in Figure A.1.2, in female rats, the the high dose group seems to have higher survival than the other study groups, but again with the remaining dose groups largely intertwined. These differences are not sufficient to result in a statistically significant, though close to significance, test of overall homogeneity (Logrank p=0.1189, Wilcoxon p=0.0656). However, there is evidence of a statistically significant test of trend in dose (Logrank p=0.0200, Wilcoxon p=0.0095), and a somewhat weaker result in the test of differences between the high dose and vehicle control (Logrank p=0.0444, Wilcoxon p=0.0380).

Figure A.1.1 Kaplan-Meier Survival Curves for Male Rats

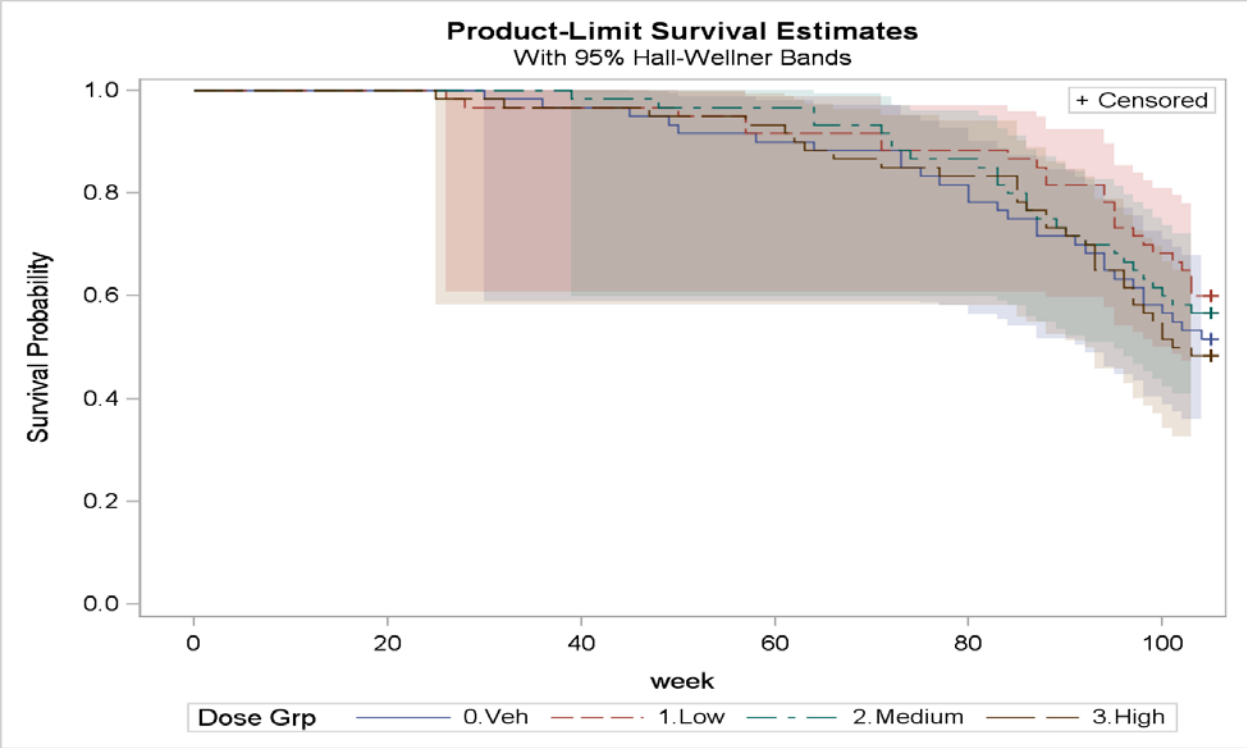
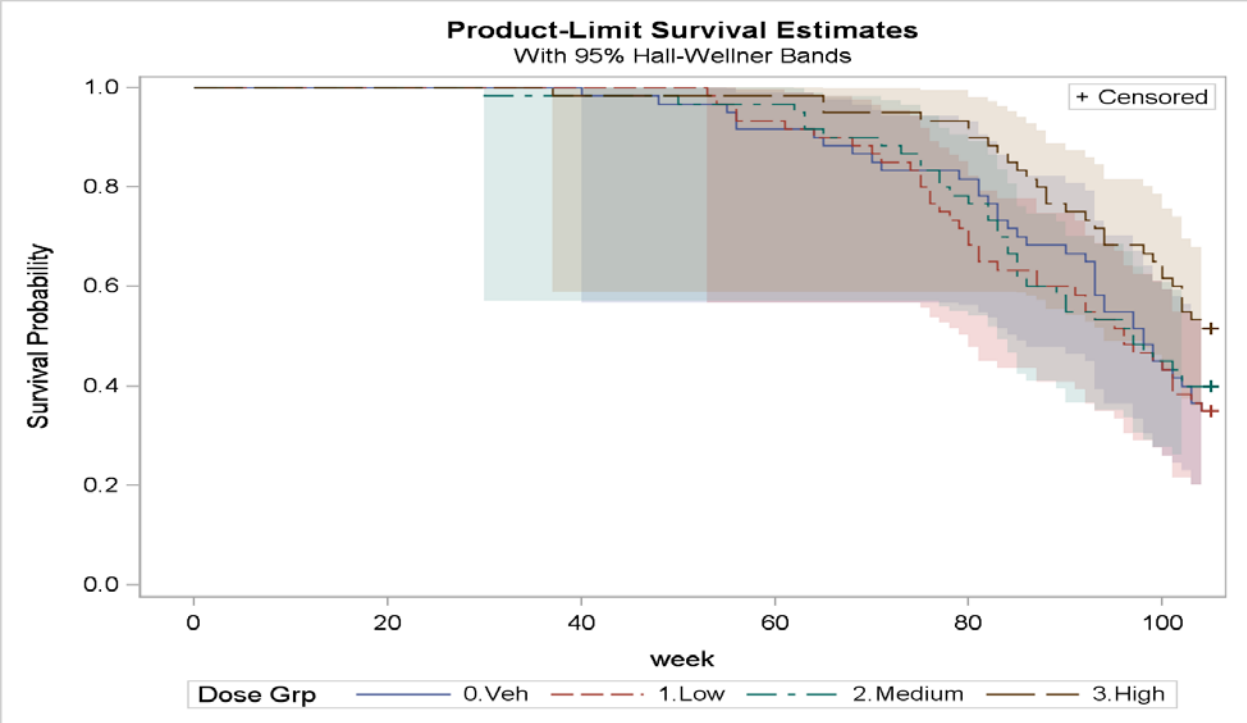


Figure A.1.2 Kaplan-Meier Survival Curves for Female Rats



The results of the same set of statistical comparisons in survival are given in Table A.1.2,, with corresponding Kaplan-Meier survival curves in Figures A.1.3 and A.1.4, below. In mice a there is a somewhat different pattern. Note that summary tables of survival are given in Tables 26 and 27 above. .

Table A.1.2. Statistical Significances of Tests of Homogeneity and Trend in Survival in Mice

Hypotheses	Males		Females	
	Logrank	Wilcoxon	Logrank	Wilcoxon
Homogeneity over all four groups	0.0002	0.0001	<0.0001	<0.0001
No Trend over all four groups	0.0100	0.0104		<0.0001
No difference between high dose and	0.0080	0.0083		<0.0001

From Figure A.1.3 in male mice the vehicle control has, by a considerable extent, the lowest survival, with eventually the medium group having the highest survival, and the low and high dose groups eventually largely intertwined between these two curves. Note these differences are sufficient to result in statistically significant differences in tests for homogeneity in both the logrank and Wilcoxon tests (both $p \leq 0.0002$), as is the test of trend in dose (both $p \leq 0.0104$), as wells as the test of no differences in the high and control low doses. Similarly, the comparison between the high dose and vehicle test of was statistically significant (both $p \leq 0.0083$). In female mice the high

dose groups have much higher mortality than the other study groups, sufficient to result in highly statistically significant tests of homogeneity, trend, and difference between high dose and control (all six $p < 0.0001$).

Figure A.1.3 Kaplan-Meier Survival Curves for Male Mice

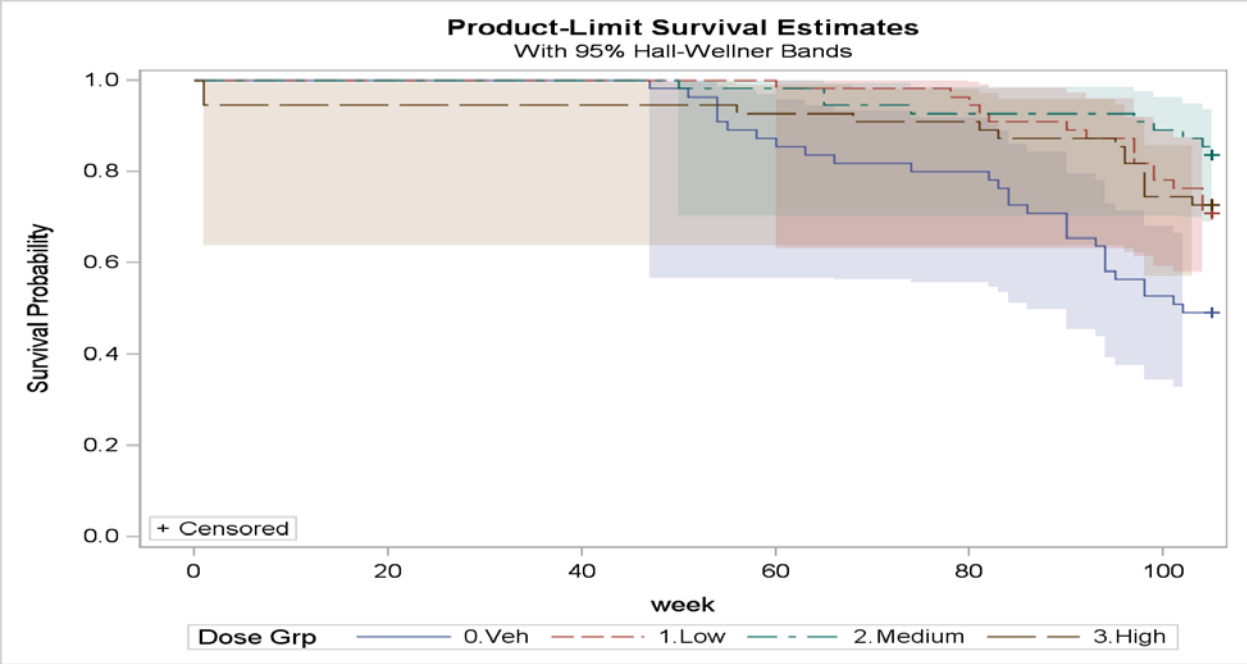
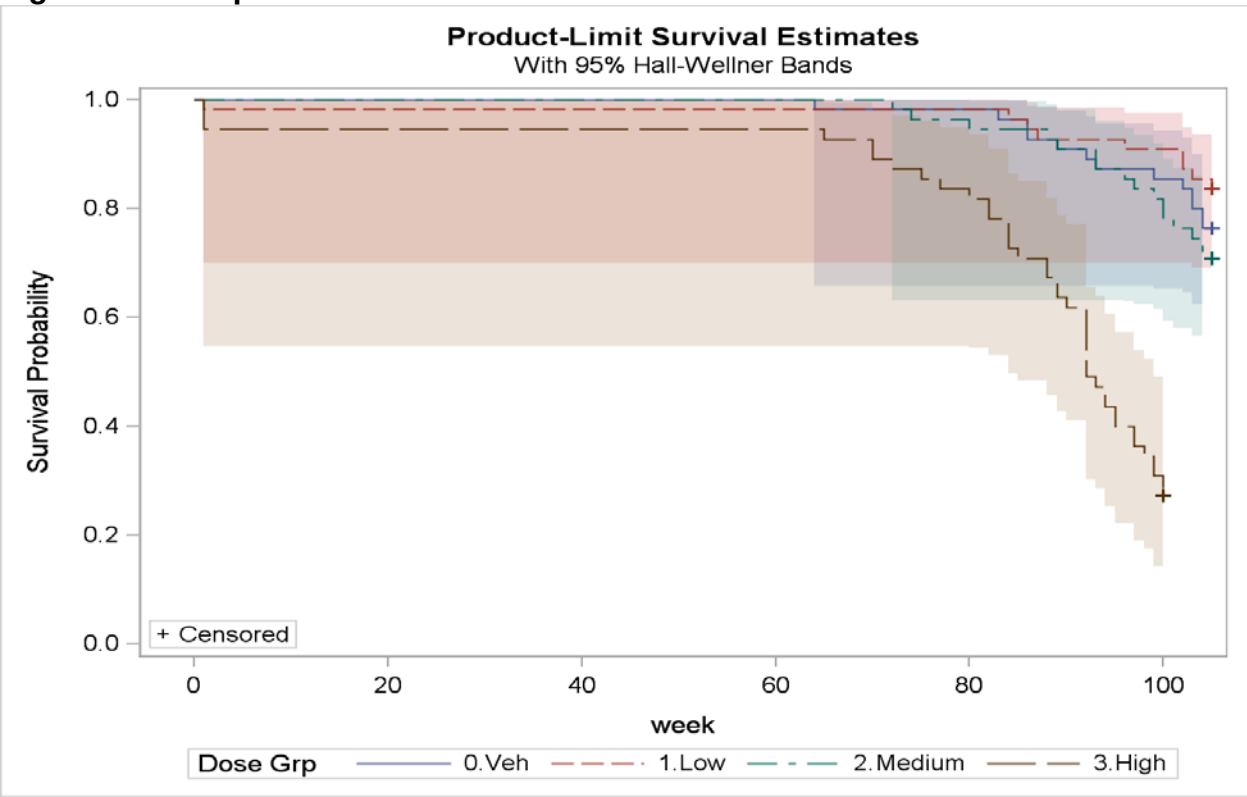


Figure A.1.4 Kaplan-Meier Survival Curves for Female Mice



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Appendix 2. FDA Poly-k Tumorigenicity Analysis

The poly-k test, here with k = 3, modifies the original Cochran-Armitage test to adjust for differences in mortality (please see Bailer & Portier, 1988, Bieler & Williams, 1993). The tests used here are small sample exact permutation tests of tumor incidence. When there were no tumors of the specific type being analyzed in either column of the 2x2 table corresponding to a pairwise comparison an argument could be made that the p-value for this test should be 1.0. However, largely for readability, in the tables below these p-values are considered as missing (i.e., corresponding to a null test), denoted by a period ".". Note that the StatXact program used for these analyses adjusts for the variance, which would be 0. Then the significance levels of the test statistics are based on the result of a division by 0, i.e., undefined, and hence StatXact codes these p-values as missing.

For each gender by organ combination the number of animals microscopically analyzed is presented first. Note that indicating an organ was not examined requires a specification in the data (please see section 2.2 above). It is possible that this specification could be missing for some organ combinations in some study groups in this data. Then the number of animals at risk could be inflated, and the proportion of animals with tumor would be artificially decreased. Thus, as discussed in Section 1.3.1.5 above, for some of these organs it is possibly more appropriate to define the actual endpoint used in the statistical analysis be the condition of being microscopically analyzed and show the tumor. This does have problems if the treatment groups are treated equally except for actual treatment applied.

The entry for each tumor is preceded by the adjusted number of animals at risk for that endpoint. It seems clear that an animal that dies early without having displaying that endpoint reduces the size of the risk set for that getting that particular endpoint. The poly-k test down weights such animals, and as also discussed in Section 1.3.1.5, above, the sum of these poly-k weights seems to be a better estimate of the number of animals at risk of getting that tumor than the simple number of animals analyzed. This sum is given in the row labeled "Adjusted # at risk". For these analyses, incidence in the control vehicle, water group is used to assess background tumor incidence, and thus whether a tumor is considered to be rare (background incidence < 1%) or common. Note that for these analyses a tumor is only classified as rare if the vehicle control group shows none of that particular tumor.

To adjust for the multiplicity of tests the so-called Haseman-Lin-Rahman (HLR) rules discussed in Section 1.3.1.6 are often applied. In this particular case we have two two-year study in rats and mice. An adjustment that seems to work is that for a roughly 10% overall error rate tests of trend would be considered significant it the tests for positive trend alone would be tested at 0.005 and 0.025 significance levels, for common and rare tumors respectively. Control-high pairwise differences would be tested at a 0.01 and 0.05 significance levels, for common and rare tumors respectively. If we require both the tests of trend and the pairwise comparison to be significant, the only

change would be that the pairwise test in the two year study be tested at a 0.10 level for rare tumors. Using these adjustments for other tests, like testing the comparisons between the low and medium dose groups versus vehicle can be expected to increase the overall type I error rate to some value above the nominal rough 10% level, possibly considerably higher than the nominal 10% rate.

Table A.2.1, below, a repeat of Table 5 above, shows those rows with at least one tumor with at least one non-multiplicity adjusted test that was statistically significant or close, to a 0.10 level.

Table A.2.1. Potentially Statistically Significant Results for Organ-Tumor Combinations in Rats

Gender	Overall Results: Tumor Incidence				Significance Levels			
Organ/Tumor	Veh	Low	Med	High	ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Male Rats								
Testis								
# Evaluated	60	60	60	60				
Adj. # at Risk	45.8	50.1	48.1	46.3				
LEYDIG CELL TUMOR	2	0	2	5	.0226	.2264	.7157	1
Thyroid								
# Evaluated	58	57	56	59				
Adj. # at Risk	44.9	47.4	46.4	45.6				
CARCINOMA,C-CELL	0	0	2	2	.0705	.2528	.2584	.
Female Rats								
Adrenal								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.4	42.4	49.5				
PHEOCHROMOCYTOMA	0	0	1	3	.0261	.1467	.4941	.
Adj. # at Risk	43.3	41.4	42.4	49.5				
Pheochromocytoma, Any	0	1	1	3	.0590	.1467	.4941	.4881
Thyroid								
# Evaluated	59	59	59	59				
Adj. # at Risk	42.5	40.9	42.9	49.5				
ADENOMA, C-CELL	1	5	5	7	.0999	.0478	.1008	.0899

Using the tumor incidence in the vehicle to determine whether a tumor should be classified as rare or common, only c-cell carcinoma and pheochromocytoma (both

above) were classified as rare tumors, the remainder common. Although some of these tumors exceed the 0.10 level, after adjusting for multiplicity using the Haseman-Lin-Rahman rules only the test of trend for Pheocromocytoma is close to statistical significance (p = 0.0261 ≈ 0.025). Complete tables of tumor incidence are given in Tables A.2.3 and A.2.4, below.

Similar results in mice are presented in Table A.2.2, below.

Table A.2.2. Potentially Statistically Significant Results for Organ-Tumor Combinations in Mice

organ/tumor	Overall Results Tumor Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Male Mice								
Testis								
# Evaluated	55	55	55	55				
Adj. # at Risk	42.4	51.5	52.0	49.0				
LEYDIG CELL TUMOR	0	0	1	2	.0644	.2816	.5484	.
Thyroid								
# Evaluated	55	55	55	55				
Adj. # at Risk	42.4	51.5	52.0	49.0				
ADENOMA,FOLLICULAR CELL	0	0	1	2	.0644	.2816	.5484	.
Adj. # at Risk	42.4	51.5	52.0	49.0				
Foll. Cell Adenoma/Carcinoma	0	0	2	2	.0749	.2816	.3034	.
Female Mice								
Thyroid								
# Evaluated	55	55	55	55				
Adj. # at Risk	52.4	52.8	52.2	41.1				
ADENOMA,FOLLICULAR CELL	1	1	1	3	.0889	.2245	.7524	.7524

Using the tumor incidence in the vehicle to determine whether a tumor should be classified as rare or common, only follicular cell adenoma would be classified as common, the remainder rare. Again, several of the tests of trend fall below the 0.10 level, after adjusting for multiplicity using the Haseman-Lin-Rahman rules no tests in

mice would be categorized as statistical significant. Complete tables of tumor incidence are given in Tables A.2.5 and A.2.6, in Appendix 2, below.

Table A.2.3. Incidence and Results for Organ-Tumor Combinations in Male Rats

Organ/tumor	Overall Results Tumor Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Abdominal cavity								
# Evaluated	60	59	60	60				
Adj. # at Risk	45.8	49.1	48.1	46.0				
FIBROMA	0	0	0	1	.2406	.5000	.	.
Adj. # at Risk	45.8	49.1	48.1	45.9				
LIPOMA	0	0	0	1	.2406	.5000	.	.
Adrenal								
# Evaluated	60	60	60	60				
Adj. # at Risk	45.8	50.1	48.1	46.7				
ADENOMA,CORTICAL CELL	0	1	1	1	.2939	.5055	.5161	.5263
Adj. # at Risk	46.4	50.7	48.8	47.5				
PHEOCHROMOCYTOMA	16	11	5	7	.9710	.9936	.9992	.9475
Adj. # at Risk	45.8	50.1	48.1	46.0				
PHEOCHROMOCYTOMA,MALIGNANT	0	1	1	1	.2939	.5055	.5161	.5263
Adj. # at Risk	46.4	50.7	48.8	47.5				

```
 Pheocromocytoma, any        16   11    6    7    .9712  .9936
.9977  .9475
Bone,vertebral
 # Evaluated                 60   60   60   60
 Adj. # at Risk              45.8 50.1 48.1 46.7
 CHONDROSARCOMA               0    0    1    1    .1825  .5055
.5161  .
 Adj. # at Risk              45.8 50.1 48.1 45.9
 OSTEOSARCOMA                 1    0    0    0    1      1
1      1
Cerebellum
 # Evaluated                 60   60   60   60
 Adj. # at Risk              45.8 50.1 48.7 45.9
 GRANULAR CELL TUMOR          0    1    1    0    .6231  .
.5161  .5263
Cerebrum
 # Evaluated                 60   60   60   60
 Adj. # at Risk              45.8 50.1 48.1 46.7
 ASTROCYTOMA,MALIGNANT        0    0    0    1    .2434  .5055
.      .
 Adj. # at Risk              45.8 50.1 48.1 45.9
 OLIGODENDROGLIOMA            1    0    0    0    1      1
1      1
Forelimb
 # Evaluated                 59   59   60   60
 Adj. # at Risk              45.5 49.2 48.1 45.9
 PAPILLOMA,SQUAMOUS CELL      1    0    0    0    1      1
1      1
 Adj. # at Risk              45.8 49.2 48.1 45.9
 SCHWANNOMA,MALIGNANT         1    0    0    0    1      1
1      1
Endo. Schwannoma, any         2    0    0    0    1      1
1      1
 Adj. # at Risk              45.8 50.1 48.1 45.9
 SCHWANNOMA,ENDOCARDIAL       1    0    0    0    1      1
1      1
 Adj. # at Risk              46.5 50.1 48.1 45.9
 SCHWANNOMA,ENDOCARDIAL,      1    0    0    0    1      1
1      1
    MALIGNANT
```

Table A.2.3. (cont.) Incidence and Results for Organ-Tumor Combinations in Male Rats

```
                                Overall Results
                                Tumor Incidence          Significance Levels
```

Organ/tumor	Veh	Low	Med	High	ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Hemolymphoreticular(all sites)								
# Evaluated	60	60	60	60				
Adj. # at Risk	46.8	50.3	48.1	45.9				
LEUKEMIA,LARGE GRANULAR	2	1	0	1	.6569	.8750	1	.8938
LYMPHO								
Adj. # at Risk	45.8	51.1	48.1	45.9				
LYMPHOMA,MALIGNANT	1	2	0	0	.9416	1	1	.5473
Adj. # at Risk	46.4	50.6	48.1	46.8				
SARCOMA,HISTIOCYTIC	3	3	2	2	.6771	.8195	.8323	.7003
Intestine,ileum								
# Evaluated	57	59	56	57				
Adj. # at Risk	44.2	49.3	45.8	44.2				
ADENOMA	0	0	1	0	.4890	.	.5056	.
Kidney								
# Evaluated	60	60	60	60				
Adj. # at Risk	45.8	50.1	48.1	45.9				
LIPOMA	0	2	0	0	.8157	.	.	.2744
Adj. # at Risk	45.8	50.1	48.1	45.9				
LIPOSARCOMA	1	0	0	0	1	1	1	1
Adj. # at Risk	45.8	50.1	48.1	45.9				
Lipoma/Liposarcoma	1	2	0	0	.9416	1	1	.5399
Liver								
# Evaluated	60	60	60	60				
Adj. # at Risk	46.1	50.1	48.1	45.9				
ADENOMA,HEPATOCELLULAR	5	4	2	0	.9960	1	.9508	.7969
Adj. # at Risk	45.8	50.1	48.1	45.9				
CARCINOMA,HEPATOCELLULAR	0	0	0	1	.2394	.5000	.	.
Adj. # at Risk	45.8	50.1	48.1	45.9				
LYMPHANGIOMA	0	1	0	0	.7606	.	.	.5263
Lung(bronchus)								
# Evaluated	60	60	60	60				
Adj. # at Risk	45.8	50.1	48.1	45.9				

```
 ADENOMA,BRONCHIOLO-ALVEOLAR  0    0    1    0    .4947  .
.5161  .
Mammary gland
 # Evaluated                 60   60   60   60
 Adj. # at Risk              45.8 50.1 48.1 45.9
 FIBROADENOMA                 1    1    0    0    .9437  1
1      .7783
 Adj. # at Risk              45.8 50.1 48.1 46.9
 Fibroadenoma/mixed tumor     1    1    0    1    .5280  .7582
1      .7783
 Adj. # at Risk              45.8 50.1 48.1 46.9
 TUMOR,MIXED,BENIGN           0    0    0    1    .2434  .5055
.      .
Mesothelium
 # Evaluated                 60   60   60   60
 Adj. # at Risk              45.8 50.1 48.1 45.9
 MESOTHELIOMA,MALIGNANT       1    0    0    1    .4224  .7528
1      1
```

Table A.2.3. (cont.) Incidence and Results for Organ-Tumor Combinations in Male Rats

```
                                Overall Results
                                Tumor Incidence          Significance Levels
Organ/tumor                    Veh  Low  Med  High   ptrend  phigh
pmed   plow
```

```
                                                                vsVeh
vsVeh  vsVeh
Oral cavity
 # Evaluated                 60   60   60   60
 Adj. # at Risk              45.8 50.1 48.6 45.9
 CARCINOMA,SQUAMOUS CELL      0    0    1    0    .4947  .
.5161  .
Origin unknown
 # Evaluated                 60   60   60   60
 Adj. # at Risk              45.8 50.1 48.1 45.9
 CHORDOMA                     0    0    1    0    .4947  .
.5161  .
Palate
 # Evaluated                 60   60   60   60
 Adj. # at Risk              45.8 51.0 48.1 45.9
 CARCINOMA,SQUAMOUS CELL      0    1    0    0    .7606  .
.      .5263
Pancreas
 # Evaluated                 60   60   60   60
 Adj. # at Risk              45.8 50.4 48.1 46.4
 ADENOMA,ACINAR CELL          1    3    3    1    .6920  .7582
.3332  .3494
```

```
 Adj. # at Risk              45.8 50.1 48.1 45.9
 ADENOMA,ACINAR-ISLET CELL    1    0    1    0    .7460  1
.7686  1
 Adj. # at Risk              47.5 50.4 49.0 45.9
 ADENOMA,ISLET CELL          22   16   10    6    .9997  .9999
.9986  .9558
 Adj. # at Risk              45.8 50.4 48.2 46.4
 Acinar Cell Adenoma/Carcin.  1    3    4    1    .7069  .7582
.2014  .3494
 Adj. # at Risk              45.8 50.1 48.2 45.9
 CARCINOMA,ACINAR CELL        0    0    1    0    .4947  .
.5161  .
 Adj. # at Risk              45.8 50.1 48.1 45.9
 CARCINOMA,ISLET CELL         2    6    2    1    .9075  .8792
.7157  .1708
 Adj. # at Risk              47.5 50.4 49.0 45.9
 Islet Cell Adenoma/Carcin.  22   16   11    6    .9997  .9999
.9970  .9558
Parathyroid
 # Evaluated                 60   60   60   60
 Adj. # at Risk              45.8 50.2 48.1 45.9
 ADENOMA                      2    5    0    1    .8932  .8792
1      .2636
Pituitary
 # Evaluated                 60   60   60   60
 Adj. # at Risk              49.2 56.3 52.3 48.6
 ADENOMA,ANTERIOR            20   40   23   22    .8277  .3846
.4423  .0014
 Adj. # at Risk              45.8 50.1 48.1 45.9
 ADENOMA,INTERMEDIATED        1    0    0    1    .4224  .7528
1      1
 Adj. # at Risk              46.4 50.1 48.5 46.1
 CARCINOMA,ANTERIOR           2    0    1    1    .5656  .8791
.8868  1
Prostate
 # Evaluated                 60   60   60   60
 Adj. # at Risk              45.8 50.1 48.1 46.0
 ADENOMA                      0    1    0    1    .3120  .5055
.      .5263
```

Table A.2.3. (cont.) Incidence and Results for Organ-Tumor Combinations in Male Rats

```
                                Overall Results
                                Tumor Incidence          Significance Levels
Organ/tumor                   Veh  Low  Med  High   ptrend  phigh
pmed   plow
```

```
                                                                  vsVeh
vsVeh  vsVeh
Skin/Subcutis
 # Evaluated                  60   60   60   60
 Adj. # at Risk              45.8 50.1 48.1 46.0
 CARCINOMA,SEBACEOUS          0    0    0    1    .2434  .5055
.      .
 Adj. # at Risk              45.8 50.1 48.3 45.9
 CARCINOMA,SQUAMOUS CELL      0    0    1    0    .4947  .
.5161  .
 Adj. # at Risk              46.0 50.3 48.1 45.9
 FIBROMA                      5    4    2    4    .5297  .7464
.9508  .7969
 Adj. # at Risk              45.9 50.4 48.1 45.9
 FIBROSARCOMA                 1    1    0    1    .5213  .7528
1      .7783
 Adj. # at Risk              46.1 50.1 48.1 46.0
 KERATOACANTHOMA              1    1    1    3    .1054  .3083
.7632  .7730
 Adj. # at Risk              45.8 50.1 48.9 45.9
 LEIOMYOSARCOMA               0    0    1    0    .4947  .
.5161  .
 Adj. # at Risk              45.8 50.1 48.1 45.9
 LIPOMA                       0    0    1    0    .4947  .
.5161  .
 Adj. # at Risk              45.8 50.1 48.5 45.9
 LIPOSARCOMA                  0    0    1    1    .1792  .5000
.5161  .
 Adj. # at Risk              45.8 50.1 48.1 45.9
 PAPILLOMA,SQUAMOUS CELL      1    1    1    0    .8294  1
.7686  .7783
 Adj. # at Risk              46.0 50.1 48.3 45.9
 SCHWANNOMA,MALIGNANT         1    0    1    0    .7433  1
.7632  1
 Adj. # at Risk              45.8 50.1 48.1 46.0
 TUMOR,HAIR FOLLICLE,BENIGN   1    2    1    1    .5992  .7528
.7686  .5399
Spleen
 # Evaluated                  60   60   60   60
 Adj. # at Risk              45.8 50.1 48.1 45.9
 HEMANGIOSARCOMA              0    1    0    0    .7606  .
.      .5263
 Adj. # at Risk              45.8 50.1 48.5 45.9
 SARCOMA,NOS                  0    0    1    0    .4947  .
.5161  .
Stomach
```

```
 # Evaluated                    60   60   60   60
 Adj. # at Risk                46.2 50.1 48.1 45.9
 PAPILLOMA,SQUAMOUS CELL        1    1    0    0    .9417  1
1      .7730
```

```
      Systemic
       # Evaluated                 60   60   60   60
       Adj. # at Risk              45.8 50.1 48.1 45.9
       HEMANGIOSARCOMA              0    1    0    0    .7606  .      .
.5263
     Testis
       # Evaluated                 60   60   60   60
       Adj. # at Risk              45.8 50.1 48.1 46.3
       LEYDIG CELL TUMOR            2    0    2    5    .0226  .2264  .7157
1
       Adj. # at Risk              45.8 50.1 48.1 45.9
       SEMINOMA                     0    1    0    0    .7606  .      .
.5263
```

Table A.2.3. (cont.) Incidence and Results for Organ-Tumor Combinations in Male Rats

```
                                   Overall Results
                                   Tumor Incidence          Significance Levels
Organ/tumor                          Veh  Low  Med  High   ptrend  phigh
pmed   plow
                                                                    vsVeh
vsVeh  vsVeh
```

```
     Thymus
       # Evaluated                   60   60   58   59
       Adj. # at Risk                45.8 50.4 46.7 45.0
       THYMOMA                        0    1    0    0    .7581  .      .
.5263
       Adj. # at Risk                45.8 50.4 47.6 45.0
       THYMOMA ([B]+[M})              0    1    1    0    .6216  .
.5109  .5263
       Adj. # at Risk                45.8 50.1 47.6 45.0
       THYMOMA,MALIGNANT              0    0    1    0    .4920  .
.5109  .
     Thyroid
       # Evaluated                   58   57   56   59
       Adj. # at Risk                44.9 47.5 46.8 45.7
       ADENOMA,C-CELL                 5    5    4    4    .6449  .7691
.7796  .6720
       Adj. # at Risk                45.0 47.4 46.4 45.4
       ADENOMA,FOLLICULAR CELL        3    1    1    0    .9725  1
.9469  .9492
       Adj. # at Risk                44.9 47.5 46.8 45.9
```

```
      C-Cell Adenoma/Carcinoma          5    5    6    6    .3610  .5161
.5319  .6720
      Adj. # at Risk                   44.9 47.4 46.4 45.6
      CARCINOMA,C-CELL                  0    0    2    2    .0705  .2528
.2584  .
    Trigeminal nerve
      # Evaluated                      60   60   60   60
      Adj. # at Risk                   45.8 50.1 48.8 45.9
      SCHWANNOMA,MALIGNANT              0    0    1    0    .4947  .
.5161  .
    Urinary bladder
      # Evaluated                      60   60   60   60
      Adj. # at Risk                   45.8 50.1 48.1 45.9
      PAPILLOMA,TRANSITIONAL CELL       0    0    1    0    .4947  .
.5161  .
    Zymbal gland
      # Evaluated                      60   60   60   60
      Adj. # at Risk                   45.8 50.1 48.1 45.9
      ADENOMA                           1    0    0    0    1      1      1
1
      Adj. # at Risk                   46.4 50.1 48.5 45.9
      Adenoma/Carcinoma                 2    0    2    0    .8206  1
.7076  1
      Adj. # at Risk                   46.4 50.1 48.5 45.9
      CARCINOMA                         1    0    2    0    .6733  1
.5161  1
```

Table A.2.4. Incidence and Results for Organ-Tumor Combinations in Female Rats

	Overall Results Tumor Incidcence				Significance Levels			
Organ/tumor	Veh	Low	Med	High	ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Adrenal								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.5	41.5	42.4	49.4				
ADENOMA,CORTICAL CELL	1	1	2	1	.5562	.7843	.4911	.7410
Adj. # at Risk	43.3	41.4	42.7	49.4				
CARCINOMA,CORTICAL CELL	0	0	1	0	.5200	.	.4941	.
Adj. # at Risk	43.5	41.5	42.7	49.4				

Cortical Adenoma/Carcinoma	1	1	3	1	.5917	.7843	.2991	.7410
Adj. # at Risk	43.3	41.4	42.4	49.5				
PHEOCHROMOCYTOMA	0	0	1	3	.0261	.1467	.4941	.
Adj. # at Risk	43.3	41.4	42.4	49.4				
PHEOCHROMOCYTOMA,MALIGNANT	0	1	0	0	.7543	.	.	.4881
Adj. # at Risk	43.3	41.4	42.4	49.5				
Pheochromocytoma, Any	0	1	1	3	.0590	.1467	.4941	.4881
Cerebrum								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.4	42.4	49.4				
ASTROCYTOMA,MALIGNANT	1	0	1	1	.4347	.7843	.7471	1
Adj. # at Risk	43.3	41.4	42.4	49.4				
PAPILLOMA,CHOROID PLEXUS	0	1	0	0	.7543	.	.	.4881
Hemolymphoreticular(all sites)								
# Evaluated	60	60	60	60				
Adj. # at Risk	44.1	41.4	42.4	49.4				
LEUKEMIA,LARGE GRANULAR LYMPHO	1	0	0	0	1	1	1	1
Adj. # at Risk	43.3	41.6	42.4	49.9				
LYMPHOMA,MALIGNANT	0	1	0	1	.3444	.5326	.	.4881
Adj. # at Risk	43.6	41.4	42.7	49.4				
SARCOMA,HISTIOCYTIC	2	0	1	0	.9036	1	.8751	1
Intestine,jejunum								
# Evaluated	58	56	57	58				
Adj. # at Risk	41.5	39.9	41.6	48.8				
LEIOMYOSARCOMA	0	1	0	0	.7574	.	.	.4875
Kidney								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.4	42.4	49.4				
ADENOMA,RENAL CELL	0	1	0	0	.7543	.	.	.4881
Adj. # at Risk	43.3	41.4	43.2	49.4				
LIPOMA	0	0	1	0	.5227	.	.5000	.
Liver								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.4	42.4	49.9				
ADENOMA,HEPATOCELLULAR	1	0	0	1	.4828	.7843	1	1

Table A.2.4. (cont.) Incidence and Results for Organ-Tumor Combinations in Female Rats

	Overall Results Tumor Incidcence				Significance Levels			
Organ/tumor	Veh	Low	Med	High	ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Mammary gland								
# Evaluated	60	60	60	60				
Adj. # at Risk	47.7	47.0	48.0	52.9				
ADENOCARCINOMA	23	18	18	18	.8704	.9506	.9088	.8941
Adj. # at Risk	45.2	44.1	43.2	49.8				
ADENOCARCINOMA IN FIBROADENOMA	6	9	2	4	.9045	.8743	.9663	.2700
Adj. # at Risk	43.3	42.5	42.8	49.4				
ADENOMA	0	3	2	1	.6138	.5326	.2412	.1162
Adj. # at Risk	49.2	49.0	50.5	53.1				
Adenoma/Fibro-/-carc/carc in	36	28	32	28	.9594	.9912	.8913	.9634
Adj. # at Risk	45.3	44.0	45.9	50.1				

FIBROADENOMA	20	14	15	15	.8651	.9527	.9029	.9139
Adj. # at Risk	43.3	41.4	42.4	49.4				
LIPOMA	0	1	0	0	.7543	.	.	.4881
Adj. # at Risk	43.8	41.4	42.4	50.2				
TUMOR,MIXED,MALIGNANT	2	0	0	1	.6356	.9049	1	1
Oral cavity								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.5	43.0	49.7				
CARCINOMA,SQUAMOUS CELL	0	1	1	1	.3339	.5326	.4941	.4881
Ovary								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.4	42.4	49.4				
GRANULOSA CELL TUMOR	0	0	1	0	.5200	.	.4941	.
Adj. # at Risk	43.3	41.4	42.9	49.4				
THECOMA,BENIGN	0	0	1	0	.5200	.	.4941	.
Pancreas								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.9	41.6	42.4	49.4				
ADENOMA,ISLET CELL	5	4	4	2	.9204	.9622	.7465	.7340
Adj. # at Risk	43.3	41.4	42.4	49.4				
CARCINOMA,ACINAR CELL	0	0	1	0	.5200	.	.4941	.
Adj. # at Risk	43.3	41.4	42.4	49.4				
CARCINOMA,ISLET CELL	0	0	0	1	.2800	.5326	.	.
Adj. # at Risk	43.9	41.6	42.4	49.4				
Islet Cell Adenoma/Carcinoma	5	4	4	3	.8239	.9045	.7465	.7340
Parathyroid								
# Evaluated	58	59	59	59				
Adj. # at Risk	41.9	41.3	41.9	49.4				
ADENOMA	1	0	2	0	.7303	1	.5000	1
Pituitary								
# Evaluated	60	60	60	60				
Adj. # at Risk	58.1	56.7	55.4	54.7				
ADENOMA,ANTERIOR	49	44	44	38	.9572	.9787	.8079	.8544
Adj. # at Risk	58.7	58.0	56.4	55.5				
Anterior Adenoma/Carcinoma	51	48	47	41	.9633	.9814	.8116	.8532
Adj. # at Risk	43.9	42.7	43.4	50.3				
CARCINOMA,ANTERIOR	2	4	3	3	.5633	.5719	.5000	.3267
Skeletal muscle,femoral								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.4	42.4	49.5				
FIBROSARCOMA	0	0	0	1	.2800	.5326	.	.

Table A.2.4. (cont.) Incidence and Results for Organ-Tumor Combinations in Female Rats

	Overall Results Tumor Incidcence				Significance Levels			
Organ/tumor	Veh	Low	Med	High	ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Skin/Subcutis								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.5	42.4	49.4				
CARCINOMA,SQUAMOUS CELL	0	1	0	0	.7543	.	.	.4881
Adj. # at Risk	43.3	42.3	42.4	49.4				
FIBROSARCOMA	0	1	0	0	.7557	.	.	.4941
Adj. # at Risk	43.3	41.4	42.4	49.4				
KERATOACANTHOMA	0	1	0	0	.7543	.	.	.4881
Adj. # at Risk	43.3	41.4	42.4	49.4				
LIPOMA	1	0	0	0	1	Thyroid		
# Evaluated	59	59	59	59				
Adj. # at Risk	42.5	40.9	42.9	49.5				
ADENOMA,C-CELL	1	5	5	7	.0999	.0478	.1008	.0899
Adj. # at Risk	42.3	40.7	42.3	48.8				
ADENOMA,FOLLICULAR CELL	1	1	2	0	.8236	1	.5000	.7407

Adj. # at Risk	42.3	40.9	42.3	49.4				
CARCINOMA,C-CELL	1	4	0	1	.8344	.7897	1	.1648
Stomach								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.4	42.6	49.4				
PAPILLOMA,SQUAMOUS CELL	0	0	1	0	.5200	.	.4941	.
Thymus								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.4	42.4	49.4				
THYMOMA	0	0	0	1	.2800	.5326	.	.
Uterus								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.4	42.4	49.4				
GRANULAR CELL TUMOR	1	0	0	1	.4828	.7843	1	1
Adj. # at Risk	43.3	41.4	42.4	49.4				
LEIOMYOMA	0	0	1	0	.5200	.	.4941	.
Adj. # at Risk	44.3	43.0	43.0	49.9				
POLYP,ENDOMETRIAL STROMAL	8	5	4	5	.8023	.9206	.9306	.8674
Adj. # at Risk	43.3	41.4	42.4	49.6				
SCHWANNOMA,MALIGNANT	0	0	0	1	.2800	.5326	.	.
Vagina								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.4	41.4	42.4	49.4				
GRANULAR CELL TUMOR	1	0	0	0	1	1	1	1
Adj. # at Risk	43.3	41.9	42.4	49.4				
POLYP,VAGINAL STROMAL	0	2	0	0	.8249	.	.	.2352
Adj. # at Risk	43.3	41.4	42.5	49.7				
SCHWANNOMA,MALIGNANT	0	0	1	1	.2124	.5326	.4941	.
Zymbal gland								
# Evaluated	60	60	60	60				
Adj. # at Risk	43.3	41.4	42.4	49.5				
CARCINOMA	1	0	0	1	.4828	.7843	1	1	1	1
Adj. # at Risk	43.3	41.4	42.4	49.5				
PAPILLOMA,SQUAMOUS CELL	0	0	0	1	.2800	.5326	.	.
Adj. # at Risk	43.3	41.5	42.4	49.5				
Sq.Cell Carc,Pap,Keratoacan-thoma	0	2	0	1	.4805	.5326	.	.2352

The following two tables give similar results in mice. Again, for each identified neoplasm within organ, the adjusted number at risk is presented first. The next row provides the tumor incidence over all five dose groups, followed by the significance levels of test of trend over the actual dose groups 1-4, and then followed by the results of the comparisons between the high dose and the high-medium dose, respectively, with the vehicle. The next row, with slightly indented p-values lined up with those of the preceding row, presents the significance levels of the comparisons between the low and positive control, respectively, with vehicle.

