Total Mortality and Moribundity, Separated by Sex						
Group	Substance	Pitavastatin dose (mg/kg)	Male mortality (dead/total)	Male mortality (%)	Female mortality (dead/total)	Female mortality (%)
1	-pitavastatin -urethane	Vehicle only	1/9	11.1	0/9	0
2	-pitavastatin +urethane	Vehicle only	3/15	20.0	3/15	20.0
3	+pitavastatin -urethane	150	0/12	0	1/12	4.2
4	+pitavastatin +urethane	150	1/15	6.7	3/15	20.0

Clinical signs:

All Clinical Observations (Days/Animals)								
Clinical signs	Males				Females			
	-U -P	+U -P	-U +P	+U +P	-U -P	+U -P	-U +P	+U +P
	n=9	n=15	n=12	n=15	n=9	n=15	n=12	n=15
Dead	1/1	3/3	-	1/1	-	3/3	1/1	2/2
Moribund	-	-	-	-	-	-	-	1/1
Fracture of hindlimb	-	-	-	-	-	-	117/1	-
Tachypnea	-	-	-	5/1	-	-	-	-
Decreased locomotor activity	-	-	4/2	9/6	-	1/1	11/4	5/3
Hindlimb swelling	10/1	-	-	-	-	-	-	-
Loss of hair	152/2	-	-	-	-	261/2	328/2	169/2

Body weights: At the end-of-study, Day 182 time point, male mice administered the vehicle-only plus urethane weighed slightly but statistically significantly greater then vehicle-only controls. At the final time point, female mice administered pitavastatin plus urethane weighed less than urethane-only controls.

Food consumption: There were no trends for any statistically significant differences in food consumption in male or female mice. Statistically significant increases tended to be closely followed by statistically significant decreases, and *vice versa.*

Water consumption: Male mice administered a single dose of urethane by i.p. injection tended to have different water consumption from controls, with no consistent effect noted. Male mice administered pitavastatin showed a significant increase in water consumption compared to control mice at almost every time point. In males administered urethane (single i.p.) and pitavastatin (daily) there were few differences in water consumption compared to controls. Females administered pitavastatin (with and without urethane) consumed highly significantly more water than controls. The clinical significance of this is unknown.

Body and organ weights: There were no clinically significant findings in body and organ weight for any group administered pitavastatin.

Notable Changes in Body and Organ Weights as a Percent (%) of Vehicle-only Controls (VC) or +Urethane-only controls (UC)								
Organ	-Urethane -Pitavastatin		+Urethane -Pitavastatin		-Urethane +Pitavastatin		+Urethane +Pitavastatin	
	-		vs. VC		vs. VC		vs. UC	
	♂	♀	♂	♀	♂	♀	♂	♀
Total body	-	-	↑7*	NC	NC	NC	NC	↓6***
Lung	-	-	NC	NC	NC	↓10	NC	↓10
Spleen	-	-	↑32	↑25**	NC	↑13	↓10	↑10

***Indicates p<0.01,** statistically significant difference by absolute weight.
****Indicates p<0.05,** statistically significant difference by % body weight.
*****Indicates p<0.05**, statistically significant difference by absolute weight.

NC indicates <10% change and not statistically significant (p>0.05)

Hematology:

Hematology data were not collected.

Gross pathology:

Scheduled necropsy- There were no histopathology correlates noted for nodules in uterus, hypertrophy in thymus, and in a mass found in ovary in animals necropsied at study termination. Among animals found dead or moribund, there were no histopathological correlates for a mass in the thoracic cavity, or in a mass associated with the portal vein of liver.

There were histopathological correlates for nodules and masses observed in spleen and in lungs (A/B). Hyperplasia was observed in the fore-stomachs of the majority of animals administered pitavastatin, however there were no correlating neoplastic lesions associated with hyperplasia.

Gross Pathology (Terminal Necropsy)									
Tissue	Finding	Males				Females			
		-U -P	+U -P	-U +P	+U +P	-U -P	+U -P	-U +P	+U +P
		n=8	n=12	n=12	n=14	n=9	n=12	n=11	n=12
Abdominal cavity	Fluid retention	-	-	-	-	-	-	-	1
Lungs (with bronchus)	Nodule, single	2	6	3	10	2	11	1	12
	Nodule, multiple	-	1	-	2	-	-	-	-
	Discoloration	-	1	-	1	1	1	-	1
	Mass	-	1	-	-	-	-	-	-
	Mottled	-	-	-	2	-	-	-	-
Thoracic cavity	Retention of pleural effusion	-	-	-	-	-	1	-	-
Uterus	Nodule, single	N/A	N/A	N/A	N/A	-	1	-	-
Skin	Hair loss, localized	-	-	-	-	-	1	2	-
Fore-stomach	Elevated area	-	-	1	1	-	-	1	
	Thickening	-	-	10	14	-	-	11	12
Femur	Atrophy of skeletal m.	-	-	-	-	-	-	1	-
Thymus	Hypertrophy	-	1	-	-	-	-	-	-
Subcutis, inguinal	Hematoma	-	-	-	1	-	-	1	-
	Hemorrhage	-	-	-	1	-	-	-	-
Ovary	Mass	N/A	N/A	N/A	N/A	-	-	1	-
Spleen	Nodule, single	1	7	-	3	-	3	-	4
	Nodule, multiple	-	-	-	1	-	-	-	1
	Discoloration	-	1	3	3	3	2	1	2
	Hematoma	-	-	1	-	-	-	-	-
	Mottled	-	-	2	-	-	1	-	-
	Mass, multiple	-	-	-	1	-	-	-	-

Unscheduled/early necropsy –

Gross Pathology (Moribund/Found Dead Necropsy)									
Tissue	Finding	Males				Females			
		-U -P	+U -P	-U +P	+U +P	-U -P	+U -P	-U +P	+U +P
		n=1	n=3	n=0	n=1	n=0	n=3	n=1	n=3
Abdominal cavity	Hemoabdomen	1	1	-	-	-	1	1	-
Adrenal	Congestion	-	1	-	-	-	-	-	-
Hindlimb	Swelling	1	-	-	-	-	-	-	-
	Hematoma	1	-	-	-	-	-	-	-
Skin	Hair loss	1	-	-	-	-	1	-	-
Fore-stomach	Thickening	-	-	-	1	-	-	1	2
Spleen	Hypertrophy	1	-	-	-	-	1	-	-
	Hematoma	-	2		-	-	1	1	-
	Adhesion of blood clot	-	-	-	-	-	1	-	-
	Nodule	-	1	-	-	-	-	-	-
	Discoloration	-	-		-	-	-	-	1
Thoracic cavity	Blood-filled	-	1	-	1	-	1	-	2
	Mass	-	1	-	-	-	-	-	-
	Pleural effusion		1	-	-	-	-	-	-
Liver	Congestion	-	-	-	-	-	1	-	1
	Mass, portal vein	-	-	-	-	-	-	-	1
Kidneys	Congestion	1	-	-	-	-	-	-	-
	Concave area and white elevated object	-	-	-	-	-	-	-	1
Lungs, with bronchus	Congestion	1	1	-	-	-	1	1	2
	White mottle	-	1	-	-	-	1	-	-
	Red discoloration	-	1	-	1	-	-	-	-
	Nodule, single	-	1	-	-	-	-	-	-
	Nodule, multiple	-	-	-	1	-	-	-	1

Histopathology:

Nodules and masses observed in spleen and lung tissues were confirmed as neoplastic lesions upon histopathological examination.

Summary of non-neoplastic findings –

Summary of Notable Non-neoplastic and Neoplastic* Findings

Tissue	Finding		Males			
			-U -P (n=9)	+U -P (n=15)	-U +P (n=12)	+U +P (n=15)
Lungs, with bronchus	Adenoma*		2	7	3	8
	Carcinoma*		-	4	-	4
	Hyperplasia	Min	4	3	5	7
	Granulation	Min	(b) (4)			
	Inflammation	Min	1	1	1	6
Spleen	Hemangiosarcoma*		-	8	1	7
	Fibrosis	Min	-	-	3	2
		Mild	4	2	-	-
Forestomach	Hyperkeratosis	Min	-	-	9	6
		Mild	-	-	3	8
	Hyperplasia	Min	-	-	9	6
		Mild	-	-	3	7
		Mod	-	-	-	1
	Erosion	Min	-	-	2	-
		Mild	-	-	-	1
	Inflammation	Min	-	-	5	6
		Mild	-	-	-	1

Summary of Notable Non-neoplastic and Neoplastic* Findings

Tissue	Finding		Females			
			-U -P (n=9)	+U -P (n=15)	-U +P (n=12)	+U +P (n=15)
Lungs, with bronchus	Adenoma*		1	6	1	10
	Carcinoma*		1	4	1	1
	Hyperplasia	Min	2	3	2	4
	Granulation	Min	(b) (4)			
	Inflammation	Min	-	6	2	6
Spleen	Hemangiosarcoma*		-	6	-	5
	Fibrosis	Min	-	-	-	1
Forestomach	Hyperkeratosis	Min	-	-	-	1
		Mild	-	-	10	11
		Mod			1	-
	Hyperplasia	Min	-	-	-	1
		Mild	-	-	10	11
		Mod	-	-	1	-
	Erosion	Min	-	-	2	3
	Inflammation	Min	-	-	7	9

Full histopathology tables follow.

Non-neoplastic:

ALL HISTOPATHOLOGY FINDINGS, TERMINAL AND UNSCHEDULED, MALES

Male

Organ		Group-No.	01	02	03	04
		Test article	Control	Control (+ Urethane)	NK-104	NK-104 (+ Urethane)
		Dose : mg/kg	0	0	150	150
Lung(with bronchus)		Total	(8)	(12)	(12)	(14)
	Alveolar /Bronchiolar adenoma	–	6	5	9	6
		P	2	7	3	8
	Alveolar /Bronchiolar carcinoma	–	8	8	12	10
		P	0	4	0	4
	Alveolar /Bronchiolar hyperplasia	–	4	9	7	7
		±	4	3	5	7
		+	0	0	0	0
		++	0	0	0	0
		+++	0	0	0	0
	Granulation					(b) (4)
	Hemorrhage	–	8	11	12	14
		±	0	1	0	0
		+	0	0	0	0
		++	0	0	0	0
		+++	0	0	0	0
	Inflammation	–	7	11	11	8
		±	1	1	1	6
		+	0	0	0	0
		++	0	0	0	0
		+++	0	0	0	0
Spleen		Total	(8)	(12)	(12)	(14)
	Hemangiosarcoma	–	8	4	11	7
		P	0	8	1	7
	Fibrosis	–	4	10	9	12
		±	3	1	3	2
		+	1	1	0	0
		++	0	0	0	0
		+++	0	0	0	0
	Angiectasis	–	8	12	11	14
		±	0	0	1	0
		+	0	0	0	0
		++	0	0	0	0
		+++	0	0	0	0

–:No abnormal changes ±:Minimal +:Mild ++:Moderate +++:Marked P:Non-graded change

(Sponsor, M4, G2536, p292)

ALL HISTOPATHOLOGY FINDINGS, TERMINAL AND UNSCHEDULED, MALES (CONT.)

Male

Organ		Group-No. / Test article / Dose : mg/kg	01 Control 0	02 Control (+ Urethane) 0	03 NK-104 150	04 NK-104 (+ Urethane) 150
Spleen	Deposit, hemosiderin	–	8	12	9	13
		±	0	0	3	1
		+	0	0	0	0
		++	0	0	0	0
		+++	0	0	0	0
Forestomach		Total	(8)	(12)	(12)	(14)
	Hyperkeratosis	–	8	12	0	0
		±	0	0	9	6
		+	0	0	3	8
		++	0	0	0	0
		+++	0	0	0	0
	Hyperplasia	–	8	12	0	0
		±	0	0	9	6
		+	0	0	3	7
		++	0	0	0	1
		+++	0	0	0	0
	Erosion	–	8	12	10	13
		±	0	0	2	0
		+	0	0	0	1
		++	0	0	0	0
		+++	0	0	0	0
	Inflammation	–	8	12	7	7
		±	0	0	5	6
		+	0	0	0	1
		++	0	0	0	0
		+++	0	0	0	0

–:No abnormal changes ±:Minimal +:Mild ++:Moderate +++:Marked P:Non-graded change

(Sponsor, M4, G2536, p292)

ALL HISTOPATHOLOGY FINDINGS, TERMINAL AND UNSCHEDULED, FEMALES

Female

Organ		Group-No. / Test article / Dose : mg/kg	01 Control 0	02 Control (+ Urethane) 0	03 NK-104 150	04 NK-104 (+ Urethane) 150
Lung(wth bronchus)		Total	(9)	(12)	(11)	(12)
	Alveolar /Bronchiolar adenoma	−	8	6	10	2
		P	1	6	1	10
	Alveolar /Bronchiolar carcinoma	−	8	8	10	11
		P	1	4	1	1
	Alveolar /Bronchiolar hyperplasia	−	7	9	9	8
		±	2	3	2	4
		+	0	0	0	0
		++	0	0	0	0
		+++	0	0	0	0
	Granulation					(b) (4)
	Inflammation	−	9	6	9	6
		±	0	6	2	6
		+	0	0	0	0
		++	0	0	0	0
		+++	0	0	0	0
Spleen		Total	(9)	(12)	(11)	(12)
	Hemangiosarcoma	−	9	6	11	7
		P	0	6	0	5
	Fibrosis	−	9	12	11	11
		±	0	0	0	1
		+	0	0	0	0
		++	0	0	0	0
		+++	0	0	0	0
	Deposit, hemosiderin	−	6	12	10	11
		±	3	0	1	1
		+	0	0	0	0
		++	0	0	0	0
		+++	0	0	0	0

−:No abnormal changes ±:Minimal +:Mild ++:Moderate +++:Marked P:Non-graded change

(Sponsor, M4, G2536, p291)

ALL HISTOPATHOLOGY FINDINGS,
TERMINAL AND UNSCHEDULED, FEMALES (CONT.)

Female						
		Group-No.	01	02	03	04
		Test article	Control	Control (+ Urethane)	NK-104	NK-104 (+ Urethane)
Organ		Dose : mg/kg	0	0	150	150
Forestomach		Total	(9)	(12)	(11)	(12)
	Hyperkeratosis	–	9	12	0	0
		±	0	0	0	1
		+	0	0	10	11
		++	0	0	1	0
		+++	0	0	0	0
	Hyperplasia	–	9	12	0	0
		±	0	0	0	1
		+	0	0	10	11
		++	0	0	1	0
		+++	0	0	0	0
	Erosion	–	9	12	9	9
		±	0	0	2	3
		+	0	0	0	0
		++	0	0	0	0
		+++	0	0	0	0
	Inflammation	–	9	12	4	3
		±	0	0	7	9
		+	0	0	0	0
		++	0	0	0	0
		+++	0	0	0	0

–:No abnormal changes ±:Minimal +:Mild ++:Moderate +++:Marked P:Non-graded change

(Sponsor, M4, G2536, p292)

Carcinogenicity Summary

Initial carcinogenicity studies were performed prior to submission of the initial IND. In a 92-week mouse carcinogenicity study at doses of 1, 12, 30, 75 mg/kg/day of pitavastatin by oral gavage, survival was impaired in males by 37% and females by 47%. High incidences of liver and forestomach hypertrophy and hyperplasia and skeletal myofiber atrophy were observed. Executive Carcinogenicity Assessment Committee (ECAC) considered the dosing inadequate in males and requested further analysis to adjust for the excess deaths in the high dose group in the first year of the study as well as a peer review of the stomach histopathology to determine if the hyperplastic lesions may have progressed over time to a neoplastic response since carcinogenicity studies are typically 104 weeks duration.

In a 92-week rat carcinogenicity study at doses of 1, 5, 25 mg/kg/day by oral gavage, ECAC found the dose selection to be adequate. Survival in females at week 92 was 29%, 33%, 62%, 60%, respectively, resulting in the early termination of the females. Male survival at week 104 was 35%, 38%, 38%, 56%, respectively. Survival in controls for each gender was similar to the lowest dose group. A high incidence of hypertrophy and hyperplasia of the liver and forestomach and skeletal myofiber atrophy were seen which might have progressed to neoplasia if the study were to have continued for the complete

104 week duration. An increased incidence of thyroid follicular cell adenocarcinomas was noted in males at 25 mg/kg/day. Pitavastatin increased thyroid follicular adenocarcinomas in male rats given ≥25 mg/kg/day in a lifetime carcinogenicity study (≥295-fold human exposure). This finding is attributed to excessive stimulation of thyroid follicular cells by elevated TSH. Pitavastatin appears to induce T_4 UDG-GT activity in male rat liver which results in decreased plasma T_4 based on increased metabolism. Decreased T_4 leads to a feedback-mediated increase in TSH release resulting in extensive follicular cell stimulation in the thyroid. This has been established for liver enzyme inducing agents, including statins. Rats are particularly sensitive to this induced metabolism because they lack T_4-binding globulin, which results in increased turnover of T_4 compared to other thyroid hormone species.

ECAC requested a transgenic mouse study to address these inadequacies. A 26-week carcinogenicity study was performed in Tg *rasH2* mice with administration of doses of 30, 75, 150 mg/kg pitavastatin by oral gavage. Males given 150 mg/kg/day had a higher frequency of alveolar/bronchiolar adenoma/carcinoma (2/25) and forestomach carcinoma (1/25). These tumors occurred at low frequency and are considered common tumors in Tg *rasH2* mice. This study was considered an adequate assessment of carcinogenicity by ECAC, without any statistically significant or clinically significant dose-related tumors.

A second Tg *rasH2* mouse carcinogenicity study was submitted using a 150 mg/kg/day pitavastatin dose with and without an initiating dose of urethane (250 mg/kg as a single IP dose on day 1) a known rodent carcinogen. Animals treated with urethane alone showed increased incidence of benign and malignant lung tumors that was unaffected by pitavastatin treatment. Thus, pitavastatin-treated animals did not show an increase in tumor incidence compared to urethane treated controls. Urethane was used as an anesthetic during the carcinogenicity studies.

Overall, the carcinogenicity assessment suggests no tumorogenicity in rats at doses up to 25 mg/kg/day (295-fold human exposure at 4 mg/day based on AUC) after 104 weeks treatment and in Tg rasH2 mice up to 150 mg/kg/day (194-fold human exposure at 4 mg/day based on AUC) after 26 weeks.

Pitavastatin lactone is not present in rodent plasma; however, rats liver microsomes produced significant amounts of pitavastatin lactone. Therefore, this metabolite has considered to have been tested in the 92-week rat carcinogenicity studies. Pitavastatin lactone was not genotoxic in an Ames assay with and without metabolic activation and was not genotoxic in a chromosomal aberration assay with and without metabolic activation.

2.6.6.6 Reproductive and developmental toxicology

Table 2.4.8: Reproductive and Developmental Toxicity Studies with Pitavastatin

Study type	Species	Dosing Period	Dose Levels (mg/kg/day)	Reference
Fertility	Rat	M: From Day 70 pm[a] F: From Day 14 pm to Day 7 pc	0, 2, 10, 30[c]	[KW92083]
	Rabbit[b]	M: From Day 28 pm F: From Day 28 pm to Day 6 pc	0, 0.5, 1	[100320P]
	Rabbit	M: From Day 28 pm F: From Day 28 pm to Day 6 pc	0, 0.5, 1, 2	[100320]
Embryofoetal development	Rat[b]	Days 7 to 17 pc	0, 2, 10, 50, 100	[R92080]
	Rat	Days 7 to 17 pc	0, 3, 10, 30	[RFG2508]
	Rabbit[b]	Days 6 to 18 pc	0, 0.3, 1, 5, 10, 20	[RF2505]
	Rabbit	Days 6 to 18 pc	0, 0.1, 0.3, 1	[RFG2507]
Peri/post-natal	Rat	Day 17 pc to Day 21 pp	0, 1, 3, 10, 30	[RFG2511]
	Rat	Day 17 pc to Day 21 pp	0, 0.1, 0.3	[RFG2512]

a: pm: Premating; pc: *Post-coitum*; pp: *Post-partum*; b: Preliminary study; c: 50 mg/kg for males until Day 35.

(Sponsor, M2.4, Nonclinical overview, p37)

Dose-limiting Toxicities in Reproductive and Developmental Toxicology Studies

Species - duration	Duration	Toxicity	NOAEL (mg/kg/day)	Multiple of human exposure at 4 mg HED
Rat Seg I	F_0 male fertility	Mortality	10	56x
	F_0 female	No toxicity	30	168x
	F_1 embryos	No toxicity	30 (maternal)	168x
Rat Seg II-III	F_1 fetus	Higher rate of developmental defects	3 (maternal)	17x
	F_1 female	No toxicity	30	168x
	F_1 pup	Decreased weight gain (>20%)	10 (maternal)	56x
	F_0 dam	Decreased weight gain (>20%)	10	56x
Rat Seg III	F_0 dams	Mortality	<1	<5.6
	F_1 pups	Decreased viability index	<1	<5.6
Rabbit Seg I	F_0 male fertility	Mortality	0.5	47x
	F_0 female	ND, due to lack of appropriate histopathology data	ND	ND
	F_1 embryo	Decreased viability index	<0.5	<47x
Rabbit Seg II	F_0 female	mortality, kidney necrosis	<0.1	<9.4x
	F_1 fetus	ND	ND	ND

ND, not determined

TOX008 (KW92083) – Study on Effects of Oral Administration of NK-104 on Fertility and Early Embryonic Development to Implantation in Rats (Segment I)

Key study findings:

- The NOAEL, fertility and early embryonic development, was 30 mg/kg, due to a lack of remarkable toxicity at the high dose of 30 mg/kg dose of NK-104
- NOAEL, males, was 10 mg/kg, due to mortality (3/24) at 30 mg/kg. Findings in early decedents were stomach whitening (2/3) and thickening (2/3), and subdural brain bleeding (1/3)
- NOAEL, females, was 30 mg/kg, due to an absence of any remarkable toxicity at 30 mg/kg dose of NK-104

Volume #, and page #: M4, TOX008 (KW92083)
Conducting laboratory and location (b) (4)
Date of study initiation: September 7, 1992
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot number, and purity: 104P-9202, 99.2% purity

Methods:

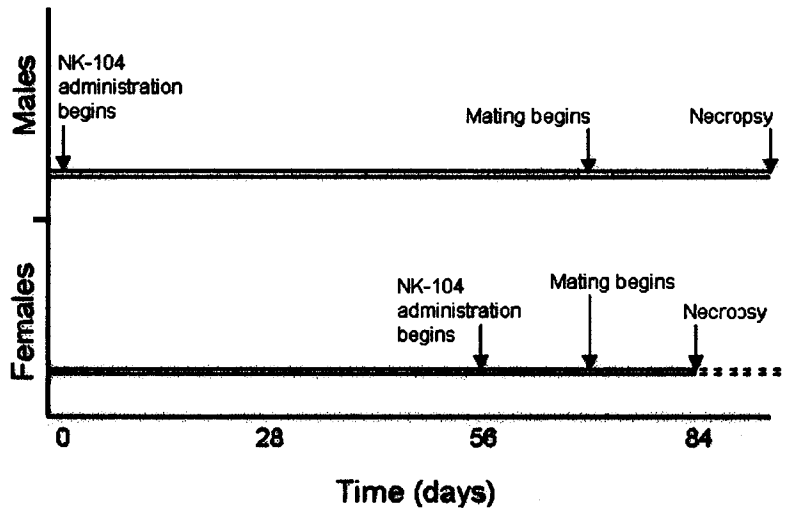
Group	Substance	Dose Level (mg/kg/day)	Conc. (% v/v)	Dose Volume (ml/kg)	Number of Animals Males	Number of Animals Females
Control	0.5% CMC	–	–	2	24	24
Minimum	NK-104	2	0.1	2	24	24
Intermediate	NK-104	10	0.5	2	24	24
Maximum	NK-104	30*	1.5	2	24	24

*50 mg/kg/day for males on Day 1-35 of administration; 30 mg/kg thereafter

(Sponsor, M4, KW92083, p22)

Doses: 0, 2, 10, and 30* mg/kg/day (*50 mg/kg/day for males Days 1-35, then 30 mg/kg/day thereafter)
Species/strain: Crj:CD (SD) Sprague-Dawley rats
Number/sex/group: 24/sex/dose
Route, formulation, volume, and infusion rate: oral gavage, 0.5% solution of carboxymethylcellulose in water, 2 mL/kg/day
Satellite groups used for toxicokinetics: Not performed.