Table A.2.5. Incidence and Results for Organ-Tumor Combinations in Male Mice

	Overall Results							
	Tumor Incidcence				Significance Levels			
Organ/Tumor	Veh	Low	Med	High	ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Adrenal								
# Evaluated	55	55	55	55				
Adj. # at Risk	42.4	51.5	52.0	49.0				
ADENOMA,SUBCAPSULAR CELL	2	0	0	0	1	1	1	1
Cranial bone								
# Evaluated	55	54	55	55				
Adj. # at Risk	42.4	50.6	52.7	49.0				

OSTEOSARCOMA	0	0	1	0	.5208	.	.5532	.
Harderian gland								
# Evaluated	55	55	55	55				
Adj. # at Risk	42.4	51.5	52.0	49.0				
ADENOCARCINOMA	0	0	0	1	.2500	.5333	.	.
Adj. # at Risk	43.1	51.5	52.0	49.0				
ADENOMA	5	7	5	3	.8807	.8992	.7334	.5053
Adj. # at Risk	42.4	51.5	52.0	49.0				
Adenoma/Adenocarcinoma	0	0	0	1	.2500	.5333	.	.
Heart								
# Evaluated	55	55	55	55				
Adj. # at Risk	42.4	51.5	52.0	49.0				
HEMANGIOSARCOMA	1	0	0	0	1	1	1	1
Hemolymphoreticular(all sites)								
# Evaluated	55	55	55	55				
Adj. # at Risk	42.6	52.0	52.7	49.0				
LYMPHOMA,MALIGNANT	7	7	5	5	.8373	.8811	.9079	.7664
Adj. # at Risk	43.6	51.5	52.0	49.1				
SARCOMA,HISTIOCYTIC	3	1	0	2	.7323	.8575	1	.9595
Intestine, overall								
# Evaluated	55	55	55	55				
Adj. # at Risk	42.4	51.5	52.0	49.0				
ADENOMA	1	1	0	0	.9530	1	1	.7987
Intestine,cecum								
# Evaluated	51	53	53	47				
Adj. # at Risk	40.2	50.3	50.7	45.7				
LEIOMYOSARCOMA	0	1	0	0	.7838	.	.	.5556
Intestine,duodenum								
# Evaluated	52	51	51	47				
Adj. # at Risk	40.6	48.7	49.7	45.7				
ADENOMA	1	0	0	0	1	1	1	1

Table A.2.5. (cont.) Incidence and Results for Organ-Tumor Combinations in Male Mice

	Overall Results							
	Tumor Incidcence				Significance Levels			
Organ/Tumor	Veh	Low	Med	High	ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Intestine,ileum								
# Evaluated	52	52	52	50				
Adj. # at Risk	40.3	49.3	49.8	47.2				
ADENOMA	0	1	0	0	.7838	.	.	.5506
Adj. # at Risk	40.8	49.3	49.8	47.2				
HEMANGIOMA	1	0	0	0	1	1	1	1
Kidney								
# Evaluated	55	55	55	55				
Adj. # at Risk	42.4	51.5	52.0	49.0				
ADENOMA,RENAL CELL	0	0	0	1	.2500	.5333	.	.
Liver								
# Evaluated	55	55	55	55				
Adj. # at Risk	43.7	52.3	52.0	49.1				
ADENOMA,HEPATOCELLULAR	15	10	7	7	.9897	.9950	.9962	.9749
Adj. # at Risk	44.0	52.5	52.6	49.6				
CARCINOMA,HEPATOCELLULAR	9	7	8	5	.9086	.9578	.8342	.8930

```
 Adj. # at Risk                42.4 51.5 52.0 49.0
 CARCINOMA,HEPATOCHOLANGIO-     0    1    0    0    .7812  .
.      .5484
    CELLULAR
 Adj. # at Risk                42.4 51.5 52.0 49.0
 HEMANGIOMA                     0    0    1    0    .5156  .
.5484  .
 Adj. # at Risk                42.4 51.6 52.0 49.0
 HEMANGIOSARCOMA                1    1    1    0    .8684  1
.7987  .7987
 Adj. # at Risk                45.3 52.9 52.6 49.7
 Hepato. Adenoma/Carc./Heman- 22   16   15   12    .9897  .9964
.9875  .9791
   gio-
Lung(bronchus)
 # Evaluated                   55   55   55   55
 Adj. # at Risk                42.6 51.5 52.0 49.1
 ADENOMA,BRONCHIOLO-ALVEOLAR   5    2    3    4    .6298  .8283
.9238  .9689
 Adj. # at Risk                42.5 51.5 52.0 49.0
 CARCINOMA,BRONCHIOLO-ALVEOLAR 2    1    1    0    .9566  1
.9115  .9115
Pancreas
 # Evaluated                   55   55   55   55
 Adj. # at Risk                42.4 51.5 52.0 49.0
 ADENOMA,ACINAR CELL           0    0    0    1    .2500  .5333
.      .
Skin+Subcutis
 # Evaluated                   55   55   55   55
 Adj. # at Risk                42.4 51.5 52.0 49.0
 HEMANGIOMA                     0    1    0    0    .7812  .
.      .5484
 Adj. # at Risk                42.4 51.5 52.0 49.0
 HEMANGIOSARCOMA                0    0    1    0    .5156  .
.5484  .
 Adj. # at Risk                42.4 51.5 52.0 49.0
 Hemangioma/-sarcoma            0    1    1    0    .6499  .
.5484  .5484
 Adj. # at Risk                42.4 51.5 52.0 49.8
 LEIOMYOSARCOMA                 0    0    0    1    .2539  .5385
.      .
```

Table A.2.5. (cont.) Incidence and Results for Organ-Tumor Combinations in Male Mice

Organ/Tumor	Overall Results Tumor Incidcence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh

```
Systemic
 # Evaluated                    55   55   55   55
 Adj. # at Risk                42.8 51.5 52.0 49.0
 HEMANGIOMA                     1    1    1    0    .8684  1
.7987  .7987
```

```
 Adj. # at Risk                42.4 51.6 52.0 49.0
 HEMANGIOSARCOMA                2    1    2    0    .9219  1
.7611  .9115
 Adj. # at Risk                42.8 51.6 52.0 49.0
 Hemangioma/-sarcoma            3    2    3    0    .9615  1
.7490  .8738
Testis
 # Evaluated                    55   55   55   55
 Adj. # at Risk                42.4 51.5 52.0 49.0
 LEYDIG CELL TUMOR              0    0    1    2    .0644  .2816
.5484  .


Thymus
 # Evaluated                    54   55   54   53
 Adj. # at Risk                42.1 51.5 51.0 47.5
 THYMOMA                        0    0    1    0    .5105  .
.5435  .
Thyroid
 # Evaluated                    55   55   55   55
 Adj. # at Risk                42.4 51.5 52.0 49.0
 ADENOMA,FOLLICULAR CELL        0    0    1    2    .0644  .2816
.5484  .
 Adj. # at Risk                42.4 51.5 52.0 49.0
 CARCINOMA,FOLLICULAR CELL      0    0    1    0    .5181  .
.5532  .
 Adj. # at Risk                42.4 51.5 52.0 49.0
 Foll. Cell Adenoma/Carcinoma   0    0    2    2    .0749  .2816
.3034  .
```

```
Tongue
 # Evaluated                    55   55   55   55
 Adj. # at Risk                42.4 51.5 52.0 49.0
 PAPILLOMA,SQUAMOUS CELL        1    0    0    0    1      1
1      1
```

Table A.2.6 Incidence and Results for Organ-Tumor Combinations in Female Mice

organ/tumor	Overall Results Tumor Incidcence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	plow vsVeh	pmed vsVeh
Adrenal								
# Evaluated	55	55	55	55				
Adj. # at Risk	52.4	52.8	52.1	41.1				
ADENOMA,CORTICAL CELL	0	0	1	0	.4721	.	.5000	.
Adj. # at Risk	52.4	52.8	52.1	41.1				
ADENOMA,SUBCAPSULAR CELL	1	1	0	0	.9313	1	1	.7524
Adj. # at Risk	52.4	52.8	52.1	41.1				
PHEOCHROMOCYTOMA	0	2	0	1	.3914	.4409	.	.2476
Adj. # at Risk	52.4	52.8	52.1	41.1				
PHEOCHROMOCYTOMA,MALIGNANT	0	1	0	0	.7360	.	.	.5000
Adj. # at Risk	52.4	52.8	52.1	41.1				
Pheochromocytoma, Any	0	3	0	1	.4791	.4409	.	.1214
Bone+Bone marrow,femoral								
# Evaluated	55	55	55	55				
Adj. # at Risk	52.4	52.8	52.1	41.1				
HEMANGIOSARCOMA	0	1	0	0	.7360	.	.	.5000
Brachium								
# Evaluated	55	55	55	55				
Adj. # at Risk	52.4	52.8	52.1	41.1				
SARCOMA,NOS	0	0	1	0	.4721	.	.5000	.
Buccal								
# Evaluated	55	55	55	55				
Adj. # at Risk	52.4	52.8	52.1	41.1				
OSTEOSARCOMA	0	1	0	0	.7360	.	.	.5000
Harderian gland								

```
 # Evaluated                    55   55   55   55
 Adj. # at Risk                52.4 52.8 52.4 41.6
 ADENOMA                        5    6    2    3    .7913  .7748
.9439  .5000
Hemolymphoreticular(all sites)
 # Evaluated                    55   55   55   55
 Adj. # at Risk                53.6 53.2 52.5 41.1
 LYMPHOMA,MALIGNANT            24   20   15    5    .9999  .9999
.9745  .8378
 Adj. # at Risk                52.7 52.8 53.2 41.1
 SARCOMA,HISTIOCYTIC            4    0    7    1    .6544  .9500
.2741  1
Intestine,duodenum
 # Evaluated                    52   55   50   52
 Adj. # at Risk                50.3 52.8 48.1 39.3
 ADENOMA                        0    1    0    0    .7354  .
.      .5098
Kidney
 # Evaluated                    55   55   54   55
 Adj. # at Risk                52.4 52.8 51.4 41.1
 ADENOMA,RENAL CELL             1    0    0    0    1      1
1      1
 Adj. # at Risk                52.4 52.8 51.4 41.1
 CARCINOMA,RENAL CELL           2    0    0    0    1      1
1      1
 Adj. # at Risk                52.4 52.8 51.4 41.1
 Renal Cell Adenoma/carcinoma   2    0    0    0    1      1
1      1
```

Table A.2.6. (cont.) Incidence and Results for Organ-Tumor Combinations in Female Mice

```
                              Overall Results
                              Tumor Incidcence      Significance Levels
organ/tumor                   Veh  Low  Med  High   ptrend  phigh
pmed  plow
                                                                vsVeh
vsVeh vsVeh
Liver
 # Evaluated                    55   55   55   55
 Adj. # at Risk                52.4 52.8 52.1 41.3
 ADENOMA,HEPATOCELLULAR        12    6    3    3    .9934  .9923
.9980  .9662
 Adj. # at Risk                52.6 52.8 52.1 41.1
 CARCINOMA,HEPATOCELLULAR       3    4    6    1    .7298  .9073
.2439  .5000
 Adj. # at Risk                52.7 52.8 52.1 41.1
```

```
 HEMANGIOSARCOMA                1    0    0    0    1      1
1      1
 Adj. # at Risk               52.6 52.8 52.1 41.3
 Hepato. Adenoma/carcinoma    15    9    9    4    .9897  .9955
.9489  .9489
Lung(bronchus)
 # Evaluated                  55   55   55   55
 Adj. # at Risk               52.6 52.8 52.1 41.1
 ADENOMA,BRONCHIOLO-ALVEOLAR   3    1    1    1    .8227  .9073
.9411  .9411
 Adj. # at Risk               52.6 52.8 52.1 41.1
 Bronch-Alv. Adenoma/Carc.     6    2    2    2    .8971  .9388
.9701  .9701
 Adj. # at Risk               52.4 52.8 52.1 41.1
 CARCINOMA,BRONCHIOLO-ALVEOLAR 3    1    1    1    .8227  .9073
.9411  .9411
Mammary gland
 # Evaluated                  55   55   55   55
 Adj. # at Risk               52.4 52.8 52.1 41.1
 ADENOACANTHOMA,MALIGNANT      0    2    0    0    .7913  .
.      .2476
 Adj. # at Risk               52.4 52.8 52.1 41.1
 ADENOCARCINOMA                0    2    0    0    .7913  .
.      .2476
 Adj. # at Risk               52.4 52.8 52.1 41.1
 ADENOMA                       1    1    0    0    .9313  1
1      .7524
 Adj. # at Risk               52.4 52.8 52.1 41.1
 Adenoma/-carc./-canthoma      1    4    0    0    .9365  1
1      .1813
Ovary
 # Evaluated                  55   55   55   55
 Adj. # at Risk               52.4 52.8 52.1 41.1
 CYSTADENOMA                   0    1    0    0    .7360  .
.      .5000
 Adj. # at Risk               52.4 52.8 52.1 41.1
 GRANULOSA CELL TUMOR          0    0    1    0    .4721  .
.5000  .
 Adj. # at Risk               52.4 52.8 52.3 41.1
 HEMANGIOMA                    0    1    1    0    .5825  .
.5000  .5000
 Adj. # at Risk               52.4 52.8 52.1 41.1
 LUTEOMA                       1    0    0    0    1      1
1      1
 Adj. # at Risk               52.4 52.8 52.1 41.1
 SERTOLI CELL TUMOR            1    1    0    0    .9313  1
1      .7524
```

```
 Adj. # at Risk                52.4 52.8 52.6 41.1
 TERATOMA                       0    0    1    0    .4721  .
.5000  .
 Adj. # at Risk                52.4 52.8 52.1 41.1
 YOLK SAC CARCINOMA             0    1    0    0    .7360  .
.      .5000
```

Table A.2.6. (cont.) Incidence and Results for Organ-Tumor Combinations in Female Mice

```
                            Overall Results
                            Tumor Incidcence      Significance Levels
organ/tumor                  Veh  Low  Med  High   ptrend  phigh
pmed  plow
                                                                vsVeh
vsVeh vsVeh
Pancreas
 # Evaluated                 55   55   54   55
 Adj. # at Risk              52.4 52.8 51.4 41.3
 ADENOMA,ISLET CELL           0    0    0    1    .2092  .4409
.      .
Pituitary
 # Evaluated                 55   55   55   55
 Adj. # at Risk              52.4 52.8 52.9 41.6
 ADENOMA,ANTERIOR            10    8   10    2    .9701  .9936
.5980  .7812
 Adj. # at Risk              52.4 52.8 52.1 41.1
 ADENOMA,INTERMEDIATED        0    1    1    0    .5825  .
.5000  .5000
Skin+Subcutis
 # Evaluated                 55   55   55   55
 Adj. # at Risk              52.4 53.2 52.1 41.1
 CARCINOMA,BASAL CELL         0    1    0    0    .7374  .
.      .5048
 Adj. # at Risk              52.4 52.8 52.1 41.1
 FIBROMA                      1    0    0    0    1      1
1      1
 Adj. # at Risk              52.4 52.8 52.2 41.6
 FIBROSARCOMA                  1    0    2    1    .3506  .6900
.5000  1
 Adj. # at Risk              52.4 52.8 52.1 41.1
 HEMANGIOSARCOMA              1    0    0    0    1      1
1      1
 Adj. # at Risk              52.4 53.2 52.1 41.1
 LIPOSARCOMA                  1    1    0    0    .9320  1
1      .7571
 Adj. # at Risk              52.4 52.8 52.1 41.1
```

```
 MELANOMA,MALIGNANT            0    1    1    0    .5825  .
.5000  .5000
 Adj. # at Risk               52.4 52.8 52.1 41.1
 SARCOMA,NOS                   0    1    1    0    .5825  .
.5000  .5000
Spleen
 # Evaluated                  55   55   55   55
 Adj. # at Risk               52.4 52.8 52.1 41.1
 HEMANGIOMA                    1    0    0    0    1      1
1      1
Stomach
 # Evaluated                  54   55   54   54
 Adj. # at Risk               51.7 52.8 51.1 40.3
 NEUROENDOCRINE TUMOR,MALIG-   1    0    0    0    1      1
1      1
     NANT
 Adj. # at Risk               51.7 52.8 51.1 40.5
 PAPILLOMA,SQUAMOUS CELL       1    0    1    2    .1498  .4088
.7525  1
Systemic
 # Evaluated                  55   55   55   55
 Adj. # at Risk               52.4 52.8 52.3 41.1
 HEMANGIOMA                    1    1    2    0    .7440  1
.5000  .7524
 Adj. # at Risk               52.7 52.8 52.1 41.1
 HEMANGIOSARCOMA               2    2    1    0    .9365  1
.8786  .6912
 Adj. # at Risk               52.7 52.8 52.3 41.1
 Hemangioma/-sarcoma           3    3    3    0    .9338  1
.6609  .6609
```

Table A.2.6. (cont.) Incidence and Results for Organ-Tumor Combinations in Female Mice

```
                              Overall Results
                              Tumor Incidcence      Significance Levels
organ/tumor                   Veh  Low  Med  High   ptrend  phigh
pmed  plow
                                                                vsVeh
vsVeh vsVeh
Thoracic cavity
 # Evaluated                  53   54   55   55
 Adj. # at Risk               50.6 51.9 52.7 41.1
 OSTEOSARCOMA                  0    0    1    0    .4794  .
.5098  .
Thyroid
 # Evaluated                  55   55   55   55
```

Adj. # at Risk	52.4	52.8	52.2	41.1		
ADENOMA,FOLLICULAR CELL	1	1	1	3	.0889	.2245
.7524	.7524					
Uterus						
# Evaluated	55	55	55	55		
Adj. # at Risk	52.4	52.8	52.1	41.1		
ADENOCARCINOMA	1	2	1	0	.8511	1
.7524	.5000					
Adj. # at Risk	52.4	52.8	52.1	41.1		
CYSTADENOMA	0	1	0	0	.7360	.
.	.5000					
Adj. # at Risk	52.4	52.8	52.1	41.1		
GRANULAR CELL TUMOR	0	1	1	0	.5825	.
.5000	.5000					
Adj. # at Risk	52.4	52.8	52.1	41.1		
HEMANGIOMA	0	0	1	0	.4721	.
.5000	.					
Adj. # at Risk	52.4	52.8	52.1	41.1		
HEMANGIOSARCOMA	0	2	0	0	.7913	.
.	.2476					
Adj. # at Risk	52.4	52.8	52.1	41.1		
LEIOMYOMA	1	0	0	0	1	1
1	1					
Adj. # at Risk	52.4	52.8	52.1	41.1		
LEIOMYOSARCOMA	0	0	1	0	.4721	.
.5000	.					
Adj. # at Risk	52.4	52.8	52.1	42.0		
POLYP,ENDOMETRIAL STROMAL	2	4	1	3	.3897	.3989
.8786	.3391					
Vagina						
# Evaluated	55	55	55	55		
Adj. # at Risk	52.4	52.8	52.1	41.1		
HEMANGIOSARCOMA	0	0	1	0	.4721	.
.5000	.					

Appendix 3. References

Bailer, A. and Portier, C. (1988), “Effects of Treatment-Induced Mortality on Tests for Carcinogenicity in Small Samples”, *Biometrics*, **44**, 4, 417-431.

Bieler, G.S., and Williams, R.L. (1993), “Ratio Estimates, the Delta Method, and Quantal Response Tests for Increased Carcinogenicity”, *Biometrics*, **49**, 4, 793-801.

Chu, K.C., Ceuto, C., and Ward, J.M. (1981), “Factors in the Evaluation of 200 National Cancer Institute Carcinogen Bioassays”, *Journal of Toxicology and Environmental Health,* **8**, 251-280.

Greaves, P. (2007), “Neoplasia of Adrenal Medulla,” In: *Histopathology of Preclinical Toxicity Studies.* 3rd edition, pp 819. Oxford, UK: Academic Press, Elsevier Inc.

Haseman, J. K. (1983), “A Reexamination of False-positive Rates for Carcinogenicity Studies”, *ndamental and Applied Toxicology,* **3**, 334-339.

Jara, A. (2007), “Applied Bayesian Non- and Semi-parametric Inference using DPpackage”, *Rnews***, 7**, 3, 17-26.

Lin, K. K. and Ali, M.W. (2006), “Statistical Review and Evaluation of Animal Tumorigenicity Studies”, *Statistics in the Pharmaceutical Industry, Third Edition,* edited by C.R. Buncher and J.Y. Tsay, Marcel Dekker, Inc. New York.

Lin, K. K. and Rahman, M.A. (1998), “Overall False Positive Rates in Tests for Linear Trend in Tumor Incidence in Animal Carcinogenicity Studies of New Drugs”, *Journal of Biopharmaceutical Statistics*. **8**, 1, 1-15.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986), “Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies”, *Journal of the National Cancer Institute*. **76**, 283-289.

Parola, A, and Jacobs, A. (2010). “Combining Tumors for Statistical Analysis”, online FDA handout.

Peto, R., Pike, M.C., Day, N.E., Gray, R.G., Lee, P.N., Parrish, S., Peto, J., Richards, S., and Wahrendorf, J. (1980). “Guidelines for sample sensitive significance tests for carcinogenic effects in long-term animal experiments”, *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, supplement 2: Long term and Short term Screening Assays for Carcinogens: A Critical Appraisal*, International Agency for Research Against Cancer, 311-426.

R Development Core Team (2009). *R: A language and environment for statistical computing. R Foundation for Statistical Computing*, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rahman, M.A. and Lin, K.K. (2008), "A Comparison of False Positive Rates of Peto and Poly-3 Methods for Long Term Carcinogenicity Data Analysis Using Multiple Comparison Adjustment Method Suggested by Lin and Rahman", *Journal of Biopharmaceutical Statistics*. **18**, 949-958.

STP Peto Working Group (2002), "Statistical Methods for Carcinogenicity Studies", *Toxicologic Pathology*. **30** (3), 403-414.

U.S. Department of Health and Human Services (2013), Guidance for Industry Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals (DRAFT GUIDANCE), Center for Drug Evaluation and Research, Food and Drug Administration

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Appendix III. CAC Rat and Mouse Study Information

Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Proposals

Application (IND/NDA) number: NDA 207947
Submission date and number: 12/22/2011, #55
Division: DCaRP
Project manager: Wayne Amchin
CAS#:475086-01-2
Drug name: Selexipag
Pharmacological Classification: Platelet aggregation inhibitors excl. heparin
Sponsor/Applicant: Actelion Pharmaceuticals
Sponsor/Applicant contact name: James B. Davis
Sponsor/Applicant telephone and fax number: 856-773-5719 (phone) 856-773-4247 (fax)
Date submitted (stamp date):12/18/14
45-day date (from submission stamp date):
P/T Reviewer(s):James M. Willard
Date Review Completed: 6/23/15
Date of CAC review: Scheduled for Dec 1, 2015
CAC members:

Summary of Proposal for Review:

Species/strain: B6C3F1/Crlj SPF mice
Number/sex/dose:

Group composition

Test group	Dose (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg/day)	Sex	Main group		Satellite group	
					No. of animals	Animal No.	No. of animals	Animal No.
Control[a)]	0	0	10	M	55	1001-1055	9	1201-1209
				F	55	1101-1155	9	1301-1309
Low	125	12.5	10	M	55	2001-2055	24	2201-2224
				F	55	2101-2155	24	2301-2324
Middle	250	25	10	M	55	3001-3055	24	3201-3224
				F	55	3101-3155	24	3301-3324
High	500	50	10	M	55	4001-4055	29	4201-4229
				F	55	4101-4155	29	4301-4329

a): 0.5 w/v% methylcellulose solution, M: Male, F: Female

Route: oral gavage

	male	**female**
Doses proposed:	0, 125, 250, 500 mg/kg/day	0, 125, 250, 500 mg/kg/day
Basis of dose selection:		
MTD	13 week study	13 week study
AUC ratio	_____	_____ saturation
MFD	_____	_____
PD	_____	_____
other	_____	_____

Kinetics submitted:	**rodent**	**human**
pharmacokinetics	_X_____	__X____
metabolism	_X_____	__X____
protein binding	_X__	__X____

Notable design features: none

Summary of Recommendations to CAC

	male	**female**
Doses recommended by reviewer:	_____	_____

Basis for recommendation (details):

CAC Concurrence (y/n):

CAC Recommendations:

Comments:

CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT
AND
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET

P/T REVIEWER(s):
DATE:

IND/NDA:
DRUG CODE#:
CAS#:
DIVISION(s):
DRUG NAME(s):

SPONSOR:
LABORATORY:
CARCINOGENICITY STUDY REPORT DATE:

THERAPEUTIC CATEGORY:
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION:
MUTAGENIC/GENOTOXIC (y/n/equivocal/na; assay):

RAT CARCINOGENICITY STUDY (multiple studies? Std1; Std2 etc.):
\\cdsesub1\evsprod\IND104504\0053\m4\datasets\t-10-649\listings

RAT STUDY DURATION (weeks):105
STUDY STARTING DATE:11/29/2007
STUDY ENDING DATE:2/8/2011
RAT STRAIN: Crl:CD(SD) SPF
ROUTE: oral gavage
DOSING COMMENTS:

NUMBER OF RATS and RAT DOSE LEVELS* (mg/kg/day):

Group composition

Test group	Dose (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg/day)	Sex	Main group		Satellite group	
					No. of animals	Animal No.	No. of animals	Animal No.
Control[a)]	0	0	5	M	60	1001-1060	5	1201-1205
				F	60	1101-1160	5	1301-1305
Low	10	2	5	M	60	2001-2060	8	2201-2208
				F	60	2101-2160	8	2301-2308
Middle	30	6	5	M	60	3001-3060	8	3201-3208
				F	60	3101-3160	8	3301-3308
High	100	20	5	M	60	4001-4060	8	4201-4208
				F	60	4101-4160	8	4301-4308

a): 0.5 w/v% methylcellulose solution, M: Male, F: Female

BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible): MTD based on a 26 week study, (b)(4) 5895.

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): no

RAT CARCINOGENICITY (conclusion: negative; positive; MF; M; F): negative

RAT TUMOR FINDINGS (details):

Text Table 11. Number of tumors and tumor bearers

Sex	Male				Female			
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of animals used	60	60	60	60	60	60	60	60
Total No. of tumors	121	124	91	84	159	153	128	125
No. of benign tumors	102	107	70	69	104	94	95	79
No. of malignant tumors	19	17	21	15	55	59	33	46
Total No. of tumor bearing animals	52	55	47	43	56	57	59	52
No. of benign tumor bearers	46	52	37	38	52	52	57	46
No. of malignant tumor bearers	18	17	18	13	29	28	23	27
No. of multiple tumor bearers	31	34	23	20	41	37	39	36

Text Table 12. Incidence summary of major tumors and hyperplasias

Sex	Male				Female			
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of animals used	60	60	60	60	60	60	60	60
Testis								
Leydig cell tumor	2$[a)]	0	2	5	NA	NA	NA	NA
Hyperplasia, Leydig cell, focal (total, ±/+)	0	2	2	6	NA	NA	NA	NA
Pituitary								
Adenoma, anterior	20	40**	23	22	49	44	44	38

Numbers in the table indicate the number of animals with lesions.
$: p<0.025 (statistically significant positive trend, rare tumor, Petoís test)
**: p<0.01 (statistically significant difference from the control group, common tumor, Petoís test)
a): There was no statistically significant positive trend in the control, 10 and 30 mg/kg groups.
±: Minimal, +: Mild, NA: Not applicable

Testis: Marginally increased incidence of Leydig cell tumor was observed in the 100 mg/kg group, and statistically significant positive trend was noted (rare tumor, p<0.025); however, there was no statistical significance in pairwise comparison between the control and 100 mg/kg groups. Also, slightly higher incidence in focal hyperplasia of Leydig cells was observed in this group, although the difference in incidence between the two groups was very small. The tumor incidence of the 100 mg/kg group (5/60 animals, 8%) was marginally higher than that in our historical data (0 to 4% in the incidence). In addition, there was no statistically significant positive trend in incidence of Leydig cell tumor in the control, 10 and 30 mg/kg groups.

In the pituitary, increased incidence of anterior adenoma was observed in males in the 10 mg/kg group with statistical significance (common tumor, p<0.01). However, it was not considered to be treatment related, since it was not dose-related.

Decreased incidence of islet cell adenoma in the pancreas was observed in males in the 10 mg/kg and higher groups; however, reduced incidence of a common spontaneous tumor was not regarded as adverse effect of treatment.

RAT STUDY COMMENTS:

MOUSE CARCINOGENICITY STUDY (multiple studies? Std1; Std2 etc.): Single study.
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MOUSE STUDY DURATION (weeks):105
STUDY STARTING DATE:12/28/2007
STUDY ENDING DATE:
MOUSE STRAIN: B6C3F1/Crlj SPF
ROUTE:oral gavage
DOSING COMMENTS:

NUMBER OF MICE:
- Control-1 (C1):55M/55F
- Low Dose (LD):55M/55F
- Middle Dose (MD):55M/55F
- High Dose-1 (HD1):55M/55F

MOUSE DOSE LEVELS* (mg/kg/day):
- Low Dose:125
- Middle Dose:250
- High Dose-1:500

BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible): MTD from 13 week study

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date):No

MOUSE CARCINOGENICITY (conclusion: negative; positive; MF; M;F):Negative

MOUSE TUMOR FINDINGS (details):

Text Table 9. Number of tumors and tumor bearers

Sex	Male				Female			
Dose (mg/kg/day)	0	125	250	500	0	125	250	500[a)]
No. of animals used	55	55	55	55	55	55	55	55
Total No. of tumors	62	46	44	35	89	81	66	30
No. of benign tumors	37	24	23	21	45	40	27	21
No. of malignant tumors	25	22	21	14	44	41	39	9
Total No. of tumor bearing animals	38	31	26	27	45	41	42	19
No. of benign tumor bearers	24	16	16	16	31	26	23	15
No. of malignant tumor bearers	21	18	17	13	32	30	32	8
No. of multiple tumor bearers	16	13	10	6	26	23	19	9

a): All surviving females in the 500 mg/kg group were sacrificed prematurely in Week 100 after administration for 99 weeks.

Text Table 10. Incidence summary of tumors and hyperplasia in the thyroid

Sex	Male				Female			
Dose (mg/kg/day)	0	125	250	500	0	125	250	500[a)]
No. of animals used	55	55	55	55	55	55	55	55
Thyroid								
Adenoma, follicular cell	0	0	1	2	1	1	1	3
Carcinoma, follicular cell	0	0	1	0	0	0	0	0
Adenoma + Carcinoma, follicular cell	0	0	2	2	1	1	1	3
Hyperplasia/hypertrophy, follicular cell (total)	1	2	17	36	2	51	52	52
(±)	1	2	17	33	2	41	20	4
(+/++)	0	0	0	3	0	10	32	48

a): All surviving females in the 500 mg/kg group were sacrificed prematurely in Week 100 after administration for 99 weeks.
Numbers in the table indicate the number of animals with lesions.
±; Minimal, +; Mild, ++; Moderate

Thyroid: Slightly higher incidence of follicular cell tumors (adenoma + carcinoma) were observed in 2 males each in the 250 and 500 mg/kg groups and in 3 females in the 500 mg/kg group, although there were no statistically significant differences in either trend analysis or pairwise comparison between the control and any dose group. At the same time, increased incidence and severity of hyperplasia/hypertrophy of the follicular cells were observed in the above groups. Follicular cell adenoma was also observed in 1 female each in the 125 and 250 mg/kg groups, respectively, and the possibility of involvement of treatment could not be completely excluded, since increased incidence and severity of hyperplasia/hypertrophy of the follicular cells were also observed in these groups. However, it was hardly judged to be treatment-related since follicular cell adenoma was also observed in 1 control female in the present study.

MOUSE STUDY COMMENTS: The sponsor identified an increase in the incidence of tumors and hyperplasia in the thyroid gland. Although there is a minor increase there, the numbers are not large. Of the 440 animals examined, there was only one thyroid carcinoma detected. Also detected were 9 adenomas, with a small dose dependency, with one of the adenomas in the control group (female). Hyperplasia/hypertrophy apparently fitted a more dose dependent incidence in the mice. In the males, 1,2,17, and 36 incidences were recorded in the, respectively, control, low, mid and high dose groups. All but 3 in the high dose group were minimal in nature. In the females, the sensitivity to hyperplasia/hypertrophy was more strongly linked to the presence of selexipag, with 2, 51, 52, 52 positive animals in the control, low, mid, and high dose groups. A higher proportion of the female mice also had mild/moderate

hyperplasia/hypertrophy, with 0, 10, 32, and 48 in the control, low, mid, and high dose groups.
However, it is important to note that in the sponsor's text table 9, it is clear that in general all types of tumors were reduced in the mice, with the exception of the thyroid gland, in a dose dependent fashion. It is important to note that these doses were at a very high multiple of the human equivalent dose.

Selexipag does not apparently increase tumors in this study in the mouse.

Appendix IV. Sponsor tables of tumor incidence in the mouse carcinogenicity study.

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Table 10-1 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice
Histopathological findings - tumor data
Kill type : All

Organs / Findings	Sex:	M	M	M	M
	Dose(mg/kg/day):	0	125	250	500
	Number:	55	55	55	55
Adrenal					
Number examined		55	55	55	55
ADENOMA,SUBCAPSULAR CELL		2	0	0	0
Harderian gland					
Number examined		55	55	55	55
ADENOMA		5	7	5	3
ADENOCARCINOMA		0	0	0	1
Heart					
Number examined		55	55	55	55
HEMANGIOSARCOMA		1	0	0	0
Intestine,duodenum					
Number examined		52	51	51	47
ADENOMA		1	0	0	0
Intestine,ileum					
Number examined		52	52	52	50
ADENOMA		0	1	0	0
HEMANGIOMA		1	0	0	0
Intestine,cecum					
Number examined		51	53	53	47
LEIOMYOSARCOMA		0	1	0	0
Kidney					
Number examined		55	55	55	55
ADENOMA,RENAL CELL		0	0	0	1
Liver					
Number examined		55	55	55	55
ADENOMA,HEPATOCELLULAR		15	10	7	7
HEMANGIOMA		0	0	1	0
CARCINOMA,HEPATOCELLULAR		9	7	8	5
CARCINOMA,HEPATOCHOLANGIOCELLULAR		0	1	0	0
HEMANGIOSARCOMA		1	1	1	0
Lung(bronchus)					
Number examined		55	55	55	55
ADENOMA,BRONCHIOLO-ALVEOLAR		5	2	3	4
CARCINOMA,BRONCHIOLO-ALVEOLAR		2	1	1	0
Pancreas					
Number examined		55	55	55	55
ADENOMA,ACINAR CELL		0	0	0	1
Skin+Subcutis					
Number examined		55	55	55	55
HEMANGIOMA		0	1	0	0
HEMANGIOSARCOMA		0	0	1	0
LEIOMYOSARCOMA		0	0	0	1
Testis					
Number examined		55	55	55	55
LEYDIG CELL TUMOR		0	0	1	2
Thymus					
Number examined		54	55	54	53
THYMOMA		0	0	1	0

M : Male
No statistically significant difference

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Table 10-2 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice
Histopathological findings - tumor data
Kill type : All

Organs	Sex:	M	M	M	M
	Dose(mg/kg/day):	0	125	250	500
Findings	Number:	55	55	55	55
Thyroid					
Number examined		55	55	55	55
ADENOMA,FOLLICULAR CELL		0	0	1	2
CARCINOMA,FOLLICULAR CELL		0	0	1	0
Total with ADENOMA,FOLLICULAR CELL and CARCINOMA,FOLLICULAR CELL		0	0	2	2
Tongue					
Number examined		55	55	55	55
PAPILLOMA,SQUAMOUS CELL		1	0	0	0
Hemolymphoreticular(all sites)					
Number examined		55	55	55	55
LYMPHOMA,MALIGNANT		7	7	5	5
SARCOMA,HISTIOCYTIC		3	1	0	2
Cranial bone					
Number examined		0	1	1	0
OSTEOSARCOMA		0	0	1	0

M : Male
No statistically significant difference

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Table 10-3 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice
Histopathological findings - tumor data
Kill type : Found dead / Moribund sacrifice

Organs	Sex:	M	M	M	M
	Dose(mg/kg/day):	0	125	250	500
Findings	Number:	28	16	9	15
Harderian gland					
Number examined		28	16	9	15
ADENOMA		3	0	0	0
Intestine,ileum					
Number examined		25	13	6	10
HEMANGIOMA		1	0	0	0
Liver					
Number examined		28	16	9	15
ADENOMA,HEPATOCELLULAR		4	3	1	1
CARCINOMA,HEPATOCELLULAR		5	5	2	4
CARCINOMA,HEPATOCHOLANGIOCELLULAR		0	1	0	0
HEMANGIOSARCOMA		0	1	0	0
Lung(bronchus)					
Number examined		28	16	9	15
ADENOMA,BRONCHIOLO-ALVEOLAR		1	0	1	1
CARCINOMA,BRONCHIOLO-ALVEOLAR		1	1	0	0
Skin+Subcutis					
Number examined		28	16	9	15
HEMANGIOSARCOMA		0	0	1	0
LEIOMYOSARCOMA		0	0	0	1
Thyroid					
Number examined		28	16	9	15
CARCINOMA,FOLLICULAR CELL		0	0	1	0
Hemolymphoreticular(all sites)					
Number examined		28	16	9	15
LYMPHOMA,MALIGNANT		3	2	1	1
SARCOMA,HISTIOCYTIC		2	0	0	1
Cranial bone					
Number examined		0	1	1	0
OSTEOSARCOMA		0	0	1	0

M : Male

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Table 10-4 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice
Histopathological findings - tumor data
Kill type : Scheduled sacrifice

Organs	Sex:	M	M	M	M
	Dose(mg/kg/day):	0	125	250	500
Findings	Number:	27	39	46	40
Adrenal					
Number examined		27	39	46	40
ADENOMA,SUBCAPSULAR CELL		2	0	0	0
Harderian gland					
Number examined		27	39	46	40
ADENOMA		2	7	5	3
ADENOCARCINOMA		0	0	0	1
Heart					
Number examined		27	39	46	40
HEMANGIOSARCOMA		1	0	0	0
Intestine,duodenum					
Number examined		27	39	46	40
ADENOMA		1	0	0	0
Intestine,ileum					
Number examined		27	39	46	40
ADENOMA		0	1	0	0
Intestine,cecum					
Number examined		27	39	46	40
LEIOMYOSARCOMA		0	1	0	0
Kidney					
Number examined		27	39	46	40
ADENOMA,RENAL CELL		0	0	0	1
Liver					
Number examined		27	39	46	40
ADENOMA,HEPATOCELLULAR		11	7	6	6
HEMANGIOMA		0	0	1	0
CARCINOMA,HEPATOCELLULAR		4	2	6	1
HEMANGIOSARCOMA		1	0	1	0
Lung(bronchus)					
Number examined		27	39	46	40
ADENOMA,BRONCHIOLO-ALVEOLAR		4	2	2	3
CARCINOMA,BRONCHIOLO-ALVEOLAR		1	0	1	0
Pancreas					
Number examined		27	39	46	40
ADENOMA,ACINAR CELL		0	0	0	1
Skin+Subcutis					
Number examined		27	39	46	40
HEMANGIOMA		0	1	0	0
Testis					
Number examined		27	39	46	40
LEYDIG CELL TUMOR		0	0	1	2
Thymus					
Number examined		27	39	45	39
THYMOMA		0	0	1	0
Thyroid					
Number examined		27	39	46	40
ADENOMA,FOLLICULAR CELL		0	0	1	2

M : Male

(b)
(4)

Table 10-5 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice
Histopathological findings - tumor data
Kill type : Scheduled sacrifice

Organs	Sex:	M	M	M	M
	Dose(mg/kg/day):	0	125	250	500
Findings	Number:	27	39	46	40
Tongue					
Number examined		27	39	46	40
PAPILLOMA,SQUAMOUS CELL		1	0	0	0
Hemolymphoreticular(all sites)					
Number examined		27	39	46	40
LYMPHOMA,MALIGNANT		4	5	4	4
SARCOMA,HISTIOCYTIC		1	1	0	1

M : Male

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Table 10-6 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice
Histopathological findings - tumor data
Kill type : All

Organs	Sex:	F	F	F	F
	Dose(mg/kg/day):	0	125	250	500
Findings	Number:	55	55	55	55
Adrenal					
Number examined		55	55	55	55
ADENOMA,CORTICAL CELL		0	0	1	0
ADENOMA,SUBCAPSULAR CELL		1	1	0	0
PHEOCHROMOCYTOMA		0	2	0	1
PHEOCHROMOCYTOMA,MALIGNANT		0	1	0	0
Bone+Bone marrow,femoral					
Number examined		55	55	55	55
HEMANGIOSARCOMA		0	1	0	0
Harderian gland					
Number examined		55	55	55	55
ADENOMA		5	6	2	3
Intestine,duodenum					
Number examined		52	55	50	52
ADENOMA		0	1	0	0
Kidney					
Number examined		55	55	54	55
ADENOMA,RENAL CELL		1	0	0	0
CARCINOMA,RENAL CELL		2	0	0	0
Liver					
Number examined		55	55	55	55
ADENOMA,HEPATOCELLULAR		12	6	3	3
CARCINOMA,HEPATOCELLULAR		3	4	6	1
HEMANGIOSARCOMA		1	0	0	0
Lung(bronchus)					
Number examined		55	55	55	55
ADENOMA,BRONCHIOLO-ALVEOLAR		3	1	1	1
CARCINOMA,BRONCHIOLO-ALVEOLAR		3	1	1	1
Mammary gland					
Number examined		55	55	55	55
ADENOMA		1	1	0	0
ADENOCARCINOMA		0	2	0	0
ADENOACANTHOMA,MALIGNANT		0	2	0	0
Ovary					
Number examined		55	55	55	55
CYSTADENOMA		0	1	0	0
GRANULOSA CELL TUMOR		0	0	1	0
HEMANGIOMA		0	1	1	0
LUTEOMA		1	0	0	0
SERTOLI CELL TUMOR		1	1	0	0
TERATOMA		0	0	1	0
YOLK SAC CARCINOMA		0	1	0	0
Pancreas					
Number examined		55	55	54	55
ADENOMA,ISLET CELL		0	0	0	1
Pituitary					
Number examined		55	55	55	55
ADENOMA,ANTERIOR		10	8	10	2
ADENOMA,INTERMEDIATED		0	1	1	0

F : Female
No statistically significant difference

(b)
(4)

Table 10-7 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice
Histopathological findings - tumor data
Kill type : All

Organs / Findings	Sex:	F	F	F	F
	Dose(mg/kg/day):	0	125	250	500
	Number:	55	55	55	55
Skin+Subcutis					
Number examined		55	55	55	55
FIBROMA		1	0	0	0
CARCINOMA,BASAL CELL		0	1	0	0
FIBROSARCOMA		1	0	2	1
HEMANGIOSARCOMA		1	0	0	0
LIPOSARCOMA		1	1	0	0
MELANOMA,MALIGNANT		0	1	1	0
SARCOMA,NOS		0	1	1	0
Spleen					
Number examined		55	55	55	55
HEMANGIOMA		1	0	0	0
Stomach					
Number examined		54	55	54	54
PAPILLOMA,SQUAMOUS CELL		1	0	1	2
NEUROENDOCRINE TUMOR,MALIGNANT		1	0	0	0
Thyroid					
Number examined		55	55	55	55
ADENOMA,FOLLICULAR CELL		1	1	1	3
Uterus					
Number examined		55	55	55	55
CYSTADENOMA		0	1	0	0
GRANULAR CELL TUMOR		0	1	1	0
HEMANGIOMA		0	0	1	0
LEIOMYOMA		1	0	0	0
POLYP,ENDOMETRIAL STROMAL		2	4	1	3
ADENOCARCINOMA		1	2	1	0
HEMANGIOSARCOMA		0	2	0	0
LEIOMYOSARCOMA		0	0	1	0
Vagina					
Number examined		55	55	55	55
HEMANGIOSARCOMA		0	0	1	0
Hemolymphoreticular(all sites)					
Number examined		55	55	55	55
LYMPHOMA,MALIGNANT		24	20	15	5
SARCOMA,HISTIOCYTIC		4	0	7	1
Brachium					
Number examined		0	0	1	0
SARCOMA,NOS		0	0	1	0
Buccal					
Number examined		0	1	0	0
OSTEOSARCOMA		0	1	0	0
Thoracic cavity					
Number examined		2	1	1	0
OSTEOSARCOMA		0	0	1	0

F : Female
No statistically significant difference

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Table 10-8 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice
Histopathological findings - tumor data
Kill type : Found dead / Moribund sacrifice

Organs / Findings	Sex:	F	F	F	F
	Dose (mg/kg/day):	0	125	250	500
	Number:	13	9	16	40
Adrenal					
Number examined		13	9	16	40
PHEOCHROMOCYTOMA, MALIGNANT		0	1	0	0
Harderian gland					
Number examined		13	9	16	40
ADENOMA		1	2	1	2
Liver					
Number examined		13	9	16	40
ADENOMA, HEPATOCELLULAR		2	0	1	1
CARCINOMA, HEPATOCELLULAR		1	1	1	0
HEMANGIOSARCOMA		1	0	0	0
Lung (bronchus)					
Number examined		13	9	16	40
ADENOMA, BRONCHIOLO-ALVEOLAR		2	0	0	0
CARCINOMA, BRONCHIOLO-ALVEOLAR		2	0	0	0
Ovary					
Number examined		13	9	16	40
HEMANGIOMA		0	0	1	0
SERTOLI CELL TUMOR		1	0	0	0
TERATOMA		0	0	1	0
YOLK SAC CARCINOMA		0	1	0	0
Pancreas					
Number examined		13	9	15	40
ADENOMA, ISLET CELL		0	0	0	1
Pituitary					
Number examined		13	9	16	40
ADENOMA, ANTERIOR		0	1	4	1
Skin+Subcutis					
Number examined		13	9	16	40
CARCINOMA, BASAL CELL		0	1	0	0
FIBROSARCOMA		0	0	1	1
LIPOSARCOMA		1	1	0	0
SARCOMA, NOS		0	1	1	0
Stomach					
Number examined		12	9	15	39
PAPILLOMA, SQUAMOUS CELL		0	0	0	1
Thyroid					
Number examined		13	9	16	40
ADENOMA, FOLLICULAR CELL		0	0	1	0
Uterus					
Number examined		13	9	16	40
POLYP, ENDOMETRIAL STROMAL		1	0	0	2
ADENOCARCINOMA		1	0	0	0
Hemolymphoreticular (all sites)					
Number examined		13	9	16	40
LYMPHOMA, MALIGNANT		7	3	3	0
SARCOMA, HISTIOCYTIC		3	0	4	0
Brachium					
Number examined		0	0	1	0
SARCOMA, NOS		0	0	1	0

F : Female

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Table 10-9 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice
Histopathological findings - tumor data
Kill type : Found dead / Moribund sacrifice

Organs	Sex:	F	F	F	F
	Dose(mg/kg/day):	0	125	250	500
Findings	Number:	13	9	16	40
Thoracic cavity					
Number examined		2	1	1	0
OSTEOSARCOMA		0	0	1	0

F : Female

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Table 10-10 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice
Histopathological findings - tumor data
Kill type : Scheduled sacrifice / Unscheduled terminal

Organs	Sex:	F	F	F	F
	Dose(mg/kg/day):	0	125	250	500
Findings	Number:	42	46	39	15
Adrenal					
Number examined		42	46	39	15
ADENOMA,CORTICAL CELL		0	0	1	0
ADENOMA,SUBCAPSULAR CELL		1	1	0	0
PHEOCHROMOCYTOMA		0	2	0	1
Bone+Bone marrow,femoral					
Number examined		42	46	39	15
HEMANGIOSARCOMA		0	1	0	0
Harderian gland					
Number examined		42	46	39	15
ADENOMA		4	4	1	1
Intestine,duodenum					
Number examined		42	46	39	15
ADENOMA		0	1	0	0
Kidney					
Number examined		42	46	39	15
ADENOMA,RENAL CELL		1	0	0	0
CARCINOMA,RENAL CELL		2	0	0	0
Liver					
Number examined		42	46	39	15
ADENOMA,HEPATOCELLULAR		10	6	2	2
CARCINOMA,HEPATOCELLULAR		2	3	5	1
Lung(bronchus)					
Number examined		42	46	39	15
ADENOMA,BRONCHIOLO-ALVEOLAR		1	1	1	1
CARCINOMA,BRONCHIOLO-ALVEOLAR		1	1	1	1
Mammary gland					
Number examined		42	46	39	15
ADENOMA		1	1	0	0
ADENOCARCINOMA		0	2	0	0
ADENOACANTHOMA,MALIGNANT		0	2	0	0
Ovary					
Number examined		42	46	39	15
CYSTADENOMA		0	1	0	0
GRANULOSA CELL TUMOR		0	0	1	0
HEMANGIOMA		0	1	0	0
LUTEOMA		1	0	0	0
SERTOLI CELL TUMOR		0	1	0	0
Pituitary					
Number examined		42	46	39	15
ADENOMA,ANTERIOR		10	7	6	1
ADENOMA,INTERMEDIATED		0	1	1	0
Skin+Subcutis					
Number examined		42	46	39	15
FIBROMA		1	0	0	0
FIBROSARCOMA		1	0	1	0
HEMANGIOSARCOMA		1	0	0	0
MELANOMA,MALIGNANT		0	1	1	0
Spleen					
Number examined		42	46	39	15
HEMANGIOMA		1	0	0	0

F : Female

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Table 10-11 Twenty-four-month oral gavage carcinogenicity study of NS-304 in mice
Histopathological findings - tumor data
Kill type : Scheduled sacrifice / Unscheduled terminal

Organs	Sex:	F	F	F	F
	Dose(mg/kg/day):	0	125	250	500
Findings	Number:	42	46	39	15
Stomach					
Number examined		42	46	39	15
PAPILLOMA,SQUAMOUS CELL		1	0	1	1
NEUROENDOCRINE TUMOR,MALIGNANT		1	0	0	0
Thyroid					
Number examined		42	46	39	15
ADENOMA,FOLLICULAR CELL		1	1	0	3
Uterus					
Number examined		42	46	39	15
CYSTADENOMA		0	1	0	0
GRANULAR CELL TUMOR		0	1	1	0
HEMANGIOMA		0	0	1	0
LEIOMYOMA		1	0	0	0
POLYP,ENDOMETRIAL STROMAL		1	4	1	1
ADENOCARCINOMA		0	2	1	0
HEMANGIOSARCOMA		0	2	0	0
LEIOMYOSARCOMA		0	0	1	0
Vagina					
Number examined		42	46	39	15
HEMANGIOSARCOMA		0	0	1	0
Hemolymphoreticular(all sites)					
Number examined		42	46	39	15
LYMPHOMA,MALIGNANT		17	17	12	5
SARCOMA,HISTIOCYTIC		1	0	3	1
Buccal					
Number examined		0	1	0	0
OSTEOSARCOMA		0	1	0	0

F : Female

Appendix V. Sponsor tables of tumor incidence in the rat carcinogenicity study.

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Table 11-1 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : All

Organs / Findings	Sex:	M	M	M	M
	Dose(mg/kg/day):	0	10	30	100
	Number:	60	60	60	60
Adrenal					
Number examined		60	60	60	60
ADENOMA,CORTICAL CELL		0	1	1	1
PHEOCHROMOCYTOMA		16	11	5	7
PHEOCHROMOCYTOMA,MALIGNANT		0	1	1	1
Cerebellum					
Number examined		60	60	60	60
GRANULAR CELL TUMOR		0	1	1	0
Cerebrum					
Number examined		60	60	60	60
ASTROCYTOMA,MALIGNANT		0	0	0	1
OLIGODENDROGLIOMA		1	0	0	0
Heart					
Number examined		60	60	60	60
SCHWANNOMA,ENDOCARDIAL		1	0	0	0
SCHWANNOMA,ENDOCARDIAL,MALIGNANT		1	0	0	0
Hemolymphoreticular(all sites)					
Number examined		60	60	60	60
LYMPHOMA,MALIGNANT		1	2	0	0
SARCOMA,HISTIOCYTIC		3	3	2	2
LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC		2	1	0	1
Intestine,ileum					
Number examined		57	59	56	57
ADENOMA		0	0	1	0
Kidney					
Number examined		60	60	60	60
LIPOMA		0	2	0	0
LIPOSARCOMA		1	0	0	0
Liver					
Number examined		60	60	60	60
ADENOMA,HEPATOCELLULAR		5	4	2	0
LYMPHANGIOMA		0	1	0	0
CARCINOMA,HEPATOCELLULAR		0	0	0	1
Lung(bronchus)					
Number examined		60	60	60	60
ADENOMA,BRONCHIOLO-ALVEOLAR		0	0	1	0
Mammary gland					
Number examined		60	60	60	60
FIBROADENOMA		1	1	0	0
TUMOR,MIXED,BENIGN		0	0	0	1
Mesothelium					
Number examined		60	60	60	60
MESOTHELIOMA,MALIGNANT		1	0	0	1
Pancreas					
Number examined		60	60	60	60
ADENOMA,ACINAR CELL		1	3	3	1
ADENOMA,ISLET CELL		22	16	10	6
ADENOMA,ACINAR-ISLET CELL		1	0	1	0
CARCINOMA,ACINAR CELL		0	0	1	0
CARCINOMA,ISLET CELL		2	6	2	1

M : Male
No statistically significant difference

Table 11-2 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : All

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Organs / Findings	Sex:	M	M	M	M
	Dose(mg/kg/day):	0	10	30	100
	Number:	60	60	60	60
Parathyroid					
Number examined		60	60	60	60
ADENOMA		2	5	0	1
Pituitary					
Number examined		60	60	60	60
ADENOMA,ANTERIOR		20	40**	23	22
ADENOMA,INTERMEDIATED		1	0	0	1
CARCINOMA,ANTERIOR		2	0	1	1
Prostate					
Number examined		60	60	60	60
ADENOMA		0	1	0	1
Skin/Subcutis					
Number examined		60	60	60	60
TUMOR,HAIR FOLLICLE,BENIGN		1	2	1	1
FIBROMA		5	4	2	4
KERATOACANTHOMA		1	1	1	3
LIPOMA		0	0	1	0
PAPILLOMA,SQUAMOUS CELL		1	1	1	0
CARCINOMA,SQUAMOUS CELL		0	0	1	0
FIBROSARCOMA		1	1	0	1
LEIOMYOSARCOMA		0	0	1	0
LIPOSARCOMA		0	0	1	1
SCHWANNOMA,MALIGNANT		1	0	1	0
CARCINOMA,SEBACEOUS		0	0	0	1
Spleen					
Number examined		60	60	60	60
HEMANGIOSARCOMA		0	1	0	0
SARCOMA,NOS		0	0	1	0
Stomach					
Number examined		60	60	60	60
PAPILLOMA,SQUAMOUS CELL		1	1	0	0
Thymus					
Number examined		60	60	58	59
THYMOMA		0	1	0	0
THYMOMA,MALIGNANT		0	0	1	0
Thyroid					
Number examined		58	57	56	59
ADENOMA,C-CELL		5	5	4	4
ADENOMA,FOLLICULAR CELL		3	1	1	0
CARCINOMA,C-CELL		0	0	2	2
Testis					
Number examined		60	60	60	60
LEYDIG CELL TUMOR		2$a)	0	2	5
SEMINOMA		0	1	0	0
Urinary bladder					
Number examined		60	60	60	60
PAPILLOMA,TRANSITIONAL CELL		0	0	1	0
Abdominal cavity					
Number examined		0	1	0	2
FIBROMA		0	0	0	1
LIPOMA		0	0	0	1

M : Male
$: p<0.025 (statistically significant positive trend, rare tumor, Peto's test)
a) : There was no statistically significant positive trend in the 0, 10 and 30 mg/kg groups.
** : p<0.01 (statistically different from the control group, common tumor, Peto's test)

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Table 11-3 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : All

Organs	Sex:	M	M	M	M
	Dose(mg/kg/day):	0	10	30	100
Findings	Number:	60	60	60	60
Bone,vertebral					
Number examined		1	0	1	1
CHONDROSARCOMA		0	0	1	1
OSTEOSARCOMA		1	0	0	0
Forelimb					
Number examined		3	1	0	0
PAPILLOMA,SQUAMOUS CELL		1	0	0	0
SCHWANNOMA,MALIGNANT		1	0	0	0
Oral cavity					
Number examined		0	0	1	0
CARCINOMA,SQUAMOUS CELL		0	0	1	0
Origin unknown					
Number examined		0	0	1	0
CHORDOMA		0	0	1	0
Palate					
Number examined		0	1	0	0
CARCINOMA,SQUAMOUS CELL		0	1	0	0
Trigeminal nerve					
Number examined		0	0	1	0
SCHWANNOMA,MALIGNANT		0	0	1	0
Zymbal gland					
Number examined		2	0	2	0
ADENOMA		1	0	0	0
CARCINOMA		1	0	2	0

M : Male
No statistically significant difference

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Table 11-4 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : Found dead Moribund sacrifice

Organs / Findings	Sex:	M	M	M	M
	Dose (mg/kg/day):	0	10	30	100
	Number:	29	26	26	31
Adrenal					
Number examined		29	26	26	31
ADENOMA, CORTICAL CELL		0	0	0	1
PHEOCHROMOCYTOMA		4	4	3	5
PHEOCHROMOCYTOMA, MALIGNANT		0	0	0	1
Cerebellum					
Number examined		29	26	26	31
GRANULAR CELL TUMOR		0	0	1	0
Cerebrum					
Number examined		29	26	26	31
ASTROCYTOMA, MALIGNANT		0	0	0	1
Heart					
Number examined		29	26	26	31
SCHWANNOMA, ENDOCARDIAL, MALIGNANT		1	0	0	0
Hemolymphoreticular (all sites)					
Number examined		29	26	26	31
LYMPHOMA, MALIGNANT		0	2	0	0
SARCOMA, HISTIOCYTIC		1	2	0	2
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC		2	1	0	0
Liver					
Number examined		29	26	26	31
ADENOMA, HEPATOCELLULAR		1	0	0	0
Lung (bronchus)					
Number examined		29	26	26	31
ADENOMA, BRONCHIOLO-ALVEOLAR		0	0	1	0
Mammary gland					
Number examined		29	26	26	31
FIBROADENOMA		0	1	0	0
TUMOR, MIXED, BENIGN		0	0	0	1
Pancreas					
Number examined		29	26	26	31
ADENOMA, ACINAR CELL		0	1	0	1
ADENOMA, ISLET CELL		4	2	2	0
CARCINOMA, ACINAR CELL		0	0	1	0
CARCINOMA, ISLET CELL		1	0	0	0
Parathyroid					
Number examined		29	26	26	31
ADENOMA		0	2	0	0
Pituitary					
Number examined		29	26	26	31
ADENOMA, ANTERIOR		9	19	11	9
CARCINOMA, ANTERIOR		2	0	1	1
Prostate					
Number examined		29	26	26	31
ADENOMA		0	0	0	1
Skin/Subcutis					
Number examined		29	26	26	31
TUMOR, HAIR FOLLICLE, BENIGN		0	0	0	1
FIBROMA		1	2	0	0
KERATOACANTHOMA		1	0	0	1
PAPILLOMA, SQUAMOUS CELL		1	1	0	0

M : Male

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Table 11-5 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : Found dead Moribund sacrifice

Organs / Findings	Sex:	M	M	M	M
	Dose (mg/kg/day):	0	10	30	100
	Number:	29	26	26	31
Skin/Subcutis (continued)					
CARCINOMA, SQUAMOUS CELL		0	0	1	0
FIBROSARCOMA		1	1	0	0
LEIOMYOSARCOMA		0	0	1	0
LIPOSARCOMA		0	0	1	0
SCHWANNOMA, MALIGNANT		1	0	1	0
CARCINOMA, SEBACEOUS		0	0	0	1
Spleen					
Number examined		29	26	26	31
SARCOMA, NOS		0	0	1	0
Stomach					
Number examined		29	26	26	31
PAPILLOMA, SQUAMOUS CELL		1	1	0	0
Thymus					
Number examined		29	26	24	30
THYMOMA		0	1	0	0
THYMOMA, MALIGNANT		0	0	1	0
Thyroid					
Number examined		27	23	22	30
ADENOMA, C-CELL		0	1	2	1
ADENOMA, FOLLICULAR CELL		1	0	0	0
CARCINOMA, C-CELL		0	0	0	1
Testis					
Number examined		29	26	26	31
LEYDIG CELL TUMOR		0	0	0	3
Abdominal cavity					
Number examined		0	0	0	1
FIBROMA		0	0	0	1
Bone, vertebral					
Number examined		0	0	1	1
CHONDROSARCOMA		0	0	1	1
Forelimb					
Number examined		3	1	0	0
PAPILLOMA, SQUAMOUS CELL		1	0	0	0
SCHWANNOMA, MALIGNANT		1	0	0	0
Oral cavity					
Number examined		0	0	1	0
CARCINOMA, SQUAMOUS CELL		0	0	1	0
Palate					
Number examined		0	1	0	0
CARCINOMA, SQUAMOUS CELL		0	1	0	0
Trigeminal nerve					
Number examined		0	0	1	0
SCHWANNOMA, MALIGNANT		0	0	1	0
Zymbal gland					
Number examined		1	0	1	0
CARCINOMA		1	0	1	0

M : Male

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Table 11-6 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : Scheduled sacrifice

Organs / Findings	Sex:	M	M	M	M
	Dose(mg/kg/day):	0	10	30	100
	Number:	31	34	34	29
Adrenal					
Number examined		31	34	34	29
ADENOMA,CORTICAL CELL		0	1	1	0
PHEOCHROMOCYTOMA		12	7	2	2
PHEOCHROMOCYTOMA,MALIGNANT		0	1	1	0
Cerebellum					
Number examined		31	34	34	29
GRANULAR CELL TUMOR		0	1	0	0
Cerebrum					
Number examined		31	34	34	29
OLIGODENDROGLIOMA		1	0	0	0
Heart					
Number examined		31	34	34	29
SCHWANNOMA,ENDOCARDIAL		1	0	0	0
Hemolymphoreticular(all sites)					
Number examined		31	34	34	29
LYMPHOMA,MALIGNANT		1	0	0	0
SARCOMA,HISTIOCYTIC		2	1	2	0
LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC		0	0	0	1
Intestine,ileum					
Number examined		31	34	34	29
ADENOMA		0	0	1	0
Kidney					
Number examined		31	34	34	29
LIPOMA		0	2	0	0
LIPOSARCOMA		1	0	0	0
Liver					
Number examined		31	34	34	29
ADENOMA,HEPATOCELLULAR		4	4	2	0
LYMPHANGIOMA		0	1	0	0
CARCINOMA,HEPATOCELLULAR		0	0	0	1
Mammary gland					
Number examined		31	34	34	29
FIBROADENOMA		1	0	0	0
Mesothelium					
Number examined		31	34	34	29
MESOTHELIOMA,MALIGNANT		1	0	0	1
Pancreas					
Number examined		31	34	34	29
ADENOMA,ACINAR CELL		1	2	3	0
ADENOMA,ISLET CELL		18	14	8	6
ADENOMA,ACINAR-ISLET CELL		1	0	1	0
CARCINOMA,ISLET CELL		1	6	2	1
Parathyroid					
Number examined		31	34	34	29
ADENOMA		2	3	0	1
Pituitary					
Number examined		31	34	34	29
ADENOMA,ANTERIOR		11	21	12	13
ADENOMA,INTERMEDIATED		1	0	0	1

M : Male

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Table 11-7 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : Scheduled sacrifice

Organs	Sex:	M	M	M	M
	Dose(mg/kg/day):	0	10	30	100
Findings	Number:	31	34	34	29
Prostate					
Number examined		31	34	34	29
ADENOMA		0	1	0	0
Skin/Subcutis					
Number examined		31	34	34	29
TUMOR,HAIR FOLLICLE,BENIGN		1	2	1	0
FIBROMA		4	2	2	4
KERATOACANTHOMA		0	1	1	2
LIPOMA		0	0	1	0
PAPILLOMA,SQUAMOUS CELL		0	0	1	0
FIBROSARCOMA		0	0	0	1
LIPOSARCOMA		0	0	0	1
Spleen					
Number examined		31	34	34	29
HEMANGIOSARCOMA		0	1	0	0
Thyroid					
Number examined		31	34	34	29
ADENOMA,C-CELL		5	4	2	3
ADENOMA,FOLLICULAR CELL		2	1	1	0
CARCINOMA,C-CELL		0	0	2	1
Testis					
Number examined		31	34	34	29
LEYDIG CELL TUMOR		2	0	2	2
SEMINOMA		0	1	0	0
Urinary bladder					
Number examined		31	34	34	29
PAPILLOMA,TRANSITIONAL CELL		0	0	1	0
Abdominal cavity					
Number examined		0	1	0	1
LIPOMA		0	0	0	1
Bone,vertebral					
Number examined		1	0	0	0
OSTEOSARCOMA		1	0	0	0
Origin unknown					
Number examined		0	0	1	0
CHORDOMA		0	0	1	0
Zymbal gland					
Number examined		1	0	1	0
ADENOMA		1	0	0	0
CARCINOMA		0	0	1	0

M : Male

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Table 11-8 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : All

Organs / Findings	Sex:	F	F	F	F
	Dose(mg/kg/day):	0	10	30	100
	Number:	60	60	60	60
Adrenal					
Number examined		60	60	60	60
ADENOMA,CORTICAL CELL		1	1	2	1
PHEOCHROMOCYTOMA		0	0	1	3
PHEOCHROMOCYTOMA,MALIGNANT		0	1	0	0
CARCINOMA,CORTICAL CELL		0	0	1	0
Cerebrum					
Number examined		60	60	60	60
PAPILLOMA,CHOROID PLEXUS		0	1	0	0
ASTROCYTOMA,MALIGNANT		1	0	1	1
Hemolymphoreticular(all sites)					
Number examined		60	60	60	60
LYMPHOMA,MALIGNANT		0	1	0	1
SARCOMA,HISTIOCYTIC		2	0	1	0
LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC		1	0	0	0
Intestine,jejunum					
Number examined		58	56	57	58
LEIOMYOSARCOMA		0	1	0	0
Kidney					
Number examined		60	60	60	60
ADENOMA,RENAL CELL		0	1	0	0
LIPOMA		0	0	1	0
Liver					
Number examined		60	60	60	60
ADENOMA,HEPATOCELLULAR		1	0	0	1
Mammary gland					
Number examined		60	60	60	60
ADENOMA		0	3	2	1
FIBROADENOMA		20	14	15	15
LIPOMA		0	1	0	0
ADENOCARCINOMA		23	18	18	18
TUMOR,MIXED,MALIGNANT		2	0	0	1
ADENOCARCINOMA IN FIBROADENOMA		6	9	2	4
Ovary					
Number examined		60	60	60	60
GRANULOSA CELL TUMOR		0	0	1	0
THECOMA,BENIGN		0	0	1	0
Pancreas					
Number examined		60	60	60	60
ADENOMA,ISLET CELL		5	4	4	2
CARCINOMA,ACINAR CELL		0	0	1	0
CARCINOMA,ISLET CELL		0	0	0	1
Parathyroid					
Number examined		58	59	59	59
ADENOMA		1	0	2	0
Pituitary					
Number examined		60	60	60	60
ADENOMA,ANTERIOR		49	44	44	38
CARCINOMA,ANTERIOR		2	4	3	3
Skeletal muscle,femoral					
Number examined		60	60	60	60
FIBROSARCOMA		0	0	0	1

F : Female
No statistically significant difference

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Table 11-9 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : All

Organs	Sex:	F	F	F	F
	Dose (mg/kg/day):	0	10	30	100
Findings	Number:	60	60	60	60
Skin/Subcutis					
Number examined		60	60	60	60
KERATOACANTHOMA		0	1	0	0
LIPOMA		1	0	0	0
PAPILLOMA, SQUAMOUS CELL		0	0	0	1
CARCINOMA, SQUAMOUS CELL		0	1	0	0
FIBROSARCOMA		0	1	0	0
Stomach					
Number examined		60	60	60	60
PAPILLOMA, SQUAMOUS CELL		0	0	1	0
Thymus					
Number examined		60	60	60	60
THYMOMA		0	0	0	1
Thyroid					
Number examined		59	59	59	59
ADENOMA, C-CELL		1	5	5	7
ADENOMA, FOLLICULAR CELL		1	1	2	0
CARCINOMA, C-CELL		1	4	0	1
Uterus					
Number examined		60	60	60	60
GRANULAR CELL TUMOR		1	0	0	1
LEIOMYOMA		0	0	1	0
POLYP, ENDOMETRIAL STROMAL		8	5	4	5
SCHWANNOMA, MALIGNANT		0	0	0	1
Vagina					
Number examined		60	60	60	60
GRANULAR CELL TUMOR		1	0	0	0
POLYP, VAGINAL STROMAL		0	2	0	0
SCHWANNOMA, MALIGNANT		0	0	1	1
Oral cavity					
Number examined		0	1	1	1
CARCINOMA, SQUAMOUS CELL		0	1	1	1
Zymbal gland					
Number examined		1	0	0	1
CARCINOMA		1	0	0	1

F : Female
No statistically significant difference

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Table 11-10 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : Found dead Moribund sacrifice

Organs / Findings	Sex:	F	F	F	F
	Dose(mg/kg/day):	0	10	30	100
	Number:	39	39	36	29
Adrenal					
Number examined		39	39	36	29
ADENOMA,CORTICAL CELL		1	1	0	0
PHEOCHROMOCYTOMA		0	0	0	1
CARCINOMA,CORTICAL CELL		0	0	1	0
Hemolymphoreticular(all sites)					
Number examined		39	39	36	29
LYMPHOMA,MALIGNANT		0	1	0	1
SARCOMA,HISTIOCYTIC		1	0	1	0
LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC		1	0	0	0
Intestine,jejunum					
Number examined		37	35	33	27
LEIOMYOSARCOMA		0	1	0	0
Kidney					
Number examined		39	39	36	29
LIPOMA		0	0	1	0
Liver					
Number examined		39	39	36	29
ADENOMA,HEPATOCELLULAR		0	0	0	1
Mammary gland					
Number examined		39	39	36	29
ADENOMA		0	3	1	0
FIBROADENOMA		8	7	7	5
ADENOCARCINOMA		16	14	11	12
TUMOR,MIXED,MALIGNANT		2	0	0	1
ADENOCARCINOMA IN FIBROADENOMA		6	7	1	2
Ovary					
Number examined		39	39	36	29
THECOMA,BENIGN		0	0	1	0
Pancreas					
Number examined		39	39	36	29
ADENOMA,ISLET CELL		2	2	0	0
Parathyroid					
Number examined		38	38	35	28
ADENOMA		1	0	0	0
Pituitary					
Number examined		39	39	36	29
ADENOMA,ANTERIOR		33	33	28	17
CARCINOMA,ANTERIOR		2	2	2	2
Skeletal muscle,femoral					
Number examined		39	39	36	29
FIBROSARCOMA		0	0	0	1
Skin/Subcutis					
Number examined		39	39	36	29
PAPILLOMA,SQUAMOUS CELL		0	0	0	1
CARCINOMA,SQUAMOUS CELL		0	1	0	0
FIBROSARCOMA		0	1	0	0
Stomach					
Number examined		39	39	36	29
PAPILLOMA,SQUAMOUS CELL		0	0	1	0

F : Female

(b) (4) 5940

Table 11-11 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : Found dead Moribund sacrifice

Organs	Sex:	F	F	F	F
	Dose(mg/kg/day):	0	10	30	100
Findings	Number:	39	39	36	29
Thyroid					
Number examined		38	38	35	28
ADENOMA,C-CELL		1	2	2	3
ADENOMA,FOLLICULAR CELL		0	1	0	0
CARCINOMA,C-CELL		0	1	0	1
Uterus					
Number examined		39	39	36	29
POLYP,ENDOMETRIAL STROMAL		5	3	1	2
SCHWANNOMA,MALIGNANT		0	0	0	1
Vagina					
Number examined		39	39	36	29
GRANULAR CELL TUMOR		1	0	0	0
POLYP,VAGINAL STROMAL		0	1	0	0
SCHWANNOMA,MALIGNANT		0	0	1	1
Oral cavity					
Number examined		0	1	1	1
CARCINOMA,SQUAMOUS CELL		0	1	1	1
Zymbal gland					
Number examined		0	0	0	1
CARCINOMA		0	0	0	1

F : Female

(b) (4) 5940

Table 11-12 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : Scheduled sacrifice

Organs / Findings	Sex:	F	F	F	F
	Dose (mg/kg/day):	0	10	30	100
	Number:	21	21	24	31
Adrenal					
Number examined		21	21	24	31
ADENOMA, CORTICAL CELL		0	0	2	1
PHEOCHROMOCYTOMA		0	0	1	2
PHEOCHROMOCYTOMA, MALIGNANT		0	1	0	0
Cerebrum					
Number examined		21	21	24	31
PAPILLOMA, CHOROID PLEXUS		0	1	0	0
ASTROCYTOMA, MALIGNANT		1	0	1	1
Hemolymphoreticular (all sites)					
Number examined		21	21	24	31
SARCOMA, HISTIOCYTIC		1	0	0	0
Kidney					
Number examined		21	21	24	31
ADENOMA, RENAL CELL		0	1	0	0
Liver					
Number examined		21	21	24	31
ADENOMA, HEPATOCELLULAR		1	0	0	0
Mammary gland					
Number examined		21	21	24	31
ADENOMA		0	0	1	1
FIBROADENOMA		12	7	8	10
LIPOMA		0	1	0	0
ADENOCARCINOMA		7	4	7	6
ADENOCARCINOMA IN FIBROADENOMA		0	2	1	2
Ovary					
Number examined		21	21	24	31
GRANULOSA CELL TUMOR		0	0	1	0
Pancreas					
Number examined		21	21	24	31
ADENOMA, ISLET CELL		3	2	4	2
CARCINOMA, ACINAR CELL		0	0	1	0
CARCINOMA, ISLET CELL		0	0	0	1
Parathyroid					
Number examined		20	21	24	31
ADENOMA		0	0	2	0
Pituitary					
Number examined		21	21	24	31
ADENOMA, ANTERIOR		16	11	16	21
CARCINOMA, ANTERIOR		0	2	1	1
Skin/Subcutis					
Number examined		21	21	24	31
KERATOACANTHOMA		0	1	0	0
LIPOMA		1	0	0	0
Thymus					
Number examined		21	21	24	31
THYMOMA		0	0	0	1
Thyroid					
Number examined		21	21	24	31
ADENOMA, C-CELL		0	3	3	4
ADENOMA, FOLLICULAR CELL		1	0	2	0
CARCINOMA, C-CELL		1	3	0	0

F : Female

(b) (4) 5940

Table 11-13 Twenty-four-month oral gavage carcinogenicity study of NS-304 in rats
Histopathological findings - tumor data
Kill type : Scheduled sacrifice

Organs / Findings	Sex:	F	F	F	F
	Dose (mg/kg/day):	0	10	30	100
	Number:	21	21	24	31
Uterus					
Number examined		21	21	24	31
GRANULAR CELL TUMOR		1	0	0	1
LEIOMYOMA		0	0	1	0
POLYP, ENDOMETRIAL STROMAL		3	2	3	3
Vagina					
Number examined		21	21	24	31
POLYP, VAGINAL STROMAL		0	1	0	0
Zymbal gland					
Number examined		1	0	0	0
CARCINOMA		1	0	0	0

F : Female

Appendix VI. Executive CAC minutes for Selexipag

Executive CAC
Date of Meeting: December 1, 2015

Committee: Karen Davis Bruno, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Tim McGovern, Ph.D., OND IO, Member
John Leighton, Ph.D., DHOT, Alternate Member
Albert De Felice, Ph.D., DCRP, Pharm Tox Supervisor
James Willard, Ph.D., DCRP, Presenting Reviewer

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 207947
Drug Name: Uptravi (selexipag)
Sponsor: Actelion Pharmaceuticals, ltd.

Background:
Selexipag is a non-prostanoid prostacyclin agonist for the treatment of pulmonary arterial hypertension. In the genotoxicity assays in bacteria, eukaryotic cell cultures and *in vivo,* selexipag was found to be negative. The sponsor did not obtain dose

concurrence from the Executive CAC.

Mouse Carcinogenicity Study
B6C3F1/Crlj SPF mice (55/sex/group) were administered 0, 125, 250 or 500 mg/kg/day of selexipag in 0.5% methylcellulose via oral gavage daily for 104 weeks. Dosages were selected to achieve an AUC exposure up to, or more than, 25x the human exposure. Significant mortality occurred in the female 500 mg/kg/day group beginning around week 72 due to severe gastric erosion, with 24 of 55 animals dying from this adverse effect. This group was prematurely sacrificed at week 100 of 104 weeks. AUC values were similar between high dose males and females, while Cmax values were much higher in the females for selexipag, the parent compound (24,900 ng/mL for the males versus 43,900 ng/mL for the females on day 1). This difference may help account for the severe gastric erosion in the high dose female group, while sparing the high dose male group. No other treatment related mortality was seen in the study. After correcting for multiplicity testing, CDER statisticians found no significant dose-related excess in any tumor incidence.

Rat Carcinogenicity Study
Sprague-Dawley rats (60/sex/group) were administered 0, 10, 30, or 100 mg/kg/day of selexipag in 0.5% methylcellulose via oral gavage for 104 weeks. As in the mice, doses were selected to achieve an AUC exposure of up to, or more than, 25x the human exposure. No significant treatment related mortality occurred in the study. There was no significant dose-related increase seen in incidence of any tumor type in the selexipag treated groups at margins of exposure of approximately 170x of the human AUC for the parent compound and >300x of the human AUC for the active metabolite at the high dosage.

Executive CAC Recommendations and Conclusions

Mouse:

- The Committee concurred that the study was acceptable.
- The Committee concurred that there were no drug-related neoplasms

Rat:

- The Committee concurred that the study was acceptable.
- The Committee concurred that there were no drug-related neoplasms.

Karen Davis Bruno, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DCRP
Albert De Felice/Team leader, DCRP
James Willard/Reviewer, DCRP
Wayne Amchin/CSO/PM, DCRP
/ASeifried, OND IO

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JAMES M WILLARD
12/11/2015

ALBERT F DEFELICE
12/11/2015

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 207947 **Applicant: Actelion** **Stamp Date: 12/18/2014**

Drug Name: Uptravi **NDA Type: Standard**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?		X	No abuse potential assumed for this drug and indication.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? _Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

No known issues at this time after initial examination of the NDA submission

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JAMES M WILLARD
02/10/2015

ALBERT F DEFELICE
02/18/2015

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

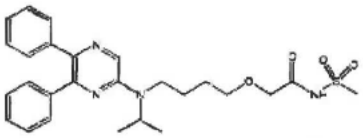
IND number: 104504
Review number: 1
Sequence number/date/type of submission:
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: Actelion Pharmaceuticals, Ltd.
Manufacturer for drug substance: (b) (4)

Reviewer name: James M. Willard, Ph.D.
Division name: **Division of Cardiovascular and Renal Products**
HFD #: **110**
Review completion date:

Drug:

Trade name:
Generic name:
Code name: ACT-293987 (also NS-304)
Chemical name: 2-{4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy}-N-(methylsulfonyl) acetamide
CAS registry number:
Molecular formula/molecular weight: /$C_{26}H_{32}N_4O_4S$/496.62

Structure:



Relevant INDs/NDAs/DMFs:

Drug class: Prostacyclin (IP) receptor agonist

Intended clinical population: Patients with Pulmonary Arterial Hypertension (PAH)

Clinical formulation:

Table 1: Quantitative compositions of ACT-293987 Tablet

Ingredient	Specification	Function	ACT-293987 Tablet 200 µg	ACT-293987 Tablet (Placebo)
ACT-293987	In house	API	0.2 mg	-
D-Mannitol	USP/NF	[redacted]	[redacted]	[redacted] (b) (4)
Corn starch	USP/NF	[redacted]	[redacted]	[redacted]
Low substituted Hydroxypropylcellulose	USP/NF	[redacted]	[redacted]	[redacted]
Hydroxypropylcellulose	USP/NF	[redacted]	[redacted]	[redacted]
Magnesium stearate	USP/NF	[redacted]	[redacted]	[redacted]
Core tablet weight		[redacted]	[redacted]	[redacted]
Hypromellose	USP/NF	[redacted]	[redacted]	[redacted]
Propylene glycol	USP/NF	[redacted]	[redacted]	[redacted]
Titanium oxide	USP/NF	[redacted]	[redacted]	[redacted]
Yellow ferric oxide	USP/NF	[redacted]	[redacted]	[redacted]
Carnauba wax	USP/NF	[redacted]	[redacted]	[redacted]
Coating weight		[redacted]	[redacted]	[redacted]
Total weight of film-coated tablet		[redacted]	[redacted]	[redacted] (b) (4)

Route of administration: oral

Proposed clinical protocol:
From the sponsor:

> "The sponsor intends to start two identical multicenter, double-blind, randomized, placebo-controlled, parallel group, event-driven clinical studies in adult patients with symptomatic PAH. Each trial will have 272 patients to be randomized into 2 groups (n = 136/group), placebo:active (1:1). The primary objective for each single study will be to demonstrate the effect of ACT-293987 on 6 minute walk distance (6MWD) from baseline to Week 16. The co-primary objective for the pool of the two studies will be to demonstrate the effect of ACT-293987 on time to clinical worsening up to the end of treatment (EOT) (expected maximum duration 4.1 years). An open-label extension (AC-065A303) of these two studies will be aimed at collecting long-term safety and tolerability in patients who will have experienced a clinical worsening of PAH or who will have completed the double-blind phase of the two studies."

Previous clinical experience: Six clinical studies have been done with ACT-293987, originally developed by Nippon Shinyaku Co. Ltd., Kyoto, Japan. Five Phase I studies were done, fulfilling a first in man study, and looking at pharmacokinetics and tolerability. One Phase 2a study has been carried out of 17 weeks duration, with an open-label extension still ongoing. Approximately 184 patients have been exposed to ACT-293987 with the following side effects seen: headache, myalgia, arthralgia, flushing, jaw pain, nausea, vomiting and diarrhea..

Disclaimer: Tabular and graphical depiction of information are constructed by the reviewer unless cited otherwise.
Note: For IND reviews, unused headings may be deleted.

Studies reviewed within this submission:
Studies not reviewed within this submission:

APPEARS THIS WAY ON ORIGINAL

TABLE OF CONTENTS

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Prostacyclin receptors are primary targets in Pulmonary Arterial Hypertension, however, this development area has had issues with most of the compounds not being orally available and having very short half-lives requiring frequent administration. ACT-293987 is a pro-drug of ACT-333679, which is produced by hydrolysis of the parent compound in the liver.. ACT-333679 is a non-prostanoid compound that acts as an agonist at the IP receptor with high affinity. Studies show ACT-333679 is highly selective for IP receptors, with some small reactivity with EP_2 receptors. In animal models of PAH, ACT-333679 improved hemodynamic parameters indicative that it has a potential to help clinical cases of PAH. One important issue is that all the non-prostanoid prostacyclin agonists have more the properties of a partial agonist and are not fully able to activate all the functions of both IP1 and IP2 receptors that endogenous prostacyclin does. (Seller, et al., 1997, Prostaglandins 53:21-35)

2.6.2.2 Primary pharmacodynamics

Mechanism of action: ACT-293987 is a pro-drug that is hydrolyzed into ACT-333679, which is a high affinity agonist at IP receptors, and intended for relief from PAH.

Drug activity related to proposed indication: ACT-293987 is a pro-drug that is hydrolyzed into ACT-333679, which is a high affinity agonist at IP receptorsintended to relieve PAH.

2.6.2.3 Secondary pharmacodynamics No studies have been conducted beyond the safety pharmacology studies noted below which are observational rather than mechanistic, and typically explore high dosages.

2.6.2.4 Safety pharmacology

Neurological effects:

Central nervous system						
Irwin screen (b) (4) 08.267]	Y	Oral	Single dose	Sprague-Dawley rats	0, 10, 30, 100 mg/kg	6 m / group
Body temperature, sleep & pain response [T-08.383]	Y	Oral	Single dose	Sprague-Dawley rats	0, 10, 30, 100 mg/kg	6 m / group

The modified Irwin Screen performed by the sponsor was designed to look for signs of behavioral or physiologic changes indicative of effects on the central or peripheral

nervous systems. The sponsor used high doses of the compound, 10, 30, and 100 mg/kg in rats. The low and mid dose had no noticeable effects on the rats, however, the high dose group exhibited hunchback position, reddened skin, struggle response to handling, tip-toe gait, problems with righting reflex, prolonged standing on the hind paws, low body temperature, deep respiration, soft stools, and difficulty with the wire maneuver. All signs disappeared within 24 hrs of drug administration.

The second study on body temperature, sleep and pain responsiveness used a control group, and doses of 10, 30, and 100 mg/kg. The high dose group saw all three parameters impacted, with a body temperature lowered7% , sleep prolonged by 77%, and pain response reduced by 26%. The mid dose group only saw a reduction in body temperature.

Summary: The neurological safety pharmacology studies indicate at high doses notable effects occur in the modified Irwin screening, along with an results of an examination of CNS effects (body temperature, sleep, and pain response).