Study design: Daily NK-104 administration for males (70 days prior to mating, throughout mating period) and females (14 days prior to mating, through 7 days of gestation), Segment I.



Parameters and endpoints evaluated: Clinical observations were obtained three times daily during the drug administration **period for males (~97 – 98 days), three** times daily during the drug administration period for females (14 days pre-mating, throughout mating, until Day 7 of gestation), and once daily during other periods. All males were weighed weekly. Females were weighed twice weekly from the first day of administration to the day of **copulation, then on gestational Days 0 –** 8, 11, 14, 17, and 20. Food intake was measured for four days prior to administration for males and females and then once weekly for males and twice weekly for females, until the first day of the mating period. After mating had begun, female food consumption **was measured daily on Days 1 – 8 of gestation and on Days 8 – 11, 11 – 14, 14 – 17, and 17 – 20 of gestation.**

Day 0 of gestation was the first day that copulation was observed to have taken place (vaginal plug, or sperm present in the vaginal smear). Copulation index [(# animals which mated / # animals paired) x 100], fertility index [(# pregnant animals / # animals which mated) x 100] were calculated.

After mating, males were euthanized and necropsies were performed. Male organs analyzed were brain, pituitary, thymus, lungs, heart, liver, spleen, adrenals, kidneys, testes, epididymis, seminal vesicles, prostate, and organs and tissues with observed pathologies.

Females were necropsied on Day 20 of gestation. Uteri were examined for pregnancy and corpora lutea were counted. Females that failed to mate after 7 days were necropsied. The organs of interest were brain, pituitary, thymus, lungs, heart, liver, spleen, adrenals, kidneys, ovaries, and uteri.

Fetal observations were performed on Day 20 of gestation. Evaluations were for the number of implants, and alive and dead fetuses were performed. Dead fetuses were categorized as implantation sites, early deaths, late deaths, or macerated fetuses. Live fetuses were examined for external abnormalities in the oral cavity,

and were sexed and weighed. Placentae and properties of amniotic fluid were inspected. Fetuses with external anomalies were fixed and photographed. Implantation [(# implants / # corpora lutea) x 100] and fetal mortality [(# dead fetuses / # implants) x 100] indices were calculated.

Results:

Mortality: There was no mortality in males of the 0, 2, 10 mg/kg dose groups of NK-104. In the 30 mg/kg group of males, there was mortality (3/24). There was no mortality in females of the no-dose control group, nor any dose group of NK-104 used in this study. The three deaths in males occurred on Days 14, 21, and 23 in the high-dose group at 50 mg/kg.

Segment I Reproductive Toxicology Study in Rats – Mortality in F_0 Males and Females				
	Dose (mg/kg)			
Sex	0	2.0	10	30*
Males	0/24	0/24	0/24	3/24
Females	0/24	0/24	0/24	0/24

*Beginning on study Day 36, the high dose in males was lowered from 50 mg/kg to 30 mg/kg

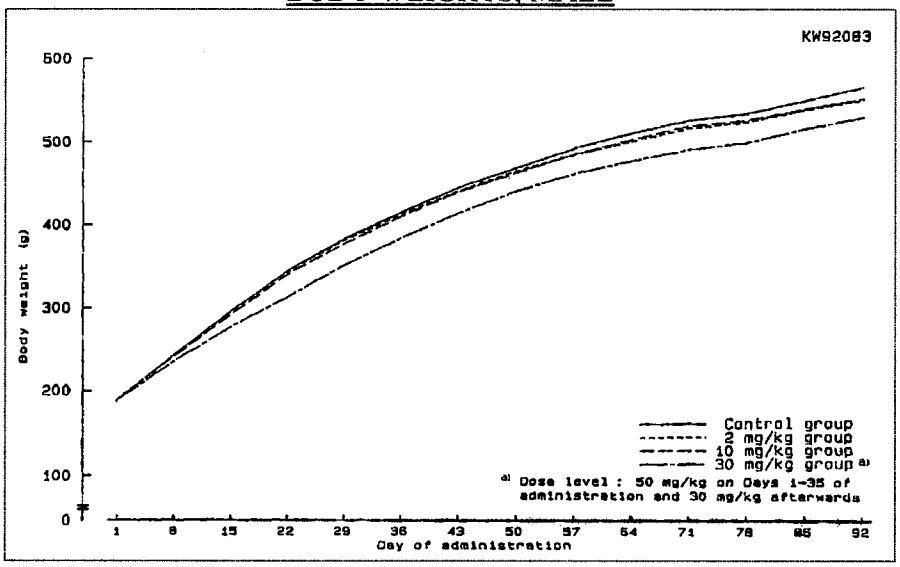
Clinical signs: Clinical signs in males that survived to complete the study in the 0, 2, 10, and 30 mg/kg groups included loss of hair around the neck, crust formation on the neck or around nose, flushing of the auricula, flushing of the extremities, flushing of the tail, and salivation. All symptoms except salivation occurred in the control group to the same or greater extent as treatment groups, and are thus probably not drug-related. Increased salivation was noted in one survivor after **dose administration on Days 15 – 18, Days 24 – 33, and Day 47. Increased salivation occurred only in 1/24 animals, and only in the 30** mg/kg dose group and may be drug-related.

In males that died early, clinical signs included decreased movement (3/3), soiled hair (3/3), red soil around eyes (1/3), piloerection (1/3), and markedly decreased fecal amount (2/3). These findings were considered related to drug-induced pathology.

Clinical signs in females were loss of hair on neck, which were transient findings and not dose-related, and not considered to be related to drug administration in female rats.

Body weight gains: Male body weight gains were dec**reased between Days 8 – 50 of drug** administration by 1.3%, 1.7%, and 8.8% in 2, 10, and 30/50mg/kg dose groups, respectively. The 50 mg/kg dose group in males was lowered to 30 mg/kg beginning on Day 36. Only the 8.8% decrement in body weight gain at 30/50 mg/kg NK-104 dose was **statistically significant ($p<0.05 – 0.01$, depe**nding on the day. However, a decrease in body weight gain of < 20% compared to controls was not considered biologically significant.

BODY WEIGHTS, MALE

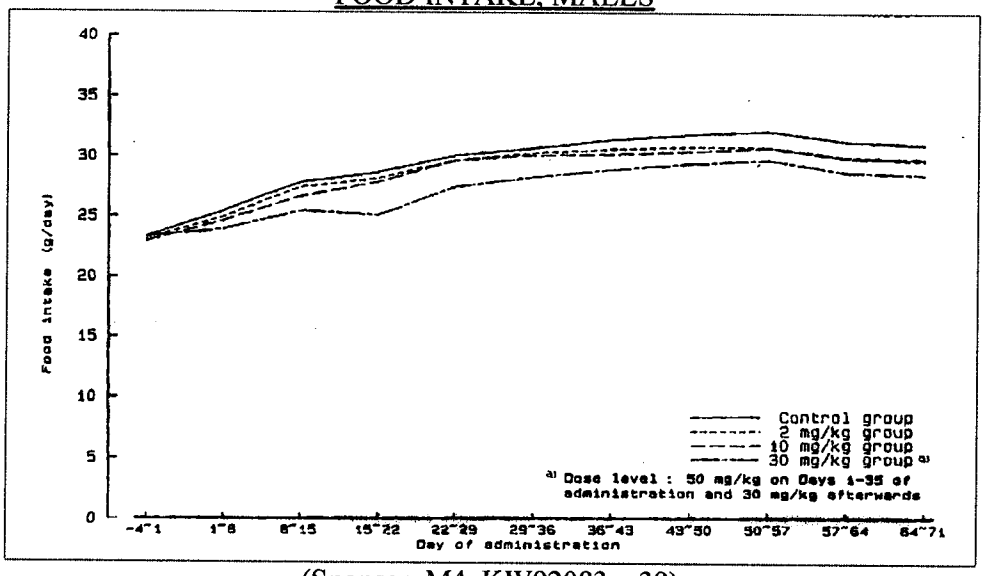


(Sponsor, M4, KW92083, p38)

Body weights in females were not remarkably different between dose groups and controls, both during administration prior to mating and during the gestational period.

Food consumption: Food consumption data were remarkable only for the 30 mg/kg dose **group of males. During Weeks 2 – 6, food** consumption was decreased an average of ~9%. During Week 10, a decrease in food consumption for this group was 8.4% compared to controls. These drops in food intake were likely responsible for the decreased weight gains measured around the same time periods in the 30 mg/kg NK-104 dose group.

FOOD INTAKE, MALES



(Sponsor, M4, KW92083, p39)

The data for the 2 mg/kg and 10 mg/kg male dose groups were unremarkable.

All food consumption data for females were unremarkable.

Toxicokinetics: Not evaluated.

Gross necropsy: There were no remarkable findings among males in the 2 and 10 mg/kg dose groups compared to controls. In surviving males in the 30 mg/kg dose group, 11/21 had a white discoloration of the forestomach and a thickening of the forestomach wall. In early decedent males, there were instances of white discolored areas of the forestomach (2/3) with erosions (1/3) and bleeding (1/3), lung congestion (1/3) and subdural brain bleeding (1/3).

In dams of the control, 2 mg/kg, and 10 mg/kg groups, there were findings in kidney, brain, thoracic cavity, and liver. However, these findings did not show a dose-response relationship to drug administration. There were no remarkable findings in the high dose, 30 mg/kg dose group for females from necropsy.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): Female estrous cycle parameters were not different between control and dose groups. Parameters were the mean number of estrous cycles and the mean length of estrous cycle.

All mating parameters, including the number of males and females that successfully mated, the number of males and females which impregnated/were impregnated in the first and second mating periods, were unremarkable.

One fetus showed a vestigial tail in the control group. Two fetuses in the 2 mg/kg group showed gross developmental abnormalities. One was micro agnathia with open eyelids,

and the other was edema. There were no abnormalities at the 10 or 30 mg/kg dose of NK-104.

2000KW100 – Histopathological examination of the effect of NK-104 on the testis and epididymis of rats

This study examined the histopathological findings in the testis and epididymes of rats in the Segment I, KW92083 study. Specimens were cut and embedded in paraffin and slides were prepared for 3 different slices of testes and stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS) staining. Epididymal slides were processed with HE stain only. There were no toxicities observed in testis of any animal. There were statistically significant decreases in Leydig cells in seminiferous tubules in Block 1 and 2 of high dose, 30(50) mg/kg group. There were decreases in Stage IX-XI spermatids (elongate) ($p<0.01$) and Stage XII spermatids (elongate) in Block 1 and 2 relative to Sertoli cells. Samples with statistically significant differences in spermatids were reanalyzed by the Sponsor and hypothesized to be due to counting error. However, all samples were not reanalyzed (just positive ones) and this type of re-analyzing only positive samples is not acceptable. However, the biological importance of these findings is unlikely, since the differences in spermatids relative to Sertoli cells were not large (10% or less) and no histopathological changes were noted.

100320P – Preliminary study of the effect of NK-104 on fertility and early embryonic development up to implantation in oral administration to rabbits

Definitive study already reviewed.

100320 (TOX078) – Study on Effects of Oral Administration of NK-104 on Fertility and Early Embryonic Development to Implantation in Rabbits (Segment I)

Key study findings:

- NOAEL (early embryonic development) was less than 0.5 mg/kg because of a strong trend for decreased viability index during the pre-implantation phase of development.
- NOAEL (males) was 0.5 mg/kg, because 1.0 and 2.0 mg/kg dose groups experienced increased mortality, 3/16 and 9/16, respectively; intermediate decreases in body weight gain of **↓55 – 94% (Day 18); intermediate decrease in** food consumption by **↓12 – 16% (Day 16).**
- Minimum lethal dose (females) was 1 mg/kg because of increased mortality, 1/16 and 6/16, at 1.0 and 2.0 mg/kg, respectively. NOAEL could not be determined because no histopathological evaluations were carried out for females.
- NK-104 showed the potential for kidney toxicity in adult animals, as males (12/12) and females (5/7) that were found dead showed whitening of the kidneys (probably related to ischemia, not drug MOA). No histopathological examinations of kidney findings were performed by Sponsor.

Volume #, and page #: M4, 100320 (TOX078)
Conducting laboratory and location(b) (4)
(b) (4)
Date of study initiation: April 20, 2001

GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot number, and purity: NK003A04-K, 99.4% purity

Methods:

DOSE GROUP ASSIGNMENTS

Test group	Dosage (concentration)	No. of animals (Animal No.)	
		Male	Female
1. Control (vehicle)	0 mg/kg (0 mg/mL)	16 (M01101 - M01116)	16 (F01151 - F01166)
2. NK-104	0.5 mg/kg (0.25 mg/mL)	16 (M02201 - M02216)	16 (F02251 - F02266)
3. NK-104	1.0 mg/kg (0.5 mg/mL)	16 (M03301 - M03316)	16 (F03351 - F03366)
4. NK-104	2.0 mg/kg (1.0 mg/mL)	16 (M04401 - M04416)	16 (F04451 - F04466)

(Sponsor, vSR100320, p22)

Doses: 0, 0.5, 1.0, 2.0 mg/kg
Species/strain: Kbl:JW rabbits (SPF)
Number/sex/group: 16/sex/group
Route, formulation, volume, and infusion rate: Oral gavage, 2 mL/kg/day

FORMULATION

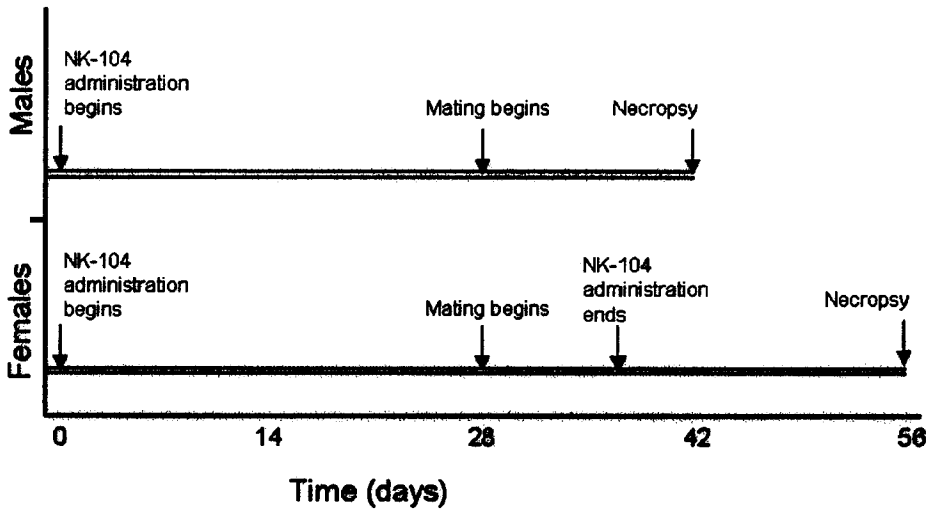
Concentration of NK-104 in dosing preparations

Test article:	NK-104 (Lot No. NK003A04-K)
Vehicle:	0.5 w/v% Carboxymethyl Cellulose Sodium Solution
Date of preparation:	July 5, 2001
Method:	HPLC method
Date of analysis:	July 5, 2001
Testing facility:	(b) (4) [redacted].

(Sponsor, vSN100320, p43)

Satellite groups used for toxicokinetics: subgroup of 4/sex/dose

Study design: males, 42-day daily administration by oral gavage (28 days pre-mating, 14 days during mating); females, 37-day daily administration by oral gavage (28 days pre-mating, 3 days during mating; 6 days after copulation)



Parameters and endpoints evaluated: For males, clinical signs were monitored once before and once after administration, and once on day of necropsy. Males were weighed twice per week and once on day of necropsy. Food consumption was measured one day per week, on Days 1 to 2, 8 to 9, 15 to 16, and 22 to 23. Approximately 0.7 mL of blood was collected every two weeks on Days 1, 15, and 28. Necropsies of males were performed on Day 42 for all surviving animals or on early decedent males as soon as possible. Male organs were removed (liver, kidneys, testes, epididymis, seminal vesicles, and prostate) and weighed (liver, kidneys, testes, and caudal epididymis), and fixed for histopathological analyses. Semen samples were prepared from the right caudal epididymis and sperm were examined for motility, morphology, and were counted.

Does were examined once daily or twice daily during drug administration (once before and once after administration). Females were weighed twice per week, or every day during Days 0-7 of pregnancy. Food consumption was measured twice per week, except daily during Days 0-7 of pregnancy. Approximately 0.7 mL of blood from females was collected every two weeks on Days 1, 15, and 28. Necropsy was performed on Day 28 of pregnancy. Liver and kidneys from females were removed, weighed, and fixed for histopathological analyses. In animals determined to have been pregnant, corpora lutea and number of implantation sites were counted. If implantation was not found, the uterus and ovaries were removed and examined for implantation scars. In early decedent females, liver, kidney, uterus, and ovaries were removed and fixed for gross pathological evaluation. In females that failed to copulate, livers and kidneys were weighed, and along with ovaries and uteruses, were fixed and examined for gross pathology.

Mating was attempted with up to two times for both males and females, on a one-to-one basis, within groups if possible, and with untreated animals if not possible. Duration of mating was 7 days (maximum) for both attempts. Vaginal smears

were taken to confirm copulation, and Day 0 of pregnancy was the first day sperm were detected.

Post-implantation losses and live fetuses were counted in all rabbit does, and classified as implantation scar only, early resorptions, late resorptions, macerated fetuses, or dead fetuses. All samples were analyzed for external anomalies and placental abnormalities. Live fetuses were weighed, sexed, and were fixed and preserved for later examination.

Toxicokinetic evaluations of blood samples for C_{max}, T_{max}, AUC, and plasma NK-104 concentration were carried out by HPLC.

Results:

Segment I Reproductive Toxicology Study in Rabbits – Mortality (including Moribundity)		
Dose Group (mg/kg)	**Males**	**Females**
0	0/16	0/16
0.5	0/16	0/16
1.0	3/16	1/16
2.0	7/16 (9/16)	3/16 (6/16)

Mortality: See table, directly above. The mean date for deaths (including moribundity) for males was Day 22.9, or approximately 6 days before the onset of mating. Two males died after Day 28 (beginning of mating), one on Day 29 and one on Day 39. For females, the mean date for deaths (including moribundity) was Day 23.5, or approximately 5.5 days before the onset of mating. No females died or were found moribund after Day 28.

Clinical signs: There were no adverse clinical signs or deaths reported in the no-dose control or 0.5 mg/kg groups in buck rabbits. Three males (3/16) in the 1.0 mg/kg NK-104 dose group died. Symptoms most often noted prior to deaths in males in the 1.0 mg/kg NK-104 dose groups were decreases in motor activity (3/3), and some instances of prone positioning (2/3), hypothermia (1/3), and bradypnea (1/3). There were instances of moribundity (2/16) and outright death (7/16) in the 2.0 mg/kg group. Symptoms most often noted prior to moribundity and deaths in males in the 2.0 mg/kg NK-104 dose groups were decrease in motor activity (9/9), with some instances of prone positioning (4/9), hypothermia (2/9), and bradypnea (2/9).

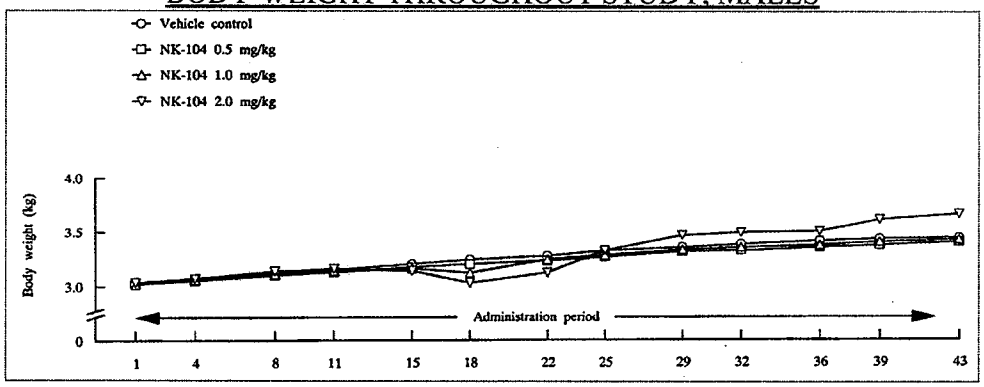
In doe rabbits, there were no adverse clinical signs among control and 0.5 mg/kg dose groups. In the 1.0 mg/kg group, there was one animal that died after showing decreased motor activity, prone positioning, and hypothermia. This death occurred on Day 28, just prior to mating. In the high-dose, 2.0 mg/kg NK-104 group, there were moribundity (3/16) and deaths (3/16). These events were accompanied by decreases in locomotor activity (6/6), prone positioning (6/6), hypothermia (2/6), and bradypnea (5/6). Outright

deaths in females occurred prior to mating, on Days 20, 22, and 23. Moribundity occurred on Days 23, 25, and 28.

In females, there were no remarkable clinical signs in any dose group during early pregnancy.

Body weight gain: The male 2.0 mg/kg dose group for NK-104 administration showed a transient trend towards decreased body weight gain decrement (**↓94%, Days 1 – 18).** There was a weaker, transient trend for the same effect in the 1.0 mg/kg group (↓55%, **Days 1 – 18). The survivors of the 2.0 mg/kg dose group appeared to add weight at a** greater rate than the other three dose groups of animals, beginning around Day 25, and continuing through the last day of drug administration on Day 43 (see following figure).