Cardiovascular effects:

Cardiovascular system						
hERG channel K^+ current (ACT-293987) [B-08.268]	Y	*In vitro*	N/A	CHO-K1 cell line expressing hERG K^+ channel	0, 3, 10, 30, 100 μM	6 replicates
hERG channel K^+ current (ACT-333679) (b) (4) 08.270]	Y	*In vitro*	N/A	CHO-K1 cell line expressing hERG K^+ channel	0, 3, 10, 30 μM	6 replicates
Contractile force & heart rate (right atria) (ACT-293987) (b) (4) 08.271]	Y	*In vitro*	N/A	Isolated right atrium from Hartley guinea pig	0, 3, 10, 30, 100 μM	6 m
Contractile force & heart rate (right atria) (ACT-333679) (b) (4) -08.382]	Y	*In vitro*	N/A	Isolated right atrium from Hartley guinea pig	0, 3, 10, 30, 100 μM	6 m
Cardiac electrophysiology (ACT-293987) (b) (4) 08.269]	Y	*In vitro*	N/A	Isolated papillary muscle from Hartley guinea pig	0, 3, 10, 30, 100 μM	6 m
Hemodynamics & cardiac electrophysiology (telemetry study) (b) (4) 08.265]	Y	Oral	Single dose x 4 (crossover design)	Conscious Beagle dogs	0, 1, 3, 10 mg/kg	4 m
Blood coagulation [T-08.387]	Y	Oral	Single dose	Sprague-Dawley rats	0, 10, 30 or 100 mg/kg	6 m / group

Several studies were done to explore potential effects of ACT-293987 on the cardiovascular system. Prostacyclin agonists are known to be vasodilators, the basis for expecting efficacy in Pulmonary Arterial Hypertension (PAH). Therefore it is important to assess any impact on the cardiovascular system.

ACT-293987 at concentrations up to 30 mcM had no effect on hERG channels, and only had effects on guinea pig atria and papillary muscle in vitro at the highest dose tested, 100 mcM, indicating it probably affects Na or Ca channels. ACT-293987 had no effect on blood coagulation in vivo in rats. However, the compound reduced blood pressure at its lowest dose, and at the mid dose it also increased heart rate and respiration rates. There were no effects on ECG parameters or hemoglobin oxygenation.

Pulmonary effects:

Respiratory system						
Whole body plethysmography (b) (4) 08.266]	Y	Oral	Single dose	Conscious, unrestrained Sprague-Dawley rats	0, 10, 30, 100 mg/kg	6 m / group

Rats were treated with control, 10, 30, 100 mg/kg of ACT 293987. Animals in the mid and high dose groups showed higher respiration rates, tidal volumes and minute volumes.

Renal effects:

Urinary system						
Water & electrolyte excretion (b) (4) -08.272]	Y	Oral	Single dose	Sprague-Dawley rats	0, 10, 30, and 100 mg/kg	6 m / group

Results indicate that ACT-293987 at all tested doses decreased chloride and sodium/potassium ratio in rats.

Gastrointestinal effects:

Gastrointestinal system						
Intestinal transport [T-08.384]	Y	Oral	Single dose	Sprague-Dawley rats	0, 10, 30, 100 mg/kg	6 m / group
Gastric secretion [T-08.384]	Y	Intra-duodenal	Single dose	Sprague-Dawley rats	0, 10, 30, 100 mg/kg	6 m / group

Prostacyclin receptors are common in the gastrointestinal tract. All doses of ACT-293987 tested inhibited GI transit of the carbon powder and decreased acid output in the stomach.

Abuse liability: n/a

Other:

Reproductive system						
Amplitude & frequency of uterine contraction (ACT-333679) [T-08.385]	Y	*In vitro*	N/A	Sprague-Dawley rats	0, 10, 30, 100 µM	6 f / group (4 f for 100 µM)

Spontaneous contractions of the uterine muscle were not affected by the low dose of ACT-293987, while the mid and high doses significantly reduced uterine muscle contractions.

2.6.2.5 Pharmacodynamic drug interactions - No studies done, but see below for anticipated interactions based on pharmacology of prostacyclin.

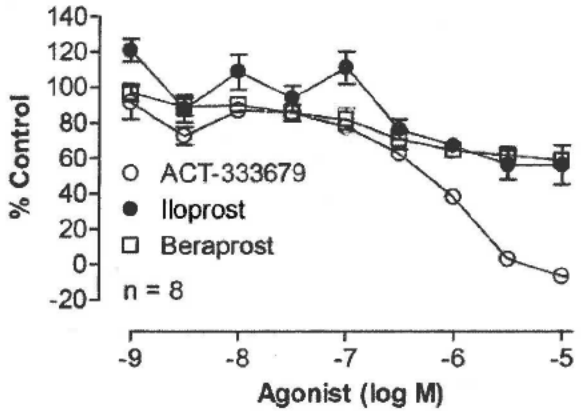
2.6.3 PHARMACOLOGY TABULATED SUMMARY

Table 1 **Affinities of ACT-293987, ACT-333679 and beraprost for human prostanoid receptors**

	Binding Affinity (K_i, μM)							
	IP	EP_1	EP_2	EP_3	EP_4	DP	FP	TP
ACT-293987	0.26	> 10	> 10	> 10	> 10	> 10	> 10	> 10
ACT-333679	0.02	> 10	5.8	> 10	4.9	2.6	> 10	> 10
Beraprost	0.039	> 10	> 10	0.68	7.2	> 10	> 10	> 10

IP = PGI_2 receptor; EP_{1-4} = PGE_2 receptor; DP = PGD_2 receptor; FP = $PGF_{2\alpha}$ receptor; TP = TXA_2 receptor

Figure 1 **Effect of ACT-333679 on proliferation of human pulmonary arterial smooth muscle cells**



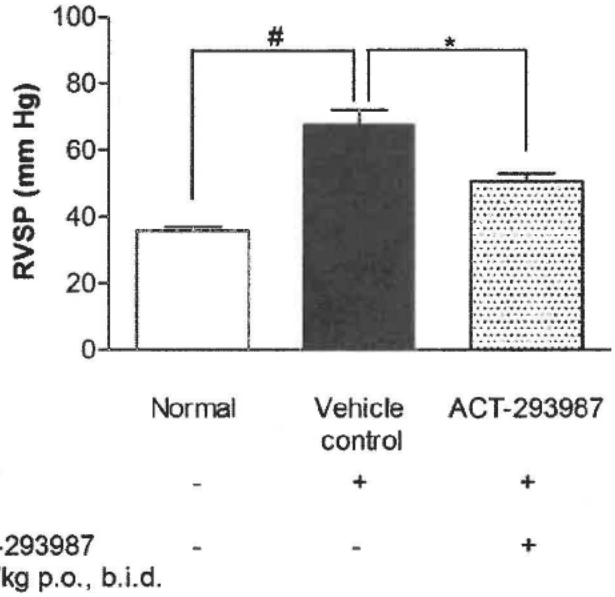
Cells were stimulated with PDGF (10 ng/ml) either in the absence or presence of test compounds for 24 h, and cellular incorporation of radioactivity was measured. Data is presented as mean ± S.E.M. (n = 8).

Table 2 **Inhibition of platelet aggregation by ACT-293987 and ACT-333679 in platelet-rich plasma from different species**

	Platelet aggregation IC_{50} (μM)			
Drug	Human	Monkey	Dog	Rat
ACT-293987	5.5 ± 0.8	3.4 ± 1.1	456 ± 23	–
ACT-333679	0.21 ± 0.04	0.21 ± 0.02	25 ± 1.5	10 ± 0.4

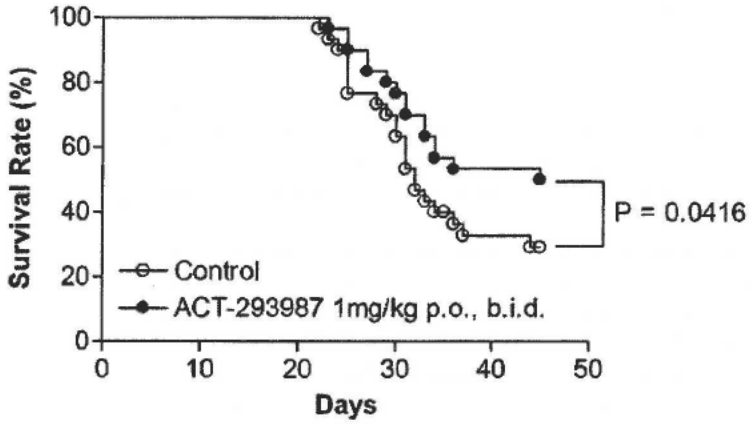
– = not determined; data is presented as mean ± S.E.M. (n = 4).

Figure 4 **Effect of ACT-293987 on RVSP in rats with MCT-induced PAH**



Data is presented as mean ± S.E.M. #, P < 0.01 vs normal rats, *, P < 0.01 vs vehicle control rats. (n = 12).

Figure 8 Effect of ACT-293987 on survival of rats with MCT-induced PAH



Survival data is presented using the Kaplan-Meier method and compared by the log-rank test. Day 0 indicates the time of injection of MCT and the beginning of administration of ACT-293987. (n = 30)

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary ACT-293987 is rapidly absorbed from the Gastro-intestinal tract with a Tmax of approximately 1 hr and a bioavailability of 80 to 90%. Radiolabeled ACT-293987 accumulates first in the liver after oral dosing, followed by accumulation in the stomach. This is probably due to the high level of activity and receptors present in the stomach, where prostacyclin agonists apparently have high activity. Most adverse events for prostacyclin agonists are related to their effects on the gastrointestinal system. ACT-293987 is primarily excreted through the biliary system, with only 2% of it's excretion due to renal pathways. ACT-293987 is a prodrug, although it also has activity at prostacyclin receptors, and is hydrolyzed to ACT-333697, the primary prostacyclin agonist. ACT-333697 is glucuronidated primarily and excreted through the biliary system, although *in toto* there are up to 15 potential metabolites of ACT-293987.

2.6.4.2 Methods of Analysis

HPLC with either analysis [redacted] (b) (4) or mass spectrophotometric methods was used to quantify the amounts of ACT-293987 and/or ACT-333697 in serum. Both methods provided a wide and useful range

2.6.4.3 Absorption

	Rat	Dog
Bioavailability	72%	84%
Dose Proportionality	Linear	Linear
Food Effects	Decreased Cmax AUC unchanged	

	Increased Tmax	
Gender Differences	No effect	Slight increases in Females vs. Males
Accumulation	None	None
Tmax	1 hr	1 hr

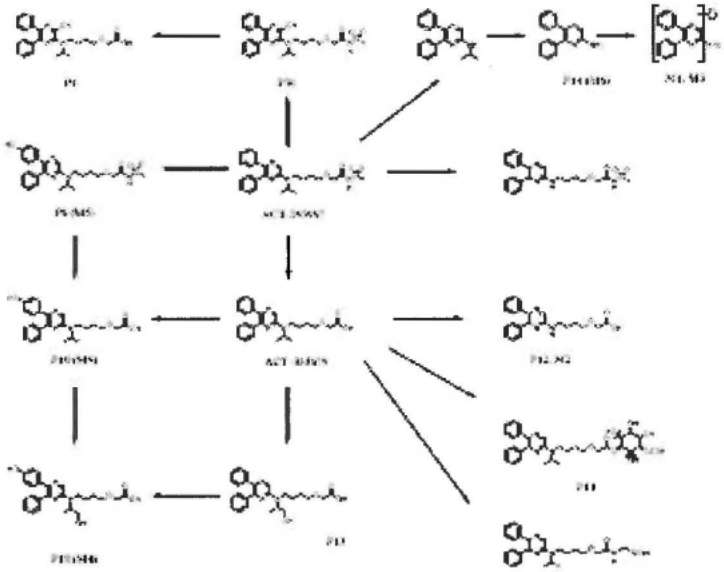
2.6.4.4 Distribution

Upon initial absorption of ACT-293987, the liver is the primary site of accumulation, presumably part of the normal first pass effect. It is also desireable with ACT-293987 since it is a prodrug and is metabolized to the active product, ACT-33679, primarily in the liver. After that, the stomach becomes the primary organ of accumulation of ACT-293987 and it's active metabolite, ACT-333679. Although the sponsor does not comment on this (and seems to ignore it), presumably this, among other possibilities, is due to the importance of prostacyclins in the gastric environment and may reflect a receptor mediated accumulation of the drug products. Since gastro-intestinal effects are the primary drivers of the adverse events seen in the dog and human studies, the issue is worth taking note of. Prostacyclins are thought to increase blood flow to the gastric mucosa and also to inhibit gastric acid secretion.

2.6.4.5 Metabolism

ACT-293987 is a pro-drug that has modest activity by itself as a prostacyclin agonist, and is hydrolyzed to ACT-333679, an active metabolite at prostacyclin receptors. As shown in the metabolic pathways figure below, ACT-333679 is not the only metabolite of ACT-293987, with a total of 15 possible metabolites.

Figure 5 Metabolic pathways of ACT-293987



2.6.4.6 Excretion In rats, biliary excretion accounted for 95% of the excretion of ACT-293987 and it's metabolites, while in dogs, 80-88% was recovered in the bile. 85% of the radiolabeled compound was eliminated within the first 24 hrs.

2.6.4.7 Pharmacokinetic drug interactions ACT-293987 and ACT-333697 are poor substrates for most of the CYP-450 enzyme systems, having only weak activity at CYP2C8 and CYP 2C9, which are more minor metabolic enzymes. Both substrates are also negligible at the multi-drug resisitance transporters. These results would predict minimal interactions with most drugs.

2.6.4.8 Other Pharmacokinetic Studies none

2.6.4.9 Discussion and Conclusions

The oral pro-drug, ACT-293987, is hydrolyzed by carboxyesterases to ACT-333697, a prostacyclin agonist. ACT-293987 is highly bioavailable, with approximately 80-90% absorbed from the gastro-intestinal tract. Hydrolysis to ACT-333697 is variable by species, rats having high serum levels of carboxyesterases, while dogs, monkeys, and man have high levels primarily in the liver. Although the half-life is approximately 14 hrs, distribution of oral doses are first primarily to the liver followed by accumulation in the stomach, an area with a significant level of prostacyclin receptors and activity. Of note is that most of the adverse reactions involving prostacyclin agonists are related to their activity in the gastro-intestinal system. ACT-293987 is primarily metabolized to ACT-333697 which is glucuronidated and excreted primarily through the biliary system. Only 2% of radiolabel from ACT-293987 is excreted through the kidneys. ACT-293987 and its metabolites are only minor substrates for 2 minor CYP enzymes, 2C8 and 2C9, and are not substrates for the multi-drug transporters, thereby minimizing the potential for major interactions with other drugs. Possible additive or synergistic effects with other vasodilators (e.g. nitrates) ,H2 blockers or other acid blockers, and anti-platelet drugs. is possible in view of the vasodilating activity of ACT-293987 and ACT-333697 and inhibition of gastric acid secretion and platelet aggregation.Reduction of acid in the stomach is known to effect the uptake of vitamin B12 in man, and it is also known to affect absorption of some drugs, for example clopidogrel. So, although drug-drug interactions at the metabolic or drug transport level are not expected to occur, there is still potential for interactions based on vasodilating, , gastro-intestinal, and/or platelet-modulating activities of ACT-293987 and ACT-333697.

2.6.4.10 Tables and figures to include comparative TK summary

Table 2 Summary of ACT-293987 and ACT-333679 exposures in the 4- and 13-week toxicity studies with ACT-293987 in mice

ACT-293987 (mg/kg)	Time		C_{max} (µg/mL) Male	C_{max} (µg/mL) Female	AUC_{0-24} (µg·h/mL) Male	AUC_{0-24} (µg·h/mL) Female
4-week toxicity study in mice [T-08.291]: NOAEL at 100 mg/kg/day						
30	Day 1	ACT-293987	1.49	1.50	1.33	1.41
		ACT-333679	2.14	1.91	2.86	2.45
100	Day 1	ACT-293987	7.77	10.1	11.3	13.9
		ACT-333679	6.53	6.93	16.2	17.0
300	Day 1	ACT-293987	14.5	22.4	64.2	70.0
		ACT-333679	10.7	13.2	65.9	62.2
30	Week 4	ACT-293987	1.45	1.54	1.07	1.41
		ACT-333679	2.16	1.76	2.25	1.99
100	Week 4	ACT-293987	8.32	11.3	9.36	10.6
		ACT-333679	6.01	6.9	11.9	10.5
300	Week 4	ACT-293987	8.33	15.1	24.8	23.2
		ACT-333679	8.03	8.02	28.7	24.3
13-week toxicity study in mice [T-08.292]: NOAEL at 100 mg/kg/day						
100	Day 1	ACT-293987	9.66	12.2	15.0	19.5
		ACT-333679	10.0	8.23	23.6	21.4
300	Day 1	ACT-293987	26.5	35.9	88.4	115
		ACT-333679	19.5	19.1	99.6	98.8
500	Day 1	ACT-293987	21.1	45.6	295	310
		ACT-333679	17.5	17.0	250	223
100	Week 13	ACT-293987	16.9	10.8	12.2	10.1
		ACT-333679	11.4	7.15	12.4	9.99
300	Week 13	ACT-293987	23.2	24.9	52.1	44.4
		ACT-333679	12.4	13.5	51.5	39.8
500	Week 13	ACT-293987	27.3	18.8	90.0	74.0
		ACT-333679	20.3	12.4	71.5	58.0

Values are means of 3 mice/sex/dose/sampling time

Table 3 **Summary of ACT-293987 and ACT-333679 exposures in the 4- and 26-week toxicity studies in rats**

ACT-293987 (mg/kg)	Time		C_{max} (µg/mL)		AUC_{0-24} (µg·h/mL)	
			Male	Female	Male	Female
4-week toxicity study in rats [T-08.275]						
20	Day 1	ACT-293987	0.28	0.63	0.29	0.46
		ACT-333679	2.76	2.8	13.3	9.53
60	Day 1	ACT-293987	1.05	2.1	2.41	3.64
		ACT-333679	7.15	9.3	64.5	55.4
180	Day 1	ACT-293987	2.5	5.3	8.22	13.7
		ACT-333679	14.7	16.68	210	226
20	Week 4	ACT-293987	0.27	0.53	0.32	0.51
		ACT-333679	3.04	3.25	12.2	9.54
60	Week 4	ACT-293987	1.2	2.3	2.14	2.92
		ACT-333679	8.99	11.9	38.8	36.3
180	Week 4	ACT-293987	2.5	6.01	8.77	24.8
		ACT-333679	14.5	27.2	137	304
4-week toxicity study in rats [T-08.276]: NOAEL at 6 mg/kg/day						
2	Day 1	ACT-293987	n.d.	n.d.	n.d.	n.d.
		ACT-333679	0.16	0.10	1.23	0.34
6	Day 1	ACT-293987	0.10	0.13	0.09	0.09
		ACT-333679	0.86	0.63	4.38	2.89
60	Day 1	ACT-293987	1.29	3.66	2.51	5.92
		ACT-333679	8.69	13.2	61.4	75.0

2	Week 4	ACT-293987	n.d.	n.d.	n.d.	n.d.
		ACT-333679	0.11	0.11	0.21	0.12
6	Week 4	ACT-293987	0.02	0.08	0.01	0.04
		ACT-333679	0.53	0.46	1.40	0.95
60	Week 4	ACT-293987	1.21	2.30	2.10	4.09
		ACT-333679	9.63	13.6	50.9	63.6
26-week toxicity study in rats [T-08.285]: NOAEL at 6 mg/kg/day						
6	Day 1	ACT-293987	0.20	0.18	0.14	0.13
		ACT-333679	1.12	0.90	6.43	3.10
25	Day 1	ACT-293987	1.27	2.08	1.73	1.72
		ACT-333679	7.06	6.85	38.5	33.2
100	Day 1	ACT-293987	4.89	5.51	13.1	12.4
		ACT-333679	22.3	20.0	192	190
6	Week 26	ACT-293987	0.4	0.40	0.23	0.29
		ACT-333679	1.26	1.78	4.77	6.79
25	Week 26	ACT-293987	0.50	2.77	1.43	2.81
		ACT-333679	4.46	11.9	22.7	45.7
100	Week 26	ACT-293987	2.07	10.1	5.33	18.8
		ACT-333679	12.7	43.7	76.3	202

Values are means of 3 rat/sex/dose/sampling time. n.d. = not determined.

Table 5 Summary of ACT-293987 and ACT-333679 exposures in the 2-, 4-, and 39-week toxicity studies in dogs

ACT-293987 (mg/kg/day)	Time		C_{max} (µg/mL) Male	C_{max} (µg/mL) Female	AUC_{0-24} (µg·h/mL) Male	AUC_{0-24} (µg·h/mL) Female
2-week toxicity study in dogs [T-08.277]: NOAEL at 2 mg/kg/day						
2	Day 1	ACT-293987	3.17	2.36	7.42	6.18
		ACT-333679	5.16	5.59	59.5	62.4
6	Day 1	ACT-293987	9.59	5.48	25.7	22.2
		ACT-333679	15.2	12.1	186	181
20	Day 1	ACT-293987	10.1	18.2	45.5	59.9
		ACT-333679	18.8	27.4	281	403
2	Week 2	ACT-293987	3.19	2.90	7.50	6.72
		ACT-333679	5.99	6.34	50.8	67.8
6	Week 2	ACT-293987	10.1	8.50	24.3	25.1
		ACT-333679	14.8	13.5	154	155
20	Week 2	ACT-293987	22.0*	23.3**	77.2*	103**
		ACT-333679	44.6*	35.9**	576*	532**
4-week toxicity study in dogs [T-08.290]: NOAEL at 1.5 mg/kg/day						
1.5	Day 1	ACT-293987	2.23	3.39	5.47	7.95
		ACT-333679	2.80	5.22	31.7	50.2
3	Day 1	ACT-293987	5.25	5.08	14.4	14.8
		ACT-333679	7.13	8.20	91.0	105
6	Day 1	ACT-293987	9.38	10.6	23.8	31.5
		ACT-333679	12.6	15.2	168	211

1.5	Week 4	ACT-293987	1.98	3.09	5.39	7.29
		ACT-333679	3.72	4.71	39.7	54.6
3	Week 4	ACT-293987	4.25	4.99	13.1	14.4
		ACT-333679	6.82	8.33	82.9	85.6
6	Week 4	ACT-293987	6.77	13.2	29.2	35.4
		ACT-333679	12.4	15.9	159	201
39-week toxicity study in dogs [T-08.286]						
1	Day 1	ACT-293987	0.98	1.53	2.55	3.36
		ACT-333679	2.50	3.05	24.6	33.2
2	Day 1	ACT-293987	2.54	2.87	8.44	7.30
		ACT-333679	5.27	5.56	49.9	54.0
4	Day 1	ACT-293987	5.77	5.21	17.4	17.6
		ACT-333679	9.17	8.62	94.4	104
1	Week 26	ACT-293987	1.83	0.97	3.49	2.85
		ACT-333679	3.29	2.52	28.8	24.6
2	Week 26	ACT-293987	4.50	3.26	10.2	9.15
		ACT-333679	6.40	6.13	55.8	52.2
4	Week 26	ACT-293987	5.66	5.03	18.1	16.2
		ACT-333679	6.89	10.4	58.4	90.8
1	Week 39	ACT-293987	1.03	1.36	2.55	2.88
		ACT-333679	2.60	3.26	23.1	35.7
2	Week 39	ACT-293987	3.10	1.79	8.15	6.54
		ACT-333679	5.29	5.87	40.5	58.9
4	Week 39	ACT-293987	8.62	6.88	21.3	18
		ACT-333679	9.54	12.5	109	120

Values are means of 3 dog/sex/dose/sampling time. *: 1 dog/sex/dose; **: 2 dog/sex/dose.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

From the mouse studies:

Study Length	Doses	Results	NOAEL
4 weeks	0, 30, 100, 300 mg/kg/day	No mortality, @300 mg/kg/day flaccidity and flushing	100 mg/kg/day

13 weeks	0, 100, 300, 500 mg/kg/day	500 mg/kg/day one mortality, CK & ALT increase; 300 mg/kg/day decrease in food consumption, flushing, flaccidity, BUN decreased, kidney tubular vacuolation	100 mg/kg/day

From the rat studies:

Study Length	Doses	Results	NOAEL
4 weeks	0, 20, 60, 180 mg/kg/day	20 mg/kg/day alveolar hemorrhage and decreased platelets, loss or black discoloration of tail tip;	Not determined
4 weeks + 4 weeks recovery	2, 6, 60 mg/kg/day	60 mg/kg/day flushing, decreased movement, piloerection, reduced body wt and food consumption. Reversible	6 mg/kg/day
26 weeks + 4 week recovery	0, 6, 25, 100 mg/kg/day	>25, 100 mg/kg/day liver and adrenal hypertrophy, hyperplasia mammary gland, salivary gland, follicular cells in thyroid; all treated animals, flushing, decreased movement, reversible; one animal died in high dose group of malignant lymphoma	6 mg/kg/day

From the dog studies:

Study length	Doses	Results	NOAEL	
2 weeks	2, 6, 20 mg/kg/day	20 mg/kg/day led to mortalities, Intussuseception, QTc prolongation; 6 mg/kg/day increased ossification, bone marrow fibrosis; 2 mg/kg/day decreased platelet, wbc, & neutrophils.	2 mg/kg/day	
4 weeks	1.5, 3, 6 mg/kg/day	6 mg/kg/day intussusception; 3 mg/kg/day intussusception, bone marrow fibrosis, ossification; 1.5 mg/kg/day vomiting, diarrhea, jelly feces	1.5 mg/kg/day	
39 weeks	1, 2, 4 mg/kg/day	4 mg/kg/day 2 mortalities, intussusception; 1mg/kg/day bone marrow fibrosis and ossification	Not determined	

Genetic toxicology: ACT-293987 was negative in most of the genotoxicity testing. The exception was a small signal of clastogenicity in the Chinese Hamster Lung cell in vitro assay. This was not present in the in vivo mouse micronucleus study.

Carcinogenicity: not done

Reproductive toxicology: For the rat studies, the NOAEL was the same as for the standard toxicity testing, 6 mg/kg/day. In the rat studies, the primary issue appeared to be low birth weight, which in humans is correlated with developmental difficulties.
In the rabbit study, the NOAEL was 10 mg/kg/day, with one animal dying at 30 mg/kg/day, reproductive function and fetal development were not effected.

Special toxicology: **Study title**: *in vitro* phototoxicity study

Key study findings: both ACT-293987 and ACT-333697 were positive for phototoxicity with UVA light, suggesting absorption in the UV spectrum.

2.6.6.2 Single-dose toxicity

Table 1 Overview of completed toxicity studies with ACT-293987

Study type [Reference] *Batch number**	GLP	Route	Species / test system	Treatment duration	Dose (mg/kg/day) / concentration	Animals / sex/ groups
Single dose						
Acute [T-08.284] *Lot 20*	Y	i.v.	Slc:ddy mice (m & f)	Single dose	0, 10, 20, and 40	5/sex/group
Acute [T-08.274] *Lot 20*	Y	Oral	SD rats (m & f)	Single dose	0, 125, 250, 500, and 1,000	5/sex/group
Acute [T-08.283] *Lot 20*	Y	i.v.	SD rats (m & f)	Single dose	0, 10, 20, and 40	5/sex/group
Acute [T-08.273] *Lot 20*	Y	Oral	Beagle dogs (m)	Single dose	20, 200, and 2,000	2/group

Single intravenous doses of up to 40 mg/kg were given to mice and rats. There were no mortalities, in mice, there was flushing at 10 mg/kg, decreased movement at 20 mg/kg, and prone position at 40 mg/kg, in rats, prone position, decreased movement, flaccidity and flush were observed at all doses above 10 mg/kg, with severity and reversibility dependent on the dose.

Single oral doses were given to rats and dogs. In rats the oral lethal dose was 500 mg/kg, in dogs 2,000 mg/kg. Reasons for mortality were different, with the cause of death evidently being severe hypotension due to peripheral vasodilation in rats, while in dogs the cause of death was intussusception. In general, the dogs showed more adverse

clinical signs related to gastrointestinal effects, with some of the animals flushing as a sign of vasodilatation., In the rats, signs were similar to those seen after 40 mg/Kg iv , but with increased severity resulting in death at 500mg/Kg po.

No serum levels were reported, making it difficult to compare the toxicities of the oral vs. intravenous routes of administration as a function of blood levels.

2.6.6.3 Repeat-dose toxicity

Study title: Twenty-six-week oral gavage toxicity study of NS-304 in rats with 4-week recovery period

Key study findings: One male died in the high dose group, however, upon necropsy it was determined the animal had malignant lymphoma. This was thought to be not drug related, but it will not be known until the carcinogenicity study is done. Doses were 6, 25, and 100 mg/kg/day, with 6 mg/kg/day being the NOAEL dose. Flushing was the primary observation, with alopecia and reduced body weights and food consumption observed in the high dose groups. . Many of the changes seen were reversible in the 4 week recovery period.

Study no.: T-08.285
Volume #, and page #: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: July 7, 2006
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity:
Test Article
The test article, NS-304, was supplied by Nippon Shinyaku Co Ltd. The lot number, appearance and other information on the test article used in the present study were as follows (Attached Data 1).
Name: NS-304 (another name; MRE-304)
Lot number: 20
Content: 100.3%
Appearance: Pale yellow crystalline powder
Stability: Characterization analysis was conducted at (b) (4) after animal experiment, and the stability during the administration period was deduced (Study No. A-1942, Attached Data 2-1).
Handling precautions: Mask and gloves were worn as the test article is a skin irritant.
Storage conditions: (b) (4)
Storage place: (b) (4)

(b) (4)

Methods

Doses:

Text Table 1. Group composition

Test group	Dose (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg/day)	Sex	Main group		Recovery group	
					No. of animals	Animal No.	No. of animals	Animal No.
Control	0	0	5	M	12	1001-1012	6	1013-1018
				F	12	1101-1112	6	1113-1118
Low	6	1.2	5	M	12	2001-2012	–	–
				F	12	2101-2112	–	–
Middle	25	5	5	M	12	3001-3012	6	3013-3018
				F	12	3101-3112	6	3113-3118
High	100	20	5	M	12	4001-4012	6	4013-4018
				F	12	4101-4112	6	4113-4118

M: Male, F: Female

(continued)

Test group	Dose (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg/day)	Sex	Satellite group	
					No. of animals	Animal No.
Control	0	0	5	M	8	1201-1208
				F	8	1301-1308
Low	6	1.2	5	M	8	2201-2208
				F	8	2301-2308
Middle	25	5	5	M	8	3201-3208
				F	8	3301-3308
High	100	20	5	M	8	4201-4208
				F	8	4301-4308

M: Male, F: Female

Species/strain: Crl:CD(SD) SPF rats (b) (4)

Route, formulation, volume, and infusion rate: oral gavage, 0.5% w/v% methyl cellulose, 5 mL/kg body weight,

Age: 4 weeks old

Weight: 195-234 g in males, 137-177 g in females

Sampling times: see observation and times

Unique study design or methodology (if any):

Observation and Times:

6.11.1 Clinical Observation

All animals were observed for clinical signs including external appearance, nutritional condition, posture, behavior and excretions 3 times a day (before, immediately after and approximately 2 hours after dosing; however twice on Saturdays, Sundays and holidays: before and immediately after dosing) during the administration period. During the recovery period, all animals were observed once a day in the morning.

6.11.2 Measurement of Body Weight

During the administration period, all animals were weighed before administration twice in week 1 (days 1 and 7) of administration, and once a week every 7 days thereafter. During the recovery period, all animals were weighed twice in week 1 (days 1 and 7) of recovery and once a week at 7-day intervals thereafter. Measurement was done between 08:30 and 11:32. Body weight gains for the entire period of administration (26 weeks) or recovery period (4 weeks) were also calculated. In order to calculate the relative organ weight of the animals sacrificed as scheduled, the body weight was also recorded on the day of necropsy after depriving the animals of food overnight (approximately 16 hours). The animal that died was weighed before carrying the animal out of the animal room for necropsy; however, this data was omitted from the table and appendix.

6.11.3 Measurement of Food Consumption

During the administration period, food consumption was measured for each animal twice in week 1 (days 1 and 7) of administration, and once a week every 7 days thereafter. During the recovery period, food consumption was measured for each animal once a week. Measurement was done between 08:57 and 12:09. On day 1 of administration, one day's food consumption was measured from the day before the start of administration and that on day 7 was calculated from 6-day's cumulative food consumption. Thereafter, 7-day's cumulative consumption was measured and one day's food consumption was calculated. On day 7 of recovery, one day's food consumption was measured from 6-day's cumulative consumption and thereafter, 7-day's cumulative consumption was measured and one day's food consumption was calculated.

6.11.4 Ophthalmology

Before the start of administration (during the quarantine/acclimatization period, 11 and 12 days before administration), all animals were examined, and the animals with pre-existing ophthalmological abnormalities that might affect the toxicity evaluation were excluded from animal grouping for the main and recovery groups (note).
During the administration period, examination was done in month 3 (week 12, days 80 and 81) and month 6 (week 25, days 171 and 172) of administration. All survivors of each sex in control and high dose group were examined after dosing on the day of examination. Examination was not done in middle and low dose groups, since no treatment-related changes were observed in the high dose group. During the recovery period, examination was done in week 4 (day 22) of recovery. All survivors of each sex in each test group were examined. The procedure for examination was as follows.
First, the light reflex was tested using an ophthalmoscope (BXα-13 type: NEIZ Inc.). Then mydriatic agent (Mydrin® P: Santen Pharmaceutical Co., Ltd., Lot Nos. MP0872, MP0931 and MP0947) was applied to dilate the pupil and the anterior portion, transparent body (optic media) and fundus oculi were examined using a hand slit lamp (SL-14 type: Kowa, Inc.) and an ophthalmoscope (Omega 200: HEINE OPTOTECNIK GmbH & Co. KG, Germany).

note: Four males and 3 females with severe ophthalmological abnormalities such as aqueous flare, hemorrhage of iris, retinal hemorrhage and impossibility of observation in transparent body and fundus were excluded from the present study. Twelve males and 19 females with ophthalmological abnormalities such as focal corneal vascularization, residue in anterior chamber, synechia, focal opacity in lens, adhesion posterior lens capsule, persistent tunica vasculosa lentis, persistent hyaloids artery, vitreous hemorrhage, persistent hyperplastic primary vitreous and retinal hold were excluded as candidates for the main and recovery groups since it was judged that the above abnormalities might affect the toxicity evaluation. However, they were included as candidates for the satellite

group.

6.11.5 Urinalysis (including water intake)

Examination was done in all survivors of each sex in months 3 (week 13, days 85 to 87) and 6 (week 25, days 169 to 171) (note) of administration and in week 4 (days 24 to 25) of recovery.

During the administration period, after the dosing, all survivors of each sex in main and recovery groups were accommodated in cages with trays attached for urine collection and 4-hour urine samples were collected under fasting conditions but withfree access to water. Then the following 20-hour urine samples were collected under free access to food and water.

In week 4 of recovery, the survivors were accommodated in cages with trays attached for urine collection, and 4-hour urine samples were collected under fasting but free access to water. The following 20-hour urine samples were collected under free access to food and water. The parameters listed in the following were examined. The 4-hour urine samples were used for examination of pH, protein, ketone body, glucose, occult blood, bilirubin, urobilinogen, color, urinary sediments and urine volume. The urinary sediments obtained by centrifugation (set at 1,500 rpm, approximately 440×g for 5 minutes) were subjected to microscopic examination without fixation or staining.

Urine volume (24h) was calculated by totaling the volume of 4-hour and 20-hour urine.

One day's output of electrolytes was calculated by their concentration and 24-hour urine volume.

One day's water intake was measured using water bottles on the day of urinalysis for each animal.

Text Table 2. Items, Methods and Equipment for Urinalysis Examinations

Item	Method	Unit
pH	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)[a]	
protein	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)[a]	
ketone body	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)[a]	
glucose	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)[a]	
occult blood	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)[a]	
bilirubin	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)[a]	
urobilinogen	Urine test strips AUTION Sticks-7EA (ARKREY, Inc.)[a]	
color	macroscopic observation	
urinary sediments	microscopic examination	
urine volume (24h)	volumetry	mL/24h
osmotic pressure	freezing point method[b]	mOsm/kg
sodium (Na)	ion selective electrode method[c]	mmol/24h
potassium (K)	ion selective electrode method[c]	mmol/24h
chloride (Cl)	coulometric titration method[c]	mmol/24h

Equipment used

a): AUTION MINI™ AM-4290 (ARKREY, Inc.)

b): Osmotic Pressure AUTO & STAT OM-6030 (ARKREY, Inc.)

c): Automatic Electrolyte Analyzer PVA-α II (Analytical Instrument Inc.)

note: At the examination in month 6, 2 males was subjected to re-collection of urine sample or re-examination was conducted (days 176 and 177).

In No. 2001, re-collection of the urine sample was conducted to examine osmotic pressure and output of electrolytes, since an initial urine sample was dropped on the floor and could not be collected. The data from re-collected urine for urine volume was adopted.

In No. 4007, re-examination of water intake was conducted, since a relatively high value compared to other animals was recorded, although there was no apparent abnormality in any other parameter. The data from re-examination were adopted, since it was judged that the re-examined value was normal for these weeks of age.

6.11.6 Hematology

At the time of necropsy conducted on the day following the end of the administration period or the end of the recovery period, the survivors were deprived of food overnight

(approximately 16 to 20 hours) prior to blood collection. Blood samples were collected from the abdominal aorta under ether anesthesia into blood collection tubes (approximately 1 mL, SB-41: Sysmex Corp.) containing an anticoagulant (EDTA-2K) and the following items were determined. May-Gruenwald-Giemsa staining smears from all animals were prepared as reserve in the case of microscopic examination, although examination was not actually conducted. However, for determining PT, APTT and fibrinogen, plasma obtained by centrifuging (set at 3100 rpm, approximately 1690 × g for 12 minutes) blood samples (approximately 0.9 mL) treated with 3.8 w/v% sodium citrate (1 vol sodium citrate solution/9 vol blood) was used.

Text Table 3. Items, Methods and Equipment for Hematological Examinations

Item Method Unit
red blood cell count (RBC) dual angle laser flow-cytometric measurement[a] $10^4/\mu L$
hemoglobin (HGB) modified cyanmethemoglobin method[a] g/dL
hematocrit (HCT) calculated from mean corpuscular volume and red blood
cell count[a]
%
mean corpuscular volume (MCV) dual angle laser flow-cytometric measurement[a] fL
mean corpuscular hemoglobin (MCH) calculated from red blood cell count and hemoglobin[a] pg
mean corpuscular hemoglobin concentration
(MCHC)
calculated from hematocrit and hemoglobin[a]
g/dL
reticulocyte ratio (Reticul.) laser flow-cytometric measurement with RNA stain[a] %
platelet count (PLT) dual angle laser flow-cytometric measurement[a] $10^4/\mu L$
white blood cell count (WBC)
dual angle laser flow-cytometric measurement[a]
$10^2/\mu L$
differential leukocyte count (note) peroxidase flow-cytometric measurement and dual angle
laser flow-cytometric measurement[a]
%
prothrombin time (PT) clot method[b] s
activated partial thromboplastin time
(APTT)
clot method[b] s
Fibrinogen (FIB) thromboplastin method[b] mg/dL
Equipment used
a): ADVIA®120 Hematology System (Bayer Corporation, New York, USA)
b): Coagulometer ACL 100 (Instrumentation Laboratory)
note: Lymphocytes (LYM), neutrophils (NE), eosinophils (EOSINO), basophils (BASO), monocytes (MONO) and large unstained cells (LUC)

6.11.7 Blood chemistry

At the same time as hematology, blood samples were collected from the abdominal aorta into blood collection tubes (approximately 5 or 6 mL, Venoject II-Autosep: Terumo Corporation) containing serum separator, and the serum was obtained by centrifugation (set at 3,100 rpm, approximately 1,690×g for 12 minutes). The following items were determined on the serum. However, for determining AST, ALT and CK, the plasma obtained by centrifuging (set at 3,100 rpm, approximately 1,690×g for 12 minutes) blood samples collected into test tubes (approximately 2 mL) containing anti-coagulant heparin sodium salt (approximately 20 units/mL blood) was used. The following parameters were determined.

Text Table 4. Items, Methods and Equipment for Blood chemistry Examinations

Item Method Unit
AST UV-rate method[a] IU/L
ALT UV-rate method[a] IU/L
AlP (ALP) Bessey-Lowry method[a] IU/L
CK UV-rate method[a] IU/L
total cholesterol (T-CHO) CEH-COD-POD method[a] mg/dL
triglyceride (TG) LPL-GK-GPO-POD method[a] mg/dL

total bilirubin (T-BIL) bilirubin oxidase method[a] mg/dL
glucose (GLU) glucose dehydrogenase method[a] mg/dL
blood urea nitrogen (BUN) urease-LEDH method[a] mg/dL
creatinine (CRNN) creatininase-creatinase-sarcosine oxidase-POD method[a] mg/dL
sodium (Na) ion selective electrode method[a] mmol/L
potassium (K) ion selective electrode method[a] mmol/L
chloride (Cl) ion selective electrode method[a] mmol/L
calcium (Ca) OCPC method[a] mg/dL
inorganic phosphorus (P) molybdic acid method[a] mg/dL
total protein (TP) Biuret method[a] g/dL
A/G ratio (A/G) calculated from protein fractions
protein fractions cellulose acetate membrane electrophoresis[b] %
Equipment used
a): Toshiba Biochemical Analyzer Model TBA-120FR (Toshiba Corp.)
b): CLINISCAN SA-V (Helena Co. Ltd.)

6.11.8 Pathology

6.11.8.1 Necropsy

After collecting blood samples, all survivors were sacrificed by exsanguination from the abdominal aorta under ether anesthesia. External appearance and all the organs/tissues in the cranial, thoracic and abdominal cavities were carefully examined and the results were recorded. The animal that was found dead was necropsied as soon as it was discovered.

6.11.8.2 Organ weights

After necropsy, the organs listed below of all survivors were weighed (absolute weight) and organ weight per 100 g body weight (relative weight) was calculated based on the fasted animal's body weight and absolute organ weight. The paired organs indicated by asterisks (*) were weighed separately; however, evaluation was done on the total value of the right and left organs. Organ weights were not measured on the animals that were found dead.

brain, pituitary, thyroids (including parathyroids)*, adrenals*, thymus, spleen, heart, lungs (including bronchus), salivary glands (submandibular + sublingual glands)*, liver, kidneys*, testes*, ovaries*, epididymides*, uterus, prostate and seminal vesicles

6.11.8.3 Histopathology

All the organs/tissues listed below of all animals were fixed and preserved in phosphate buffered 10 vol% formalin. However, the eyeballs and optic nerves were fixed with a mixture containing 3 w/v% glutaraldehyde and 2.5 vol% formalin, and the testes and epididymides of all survivors were fixed with Bouin's solution, and then preserved in phosphate buffered 10 vol% formalin. All organs/tissues of all animals were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H.E). Of these, all organs/tissues from the control and the high dose groups were examined histopathologically in the main group (26-week sacrificed group). In addition, sections of the adrenal and liver in both sexes and mammary gland, submandibular gland and thyroid in females from all other groups were subjected to histopathological examination, since treatment-related lesions were suspected in these organs. The submandibular gland in males was also suspected to be a target organ and it was also examined; however, it was ultimately judged that there were no treatment-related changes. In the recovery groups, the above organs/tissues suspected to be the target organ from all animals were examined. The paired organs indicated by asterisks (*) were examined bilaterally; however, the organs marked with # were examined unilaterally.

Deleted:

cerebrum, cerebellum, spinal cord (cervical, thoracic and lumbar), sciatic nerves#, eyeballs*, optic nerves*, Harderian glands*, pituitary, thyroids*,

parathyroids*, adrenals*, thymus, spleen, submandibular lymph node, mesenteric lymph node, heart, thoracic aorta, trachea, lungs (including bronchus), tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, submandibular glands*, sublingual glands*, parotid glands*, liver, pancreas, kidneys*, urinary bladder, testes*, epididymides*, prostate, seminal vesicles*, ovaries*, uterus*, vagina, mammary glands (inguinal region, both sides)#, sternum (including bone marrow), femurs (including bone marrow)#, femoral skeletal muscles#, skin (inguinal region, both sides)#, preputial glands*, clitoral glands* and gross lesions
Besides the organs/tissues listed above the oviducts, extraorbital lacrimal glands, Zymbal's glands, larynx, nasal cavity and the site of animal identification (ear auricle) were preserved.

6.11.8.4 Electron microscopy

At the time of necropsy conducted on the day following the end of the administration period or the end of the recovery period, liver and kidney (cortex and medulla) from 2 animals in each sex in the control and 100 mg/kg group (note) were fixed with phosphate buffered 0.5 w/v% glutaraldehyde and 1.5 w/v% paraformaldehyde, post fixed with 1 w/v% tetroxide osmium and embedded in epoxy resin.
note: animal Nos. 1001, 1003, 4002, 4004, 1101, 1103, 4101 and 4103 at week 26 of administration, animal Nos. 1013, 1015, 4013, 4015, 1113, 1115, 4113 and 4115 at week 4 of recovery

Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )

Results:

Mortality: below is the sponsor table on the progression for the one animal that died in the study in the high dose group.

Table 1-1 Twenty-six week oral gavage toxicity study of XS-301 in rats with 4 week recovery period (Clinical signs (Administration period, dead animals))

Sex	Dose mg/kg	Animal number	Day of death	Week of administration 1	2-14	15	16	17	18-26
Male	100	1001	113	AB	A	AJK	AKL		

A : Flaskspirans, Tinted
B : Flaskfabelle body
J : Decrease, spontaneous movement
K : Deep breathing
L : Unkempt fur
: Dead

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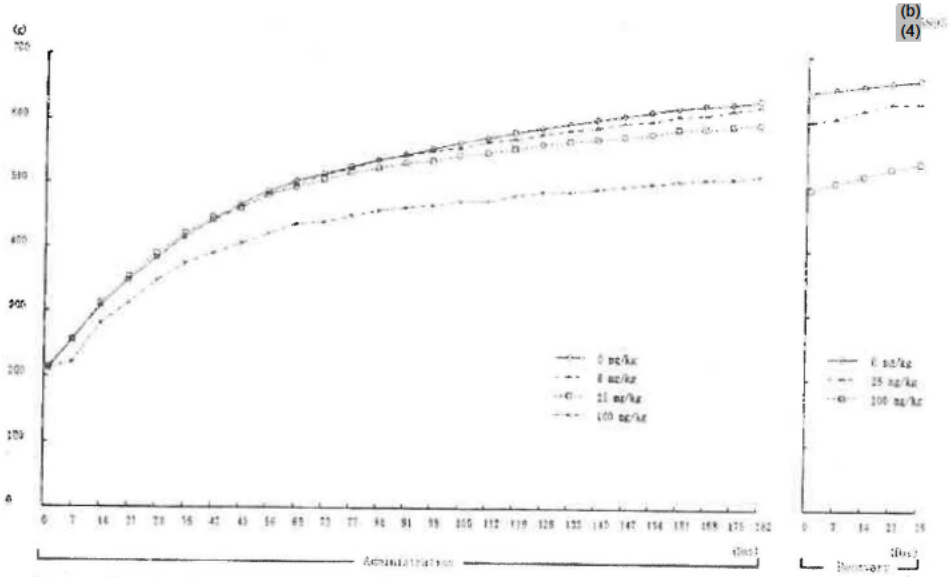
Clinical signs:

Body weights:

(b) (4)



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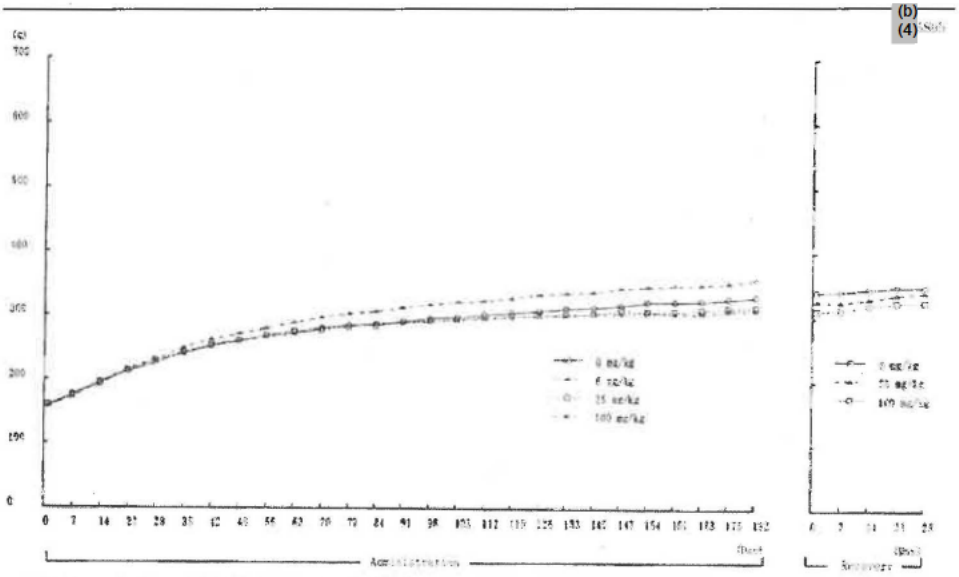
(b) (4)



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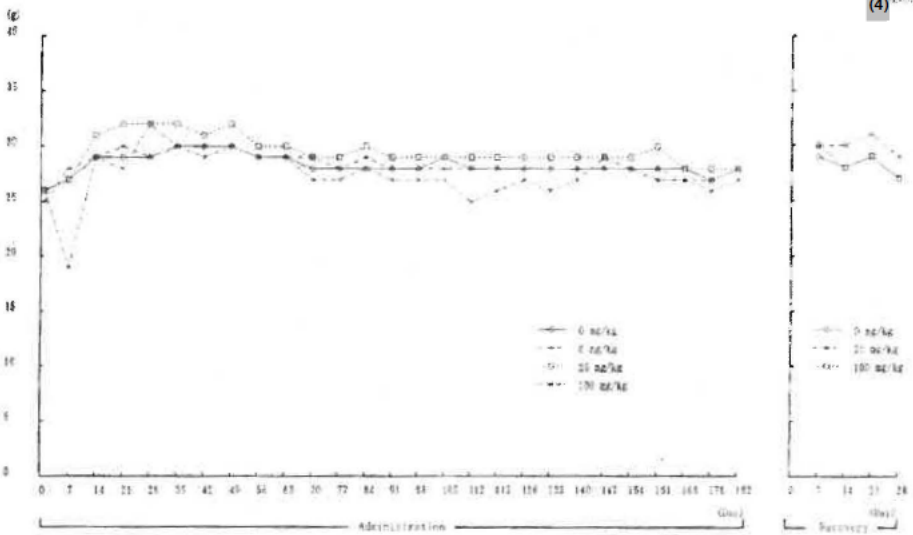
Food consumption:

(b) (4)



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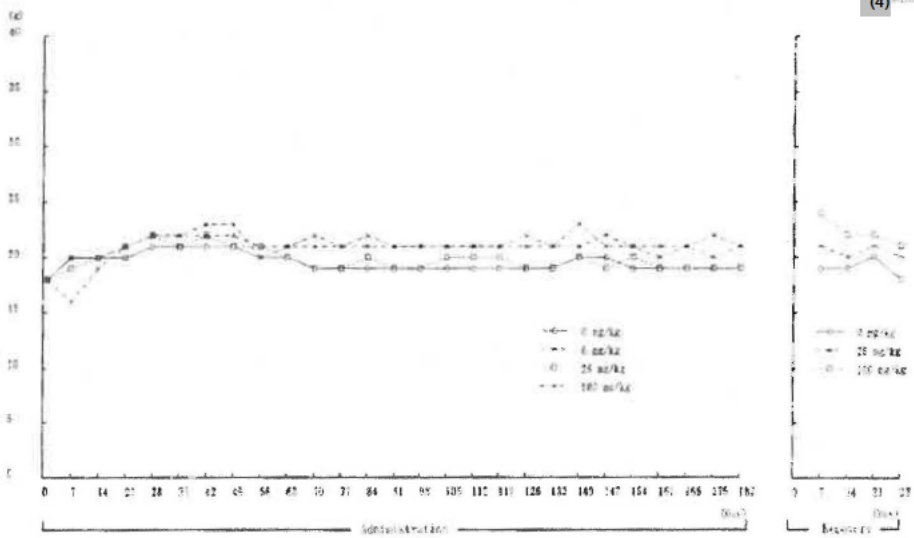
(b) (4)



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Ophthalmoscopy: no treatment related abnormalities were reported

EKG: not done

Hematology:

Text Table 3-1. Summary of hematology –End of the administration period–

Sex	Male			Female		
Dose (mg/kg/day)	6	25	100	6	25	100
No. of animals	12	12	11	12	12	12
HGB	N	N	N	N	N	-4%**
HCT	N	N	N	N	N	-4%*
MCV	N	N	N	N	-4%*	N
WBC	-31%**	N	-23%*	N	N	N
Differential leukocyte ratio						
Lymphocyte ratio	N	-23%**	-16%**	N	N	N
Neutrophil ratio	N	-41%**	-27%*	N	N	N
Monocyte ratio	N	N	-34%*	N	N	-35%*
Platelet	-15%**	-16%**	-32%**	N	N	-17%**
Fibrinogen	N	N	N	N	-23%*	-46%**

Values in the table indicate percentage of change against the control mean (-: decrease, +: increase).
N: No remarkable changes
* (**): p≤0.05 (0.01) (significantly different from the control group)

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Text Table 3-2. Summary of WBC and differential leukocyte count[a)]

–End of the administration period–

Sex	Male				Female			
Dose (mg/kg/day)	0	6	25	100	0	6	25	100
No. of animals	12	12	12	11	12	12	12	12
WBC (10^2/μL)	107.2	74.3**	84.2	82.1*	60.9	59.2	54.3	71.9
Lymphocyte ratio	62.6%	N	76.8%**	72.8%**	71.0%	N	N	N
actual number	67.1	NC	64.7	59.8	NC	NC	NC	NC
Neutrophil ratio	30.8%	N	18.2%**	22.6%*	22.8%	N	N	N
actual number	33.0	NC	15.3	18.6	NC	NC	NC	NC
Monocyte ratio	3.8%	N	N	2.5%*	3.4%	N	N	2.2%*
actual number	4.1	NC	NC	2.1	2.1	NC	NC	1.6

Values are group mean
a): Actual number of each cell type was calculated from WBC and differential leukocyte ratio (unit: 10^2/μL).
N: No remarkable changes
* (**): p≤0.05 (0.01) (significantly different from the control group)
NC: Not calculated, since there were no statistical differences from the control group in the ratio

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Text Table 3-3. Summary of hematology –End of the recovery period–

Sex	Male		Female	
Dose (mg/kg/day)	25	100	25	100
No. of animals	6	6	6	6
Differential leukocyte ratio				
Basophil ratio	-25%*	-50%**	N	N
PT	N	-4%*	N	N
APTT	-10%*	-10%*	N	N

Values in the table indicate percentage of change against the control mean (-: decrease).
N: No remarkable changes
* (**): p≤0.05 (0.01) (significantly different from the control group)

Clinical chemistry:

Text Table 4-1. Summary of blood chemistry –End of the administration period–

Sex	Male			Female		
Dose (mg/kg/day)	6	25	100	6	25	100
No. of animals	12	12	11	12	12	12
AST	N	N	N	N	-49%**	-56%**
ALT	-51%*	N	N	N	-48%**	-52%**
CK	N	N	N	N	-14%*	-18%**
AlP	N	N	N	N	N	+90%**
T-CHO	N	N	N	N	N	+29%*
TG	N	N	-51%**	N	N	-62%*
Glucose	N	N	-18%**	N	N	N
BUN	N	N	N	N	N	-20%*
K	N	N	-8%**	N	N	N
Protein fractions						
Albumin ratio	N	+6%*	N	N	N	N
α_2-globulin ratio	N	N	N	N	N	+20%**
β-globulin ratio	-6%*	-7%**	N	N	N	+10%*
γ-globulin ratio	N	N	+17%*	N	N	N
A/G ratio	N	+14%*	N	N	N	-11%*

Values in the table indicate percentage of change against the control mean (-: decrease, +: increase)
N: No remarkable changes
* (**): p≤0.05 (0.01) (significantly different from the control group)

Text Table 4-2. Summary of blood chemistry –End of the recovery period–

Sex	Male		Female	
Dose (mg/kg/day)	25	100	25	100
No. of animals	6	6	6	6
CK	-21%*	-22%*	N	N
BUN	N	+17%**	N	N
P	+14%*	N	N	N
Protein fractions				
β-globulin ratio	N	-10%*	N	N
γ-globulin ratio	N	N	N	+28%*

Values in the table indicate percentage of change against the control mean (-: decrease, +: increase).
N: No remarkable changes
* (**): p≤0.05 (0.01) (significantly different from the control group)

Urinalysis:

Text Table 2-1. Summary of urinalysis –Month 3 of administration–

Sex	Male			Female		
Dose (mg/kg/day)	6	25	100	6	25	100
No. of animals	12	18	18	12	18	18
Urine volume	N	+54%*	+36%	N	+166%**	+305%**
Water intake	N	+30%	+24%	N	+24%	+82%**
Osmolality	N	-23%	-28%*	N	-46%**	-60%**
Na	N	N	N	N	+44%**	+33%*
K	N	N	N	N	+53%**	+35%*
Cl	N	N	N	N	+55%**	+36%*

Values in the table indicate percentage of change against the control mean (-: decrease, +: increase).
N: No remarkable changes
* (**): p≤0.05 (0.01) (significantly different from the control group)

Gross pathology:

1) Dead animal (one male in the 100 mg/kg group, Animal No. 4001)

Nodule in the thymus, enlargement of the submandibular, mesenteric and other lymph node, excess fluid in abdominal and thoracic cavities, enlargement of the spleen and liver, dark red focus in the femur and unkempt fur were observed.

2) End of the administration period

(1) Males

There were no treatment-related changes in any animal.

(2) Females

Hair loss (alopecia) was observed in 2/12 females in the 100 mg/kg group.

3) End of the recovery period

There were no treatment-related changes in any animal in either sex.

Organ weights (specify organs weighed if not in histopath table):

Text Table 5-1. Summary of organ weights –End of the administration period–

Sex	Male			Female		
Dose (mg/kg/day)	6	25	100	6	25	100
No. of animals	12	12	11	12	12	12
Body weight at necropsy	N	-5%	-17%**	N	N	N
Brain						
absolute	N	N	N	N	N	N
relative	N	N	+22%**	N	N	N
Thyroid						
absolute	N	N	N	N	N	N
relative	N	N	N	N	+26%*	+31%**
Salivary gland						
absolute	N	N	N	N	N	+21%**
relative	N	N	+23%**	N	N	+26%**
Heart						
absolute	N	N	N	N	N	+23%**
relative	N	+12%*	+23%**	N	+16%**	+29%**
Lung						
absolute	N	N	N	N	N	+15%**
relative	N	+15%**	+31%**	N	+14%*	+22%**
Liver						
absolute	N	N	N	N	N	+25%**
relative	N	N	+21%**	N	+13%**	+30%**
Kidney						
absolute	N	N	-11%*	N	N	N
relative	N	N	N	N	N	+8%*
Adrenal						
absolute	N	+15%*	+22%**	N	+23%**	+42%**
relative	N	+22%**	+56%**	N	+29%**	+48%**
Testis						
absolute	N	N	N	NA	NA	NA
relative	N	N	+21%**	NA	NA	NA
Prostate						
absolute	N	N	-19%**	NA	NA	NA
relative	N	N	N	NA	NA	NA
Epididymis						
absolute	N	N	-10%*	NA	NA	NA
relative	N	N	N	NA	NA	NA
Seminal vesicle						
absolute	N	N	-19%**	NA	NA	NA
relative	N	N	N	NA	NA	NA

Values in the table indicate percentage of change against the control mean (-: decrease, +: increase).
N: No remarkable changes
NA: Not applicable
* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)

Text Table 5-2. Summary of organ weights –End of the recovery period–

Sex	Male		Female	
Dose (mg/kg/day)	25	100	25	100
No. of animals	6	6	6	6
Body weight at necropsy	-5%	-20%**	N	N
Brain				
absolute	N	N	N	N
relative	N	+22%**	N	N
Pituitary				
absolute	-18%**	-24%**	N	N
relative	-12%**	N	N	N
Thyroid				
absolute	N	N	+41%*	N
relative	N	+49%*	+45%*	N
Salivary gland				
absolute	N	-16%**	N	N
relative	N	N	N	N
Thymus				
absolute	N	N	N	+71%*
relative	N	N	N	+88%*
Heart				
absolute	N	N	N	N
relative	+8%*	+12%**	N	+29%*
Lung				
absolute	+10%*	N	N	+14%*
relative	+15%*	+19%**	N	+19%**
Liver				
absolute	N	N	N	N
relative	N	N	N	+17%*
Spleen				
absolute	N	N	N	N
relative	+14%*	N	N	N
Kidney				
absolute	N	-18%**	N	N
relative	N	N	N	N
Adrenal				
absolute	N	-21%*	N	N
relative	N	N	N	+42%*

Values in the table indicate percentage of change against the control mean (-: decrease, +: increase).
N: No remarkable changes
* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )

Text Table 6. Incidence summary of histopathological lesions –End of the administration period–

Sex	Male				Female			
Dose (mg/kg/day)	0	6	25	100	0	6	25	100
No. of animals	12	12	12	11	12	12	12	12
Adrenal								
Hypertrophy, zona glomerulosa (±, +)	2	1	4	11	1	3	10	12
Liver								
Hypertrophy, hepatocytic, central (+)	0	0	0	5	0	0	0	9
Mammary gland								
Hyperplasia, acinar cell, diffuse (total)	0	NE	NE	0	8	6	9	11
(±)	0	NE	NE	0	7	5	9	1
(+)	0	NE	NE	0	1	1	0	10
Submandibular gland								
Hypertrophy, acinar cell (+)	0	1	1	1	0	0	7	10
Thyroid								
Hyperplasia, follicular cell, diffuse (±)	0	NE	NE	0	0	0	1	4

Values in the table indicate the number of animals with lesions.
±: Minimal, +: Mild
NE: Not examined

Toxicokinetics:

Text Table 7. Summary of TK parameters

Sex	Male (n=3)			Female (n=3)		
Dose (mg/kg/day)	6	25	100	6	25	100
NS-304						
T_{max} (h)						
Day 1	0.5	0.5	1.0	0.5	0.5	0.5
Week 26	0.5	0.5	1.0	0.5	0.5	0.5
C_{max} (μg/mL)						
Day 1	0.204	1.27	4.89	0.181	2.08	5.51
Week 26	0.398	0.498	2.07	0.405	2.77	10.1
C_{1h} (μg/mL)						
Day 1	0.0462	0.787	4.89	0.0476	0.642	3.60
Week 13	0.0626	0.415	1.37	0.0609	0.368	3.59
AUC_{0-24h} (μg·h/mL)						
Day 1	0.137	1.73	13.1	0.126	1.72	12.4
Week 26	0.229	1.43	5.33	0.286	2.81	18.8
MRE-269						
T_{max} (h)						
Day 1	1.0	1.0	1.0	1.0	1.0	1.0
Week 26	1.0	1.0	1.0	1.0	1.0	1.0
C_{max} (μg/mL)						
Day 1	1.12	7.06	22.3	0.904	6.85	20.0
Week 26	1.26	4.46	12.7	1.78	11.9	43.7
C_{1h} (μg/mL)						
Day 1	1.12	7.06	22.3	0.904	6.85	20.0
Week 13	1.31	5.07	8.13	1.71	6.01	24.8
AUC_{0-24h} (μg·h/mL)						
Day 1	6.43	38.5	192	3.10	33.2	190
Week 26	4.77	22.7	76.3	6.79	45.7	202

Value in the table (C_{max} and AUC_{0-24h}) indicates the mean value of 3 animals.

Other:

Study title: 39-week oral (capsule) toxicity study in the Beagle dog

Key study findings: dogs were placed in 4 groups of 6 males and 6 females receiving 0, 1, 2 or 4 mg/kg/day. 2 Females in the high dose group were sacrificed moribund on day 24 and 124, respectively.

Study no.: T-08.286
Volume #, and page #: eCTD 4.2.3.2
Conducting laboratory and location: (b) (4)

Date of study initiation: July 25, 2006
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: NS-304 (MRE-304)/ Lot # 20/ 100.3% purity

Methods

Doses:

Text Table 1. Group Composition

Test group	Dose level (mg/kg)	Sex	26-week necropsy group		39-week necropsy group	
			No. of animals	Animal number	No. of animals	Animal number
Control group	0	Male	3	1001-1003	3	1004-1006
		Female	3	1101-1103	3	1104-1106
Low dose group	1	Male	3	2001-2003	3	2004-2006
		Female	3	2101-2103	3	2104-2106
Middle dose group	2	Male	3	3001-3003	3	3004-3006
		Female	3	3101-3103	3	3104-3106
High dose group	4	Male	3	4001-4003	3	4004-4006
		Female	3	4101-4103	3	4104-4106

Species/strain: Beagle dogs

Route, formulation, volume, and infusion rate: Oral/gelatin capsules packed with drug compound.

Satellite groups used for toxicokinetics or recovery: none

Age: 6 months

Weight: 6.6 to 9.4 kg in males and 6.1 to 8.3 kg in females

Sampling times:

Text Table 2. Items and Schedule for Observation and Examination (In-life)

Item	Schedule / Frequency
Observation of general condition	Week −1 of administration: once daily (morning) Administration period: before and 1 hour after dosing (every day) and 3 hours after dosing (once a week)
Measurement of body weight	Once in Week −1 of administration (morning) Until Week 13 of administration: On Day 1 of administration and once a week thereafter (before dosing of the day) From Week 14 of administration: Once every 2 weeks (morning) and before necropsy on the day of necropsy
Measurement of food consumption	Every day from Week −1 of administration until the end of the administration period
Measurement of water intake	Once in Week −2 of administration Administration period: Once each in Weeks 4, 13, 26 and 39 of administration
Ophthalmological examination	Week −2 of administration: Once Administration period: Once each in Weeks 12, 25 and 38 of administration (1 to 4 hours after dosing)
Electrocardiography	Week −2 of administration: Once (morning) Administration period: Once each in Weeks 13, 26 and 39 of administration (before dosing and 1 to 4 hours after dosing)
Urinalysis	Week −2 of administration: Once Administration period: Once each in Weeks 4, 13, 26 and 39 of administration
Hematological examination	Week −1 of administration: Once Administration period: Once each in Weeks 13, 26 and 39 of administration (before dosing)
Blood chemistry examination	Week −1 of administration: Once Administration period: Once each in Weeks 13, 26 and 39 of administration (before dosing)

Unique study design or methodology (if any):

Observation and Times: (this information can be provided in a separate section OR evaluation times can be described for each parameter in the results section).

6.13.1 Clinical Observation

All animals were observed for clinical abnormalities such as the condition of visual mucosa and appearance of excreta and abnormal behaviors and abnormalities observed were recorded.

6.13.2 Measurement of Body Weight

All animals were weighed between 08:12 and 9:50 on the days of measurement (before dosing of the day during the administration period). On the day of necropsy, animals were weighed after fasting for at least 16 hours from the previous day. Moribund animals were weighed before necropsy.

6.13.3 Measurement of Food Consumption

For all animals, feed was supplied and removed as described in 6.5, one-day food consumption was calculated from the residual amount, and mean daily food consumption was calculated from the cumulative food consumption for 1 week.

6.13.4 Measurement of Water Intake

For the animals that were alive at the time of urinalysis, 1000 mL of water was put into a 1000-mL polyethylene container for each animal, and the amount of water that remained was measured (by weight and recorded by volume) at 08:40-09:44 on the following day to calculate daily intake. During the measurement of water intake, the automatic water supply system was stopped. For the animal (No. 3001) for

which contamination of the urine collected in Week 13 of administration with drinking water was suspected and for the animal (No. 2001) which took all the water given and thus correct water intake could not be measured, the amount of water intake was measured again and urinalysis was done again using the urine re-collected.

6.13.5 Ophthalmological Examination

For all animals, using the equipments listed in Text Table 3, each item was examined macroscopically, or using a slit lamp, binocular indirect ophthalmoscope or fundus camera. Light reflex test was done first, and the anterior portion and fundus of the eye were examined after application of Mydrin® P (Lot Nos. MP0932, MP0940, MP0935, Santen Pharmaceutical Co., Ltd.), a mydriatic agent, to the eyes. Fundus oculi was photographed for all animals before the start of administration and only for one animal (No. 2002) in Week 25 of administration. For this animal, fundus oculi was also examined and photographed in Week 26 of administration to examine progress of the changes.

Text Table 3. Ophthalmological Examinations

Examination	Equipment	Items
Macroscopic observation	Pen light	Macroscopic examination of external appearance of the eyes
Examination of anterior portion, optic media and fundus oculi	Binocular indirect ophthalmoscope [a] and slit lamp [b]	Observation of cornea, conjunctiva, lens, iris, vitreous body and fundus oculi
Photography of fundus oculi	Fundus camera [c]	Photography of fundus oculi
Instruments used a): Omega 200, HEINE OPTOTECHNIK, Germany b): Slitlamp (SL-14, Kowa Co., Ltd.) c): Fundus camera (Kowa GENESIS, Kowa Co., Ltd.)		

6.13.6 Electrocardiography

For all animals, items listed in Text Table 4 were recorded and calculated. During the administration period, the examination after dosing was performed in such a way that the examination time was approximately the same time for each group.

Text Table 4. Electrocardiography

Recording conditions	Position of animals etc.: Unanesthetized, right recumbent position, standard limb lead Equipment used: Electrocardiograph (LABO-SYSTEM ZM-5012: Fukuda M.E. Co., Ltd.)
Items calculated	Heart rate, P-R, Q-T and QRS intervals. QTc: Q-T interval (s) / $\sqrt{\text{R-R interval (s)}}$ × 1,000

6.13.7 Urinalysis

For all animals, fresh urine and cumulative urine were collected and items listed in Text Table 5 were examined by the method described in the same table. A urine collector was placed under each cage and fresh urine (comparatively fresh urine after urination) was collected between 08:50 and 13:20 before dosing during the administration period under deprivation of feed and drinking water. Cumulative urine was collected thereafter for approximately 20 hours from 13:00 (after dosing during the administration period) to 09:20 next morning with free access to feed and drinking water. At the same time as for cumulative urine collection, water intake was measured.

Text Table 5. Items, Methods and Equipment in Urinalysis

1) Examination on fresh urine		2) Examination on cumulative urine	
Item	Method	Item	Method
pH protein glucose ketones occult blood urobilinogen bilirubin	Multistix Test Paper (Bayer Medical Ltd., Lot Nos. 5K11C, 6B10C, 6C15D, 6H19C, 6L09D)	urine volume	measuring cylinder (Unit: mL)
sediment	microscopic examination	sodium potassium chloride	ion selective electrode method[b] (Unit: mmol/20h)
specific gravity	refractometry[a]		
color	macroscopic examination		
Equipment used a): Clinical Refractometer (Erma Inc.) b): Clinical Laboratory System TBA-120 FR (Toshiba Corporation)			

For the animal (No. 2001) which took all the water given and thus accurate water intake could not be measured and for the animal (No. 3001) for which contamination of the urine collected with drinking water was suspected in Week 13 of administration, the amount of water intake was measured again and urinalysis was done again using the re-collected urine. The data of re-urinalysis were adopted since they were all within the range of the background data of the testing facility. For the animals that showed occult blood (± to ++) or protein (++) in the urinalysis of fresh urine in Week –2 of administration and Week 13 of administration, re-examination was done (2 to 4 times) for confirmation. However, since the values obtained in the re-examination were similar to the initial values and the initial data were adopted, the data obtained in the re-examinations were regarded as supporting data.

6.13.8 Hematological Examination

After deprivation of feed for at least 16 hours from the previous day, blood samples (approximately 2 mL) were collected via the cephalic vein of all animals. Blood samples (approximately 1 mL) that were put into blood collecting tubes containing EDTA-2K (SB-41: Sysmex Corporation, Lot Nos. G5090, G6002, G6040) and items listed in Text Table 6-1) were measured by the method specified in the same table. Blood smear specimens were prepared by the May-Gruenwald-Giemsa staining method for all animals. In addition, blood samples (approximately 1 mL) that were collected into blood collecting tubes containing 3.8 w/v% sodium citrate solution (volume ratio of blood to citrate solution = 9:1) were centrifuged (approximately 1,600 × g, 10 minutes) and plasma samples were examined for items listed in Text Table 6-2) by the method specified in the same table. For moribund animals (Nos. 4102, 4106), blood was collected in the same manner and examined, but these animals were not fasted before blood collection.

Text Table 6. Items, Methods and Equipment for Hematological Examinations

1) Examination on EDTA-2K treated blood samples		
Item	Method	Unit
red blood cell count (RBC)	dual-laser flow cytometry[a)]	10^4/μL
hemoglobin (Hb)	modified cyanmethemoglobin method[a)]	g/dL
hematocrit (Ht)	calculated from red blood cell count and mean corpuscular volume[a)]	%
mean corpuscular volume (MCV)	dual-laser flow cytometry[a)]	fL
mean corpuscular hemoglobin (MCH)	calculated from red blood cell count and hemoglobin[a)]	pg
mean corpuscular hemoglobin concentration (MCHC)	calculated from hematocrit and hemoglobin[a)]	g/dL
reticulocyte percentage (Reticulocyte) and count	laser flow cytometry by RNA staining[a)]	%, 10^4/μL
platelet count (Platelet)	dual-laser flow cytometry[a)]	10^4/μL
white blood cell count (WBC)	dual-laser flow cytometry[a)]	10^2/μL
differential white blood cell percentage and differential white blood cell count	flow cytometry by peroxidase staining + dual-laser flow cytometry[a)]	%, 10^2/μL
2) Examination on plasma samples separated from sodium citrate-treated blood samples		
Item	Method	Unit
prothrombin time (PT)	clot method[b)]	s
activated partial thromboplastin time (APTT)	clot method[b)]	s
fibrinogen	thromboplastin method[b)]	mg/dL
Equipment used		
a): Advia 120 Hematology System (Bayer Corporation, New York, USA)		
b): Coagulometer ACL 100 (Instrumentation Laboratory)		

White blood cells were classified as monocytes, neutrophils, eosinophils, basophils, lymphocytes and large unstained cells.

6.13.9 Blood Chemistry Examination

Deleted: (b) (4)

Portions of blood samples (approximately 4 mL) that were collected for hematology were put into test tubes, and allowed to stand at room temperature. The serum samples obtained by centrifugation (approximately 1,600 × g, 10 minutes) were examined for items listed in Text Table 7-1) by the method specified in the same table. Blood samples (approximately 2 mL) that were collected into blood collecting tubes containing heparin (approximately 20 units of heparin per 1 mL blood, Heparin sodium for injection "Simizu", Ajinomoto Pharma Co., Ltd., Lot No. 40231) were centrifuged (approximately 1,600 × g, 10 minutes) and the plasma samples obtained were examined for items listed in Text Table 7-2) by the method specified in the same table. For moribund animals, examination was done as far as possible. Fractions for protein electrophoresis were evaluated as _ ($_1$+$_2$+$_3$), _ ($_1$+$_2$), and _. The serum samples remaining after examination were sent to the Sponsor in a freezing condition for their use.

Text Table 7. Items, Methods and Equipment for Blood Chemistry Examinations

1) Examination on sera separated after standing at room temperature		
Item	Method	Unit
ALP	Bessey-Lowry method [a)]	IU/L
total cholesterol (CHO)	CEH-COD-POD method [a)]	mg/dL
triglyceride (TG)	LPL-GK-GPO-POD method [a)]	mg/dL
total bilirubin (BIL)	bilirubin oxidase method [a)]	mg/dL
glucose (GLU)	glucose dehydrogenase method [a)]	mg/dL
blood urea nitrogen (BUN)	urease-LEDH method [a)]	mg/dL
creatinine (CRE)	creatininase-creatinase-sarcosine-oxidase-POD method [a)]	mg/dL
sodium (Na)	ion selective electrode method [a)]	mmol/L
potassium (K)	ion selective electrode method [a)]	mmol/L
chloride (Cl)	ion selective electrode method [a)]	mmol/L
calcium (Ca)	OCPC method [a)]	mg/dL
inorganic phosphorus (IP)	molybdic acid method [a)]	mg/dL
total protein (TP)	biuret method [a)]	g/dL
albumin (ALB)	BCG method [a)]	g/dL
A/G ratio	calculated from total protein and albumin	
protein fractions	electrophoresis using cellulose acetate membrane [b)]	%
2) Examination of plasma samples from heparin treated blood sample		
Item	Method	Unit
AST (GOT)	UV-rate method [a)]	IU/L
ALT (GPT)	UV-rate method [a)]	IU/L
LDH	UV-rate method [a)]	IU/L
CPK	UV-rate method [a)]	IU/L
Equipment used a): Clinical Laboratory System TBA-120FR (Toshiba Corporation) b): Cliniscan 2 (K.K. Helena Kenkyujyo)		

6.13.10 Pathological Examinations

1) Necropsy

All animals subjected to necropsy were fasted for at least 16 hours from the previous day. They were sacrificed humanely by exsanguination via the cervical artery under anesthesia by intravenous injection of pentobarbital sodium (30 mg/kg, Tokyo Kasei Kogyo Co., Ltd., Lot No. GM01). They were subjected to necropsy as soon as possible after sacrifice. The organs/tissues weighed and collected for histopathological examination are listed in Text Table 8.

2) Measurement of Organ Weight

For the organs listed in Text Table 8, absolute weight was measured and the weight per body weight (relative weight) was calculated from the animal's terminal body weight and the absolute organ weight. For the paired organs, organ weight was measured separately but evaluation was done on the total weight.

3) Histopathological Examination

The subject organs/tissues are shown in Text Table 8. For all animals, the organs and tissues were removed and fixed in phosphate buffered 10 vol% formalin. However, the eyeballs and optic nerves were fixed in phosphate buffered 3 vol% glutaraldehyde/2.5 vol% formalin and the testes were fixed in Bouin's solution and these organs/tissues were preserved in phosphate buffered 10 vol% formalin. All the organs/tissues of all animals were embedded in paraffin, and sections were prepared, stained with hematoxylin/eosin (H&E) and subjected to microscopy.

Text Table 8. List of Organs/tissues for Pathological Examination

Organ/tissue	Histopathology H&E	Organ weight
cerebrum	√	√ (together)
cerebellum	√	
medulla oblongata	√	
spinal cord	√	
optic nerve	√	
sciatic nerve	√	
eyeball	√	
lacrimal gland	√	
pituitary	√	√
thyroid	√	√
parathyroid	√	
adrenal	√	√
thymus	√	√
spleen	√	√
submandibular lymph node	√	
mesenteric lymph node	√	
heart	√	√
aorta (aortic arch)	√	
larynx	√	
trachea	√	
lung (including bronchus)	√	√
tongue	√	
esophagus	√	
stomach	√	
duodenum	√	
jejunum	√	
ileum	√	
cecum	√	
colon	√	
rectum	√	
submandibular gland	√	√
sublingual gland	√	
parotid gland	√	
liver	√	√ (together)
gallbladder	√	
pancreas	√	√
kidney	√	√
ureter	√	
urinary bladder	√	
testis/ovary	√ / √	√ / √
epididymis/uterus	√ / √	√ / √
prostate/vagina	√ / √	√ /
mammary gland	√	
sternum (including bone marrow)	√	
femur (including bone marrow)	√	
femoral skeletal muscle	√	
skin (abdominal region)	√	
part for individual identification (ear auricle)	(preservation only)	

Adequate Battery: yes (x), no ( )—explain

Peer review: yes (), no (x)

Results:

Mortality:

Clinical signs:

Body weights:

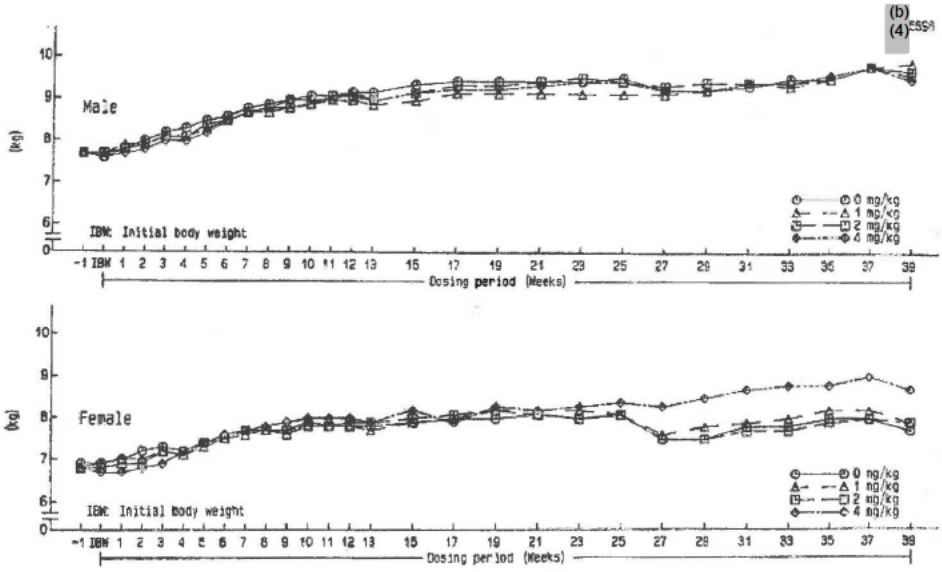

Fig.1 39-week repeated dose oral toxicity study of KS-304 in dogs
Body weight changes

Food consumption:

(b) (4)

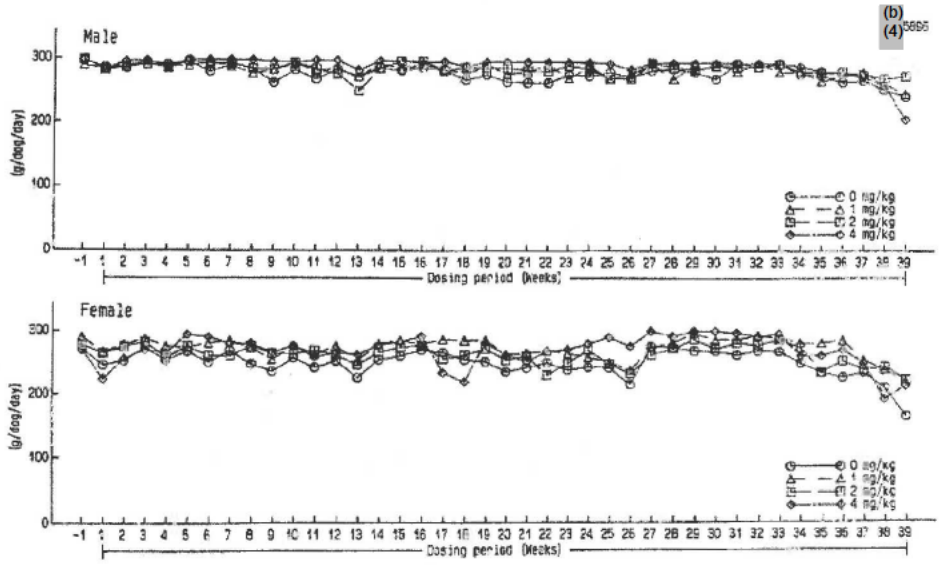

Fig.2 39-week repeated dose oral toxicity study of NS-304 in dogs
Food consumption changes

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )

Toxicokinetics:

Text Table 9-1 TK Parameters of NS-304 in Plasma (Mean Values)

Dose (mg/kg)	Week		Male/Female C_{max} (μg/mL)	T_{max} (h)	AUC_{0-24h} (μg·h/mL)
1	Day 1		0.977 / 1.53	1.1 / 1.3	2.55 / 3.36
	13W	4h	0.165 / 0.226		
		24h	0 / 0		
	26W		1.83 / 0.674	2.2 / 1.8	3.49 / 2.85
	39W		1.03 / 1.36	1.2 / 1.5	2.55 / 2.88
2	Day 1		2.55 / 2.87	2.3 / 1.6	8.44 / 7.30
	13W	4h	0.946 / 0.726		
		24h	0 / 0		
	26W		4.50 / 3.26	1.8 / 1.5	10.2 / 9.15
	39W		3.10 / 1.79	1.7 / 2.7	8.15 / 6.54
4	Day 1		5.77 / 5.21	1.3 / 1.4	17.4 / 17.6
	13W	4h	1.53 / 1.36		
		24h	0 / 0		
	26W		5.66 / 5.03	1.7 / 2.0	18.1 / 16.2
	39W		8.62 / 6.88	2.0 / 2.0	21.3 / 18.0

Text Table 9-2 TK Parameters of MRE-269 in Plasma (Mean Values)

Dose (mg/kg)	Week		Male/Female C_{max} (μg/mL)	T_{max} (h)	AUC_{0-24h} (μg·h/mL)
1	Day 1		2.50 / 3.05	3.7 / 2.7	24.6 / 33.2
	13W	4h	2.75 / 2.06		
		24h	0.154 / 0.213		
	26W		3.20 / 2.52	3.7 / 4.3	28.8 / 24.6
	39W		2.60 / 3.26	2.7 / 3.3	23.1 / 35.7
2	Day 1		5.27 / 5.56	4.0 / 3.7	49.9 / 54.0
	13W	4h	4.92 / 3.45		
		24h	0.239 / 0.238		
	26W		6.40 / 6.13	3.3 / 3.3	55.8 / 51.2
	39W		5.26 / 3.87	3.5 / 4.0	40.5 / 58.9
4	Day 1		9.17 / 8.62	3.2 / 4.0	94.4 / 104
	13W	4h	6.60 / 3.87		
		24h	0.380 / 0.548		
	26W		6.89 / 10.4	3.0 / 3.5	58.4 / 90.8
	39W		9.54 / 12.5	2.7 / 4.0	106 / 120

Other:

Study title: Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice

Key study findings: Study was primarily to determine doses for the carcinogenicity study. Doses of 0, 100, 300, and 500 mg/kg/day were administered. The top dose of 500 mg/kg/day had one animal die, and liver and kidney effects after only 13 weeks. That may be too high a dose for a 104 week carcinogenicity study, leading to loss of the high dose group.