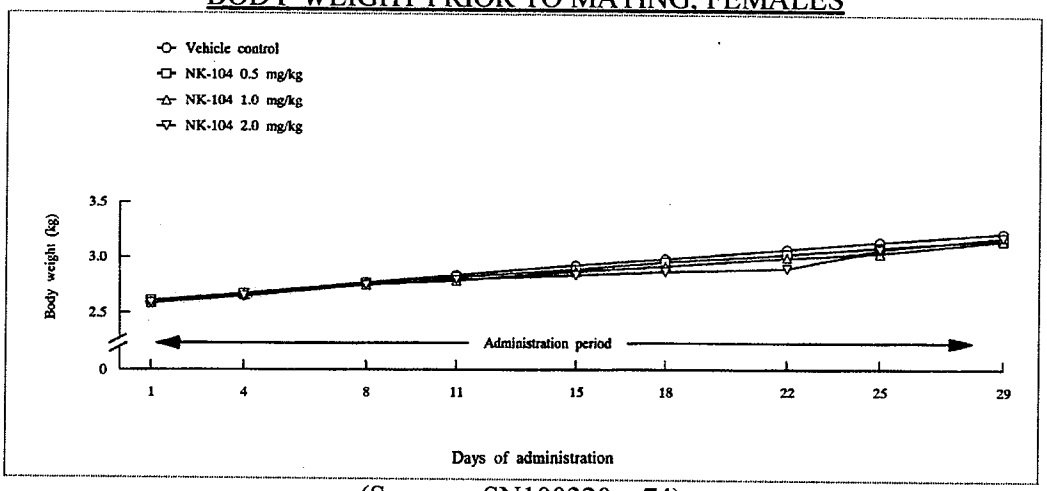
BODY WEIGHT THROUGHOUT STUDY, MALES



(Sponsor, vSN100320, p72)

Female body weight gains in the 2.0 mg/kg dose groups of NK-104 were reduced by a statistically significant 26% decrement ($p<0.05$) and 32% decrement ($p<0.01$) on Days 15 and 18, respectively, as compared to Day 1. These females subsequently recovered weight gain, and at Day 28, females in the 2.0 mg/kg dose group had gained 95% of the weight of the no-dose controls. All other **dose groups' weight gains were unremarkable.**

BODY WEIGHT PRIOR TO MATING, FEMALES

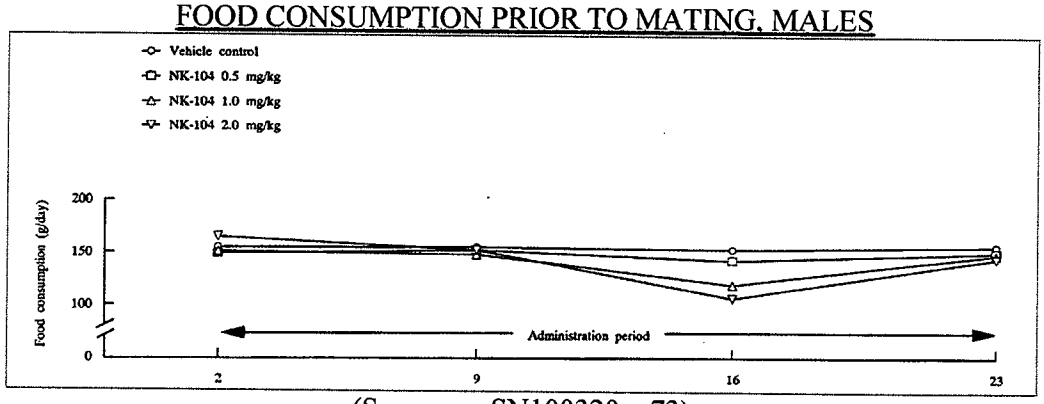


(Sponsor, SN100320, p74)

Female body weight gains during pregnancy were unremarkable.

Food consumption: There was a statistically significant reduction ($p<0.05$) in food intake in males measured on Day 16 only in the 1.0 mg/kg (↓22%) and 2.0 mg/kg (↓31%) groups of buck rabbits. This correlated with the reduction in body weight gain seen in 1.0 mg/kg (↓26%) and 2.0 mg/kg (↓32%) dose group males during this period.

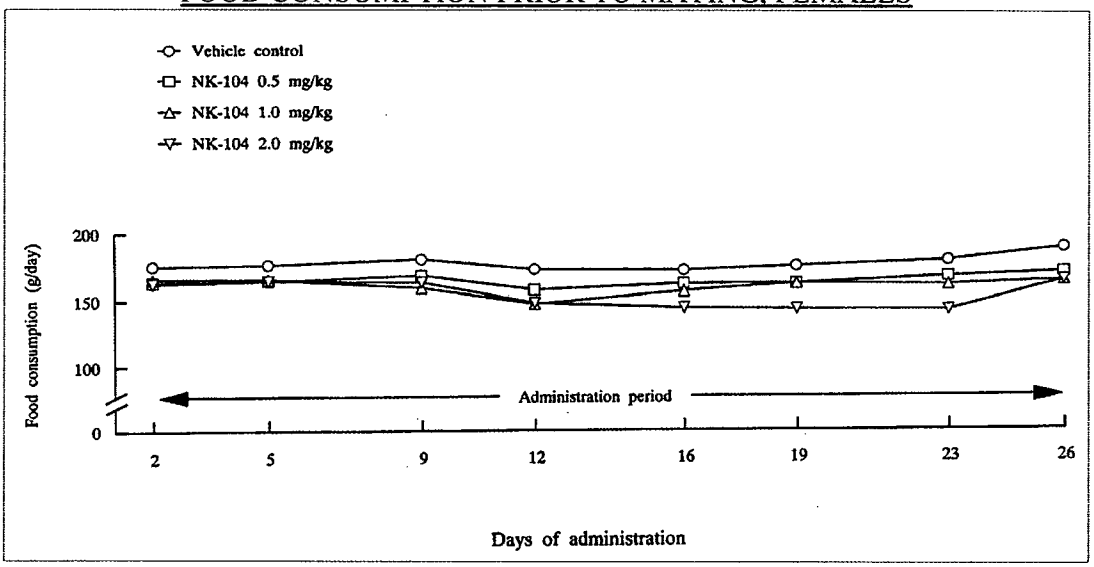
FOOD CONSUMPTION PRIOR TO MATING, MALES



(Sponsor, vSN100320, p73)

In female rabbits of the 1.0 mg/kg NK-104 group prior to mating, there were statistically significant decreases in food consumption measured around Days 9 and 12, of 12% and 15%, respectively. In the high dose, 2.0 mg/kg dose group, there were also decreases in food consumption on Days 9, 12, and 16, of 9%, 14%, and 16%, respectively. All other deviations in weight gain were unremarkable.

FOOD CONSUMPTION PRIOR TO MATING, FEMALES



(Sponsor, vSN100320, p76)

Food consumption data for females during pregnancy were unremarkable.

Clinical chemistry related to drug pharmacology: Total cholesterol was measured before administration of the first dose, after 15 days, and after 28 days of NK-104 administration. There was a significant decrease in total cholesterol in males in the 0.5 mg/kg dose group (↓23%, $p<0.05$), with a more significant decrease in the 1.0 (↓33%) and 2.0 mg/kg (↓38%) dose groups, at Day 28 ($p<0.01$).

Total cholesterol counts in females were decreased in a statistically significant manner on Day 28 for the 0.5 mg/kg (↓10%), 1.0 (↓22%) and 2.0 mg/kg (↓16%) dose groups ($p<0.01$).

Necropsy: Findings during necropsy were unremarkable in all buck rabbits that survived to Day 43 of NK-104 administration. There was no mortality in males of the control and 0.5 mg/kg dose group. For males that were found dead or moribund in the 1.0 and 2.0 mg/kg dose groups, there were findings in kidneys in 3/3 and 9/9 (white in color, probably relates ischemia), respectively. There was one finding of a dark red spot in the mucosa of the stomach of an early decedent male in the 2.0 mg/kg dose group (1/9).

In females, all findings during necropsy for animals that survived to study Day 43 were unremarkable. For early decedent does and those found moribund, kidneys were white in color (5/6) and a liver showed a white spot (1/6), which may have been due to ischemia.

Organ weights: Organ weights (liver and kidney) were collected from all males that survived to study Day 43; data were unremarkable. Likewise, all organ weight data (liver and kidney) from female rabbits were unremarkable.

Histopathology: A variety of histopathological findings were made in males (in testes, epididymis, and prostate), however all findings were present at equal or greater frequency and severity in the control group. Therefore, findings were not considered drug-related.

No histopathological examinations were carried out in doe rabbit.

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

Reproductive performance: Differences in copulation and fertilization indices between controls and all dose groups of NK-104 for males and females, were unremarkable.

Females from the 1.0 and 2.0 mg/kg dose groups were more likely to need a second mating session (2/12 and 3/9, respectively). Males from the 1.0 and 2.0 mg/kg dose groups were more likely to require a second mating session (paired with non-treated females, 1/11 and 1/7, respectively). One female failed to copulate in the 1.0 mg/kg NK-104 group during the first and second mating. Two control animals of each sex, one animal of each sex at 0.5 mg/kg, 2 animals of each sex at 1.0 mg/kg, and 1 animal of each sex at 2.0 mg/kg copulated but fertility was absent.

Fetal analyses: Does administered NK-104 had less favorable reproductive parameters, as outlined in the following figure. While there were no statistically significant differences between controls and dose groups, but there were consistent trends noted among these data. There was a rise in the number of pre-implantation losses from 0.9 per dam in controls, to 1.7, 1.5, and 1.8 per dam in 0.5, 1.0, and 2.0 mg/kg dose groups, respectively. This correlated with a decrease in the number of deaths or resorptions, decreased from 7.2 mean percent per dam in controls to 4.7, 4.8 and 2.4 mean percent per dam in the 0.5, 1.0, and 2.0 mg/kg dose group during later stages of pregnancy, respectively. That is, fetuses that would have died later, died earlier in development. There was a decrease in the number of live fetuses in the high dose, 2.0 mg/kg group prior to implantation.

Segment I Reproductive Toxicology Study in Rabbits – Effects on Reproductive Parameters (% difference from controls)			
Parameter	**NK-104 Dose Group (mg/kg)**		
	0.5	**1.0**	**2.0**
Copulation index (male and female)	NC	NC	NC
Fertilization index (male and female)	NC	NC	NC
Corpora lutea (mean per doe)	NC	NC	↓6%
Implantation sites (mean per doe)	↓8%	↓5%	↓17%
Implantation rate (mean per doe)	↓8%	↓5%	↓11%
Number of pre-implantation losses (mean % per doe)	↑89%	↑67%	↑100%
Number of deaths or resorptions (mean per doe)	↓33%	↓17%	↓67%
Number of live fetuses (mean per doe)	↓6%	NC	↓12%
Sex ratio (% males per doe)	NC	NC	↓6%
Mean male fetal body weight (mean per doe)	NC	NC	NC
Mean female fetal body weight (mean per doe)	NC	NC	NC

NC, no change from controls (<5% difference)

There were no instances of fetuses with external abnormalities, and no examples of abnormalities in placentae, at any dose level.

Sperm health: All analyses of buck rabbit sperm quality were unremarkable.

Toxicokinetics: Toxicokinetic analyses revealed that there were no significant differences between males and females for T_{max}, C_{max} and AUC for the dose range selected. T_{max} following NK-104 administration was approximately the same on Day 42 as on Day 1 of drug administration for each dose. NK-104 shows a doubling of C_{max} and AUC with each doubling of the dose, for males and females. These data indicated that, **during repeat dosing of 0.5 – 2.0 mg/kg/day for 28 days in rabbits, NK-104 exhibited** predictable, dose-related increases in C_{max} and AUC, unchanging T_{max} with increasing dose, and showed that accumulation upon repeat dosing did not occur.

NK-104 TOXICOKINETICS

Dose (mg/kg)	Sex	Period	n		Plasma concentration (ng/mL) Time after dosing (hr) 1	2	4	8	24	Toxicokinetic parameters Tmax (hr)	Cmax (ng/mL)	AUC (ng·hr/mL)
0.5 [Group 2]	Male	1st day	4	Mean	368	350	234	62	5	1.3	392	2253
				S.D.	121	49	53	14	6	0.5	86	225
		42 day	4	Mean	443	333	163	58	2	1.3	457	2023
				S.D.	88	68	13	2	3	0.5	77	155
	Female	1st day	4	Mean	336	342	233	61	9	1.5	358	2233
				S.D.	57	34	42	21	3	0.6	49	289
		28 day	4	Mean	310	342	249	95	16	1.5	368	2648
				S.D.	145	99	71	65	16	0.6	104	1260
1 [Group 3]	Male	1st day	4	Mean	844	752	440	103	8	1.5	866	4382
				S.D.	180	197	182	47	1	0.6	188	1210
		42 day	4	Mean	918	765	449	124	12	1.3	1021	4758
				S.D.	245	196	107	34	7	0.5	75	734
	Female	1st day	4	Mean	817	706	418	95	8	1.0	817	4140
				S.D.	149	173	139	32	2	0.0	149	1129
		28 day	4	Mean	924	739	408	108	17	1.3	937	4478
				S.D.	68	172	104	20	10	0.5	70	692
2 [Group 4]	Male@	1st day	3	Mean	1672	1458	934	233	10	1.3	1734	9070
				S.D.	650	698	583	175	12	0.6	705	4911
		42 day	3	Mean	2369	1496	606	186	18	1.0	2369	8443
				S.D.	996	922	273	114	14	0.0	996	4409
	Female#	1st day	3	Mean	1833	1451	602	116	12	1.0	1833	7078
				S.D.	164	253	161	29	5	0.0	164	1073
		28 day	3	Mean	2130	1366	617	139	19	1.0	2130	7585
				S.D.	314	197	100	62	12	0.0	314	1228

@ : Calculate with M04402 , M04403 and M04404
# : Calculate with F04451 , F04452 and F04454

(Sponsor, vSN100320, p18)

Embryo-fetal development

R92080 – Teratogenicity study of NK-104 in rats by oral administration (preliminary study)

Definitive study already reviewed.

RFG2508 – Study by Oral Administration of NK-104 during Period of Fetal Organogenesis in Rats (Segment II)

Key study findings:

- NOAEL (F_1 fetuses/pups) was the 3 mg/kg dose, due to agnathia at 10 mg/kg in 1/347 pups, and exencephaly and open eyelids and anal atresia with anury at 30 **mg/kg in 2/332 pups. There was decreased weight gain during postnatal Days 7 –** 21 in female pups born to 10 mg/kg/day dams, though there was no developmental delay at 30 mg/kg/day.
- No adverse effects were observed on F_1 mating at NK-104 doses up to 30 mg/kg, but there were sporadic effects on F_2 fetuses (see below).
- NOAEL (F_0 females during gestation) was the high-dose of 10 mg/kg, due to weight gain that was slightly decreased in the high-dose, 30 mg/kg group during the period of drug administration (days 7 – **18 of gestation). This was likely due** to decreased food consumption by the **same group (day 9 – 14) during drug** administration period (days **7 – 18 of gestation).**
- One case of agnathia (absence or partial absence of the lower jaw) and irregular alignment of caudal vertebrae in one F_1 fetus in the mid-dose, 10 mg/kg group (1/370). One observation of fetal exencephaly (defective skull, brain extrusion) with open eyelids in one F_1 high-dose, 30 mg/kg, fetus (1/342) with irregular

alignment of the caudal vertebrae, and one case of anal atresia with irregular alignment of caudal vertebrae in another F_1 fetus in the 30 mg/kg group (1/342).

- One case of epigastrius was noted in the mid-dose, 3 mg/kg group of F_2 fetuses (1/181), and one case of exencephaly (brain outside the skull) and myeloschisis (spina bifida resulting in flattened mass of neural tissue along the spine) in the high-dose, 30 mg/kg group of F_2 fetuses (1/207).

Volume #, and page #: v19, p2
Conducting laboratory and location: Fuji Research Laboratories, Kowa Co., Ltd., Shizuoka, Japan
Date of study initiation: February 15, 1994
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot number, and purity: 104P-9202, 99.2% purity

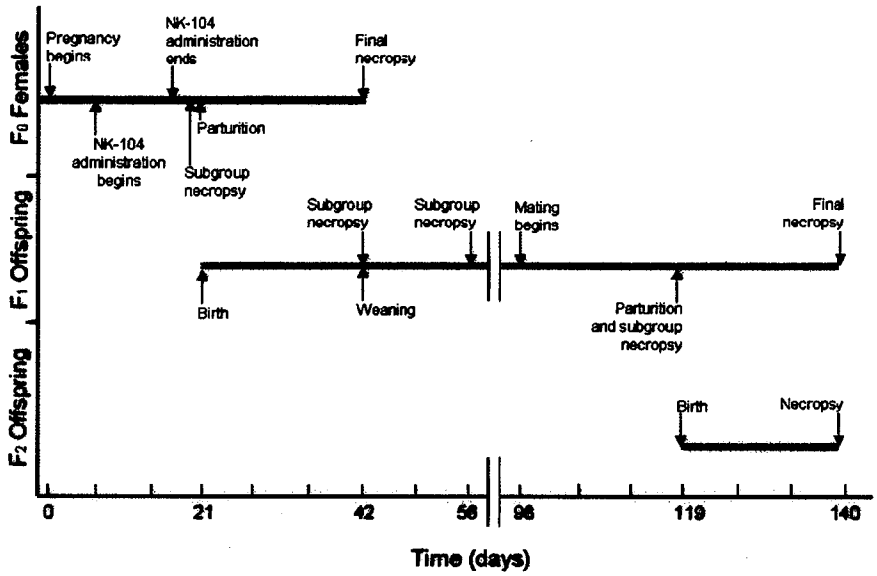
Methods:

Group No.	Drug	Dose (mg/kg)	Conc. (W/V%)	No. of Copulated Animals		No. of Pregnant Females	
				Cesarean	Spontaneous	Cesarean	Spontaneous
1	0.5% CMC	-	0	25	15	22	13
2	NK-104	3	0.15	25	15	23	14
3	NK-104	10	0.5	25	15	23	14
4	NK-104	30	1.5	25	15	23	15

Conc.=Concentration; Cesarean=Cesarean section; Spontaneous=spontaneous delivery

(Sponsor, v19, p4)

Doses: 0, 3, 10, 30 mg/kg/day NK104
Species/strain: Crj:CD (SD) Sprague-Dawley rats (SPF)
Number/sex/group: 40 females/dose
Route, formulation, volume, and infusion rate: oral gavage, 2mL/kg
Satellite groups used for toxicokinetics: None included.



Study design: Daily dosing by oral gavage on gestational Days 7 – 17, cesarean/necropsy Day 20 of gestation or Day 42 (21 days postpartum). F_1 offspring were mated at ~Day 98 (11 weeks old). F_1 fertility/reproductive parameters (~Day 118) and F_2 fetuses (~Day 140) were also evaluated.

Parameters and endpoints evaluated: Clinical evaluations were made daily, **except twice daily on days 7 – 17. Body weights were recorded on Day 0 of gestation, daily Days 7 – delivery, and on days 0, 7, 14, 21 postpartum. Food** consumption was measured on Day 1 of gestation, daily Days 7- 20 of gestation, and on Days 1, 7, 14, 21 postpartum.

Gross/histopathology/cesarean (subgroup, Day 20 of gestation) and gestational index [(# females with live pups / # pregnant females) x 100], birth index [(# live newborns / # implants) x 100] for another subgroup after spontaneous delivery, followed by gross/histopathology, were evaluated on Day 21 postpartum. Dams whose offspring died were euthanized and examined as soon as possible. Reproductive parameters, including corpora lutea, implantations, implantation marks, early resorptions, late resorptions, dead fetuses, were evaluated. Body/placental weight of live fetuses were measured. Gross/histopathology of live fetuses was performed (final subgroup).

Approximately 1/2 of live fetuses were fixed with isopropanol denatured ethanol. Subsequently, skeletal samples were removed, stained with alizarin red S, and examined for abnormalities. Approximately 1/2 of live fetuses were fixed in **Blouin's fluid and examined for morphological abnormalities by Wilson's free** hand razor blade method (gross evaluation) **and Nishimura's thoracic visceral** observation method (microdissection technique).

Offspring (F_1) were examined. Live newborns/stillborns were counted, sex ratio determined, and gross/histopathological examinations performed. Viability index calculated (Day 4). Body weights measured weekly (for 8 weeks). Separation of the auricular (Day 3), appearance of abdominal hair (Day 10), incisor eruption (Day 12), and separations of the eyelids (Day 14) were monitored. Testicular decent (Day 20) and vaginal opening (Day 33) were evaluated.

In the F_1 generation (Day 21) gross/histopathological examinations (subgroup) were performed in offspring and soft X-ray skeletal observations made. Gait, corneal reflex, pain response, righting reflex, and air righting reflex measured. Auditory function/auricular response/Preyer reflex examined.

Behaviors tested included influences on emotional expression (open field **behavior test/Bond's me**thod, latency and various behaviors in 3 minutes), neuromuscular activity (rotor rod performance/falls from a rotating drum), and learning performance (water multiple T-maze test on two consecutive days).

Gross pathology (F_1 generation) was examined (subgroup, Day 56). Organs were weighed (brain, heart, lung, liver, kidney, spleen, thymus and testis/ovary).

Non-sibling males and females (F_1 generation) of the same dose group were paired for mating (subgroup, week 11 after birth). Copulated females were weighed (day 0 and 20 of gestation). Copulation [(# copulated males / # mated males) x 100] and fertility [(# impregnating males / # copulated males) x 100] indices were calculated. Copulation [(# copulated females / # mated females) x 100] and fertility [(# impregnating females / # copulated females) x 100] indices were calculated, and time to copulation noted. Histopathological evaluations of testes/ovaries were carried out for failed mating pairs. Successfully copulated females were delivered by cesarean section and examined for reproductive outcomes (corpora lutea, implants, live fetuses, implantation marks, early and late resorptions, and dead fetuses). Fetal body and placental weights (F_2 generation) were measured for live fetuses, and gross morphological anomalies and sexual difference were noted.

Results:

Clinical evaluations of F_0 dams:

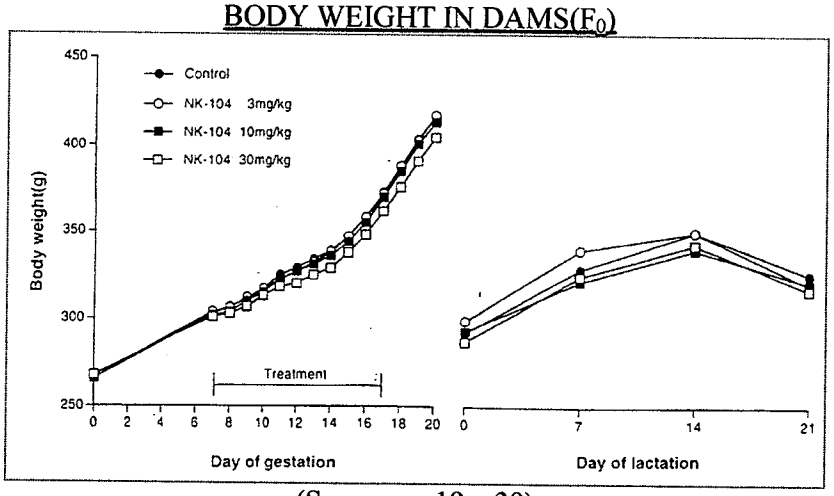
F_0 mortality: No mortality was observed.

F_0 clinical signs: During gestational period, two F_0 dams showed loss of hair (chest) in the vehicle-only control group. One instance (Day 19, observation #2) of decrease in locomotor activity was noted in the 10 mg/kg group. A single instance of loss of hair (hind paw) occurred in the 30 mg/kg group. Other clinical observations during gestation were unremarkable. These clinical findings did not appear dose-related, and were not considered to be drug-related.

In the lactation period (F_0), one dam in the vehicle-only control group was determined lactation insufficient. One dam in the 30 mg/kg group was also lactation deficient. Other clinical observations during lactation were unremarkable. Since there were similar findings in the high-dose and control groups, these findings were not likely drug-related.

F_0 body weight gain: Dam (F_0) weight gain was slightly decreased in the 30 mg/kg group during the period of drug administration (Days **7 – 18 of gestation)** leading overall to lower weight gain (↓9.9% decrement compared to control group) during gestation (days 0 **– 20). This effect was statistically significant (p<0.01). All other weight data** comparisons were unremarkable. These decreases in weight gains were likely drug-related.

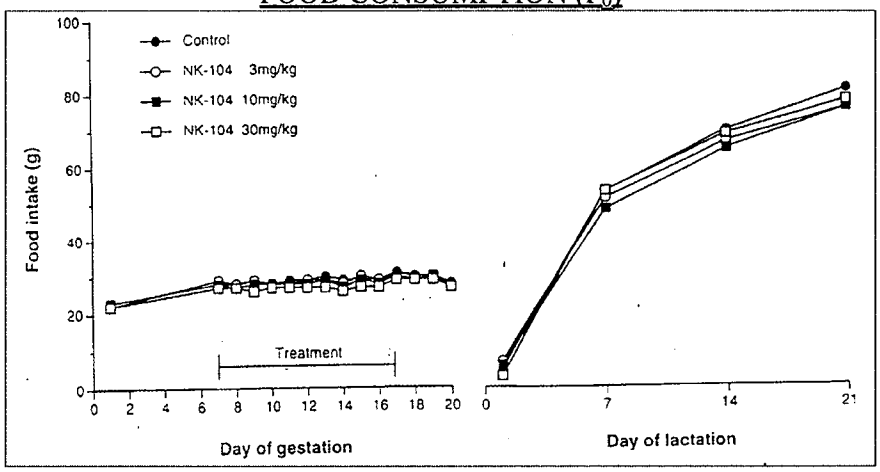
BODY WEIGHT IN DAMS(F_0)



(Sponsor, v19, p39)

F_0 dam food consumption: Food consumption was slightly decreased in dams of the 30 mg/kg group (Day 9 and Day 14 of gestation, decreased 10% from controls on both days). This effect was statistically significant (p<0.05). All other food consumption comparisons were unremarkable.

FOOD CONSUMPTION (F_0)



(Sponsor, v19, p40)

F_0 gross pathology: Necropsy findings from dams (F_0) euthanized at the end of the gestation period (Day 20), were unremarkable.

F_0 reproductive outcome:

Fertility of F_0 generation: Differences in gestation and birth indices (F_0) were unremarkable between dose groups of NK-104 and vehicle-only controls.

Prenatal observations of F_1 fetuses: Prenatal observations of gross external abnormalities in fetuses (F_1) included one case of agnathia (absence or partial absence of the lower jaw) in the 10 mg/kg group. In the 30 mg/kg group, one observation of anal atresia (absence/misplacement) with anury (missing tail) was noted, and findings of exencephaly (defective skull, brain extrusion) with open eyelids were recorded in another. These cases comprised just 0.3% and 0.6% of the total number of fetuses in 10 and 30 mg/kg groups, respectively. All other observations of gross visceral and skeletal pathologies in fetuses (F_1) were unremarkable.

GROSS EXTERNAL OBSERVATIONS IN FETUSES (F_1)

Dose (mg/kg)		Control	3	10	30
No. of dams		22	23	23	23
No. of corpora lutea		401	413	398	398
		18.2±2.2	18.0±2.7	17.3±1.8	17.3±1.7
No. of implants		364	375	370	342
		16.5±1.8	16.3±2.3	16.1±1.7	14.9±3.1
No. of dead fetuses (%)		16(4.6)	16(4.2)	23(6.3)	10(2.8)
Implantation mark		0	0	0	0
Early resorption		14(4.0)	15(3.9)	23(6.3)	9(2.6)
Late resorption		2(0.6)	1(0.3)	0	1(0.2)
Dead fetus		0	0	0	0
No. of live fetuses		348	360	347	332
		15.8±2.3	15.7±2.2	15.1±2.1	14.4±3.0
Male:female		181:167	176:184	170:177	161:171
(Male%)		(52.0)	(48.9)	(49.0)	(48.5)
Body weight(g)	male	3.36±0.25	3.38±0.24	3.52±0.51	3.49±0.30
	female	3.22±0.28	3.23±0.25	3.31±0.43	3.32±0.22
Placental weight(g)	male	0.45±0.04	0.45±0.06	0.46±0.06	0.46±0.04
	female	0.43±0.05	0.43±0.06	0.43±0.05	0.44±0.04
No. of fetuses with external anomalies		0	0	1(0.3)[a]	2(0.6)[b]

Mean±S.D. [a] : Agnathia [b] : Exencephaly & open eyelids, Anal atresia & anury

(Sponsor, v19, p44)

Visceral observations in F_1 fetuses included instances of dextrocardia, right aortic arch, and abnormal lobation of the lung in 1/173 fetuses in dams of the 3 mg/kg dose group for each instance. Fetuses from dams administered 10 mg/kg doses of NK-104 showed dilatation of the cerebral ventricle, hypoplasia of the lung, diaphragmatic hernia (1/169 for each abnormality), and 2/169 fetuses were found to have a persistent umbilical left artery. At 30 mg/kg maternal doses, 1/161fetuses exhibited anophthalmia. Fetuses of control dams exhibited situs inversus (1/169). Fetuses of control, 3, 10, and 30 mg/kg dose groups showed thymic remnant in neck (8.9%, 5.8%, 10.7%, and 8.1%, respectively, and dilatation of renal pelvis (2.4%, 1.7%, 1.2%, and 2.5%, respectively).

VISCERAL OBSERVATIONS IN FETUSES (F_1)

Dose (mg/kg)	Control	3	10	30
No. of dams	22	23	23	23
No. of fetuses examined	169	173	169	161
No. of fetuses with anomalies (%)	18(10.7)	12(6.9)	23(13.6)	17(10.6)
No. of fetuses with anomaly (%)				
Dilatation of cerebral ventricle	0	0	1(0.6)	0
Anophthalmia	0	0	0	1(0.6)
Dextrocardia	0	1(0.6)	0	0
Right aortic arch	0	1(0.6)	0	0
Abnormal lobation of lung	0	1(0.6)	0	0
Hypoplasia of lung	0	0	1(0.6)	0
Thymic remnant in neck	15(8.9)	10(5.8)	18(10.7)	13(8.1)
Diaphragmatic hernia	0	0	1(0.6)	0
Dilatation of renal pelvis	4(2.4)	3(1.7)	2(1.2)	4(2.5)
Persistent left umbilical artery	0	0	2(1.2)	0
Situs inversus	1(0.6)	0	0	0

(Sponsor, v19, p45)

Skeletal anomalies were found in F_1 fetuses in the control (0.6%), 3 mg/kg (0%), 10 mg/kg (1.1%), and 30 mg/kg (0.6%) maternal doses. Anomalies were bifurcation of the ribs of the control fetus, and wavy ribs in the treatment groups. Skeletal variations were found in F_1 fetuses in the control (4.5%), 3 mg/kg (1.6%), 10 mg/kg (6.2%), and 30 mg/kg (2.9%) maternal doses. Findings of a cervical rib were more likely in treatment fetuses (3.0 mg/kg, 0.5%; 10 mg/kg, 1.7%; 30 mg/kg, 1.2%) than in controls (0%).

SKELETAL OBSERVATIONS IN FETUSES (F_1)

Dose (mg/kg)	Control	3	10	30
No. of dams	22	23	23	23
No. of fetuses examined	179	187	178	171
No. of fetuses with anomalies (%)	1(0.6)	0	2(1.1)	1(0.6)
No. of fetuses with anomaly (%)				
Bifurcation of ribs	1(0.6)	0	0	0
Wavy ribs	0	0	2(1.1)	1(0.6)
No. of fetuses with variations (%)	8(4.5)	3(1.6)	11(6.2)	5(2.9)
No. of fetuses with variation (%)				
Cervical rib	0	1(0.5)	3(1.7)	2(1.2)
14th rib	1(0.6)	2(1.1)	1(0.6)	0
Shortened 13th rib	1(0.6)	0	1(0.6)	0
25 presacral vertebrae	4(2.2)	0	1(0.6)	1(0.6)
Lumbarization of 1st sacral vertebra	1(0.6)	0	1(0.6)	1(0.6)
Splitting of vertebral bodies	2(1.1)	0	5(2.8)	2(1.2)
Ossification				
No. of cervical vertebral bodies	0.5±0.6	0.3±0.4	0.6±0.9	0.4±0.4
No. of sacral and caudal vertebrae	7.8±0.7	7.7±0.3	8.1±0.9	8.0±0.5
No. of sternebrae	5.1±0.8	5.1±0.5	5.2±0.6	5.3±0.5
No. of metacarpals	3.5±0.4	3.4±0.3	3.5±0.4	3.5±0.3
No. of proximal phalanges (forepaw)	0.1±0.4	0.0±0.1	0.2±0.7	0.0±0.1
No. of metatarsals	4.0±0.1	4.0±0.0	4.0±0.2	4.0±0.0

Mean±S.D.

(Sponsor, v19, p46)

Delivery observations of F_0 dams: All pregnant dams produced live newborns, and had ~equivalent gestation periods (~22 days). The 30 mg/kg dose group had slightly fewer average implants (1 fewer per litter), but the number of live newborns and the birth index was basically equivalent to control animals.

Newborn F_1 observations: Findings in newborns (F_1) for percent live newborns per litter, percent stillborns per litter, sex ratio, body weight, viability and weaning indices versus controls, were unremarkable.

Body weight gain was slightly higher F_1 pups of dams in the 30 mg/kg dose group than those of the control dams at the final time point (9.9% increment, Day 28 to Day 56, $p<0.01$ on Day 56). All weights gains were similar at Day 28 between the four groups. No other biologically significant differences in body weight gains were found between 3 mg/kg group. Female F_1 pups at the 10 mg/kg maternal dose showed decreased weight gain between Days 7-21 postnatal, and male F_1 pups showed lower weights than control males at the Day 14 weighing. These decreases in weight gain were slight and were only significant when compared to controls as body weight gain decrements were not significantly different between treatment groups and controls.

BODY WEIGHT GAIN (F_1)

Dose (mg/kg)		Control	3	10	30
Body weight(g), male					
Days after birth	7	16±2 (46)	16±1 (57)	15±2 (52)	16±2 (55)
	14	36±2 (46)	36±2 (57)	34±3 (52)@@	35±3 (55)
	21	57±4 (46)	58±3 (57)	55±4 (52)	57±4 (55)
	28	96±5 (24)	100±5 (28)	94±8 (26)	97±5 (27)
	35	151±8 (24)	158±7 (28)#	149±13(26)	156±8 (27)
	42	214±14(24)	225±14(28)	214±20(26)	225±11(27)
	49	276±19(24)	290±20(28)	279±27(26)	293±16(27)#
	56	331±24(24)	348±23(28)	335±32(26)	355±19(27)@@
Body weight(g), female					
Days after birth	7	16±1 (50)	15±2 (55)	14±2 (51)#	16±2 (56)
	14	35±2 (50)	34±3 (55)	33±3 (51)##	34±2 (56)
	21	56±3 (50)	55±4 (55)	54±5 (51)#	54±3 (56)
	28	91±4 (24)	90±6 (28)	87±8 (27)	89±5 (28)
	35	134±8 (24)	132±8 (28)	130±11(27)	133±8 (28)
	42	170±15(24)	168±13(28)	169±16(27)	168±12(28)
	49	196±15(24)	190±19(28)	198±20(27)	194±15(28)
	56	222±18(24)	215±18(28)	225±23(27)	218±18(28)

Mean±S.D. ( ) : No. of offspring
Significantly different from control @@ : p<0.01 (Scheffé's d-test)
Significantly different from control # : p<0.05, ## : p<0.01 (Scheffé's rank test)

(Sponsor, v18, p48)

Postnatal development of F_1 pups: Fewer eyelid separations had occurred in 30 mg/kg group (70.2%) than in controls (84.4%) by Day 14. This difference was not statistically significant, but indicates possible developmental delay. All other developmental benchmark data (F_1) were unremarkable.

DEVELOPMENTAL BENCHMARKS (F_1)

Dose (mg/kg)		Control	3	10	30
No. of dams		12	14	14	14
Separation of auricula	day 3	132/154	197/205	157/175	156/167
(%)		(84.9)	(96.3)	(83.2)	(93.7)
Appearance of hair	day 10	73/96	90/112	83/105	87/111
(%)		(76.0)	(80.4)	(74.1)	(78.4)
Eruption of incisors	day 12	93/96	111/112	102/105	104/111
(%)		(96.9)	(99.1)	(97.3)	(93.6)
Separation of eyelids	day 14	81/96	103/112	83/105	78/111
(%)		(84.4)	(92.0)	(80.4)	(70.2)
Descent of testis	day 20	34/46	49/57	43/52	45/55
(%)		(72.9)	(85.7)	(83.8)	(78.2)
Opening of vagina	day 33	22/24	28/28	27/27	25/28
(%)		(91.7)	(100.0)	(100.0)	(89.3)

(Sponsor, v19, p49)

Reflex response, auditory function, open field behavior, and rotor rod performance, and water multiple T-maze test data (F_1) were unremarkable.

Organ weights of F_1 offspring: Organ weight data in F_1 offspring were unremarkable.

Skeletal observations of F_1 offspring: Skeletal observations indicated irregular alignment of caudal vertebrae in one offspring (F_1) in the 10 mg/kg group and in two offspring (F_1) in the 30 mg/kg group. These findings were 1.9% and 3.6% of offspring examined per group, respectively. These findings may be drug-related. All other skeletal data were unremarkable.

SKELETAL OBSERVATIONS (F_1) OFFSPRING

Dose (mg/kg)	Control	3	10	30
No. of offspring examined	48	56	52	56
No. of offspring with anomalies (%)	0	0	0	0
No. of offspring with variations (%)	5(10.4)	3(5.4)	7(13.5)	5(8.9)
No. of offspring with variation (%)				
Accessory sternebra	3(6.3)	2(3.6)	5(9.6)	4(7.1)
Shortened 13th rib	0	1(1.8)	0	0
25 presacral vertebrae	1(2.1)	1(1.8)	1(1.9)	0
Lumbarization of 1st sacral vertebra	1(2.1)	1(1.8)	0	0
Irregular alignment of caudal vertebrae	0	0	1(1.9)	2(3.6)
No. of caudal vertebrae (Mean±S.D.)	28.2±0.3	28.0±0.4	28.0±0.4	28.0±0.6

(Sponsor, v19, p56)

Note: Visceral observations were not reported for F_1 offspring.

F_1 reproduction:

Fertility/reproductive parameters of F_1 generation: F_1 animals were mated at 11 weeks of age. Fertility data (F_1), including copulation and fertility indices, and length of time until copulation, were unremarkable.

Prenatal observations of F_2 pups: Body weight data for fetal offspring (F_2) were unremarkable. One example of epigastrius (underdeveloped twin attached to the epigastric region of the other) was found in the 3 mg/kg group (F_2) and one case of exencephaly (brain outside the skull) and myeloschisis (spina bifida resulting in flattened mass of neural tissue along the spine) in the 30 mg/kg group. Other prenatal observations of fetuses (F_2) were unremarkable. These findings may be drug-related.

PRENATAL OBSERVATIONS (F_2)

Dose (mg/kg)		Control	3	10	30
No. of dams		11	12	10	13
No. of corpora lutea		217	202	193	229
		19.7±2.3	16.8±4.1	19.3±3.5	17.6±2.7
No. of implants		191	181	156	207
		17.4±1.0	15.1±5.5	15.6±3.9	15.9±2.2
No. of dead fetuses (%)		16(8.2)	8(3.7)	13(8.8)	18(8.8)
Implantation mark		0	0	0	0
Early resorption		15(7.7)	8(3.7)	12(7.4)	16(7.8)
Late resorption		1(0.6)	0	1(1.4)	2(1.0)
Dead fetus		0	0	0	0
No. of live fetuses		175	173	143	189
		15.9±1.2	14.4±5.1	14.3±4.8	14.5±2.6
Male:female		88:87	94:79	65:78	90:99
(Male%)		(50.3)	(54.3)	(45.5)	(47.6)
Body weight(g)	Male	3.26±0.22	3.55±0.84	3.11±0.17	3.44±0.28
	Female	3.11±0.19	3.39±0.81	2.96±0.20	3.31±0.33
Placental weight(g)	Male	0.45±0.03	0.49±0.15	0.48±0.11	0.49±0.06
	Female	0.43±0.04	0.46±0.10	0.45±0.07	0.46±0.06
No. of fetuses with external anomalies (%)		0	1(0.6)[a]	0	1(0.5)[b]

Mean±S.D. [a] : Epigastrius [b] : Exencephaly & myeloschisis

(Sponsor, v19, p58)

F_1/F_2 Histopathology during/after reproduction: No histopathology performed.

Toxicokinetics: Not studied.

RF2505 – Teratogenicity study of NK-104 in rabbits by oral administration (preliminary study)

Definitive study already reviewed.

RFG2507 – Study by Oral Administration of NK-104 during Fetal Organogenesis in Rabbits (Segment II)

Key study findings:

- Non-dose dependent increase in skeletal variation (27 presacral vertebrae) at ≥ 0.1 mg/kg
- Maternal toxicity ≥ 0.3 mg/kg (severe necrosis of the tubular epithelium, severe dilatation of proximal tubule and flatness, and severe calcification renal tubuli and moderate single cell liver necrosis)
- Dose-related increase in maternal mortality in mid-dose, 0.3 mg/kg group (1/14) and high-dose, 1.0 mg/kg group (3/14)
- Dose-dependent decreases in maternal fecal volumes observed in the mid-dose, 0.3 mg/kg group (4/14) and high-dose, 1.0 mg/kg group (8/14). This observation was noted prior to all deaths and correlates with decreases in food consumption.
- Severe fatty deposition and moderate necrosis of hepatocytes and paleness of appearance of livers were observed in the high-dose, 1.0 mg/kg group (7/14), which is likely related to the mechanism of action of NK-104
- Severe epithelial cell necrosis of gallbladder with appearance of white materials were observed in the high-dose, 1.0 mg/kg group (4/14)
- Findings of a general hypersensitivity to statin drugs in rabbits compared to humans and other test species, characterized by severe adult/maternal toxicity, have been reported for other HMG-CoA reductase inhibitors. These include (but are not limited to): lovastatin[1], atorvastatin[2]

[1]**Kornbrust, DJ et al "Toxicity of the HMG-coenzyme A reductase inhibitor, lovastatin, to rabbits"** J Pharmacol Exp Ther (1989) **248**, 498-505.
[2]**Dostal, LA et al "Developmental toxicity of the** HMG-CoA reductase inhibitor, atorvastatin, in rats and **rabbits" Teratology (1994) 50**, 387-94.

Volume #, and page #: v18, p437
Conducting laboratory and location: Fuji Research Laboratories, Kowa Co., Ltd., Shizuoka, Japan
Date of study initiation: September 28, 1993
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot number, and purity: 104P-9202, 99.2% purity

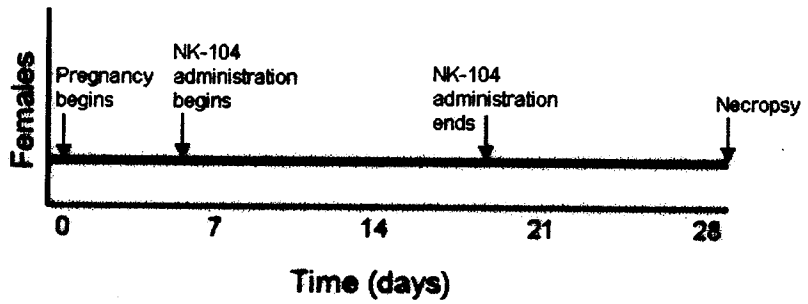
Methods:

Group No.	Drug	Dose (mg/kg)	Concentration (W/V%)	No. of Copulated Animals	No. of Pregnant Females
1	0.5% CMC	-	0	16	15
2	NK-104	0.1	0.01	16	15
3	NK-104	0.3	0.03	16	14
4	NK-104	1	0.1	16	14

(Sponsor v18, p440)

Doses: 0, 0.1, 0.3, 1.0 mg/kg/day NK104, **days 6 – 18 of gestation (13 doses** total)
Species/strain: Kbl:JW rabbits (SPF)
Number/sex/group: 16 females/dose
Route, formulation, volume, and infusion rate: oral gavage, 1 mL/kg
Satellite groups used for toxicokinetics: not included.

Study design: daily dosing by oral gavage on gestational Days 6 – 18, cesarean/necropsy Day 29
Breeding: 9 males age 27-33 weeks **or 9 males age 19 – 25 weeks were** acclimatized for at least 10 days, and utilized for breeding. Animals were observed for evidence of copulation, which if present was designated Day 0 of gestation.



Parameters and endpoints eval**uated: clinical evaluations (daily, except Days 6 – 18, twice daily), body weight (gestational Days 0, 6 – 19, 23, 26, and 29), food intake (gestational Days 1, 6 – 19, 23, 26,** and 29), gross/histopathology (Day 29), reproductive parameters (corpora lutea, implantations, early/late resorptions, dead embryos/fetuses), sex ratio, viable fetuses (body weight, placental weight, morphological/skeletal/visceral malformations).

Gross pathological examination of rabbit does that aborted or died prior to end of the study were conducted as soon as possible. Organs with observed abnormalities from does that aborted or died were fixed and examined. Livers (including gallbladder), and kidneys, in animals in which abnormalities were observed were also examined histopathologically.

Approximately 2/3 of live fetuses were fixed with isopropanol denatured ethanol. Subsequently, skeletal samples were removed, stained with alizarin red S, and examined for abnormalities. Approximately 1/3 of live fetuses were fixed in **Blouin's fluid and examined for morphological abnormalities by Wilson's free** hand razor blade method (gross evaluation) **and Nishimura's thoracic visceral** observation method (microdissection technique).

Results:

Mortality (F_0 does): Dose-related mortality occurred in females in the 0.3 mg/kg group (1/14) and the 1.0 mg/kg group (3/14). The single death in the no-dose control group was consistent with gavage error.