Study no.: T-08.292
Volume #, and page #: eCTD 4.2.3.2
Conducting laboratory and location: (b) (4)

Date of study initiation: Oct. 24, 2006
GLP compliance: Yes
QA report: yes (x) no ( )

Drug, lot #, and % purity: NS-304, lot # 20, 100.3% pure

Methods

Species/strain: B6C3F1/Crlj SPF mice
Route, formulation, volume, and infusion rate: Oral Gavage
Age: 5 weeks of age
Weight:

group, respectively, to evaluate toxicity and systemic exposure. Individual body weight on the starting day of administration ranged from 21.1 to 24.8 g in males and 17.2 to 20.3 g in females for the main group, and that for the satellite group ranged from 21.2 to 25.4 g in males and 17.2 to 21.3 g in females.

Sampling times: see observation & times

Text Table 1. Group composition

Test group	Dose (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg/day)	Sex	Main group		Satellite group	
					No. of animals	Animal No.	No. of animals	Animal No.
Control	0	0	10	M	10	1001-1010	20	1201-1220
				F	10	1101-1110	20	1301-1320
Low	100	10	10	M	10	2001-2010	42	2201-2242
				F	10	2101-2110	42	2301-2342
Middle	300	30	10	M	10	3001-3010	42	3201-3242
				F	10	3101-3110	42	3301-3342
High	500	50	10	M	10	4001-4010	42	4201-4242
				F	10	4101-4110	42	4301-4342

M: Male, F: Female

Observation and Times: (this information can be provided in a separate section OR evaluation times can be described for each parameter in the results section).

Clinical signs:

All animals were observed for clinical signs including external appearance, nutritional condition, posture, behavior and excretions 3 times a day (before, immediately after and approximately 2 hours after dosing; however twice on Saturdays, Sundays and holidays: before and immediately after dosing) during the administration period. In addition, the observation of 2 hours after dosing was done at days 3 (Saturday) and 4 (Sunday).

Body weights:

All animals were weighed twice in week 1 (days 1 and 7) of administration, and once a week every 7 days thereafter. Measurement was done before administration between 08:37 and 10:23. In order to calculate the relative organ weight, the body weight was also recorded on the day of necropsy (not fasted).

Food consumption:

Food consumption was measured for each animal twice in week 1 (days 1 and 7) of administration, and once a week every 7 days thereafter. Measurement was done between 08:50 and 11:12. On day 1 of administration, one day's food consumption was measured from the day before the start of administration and that on day 7 was calculated from 6-day's cumulative food consumption. Thereafter, 7-day's cumulative consumption was measured and one day's food consumption was calculated.

Ophthalmoscopy:

Before the start of administration (during the quarantine/acclimatization period, 3 days before administration), all candidate animals for main group were examined, and the animals with ophthalmological abnormalities that might affect the toxicity evaluation were excluded from animal grouping for the main group (note).

In month 3 of administration (week 13, day 85), all animals of each sex in the control and 500 mg/kg groups were examined after dosing on the day of examination. The examination in the 100 and 300 mg/kg groups were not done, since no treatment-related changes were observed in the 500 mg/kg group. The procedure for examination was as follows.

The mydriatic agent (Mydrin® P: Santen Pharmaceutical Co., Ltd., Lot No. MP0947) was applied to dilate the pupil and the anterior portion, transparent body (optic media) and fundus oculi were examined an ophthalmoscope (Omega 200: HEINE OPTOTECNIK GmbH & Co. KG, Germany).

note: Two males and one female with severe ophthalmological abnormalities such as focal opacity in lens, persistent tunica vasculosa lentis and myelinated nerve fiber were excluded from the present study.

EKG: not done

Hematology:

At the time of necropsy on the day following the end of the administration period, blood samples were collected from the vena cava inferior of all animals (not fasted) using syringes treated with heparin sodium under ether anesthesia into blood collection tubes (approximately 0.35 mL) containing an anticoagulant (EDTA-2K, Microtainer® Tube: Japan Becton Dickinson Inc.). The following parameters were determined. May-Gruenwald-Giemsa staining smears from all animals were prepared as reserve in the case of microscopic examination. (Ultimately, the microscopic examination was not conducted.)

Text Table 2. Items, Methods and Equipment for Hematological Examinations

Item	Method	Unit
red blood cell count (RBC)	dual angle laser flow-cytometric measurement[a)]	10^4/μL
hemoglobin (Hb)	modified cyanmethemoglobin method[a)]	g/dL
hematocrit (HCT)	calculated from mean corpuscular volume and red blood cell count[a)]	%
mean corpuscular volume (MCV)	dual angle laser flow-cytometric measurement[a)]	fL
mean corpuscular hemoglobin (MCH)	calculated from red blood cell count and hemoglobin[a)]	pg
mean corpuscular hemoglobin concentration (MCHC)	calculated from hematocrit and hemoglobin[a)]	g/dL
reticulocyte ratio (Reticul.)	laser flow-cytometric measurement with RNA stain[a)]	%
platelet count (PLT)	dual angle laser flow-cytometric measurement[a)]	10^4/μL
white blood cell count (WBC)	dual angle laser flow-cytometric measurement[a)]	10^2/μL
differential leukocyte count (note)	peroxidase flow-cytometric measurement and dual angle laser flow-cytometric measurement[a)]	%

Equipment used

a): ADVIA® 120 Hematology System (Bayer Corporation, New York, USA)

note: Lymphocytes (LYM), neutrophils (NE), eosinophils (EOSINO), basophils (BASO), monocytes (MONO) and large unstained cells (LUC)

Clinical chemistry:

At the same time as hematology, blood samples were collected from the vena cava inferior into blood collection tubes (approximately 0.45 or 0.55 mL) containing anticoagulant heparin lithium (Capiject®: Capillary blood collection tubes, Terumo Corporation). The plasma samples were obtained by centrifugation (set at 3,100 rpm, approximately 1,690×g for 12 minutes) and the following items were determined.

Text Table 3. Items, Methods and Equipment for Blood chemistry Examinations

Item	Method	Unit
AST	UV-rate method[a)]	IU/L
ALT	UV-rate method[a)]	IU/L
ALP	Bessey-Lowry method[a)]	IU/L
CK	UV-rate method[a)]	IU/L
total cholesterol (T-CHO)	CEH-COD-POD method[a)]	mg/dL
triglyceride (TG)	LPL-GK-GPO-POD method[a)]	mg/dL
total bilirubin (T-BIL)	bilirubin oxidase method[a)]	mg/dL
glucose (GLU)	glucose dehydrogenase method[a)]	mg/dL
blood urea nitrogen (BUN)	urease-LEDH method[a)]	mg/dL
creatinine (CRNN)	creatininase-creatinase-sarcosine oxidase-POD method[a)]	mg/dL
sodium (Na)	ion selective electrode method[a)]	mmol/L
potassium (K)	ion selective electrode method[a)]	mmol/L
chloride (Cl)	ion selective electrode method[a)]	mmol/L
calcium (Ca)	OCPC method[a)]	mg/dL
inorganic phosphorus (P)	molybdic acid method[a)]	mg/dL
total protein (TP)	Biuret method[a)]	g/dL
albumin (ALB)	BCG method[a)]	g/dL

Equipment used

a): Toshiba Biochemical Analyzer Model TBA-120FR (Toshiba Corp.)

Urinalysis: not done

Gross pathology:

After collecting blood samples, all animals were sacrificed by exsanguination from the abdominal aorta under ether anesthesia. External appearance and all the organs/tissues in the cranial, thoracic and abdominal cavities were carefully examined and the results were recorded.

Organ weights (specify organs weighed if not in histopath table):

After necropsy, the organs listed below of all animals were weighed (absolute weight) and organ weight per 100 g body weight (relative weight) was calculated based on the animal's body weight (not fasted) and absolute organ weight. The paired organs indicated by asterisks (*) were weighed separately; however, evaluation was done on the total value of the right and left organs.

brain, spleen, heart, lungs (including bronchus), salivary glands (submandibular + sublingual glands)*, liver (including gall bladder), kidneys*, testes* and uterus

Histopathology:

All the organs/tissues listed below of all animals were fixed and preserved in phosphate buffered 10 vol% formalin. However, the eyeballs and optic nerves were fixed with a

mixture containing 3 w/v% glutaraldehyde and 2.5 vol% formalin, and the testes and epididymides were fixed with Bouin's solution, and then preserved in phosphate buffered 10 vol% formalin. The organs/tissues marked with # listed below of all animals were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H.E). Of these, all organs/tissues from the control and the high dose groups were examined histopathologically. In addition, sections of the liver in both sexes and kidney in males from all other groups were subjected to histopathological examination, since treatment-related lesions were suspected in these organs. In addition, the mammary gland in females from all groups was observed, since treatment-related changes were observed in a 26-week oral gavage toxicity study of NS-304 in rats with 4-week recovery period [redacted] (b) (4) Study No. (b) (4) 5895)[3]. Ultimately, no treatment-related changes were observed in mammary gland in the present study. Other than the above, the animal that was found dead on day 3 in the 500 mg/kg group (No. 4328, satellite group) was necropsied and all the organs/tissues listed below were fixed, preserved and examined histopathologically to presume the cause of death, since no treatment-related deaths were observed in the main or satellite groups during the administration period except for this animal. The paired organs indicated by asterisks (*) were examined unilaterally; however, these were preserved bilaterally.

cerebrum, cerebellum, spinal cord (cervical, thoracic and lumbar), sciatic nerves*, eyeballs#*, optic nerves#*, Harderian glands*, pituitary#, thyroids#*, parathyroids*, adrenals#*, thymus#, spleen#, submandibular lymph node, mesenteric lymph node, heart#, thoracic aorta, trachea, lungs (including bronchus)#, tongue, esophagus, stomach#, duodenum#, jejunum, ileum, cecum#, colon, rectum, submandibular glands#*, sublingual glands#*, parotid glands*, liver#, gallbladder#, pancreas, kidneys#*, urinary bladder#, testes#*, epididymides*, prostate, seminal vesicles*, ovaries#*, uterus#*, vagina, mammary glands (inguinal region, females only)#*, sternum (including bone marrow), femurs (including bone marrow)#*, femoral muscles*, skin (inguinal region)*, oviducts*, extraorbital lacrimal glands*, Zymbal's glands*, larynx, nasal cavity, preputial glands*, clitoral glands* and gross lesions (note)

Besides the organs/tissues listed above, the site of animal identification (ear auricle) was preserved.

note: Gross lesions were observed only in the animal that was found dead in the satellite group.

Adequate Battery: yes (x), no ( )—explain

Peer review: yes ( ), no ( )

Results:

Mortality: one female in the high dose group died of treatment related causes on treatment day 3, but this individual was in the toxicokinetics group. One male died in the low dose group, but it was not thought to be due to treatment.

Clinical signs:

(b) (4)

Table 1 Thirteen-week oral gavage range-finding toxicity study of Na-304 in mice
Clinical signs

Sex	Dose mg/kg/day	Findings	Week of administration												
			1	2	3	4	5	6	7	8	9	10	11	12	13
Male	0	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	10	10	10	10	10	10	10	10	10	10	10	10	10
	100	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	10	10	10	10	10	10	10	10	10	10	10	10	10
	300	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	0	9	6	6	7	5	5	5	6	4	5	0	4
		Flaccidity	7	0	0	0	0	0	0	0	0	0	0	0	0
		Flush(limbs)	10	1	2	4	3	5	5	5	4	6	5	4	6
	500	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	0	2	1	2	1	1	1	0	2	1	1	0	0
		Flaccidity	10	0	0	0	0	0	0	0	0	0	0	0	0
		Flush(limbs)	10	8	9	8	9	9	9	10	8	9	9	10	10
		Creeping position	1	0	0	0	0	0	0	0	0	0	0	0	0
Female	0	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	10	10	10	10	10	10	10	10	10	10	10	10	10
	100	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	10	10	10	10	10	10	10	10	10	10	10	10	10
	300	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	2	6	6	2	3	2	2	4	4	1	3	3	4
		Flaccidity	6	0	0	0	0	0	0	0	0	0	0	0	0
		Flush(limbs)	6	4	4	8	7	8	8	6	6	9	7	7	6
	500	No. of animals	10	10	10	10	10	10	10	10	10	10	10	10	10
		No abnormality	0	1	1	2	1	1	0	1	1	0	1	1	3
		Flaccidity	10	0	0	0	0	0	0	0	0	0	0	0	0
		Flush(limbs)	10	9	9	8	9	9	10	9	9	10	9	9	7
		Creeping position	2	0	0	0	0	0	0	0	0	0	0	0	0
		Eye discharge	2	0	0	0	0	0	0	0	0	0	0	0	0

Body weights:

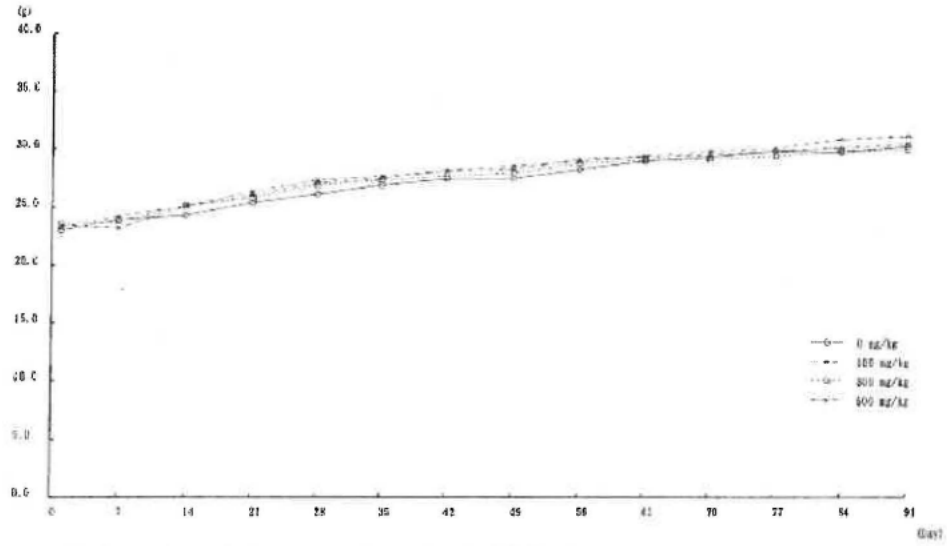

Fig. 1 Thirteen week oral gavage range-finding toxicity study of [illegible] in mice
Body Weight - Male

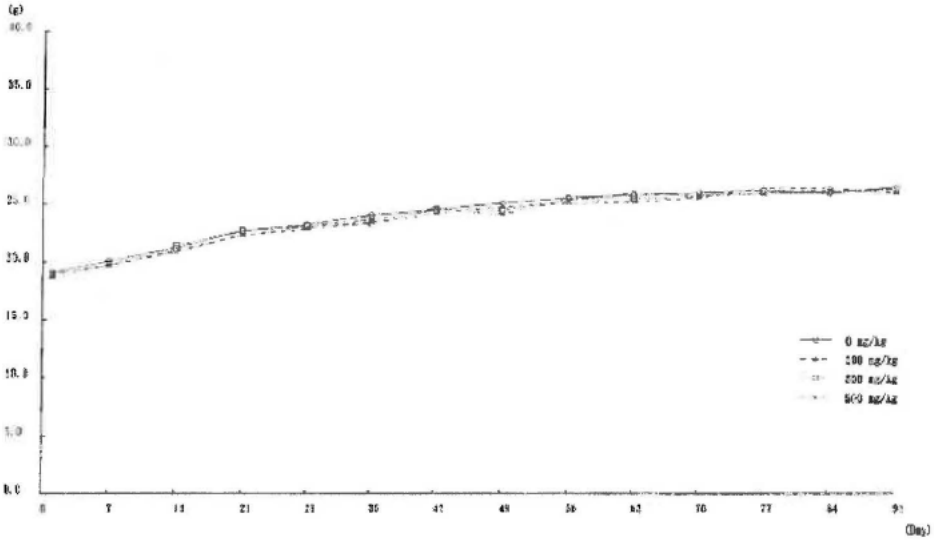

Fig. 2 Thirteen week oral gavage range-finding toxicity study of [illegible] in mice
Body Weight - Female

Food consumption:

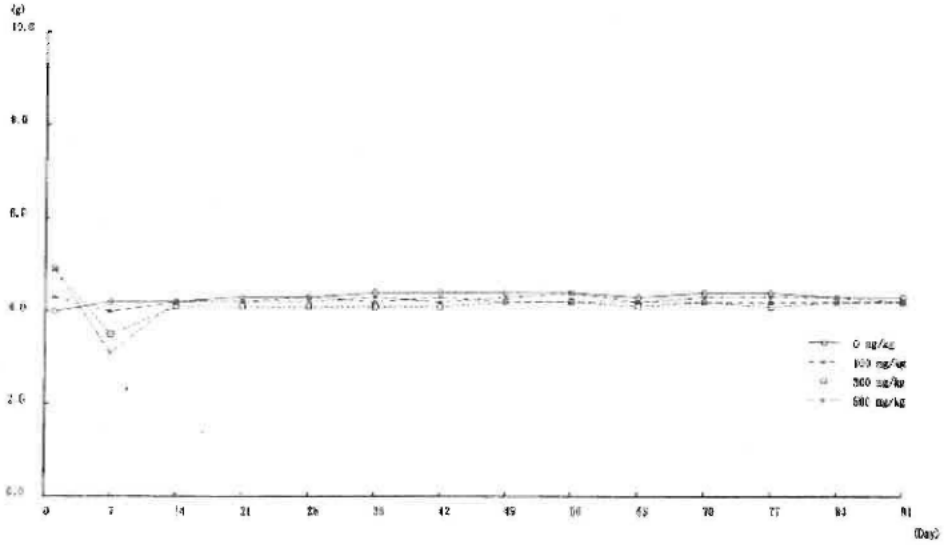

Fig. 3 Thirteen-week oral gavage range-finding toxicity study of KS-304 in mice
Food Consumption - Male

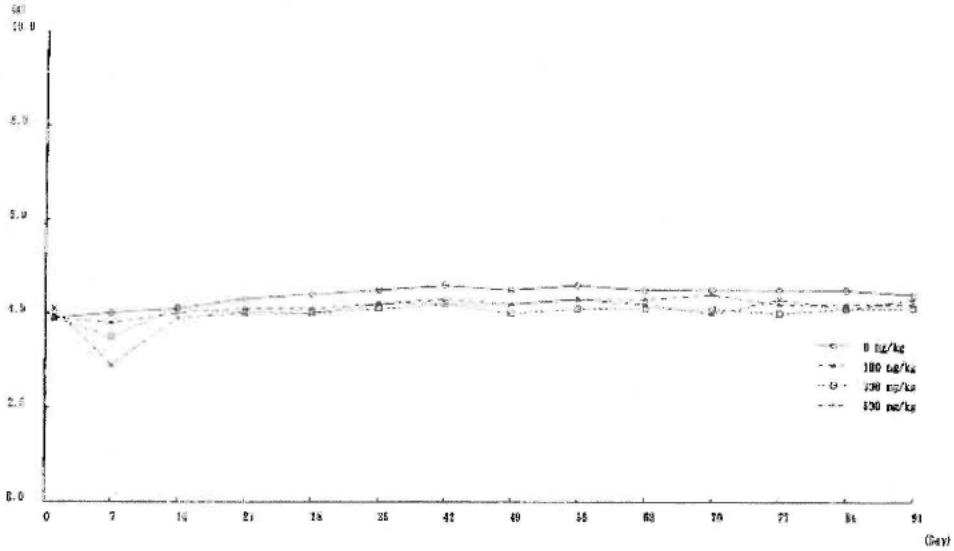

Fig. 4 Thirteen-week oral gavage range-finding toxicity study of KS-304 in mice
Food Consumption - Female

Ophthalmoscopy: no abnormalities reported

EKG: not done

Hematology:

Table 5 - 1 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice (b)(4)-938
Item : Hematology Stage : Week 13
Sex : Male

Test Article Dose		RBC 10E4/uL	HB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL
NS-304 0 mg/kg	Mean	979	15.7	44.2	45.1	16.0	35.5
	S.D.	27	0.5	1.4	0.3	0.1	0.4
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	997	16.2	45.8	45.9D2**	16.2D2**	35.4
	S.D.	43	0.6	1.8	0.5	0.2	0.2
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	1013	16.6D2**	46.8D2**	46.2D2**	16.4D2**	35.5
	S.D.	45	0.8	2.3	0.6	0.1	0.4
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	1009	16.5D2*	46.7D2*	46.3D2**	16.4D2**	35.4
	S.D.	35	0.6	1.8	0.5	0.2	0.2
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side

Table 5 - 4 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice (b)(4)-938
Item : Hematology Stage : Week 13
Sex : Female

Test Article Dose		RBC 10E4/uL	HB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL
NS-304 0 mg/kg	Mean	980	16.1	45.4	45.8	16.2	35.4
	S.D.	27	0.4	1.0	0.5	0.2	0.6
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	1018	16.9D2**	48.0D2**	47.1D2**	16.6D2**	35.3
	S.D.	42	0.7	1.8	0.5	0.1	0.2
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	1040D2**	17.1D2**	48.8D2**	46.9D2**	16.5D2*	35.1
	S.D.	36	0.6	1.6	0.5	0.1	0.4
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	1040D2**	17.3D2**	49.4D2**	47.6D2**	16.5D2*	35.0
	S.D.	34	0.5	1.4	0.4	0.2	0.5
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side

Table 5 - 5 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice (b)(4)-939
Item : Hematology Stage : Week 13
Sex : Female

Test Article Dose		Retirulocyte %	PLT 10E4/uL	WBC 10E2/uL	LYM %	NE %	EOSINO %
NS-304 0 mg/kg	Mean	2.5	105.8	22.0	83.0	15.5	1.3
	S.D.	0.5	5.0	9.3	4.3	4.6	0.7
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	2.4	110.4	18.4	84.8	12.2	1.3
	S.D.	0.3	3.5	8.4	3.8	4.1	1.0
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	2.6	118.0D2**	23.5	82.2	14.9	1.2
	S.D.	0.2	3.8	7.4	5.1	5.6	0.9
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	2.0D2*	116.3D2**	29.5	85.1	12.8	0.8
	S.D.	0.3	5.8	16.1	1.9	2.2	0.2
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side

Clinical chemistry:

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(b)(4)-938

Table 6 - 1 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Blood Chemistry Stage : Week 13
Sex : Male

Test Article Dose		AST IU/L	ALT IU/L	CK IU/L	ALP IU/L	T-CHO mg/dL	TG mg/dL
NS-304 0 mg/kg	Mean	41	25	37	259	100	60
	S.D.	2	3	15	17	9	13
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	42	24	66	262	112D2*	54
	S.D.	4	2	41	20	10	23
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	44	24	93	269	121D2**	55
	S.D.	6	2	78	21	10	18
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	42	26	6352*	274	133D2**	67
	S.D.	5	5	29	23	6	15
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side ,S2:Steel Test Two-Side

(b)(4)-938

Table 6 - 2 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Blood Chemistry Stage : Week 13
Sex : Male

Test Article Dose		T-BIL mg/dL	CLT mg/dL	BUN mg/dL	CREN mg/dL	Na mmol/L	K mmol/L
NS-304 0 mg/kg	Mean	0.1	202	29	0.07	154	4.6
	S.D.	0.0	20	5	0.01	1	0.4
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	0.1	196	27	0.08	153	4.8
	S.D.	0.0	12	6	0.01	1	0.4
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	0.1	196	28	0.09	155	4.5
	S.D.	0.0	19	5	0.01	1	0.3
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	0.1	186	2802**	0.09	155	4.4
	S.D.	0.0	17	3	0.01	2	0.2
	n	10	10	10	10	10	10

Significantly different from control : ** P≦0.01
D2:Dunnett Test Two-Side

Table 6 - 4 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice (b)(4)-5938
Item : Blood Chemistry Stage : Week 12
Sex : Female

Test Article Dose		AST IU/L	ALT IU/L	CK IU/L	ALP IU/L	T-CHO mg/dL	TG mg/dL
NS-304 0 mg/kg	Mean	48	23	46	441	98	44
	S.D.	9	3	10	48	11	6
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	47	25	66	439	103	52
	S.D.	7	3	31	34	9	20
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	49	26	53	434	115D2**	49
	S.D.	11	6	17	48	11	10
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	58	41S2*	51	440	136D2**	58
	S.D.	24	23	22	36	9	17
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side ,S2:Steel Test Two-Side

Table 6 - 5 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice (b)(4)-5938
Item : Blood Chemistry Stage : Week 12
Sex : Female

Test Article Dose		T-BIL mg/dL	GLU mg/dL	BUN mg/dL	CRNN mg/dL	Na mmol/L	K mmol/L
NS-304 0 mg/kg	Mean	0.1	192	18	0.05	154	4.1
	S.D.	0.0	16	3	0.02	1	0.6
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	0.1	202	16	0.11	155	4.1
	S.D.	0.0	11	2	0.01	1	0.4
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	0.1	210	15D2**	0.11	154	4.6
	S.D.	0.0	13	2	0.01	3	1.1
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	0.1	198	15D2**	0.11	154	4.0
	S.D.	0.0	23	1	0.02	2	0.3
	n	10	10	10	10	10	10

Significantly different from control : ** P≦0.01
D2:Dunnett Test Two-Side

Urinalysis: not done

Gross pathology: (b)(4)-5938

Table 8-1 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Gross pathological findings (Used animal, Satellite group)

Organs / Findings	Sex: / Dose (mg/kg/day): / Number:	F 500 1
Stomach		
Focus, dark red, glandular stomach		1
Tongue		
Focus, dark red		1

(b)(4)-5938

Table 8-2 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Gross pathological findings (Survivors, Main group)

Organs / Findings	Sex:	M	M	M	M	F	F	F	F
	Dose (mg/kg/day):	0	100	300	500	0	100	300	500
	Number:	10	10	10	10	10	10	10	10
All tissues									
Not remarkable		10	10	10	10	10	10	10	10

Organ weights (specify organs weighed if not in histopath table):

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Table 7 - 1 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Absolute Organ Weight Stage : Week 13
Sex : Male

Test Article Dose		Body weight g	Brain mg	Salivary gland-RL mg	Heart mg	Lung mg	Liver g
NS-304 0 mg/kg	Mean	30.3	505	201.6	148	152	1.37
	S.D.	1.5	14	16.0	12	10	0.12
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	31.1	513	224.6D3**	141	151	1.39
	S.D.	1.4	11	11.8	10	7	0.14
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	30.1	497	222.1D2**	141	154	1.53D2**
	S.D.	1.2	7	9.3	16	9	0.11
	n	10	10	10	10	10	10
NS-304 600 mg/kg	Mean	30.3	496	225.3D2**	1290D**	155	1.58D2**
	S.D.	0.8	7	11.0	9	10	0.07
	n	10	10	10	10	10	10

Significantly different from control : ** P≦0.01
D2:Dunnett Test Two-Side

(b) (4)-938

Table 7 - 2 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Absolute Organ Weight Stage : Week 13
Sex : Male

Test Article Dose		Spleen mg	Kidney-RL mg	Testis-RL mg
NS-304 0 mg/kg	Mean	63	477	187
	S.D.	7	21	19
	n	10	10	10
NS-304 100 mg/kg	Mean	58	461	194
	S.D.	3	38	15
	n	10	10	10
NS-304 300 mg/kg	Mean	57	419D2**	189
	S.D.	2	24	15
	n	10	10	10
NS-304 600 mg/kg	Mean	59D2*	467D2**	187
	S.D.	3	15	17
	n	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side

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Table 7 - 3 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice
Item : Absolute Organ Weight Stage : Week 13
Sex : Female

Test Article Dose		Body weight g	Brain mg	Salivary gland-RL mg	Heart mg	Lung mg	Liver g
NS-304 0 mg/kg	Mean	36.0	521	121.6	127	142	1.22
	S.D.	0.8	7	8.8	8	8	0.07
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	36.2	518	135.7D2**	124	142	1.26
	S.D.	1.2	12	7.1	7	9	0.10
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	36.0	530D2*	137.8D2**	121	142	1.25D2**
	S.D.	0.8	12	10.0	6	8	0.06
	n	10	10	10	10	10	10
NS-304 600 mg/kg	Mean	36.2	508D2*	140.4D2**	114D2**	147	1.72D2**
	S.D.	0.9	12	8.6	3	10	0.11
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D2:Dunnett Test Two-Side

Table 7 - 4 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice (b)(4)5938
Item : Absolute Organ Weight Stage : Week 13
Sex : Female

Test Article Dose		Spleen mg	Kidney-RL mg	Uterus mg
NS-304 0 mg/kg	Mean	90	330	184
	S.D.	11	12	36
	n	10	10	10
NS-304 100 mg/kg	Mean	730D**	321	148
	S.D.	8	10	25
	n	10	10	10
NS-304 300 mg/kg	Mean	760D**	300D**	137
	S.D.	6	13	32
	n	10	10	10
NS-304 500 mg/kg	Mean	730D**	290D**	141
	S.D.	6	14	36
	n	10	10	10

Significantly different from control : ** P≦0.01
D:Dunnett Test Two-Side

Table 7 - 5 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice (b)(4)5938
Item : Relative Organ Weight Stage : Week 13
Sex : Male

Test Article Dose		Body weight g	Brain mg/100g	Salivary gland-RL mg/100g	Heart mg/100g	Lung mg/100g	Liver g/100g
NS-304 0 mg/kg	Mean	30.3	1672	666.1	486	502	4.53
	S.D.	1.5	107	28.9	37	20	0.35
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	31.1	1681	724.6D*	484	496	4.46
	S.D.	1.4	61	45.4	35	25	0.23
	n	10	10	10	10	10	10
NS-304 300 mg/kg	Mean	30.1	1653	742.9D**	468	512	5.08D**
	S.D.	1.2	69	42.0	32	28	0.34
	n	10	10	10	10	10	10
NS-304 500 mg/kg	Mean	30.5	1625	736.3D**	424D**	509	5.17D**
	S.D.	0.8	50	37.0	32	26	0.21
	n	10	10	10	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D:Dunnett Test Two-Side

Table 7 - 6 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice (b)(4)5938
Item : Relative Organ Weight Stage : Week 13
Sex : Male

Test Article Dose		Spleen mg/100g	Kidney-RL mg/100g	Testis-RL mg/100g
NS-304 0 mg/kg	Mean	294	1377	621
	S.D.	22	65	72
	n	10	10	10
NS-304 100 mg/kg	Mean	157	1462D*	625
	S.D.	12	106	55
	n	10	10	10
NS-304 300 mg/kg	Mean	188	1375D**	636
	S.D.	11	66	46
	n	10	10	10
NS-304 500 mg/kg	Mean	183D*	1325D**	613
	S.D.	16	37	56
	n	10	10	10

Significantly different from control : * P≦0.05, ** P≦0.01
D:Dunnett Test Two-Side

Table 7 - 7 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice (b)(4) 5936
Item : Relative Organ Weight Stage : Week 13
Sex : Female

Test Article Dose		Body weight g	Brain mg/100g	Salivary gland-R. mg/100g	Heart mg/100g	Lung mg/100g	Liver g/100g
NS-304 0 mg/kg	Mean	26.0	2036	467.4	469	548	4.80
	S.D.	0.8	74	31.8	36	22	0.26
	n	10	10	10	10	10	10
NS-304 100 mg/kg	Mean	26.2	1981	518.4D2**	473	544	4.80
	S.D.	1.3	75	23.5	23	22	0.23
	n	10	10	10	10	10	10
NS-304 200 mg/kg	Mean	26.0	1902	529.7D2**	466	521	5.2D2**
	S.D.	0.6	69	41.5	21	36	0.23
	n	10	10	10	10	10	10
NS-304 400 mg/kg	Mean	26.2	1924	533.7D2**	437D2**	501	6.4D2**
	S.D.	0.9	51	22.8	28	21	0.21
	n	10	10	10	10	10	10

Significantly different from control : ** P≦0.01
D2:Dunnett Test Two-Side

Table 7 - 8 Thirteen-week oral gavage range-finding toxicity study of NS-304 in mice (b)(4) 5936
Item : Relative Organ Weight Stage : Week 13
Sex : Female

Test Article Dose		Spleen mg/100g	Kidney-R. mg/100g	Uterus mg/100g
NS-304 0 mg/kg	Mean	340	1206	892
	S.D.	49	48	147
	n	10	10	10
NS-304 100 mg/kg	Mean	2875D2**	1224	927
	S.D.	29	54	110
	n	10	10	10
NS-304 200 mg/kg	Mean	2922D2**	1176D2**	825
	S.D.	26	44	126
	n	10	10	10
NS-304 400 mg/kg	Mean	2769D2**	1166D2**	905
	S.D.	23	27	127
	n	10	10	10

Significantly different from control : ** P≦0.01
D2:Dunnett Test Two-Side

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

Table 9-2 Thirteen-week oral gavage range-finding toxicity study of NS-304 in (b)(4) Histopathological findings (Survivors, Main group)

Organs / Findings	Sex: M	M	M	M	F	F	F	F
Dose(mg/kg/day):	0	100	300	500	0	100	300	500
Number:	10	10	10	10	10	10	10	10
Adrenal								
Number examined	10	0	0	10	10	0	0	10
Hyperplasia, zirconular cell	1	0	0	1	10	0	0	9
minimal	1	0	0	1	10	0	0	9
Eye								
Number examined	10	0	0	10	10	0	0	10
Dysplasia, retinal	0	0	0	0	1	0	0	0
minimal	0	0	0	0	1	0	0	0
Kidney								
Number examined	10	10	10	10	10	0	0	10
Vacuolation, tubular cell	8	5	0	0	0	0	0	0
minimal	5	5	0	0	0	0	0	0
mild	3	0	0	0	0	0	0	0
Regeneration, tubular	0	0	0	0	2	0	0	4
minimal	0	0	0	0	2	0	0	4
Urinary cast, hyaline	1	1	0	0	2	0	0	3
minimal	1	1	0	0	2	0	0	3
Cell infiltration	1	0	0	0	2	0	0	0
minimal	1	0	0	0	2	0	0	0
Liver								
Number examined	10	10	10	10	10	10	10	10
Necrosis, focal	0	0	0	0	1	0	0	0
minimal	0	0	0	0	1	0	0	0
Microgranuloma	1	3	0	1	4	3	5	5
minimal	1	3	0	1	4	3	5	5
Hypertrophy, hepatocyte, centrilobular	0	0	6	10	0	0	6	10
minimal	0	0	6	2	0	0	6	2
mild	0	0	0	8	0	0	0	8
Lung(bronchus)								
Number examined	10	0	0	10	10	0	0	10
Cell infiltration	0	0	0	0	1	0	0	0
minimal	0	0	0	0	1	0	0	0
Thickening, arterial wall	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Pituitary								
Number examined	10	0	0	10	10	0	0	10
Cyst	1	0	0	0	0	0	0	0
minimal	1	0	0	0	0	0	0	0
Spleen								
Number examined	10	0	0	10	10	0	0	10
Hematopoiesis, extramedullary	0	0	0	0	3	0	0	3
minimal	0	0	0	0	3	0	0	3
Stomach								
Number examined	10	0	0	10	10	0	0	10
Ectopic tissue	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Thymus								
Number examined	10	0	0	10	10	0	0	10
Cyst	0	0	0	1	0	0	0	0
minimal	0	0	0	1	0	0	0	0
Thyroid								
Number examined	10	0	0	10	10	0	0	10
Dilatation, follicular	0	0	0	0	1	0	0	0
mild	0	0	0	0	1	0	0	0
Hyperplasia, C cell, focal	0	0	0	0	1	0	0	0
minimal	0	0	0	0	1	0	0	0
Urinary bladder								
Number examined	10	0	0	10	10	0	0	10
Cell infiltration	2	0	0	0	1	0	0	1
minimal	2	0	0	0	1	0	0	1

Toxicokinetics:

Text Table 5. Summary of TK parameters

Sex	Male (n=3)			Female (n=3)		
Dose (mg/kg/day)	100	300	500	100	300	500
NS-304						
T_{max} (h)						
Day 1	0.5	0.5	8	0.5	0.5	0.5
Week 13	0.5	0.5	2	0.5	0.5	0.5
C_{max} (ng/mL)						
Day 1	9660	26500	21100	12200	35900	45600
Week 13	16900	23200	27300	10800	24900	18800
AUC_{0-24h} (ng·h/mL)						
Day 1	15000	88400	295000	19500	115000	310000
Week 13	12200	52100	90000	10100	44400	74000
MRE-269						
T_{max} (h)						
Day 1	1	1	8	0.5	1	0.5
Week 13	0.5	0.5	2	0.5	0.5	2
C_{max} (ng/mL)						
Day 1	10000	19500	17500	8230	19100	17000
Week 13	11400	12400	20300	7150	13500	12400
AUC_{0-24h} (ng·h/mL)						
Day 1	23600	99200	250000	21400	98800	223000
Week 13	12400	51500	71500	9990	39800	58000

Value in the table (C_{max} and AUC_{0-24h}) indicates the mean value of 3 animals.
T_{max} indicates the time shown C_{max}.

Other:

Histopathology inventory

Study				
Species				
Adrenals				
Aorta				
Bone Marrow smear				
Bone (femur)				
Brain				
Cecum				
Cervix				
Colon				
Duodenum				
Epididymis				
Esophagus				
Eye				
Fallopian tube				
Gall bladder				
Gross lesions				
Harderian gland				
Heart				
Ileum				
Injection site				
Jejunum				

Kidneys				
Lachrymal gland				
Larynx				
Liver				
Lungs				
Lymph nodes, cervical				
Lymph nodes mandibular				
Lymph nodes, mesenteric				
Mammary Gland				
Nasal cavity				
Optic nerves				
Ovaries				
Pancreas				
Parathyroid				
Peripheral nerve				
Pharynx				
Pituitary				
Prostate				
Rectum				
Salivary gland				
Sciatic nerve				
Seminal vesicles				
Skeletal muscle				
Skin				
Spinal cord				
Spleen				
Sternum				
Stomach				
Testes				
Thymus				
Thyroid				
Tongue				
Trachea				
Urinary bladder				
Uterus				
Vagina				
Zymbal gland				

X, histopathology performed ??
*, organ weight obtained ??

2.6.6.4 Genetic toxicology

Study title: Reverse mutation test of NS-304

Key findings: ACT-293987 and ACT-333679 were non-mutagenic under the conditions used in this Ames bacterial assay

Study no.: T-08.279, T-08.278

Volume #, and page #: eCTD
Conducting laboratory and location: Name of facility where the test was conducted: Toxicology Department, Research and Development Division, Nippon Shinyaku Co., Ltd.
Address:
14 Kisshoin-Nishinosho-Monguchi-cho, Minami-ku, Kyoto
Date of study initiation: Study initiation date: 24-Aug-2004
GLP compliance: yes
QA reports: yes ( ) no ( )
Drug, lot #, and % purity: 1.1 Compound code: MRE-269
1.2 Lot number: 9
1.3 Date received: 31-Aug-2004
1.4 Source and responsible person: H. Yamashita, Test Article Management Unit
1.5 Quantity received: 3.00 g
1.6 Characteristics1)
1.6.1 Description: A pale yellow crystalline powder, meeting the specified requirements
1.6.2 Identification (ultraviolet spectrophotometry): MRE-269 proved to meet the specified requirements.
1.6.3 Purity (liquid chromatography): The total area of the peaks (of related substances) other than the peak of MRE-269 was (b) (4)%, thus meeting the specified requirements (not more than (b) (4)%).
Study No.: TX-1298
1.6.4 Assay: The assay value was (b) (4)%, hence meeting the specified requirements (98.0 to 101.0%).

Methods

Strains/species/cell line:

Strain designation	Genetic characteristics
TA100	His⁻, uvrB⁻, rfa, R
TA1535	His⁻, uvrB⁻, rfa
TA98	His⁻, uvrB⁻, rfa, R
TA1537	His⁻, uvrB⁻, rfa
WP2*uvr*A	Trp⁻, uvrA⁻

His⁻ : Histidine-requirement
Trp⁻ : Tryptophan- requirement
uvrB⁻ : Defective of DNA repair system (sensitive to ultraviolet rays)
uvrA⁻ : Defective of DNA repair system (sensitive to ultraviolet rays)
rfa: Membrane mutation (sensitive to crystal violet)
R: Ampicillin-resistant

Doses used in definitive study: see tables below

Basis of dose selection: dosing study done

Negative controls: see tables below

Positive controls: see tables below

Incubation and sampling times: from the sponsor:

13 Test Procedure

13.1 Test method

The test was performed by the preincubation method with and without a metabolic activation system (S9 liver microsomal fraction from arochlor-treated rats).

13.2 Number of assay plates

In both the dose-finding assay and the main assay, the test was conducted with a negative control (DMSO), the test article and each positive control by treatment (incubation) with and without metabolic activation using duplicate assay plates per dose except the negative control for which triplicate plates were used.

13.3 Identification of plates

Each plate was marked with the study number, name of tester strain, presence or absence of S9, name of test article or control article, concentration (treatment concentration and stock solution number) of test article or control article, and intra-treatment identification number.

13.4 Treatment with test article or control article, and incubation

To each tube, 0.1 mL of the test article solution or control article solution, 0.5 mL of S9 mix, and 0.1 mL of the bacterial suspension were added, mixed, and agitated for 20 minutes at 37°_C. Two mL of the top agar was then added to the mixture and poured evenly over a minimum glucose agar plate, and the plate was observed for test article precipitation. After the top agar hardened, the plates were incubated in a T.H. Type Digital Incubator Model HD-12-b (Hirasawa Works Co., Ltd.) at 37_C for about 48 hours.

13.5 Observation of revertant colonies

Plates were observed for inhibition of bacterial growth of the tester strain under the inverted microscope to check for antibacterial (cytotoxic) effect of the test article. They were then examined for the number of colonies formed by prototrophic revertants that emerged, and observed for test article precipitation. Colonies were counted with an automated colony counter (Bio-Multiscanner BMS-400; Toyo Sokki Co., Ltd.). Colony enumeration data were tallied using the Genotoxicity Study System MUTAPACK, of which protocol registration was made by the study director.

13.6 Sterility test

Top agar was added, 2 mL each, to 0.1 mL of the test article solution and to 0.5 mL of S9 mix, and these were poured evenly over nutrient agar plates. After the top agar layer hardened, the plates were incubated in the same manner as indicated in Section 13.4, and examined for bacterial contamination.

13.7 Interpretation of results

Plates were subjected to interpretation of results for mutagenic activity of the test article providing that the sterility test showed no bacterial contamination and that the numbers of revertant colonies on plates treated with the negative control and those on plates

treated with positive control articles were within the limits (mean_3SD) of background data[10].
The test was interpreted as positive when the number of revertant colonies (mean per plate) on plates treated with the test article was twice or more than that on negative control plates and when the increase was dose-dependent. No statistical processing of the data was performed.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): studies followed guidelines, had sufficient replicates, used typical criteria, and positive and negative controls behaved as expected.