Segment II Reproductive Toxicology Study in Rabbits – Mortality and Major Clinical Findings				
Dose group (mg/kg)	**0**	**0.1**	**0.3**	**1.0**
Mortality	1/15*	0/15	1/14	3/14
Abortion	0/15	0/15	2/14	2/14
Vaginal hemorrhage	0/15	0/15	1/14	1/14
Weakness	0/15	0/15	0/14	1/14
↓ Fecal volume	1/15	1/15	4/14	8/14

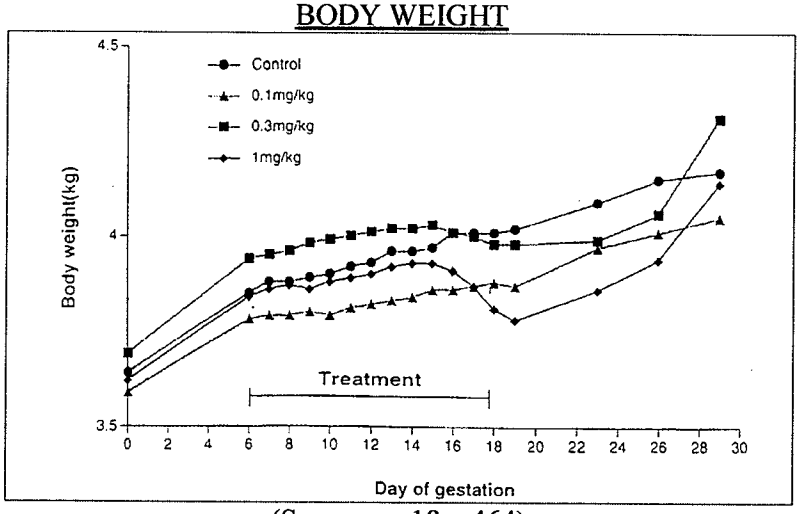
*Pathological findings in the control-group mortality were consistent with gavage error

Clinical signs (F_0 does): Decreased fecal volume measured with increasing dose in female rabbits. Does aborted in the 0.3 mg/kg group (2/14) and the 1.0 mg/kg (2/14) group. One doe exhibited weakness prior to death in the 1.0 mg/kg group (1/14). One doe showed vaginal hemorrhage prior to abortion in both the 1.0 mg/kg group (1/14) and 3.0 mg/kg (1/14) dose groups. There were decreases in fecal volumes for 0.3 mg/kg (4/14) and 1.0 mg/kg (8/14) NK-104 dose groups.

Clinical signs in the 0.1 mg/kg dose group were unremarkable compared to vehicle-only controls.

Body weight gains (F_0 does): The high dose group (1.0 mg/kg) exhibited a transient ~4% loss of body weight towards the end of the NK-104 treatment phase between Days 15 and 19. Surviving 1.0 mg/kg group member body weight gains appeared to recover in the **post-treatment phase (Days 20 – 29). This effect** lacked statistical significance, may have been drug-related, but was considered potentially biologically significant. It was possible that continued NK-104 administration may have had significant effects; drug administration ended on Day 18.

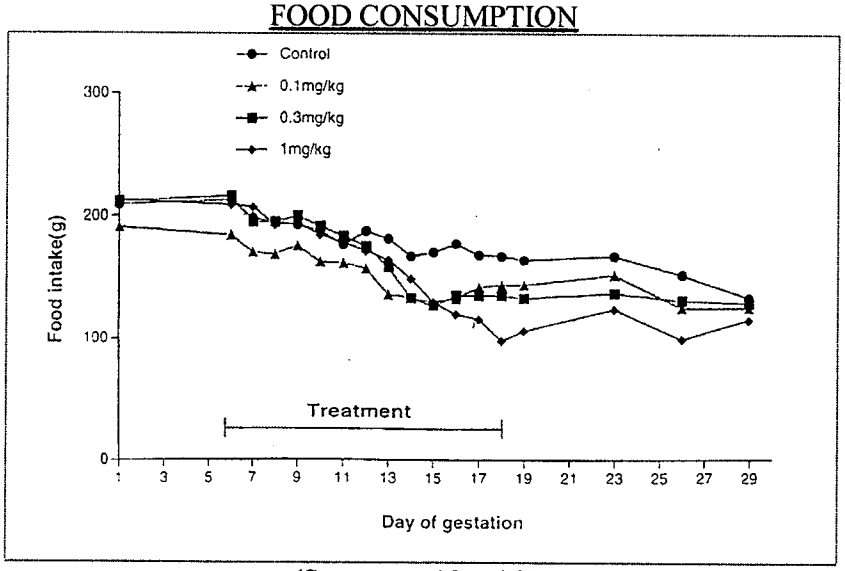
BODY WEIGHT



(Sponsor, v18, p464)

Food consumption (F_0 does): **Decreased body weight of 1.0 mg/kg group (days 15 – 19)** appeared to correlate with moderate decrease in food consumption (↓33%), as compared to controls during the same time period. This effect lacked statistical significance.

FOOD CONSUMPTION



(Sponsor, v18, p465)

Toxicokinetics: Not studied.

Pathological evaluations (F_0 does): Observed pathologies were correlated with death and abortion, and included predominantly liver, kidney and gallbladder findings. Liver pathologies in the 1.0 mg/kg group were paleness of color (7/14) and accentuated lobular appearance (4/14). Kidney pathologies in the 0.3 mg/kg group (2/14) and 1.0 mg/kg group (7/14) were paleness of color (likely related to ischemia). One case of hemorrhage of the thymus occurred in the 1.0 mg/kg group (1/14).

GROSS PATHOLOGY, F_0 DOES

organ	Dose	Control	NK-104		
finding (part)	mg/kg		0.1	0.3	1
Lung					
Hemorrhage		1(1)/15	0/15	0/14	0/14
Liver					
Pale in color		1/15	1/15	0/14	7(5)/14
Diffuse white regions		0/15	0/15	1(1)/14	0/14
Accentuated lobular pattern		0/15	0/15	0/14	4(4)/14
Kidney					
Pale in color		0/15	0/15	2(2)/14	7(5)/14
Thymus					
Hemorrhage		0/15	0/15	0/14	1(1)/14
Gallbladder					
Appearance of white materials		0/15	0/15	1(1)/14	4(3)/14
Trachea					
Hemorrhage		1(1)/15	0/15	1(1)/14	0/14
Stomach					
Petechia (Glandular stomach)		0/15	0/15	0/14	1(1)14
Hemorrhage (Glandular stomach)		0/15	0/15	1(1)/14	0/14
Colon					
Hemorrhage		0/15	0/15	1(1)/14	0/14

(Sponsor, v18, p466)

Histopathological evaluations (F_0 does): Does were examined after cesarean section, spontaneous abortion, or death, as follows: control, 1/15; 0.1 mg/kg, 1/15; 0.3 mg/kg, 1/14; 1.0 mg/kg, 7/14.

There were two animals that aborted in the 0.3 mg/kg group that were not examined histopathologically. Likewise there were two animals in the 1.0 mg/kg group that did not abort, undergo cesarean section, or that died, which were examined histopathologically. There is was no indication that animal examination findings were switched between groups (transposition error). There was no indication what the rationale might have been for excluding two animals from the 0.3 mg/kg group that met stated criteria for examination, while two animals that did not meet stated criteria for examination were included in the other.

Liver: Moderate degeneration and/or necrosis of the liver were observed in the early decedent/aborted/cesarean sectioned does in the 0.3 mg/kg group. Fatty deposition of hepatocytes, severe, fine droplets (centrilobular) and large droplets (perilobular), 7/7 (6/7, severe) were seen in the 1 mg/kg group of early deaths, which was likely related to HMG-CoA reductase inhibition. Degeneration and/or necrosis were seen in 4/7 in the high dose group (3/4, mild; 1/4, severe). Necroses of the epithelial cells of the gallbladder were seen in 5/7 does (4/7, severe) in the 1.0 mg/kg group. Minimal or slight calcification of hepatocytes was described in the high dose, 1.0 mg/kg NK-104 dose group (3/7).

LIVER HISTOPATHOLOGY, F_0 DOES

		Control	NK-104 (mg/kg)										
			0.1		0.3				1				
No. of dams examined		1	1		1				7				
Organ	findings	−	−+	+++	−	+	++	+++	−	±	+	++	+++
Liver	Fatty deposition of hepatocytes (Fine droplets, centrilobular and large droplets, perilobular)	1		1		1						1	6
	Fatty deposition of hepatocytes (Diffuse fine droplets)		1	1		1			7				
	Degeneration and/or necrosis of hepatocytes	1		1			1		3		3		1
	Calcification of hepatocytes	1		1		1			4	1	2		
Gallbladder	Necrosis of epithelial cells	1		1				1	3				4
	Hemorrhagic necrosis of epithelial cells	1		1		1			6			1	

− : Negative ± : Slight + : Mild ++ : Moderate +++ : Severe

(Sponsor, v18, p467)

Kidney: In the 0.3 mg/kg group, necrosis of the tubular epithelium (1/2), dilatation of proximal tubule and flatness (1/2), and calcification (1/2), all severe, were noted in early decedent/aborted/cesarean sectioned females. Histopathological examinations of the kidneys upon early death in the 1.0 mg/kg group revealed necrosis of the tubular epithelium (3/7, severe), casts, tubule (3/7, moderate), dilatation of proximal tubules and flatness (3/7, severe), calcification of tubuli (1/7, severe), congestion (1/7, moderate), perivascular infiltration (3/7, slight to mild).

KIDNEY HISTOPATHOLOGY, F_0 DOES

		NK-104 (mg/kg)								
		0.3				1				
No. of dams examined		2				7				
Organ	findings	−	+	++	+++	−	±	+	++	+++
Kidney	Necrosis of renal tubular epithelium	1			1	4				3
	Cast in renal tubuli	1	1			3		1	3	
	Dilatation of proximal tubules and flatness	1			1	2	1	1		3
	Calcification of renal tubuli	1			1	3	2	1		1
	Congestion	2				6			1	
	Perivascular cell infiltration		1	1		4	1	2		

− : Negative ± : Slight + : Mild ++ : Moderate +++ : Severe

(Sponsor, v18, p468)

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

Offspring (malformations, variations, etc.): Gross pathological examinations of all fetuses were unremarkable.

Fetal visceral and skeletal observations: Unremarkable.

Prenatal and postnatal development

RFG2511 – Study by Oral Administration of NK-104 in Rats during the Perinatal and Lactation Periods (Segment III)

Key study findings:

- NOAEL (F_1 pups) was <1 mg/kg, due to decreased viability indices in the 1 mg/kg (↓28%), 3 mg/kg (↓19%), 10 mg/kg (↓29%) and 30 mg/kg group (↓100%) compared to controls.
- NOAEL (F_0 dams) was <1 mg/kg, due to death during administration of 1 mg/kg NK-104 during gestation. There were no deaths at the 3 mg/kg dose. The 10 mg/kg group also had dam deaths (3/23), [paleness of kidneys (2/3), stomach petechia or hemorrhage (2/3), and vaginal discharge or bleeding (2/3), paleness of liver (1/3).
- At the 30 mg/kg NK-104 dose, gestation index was decreased 53% ($p<0.01$), the number of newborns per litter were decreased 26%, the number of stillborns per litter data trended higher with increasing dose (30 mg/kg, ↑~7-fold), and the birth index was decreased by 22%.
- During the lactation period, in the early decedent/lactation insufficient dams of the 10 mg/kg NK-104 group (2/20) [paleness of liver (2/2), paleness of kidney (1/2), thickening of the forestomach wall (2/2), and vaginal discharge (2/2)].
- Pathologies in early decedent animals during 30 mg/kg NK-104 administration during gestation (8/23) were [liver paleness (6/8), kidney paleness (5/8), petechia or hemorrhage of the stomach (8/8), undelivered fetuses or hemorrhage of the uterus (2/8), vaginal bleeding (3/8)].
- In the 30 mg/kg group during lactation, in dams that died or were lactation deficient (7/7) [necropsy findings were paleness of liver (3/7), and atrophy (2/7) and hemorrhage (1/7) of the thymus.]
- Histopathology was not evaluated.
- NOAEL (F_2 fetuses) was 10 mg/kg, due to zero F_1 survival at the 30 mg/kg dose.
- There was a transient, drug-related decrease in weight gain (51% decrement) during **gestational Days 17 – 21 in F_0** dams at the 30 mg/kg dose of NK-104.
- Lactation insufficiency was present in each NK-104 dose group of dams, 0 mg/kg (0/24), 1 mg/kg, (6/23), 3 mg/kg (5/24), 10 mg/kg (4/18), and 30 mg/kg (1/5).
- The weaning index was decreased slightly for dams dosed with 10 mg/kg NK-104 (↓8%), while no F_1 pups survived to weaning in the 30 mg/kg NK-104 group.
- The percent of F_1 pups meeting developmental benchmarks was decreased when dams were administered NK-104 during gestation and lactation, as follows: the percent of F_1 pups with unseparated auricula at 10 mg/kg (↓19%) and 30 mg/kg (↓100%, all pups later died), the percent of F_1 offspring with eruption of incisors at 10 mg/kg, (↓8%, $p<0.05$), and the percent of pups with separation of eyelids at 10 mg/kg (↓13%), compared to controls. No F_1 pups in the 30 mg/kg NK-104 group survived to evaluate the later benchmarks. Other benchmarks for all dose groups, including appearance of hair, descent of testis, and opening of the vagina, were unremarkable.

Volume #, and page #: v19, p189

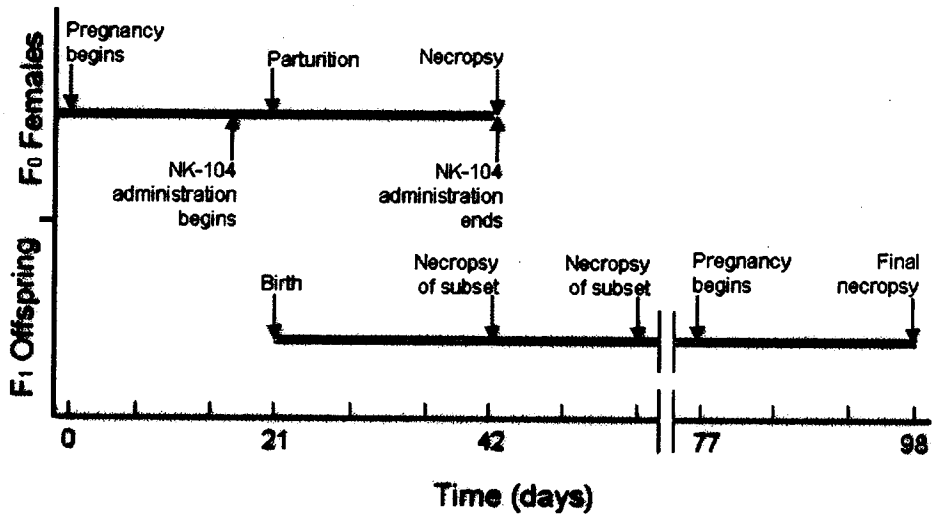
Conducting laboratory and location: Fuji Research Laboratories, Kowa Co., Ltd., Shizuoka, Japan
Date of study initiation: January 1, 1995
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot number, and purity: 104P-9401, 99.3% purity

Methods:

Group No.	Test substance	Dose (mg/kg)	Concentration (W/V%)	Number of animals with successful copulation	Number of pregnant animals
1	0.5% CMC	–	0	25	24
2	NK-104	3	0.15	25	24
3	NK-104	10	0.5	25	23
4	NK-104	30	1.5	16	15
5	NK-104	1	0.05	25	24

(Sponsor, v19, p215)

Doses: 0, 1, 3, 10, 30 mg/kg/day NK104
Species/strain: Crj:CD(SD) Sprague-Dawley rats (SPF)
Number/sex/group: 25/female/dose
Route, formulation, volume, and infusion rate: oral gavage, 2 mL/kg
Satellite groups used for toxicokinetics: None.



Study design: Daily dosing by oral gavage from gestational Day 17 to postpartum Day 21. Necropsy of F_0 dams was carried out on postpartum Day 21, necropsy of a subset of F_1 offspring was performed on study Day 56, a subset F_1 offspring were bred at 11 weeks of age, and cesarean/necropsy procedures were carried out on Day 20 of F_1 gestation and F_2 fetuses were evaluated.

Parameters and endpoints evaluated: Clinical signs (F_0) Days 0, 7, and 14 of **gestation, twice daily Days 17 – 21 of** gestation (administration period), daily **Days 0 – 21 postpartum. Body weight (F_0) weekly until Day 17, daily Days 17 –** delivery, and Days 0, 4, 7, 14, 18 and 21 postpartum. Food intake (F_0) weekly **until Day 17, daily Days 17 – delivery, and Days 1, 4, 7,11, 14, 18 and 21** postpartum. Gestation [(# females with live newborns / # pregnant females) x 100] and birth indices [(# live newborns / # implants) x 100] calculated. Gross examination was performed on dead dams and dams of which all pups died as soon as possible.

Gross fetal (F_1) anomalies, live and dead newborns, and sexes were counted. Viability and weaning indices were calculated. F_1 body weights were measured weekly until Day 56. Viability index [(# live pups on day 4 postpartum / # live pups born) x 100] and weaning index [(# pups weaned / # live pups born) x 100]. Development of newborns was monitored, including separation of auricula (Day 3 postpartum), appearance of abdominal hair (Day 10), eruption of incisors (Day 12), separation of the eyelids (Day 14), and decent of testes (Day 20)/opening of the vagina (Day 33).

Subset of F_1 offspring were necropsied, and examined for visceral and skeletal abnormalities (soft X-ray) at Day 21 after birth.

Reflex function tests included presence of gait, corneal reflex, pain response, **righting reflex, and air righting reflex on Day 21 – 23 after birth. Presence of** pinna reflex (auditory test/Preyer reflex) was assessed.

Behaviors tested were influences on emotional expression (open field test, ambulation-number section crossed, rearing frequency, preening frequency, grooming frequency, defecation frequency, and frequency of urination), nervous/muscular activities (rotarod test) and learning performance (water multiple T-maze test) at 5-6 weeks of age.

One male and one female (F_1) were euthanized and gross pathology was examined. Organ weights (brain, heart, lung, liver, kidney, spleen, thymus, and testis or ovary) were recorded.

F_1 reproductive performance was evaluated, including copulation index [(# successfully copulated males / # mated males) x 100], fertility index [(# males successfully impregnating females / # mated males) x 100] for males, and the copulation index [(# successfully copulated females / # mated females) x 100], fertility index [(# females successfully impregnated / # mated females) x 100] for females. The days to copulation were counted. After the mating period, males were euthanized and visceral observations were made. For males and females with no success with copulation or where males for copulation died, histopathological evaluation of the testis/ovaries was carried out. After cesarean

section, females (F_1) were euthanized and corpora lutea implants, live fetuses, dead embryos, implantation sites, early resorbed embryos, late resorbed embryos, dead fetuses were counted. Body and placental weights were measured, and the presence of any gross anomalies in fetuses (F_2) was recorded.

Results:

F_0 females, in-life:

Maternal mortality: There was a dose-related increase in dam mortality with increasing NK-104. In the high-dose group (30 mg/kg), 14/15 dams died between Days 22 of gestation and Day 4 of lactation (treatment was Day 17 of gestation to Day 21 of lactation), including 8/14 prior to parturition. The 30 mg/kg dose group was halted due to high mortality. A 1 mg/kg was added to **the protocol as a new "low dose" group. 5/23** dams died in the 10 mg/kg group. The only other death was in the 1 mg/kg group (1/24). There were no deaths in the vehicle-only control and 3 mg/kg groups.

Of the 14 deaths in the 30 mg/kg group of NK-104, 8/14 deaths occurred during the gestational period. The remaining dam deaths (6/14) **occurred on Day 2 – 4 of lactation.**

Care of F_1 neonates: Lactation insufficiency occurred in each dose group of NK-104 of the following groups of dams (confounding analyses of pup development/indicates that dams are sick): 1 mg/kg, 6/23; 3 mg/kg, 5/24; 10 mg/kg, 4/18; 30 mg/kg, 1/5; no-dose control group, 0/24.

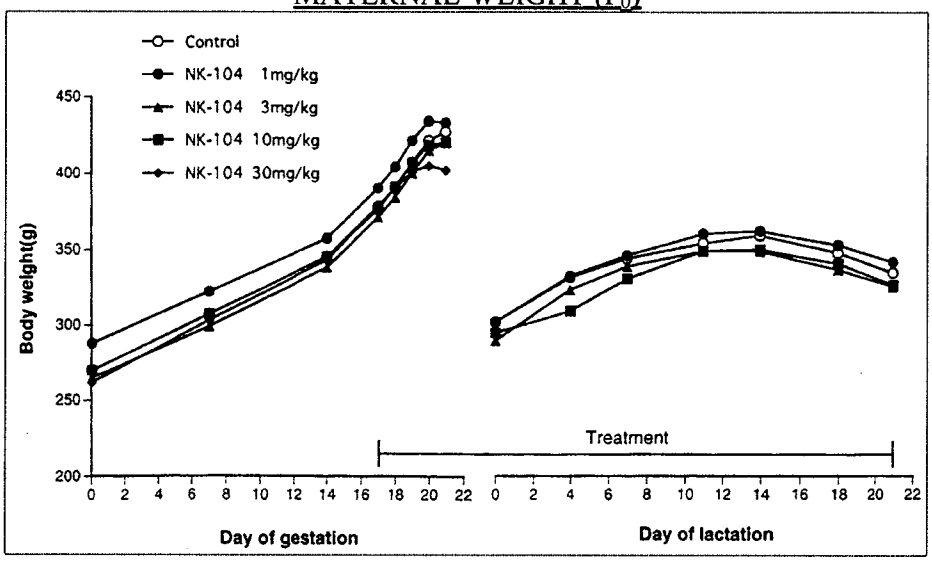
MATERNAL MORTALITY/LACK OF CARE (F_0)

Dose (mg/kg)		Control	1	3	10	30
No. of dams		24	24	24	23	15
No. of dead dams		0	1	0	5	14
Day of gestation	Day 22	0	0	0	1	5
	Day 23	0	1	0	2	3
Day of lactation	Day 2	0	0	0	1	2
	Day 3	0	0	0	0	1
	Day 4	0	0	0	1	3
Lack of care		0	6	5	4	1
Day of lactation	Day 1	0	2	0	1	0
	Day 2	0	3	5	2	1
	Day 3	0	1	0	0	0
	Day 7	0	0	0	1	0

(Sponsor, v19, p232)

Body weight gains: There was a significant decrease in rate of body weight gain in the 30mg/kg group ($p<0.001$) compared to control dams (↓51% decrement), which was detected in the first 5 days of drug administ**ration (gestational Days 17 – 21). Weigh gain** data for the 0, 3, and 10 mg/kg dose groups of NK-104 were unremarkable.

MATERNAL WEIGHT (F_0)

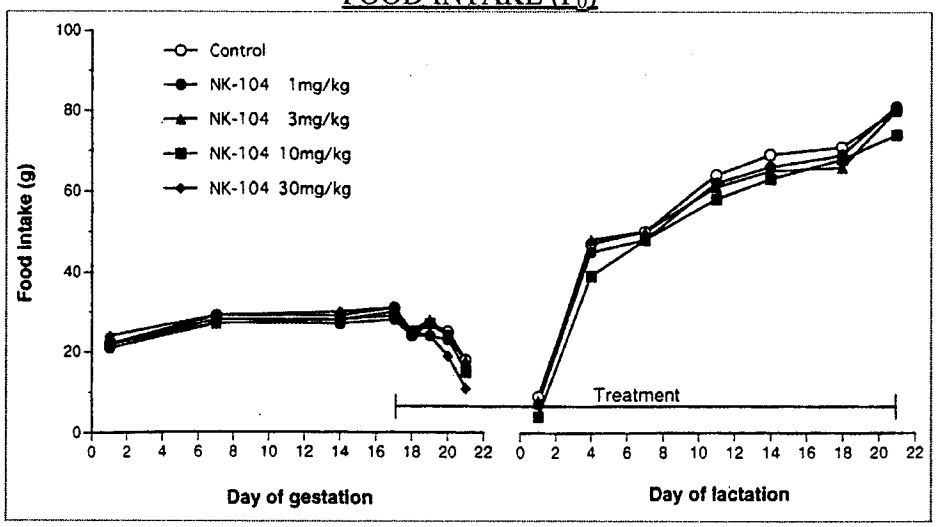


(Sponsor, v19, p230)

Food Intake: The 30 mg/kg dose group experienced a significant decrease in food intake on gestational Day 19, ↓11%; Day 20, ↓24% and Day 21, ↓39%. Decreased weight gain in the 30 mg/kg NK-104 group was correlated with decreased food intake during the same period.

In the 1 mg/kg dose group, there was a single day with a significant decrease in food intake (Day 19) compared to controls ↓11%, but was an isolated finding and not considered drug-related or biologically relevant. (The additional 1 mg/kg group was begun when high mortality caused the 30 mg/kg group to be halted.)

FOOD INTAKE (F_0)



(Sponsor, v19, p231)

MATERNAL FOOD INTAKE (F_0)

Dose (mg/kg)		Control	1	3	10	30
No. of dams		24	24	24	23	15
Food intake (g)						
Day of gestation	1	22±3	21±3	22±3	22±4	24±2
	7	29±4	27±3	27±5	28±3	29±3
	14	29±3	27±3	28±4	28±3	30±3
	17	31±3	28±4	30±3	29±5	31±3
	18	24±3	24±4	25±3	25±3	25±5
	19	27±2	24±3***	28±2	27±3	24±3*
	20	25±3	23±4	24±2	24±4	19±3***
	21	18±6	16±8	18±5	15±6	11±5*
No. of dams		24	21	24	19	7
Food intake (g)						
Day of lactation	0	9±8	7±8	8±8(23)	4±5	1±2
	4	47±7	45±9(17)	48±6(19)	39±13(15)	
	7	50±5	48±6(17)	50±4(19)	48±5 (14)	
	11	64±5	62±8(17)	61±5(19)	58±8 (14)	
	14	69±5	66±6(17)	65±7(19)	63±7 (14)	
	18	71±4	69±4(17)	66±7(19)	68±7 (14)	
	21	80±8	81±9(17)	80±7(19)	74±8 (14)	

Mean±S.D. ( ) : No. of dams examined
Significantly different from control : * $p<0.05$, *** $p<0.001$ (Scheffé's d-test)
Significantly different from control : *** $p<0.001$ (Scheffé's rank test)

(Sponsor v19, p235)

Reproductive findings in F_0 dams: The gestational indices were similar between 0, 1, 3, and 10 mg/kg groups, with a slight trend towards a decrease in the 10 mg/kg group (↓17%) and were due to dam death (3/23) prior to parturition. The 30 mg/kg group showed a highly significant ($p<0.001$) decrease in gestation index (↓53%), which was due to dam deaths (8/15) prior to parturition.

The gestational periods and # implants/litter data were unremarkable for all dose levels.

The number of newborns/litter data showed trend towards fewer newborns per litter with increased dose in the 30 mg/kg dose group (↓26%).

The birth index was highest in the control group (88.4), while birth indices were similar at 1, 3, and 10 mg/kg doses (76.3, 78.9, and 78.9, respectively). The birth index was lowest in the 30 mg/kg group (↓22 percentage points), but was not statistically significant.

DELIVERY FINDINGS IN DAMS (F_0)

Dose (mg/kg)		Control	1	3	10	30
No. of dams		24	24	24	23	15
No. of dams with live newborns		24	23	24	20	7
Gestation index (%)		100	95.8	100	87.0	46.7***
Gestation period (day)		22.3±0.4	22.3±0.5	22.4±0.5	22.4±0.5	22.4±0.5
No. of implants	(Total)	391	390	389	331	110
	(Per litter)	16.3±1.2	17.0±1.8	16.2±1.3	16.6±1.6	15.7±1.9
No. of newborns	(Total)	346	295	303	259	75
	(Per litter)	14.4±1.7	12.8±4.1	12.6±3.3	13.0±2.6	10.7±3.8
Birth index (%)		88.4	76.3	78.9	78.9	68.9

Mean±S.D. Significantly different from control : *** $p<0.001$ (Chi-Square test)

(Sponsor, v19, p235)

F_0 necropsy:

Necropsy during gestation: In the 0 and 3 mg/kg NK-104 dose groups during the gestational period, no animals (F_0 dams) died or were necropsied. One dam in the 1 mg/kg group succumbed to dystocia on day 23 of gestation. Pathologies in the 10 mg/kg group were: paleness of kidneys (2/3), stomach petechia or hemorrhage (2/3), and vaginal discharge or bleeding (2/3); paleness of liver (1/3). Pathologies in the 30 mg/kg group were predominantly liver paleness (6/8), kidney paleness (5/8), petechia or hemorrhage of the stomach (8/8), undelivered fetuses or hemorrhage of the uterus (2/8), vaginal bleeding (3/8), and hemorrhage of the ileum (1/8). Organ paleness is probably related to the statin MOA.

NECROPSY FINDINGS IN DAMS DURING GESTATION (F_0)

	Findings	Dam-No.							
		3F04*	3F07*	3F12*					
NK-104 10 mg/kg	Liver								
	Pale in color			+					
	Kidney								
	Pale in color	+		+					
	Stomach								
	Petechia (Glandular stomach)	+							
	Hemorrhage (Glandular stomach)		+						
	Vagina								
	Vaginal discharge	+							
	Vaginal bleeding		+						
		4F03*	4F05*	4F06*	4F07*	4F09*	4F10*	4F12*	4F16*
NK-104 30 mg/kg	Liver								
	Pale in color		+	+	+	+	+	+	
	Kidney								
	Pale in color		+	+		+	+	+	
	Stomach								
	Petechia (Glandular stomach)	+	+	+	+	+	+	+	
	Hemorrhage (Glandular stomach)								+
	Ileum								
	Hemorrhage								+
	Uterus								
	Hemorrhage		+						
	Undelivered fetuses in the uterus							+	
	Vagina								
	Vaginal bleeding		+					+	+
		5F13*							
NK-104 1 mg/kg	Uterus								
	Undelivered fetuses in the uterus	+							

* : Dead

(Sponsor, v19, p296)

Necropsy during lactation: Necropsy findings in control, 1, and 3 mg/kg dose groups during lactation were unremarkable. In the 10 mg/kg NK-104 group, there was paleness of liver (2/20), paleness of kidney (1/20), thickening of the forestomach wall (2/20), and vaginal discharge (2/20). All of these findings occurred in dams that died or were determined to be lactation deficient. Organ paleness was probably due to MOA if NK-104.

NECROPSY FINDINGS IN DAMS DURING LACTATION (F_0) 10 mg/kg NK-104

	Findings	Dam-No.														
		3F01*	3F02*	3F03*	3F06*	3F08*	3F09*	3F10*	3F11*	3F14*	3F15*	3F16*	3F17*	3F18*	3F19*	3F20*
NK-104 10 mg/kg		-	-	-	-	-	-	-	-	-		-	-			-
	Liver															
	Pale in color										+				+	
	Kidney															
	Pale in color										+					
	Stomach															
	Thickening of wall (Forestomach)														+	
	Vagina															
	Vaginal discharge										+			+		
		3F21*	3F22*	3F23*	3F24*	3F25*										
			-	-	-	-										
	Liver															
	Pale in color															
	Kidney															
	Pale in color															
	Stomach															
	Thickening of wall (Forestomach)	+														
	Vagina															
	Vaginal discharge															

* : Scheduled kill * : Dead (j: lactation insufficiency

(Sponsor, v19, p298)

In the 30 mg/kg group during lactation, necropsy findings was paleness of liver (3/7, statin MOA) atrophy (2/7) and hemorrhage (1/7) of the thymus, hemorrhage (1/7) or undelivered fetuses (1/7) in the uterus, adrenal congestion (1/7), staining of anus (1/7); vaginal discharge (1/7) and bleeding (1/7). All of these findings were in animals that died; the lone dam in the 30 mg/kg group that survived to study end (lactation Day 21) showed no remarkable pathology during necropsy evaluation.

NECROPSY FINDINGS IN DAMS DURING LACTATION (F_0) 30 mg/kg NK-104

	Findings	Dam-No.						
		4F01*	4F02*	4F08'	4F11*	4F13'	4F14'	4F15*
		-						
	Liver							
	Pale in color		+		+			+
	Thymus							
NK-104 30 mg/kg	Atrophy					+	+	
	Hemorrhage					+		
	Uterus							
	Hemorrhage		+					
	Undelivered fetuses in the uterus							+
	Adrenal							
	Congestion					+		
	Anus							
	Staining					+		
	Vagina							
	Vaginal discharge		+					
	Vaginal bleeding			+				+

*: Dead ': Found dead (J: lactation insufficiency

-:no notable changes were observed

(Sponsor, v19, p299)

F_1 physical development:

Newborn (F_1) observations: The data for the number of newborns per litter showed a trend for fewer newborns per litter with increased dose of NK-104 (30 mg/kg, ↓26%), compared to controls. The number of stillborns per litter data trended higher with increased dose (30 mg/kg, ↑~7-fold) compared to controls. These differences were not statistically significant.

Sex ratio data were unremarkable.

Body weights of newborns with increased dose were lower (30 mg/kg NK-104, ~↓7%) compared to controls, but this effect did not reach statistical significance.

There were no external anomalies noted at any dose.

Viability index [(# live pups on day 4 postpartum / # live pups born) x 100] on Day 4 of lactation was decreased at 1 mg/kg (↓28%), 3 mg/kg (↓19%), 10 mg/kg (↓29%), and 30 mg/kg (↓100%, all pups later died) compared to controls. Weaning index [(# pups weaned / # live pups born) x 100] was decreased slightly for the 10 mg/kg group (↓8%),

though not statistically different from control. No pups survived to weaning in the 30 mg/kg NK-104 group.

FINDINGS IN F_1 NEWBORNS BORN TO F_0 DAMS

Dose (mg/kg)		Control	1	3	10	30
No. of newborns	(Total)	346	295	303	259	75
	(Per litter)	14.4±1.7	12.8±4.1	12.6±3.3	13.0±2.6	10.7±3.8
No. of stillborns	(Total)	13	59	50	39	25
	(Per litter)	0.5±0.8	2.6±3.8	2.1±3.5	2.0±2.4	3.6±3.4
Sex ratio on newborns	Male	52.3	49.8	51.2	53.3	54.7
	Male/Female	181/165	147/148	155/148	138/121	41/34
Body weight(g) of newborns	(Male)	6.3±0.6	6.2±0.3	6.4±0.6	6.1±0.5	5.9±0.5
	(Female)	6.0±0.6	5.9±0.5	6.0±0.4	5.8±0.5	5.6±0.4
No. of newborns with external anomalies		0	0	0	0	0
Viability index on day 4 (%)		97.3	70.5*	78.9	68.6**	0.0***
Weaning index (%)		100.0	100.0	100.0	92.0	

Mean±S.D. Significantly different from control : * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (Wilcoxon's rank test)

(Sponsor v19, p236)

F_1 body weight gain: Body weight gains were unremarkable among 0, 1, 3, and 10 mg/kg dose groups of NK-104. No pups in the 30 mg/kg dose group survived to Day 7 of lactation, which was the first day pup weights were recorded.

Table 7 Postnatal development in offspring(F_1) born of rat dams(F_0) treated orally with NK-104

Dose (mg/kg)		Control	1	3	10	30
No. of dams		24	17	19	14	3
Separation of auricula	day 3	304/338	225/232	242/263	152/193	0/35
(%)		(90.1)	(95.4)	(91.3)	(73.0)	(0)**
Appearance of hair	day 10	148/192	129/133	138/152	94/108	
(%)		(77.1)	(97.1)	(90.8)	(87.5)	
Eruption of incisors	day 12	185/192	124/133	148/152	96/108	
(%)		(96.4)	(93.4)	(97.4)	(88.4)*	
Separation of eyelids	day 14	183/192	124/133	148/152	89/108	
(%)		(95.3)	(93.4)	(97.4)	(83.0)	
Descent of testis	day 20	66/98	44/66	63/78	41/55	
(%)		(68.1)	(67.6)	(80.5)	(75.0)	
Opening of vagina	day 33	42/48	31/32	36/38	25/27	
(%)		(87.5)	(96.9)	(94.7)	(92.9)	

Significantly different from control : * $p<0.05$, ** $p<0.01$ (Wilcoxon's rank test)

(Sponsor, v19, p238)

F_1 postnatal development: The percent of pups with separation of auricula were decreased at 10 mg/kg (↓19%) and 30 mg/kg (↓100%), as measured at Day 3. The percent of F_1 offspring with eruption of incisors was decreased at 10 mg/kg (↓8%, $p<0.05$), as measured on Day 12, and the percent of pups with separation of eyelids was decreased at 10 mg/kg, ↓13%, as measured on Day 14. No pups in the 30 mg/kg NK-104 group survived to Day12. Data for appearance of hair, descent of testis, and opening of the vagina were unremarkable.

F_1 behavioral evaluation: Measured at 5-6 weeks of age, reflex responses and auditory function data (appearance of normal gait, corneal reflex, pain response, righting reflex, air righting reflex, and Preyer reflex) were unremarkable. Open field behavior data were unremarkable.

Rotarod performance was decreased in male offspring of 10 mg/kg, as measured by the average number of falls from the rod for 3 minutes (5.6 falls versus 3.3 falls in controls); this finding was not statistically significant. Female rotarod performance data were unremarkable.

Water multiple T-maze test data were unremarkable in male and female F_1 offspring of dams treated orally with NK-104.

F_1 gross external observations: Not evaluated.

F_1 skeletal observations: Skeletal observations were unremarkable.

F_1 visceral observation: Not evaluated.

F_1 organ weights: Absolute and relative organ weight data in male and female F_1 offspring were unremarkable.

F_1 reproduction:

Findings in F_1 dams: Upon mating of F_1 dams, the number of copulated animals, the number of impregnating males, the number of pregnant females, the occurrence time of copulation after mating was started, and body weight gain data were unremarkable.

Findings in F_2 fetuses: The number of corpora lutea per F_1 dam, number of implants per F_1 dam, number of dead F_2 fetuses (implantation marks, early and late resorptions, and dead fetuses), male-to-female ratio, number of live F_2 fetuses, body and placental weight, and the number of F_2 fetuses with external anomalies were unremarkable.

Matings in the 1 mg/kg group average 4.9 days to copulation, but were 3.0, 2.9, and 2.8 days to copulation in control, 3 mg/kg and 10 mg/kg dose groups.

RFG2512 – Study by oral administration of NK-104 in rats during the perinatal and lactation periods (Segment III – Additional Study)

Key study findings:

- The F_1 pups for oral administration of NK-104 during the perinatal and lactation periods was <0.1 mg/kg NK-104, due increased pup mortality in the 0.1 mg/kg dose group. This effect was not dose-related, but was probably related to lactation insufficiency in F_0 dams (see next item).
- NOAEL <0.1 mg/kg for pups, due to one dam in the 0.1 mg/kg group that had all dead pups, but all dams in the 0.3 mg/kg treatment group gave birth to live pups.
- Lactation insufficiency was present in both controls and both dose groups: 0 mg/kg, no-dose controls (1/25), 0.1 mg/kg (5/25), and 0.3 mg/kg (2/25), but did not show a clear dose-related trend.

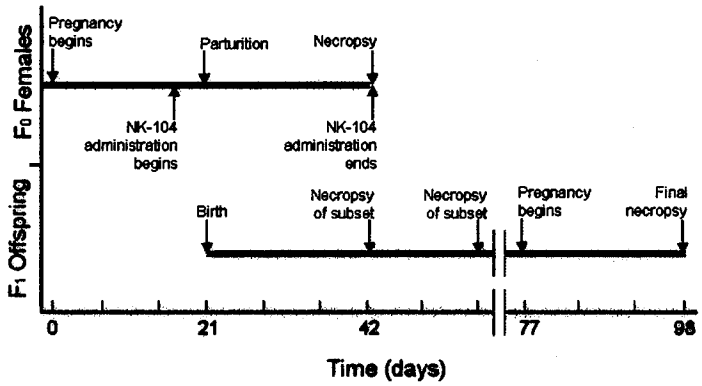
Volume #, and page #: v20, p3
Conducting laboratory and location: Fuji Research Laboratories, Kowa Co., Ltd., Shizuoka, Japan
Date of study initiation: September 13, 1995
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot number, and purity: 104P-9401, 99.3% purity

Methods:

Group No.	Test Substance	Dose (mg/kg)	Concentration (W/V%)	Number of animals with successful copulation	Number of pregnant animals
1	0.5% CMC	–	0	25	25
2	NK-104	0.1	0.005	25	23
3	NK-104	0.3	0.015	25	22

(Sponsor v20, p4)

Doses: 0, 0.1, and 0.3 mg/kg NK-104
Species/strain: Crj:CD (SD) Sprague-Dawley rats (SPF)
Number/sex/group: 25/females/group
Route, formulation, volume, and infusion rate: Oral gavage, 2 mL/kg
Satellite groups used for toxicokinetics: None.



Study design: Daily dosing of NK-104 by oral gavage, from gestational Day 17 to postpartum Day 21, with necropsy of F_0 dams and a subset of F_1 pups on Day 21 postpartum, necropsy of a subset of F_1 offspring on Day 56, breeding of a subset of F_1 offspring, and cesarean/necropsy of F_1 dams and F_2 fetuses) on Day 20 of gestation.

Parameters and endpoints evaluated: Clinical signs (F_0) Days 0, 7, 14, twice daily **Days 17 – 21 of gestation (administration period), daily Days 0 – 21 postpartum.** Body weight (F_0) **weekly until Day 17, daily Days 17 – delivery, and Days 0, 4, 7,** 14, 18 and 21 postpartum. Food intake (F_0) **weekly until Day 17, daily Days 17 –** delivery, and Days 1, 4, 7,11, 14, 18 and 21 postpartum. Gestation [(# females with live newborns / # pregnant females) x 100] and birth indices [(# live newborns / # implants) x 100] calculated. Gross examination was performed on dead dams and dams of which all pups died as soon as possible.

Gross fetal (F_1) anomalies, live and dead newborns, and sexes were counted. Viability and weaning indices were calculated. F_1 body weights were measured weekly until day 56. Viability index [(# live pups on day 4 postpartum / # live pups born) x 100] and weaning index [(# pups weaned / # live pups born) x 100]. Development of newborns was monitored, including separation of auricula (Day 3 postpartum), appearance of abdominal hair (Day 10), eruption of incisors (Day 12), and separation of the eyelids (Day 14).

Reflex function tests included presence of gait, corneal reflex, pain response, **righting reflex, and air righting reflex on Days 21 – 23 after birth. Presence of** pinna reflex (auditory test/Preyer reflex) was assessed.

Results:

F_0 dams in-life:

Body weight gain and food intake: Differences between dose groups of NK-104 and no-dose controls for body weight gain and food intake during gestation and lactation were unremarkable.

Delivery findings: All pregnant dams produced live newborns. Gestational index, gestation period, number of implants per F_0 dam, number of live newborns per F_0 dam, and birth index data were not different from controls.

F_0 dams were more likely to show lactation insufficiency at 0.1 mg/kg (4/25) and 0.3 mg/kg (2/25) than were control animals (1/25).

Necropsy of F_0 dams: Findings were unremarkable.

F_1 physical development:

Findings in F_1 neonates: The number of newborns per F_0 dam, stillborns per F_0 dam, sex ratio, body weight, and the number of newborns with external anomalies per F_0 dam, viability index, and weaning index after NK-104 administration were not remarkably different from controls.

Body weight gain and postnatal development in F_1 offspring: Body weight gain and developmental indicators were not different between dose groups and controls.

F_1 behavioral evaluation:

Reflex responses: Gait, corneal reflex, pain response, righting reflex, air righting reflex, and Preyer reflex in NK-104 dose groups were not different from controls.

F_1 reproduction: Not studied here. Covered in main study, RFG2511, and data were not remarkable.

F_2 findings: Not studied here. Covered in main study, RFG2511, and data were not remarkable.

Reproductive Toxicology Summary

Segment I

Pitavastatin caused no apparent adverse effects on male and female rat fertility at oral doses of 10 and 30 mg/kg/day respectively at systemic exposures 56-fold and 354-fold clinical exposure at 4 mg/day based on AUC.

Pitavastatin treatment in rabbits resulted in mortality in males and females administered 1 mg/kg/day (102-fold clinical systemic exposure) during a fertility study. Although the cause of death was not determined, rabbits had gross signs of renal toxicity (kidneys whitened), perhaps indicating ischemia. Lower doses (28-fold human systemic exposure) did not show significant toxicity in adult males and females. However decreased implantations, increased resorptions as well as decreased viable fetuses were observed.

Segment II
Pitavastatin crosses the placental barrier into the fetus. Rat fetus concentrations were ≤36% of maternal plasma pitavastatin concentrations. Pitavastatin and 5-keto-pitavastatin are secreted by rats into milk at 7.2-fold greater pitavastatin levels than are present in maternal plasma. The predominant form of pitavastatin in the milk form is the unchanged parent molecule.

Embryo-fetal developmental studies were conducted in pregnant rats treated with 3, 10, 30 mg/kg/day pitavastatin by oral gavage during organogenesis. Evidence of maternal toxicity was indicated by decreased body weight gain and decreased food consumption at the 30 mg/kg/day level. The maternal NOAEL was established at 10 mg/kg/day (56-fold human systemic exposure at 4 mg/day dose by AUC). A neonatal malformation of agnathia (absence/partial lower jaw) 1/347 neonates, and irregular alignment of caudal vertebrae 1/347 neonates was observed at this dose. The developmental NOAEL is established at 3 mg/kg/day (17-fold human systemic exposure at 4 mg/day based on AUC).

Embryo-fetal developmental studies were conducted in pregnant rabbits treated with 0.1, 0.3, 1 mg/kg/day pitavastatin by oral gavage during organogenesis. Body weight loss was seen at all doses (6.7-fold human systemic exposure at 4 mg/day dose based on AUC). At maternal doses ≥0.3 mg/kg/day mortality and spontaneous abortions were seen (28-fold human systemic exposure). Significant maternal liver and renal toxicity appears to have contributed to the toxicity. A skeletal variation of 27 presacral vertebrae was seen with 0.1 mg/kg/day litters.

Segment III
In peri-postnatal studies in pregnant rats given oral gavage doses of pitavastatin at 0.1, 0.3, 1, 3, 10, 30 mg/kg/day from organogenesis through weaning, mortality was observed at 1 mg/kg/day. As a result of the maternal mortality an additional peri-postnatal study was performed in pregnant rats given oral gavage doses of pitavastatin at 0.1, 0.3 and 1 mg/kg/day from organogenesis through weaning. Insufficient lactation was noted at all doses which contributed to the decreased survival of the neonates in these dose groups. This occurs at 6.7-fold human systemic exposure at 4 mg/day dose based on AUC. Therefore, a developmental NOAEL could not be established even at the lowest dose tested of 0.1 mg/kg/day.