Study outcome: Findings were interpreted as negative

Table 2 Reverse mutation test of NKE-269
(Main assay)

Compound (μg/plate)	S9mix	Revertant colonies/plate: Base exchange type TA100	TA1535	WP2uvrA	Frameshift type TA98	TA1537
0	−	79 95 87 (87)	6 7 7 (7)	29 17 16 (21)	18 14 16 (16)	8 5 5 (6)
78.13	−	91 89 (90)	15 7 (11)	17 17 (17)	11 16 (14)	6 5 (6)
156.25	−	84 86 (85)	6 5 (7)	18 19 (19)	11 11 (11)	6 2 (4)
312.5	−	94 95 (95)	5 5 (5)	25 17 (21)	17 17 (17)	7 2 (5)
625	−	99 85 (92)	8 11 (10)	17 17 (17)	13 17 (15)	4 7 (6)
1250	−	83 83 (83)	8 6 (7)	11 13 (12)	6 12 (9)	2 2 (2)
2500	−	71 66 (69)	4 5 (5)	24 19 (22)	11 10 (11)	3 2 (3)
5000	−	26 29 (29)*	11 10 (11)	17 19 (18)	10 6 (8)	2 4 (3)
PC	−	497 568 (533)	355 331 (345)	81 86 (84)	334 335 (335)	805 1037 (946)
0	+	111 112 97 (107)	8 10 10 (9)	22 23 13 (19)	33 28 22 (28)	14 9 11 (11)
78.13	+	99 96 (99)	6 8 (7)	21 24 (23)	25 26 (26)	5 4 (5)
156.25	+	86 108 (97)	5 7 (7)	25 27 (26)	21 24 (23)	8 13 (11)
312.5	+	104 107 (106)	6 12 (9)	31 31 (31)	21 20 (21)	16 10 (13)
625	+	100 95 (98)	11 11 (11)	30 20 (25)	16 15 (16)	2 7 (5)
1250	+	80 88 (84)	6 6 (6)	21 20 (21)	17 28 (23)	7 10 (9)
2500	+	91 104 (98)	6 5 (6)	12 16 (14)	11 12 (12)	7 5 (6)
5000 †	+	41 33 (37)*	5 11 (8)	11 19 (15)	15 13 (14)	6 6 (6)
PC	−	1140 1226 (1183)	265 278 (272)	611 445 (528)	305 324 (315)	290 331 (311)

Vehicle control : Dimethyl sulfoxide
PC(positive controls) (μg/plate) :

	Without S9mix	With S9mix
TA100	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (0.01)	2-aminoanthracene (1)
TA1535	Sodium azide (0.5)	2-aminoanthracene (2)
WP2uvrA	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (0.01)	2-aminoanthracene (10)
TA98	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (0.1)	2-aminoanthracene (0.5)
TA1537	9-aminoacridine (80)	2-aminoanthracene (2)

* : Toxic effect was observed.
† : Precipitates of test chemical were observed.
The number in parentheses means the average of plates.

Table 2 Reverse mutation test of NS 304
(Main assay)

Compound (μg/plate)	S9mix	Revertant colonies/plate: Base exchange type TA100	TA1535	WP2 uvrA	Frameshift type TA98	TA1537
0	−	96 83 112 (97)	11 10 9 (10)	32 30 30 (30)	20 19 19 (19)	8 10 11 (10)
78.13	−	93 82 (88)	11 11 (11)	28 24 (26)	13 10 (12)	8 10 (9)
156.25	−	78 91 (85)	8 7 (8)	23 23 (23)	11 10 (11)	10 10 (10)
312.5	−	91 106 (99)	7 6 (7)	28 33 (31)	12 12 (12)	6 7 (7)
625	−	100 104 (102)	6 6 (6)	33 25 (29)	18 20 (19)	5 7 (6)
1250	−	87 93 (90)	6 4 (5)	32 33 (33)	18 17 (18)	7 10 (9)
2500	−	78 88 (83)	6 7 (7)	25 27 (26)	17 22 (20)	9 10 (10)
5000	−	40 40 (40)*	7 7 (7)	27 27 (27)	20 20 (20)	9 8 (9)
PC	−	544 560 (552)	298 300 (299)	95 96 (96)	327 345 (326)	941 1020 (981)
0	+	108 122 110 (113)	11 8 8 (9)	29 30 28 (29)	25 27 28 (26)	12 14 14 (13)
78.13	+	122 131 (127)	12 12 (12)	30 28 (29)	16 25 (21)	13 13 (13)
156.25	+	123 116 (120)	11 11 (11)	34 32 (33)	29 23 (26)	12 11 (12)
312.5	+	112 120 (116)	13 7 (10)	31 33 (32)	23 18 (21)	18 19 (19)
625	+	105 114 (110)	8 8 (8)	32 31 (32)	13 12 (13)	12 11 (12)
1250	+	91 80 (86)	10 11 (11)	32 30 (31)	13 16 (15)	11 11 (11)
2500	+	80 86 (83)	10 10 (10)	32 34 (33)	14 16 (15)	8 11 (10)
5000	+	96 85 (91)	10 11 (11)	28 28 (28)	16 16 (16)	12 11 (12)
PC	+	1271 1101 (1186)	250 275 (263)	582 505 (544)	327 341 (334)	291 250 (271)

Vehicle control : Dimethyl sulfoxide
PC(positive controls) (μg/plate) :

	Without S9mix	With S9mix
TA100	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (0.01)	2-aminoanthracene (1)
TA1535	Sodium azide (0.5)	2-aminoanthracene (2)
WP2 uvrA	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (0.01)	2-aminoanthracene (10)
TA98	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (0.1)	2-aminoanthracene (0.5)
TA1537	9-aminoacridine (80)	2-aminoanthracene (2)

* : Toxic effect was observed
The number in parentheses means the average of plates.

Study title: Chromosome Aberration test for MRE-269 and MRE-304

Key findings: ACT-269987 in the absence of S9 was clastogenic at a high dose of 250 mcg/mL, but negative in the presence of S9, ACT-333697 was negative for chromosomal aberrations in Chinese hamster lung cells.

Study no.: T-08.280 and T-08.281

Volume #, and page #: eCTD
Conducting laboratory and location:
Name of facility where the test was conducted:
Toxicology Department, Research and Development Division, Nippon Shinyaku Co., Ltd.
Address:
14 Kisshoin-Nishinosho-Monguchi-cho, Minami-ku, Kyoto
Date of study initiation: Study initiation date: 16-Sep-2004
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:
1.1 Compound code: MRE-269
1.2 Lot number: 9
1.3 Date received: 30-Sep-2004
1.4 Quantity received: 8.0 g
1.5 Source and responsible person: H. Yamashita, Test Article Management Unit
1.6 Characteristics1)
1.6.1 Description: A pale yellow crystalline powder, meeting the specified requirements
1.6.2 Identification by ultraviolet spectrophotometry: MRE-269 proved to meet the specified requirements.
1.6.3 Purity by liquid chromatography: The total area of the peaks (of related substances) other than the peak of MRE-269 was (b)(4)%, thus meeting the specified requirements (not more than (b)(4)%).
1.6.4 Assay: The assay value was (b)(4)%, hence meeting the specified requirements (98.0 to 101.0%)

Methods

Strains/species/cell line: A cell line derived from newborn Chinese hamster lung (CHL/IU) was used.

Doses used in definitive study:

Basis of dose selection: cytotoxicity and solubility

Negative controls: DMSO

Positive controls: Mitomycin C (-S9), Benzo(a)pyrene (+S9)

Incubation and sampling times: see below tables

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): study used typical criteria for positive and negative results, counting methods and a typical number of replicates.

Study outcome:

Table 1 Chromosome aberration test of NS-304 : Short Time Treatment Method

Substance	Dose (μg/mL)	Treatment time (hr)	-	Recovery time (hr)	S9 mix	Number of analyzed cells	Number of structural aberrant cells: gap	ctb	cte	csb	cse	frg	Total (%) -gap	Number of polyploid cells (%)	Cell proliferation rate (%)
DMSO	-	6	-	18	-	200	0	2	0	0	0	0	2 (1.0)	0 (0.0)	100.0
NS-304	31.25	6	-	18	-	200	0	1	1	0	0	0	2 (1.0)	0 (0.0)	100.6
	62.5	6	-	18	-	200	0	1	1	0	0	0	2 (1.0)	0 (0.0)	91.7
	125	6	-	18	-	200	0	0	0	0	0	0	0 (0.0)	1 (0.5)	72.0
	250	6	-	18	-	131	1	19	45	0	0	0	53 (40.5) **	0 (0.0)	54.7
	500	6	-	18	-	0	-	-	-	-	-	-	- (-)	- (-)	8.1
	1000 P	6	-	18	-	0	-	-	-	-	-	-	- (-)	- (-)	2.8
MMC	0.1	6	-	18	-	200	1	58	103	0	0	0	123 (61.5) **	0 (0.0)	94.7
DMSO	-	6	-	18	+	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	100.0
NS-304	25	6	-	18	+	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	84.8
	50	6	-	18	+	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	80.8
	100	6	-	18	+	200	0	0	2	0	0	0	2 (1.0)	0 (0.0)	80.2
	200	6	-	18	+	200	0	1	3	0	0	0	3 (1.5)	0 (0.0)	48.3
	400	6	-	18	+	0	-	-	-	-	-	-	- (-)	- (-)	8.0
	800 P	6	-	18	+	0	-	-	-	-	-	-	- (-)	- (-)	2.0
B(a)P	20	6	-	18	+	200	0	30	106	0	1	0	116 (58.0) **	0 (0.0)	62.4

ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange; frg, fragmentation
MMC, mitomycinC; B(a)P, Benzo(a)pyrene
* Significantly different from negative control (p<0.05)
** Significantly different from negative control (p<0.01)
P, Precipitation

Table 2 Chromosome aberration test of NS-304 : Long Time Treatment Method

Substance	Dose (μg/mL)	Treatment time (hr)	-	Recovery time (hr)	Number of analyzed cells	Number of structural aberrant cells: gap	ctb	cte	csb	cse	frg	Total (%) -gap	Number of polyploid cells (%)	Cell proliferation rate (%)
DMSO	-	24	-	0	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	100.0
NS-304	15	24	-	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	103.6
	30	24	-	0	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	105.3
	60	24	-	0	200	0	1	1	0	0	0	2 (1.0)	0 (0.0)	88.4
	120	24	-	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	64.1
	240	24	-	0	0	-	-	-	-	-	-	- (-)	- (-)	39.1
	480	24	-	0	0	-	-	-	-	-	-	- (-)	- (-)	31.2
	960 P	24	-	0	0	-	-	-	-	-	-	- (-)	- (-)	0.9
MMC	0.05	24	-	0	200	0	33	62	0	0	0	83 (41.5) **	0 (0.0)	83.6
DMSO	-	48	-	0	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	100.0
NS-304	6.25	48	-	0	200	0	1	1	0	0	0	2 (1.0)	0 (0.0)	100.9
	12.5	48	-	0	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	99.7
	25	48	-	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	101.2
	50	48	-	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	83.7
	100	48	-	0	200	0	2	4	0	0	0	6 (3.0)	0 (0.0)	39.1
	200	48	-	0	0	-	-	-	-	-	-	- (-)	- (-)	20.0
	400	48	-	0	0	-	-	-	-	-	-	- (-)	- (-)	18.1
MMC	0.05	48	-	0	200	0	45	111	0	0	0	123 (61.5) **	0 (0.0)	74.5

ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange; frg, fragmentation
MMC, mitomycinC
* Significantly different from negative control (p<0.05)
** Significantly different from negative control (p<0.01)
P, Precipitation

Table 1 Chromosome aberration test of MRE-269 : Short Time Treatment Method

Substance	Dose (μg/mL)	Treatment time (hr)	–	Recovery time (hr)	S9 mix	Number of analyzed cells	Number of structural aberrant cells: gap	ctb	cte	csb	cse	frg	Total (%) –gap	Number of polyploid cells (%)	Cell proliferation rate (%)
DMSO	–	6	–	18	–	200	0	3	1	0	0	0	4 (2.0)	0 (0.0)	100.0
MRE-269	36.25	6	–	18	–	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	98.8
	72.5	6	–	18	–	200	0	2	1	0	0	0	3 (1.5)	0 (0.0)	109.0
	145	6	–	18	–	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	94.9
	290 P	6	–	18	–	200	0	4	4	0	0	0	7 (3.5)	0 (0.0)	47.9
	580 P	6	–	18	–	0	–	–	–	–	–	–	– (–)	– (–)	6.5
	1160 P	6	–	18	–	0	–	–	–	–	–	–	– (–)	– (–)	7.8
MMC	0.1	6	–	18	–	200	1	48	96	0	0	0	112 (56.0) **	0 (0.0)	88.9
DMSO	–	6	–	18	+	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	100.0
MRE-269	17.5	6	–	18	+	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	101.2
	35	6	–	18	+	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	92.5
	70	6	–	18	+	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	86.7
	140	6	–	18	+	200	0	1	1	0	0	0	2 (1.0)	0 (0.0)	80.6
	280	6	–	18	+	22	–	–	–	–	–	–	– (–)	– (–)	24.2
	560 P	6	–	18	+	0	–	–	–	–	–	–	– (–)	– (–)	7.5
B(a)P	20	6	–	18	+	200	0	21	69	0	1	0	75 (37.5) **	0 (0.0)	73.5

ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange, frg, fragmentation
MMC, mitomycinC; B(a)P, Benzo(a)pyrene
* Significantly different from negative control (p<0.05)
** Significantly different from negative control (p<0.01)
P, Precipitation

Table 2 Chromosome aberration test of MRE-269 : Long Time Treatment Method

Substance	Dose (μg/mL)	Treatment time (hr)	–	Recovery time (hr)	Number of analyzed cells	Number of structural aberrant cells: gap	ctb	cte	csb	cse	frg	Total (%) –gap	Number of polyploid cells (%)	Cell proliferation rate (%)
DMSO	–	24	–	0	200	0	0	0	0	0	0	0 (0.0)	1 (0.5)	100.0
MRE-269	22.5	24	–	0	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	90.3
	45	24	–	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	93.3
	90	24	–	0	200	0	1	0	0	0	0	1 (0.5)	0 (0.0)	74.6
	180	24	–	0	200	0	2	0	0	0	0	2 (1.0)	0 (0.0)	54.2
	360 P	24	–	0	0	–	–	–	–	–	–	– (–)	– (–)	32.4
	720 P	24	–	0	0	–	–	–	–	–	–	– (–)	– (–)	11.7
MMC	0.05	24	–	0	200	0	32	90	0	0	0	104 (52.0) **	0 (0.0)	77.1
DMSO	–	48	–	0	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	100.0
MRE-269	13.75	48	–	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	104.4
	27.5	48	–	0	200	0	2	0	0	0	0	2 (1.0)	0 (0.0)	98.7
	55	48	–	0	200	0	0	1	0	0	0	1 (0.5)	0 (0.0)	93.3
	110	48	–	0	200	0	0	0	0	0	0	0 (0.0)	0 (0.0)	48.0
	220	48	–	0	0	–	–	–	–	–	–	– (–)	– (–)	21.2
	440 P	48	–	0	0	–	–	–	–	–	–	– (–)	– (–)	15.3
MMC	0.05	48	–	0	200	1	59	140	0	0	0	148 (74.0) **	0 (0.0)	57.7

ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange; frg, fragmentation
MMC, mitomycinC
* Significantly different from negative control (p<0.05)
** Significantly different from negative control (p<0.01)
P, Precipitation

Study title: Micronucleus test of NS-304 (MRE-304) with mouse bone marrow cells

Key findings: No indications of *in vivo* clastogenic activity by ACT-293987 or ACT-333679 were seen in the assay.

Study no.: T-08.282

Volume #, and page #: eCTD

Conducting laboratory and location:
Date of study initiation:
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Methods

Strains/species/cell line: male S1c:ddY mice

Doses used in definitive study: 125, 250, and 500 mg/kg/day

Basis of dose selection: previous dose and toxicity studies

Negative controls: 0.5% methylcellulose

Positive controls: mitomycin C at 2 mg/kg

Incubation and sampling times: from the sponsor:

5. Preparation and Administration of the Test Article and Negative Control
5.1 Route of Administration and Rationale
5.1.1 Route of Administration: Oral
5.1.2 Rationale for Selection: Oral route of administration was selected according to the envisaged administration route for humans and the Guideline for Genotoxicity Studies.
5.2 Dosing Volume: 10 mL/kg. The animals were weighed immediately before administration on the first day of administrating using an LP2200S electronic balance (Sartorius K.K.) and a computer system for genotoxicity studies MUTAPACK. Based on the body weight measured, the dosing volume for each animal was determined at 10
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Actelion Research Report T-08.282
Received May 2008
Study No.: TX-1321
mL/kg of body weight. The dosing volume was calculated to 2 decimal places by rounding up the third decimal place.
5.3 Method of Administration and Rationale
5.3.1 Method of Administration: The test solution was administered to mice by oral gavage using a sterile disposable syringe (Terumo Corporation) attached with a metallic oral sonde.
5.3.2 Rationale for Selection: The method was selected because it is generally used for oral administration of drugs to mice, and, as documented by Sponsor, it is orally absorbed, and metabolized to active drug in the mouse .
5.4 Number of Administration and Rationale
5.4.1 Number of Administration: Once daily for two days.
5.4.2 Rationale for Selection: The frequency and number of administration was decided according to the Guideline for Genotoxicity Studies.
5.5 Time of Administration
5.5.1 Dose Finding Study: 9:30 to 9:55 a.m. on January 25, 2005 and 9:36 to 9:56 a.m.

on January 26, 2005
5.5.2 Main Study: 9:43 to 10:23 a.m. on February 15, 2005 and 9:44 to 10:25 a.m. on February 16, 2005
5.5.3 TK Measurement Study: 10:00 a.m. to 4:20 p.m. on March 1, 2005 and 10:01 a.m. to 4:20 p.m. on March 2, 2005
5.6 Doses and Rationale for Selection
5.6.1 Dose Finding Study
Doses: 62.5, 125, 250, 500, 1000 and 2000 mg/kg
The highest dose was set at 2000 mg/kg according to the Guideline for Genotoxicity Studies. Totally 6 doses were set using a common ratio of 1/2.
5.6.2 Main Study
Doses: 62.5, 125, 250, 500 and 1000 mg/kg
More than half the animals died at 1000 mg/kg in the dose finding study (See Results).
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Results

Study validity: Study followed OECD approved methods, criteria, and replicates.

Study outcome: Study was considered to be negative for clastogenicity.

Table 4 Micronucleus test of NS-304 (male mice) Study No. TX-1221

Compound	Dose (mg/kg)	Number of animals	Frequency of MNPCE [%] (Mean ± S.D.)	Range of MNPCE/1000PCE (Min - Max)	Ratio of PCE [%] (Mean ± S.D.)
0.5%MC	0	6	0.26 ± 0.35	4 - 7	52.3 ± 1.5
NS-304	125	6	0.24 ± 0.37	3 - 7	51.8 ± 2.2
	250	6	0.25 ± 0.37	3 - 7	51.2 ± 1.9
	500	5	0.19 ± 0.38	1 - 6	48.6 ± 4.9
MMC	2.0	6	6.27 ± 2.76 **	56 - 166	51.4 ± 1.4

Kasten-Bann Significant difference from control *:p<0.05, **:p<0.01
Student t-test Significant difference from control *:p<0.05, **:p<0.01

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2.6.6.5 Carcinogenicity not done

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: study of fertility and early embryonic development to implantation in rats treated orally with ns-304.

Key study findings: The high dose group females experienced a brief weight drop on day 4, but then recovered and gained weight at the same rate as the other groups.

The high dose group (60 mg/kg) was delayed in time to copulation, and all the treated animals had reduced litter size, however, the reduction was not statistically significant.and there was no effect on number of corpora lutea, or pre or post-implantation loss.

Study no.: R-950
Volume #, and page #: eCTD
Conducting laboratory and location:

(b) (4)

Date of study initiation: October 3, 2006
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Name:	**NS-304 (MRE-304)**
Lot number:	**20**
Assay:	**100.3%**

Methods

Test group	Dose level (mg/kg)	Dose concentration (mg/mL)	Dose volume (mL/kg)	Sex	Main group		Satellite group	
					Number of animals	Animal number	Number of animals	Animal number
Control	0	0	5	Male	20	1001-1020	4	1021-1024
				Female	20	1101-1120	4	1121-1124
Low dose	6	1.2	5	Male	20	2001-2020	8	2021-2028
				Female	20	2101-2120	8	2121-2128
Middle dose	20	4	5	Male	20	3001-3020	8	3021-3028
				Female	20	3101-3120	8	3121-3128
High dose	60	12	5	Male	20	4001-4020	8	4021-4028
				Female	20	4101-4120	8	4121-4128

Doses:
Species/strain: Sprague-Dawley rats
Number/sex/group:
Route, formulation, volume, and infusion rate: oral gavage,
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality: none

Clinical signs: At all doses, flushing occurred in the ears and extremities.

Body weight:

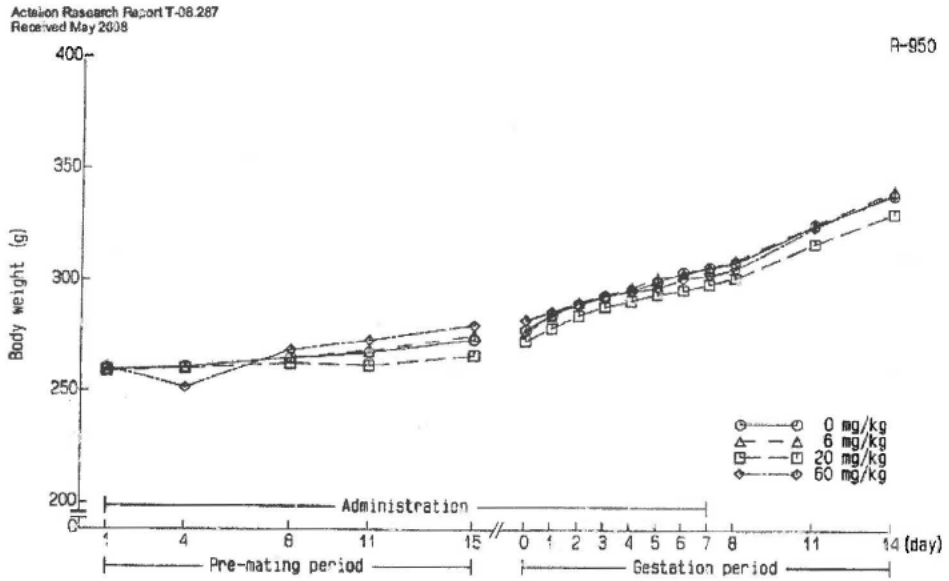

Fig.3 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Body weight of female rats

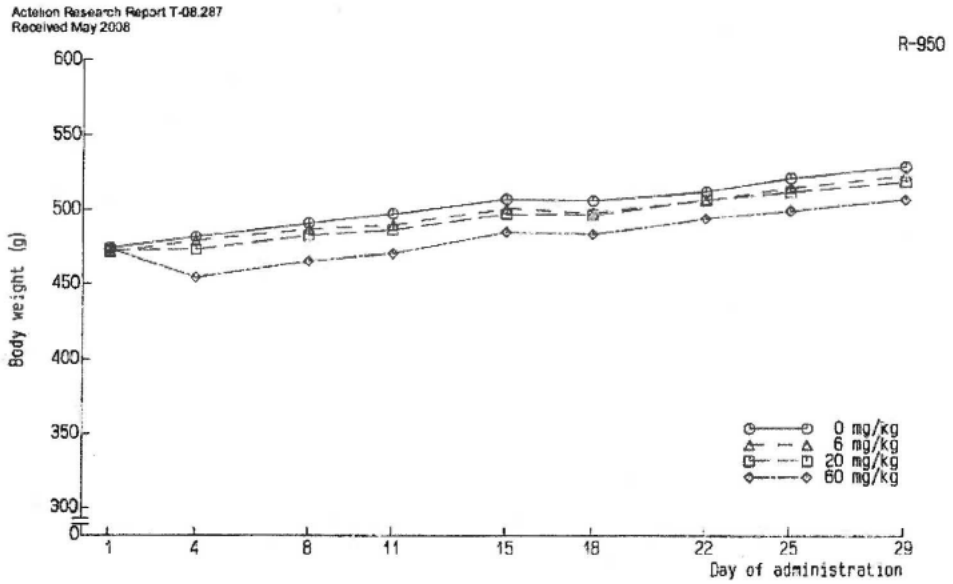

Fig.1 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Body weight of male rats

Food consumption:

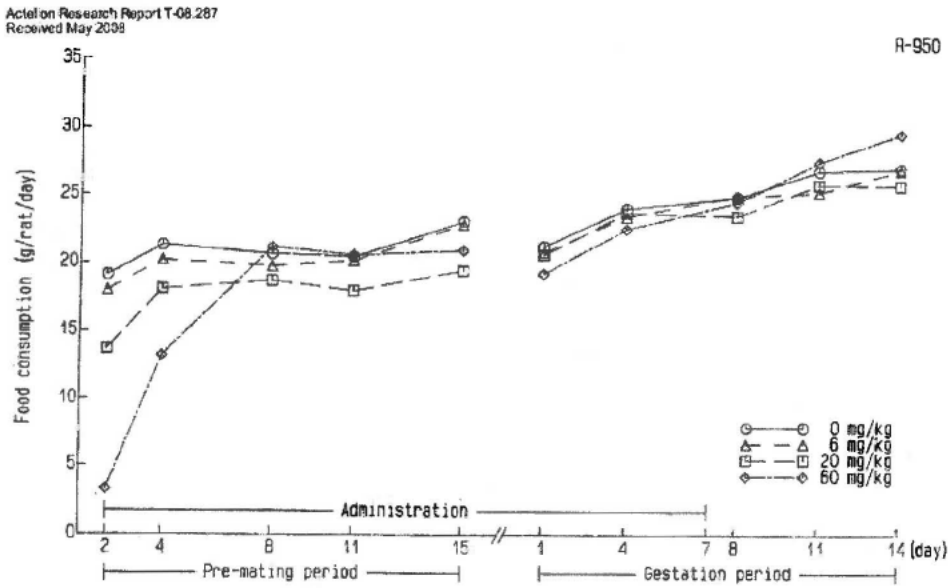

Fig.4 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Food consumption of female rats

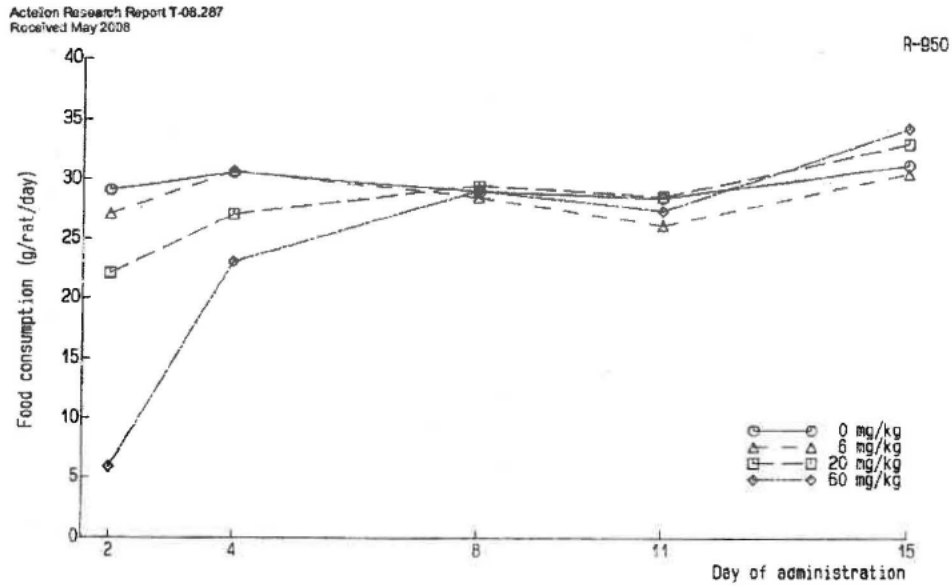

Fig.2 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Food consumption of male rats

Toxicokinetics:

K-0003

Table 15-1 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Plasma concentration of NS-304 (Day 1)

Male

Dose mg/kg	Plasma concentration (μg/mL)															
	Animal number	0.5h	Animal number	1h	Animal number	2h	Animal number	4h	Animal number	8h	Animal number	24h	Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
0			1021 1025 1026	N.D. N.D. N.D.											
	Mean S.D.			0											
6	2021 2022 2023	N.D. 0.118 0.111	2024 2025 2026	N.D. 0.0695 0.0976	2021 2022 2023	N.D. N.D. N.D.	2024 2025 2026	N.D. N.D. N.D.	2021 2022 2023	N.D. N.D. N.D.	2024 2025 2026	N.D. N.D. N.D.			
	Mean S.D.	0.0763 0.0642		0.0837 0.0420		0		0		0		0	0.0795	0.5	0.0777
20	3021 3022 3023	0.123 0.177 0.222	3024 3025 3026	0.0738 0.129 0.224	3021 3022 3023	N.D. N.D. N.D.	3024 3025 3026	N.D. N.D. N.D.	3021 3022 3023	N.D. N.D. N.D.	3024 3025 3026	N.D. N.D. N.D.			
	Mean S.D.	0.174 0.050		0.246 0.080		0		0		0		0	0.174	0.5	0.197
60	4021 4022 4023	0.520 0.949 4.41	4024 4025 4026	1.70 1.50 1.86	4021 4022 4023	N.D. 0.0617 0.329	4024 4025 4026	0.784 0.490 1.16	4021 4022 4023	N.D. N.D. N.D.	4024 4025 4026	0.110 N.D. N.D.			
	Mean S.D.	1.96 2.14		1.72 0.23		0.127 0.171		0.785 0.342		0		0.0367 0.0635	1.96	0.5	3.12

N.D. : Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean± S.D.

Table 15-2 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Plasma concentration of NS-304 (Day 15)

Male

Dose mg/kg	Plasma concentration (μg/mL)															
	Animal number	Pre	Animal number	0.5h	Animal number	1h	Animal number	2h	Animal number	4h	Animal number	8h	Animal number	24h	Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
0					1021 1024 1026	N.D. N.D. N.D.											
	Mean S.D.					0											
6	2024 2025 2026	N.D. N.D. N.D.	2021 2022 2023	0.243 0.170 0.236	2024 2025 2026	0.0673 0.0367 0.0696	2021 2022 2023	N.D. N.D. N.D.	2024 2025 2026	N.D. N.D. N.D.	2021 2022 2023	N.D. N.D. N.D.	2024 2025 2026	N.D. N.D. N.D.			
	Mean S.D.	0		0.206 0.034		0.0745 0.0136		0		0		0		0	0.256	0.5	0.360
20	3024 3025 3026	N.D. N.D. N.D.	3021 3022 3023	0.428 0.850 0.870	3024 3025 3026	0.193 0.172 0.191	3021 3022 3023	N.D. N.D. N.D.	3024 3025 3026	0.0553 N.D. N.D.	3021 3022 3023	N.D. 0.0728 N.D.	3024 3025 3026	N.D. N.D. N.D.			
	Mean S.D.	0		0.849 0.127		0.185 0.011		0		0.0185 0.0320		0.0243 0.0421		0	0.849	0.5	2.709
60	4024 4025 4026	N.D. N.D. N.D.	4021 4022 4023	2.31 2.19 1.41	4021 4022 4023	1.19 1.74 1.32	4021 4022 4023	0.0803 0.216 0.459	4024 4025 4026	0.3784 0.274 0.121	4021 4022 4023	0.168 0.0865 0.190	4024 4025 4026	N.D. N.D. N.D.			
	Mean S.D.	0		1.97 0.49		1.52 0.28		0.254 0.189		0.157 0.189		0.132 0.050		0	1.97	0.5	4.28

N.D. : Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean± S.D.

Reviewer: IND No.

Table 15-3 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Plasma concentration of NS-304 (Day 1)

Female

Dose mg/kg	Animal number	0.5h	Animal number	1h	Animal number	2h	Animal number	4h	Animal number	8h	Animal number	24h	Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
				Plasma concentration (μg/mL)											
0			1121	N.D.											
			1122	N.D.											
			1123	N.D.											
	Mean			0											
	S.D.														
6	2121	0.221	2124	N.D.	2121	N.D.	2124	N.D.	2121	N.D	2124	N.D.			
	2122	0.139	2125	N.D.	2122	N.D.	2125	N.D.	2122	N.D	2125	N.D.			
	2123	0.204	2126	N.D.	2123	N.D.	2126	N.D.	2123	N.D	2126	N.D.			
	Mean	0.188		0		0		0		0		0	0.188	0.5	0.0940
	S.D.	0.043													
20	3121	0.295	3124	0.384	3121	0.0647	3124	0.0217	3121	N.D.	3124	N.D.			
	3122	0.225	3125	0.262	3122	N.D.	3125	N.D.	3122	N.D.	3125	N.D.			
	3123	0.285	3126	0.719	3123	N.D.	3126	N.D.	3123	N.D.	3126	N.D.			
	Mean	0.251		0.555		0.0216		0.0206		0		0	0.555	1.0	0.621
	S.D.	0.034		0.252		0.0374		0.0356							
60	4121	6.24	4124	2.85	4121	2.30	4124	1.10	4121	0.225	4124	0.0717			
	4122	5.25	4125	4.55	4122	1.52	4125	1.86	4122	0.122	4125	N.D.			
	4123	7.06	4126	6.91	4123	1.21	4126	2.61	4123	0.194	4126	0.0921			
	Mean	6.26		5.80		1.66		1.76		0.180		0.0549	6.58	0.5	17.8
	S.D.	1.21		3.88		0.47		0.71		0.052		0.0484			

N.D. : Not detectable (<0.05 μg/mL)

Table 15-4 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Plasma concentration of NS-304 (Day 15)

Female

Dose mg/kg	Animal number	Pre	Animal number	0.5h	Animal number	1h	Animal number	2h	Animal number	4h	Animal number	8h	Animal number	24h	Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
						Plasma concentration (μg/mL)											
0					1121	N.D.											
					1122	N.D.											
					1123	N.D.											
	Mean					0											
	S.D.																
6	2124	N.D.	2121	0.262	2124	N.D.	2121	N.D.	2124	N.D.	2121	N.D.	2124	N.D.			
	2125	N.D.	2122	0.241	2125	N.D.	2122	N.D.	2125	N.D.	2122	N.D.	2125	N.D.			
	2126	N.D.	2123	0.146	2126	N.D.	2123	N.D.	2126	N.D.	2123	N.D.	2126	N.D.			
	Mean	0		0.214		0		0		0		0		0	0.214	0.5	0.107
	S.D.			0.057													
20	3124	N.D.	3121	0.134	3124	0.325	3121	0.0902	3124	N.D.	3121	N.D.	3124	N.D.			
	3125	N.D.	3122	0.953	3125	0.0686	3122	N.D.	3125	N.D.	3122	N.D.	3125	N.D.			
	3126	N.D.	3123	0.845	3126	0.287	3123	N.D.	3126	N.D.	3123	N.D.	3126	N.D.			
	Mean	0		0.574		0.226		0.0301		0		0		0	0.574	0.5	0.502
	S.D.			0.410		0.122		0.0521									
60	4124	N.D.	4121	0.563	4124	3.04	4121	0.136	4124	N.D.	4121	0.0721	4124	N.D.			
	4125	N.D.	4122	1.20	4125	0.652	4122	0.0524	4125	0.152	4122	N.D.	4125	N.D.			
	4126	0.124	4123	2.21	4126	0.596	4123	0.564	4126	0.232	4123	0.0591	4126	N.D.			
	Mean	0.0413		1.32		1.36		0.241		0.120		0.0441		0	1.32	1.0	2.88
	S.D.	0.0716		0.84		1.46		0.257		0.120		0.0386					

N.D. : Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean ± S.D.

APPEARS THIS WAY ON ORIGINAL

Necropsy:

Table 12 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Gross pathological findings in female rats

Dose (mg/kg)	0	6	20	60
No. of animals examined	20	20	20	20
No. of animals with abnormal findings	0	0	0	0

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Table 13 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Mating and fertility of animals

Dose mg/kg	No. of males	No. of females	Days until copulation Mean±S.D.	Copulation index (%) a)	Fertility index (%) b)
0	20	20	2.3±1.1	20/20(100.0)	20/20(100.0)
6	20	20	2.0±0.9	20/20(100.0)	19/20(95.0)
20	20	20	2.7±1.5	20/20(100.0)	19/20(95.0)
60	20	20	3.3±1.4*D	20/20(100.0)	20/20(100.0)

a): (No. of copulated animals / No. of mated animals) X 100
b): (No. of pregnant animals / No. of copulated animals) X 100
*: p<0.05 (Significant difference from control group)
D: Dunnett's test

Table 14 Study of fertility and early embryonic development to implantation in rats treated orally with NS-304

Findings at examination at the middle of gestation in dams

Dose mg/kg	No. of dams		No. of corpora lutea	No. of implan-tations	Implan-tation index % a)	No. of dead embryos (%)b)	No. of live embryos
0	20	Total	326	300		15	285
		Mean	16.3	15.0	93.6	(5.4)	14.3
		S.D.	2.0	2.1	13.3	(8.0)	2.6
6	19	Total	304	287		23	264
		Mean	16.0	15.1	94.0	(7.5)	13.9
		S.D.	3.3	3.6	11.1	(7.9)	3.4
20	19	Total	315	290		21	269
		Mean	16.6	15.3	93.2	(7.4)	14.2
		S.D.	3.2	1.9	8.7	(7.1)	2.2
60	20	Total	329	290		23	267
		Mean	16.5	14.5	88.2	(7.9)	13.4
		S.D.	2.4	3.6	19.2	(9.1)	3.7

a): (No. of implantations / No. of corpora lutea) X 100
b): (No. of dead embryos / No. of implantations) X 100
No significant difference in any treated groups from control group.

Embryofetal development

Study title: Study for effects on embryo-fetal development in rats treated orally with NS-304

Key study findings: The high dose group (20 mg/kg) experienced slightly, but statistically significantly, decreased birth weights of the fetuses.
Study no.: R-951
Volume #, and page #: eCTD
Conducting laboratory and location:
(b) (4)

Date of study initiation: November 14, 2006
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Name:	NS-304 (MRE-304)
Lot number:	20
Assay:	100.3%

Methods

Doses:
Species/strain: Sprague-Dawley rats
Number/sex/group:

Test group	Dose level (mg/kg)	Dose concentration (mg/mL)	Dose volume (mL/kg)	Main group		Satellite group	
				Number of copulated animals*	Animal number	Number of copulated animals*	Animal number
Control	0	0	5	20 (20)	1101-1120	4 (4)	1121-1124
Low dose	2	0.4	5	20 (20)	2101-2120	8 (8)	2121-2128
Middle dose	6	1.2	5	20 (20)	3101-3120	8 (8)	3121-3128
High dose	20	4	5	20 (20)	4101-4120	8 (8)	4121-4128

*: The number between the parentheses indicates the number of pregnant animals.

Route, formulation, volume, and infusion rate: oral gavage
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality (dams): none

Clinical signs (dams): flushing of ears and extremities, sporadic flaccidity in the high dose group (20 mg/kg)

Body weight (dams): minor reductions in body weight in the high dose dams (20 mg/kg)

Food consumption (dams): food consumption reduced for one day in the 6 mg/kg group, for approximately 4 days in the 20 mg/kg group.

Toxicokinetics:

Table 9-1 Study for effects on embryo-fetal development in rats treated orally with NS-304
Plasma concentration of NS-304 (Day 1)

Dose mg/kg	Plasma concentration (μg/mL)														
	Animal number	0.5h	Animal number	1h	Animal number	2h	Animal number	4h	Animal number	8h	Animal number	24h	Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg h/mL)
0			2121 2122 2123	N.D. N.D. N.D.											
	Mean S.D.			0											
2	2121 2122 2123	N.D. N.D. 0.0597	2125 2126 2127	N.D. N.D. N.D.	2121 2122 2123	N.D. N.D. N.D.	2125 2126 2127	N.D. N.D. N.D.	2121 2122 2123	N.D. N.D. N.D.	2125 2126 2127	N.D. N.D. N.D.			
	Mean S.D.	0.0199 0.0345		0		0		0		0		0	0.0199	0.5	0.00895
6	2121 2122 2123	0.216 0.101 0.343	2125 2126 2127	0.0577 N.D. N.D.	2121 2122 2123	N.D. N.D. N.D.	2125 2126 2127	N.D. N.D. N.D.	2121 2122 2123	N.D. N.D. N.D.	2125 2126 2127	N.D. N.D. N.D.			
	Mean S.D.	0.301 0.048		0.0272 0.0298		0		0		0		0	0.191	0.1	0.066
20	2121 2122 2123	1.19 1.02 1.65	2125 2126 2127	0.458 0.418 0.587	2121 2122 2123	0.0570 0.0627 0.0752	2125 2126 2127	0.0336 N.D. 0.0590	2121 2122 2123	N.D. N.D. N.D.	2125 2126 2127	N.D. N.D. N.D.			
	Mean S.D.	1.36 0.27		0.463 0.088		0.0650 0.0124		0.0419 0.0076		0		0	1.36	0.5	1.29

N.D. : Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean ± S.D.