2.6.6.7 Local tolerance

2.6.6.8 Antigenicity, mechanistic studies, qualification of metabolites, impurities

Antigenicity:

Table 11. NK-104 Antigenicity Testing

Study Number	Species	Test Type Route	Key Findings
SR9241	Guinea pig	ASA p.o. or s.c.	• ASA reaction negative to challenge with NK-104 or NK-104-BSA in animals sensitized p.o. with NK-104 or s.c. with NK-104+FCA • ASA reaction positive to challenge with either NK-104-BSA or NK-104-OA but negative with NK-104 alone in animals sensitized s.c. with NK-104-OA+FCA
SR9241	Guinea pig	PCA i.d.	• PCA reaction negative to challenge with NK-104 or NK-104-BSA in animals sensitized i.v. with NK-104 or NK-104-OA+FCA • PCA reaction positive to challenge with NK-104-BSA or NK-104-OA, but negative with NK-104 alone in animals that received i.d. sera. from animals sensitized s.c. with NK-104-OA+FCA

SR9241 – Antigenicity study of NK-104 in guinea pigs

Active systemic anaphylaxis (ASA) and passive cutaneous anaphylaxis (PSA) demonstrate no antigenicity in guinea pigs.

GROUP ASSIGNMENTS

Group	No. of animals	Antigen	Administration route	Dose	Frequency and duration
Test group 1	12	NK-104 suspension	p.o.	0.1 mg/kg/ frequency	5 times/week, 3 weeks
Test group 2	12	NK-104 suspension	p.o.	1 mg/kg/ frequency	5 times/week, 3 weeks
Test group 3	12	NK-104+FCA	s.c.	1 mg/head/ frequency	Once/week, 3 weeks
Test group 4	16	NK-104-OVA+FCA	s.c.	1 mg/head/ frequency	Once/week, 3 weeks

(Sponsor, M4, SR9241, p13)

Mechanistic studies:

TSH was increased in NK-104 treated rats, which was likely due to increased metabolism of T_4 by UDP-GT activity in rats. This may be responsible for induction of TSH-dependent hypertrophy and subsequent neoplasia in rats. Humans are understood to be able to maintain more robust T_4 concentrations than rats.

A single administration of 1 mg/kg to Beagle dogs results in lens levels of 18 ng/g or 25- to 30-fold increase with multiple dosing. This suggests a correlation between lens opacity and NK-104 distribution. Concentrations of NK-104 lactone were very low to undetectable in ocular tissue.

Binding to plasma proteins was greatest in humans > rabbits > rats > monkey, and the weakest plasma protein-binding for NK-104 was seen in dogs.

Dogs were given oral doses of NK-104±mevalonic acid. Although the formation of cataracts was not affected by coadministration of mevalonate the perturbations in plasma GPT and lung findings by NK-104 were suppressed indicating their possible association with HMG-CoA reductase inhibition. Mevalonate administration to rats appeared to protect against hyperkeratosis. (i.e. from GD 17 to lactation Day 20, 10 or 30 mg/kg NK-104 and mevalonic acid lactone 1000 mg/kg/d were administered to pregnant female rats.)

RF9811 - Study on the effect of NK-104 on the thyroid gland in rats

Key study findings:

- These data are consistent with increased TSH, which is likely due to increased metabolism of T_4 by UDP-GT activity in rats, may be responsible for induction of TSH-dependent hypertrophy and subsequent neoplasia in rats.
- Humans are understood to be able to maintain more robust T_4 concentrations than rats.

There were increases in follicular cell tumors in the thyroid glands of rats in a 92 week carcinogenicity study. Similar findings were observed in the cases of simvastatin, fluvastatin, and lovastatin. The mechanism by which NK-104 induced thyroid tumors in rats was investigated using males only. Dosing was based on the doses between the LOAEL (25 mg/kg) for tumors, the MTD based on mortality (50 mg/kg), and a dose that allowed some animals to survive (75 mg/kg) in the 92-week rat carcinogenicity study.

DOSE GROUP ASSIGNEMENTS

Group No.	Test article	Dose level (mg/kg)	Volume (mL/kg)	Concentration (w/v%)	Number of animals *1 Male	Dosing period (days)
1	Control	-	5	-	10	7
2	NK-104	25	5	0.5	10	7
3	NK-104	50	5	1.0	10	7
4	NK-104	75 (37.5 × 2)	5	0.75	10 [a]	7
5	Phenobarbital	80	5	1.6	5	7
6	Control	-	5	-	10	14
7	NK-104	25	5	0.5	10	14
8	NK-104	50	5	1.0	10 [b]	14
9 *2	NK-104	75 (37.5 × 2)	5	0.75	0 [c]	14
10	Phenobarbital	80	5	1.6	5	14
11	Control	-	5	-	10	28 or 29
12	NK-104	25	5	0.5	10	28 or 29
13 *2	NK-104	50	5	1.0	0 [c]	28 or 29
14 *2	NK-104	75 (37.5 × 2)	5	0.75	0 [c]	28 or 29
15	Phenobarbital	80	5	1.6	5	28 or 29
16 *3	Control	-	5	-	10	28
17 *3	NK-104	50	5	1.0	10 [d]	28

*1: Number of animals used for the examinations related to the thyroid gland
*2: Excluded as subjects of the study because of an insufficient number of animals due to deaths or use of the animals to supplement another group.
*3: Drug administration was initiated at the age of 7 weeks.

(Sponsor, M4, RF9811, p8)

Terminal blood samples were obtained for analysis of thyroid hormone (T_4, total free), and thyroid stimulating hormone (TSH). Livers and thyroid glands were weighed. Hepatic UDP-glucuronosyltransferase activity was determined. Histopathology was performed for thyroid glands. Clinical signs and body weights were recorded.

Mortality: There were 9/30 deaths at the 50 mg/kg dose level and 18/30 deaths in the 75 mg/kg dose group.

Clinical observations: There was 1/5 animal that showed decreased locomotor activity at the 50 mg/kg dose, and decreased locomotor activity (4/5) with hypothermia (4/5) animals were observed at 75 mg/kg NK-104 during Week 1, while no drug-related clinical observations were noted from Weeks 2 to 4.

Body weights: Body weight gain was not significantly different between dose groups and controls up to 50 mg/kg NK-104.

Hormone effects: There were immediate effects of NK-104 on TSH, total T_4 and free T_4 levels (≤1 week); TSH was decreased at the 75 mg/kg dose, T_4 was decreased at the 50-75 mg/kg doses and HD, while free T_4 was decreased at all doses.

By Week 2, TSH and free T_4 levels had rebounded to control levels, however total T_4 was still significantly lower at 25 and 50 mg/kg dose levels (the 75 mg/kg dose level showed excess mortality and data were not provided for this group at Week 2).

At Week 4, TSH levels were increased, while total T_4 and free T_4 were decreased at the 50 mg/kg dose. There was no statistically significant difference between TSH and T_4 levels measured at the 25 mg/kg dose at the Week 4 time-point.

Thyroid/liver weights: Thyroid weights were significantly increased at the 50 and 75 mg/kg dose levels at Week 1, and were increased at all remaining dose levels by Week 2 and again at Week 4 (75 mg/kg dose group was halted due to excess mortality). Liver weights were not different.

UDP-GT activity: While testosterone UDP-GT activity was not different between NK-104 dose groups, L-thyroxine UDP-GT activity was increased at the 50 mg/kg dose level at Week 2 of NK-104 administration, and was increased at all doses by Week 4.

Histopathology: At Week 1, thyroid follicular cell hypertrophy was noted at 50 mg/kg NK-104. By Week 2, it was present at all dose levels.

RF9812 – Study on the effect of NK-104 on the thyroid gland in rats: 2. Evaluation of non-effect dose with regard to thyroid hormones

NK-104 was administered to male rats for 14 and 28 days at the 5 mg/kg dose. There were no effects of drug administration on body weight gain, liver or thyroid weight, T_4, TSH, T_4-UDP-GT enzyme activity at 5 mg/kg at any time point. A 25 mg/kg dose of NK-104 was used as a positive control for earlier findings.

RF9731 – Study on the effect of NK-104 to thyroxine clearance in rats by multiple oral administrations

Mechanistic studies designed to explore the development of thyroid follicular cell tumors in rats as a function of increased TSH and decreased thyroxine (increased clearance) has been performed. This is the mechanism reported for simvastatin. The Sponsor reports that NK-104 increases UDP-GT activity in the liver and infers that enhancement of thyroxine clearance following 8-day treatment at 25 or 50 mg/d with NK-104 occurs. TSH levels increase by negative feedback accompanied by enhancement of thyroxine clearance. The establishment of TSH induced thyroid tumors by long-term administration has been reported previously.

G2533 – Immunohistochemical examination of cell-proliferating activity in the thyroid gland from "Six-month repeated oral administration toxicity study of NK-104 in rats"

This study examined the rat thyroid gland samples from RFG2506, a six-month toxicity study of NK-104 in rats. No differences were detected in weight measurements and histopathology of thyroid glands. Slides were prepared and probed with an anti-

proliferating cell nuclear antigen (PCNA) antibody. There were no differences detected between control and 10 mg/kg dose groups versus controls in this assay.

G2532 – Immunohistochemical examination of cell-proliferating activity in specimens from "Two-stage carcinogenicity study of NK-104 in rat thyroid" (additional examination)

This study examined the control rat thyroid gland samples from G2528, a carcinogenicity study of NK-104 in rat thyroid. Slides were prepared and probed with an anti-proliferating cell nuclear antigen (PCNA) antibody. There was a 6.61% PCNA-positive cell index in G2528 at 25 mg/kg dose groups versus 3.3% controls in this assay. Therefore, NK-104 was determined to cause increased proliferating activity at 25 mg/kg in the earlier assay.

RF2001/06 – Immunohistochemical study of cell proliferative activity in specimens used for evaluation of the effects of NK-104 on rat thyroid

This study examined the rat thyroid gland samples from RF9811, a two-week study of NK-104 administration in rats at 25 mg/kg and 50 mg/kg. Here, slides of thyroid glands from study RF9811 were prepared and probed with an anti-proliferating cell nuclear antigen (PCNA) antibody. In this assay, there was no statistically significant difference between PCNA positive cells (%) in dose groups versus controls, but there was a trend towards an increase with increasing dose. NK-104 could not be shown to induce weight increase by a proliferative mechanism, by using an anti-PCNA-antibody for analysis. This points to hypertrophy as the reason for increased thyroid weight at 2 weeks of NK-104 administration at 25 mg/kg.

RF9225 – Effects of a single oral dose of NK-104 on dog eyes

This study was carried out to examine the distribution of NK-104 in eye tissue after 4 and 24 hours post-administration of 10 mg/kg in 2 male beagle dogs per time-point. There were no effects after a single dose.

A92012 – Concentration of NK-104 in eye tissues after an oral administration of NK-104 to dogs

The following tables illustrate that the parent NK-104 was higher at 24 hours in the lens of dogs than at 4 hours. NK-104 lactone decreased by 24 hours and was undetectable in dog eye lens. Parent NK-104 and NK-104 lactone were lower at 24 hours than at 4 hours in plasma, heart and liver, and in the eye, including aqueous humor and vitreous body. These data indicate the potential for NK-104 to be accumulated in the lens of dogs.

Group	Animal-No.	Measuring object	Plasma level (ng/mL) 0 hr	1 hr	2 hr	4 hr	8 hr	24 hr
1	No. 1	NK-104	n.d.	14760	4400	1761	-	-
	No. 2	NK-104	n.d.	10221	2119	3857	-	-
2	No. 1	NK-104	n.d.	7001	2139	981	209	32
	No. 2	NK-104	n.d.	1975	989	975	161	12
	Mean		n.d.	8489	2412	1894	185	22
1	No. 1	NK-104 lactone	n.d.	1271	1658	1152	-	-
	No. 2	NK-104 lactone	n.d.	589	849	864	-	-
2	No. 1	NK-104 lactone	n.d.	856	1134	858	465	61
	No. 2	NK-104 lactone	n.d.	265	293	281	205	n.d.
	Mean		n.d.	745	984	789	335	31

(Sponsor, M4, A92012, p6)

Group	Animal-No.	Measuring object	Tissue level: Aqueous humor (ng/mL) Right	Left	Lens (ng/g) Right	Left	Vitreous body (ng/g) Right	Left	Heart (ng/g)	Liver (ng/g)
1	No. 1	NK-104	6.57	5.96	8.81	8.35	5.07	5.35	533	8450
(Sacrificed 4 hours)	No. 2	NK-104	4.66	5.84	4.83	5.72	4.57	5.80	643	11498
	Mean		5.62	5.90	6.82	7.04	4.82	5.58	588	9974
2	No. 1	NK-104	0.88	0.93	24.01	23.67	0.45	n.d.	36	542
(Sacrificed 24 hours)	No. 2	NK-104	0.72	0.64	12.04	13.00	n.d.	2.05	10	71
	Mean		0.80	0.79	18.03	18.34	0.23	1.03	23	307
1	No. 1	NK-104 lactone	1.85	1.91	9.76	9.57	1.69	1.78	1850	5487
(Sacrificed 4 hours)	No. 2	NK-104 lactone	1.41	1.47	4.72	5.49	0.93	1.10	1229	4043
	Mean		1.63	1.69	7.24	7.53	1.31	1.44	1540	4765
2	No. 1	NK-104 lactone	n.d.	0.54	n.d.	n.d.	n.d.	n.d.	49	162
(Sacrificed 24 hours)	No. 2	NK-104 lactone	n.d.	0.74	n.d.	n.d.	n.d.	n.d.	16	30
	Mean		n.d.	0.64	n.d.	n.d.	n.d.	n.d.	33	96

(Sponsor, M4, A92012, p7)

AG25002 – Concentration of NK-104 in lens after repeated oral administration of NK-104 for three months to dogs

Lens opacity was seen in some dogs given 1, 3 and 10 mg/kg/day NK-104 for 12, 3 and 1 months respectively. Therefore a study was designed to look at tissue distribution in the eye (vitreous, lens, aqueous). In a 3 month oral study dogs were given 1, 3, 10 mg/kg/day and selected ocular tissues excised at day 91 or following recovery on day 140 (~50 day recovery). NK-104 is distributed to the lens. In the groups given 1 mg/kg/day, 50 ng/g NK-104 was found in the lens whereas 200 ng/g was seen in the 3 mg/kg/day group and 500 ng/g was seen in the 10 mg/kg/day group on day 91. Lens opacity was not seen in the low-dose, but was present in 1/3 dogs in the mid-dose and all of the high-dose dogs. A single administration of 1 mg/kg to Beagle dogs results in lens levels of 18 ng/g or 25- to 30-fold increase with multiple dosing. This suggests a correlation between lens opacity and NK-104 distribution. The vitreous and aqueous humor contain 1/50th the drug concentration of the lens except for one high-dose male. This one male with distribution to the vitreous was thought to occur as a function of progressive lens degeneration. Concentrations of NK-104 lactone were very low to undetectable in ocular tissue. Following the 50 day recovery period the 10 mg/kg/day group still had NK-104

levels of 18 ng/g in males and 71 ng/g in females indicating a very slow release from the lens.

R93055 – Distribution of NK-104 in eye tissues after single and repeated oral administration of NK-104 to various animals

A subsequent study evaluated an oral dose of 10 mg/kg in monkey and rabbit and examined lens, vitreous, aqueous humor in addition to plasma, liver and heart levels of NK-104 and its lactone. In the monkey C_{max} was reached 1-2 hr post-dose achieving levels of 0.2-1.8 μg/mL and decreased to $1/100^{th}$ of the C_{max} by 24h post-dose. In the rabbit C_{max} was reached 1-2 h post dose achieving levels of 29-47 μg/mL and as with the monkey rapidly declined to $1/100^{th}$ C_{max} by 24h. In the monkey the concentration in ocular tissues was: aqueous humor (0.9 ng/mL) > vitreous (0.74 ng/mL) > lens (undetectable) at 4 hr post-dose. At 24 hr after dosing the lens (0.68 ng/mL) had detectable levels, vitreous levels remained the same and aqueous level were now undetectable. Little lactone was present in ocular tissues.

Hepatic levels of parent were higher than in plasma and lactone levels were about half that of parent. This is consistent with the extensive metabolism observed in the monkey. Heart levels were similar to that of the liver.

In the rabbit the ocular distribution at 4 h post dose was aqueous (22.5 ng/mL)>vitreous (8.3 ng/mL)>lens (4.85 ng/mL). At 24 h post-dose the levels changed such that the lens (5.4 ng/mL) stayed the same but the aqueous (1.7 ng/mL) and vitreous (0.98 ng/mL) levels decreased. Little lactone was present.

Lens opacity has been observed with other statins except for pravastatin. These studies suggest that the dog lens is more susceptible to distribution of NK-104 and that drug remains in this tissue to a greater extent than the monkey or rabbit. Comparisons of 4 hr versus 24 hour lens tissue levels in the dog indicates a retention of approximately 3-fold whereas rabbit lens levels did not change appreciably.

R96010 – Binding of NK-104 with lens protein of various animal

Key study findings:

- The order binding affinity constants (K) for NK-104 for plasma proteins are: human (230)>rabbit (187)>rat (130)>monkey (76)>dog (61)>mouse (29).
- For lens protein: dog (34-38)>rat (21)>mouse (15)>monkey (13)>rabbit (10-11)
- For partition coefficients (lens-to-plasma) dog(0.6)>mouse(0.54)>monkey (0.18)≥rat (0.17)>rabbit(0.06).

This study evaluated the lens/plasma ratio of NK-104 after an *in vitro* analysis of NK-104 to a homogenate of lens proteins. Plasma levels were known from other studies. The lens-to-plasma partition coefficient was modeled assuming dynamic equilibrium between lens and plasma. In addition to ocular distribution studies across species (see PK section) the Sponsor also determined binding affinities to plasma and lens proteins (report R96010). NK-104 distributes readily into dog and mouse lens. The partition coefficient

rank order does not necessarily agree with the binding constant for lens proteins because competition between plasma and lens proteins for NK-104 needs to be considered.

Animal	Amount spiked (ng/ml)	Sample No.	Lens Wt. (g)	Homogenate Wt. (g)	NK-104 concentration Protein side (ng/ml)	NK-104 concentration Buffer side (ng/ml)	K_{EYE}	$f_{u,EYE}$ (%)	K_{PLASMA} [a]	Lens-to plasma partition coefficient [b]
Rat	300	1	0.153	1.540	212.8	78.5	17.2	5.5		
		2	0.158	1.563	225.4	70.6	21.7	4.4		
		3	0.126	1.269	231.1	65.9	25.2	3.8		
		Mean	-	-	-	-	21.4	4.6	130	0.17
		S.D.	-	-	-	-	4.0	0.9		
		S.E	-	-	-	-	2.3	0.5		
Mouse	300	1	0.114	1.127	224.2	83.3	16.7	5.6		
		2	0.149	1.495	214.5	89.6	14.0	6.7		
		3	0.164	1.671	220.6	90.9	14.5	6.4		
		Mean	-	-	-	-	15.1	6.3	29	0.54
		S.D.	-	-	-	-	1.4	0.5		
		S.E	-	-	-	-	0.8	0.3		
Rabbit	30	1R [c]	0.295	2.952	20.6	9.9	10.8	8.5		
		1L [d]	0.315	3.133	21.0	10.0	10.9	8.4		
		2R	0.274	2.711	20.6	9.6	11.3	8.1		
		2L	0.269	2.655	21.4	10.0	11.3	8.2		
		3R	0.291	2.947	20.5	9.6	11.5	8.0		
		3L	0.286	2.858	19.4	9.3	10.9	8.4		
		Mean	-	-	-	-	11.1	8.3	187	0.06
		S.D.	-	-	-	-	0.3	0.2		
		S.E	-	-	-	-	0.1	0.1		
	300	1R	0.295	2.952	201.2	93.2	11.6	7.9		
		1L	0.315	3.133	198.5	94.4	11.0	8.4		
		2R	0.274	2.711	198.3	98.5	10.0	9.1		
		2L	0.269	2.655	197.9	95.5	10.6	8.6		
		3R	0.291	2.947	196.6	98.4	10.1	9.0		
		3L	0.286	2.858	200.9	96.8	10.7	8.5		
		Mean	-	-	-	-	10.7	8.6	187	0.06
		S.D.	-	-	-	-	0.6	0.4		
		S.E	-	-	-	-	0.2	0.2		
Dog	30	1R	0.613	6.173	24.4	5.6	33.8	2.9		
		1L	0.656	6.522	23.9	6.0	29.7	3.3		
		2R	0.577	5.749	24.2	5.8	31.6	3.1		
		2L	0.570	5.693	25.1	5.4	36.4	2.7		
		3R	0.599	5.908	24.7	5.5	34.4	2.8		
		3L	0.601	5.997	24.5	5.1	38.0	2.6		
		Mean	-	-	-	-	34.0	2.9	61	0.56
		S.D.	-	-	-	-	3.0	0.3		
		S.E	-	-	-	-	1.2	0.1		
	300	1R	0.613	6.173	242.9	45.0	44.3	2.2		
		1L	0.656	6.522	239.1	55.7	32.7	3.0		
		2R	0.577	5.749	239.1	55.0	33.4	2.9		
		2L	0.570	5.693	246.6	53.2	36.3	2.7		
		3R	0.599	5.908	241.2	49.8	37.9	2.6		
		3L	0.601	5.997	244.1	47.8	41.0	2.4		
		Mean	-	-	-	-	37.6	2.6	61	0.62
		S.D.	-	-	-	-	4.5	0.3		
		S.E	-	-	-	-	1.8	0.1		
Monkey	30	1R	0.100	1.045	194.3	87.5	12.8	7.3		
		1L	0.113	1.139	194.6	89.1	11.9	7.7		
		2R, L	0.129	1.296	200.4	85.6	13.5	6.9		
		Mean	-	-	-	-	12.7	7.3	76	0.18
		S.D.	-	-	-	-	0.8	0.4		
		S.E	-	-	-	-	0.4	0.2		
Human	-	-	-	-	-	-	-	-	230	-

a): Refer to the values of Tokyo laboratory report No. R93017 and R92086.
b): $(K_{EYE} + 1) / (K_{PLASMA} + 1)$. c) and d), Lens tissue of right side and left side, respectively

(Sponsor, M4, R96010, p10)

R93017 – Binding of NK-104 with plasma protein of various animals by equilibrium dialysis method

Key study findings:

- Binding to plasma proteins was greatest in humans > rabbits > rats > monkey, and the weakest plasma protein-binding for NK-104 was seen in dogs.

Binding of NK-104 to plasma proteins of human, monkey, rabbit and rat was examined by the equilibrium dialysis method. Using *in vitro*-derived data, the Sponsor calculated

that serum albumin and α_1-acid glycoprotein may provide all of the necessary protein-binding components necessary the protein-binding ratios for NK-104 observed *in vivo*.