Table 9-2 Study for effects on embryo-fetal development in rats treated orally with NS-304
Plasma concentration of NS-304 (Day 11)

Dose mg/kg	Plasma concentration (μg/mL)																
	Animal number	Pre	Animal number	0.5h	Animal number	1h	Animal number	2h	Animal number	4h	Animal number	8h	Animal number	24h	Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg h/mL)
0					2121 2122 2123	N.D. N.D. N.D.											
	Mean S.D.					0											
2	2125 2126 2127	N.D. N.D. N.D.	2121 2122 2123	0.0814 0.0707 0.0814	2125 2126 2127	N.D. N.D. N.D.	2121 2122 2123	N.D. N.D. N.D.	2125 2126 2127	N.D. N.D. N.D.	2121 2122 2123	N.D. N.D. N.D.	2125 2126 2127	N.D. N.D. N.D.			
	Mean S.D.	0		0.0812 0.0104		0		0		0		0		0	0.0812	0.5	0.0409
6	2125 2126 2127	N.D. N.D. N.D.	2121 2122 2123	0.180 0.299 0.216	2125 2126 2127	0.0812 0.0696 0.0952	2121 2122 2123	N.D. N.D. N.D.	2125 2126 2127	N.D. N.D. N.D.	2121 2122 2123	N.D. N.D. N.D.	2125 2126 2127	N.D. N.D. N.D.			
	Mean S.D.	0		0.232 0.059		0.0703 0.0105		0		0		0		0	0.207	0.5	0.152
20	2125 2126 2127	N.D. N.D. N.D.	2121 2122 2123	0.634 0.790 0.568	2125 2126 2127	0.202 0.286 0.0608	2121 2122 2123	0.0707 N.D. N.D.	2125 2126 2127	N.D. 0.0563 N.D.	2121 2122 2123	0.0930 N.D. 0.0402	2125 2126 2127	N.D. N.D. N.D.			
	Mean S.D.	0		0.664 0.161		0.205 0.095		0.0256 0.0243		0.0188 0.0325		0.0577 0.0434		0	0.634	0.5	1.16

N.D. : Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean ± S.D.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Table 4 Study for effects on embryo-fetal development in rats treated orally with NS-304

Gross pathological findings in dams

Dose (mg/kg)	0	2	6	20
No. of dams examined	22	20	20	20
No. of dams with abnormal findings	0	0	0	0

Table 5 Study for effects on embryo-fetal development in rats treated orally with NS-304

Cesarean section data

Dose mg/kg	No. of dams		No. of corpora lutea	No. of implantations	Implantation index (%) a)	No. of embryo fetal deaths: Total (%) b)	Implantation trace	Resorbed embryo	Placental remnant	Early resorbed fetus	Late resorbed fetus	Dead fetus	No. of live fetuses
0	22	Total	336	308		11 (3.6)	0	10	1	0	0	0	297
		Mean	14.9	13.5	92.8								13.5
		S.D.	1.5	1.3	5.0								1.4
2	20	Total	322	309		19 (6.1)	0	15	2	1	0	0	290
		Mean	16.1	15.5	96.4								14.5
		S.D.	2.0	1.5	5.5								1.0
6	20	Total	307	286		25 (8.3)	0	17	2	5	0	0	279
		Mean	15.4	14.3	97.3								13.9
		S.D.	1.6	1.3	4.5								1.3
20	20	Total	302	285		15 (5.3)	0	12	0	0	0	0	274
		Mean	15.1	14.3	93.1								13.7
		S.D.	2.6	2.7	19.2								3.2

a): (No. of implantations / No. of corpora lutea) X 100
b): (No. of embryo-fetal deaths / No. of implantations) X 100
No significant difference in any treated groups from control group.

Table 6 Study for effects on embryo-fetal development in rats treated orally with NS-304

Examination of live fetuses

Dose mg/kg	No. of dams		No. of males	No. of females	Sex ratio a)	Fetal body weight (g): Male	Female	No. of fetuses with external abnormalities (%) b)	Gross evaluation of placenta
0	20	Total	158	139	0.53			0	
		Mean	7.9	7.0		4.12	3.96	(0.0)	No abnormal findings
		S.D.	2.4	2.3		0.30	0.27	(0.0)	
2	20	Total	148	142	0.51			0	
		Mean	7.4	7.1		3.95	3.85	(0.0)	No abnormal findings
		S.D.	1.2	1.8		0.11	0.27	(0.0)	
6	20	Total	147	132	0.50			0	
		Mean	7.3	7.0		4.09	3.83	(0.0)	No abnormal findings
		S.D.	1.7	1.4		0.24	0.32	(0.0)	
20	20	Total	133	134	0.50			0	
		Mean	6.8	6.9		3.52**	3.67**	(0.0)	No abnormal findings
		S.D.	2.2	2.5		0.280	0.398	(0.0)	

a): No. of males / No. of live fetuses
b): (No. of live fetuses with external abnormalities / No. of live fetuses) X 100
**: p<0.01 (Significant difference from control group)
D: Dunnett's test

Offspring (malformations, variations, etc.):

Table 7 Study for effects on embryo-fetal development in rats treated orally with NS-304

Visceral examination of live fetuses

Dose (mg/kg)	0	10
No. of fetuses examined	142	134
No. of fetuses with abnormality (%)	4(2.8± 7.5)	10(7.2±11.0)
Situs inversus totalis	0(0.0± 0.0)	1(0.7± 2.9)
Ventricular septal defect	2(1.4± 4.4)	4(3.1± 7.5)
Abnormal origin of left pulmonary artery	1(0.7± 5.2)	0(2.6± 0.0)
Abnormal lobation of liver	1(0.7± 5.2)	1(0.8± 3.8)
Dilatation of renal pelvis and ureter	0(0.0± 0.0)	1(0.7± 2.9)
No. of fetuses with variation (%)	11(8.2± 8.1)	8(2.8± 6.0)*
Thymic remnant in neck	7(5.1± 7.1)	1(0.7± 2.9)*
Convoluted ureter	2(1.7± 5.4)	1(1.1± 4.0)
Left umbilical artery	2(1.4± 4.4)	1(0.8± 3.5)

*: p<0.05 (Significant difference from control group)
J: Wilcoxon's rank sum test

Table 8 Study for effects on embryo-fetal development in rats treated orally with NS-304

Skeletal examination of live fetuses

Dose (mg/kg)	0	2	7	20
No. of fetuses examined	154	150	145	140
No. of fetuses with abnormality (%)	0(0.0± 0.0)	1(0.0± 0.0)	0(0.0± 0.0)	0(0.0± 0.0)
No. of fetuses with variation (%)	15(9.8±15.1)	14(9.3±13.3)	26(18.1±15.5)	34(24.3±35.1)
Wavy ribs	0(0.0± 0.0)	1(0.6± 3.7)	1(0.6± 3.6)	0(0.0± 0.0)
14th rib	15(9.8±15.1)	13(8.7±13.4)	25(17.3±13.6)	32(22.8±33.5)
Splitting of thoracic vertebral body	7(5.7± 5.2)	0(0.0± 0.0)	0(0.0± 0.0)	2(1.4± 4.1)
Lumbarization of sacral vertebra	1(0.6± 2.0)	0(0.0± 0.0)	0(0.0± 0.0)	0(0.0± 0.0)
Progress of ossification				
No. of ossified sternebrae (%)				
1st	154(100.0± 0.0)	150(100.0± 0.0)	145(100.0± 0.0)	140(100.0± 0.0)
2nd	154(100.0± 0.0)	149(99.3± 3.7)	145(100.0± 0.0)	140(100.0± 0.0)
3rd	154(100.0± 0.0)	150(100.0± 0.0)	145(100.0± 0.0)	140(100.0± 0.0)
4th	154(100.0± 0.0)	148(98.7± 3.7)	145(100.0± 0.0)	140(100.0± 0.0)
5th	149(96.1±13.1)	147(98.0±10.5)	122(91.2±17.1)	129(85.2±19.3)
6th	154(100.0± 0.0)	149(99.3± 3.7)	144(95.2± 3.7)	139(99.4± 2.8)
No. of ossified metacarpal (Mean±S.D.)				
Right	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Left	3.99±0.09	3.99±0.08	4.00±0.00	4.00±0.00
No. of ossified metatarsal (Mean±S.D.)				
Right	4.93±0.12	4.94±0.23	4.93±0.32	4.79±0.31
Left	4.96±0.19	4.97±0.21	4.86±0.36	4.85±0.37
No. of ossified sacral and caudal vertebrae (Mean±S.D.)	8.45±0.31	8.32±0.47	8.42±0.49	8.27±0.35

No significant difference in any treated groups from control group.

Study title: Study for effects on embryo-fetal development in rabbits treated orally with NS-304

Key study findings: Fetuses from the high dose group (30 mg/kg) experienced increased incidence of right retrocaval ureter. No other statistically significant excess soft tissue or skeletal anomalies were detected. Fertility parameters were not affected.

Study no.: R-952
Volume #, and page #: eCTD
Conducting laboratory and location:

(b) (4)

Date of study initiation: December 14, 2006
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Name:	NS-304 (MRE-304)
Lot number:	20
Assay:	100.3%

Methods

Doses:
Species/strain: New Zealand White Rabbits
Number/sex/group:

Text Table 1. Group Composition Table

Test Group	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Copulated Females	Animal Number
Control	0	0	5	22	1101 to 1122
Low dose	3	0.6	5	22	2101 to 2122
Middle dose	10	2	5	22	3101 to 3122
High dose	30	6	5	22	4101 to 4122

Route, formulation, volume, and infusion rate: oral gavage
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality (dams): one dam in the high dose group died one hour post-dose, probably test article related.

Clinical signs (dams):

Table 1 Study for effects on embryo-fetal development in rabbits treated orally with NS-304

Clinical signs in dams

Dose mg/kg	Signs	0-5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28-a)
		Administration																							
0	No. of dams	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0
	Decrease in the amount of feces	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0
3	No. of dams	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
	No. of dams with abnormal findings	0	0	1	0	0	0	0	0	1	1	2	2	1	1	1	1	0	0	0	0	0	0	0	0
	Decrease in the amount of feces	0	0	1	0	0	0	0	0	1	1	2	2	1	1	1	1	0	0	0	0	0	0	0	0
10	No. of dams	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
	No. of dams with abnormal findings	0	21	21	19	19	20	20	16	10	14	11	11	7	5	0	0	0	0	0	0	0	0	0	0
	Flushing(redness)	0	10	10	15	15	14	8	6	4	0	3	4	3	3	0	0	0	0	0	0	0	0	0	0
	Flushing around upper and lower lip, around eyelid	0	15	14	17	18	14	14	11	8	10	11	5	3	3	0	0	0	0	0	0	0	0	0	0
	Decrease in the amount of feces	0	0	0	0	0	0	1	0	0	1	1	2	2	2	0	0	0	0	0	0	0	0	0	0
30	No. of dams	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
	No. of dams with abnormal findings	0	21	21	21	21	21	21	21	21	21	21	21	21	21	1	1	0	0	0	0	0	0	0	0
	Flushing(redness)	0	21	21	21	20	21	21	21	21	21	21	21	21	19	0	0	0	0	0	0	0	0	0	0
	Flushing around upper and lower lip, around eyelid	0	21	21	21	20	21	21	21	21	21	21	21	21	21	0	0	0	0	0	0	0	0	0	0
	Hyperpnea	0	21	15	11	14	14	11	7	5	5	4	6	1	1	0	0	0	0	0	0	0	0	0	0
	Decrease in the amount of feces	0	0	11	5	4	3	2	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
	Stagnant around the anus region	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Decrease in excretion activity	0	0	1	0	1	1	1	1	0	0	1	1	1	2	0	0	0	0	0	0	0	0	0	0
	Soft feces	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Death	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

a): Gestational day

Body weight (dams):

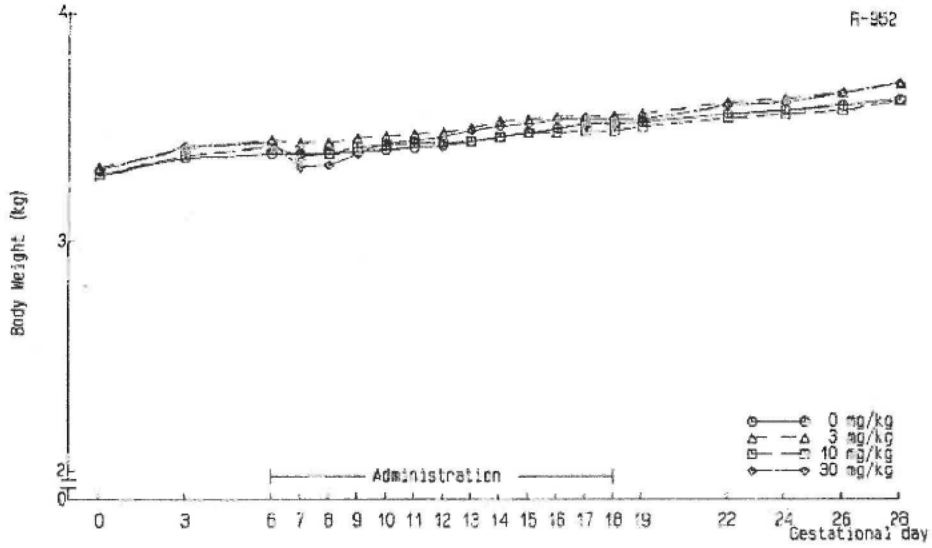

Fig. 1 Study for effects on embryo-fetal development in rabbits treated orally with NS-304
Body weight changes of dams

Food consumption (dams):

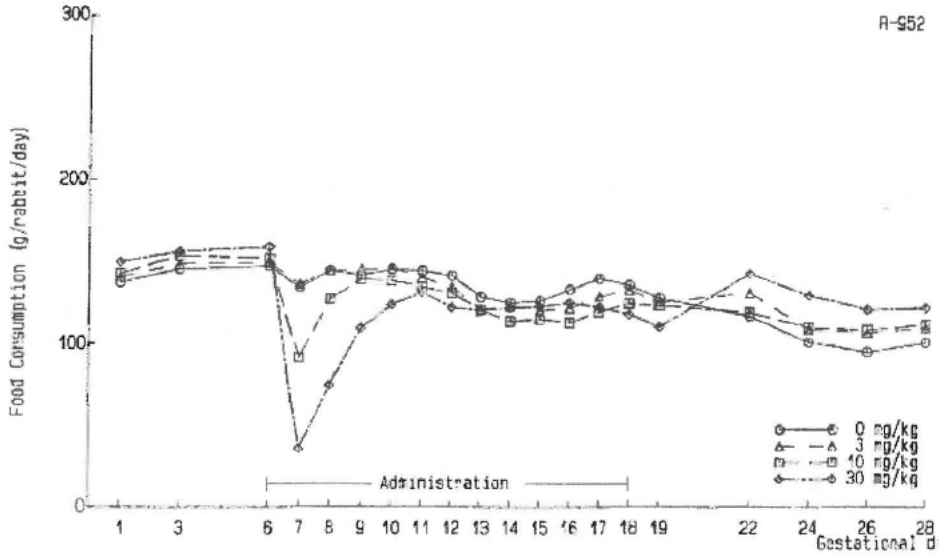

Fig. 2 Study for effects on embryo-fetal development in rabbits treated orally with NS-304
Food consumption of dams

Toxicokinetics:

Table II-1 Study for effects on embryo-fetal development in rabbits treated orally with NS-304
Plasma concentration of NS-304 (Gestational day 18)

Dose (mg/kg)	Animal number	Plasma concentration (μg/mL) Pre	0.5h	1h	2h	4h	8h	24h	Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg·h/mL)
0	1118	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	-
	1119	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	-
	1120	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	-
	Mean	0	0	0	0	0	0	0			
	S.D.										
3	2118	N.D.	0.137	N.D.	N.D.	N.D.	N.D.	N.D.	0.137	0.5	0.0685
	2119	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	-	0
	2120	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	-	0
	Mean	0	0.0457	0	0	0	0	0	0.0457	0.5	0.0228
	S.D.		0.0791						0.0791		0.0395
10	3118	N.D.	0.0665	N.D.	N.D.	N.D.	N.D.	N.D.	0.0665	0.5	0.0333
	3119	N.D.	0.336	0.0784	N.D.	N.D.	N.D.	N.D.	0.336	0.5	0.320
	3120	N.D.	0.233	0.0820	N.D.	N.D.	N.D.	N.D.	0.233	0.5	0.173
	Mean	0	0.208	0.0535	0	0	0	0	0.208	0.5	0.144
	S.D.		0.135	0.0463					0.135	0.0	0.106
30	4118	N.D.	2.55	1.81	0.394	N.D.	N.D.	N.D.	2.55	0.5	2.75
	4121	N.D.	0.921	0.492	0.0981	N.D.	N.D.	N.D.	0.921	0.5	0.974
	4122	N.D.	0.740	0.554	0.120	N.D.	N.D.	N.D.	0.740	0.5	0.868
	Mean	0	1.84	0.783	0.203	0	0	0	1.84	0.5	1.56
	S.D.		0.88	0.651	0.166				0.88	0.0	1.09

N.D.: Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean±S.D. and AUC.

Table 10-2 Study for effects on embryo-fetal development in rabbits treated orally with NS-304
Plasma concentration of MRE-289 (Gestational day 18)

Dose (mg/kg)	Animal number	Plasma concentration (μg/mL) Pre	0.5h	1h	2h	4h	8h	24h	Cmax (μg/mL)	Tmax (h)	AUC 0-24h (μg h/mL)
0	1118	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	-
	1119	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	-
	1120	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	-	-
	Mean S.D.	0	0	0	0	0	0	0			
3	2118	N.D.	0.490	0.306	0.204	0.192	N.D.	N.D.	0.506	1.0	1.20
	2119	N.D.	0.440	0.380	0.148	0.0703	N.D.	N.D.	0.440	0.5	0.964
	2120	N.D.	0.176	0.221	0.106	0.0713	0.0674	N.D.	0.221	1.0	1.30
	Mean S.D.	0	0.346 0.148	0.304 0.147	0.153 0.049	0.0812 0.0180	0.0225 0.0389	0	0.388 0.147	0.8 0.5	1.16 0.18
10	3118	N.D.	0.455	0.552	0.512	0.332	0.348	N.D.	0.552	1.0	5.06
	3119	N.D.	0.032	1.08	0.172	0.140	0.168	N.D.	1.08	1.0	8.01
	3120	N.D.	0.895	1.07	0.065	0.161	0.112	N.D.	1.07	1.0	3.46
	Mean S.D.	0	0.744 0.294	0.884 0.288	0.350 0.171	0.211 0.105	0.190 0.137	0	0.884 0.288	1.0 0.0	4.09 1.53
30	4118	N.D.	3.26	3.17	3.77	0.798	0.344	N.D.	3.17	1.0	19.4
	4121	N.D.	2.11	3.41	1.27	0.506	0.069	N.D.	3.41	1.0	13.1
	4122	N.D.	1.37	2.36	1.42	0.657	0.516	N.D.	2.39	1.0	11.7
	Mean S.D.	0	2.23 0.98	3.09 1.41	2.16 1.35	0.653 0.118	0.475 0.155	0	3.09 1.41	1.0 0.0	15.7 2.4

N.D.: Not detectable (<0.05 μg/mL)
N.D. was calculated as zero(0) for Mean±S.D. and AUC

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Table 4 Study for effects on embryo-fetal development in rabbits treated orally with NS-304
Gross pathological findings in dams

Dose mg/kg	0	3	10	30
No. of dams examined	22	21	22	21
No. of dams with abnormal findings	0	0	0	1 a)
Thymus : Discoloration, dark red	0	0	0	1
Lung : Discoloration, dark red	0	0	0	1
Stomach : Discoloration, dark red, mucosal	0	0	0	1
Subcutaneous : Hemorrhage, around the mammary gland region, many	0	0	0	1
: Hemorrhage, around the neck	0	0	0	1

a): Finding in dead dam

R-952

Table 5 Study for effects on embryo-fetal development in rabbits treated orally with NS-304

Cesarean section data on dams

Dose mg/kg	No. of dams		No. of corpora lutea	No. of implantations	Implantation index % a)	No. of resorbed or dead fetuses Total(%) b)	Early c)	Late d)	No. of live fetuses	Placental weight(g) Male	Female	Gross evaluation of placenta
0	22	Total	222	181		14	11	3	167			
		Mean	10.1	8.1	80.2	(8.0)			7.6	3.24	3.20	No abnormal findings
		S.D.	2.9	2.8	22.2	(10.1)			2.8	0.78	0.88	
3	21	Total	205	175		9	3	6	159			
		Mean	9.8	8.0	85.0	(5.4)			7.6	3.40	3.32	No abnormal findings
		S.D.	4.9	2.7	22.0	(8.5)			2.7	0.84	0.85	
10	22	Total	192	155		15	8	7	140			
		Mean	8.5	7.0	84.0	(12.0)			6.6	3.38	3.37	No abnormal findings
		S.D.	2.0	0.0	19.0	(21.4)			2.7	0.05	0.05	
30	20	Total	200	176		17	13	4	159			
		Mean	10.0	8.8	87.0	(10.4)			8.0	3.30	3.25	No abnormal findings
		S.D.	2.2	2.4	14.6	(18.4)			2.7	0.47	0.46	

a): (No. of implantations / No. of corpora lutea) X 100
b): (No. of resorbed or dead fetuses / No. of implantations) X 100
c): Implantation trace, resorbed embryo and placental remnant
d): Early macerated fetus, late macerated fetus and dead fetus
No significant difference from control group in any treated group

Offspring (malformations, variations, etc.):

R-953

Table 6 Study for effects on embryo fetal development in rabbits treated orally with NS-304

External examination of live fetuses

Dose mg/kg	No. of dams		No. of males	No. of females	Sex ratio a)	Fetal body weight(g) Male	Female	No. of fetuses with external abnormalities (%) b)
0	22	Total	75	92				0
		Mean	3.4	4.2	0.45	34.80	33.68	(0.0)
		S.D.	1.7	2.0	0.15	3.90	4.32	(0.0)
3	21	Total	64	94				c) 1
		Mean	3.0	4.5	0.40	35.98	34.41	(2.4)
		S.D.	1.7	2.0	0.19	3.33	4.38	(10.9)
10	21	Total	75	72				d) 3
		Mean	3.6	3.4	0.51	36.31	34.80	(1.8)
		S.D.	1.8	1.6	0.24	3.54	4.31	(4.6)
30	20	Total	84	75				0
		Mean	4.2	3.8	0.57	33.82	33.77	(0.0)
		S.D.	1.7	2.2	0.21	3.43	3.48	(0.0)

a): No. of males / No. of live fetuses
b): (No. of live fetuses with external abnormalities / No. of live fetuses) X 100
c): Brachyury
d): Hypoplasia in cavity, Amelia in forelimbs, Spina bifida, Paw hyperflexion in forelimb (left), Gastroschisis
No significant difference from control group in any treated group

Table 7 Study for effects on embryo-fetal development in rabbits treated orally with NS-304

Visceral examination of live fetuses

Dose (mg/kg)	0	30
No. of fetuses examined	167	159
No. of fetuses with abnormalities (%:Mean±S.D.)	0(0.0± 0.0)	2(1.1± 5.9)
Absent gallbladder	0(0.0± 0.0)	2(1.1± 5.9)
No. of fetuses with variation (%:Mean±S.D.)	8(6.1±17.4)	10(5.1±15.6)
Abnormal lung lobation (Absent accessory lobe)	3(5.8±10.8)	3(1.5± 4.9)
Abnormal origin of right subclavian artery	5(3.0± 7.3)	1(0.5± 2.2)
Retrocaval ureter (right)	0(0.0± 0.0)	6(3.1± 7.5)*J

*: p<0.05 (Significant difference from control group)
J: Wilcoxon's rank sum test

Table 8-1 Study for effects on embryo-fetal development in rabbits treated orally with NS-304 8-852

Skeletal examination of live fetuses

Dose (mg/kg)	0	30
No. of fetuses examined	167	159
No. of fetuses with abnormalities (%:Mean±S.D.)	8(5.8±10.0)	3(1.5± 4.9)
Fused sternebra	5(3.7± 9.0)	2(1.0± 3.1)
Branched rib	1(1.1± 5.3)	1(0.5± 2.2)
Fused rib	1(1.1± 5.3)	0(0.0± 0.0)
Absent thoracic vertebral arch	0(0.0± 0.0)	1(0.5± 2.2)
Absent lumbar arch	1(0.5± 2.1)	0(0.0± 0.0)
Lumbar hemivertebra	1(0.5± 2.1)	0(0.0± 0.0)
Malpositioned caudal vertebra	1(0.5± 2.1)	1(0.5± 2.2)
No. of fetuses with variation (%:Mean±S.D.)	7(4.3± 9.4)	10(5.5± 8.1)
Cervical rib	1(0.9± 4.3)	2(1.8± 4.8)
Asymmetric sternebra	1(1.1± 5.3)	1(0.5± 2.2)
Splitting of sternebra	1(0.4± 1.8)	2(1.0± 3.1)
Ossaitied talus	4(3.8± 6.0)	4(2.5± 4.5)
No. of fetuses with 13th rib (%:Mean±S.D.)	138(76.6±27.7)	117(74.4±23.7)

No significant difference from control group in any treated group

Prenatal and postnatal development: Not done

2.6.6.7 Local tolerance not done

2.6.6.8 Special toxicology studies

Study title: *in vitro* phototoxicity study

Volume #, and page #: eCTD 2.6.6.8.1 or 4.2.3.7.7
Conducting laboratory and location: not provided
Date of study initiation: not provided
GLP compliance: no
QA reports: yes ( ) no (x)
Drug, lot #, and % purity: ACT-333679 (code: MRE-269; batch number: ELB0066-1217.00) and ACT-293987 (code: NS-304; batch number: ELB0066-1216.00)

Formulation/vehicle: 10 mM stock solution in DMSO, final dilution in phosphate buffered saline.

Methods

Doses:
ACT-333679; +UVA: 100.0, 33.3, 11.1, 3.6, 1.2, 0.4, 0.14, 0.05 µM
ACT-333679; -UVA: 100.0, 33.3, 11.1, 3.6, 1.2, 0.4, 0.14, 0.05 µM
ACT-333679; +UVA: 1,000.0, 333.3, 111.1, 37, 12.3, 4.1, 1.4, 0.5 nM
ACT-333679; -UVA: 1,000.0, 333.3, 111.1, 37, 12.3, 4.1, 1.4, 0.5 nM

ACT-293987; +UVA: 100.0, 33.3, 11.1, 3.6, 1.2, 0.4, 0.14, 0.05 µM
ACT-293987; -UVA: 100.0, 33.3, 11.1, 3.6, 1.2, 0.4, 0.14, 0.05 µM
ACT-293987; +UVA: 1,000.0, 333.3, 111.1, 37, 12.3, 4.1, 1.4, 0.5 nM
ACT-293987; -UVA: 1,000.0, 333.3, 111.1, 37, 12.3, 4.1, 1.4, 0.5 nM

Study design:

Results:

Table 1 Survival data

ACT-293987				ACT-333679			
ACT-293987 [uM]	ACT-293987 [nM]	Presence of UV (% Survival)	Absence of UV (% Survival)	ACT-333679 [uM]	ACT-333679 [nM]	Presence of UV (% Survival)	Absence of UV (% Survival)
100.00		25.96	21.05	100.00		25.24	24.14
33.33		26.35	21.39	33.33		26.19	24.38
11.11		25.45	22.68	11.11		24.88	25.09
3.60		26.99	55.32	3.60		25.48	76.45
1.20		26.09	97.65	1.20		25.46	96.22
0.40		26.22	93.39	0.40		25.71	96.33
0.14		26.99	95.07	0.14		24.40	97.87
0.05		29.95	100.00	0.05		23.57	100.00
	1000.00	24.31	92.55		1000.00	23.73	87.58
	333.30	32.67	94.38		333.30	25.90	87.20
	111.10	72.94	95.99		111.10	73.49	92.42
	37.00	92.85	98.05		37.00	96.75	94.41
	12.30	95.60	93.46		12.30	98.92	97.89
	4.10	97.14	98.22		4.10	96.39	92.17
	1.40	96.15	98.51		1.40	100.00	93.42
	0.50	95.05	99.09		0.50	96.27	98.39

% Survival was calculated with respect to mean maximal value of hexaplicates obtained across all treated and non-treated samples.

2.6.6.9 Discussion and Conclusions: Although both substances, at concentrations of approx 100nM and higher, were positive for phototoxicity enabled by UVA, the drug does not accumulate in the skin or pigmented cells near the surface, so risk of such toxicity may be low.

2.6.6.10 Tables and Figures

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2.6.7 TOXICOLOGY TABULATED SUMMARY

Table 8 Incidence of intussusception in the dog

Study	LOEL	Mortality (Found dead or humanely killed)	Day	Gender
Single-Dose (plus 15 days observation) [T-08.273]	200 mg/kg	N = 1 out of 6	7	(FD)
2-week study [T-08.277]	20 mg/kg/day	N = 3 out of 6	6 10 13	(FD) (UN) (FD)
4-week study + 4 week recovery [T-08.290]	6 mg/kg/day	N = 1 out of 10 Intussusception observed during routine necropsy	29	
39-week study [T-08.286]	4 mg/kg/day	N = 2 out of 12	24 124	(UN) (UN)

FD = found dead; UN = unscheduled necropsy

Table 9 Safety margins for intussusception based on AUC data

Drug	Dog study	NOEL (mg/kg/day/day)	Mean AUC_{0-24} (ng.h/mL)	Safety* margins	Corrected safety margins**
ACT-333679	2-week study	6	154,000	562	5
ACT-293987	[T-08.277]		24,675	355	4
ACT-333679	4-week study	3	84,255	308	3
ACT-293987	recovery period (4-week) [T-08.290]		13,720	197	2
ACT-333679	39-week study	2	49,700	181	2
ACT-293987	[T-08.286]		7,345	106	1

*Achieved plasma exposure after administration of 1,600 µg ACT-293987 b.i.d. at steady state in humans subjects: ACT-293987 AUC_{0-24h} of 69.6 ng·h/ml ; ACT-333679 (active metabolite) AUC_{0-24h} of 274 ng·h/ml.

**Taking into account the species difference between dogs and humans of *in vitro* inhibition of ADP-induced platelet aggregation by ACT-333679 (b)(4)[B-08.253]. The exposure at the respective NOAEL is corrected for the species difference of *in vitro* IC_{50} values on platelet aggregation inhibition. The ratio $IC_{50\,dog}/IC_{50\,human}$ is 119 with ACT-333679 and 83 with ACT-293987. The safety margin is corrected by this factor.

Table 10 Safety margins for intussusception based on C_{max} data

Drug	Dog Study	NOEL (mg/kg/day/day)	Mean C_{max} (ng/mL)	Safety* margins	Corrected Safety margins**
ACT-333679	2-week study	6	14,135	790	7
ACT-293987	[T-08.277]		9,290	845	7
ACT-333679	4-week study	3	7,575	423	4
ACT-293987	recovery period (4-week) [T-08.290]		4,620	387	3
ACT-333679	39-week study	2	5,580	312	3
ACT-293987	[T-08.286]		2,445	222	2

* Achieved plasma exposure after administration of 1,600 µg ACT-293987 b.i.d. at steady state in human subjects: ACT-333679 (active metabolite): C_{max} 17.9 ng/ml.; ACT-293987 (drug): C_{max} 11 ng/ml.

** Taking into account the species difference between dogs and humans of *in vitro* inhibition of ADP-induced platelet aggregation by ACT-333679 [B-08.253]. The ratio of inhibition of ADP induced platelet aggregation by ACT-333679 between dogs and humans is 119. The safety margin is corrected by this factor.

Table 11 Inhibition of platelet aggregation in platelet-rich plasma (b)(4)[B-08.253]

	IC_{50} of platelet aggregation (µM)		
Drug	Human	Dog	Rat
ACT-333679	0.21 (88.1 ng/mL)	25 (10,488 ng/mL)	10 (4,195 ng/mL)
ACT-293987	5.5 (2,731 ng/mL)	456 (226,459 ng/mL)	–

– = not tested

ACT-333679 (active metabolite): C_{max} 17.9 ng/ml.; ACT-293987 (drug): C_{max} 11 ng/ml.

Table 12 Safety margins for drug-related bleeding potential based on AUC data

Compound	Human[1]		Dog[2]		Rat[3]		
	AUC (ng.h/mL)	Mean AUC (ng.h/mL)	Safety margin	Corrected safety margin[4]	Mean AUC (ng.h/mL)	Safety margin	Corrected safety margin[4]
ACT-333679	274	554,000	2,022	17	139,000	507	11
ACT-293987	69.6	90,000	1,293	16	12,000	172	na

1 Achieved plasma exposure after administration of 1,600 µg ACT-293987 b.i.d. at steady state in human subjects: ACT-293987 AUC_{0-24h} of 69.6 ng·h/mL; ACT-333679 (active metabolite) AUC_{0-24h} of 275 ng·h/mL.
2 Dog NOAEL 20 mg/kg/day/day, 2-week oral toxicity study [T-08.277]
3 Rat NOAEL 100 mg/kg/day/day, 26-week oral toxicity study [T-08.285]
4 The exposure at the respective NOAEL is corrected for the species difference of *in vitro* IC_{50} values on platelet aggregation inhibition. The ratio $IC_{50\,dog}/IC_{50\,human}$ is 119 with ACT-333679 and 83 with ACT-293987. The ratio $IC_{50\,rat}/IC_{50\,human}$ is 48 with ACT-333679 [Table 11].

Table 13 Safety margins for bleeding based on C_{max} data

Compound	Human[1]		Dog[2]		Rat[3]		
	C_{max} (ng/mL)	Mean C_{max} (ng/mL)	Safety margin	Corrected safety margin[4]	Mean C_{max} (ng/mL)	Safety margin	Corrected safety margin[4]
ACT-333679	17.9	40,000	2,235	19	28,175	1,574	33
ACT-293987	11	22,630	2,057	25	6,070	552	na

1 Achieved plasma exposure after administration of 1600 µg ACT-293987 b.i.d. at steady state in human subjects: ACT-333679 (active metabolite): C_{max} 17.9 ng/mL; ACT-293987 (drug): C_{max} 11 ng/mL
2 Dog NOAEL 20 mg/kg/day/day, 2-week oral toxicity study [T-08.277]
3 Rat NOAEL 100 mg/kg/day/day, 26-week oral toxicity study [T-08.285]
4 The exposure at the respective NOAEL is corrected for the species difference of IC_{50} values on platelet aggregation inhibition. The ratio $IC_{50\,dog}/IC_{50\,human}$ is 119 with ACT-333679 and 83 with ACT-293987.
The ratio $IC_{50\,rat}/IC_{50\,human}$ is 48 with ACT-333679 [Table 11].

Table 14 Safety margin for increased ossification in dogs based on AUC data

Study	Bone finding in dogs	NOEL (mg/kg/day)	Mean AUC_{0-24} (ng·h/mL)	Safety margins for ACT-333679*	Corrected safety margins**
2-week study [T-08.277]	Bone (femur) increased ossification in the trabeculae (males and females) and/or increased ossification of the periosteum (females)	2	59.290	216	2
4-week study [T-08.290]	Bone (femur) increased ossification in the trabeculae (males and females) and/or increased ossification of the periosteum (males)	1.5	47.110	172	1
39-week study [T-08.286]	Bone (femur) increased ossification in the trabeculae (males and females)	**LOEL** 1	29.400	107	1
39-week study interim evaluation (26-week) [T-08.286]	Bone (femur) increased ossification in the trabeculae (females)	2	49.700***	181	2

* Achieved plasma exposure after administration of 1.600 µg ACT-293987 b.i.d. at steady state in humans subjects: ACT-333679 (active metabolite) AUC_{0-24h} of 274 ng·h/ml.

** Taking into account the species difference between dogs and humans of *in vitro* inhibition of ADP-induced platelet aggregation by ACT-333679 [(b)(4) 08.253]. The ratio of inhibition of ADP-induced platelet aggregation by ACT-333679 between dogs and humans is 119. The safety margin is corrected by this factor

*** Exposures at the end of the study (39-week).

LOEL = lowest observed effect level

Table 15 Binding affinity for human receptors

	Binding affinity (Ki, μM) for human receptor			
Drug	IP	EP_2	EP_4	Fold differences for EP vs IP receptor affinities (worst case scenario)
ACT-333679 (MW 419)	0.02 (8.4 ng/mL)	5.8 (2,436 ng/mL)	4.9 (2,058 ng/mL)	245
ACT-293987 (MW 496)	0.26 (129 ng/mL)	>10 (4,960 ng/mL)	>10 (4,960 ng/mL)	38

References (b)(4)-08.217] to (b)(4)-08.222] and (b)(4)-08.255]
MW = molecular weight

Table 16 Human safety margin for EP_2/EP_4 subtype receptor binding

Drug	EP_2 (Ki)	Margins EP_2 vs human achieved** C_{max} at 1600μg b.i.d.	EP_4 (*Ki*)	Margins EP_2 vs human achieved** C_{max} at 1,600 μg b.i.d.
ACT-333679**	5.8 μM (2,436 ng/mL)	136	4.9 μM (2,058 ng/mL)	115
ACT-293987**	>10 μM (4,960 ng/mL)	451	>10 μM (4,960 ng/mL)	451

**Achieved concentration at 1,600 μg b.i.d: C_{max} 17.9 ng/mL (ACT-333679) or C_{max} 11 ng/mL (ACT-293987)

Table 17 Incidence of bone marrow fibrosis and associated findings in dogs

Study	Dose (mg/kg/day)	Incidence: Bone marrow fibrosis	Incidence: White/red cell counts decrease	Incidence: Extramedullary hematopoiesis
2-week study [T-08.277]	6	5 out of 6	√	X
	20	3 out of 3	√	X
4-week study [T-08.290]	3	4 out of 6	X	X
	6	6 out of 6	√	3 out of 6
4-week recovery period (4-week study) [T-08.290]	3	0 out of 4	X	X
	6	0 out of 4	X	X
39-week study [T-08.286]	4	3 out of 5	X	X

√ = sign present
X = not observed

Table 18 Safety margin for bone marrow fibrosis

Study	Bone marrow finding in dogs	NOEL (mg/kg/day)	Mean AUC_{0-24} (ng·h/mL)	Safety margins* ACT-333679	Corrected Safety margins** ACT-333679
2-week study [T-08.277]	Bone marrow fibrosis	2	59,290	216	2
4-week study [T-08.290]	Bone marrow fibrosis	1.5	47,110	172	1
4-week study + 4-week recovery period [T-08.290]	Bone marrow fibrosis	6	180,250	658	6
39-week study [T-08.286]	Bone marrow fibrosis	2	49,700	181	2

*Achieved plasma exposure after administration of 1,600 µg ACT-293987 b.i.d. at steady state in human subjects: ACT-333679 (active metabolite) AUC_{0-24} of 274 ng·h/mL.
** Taking into account the species difference between dogs and humans of *in vitro* inhibition of ADP-induced platelet aggregation by ACT-333679 [(b) -08.253]. The ratio of inhibition of ADP-induced platelet aggregation by ACT-333679 between dogs and humans is 119. The safety margin is corrected by this factor.
NOEL = No observed effect level

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary: ACT-293987 is a prostacyclin agonist that has an ester group conjugated to it to improve oral bioavailability .It is rapidly metabolized to ACT-333679, the primary active compound. For this submission, the indication is for treatment of pulmonary arterial hypertension (PAH).

The primary pharmacology studies project efficacy in PAH based on appreciable relief in models of PAH and an ability to reduce right ventricular hypertrophy (RVH)in such. The receptor binding profile suggests that the basis for this efficacy is selective binding to the IP receptor..

Some of the positive findings in the safety pharmacology studies reflect prostacyclin pharmacology. The cardiovascular safety studies indicated flushing, hypotension, and increased bleeding times - issues to be aware of, although platelet inhibiton and vasodilatation are expected of prostacyclin agonists.r There were also positive CNS findings i.e., in the modified Irwin screening, and manifest as changes in body temperature, sleep, pain response, and respiration rates. . ACT-293987 also decreased urinary sodium and potassium excretion.. Prostacyclin receptors mediating smooth muscle tone are common in the gastrointestinal tract, and intussusception was observed at necropsy, especially in the dog. Most adverse events for prostacyclin agonists are related to their effects on the gastrointestinal system.

ACT-293987 is rapidly and extensively absorbed from the gastro-intestinal tract with a Tmax of approximately 1 hr and a bioavailability of 80 to 90%. Radiolabeled ACT-293987 accumulates first in the liver after oral dosing, followed by accumulation in the stomach. This is probably due to the high concentration of receptors present in the stomach, where prostacyclin agonists apparently have high activity. ACT-293987 is primarily.,excreted through the biliary system (i.e., enterohepatic) with only 2% being excreted by the kidneys. ACT-293987 is a pro-drug, although it also has activity at prostacyclin receptors, and is hydrolyzed to ACT-333697, the primary prostacyclin agonist. ACT-333697, one of up to 15 potential metabolites of ACT-293987, is glucuronidated primarily and excreted through the biliary system..

General toxicology:

From the mouse studies:

Study Length	Doses	Results	NOAEL
4 weeks	0, 30, 100, 300 mg/kg/day	No mortality, @300 mg/kg/day flaccidity and flushing	100 mg/kg/day
13 weeks	0, 100, 300, 500 mg/kg/day	500 mg/kg/day one mortality, CK & ALT increase; 300 mg/kg/day decrease in food consumption, flushing, flaccidity, BUN decreased, kidney tubular vacuolation	100 mg/kg/day

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From the rat studies:

Study Length	Doses	Results	NOAEL

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4 weeks	0, 20, 60, 180 mg/kg/day	20 mg/kg/day alveolar hemorrhage and decreased platelets, loss or black discoloration of tail tip;	Not determined
4 weeks + 4 weeks recovery	2, 6, 60 mg/kg/day	60 mg/kg/day flushing, decreased movement, piloerection, reduced body wt and food consumption. Reversible	6 mg/kg/day
26 weeks + 4 week recovery	0, 6, 25, 100 mg/kg/day	>25, 100 mg/kg/day liver and adrenal hypertrophy, hyperplasia mammary gland, salivary gland, follicular cells in thyroid; all treated animals, flushing, decreased movement, reversible; one animal died in high dose group of malignant lymphoma	6 mg/kg/day

From the dog studies:

Study length	Doses	Results	NOAEL
2 weeks	2, 6, 20 mg/kg/day	20 mg/kg/day led to mortalities, Intussuseception, QTc prolongation; 6 mg/kg/day increased ossification, bone marrow fibrosis; 2 mg/kg/day decreased platelet, wbc, & neutrophils.	2 mg/kg/day
4 weeks	1.5, 3, 6 mg/kg/day	6 mg/kg/day intussusception; 3 mg/kg/day intussusception, bone marrow fibrosis, ossification; 1.5 mg/kg/day vomiting, diarrhea, jelly feces	1.5 mg/kg/day
39 weeks	1, 2, 4 mg/kg/day	4 mg/kg/day 2 mortalities, intussusception; 1mg/kg/day bone marrow fibrosis and ossification	Not determined

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Genetic toxicology: ACT-293987 was negative in most testing except for a small signal of clastogenicity in the Chinese Hamster Lung cell in vitro assay. However, the in vivo mouse micronucleus assay for clastogenicity was negative..

Carcinogenicity: not done

Reproductive toxicology: For the rat studies, the NOAEL was the same as for the standard toxicity testing, 6 mg/kg/day. In this species, but not the rabbit, the primary issue appeared to be low birth weight, which in humans has been correlated with developmental difficulties.
In the rabbit study, the NOAEL was 10 mg/kg/day,and one animal died at the highest dose tested i.e., 30 mg/kg/day, Reproductive function and fetal development were not affected.

Special toxicology: **In an** *in vitro* phototoxicity study both ACT-293987 and ACT-333697 were positive for phototoxicity with UVA light. Phototoxic concentrations were approx 100 nm (0.1uM) and above, relatively high exposures unlikely to be achieved at therapeutic dosages since no accumulation was observed in skin or eye.

Based on the animal safety studies, ACT-293987 and its active metabolite ACT-333697 primarily carry, risk of intussusception, and bleeding due to platelet effects. and. However, PAH is a high risk disease state, and with the appropriate safety measures in place , it should be reasonably safe to begin clinical trials at the proposed starting dose . Also, the prospect of an orally available prostacyclin agonist may be attractive for the treatment of these patients considering the other prostacyclin agonists are only available in intravenous or nebulizer formulations.

Internal comments:

External comments (to sponsor):

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes _x__ No ___

APPENDIX/ATTACHMENTS

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-104504	ORIG-1	ACTELION PHARMACEUTICALS LTD	ACT-293987 (Prostacyclin Receptor Agonist)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JAMES M WILLARD
05/20/2010

ALBERT F DEFELICE
06/28/2010