Table 6 Protein binding of NK-104 to plasma of different animal species (in vitro)

Amount added (ng/ml)	Unbound fraction (%)					
	Dog [a)] Mean ± S.E.	Rabbit Mean ± S.E.	Monkey Mean ± S.E.	Human Mean ± S.E.	M. Rat Mean ± S.E.	F. Rat Mean ± S.E.
100	1.6 ± 0.1	0.6 ± 0.1	1.3 ± 0.0	0.5 ± 0.0	0.8 ± 0.0	0.5 ± 0.0
300	1.7 ± 0.1	0.5 ± 0.0	1.3 ± 0.0	0.4 ± 0.0	0.8 ± 0.0	0.6 ± 0.0
1000	1.7 ± 0.1	0.5 ± 0.0	1.3 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	0.7 ± 0.0

a_1 Unbound fraction of dog were obtained from the report of Kojima[1)]

(Sponsor)

RF9615 – Investigation of NK-104-induced cataract: Concentration of NK-104 in plasma and lens in rats by single oral administration

Investigation of cataract formation: Concentration of NK-104 in plasma and lens in rat following single oral administration

Table 1. Lens and plasma concentration of NK-104 after single oral administration of NK-104 in male and female rats

Matrix	Group	Sex	Dose (mg/kg)	Period	Concentration (ng/g or ng/mL) Animal Number 1	2	3	4	5	6	7	8	9	10	Mean	S.D.
Lens	1	Male	10	4 h	17.8	31.1	28.1	72.6	-	11.5	190.2	73.5	353.3	47.9	91.8	112.0
		Female	10	4 h	285.2	33.4	20.8	28.9	18.6	7.8	17.7	71.8	15.8	21.7	51.4	84.0
	2	Male	10	24 h	13.9	24.5	15.0	23.5	7.3	68.3	14.9	11.8	16.0	24.8	23.0	17.3
		Female	10	24 h	14.6	14.2	13.6	12.4	19.1	24.0	172.2	28.3	26.9	18.9	34.4	48.7
	3	Male	30	4 h	37.4	38.6	86.0	41.3	40.5	49.5	115.9	38.0	86.1	112.1	63.8	32.6
		Female	30	4 h	44.9	59.4	41.6	25.9	39.0	32.7	127.9	72.8	75.8	113.0	63.3	34.4
	4	Male	30	24 h	28.3	48.3	38.2	24.7	25.3	17.9	80.9	56.5	40.3	72.0	43.2	21.1
		Female	30	24 h	28.3	41.1	57.7	34.9	21.3	21.9	563.4	38.5	74.6	491.0	136.5	207.3
Plasma	1	Male	10	4 h	280.7	354.1	216.8	436.1	318.9	344.7	378.2	908.5	510.1	516.5	388.9	194.3
		Female	10	4 h	329.8	1035.7	1009.9	512.4	545.4	884.5	360.1	1054.7	294.1	368.8	639.5	319.4
	2	Male	10	24 h	8.9	7.7	4.0	8.3	4.2	25.3	2.8	3.8	3.5	6.3	7.5	6.6
		Female	10	24 h	14.7	9.3	12.6	7.6	5.2	13.5	7.8	12.9	28.7	4.7	11.7	6.9
	3	Male	30	4 h	1997.5	1810.9	2997.3	2874.2	3226.3	2946.8	1198.8	1232.4	2837.0	3173.6	2429.5	793.0
		Female	30	4 h	1963.1	3422.8	2461.1	504.2	1235.7	1643.7	1753.3	5144.6	2146.7	3134.7	2341.2	1382.8
	4	Male	30	24 h	4.7	10.5	8.8	15.1	27.1	11.1	11.9	22.0	9.7	11.4	13.2	6.6
		Female	30	24 h	46.8	18.6	15.2	11.3	12.7	14.2	33.0	24.4	11.5	5.6	19.3	12.3

(Sponsor)

In contrast to dogs, rats do not develop cataracts upon chronic dosing of pitavastatin.

R98056 - Binding of NK-104 with human lens protein by equilibrium dialysis method

Lens opacity was a common toxicological finding in repeat-dose dog studies. Here the Sponsor attempted to evaluate the risk of NK-104 to humans for development of lens opacity. The Sponsor utilized lens proteins from humans in an *in vitro* protein-binding study. The ratio of lens/plasma protein-binding was calculated *in vitro* and measured *in vivo* in non-clinical species, including dogs. Human lens/plasma ratios were generated using *in vitro* data only. The safety assessment was based on a comparison of these data. There was good agreement between *in vitro* and *in vivo* lens/plasma ratios for all non-clinical species, except mouse. If the *in vitro* human data are accurate, this would indicate a 20-50-fold safety margin for lens opacity in humans administered the 4 mg

NK-104 daily. It is not clear why the *in vivo* and *in vitro* mouse data sets were not in good agreement, although the *in vivo* numbers are lower than the lens/plasma protein-binding ratio calculated *in vitro*.

Animal	Sex	*in vitro* K_{EYE}	fp (%)	K_{PLASMA}	$P_{EYE,VITRO}$
Human	-	25.8	0.4 - 0.5	230[2)]	0.12
Monkey	M	12.7[1)]		76[1,2)]	0.18
	F	-		-	-
Dog	M	35.8[1)]		61[1,3)]	0.59
	F	-		-	-
Rabbit	M	10.9[1)]		187[1,2)]	0.06
Rat	M	21.4[1)]		130[1,2)]	0.17
	F	-		167[2)]	-
Mouse	M	15.1[1)]		29[1)]	0.54
	F	-		-	-

$P_{EYE,VITRO} = (K_{EYE}+1)/(K_{PLASMA}+1)$
These data were quoted from (b) (4) [redacted] es report R96010;
2) (b) (4) [redacted] report R93017; 3) (b) (4) [redacted] report R92086.

(Sponsor, M4, R98056, p15)

Animal	Sex	Dose (mg/kg)	C_{MAX} n	Conc. (ng./ml)	Range (ng/ml)	C_{EYE} n	Conc. (ng/ml)	Range (ng/ml)	$P_{EYE,VIVO}$	Mean[a)] $P_{EYE,VIVO}$
Monkey	M	3	4	109.9	(42.4 - 234.1)	4	8.79	(6.13 - 11.20)	0.115	0.142
		6	4	177.4	(131.0 - 215.9)	4	29.59	(24.31 - 41.59)	0.169	
	F	3	2	90.5	(34.4 - 146.6)	2	7.40	(2.88 - 11.92)	0.083	0.108
		6	3	154.3	(111.2 - 274.3)	3	17.52	(8.03 - 30.30)	0.134	
Dog	M	0.3	4	107.3	(43.3 - 164.4)	4	40.4	(32.1 - 50.3)	0.500	0.448
		1	4	382.2	(355.2 - 396.8)	4	150.5	(119.9 - 183.1)	0.396	
	F	0.3	4	60.4	(18.9 - 101.5)	4	25.3	(22.3 - 30.5)	0.615	0.586
		1	4	324.5	(154.8 - 505.7)	4	148.9	(121.5 - 196.0)	0.557	
Rabbit	M	0.5	3[b)]	44.4	(40.3 - 46.8)	3	2.6	(2.5 - 2.6)	-	0.058
Rat	M	10	5	880	(820 - 910)	10	118.5	(87.2 - 150.7)	0.135	0.089
		30	5	5900	(3650 - 9610)	10	248.1	(140.3 - 484.9)	0.042	
	F	10	5	2400	(1930 - 3190)	10	120.5	(77.3 - 194.5)	0.049	0.039
		30	5	8080	(5050 - 12380)	10	230.8	(137.4 - 317.9)	0.029	
Mouse	M	25		448.0	- -		4.4	- -	-	0.012
		75		2062.4	- -		29.5	- -	-	
		225		24060.1	- -		265	- -	-	
	F	25		699.4	- -		13.3	- -	-	0.013
		75		7347.7	- -		35.3	- -	-	
		225		29305.8	- -		402.1	- -	-	

$P_{EYE,VIVO} = C_{EYE}/C_{MAX}$ or C_{EYE}/C_{SS}
a), Mean value was obtained by averaging $P_{EYE,VIVO}$ of different dose.
b), In rabbit, C_{SS} values were used for the relational analysis instead of C_{MAX} values.

(Sponsor, M4, R98056, p15)

RF9719 – Investigation of NK-104-induced cataract: 4. Accumulation of NK-104 in lens by a 3-month oral administration to rats

NK-104 was administered to male and female rats at doses of 10 and 30 mg/kg body weight daily for 3 months by oral gavage. On Days 28 and 92, rats were sacrificed at 4 or 24 hours post-administration of NK-104. Lenses were removed from the eyes, and lens and plasma concentrations of NK-104 were calculated. On Days 1, 28, and 92, plasma NK-104 concentrations were basically unchanged at each dose, and dose-proportional with increasing dose. NK-104 concentrations in the lens, in contrast, continued to increase over time with repeated administration. This indicates the potential

for accumulation of NK-104 in lens of species that have not already exhibited lens opacity in repeat-dose toxicity studies.

Dose	Sex	Treatment		Plasma Concentration (ng/mL) Period (month) 0*	1	3	Lens Concentration (ng/mL) Period (month) 0*	1	3
10 mg/kg	Male	4h	Mean	385.9	307.0	446.9	91.8	57.9	118.5
			S.D.	104.3	101.4	238.8	112.0	15.2	24.3
			(S.E.)	(33.0)	(32.1)	(75.5)	(37.3)	(4.8)	(7.7)
		24h	Mean	7.5	12.5	20.7	22.0	63.3	86.0
			S.D.	6.6	4.9	13.4	17.3	23.7	22.4
			(S.E.)	(2.1)	(1.6)	(4.2)	(5.5)	(7.5)	(7.1)
	Female	4h	Mean	639.5	180.7	365.5	51.4	84.8	120.5
			S.D.	319.4	60.8	280.4	84.0	33.4	33.4
			(S.E.)	(101.0)	(19.2)	(88.7)	(26.6)	(10.6)	(10.6)
		24h	Mean	11.7	15.5	15.8	34.4	70.0	103.6
			S.D.	6.9	8.9	10.9	48.7	20.3	28.3
			(S.E.)	(2.2)	(2.8)	(3.4)	(15.4)	(6.4)	(8.9)
30 mg/kg	Male	4h	Mean	2429.5	2131.0	2122.6	63.8	137.9	248.1
			S.D.	793.0	1795.7	1090.1	32.6	39.6	99.5
			(S.E.)	(250.8)	(598.6)	(344.7)	(10.3)	(12.5)	(31.5)
		24h	Mean	13.2	25.6	19.8	43.2	182.6	230.8
			S.D.	6.6	15.7	13.4	21.1	75.2	89.2
			(S.E.)	(2.1)	(5.5)	(4.5)	(6.7)	(23.8)	(28.2)
	Female	4h	Mean	2341.2	1046.4	2386.2	63.3	158.4	230.8
			S.D.	1302.8	665.9	1903.9	34.4	42.2	64.3
			(S.E.)	(412.0)	(210.6)	(602.1)	(10.9)	(13.3)	(20.3)
		24h	Mean	19.3	26.6	26.9	136.5	177.8	222.8
			S.D.	12.3	12.5	18.1	207.3	47.6	50.7
			(S.E.)	(3.9)	(4.2)	(5.7)	(65.6)	(15.1)	(16.0)

*: Month 0 (single dosing) data quoted from Ref. 7

(Sponsor, M4, Rf9719, p14)

RF9725 – Accumulation of NK-104 in lens by multiple oral administration[s] to monkeys

Accumulation of NK-104 in lens by multiple oral administrations to monkeys (1, 6 month studies). Pitavastatin does appear to be absorbed by the lens of monkeys, but not at high enough levels to cause lens opacity upon chronic administration. Pitavastatin also appears to leave the eye upon cessation of pitavastatin administration.

Table 1. NK-104 concentration in the monkey ocular humor in the repeated oral administration of NK-104 for 1 month

Dose (mg/kg)	Sex	Animal No.	Concentration (ng/mL or ng/g)		
			Aqueous Humor	Crystalline Lens	Vitreous Humor
3	Male	5	n.d.	n.d.	n.d.
		6	n.d.	n.d.	1.50
		Mean	n.d.	n.d.	0.75
	Female	7	n.d.	n.d.	1.03
		8	n.d.	7.62	n.d.
		Mean	n.d.	3.81	0.52
	Mean		n.d.	1.90	0.63
8	Male	9	n.d.	10.17	2.66
		10	n.d.	9.49	1.17
		Mean	n.d.	9.83	1.92
	Female	11	n.d.	13.37	14.31
		12	n.d.	13.95	1.18
		Mean	n.d.	13.66	7.75
	Mean		n.d.	11.75	4.83
15	Male	13	n.d.	56.77	n.d.
		14	n.d.	40.03	5.31
		Mean	n.d.	48.40	2.65
	Female	15	n.d.	79.93	1.48
		16	n.d.	16.51	2.49
		Mean	n.d.	48.22	1.98
	Mean		n.d.	48.31	2.32

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Table II. NK-104 concentration in the monkey ocular humor in the repeated oral administra NK-104 for 6 month

Dose (mg/kg)	Sex	Animal No.	Concentration (ng/mL or ng/g)		
			Aqueous Humor	Crystalline Lens	Vitreous Hu
0.5	Male	13	n.d.	n.d.	n.d.
		14	n.d.	n.d.	n.d.
		15	n.d.	n.d.	n.d.
		16	n.d.	n.d.	n.d.
		Mean	n.d.	n.d.	n.d.
	Female	17	n.d.	n.d.	n.d.
		18	n.d.	n.d.	n.d.
		19	n.d.	n.d.	n.d.
		20	n.d.	n.d.	n.d.
		Mean	n.d.	n.d.	n.d.
	Mean		n.d.	n.d.	n.d.
1	Male	21	n.d.	n.d.	n.d.
		22	n.d.	n.d.	n.d.
		23	n.d.	n.d.	n.d.
		24	n.d.	n.d.	n.d.
		Mean	n.d.	n.d.	n.d.
	Female	25	n.d.	n.d.	n.d.
		26	n.d.	n.d.	n.d.
		27	n.d.	n.d.	n.d.
		28	n.d.	n.d.	n.d.
		Mean	n.d.	n.d.	n.d.
	Mean		n.d.	n.d.	n.d.
3	Male	29	n.d.	10.42	n.d.
		30	n.d.	11.20	n.d.
		31	n.d.	7.39	n.d.
		32	n.d.	6.13	n.d.
		Mean	n.d.	8.78	n.d.
	Female	33	n.d.	n.d.	n.d.
		34	n.d.	2.88	n.d.
		35	n.d.	11.92	n.d.
		36	n.d.	n.d.	n.d.
		Mean	n.d.	3.70	n.d.
	Mean		n.d.	6.24	n.d.
6	Male	39	n.d.	24.31	9.93
		40	n.d.	25.67	n.d.
		41	n.d.	26.77	3.42
		42	n.d.	41.59	0.80
		Mean	n.d.	29.58	3.54
	Female	45	n.d.	5.03	3.11
		46	n.d.	n.d.	n.d.
		47	n.d.	30.30	n.d.
		48	n.d.	14.24	n.d.
		Mean	n.d.	13.14	0.78
	Mean		n.d.	21.34	2.16

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Table III. NK-104 concentration in the monkey ocular humor in the repeated oral administration of NK-104 for 6 month with 8 weeks of withdrawal

Dose (mg/kg)	Sex	Animal No.	Concentration (ng/mL or ng/g)		
			Aqueous Humor	Crystalline Lens	Vitreous Humor
6	Male	37	n.d.	10.30	n.d.
		38	n.d.	15.99	n.d.
		Mean	n.d.	13.14	n.d.
	Female	43	n.d.	26.22	n.d.
		44	n.d.	6.10	n.d.
		Mean	n.d.	16.16	n.d.
	Mean		n.d.	14.65	n.d.

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RF9727 – Investigation of NK-104-induced cataract: 5. A 3-month oral toxicity study of NK-104 and mevalonic acid in combination in dogs

Dogs were given oral doses of NK-104±mevalonic acid. Although the formation of cataracts was not affected by coadministration of mevalonate the perturbations in plasma GPT and lung findings by NK-104 were suppressed indicating their possible association with HMG-CoA reductase inhibition.

3842 (144-015) – Examination of hepatocyte-injuring and drug-metabolizing enzyme inducing activities of NK-104 in mice (non GLP study)

Study design: CD-1 mice were utilized to study the effects of NK-104 on liver and liver metabolizing enzymes. Doses were 30, 75, and 225 mg/kg/day NK-104 by oral gavage for one week.

Endpoints: Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (γ-GTP), and alkaline phosphatase (ALP) were measured in serum. Livers were weighed and gross/histopathology (light and electron microscopy) were performed. Livers were probed for P450 3A2 and anti-PCNA reactivity. Liver microsomes were prepared and P450 content was measured by difference spectroscopy at 450 nm in the reduced form plus and minus carbon monoxide. Liver S9 was prepared and PCNA was measured by ELISA.

Results: Changes in blood chemistry were unremarkable. Liver weights were significantly increased (absolute and relative to body weight) at the 225 mg/kg/day dose in males and females. Liver gross pathological examination was unremarkable in males; in females, 1/5 livers were enlarged and pale. Males (3/10) and females (2/10) showed an increase in hepatocyte swelling at the 225 mg/kg/day dose. One male in the mid-dose, 75 mg/kg/day NK-104 group showed swelling of hepatocytes. Females showed a trend towards an increase in microgranuloma in the liver. All histopathological findings were slight in severity. There were no remarkable differences in PCNA-labeling between dose groups or P450 3A2 distribution or intensity. NK-104 did not appear to induce P450 enzyme content in liver.

RF2001/28 – Effect of NK-104 by concomitant administration of mevalonic acid on rat forestomach (morphological examination)

NK-104 inhibits HMG-CoA reductase, the product of which is mevalonate. The Sponsor tested the effect of mevalonate feeding on the forestomach morphology of rats, where there were findings of thickening and hyperkeratosis. The Sponsor utilized animals from RF9834, where a subset of animals received mevalonate in addition to NK-104. Mevalonate was able to alleviate the thickening of the corneum of the forestomach in rats, which indicates that the hyperkeratosis and thickening in the forestomach of rats is likely due to a pharmacodynamic effect of NK-104 on this organ.

RF2001/35 – Evaluation of the effects of NK-104, used in combination with mevalonic acid, on rat fore-stomach (a preliminary study)

This study was a dose range-findings study to determine the optimal NK-104 concentration needed to induce forestomach hypertrophy for further study on the

mechanism by which this thickening was induced by NK-104 in rats. Daily oral gavage doses of 10, 30, 50 and 100 mg/kg/day for up to 14 days showed that a reliable severity of thickening of the forestomach could be achieved at 30 mg/kg/day for 7 days.

G2534 – Evaluation of the effects of NK-104, used in combination with mevalonic acid, on rat fore-stomach

GLP-study to examine the effects of mevalonate administration on the forestomach thickening, hyperplasia and hyperkeratosis observed in rats in prior studies.

Group No.	Test article	Dosage (mg/kg)	Volume (mL/kg)	Concentration (mg/mL)	Concomitant article	Dosage, frequency/day and volume/dose	No. of animals	Dosing period (days)
1	0.5%CMC-Na	0	2	0	Water for injection	0 mg/kg x 4 doses, 2 mL/kg	10	7
2	NK-104	30	2	15	Water for injection	0 mg/kg x 4 doses, 2 mL/kg	10	7
3	NK-104	30	2	15	Mevalonic acid	500 mg/kg x 2 doses, 2 mL/kg	10	7
4	NK-104	30	2	15	Mevalonic acid	500 mg/kg x 4 doses, 2 mL/kg	10	7
5	NK-104	50	2	25	-	-	5	7

(Sponsor, M4, G2534, p14)

There were no notable clinical observations, body weights, or deaths in any group.

Stomach Hypertrophy				
Group	NK-104 Dose (mg/kg/day)	Mevalonate Dose (mg/kg/day)	Incidence of hypertrophy	Other findings
1	0	0	0/10	0/10
2	30	0	7/10	1/10, cyst; 1/10 spot in mucosa
3	30	500 x 2	1/10	0/10
4	30	250 x 2	3/10	0/10
5	50	0	3/5	1/5, cyst; 1/5, spot in mucosa

Upon histopathological evaluation, it was determined that mevalonate was partially protective against NK-104-induced hypertrophy as measured in mucosal and horny layer length. These results argue that NK-104 causes forestomach hypertrophy in rats due to pharmacodynamic effects of HMG-CoA reductase inhibition.

RF9834 – Prevention of NK-104 induced toxicity in pregnant rats by mevalonic acid

Studies with NK-104 given during perinatal and lactation periods in rats demonstrate greater maternal toxicity than those during administration during organogenesis. Fluvastatin has been reported to cause maternal death and cardiomyopathy during perinatal administration and that coadministration of mevalonic acid can prevent this suggesting some relationship with HMG-CoA reductase inhibition according to the Sponsor. From GD 17 to lactation day 20, NK-104 at 10 or 30 mg/kg and mevalonic acid lactone 1000 mg/kg/d was given to pregnant female rats. Administration of NK-104

alone caused suppression of weight gain, decreased food intake, increased death and histopathology in forestomach, skeletal muscle, liver and kidney in dams. Increased numbers of stillborns, decreased viability index and suppression of weight gain in F1 generation rats was observed. These effects were markedly reduced by coadministration of mevalonic acid suggesting that the maternal toxicity observed during perinatal administration is likely attributed to HMG-CoA reductase inhibition.

		0.5% CMC	0.5% CMC + Mevalonic acid lactone 500 mg/kg × 2	NK-104 10 mg/kg + Mevalonic acid lactone 500 mg/kg × 2	NK-104 30 mg/kg + Mevalonic acid lactone 500 mg/kg × 2	NK-104 10 mg/kg	NK-104 30 mg/kg
No. of dams		11	12	10	12	12	12
No. of dams with live newborns		11	12	10	12	9	4
Gestation index (%)[a]		100	100	100	100	75	33[b]
No. of dead dams		0	0	0	0	3	10
	Gestation day 22	0	0	0	0	1	0
	23	0	0	0	0	1	8
	Lactation day 1	0	0	0	0	1	0
	3	0	0	0	0	0	1
	4	0	0	0	0	0	1
No. of lack of care		0	1	0	2	2	1
	Lactation day 0	0	0	0	0	1	0
	1	0	1	0	0	0	1
	5	0	0	0	0	1	0
	6	0	0	0	1	0	0
	11	0	0	0	1	0	0
Gestation period (day)		22.5±0.5	22.5±0.5	22.4±0.5	22.3±0.5	22.3±0.5	22.5±0.6
No. of implants	Total	178	206	164	205	173	63
	Per litter	16.2±3.2	17.2±1.9	16.4±2.6	17.1±1.9	17.3±1.1	15.8±3.3
No. of live newborns	Total	155	166	138	168	133	38
	Per litter	14.1±3.0	13.8±2.7	13.8±2.4	14.0±1.9	13.3±4.9	9.5±6.4
Birth index[b]		87.7	80.5	84.6	82.8	77.8	64.6

[a] (No. of dams with live newborns / No. of dams) × 100 [b] (No. of live newborns / No. of implants) × 100

Significantly different from 0.5% CMC group [b]P < 0.05 (Chi-square test)

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Table 4 Body weight in rat dams Protocol No. 97028

	0.5% CMC	0.5% CMC + Mevalonic acid lactone 500 mg/kg × 2	NK-104 10 mg/kg + Mevalonic acid lactone 500 mg/kg × 2	NK-104 30 mg/kg + Mevalonic acid lactone 500 mg/kg × 2	NK-104 10 mg/kg	NK-104 30 mg/kg
Gestation period						
No. of dams	11	12	10	12	12	12
Day 0	284±26	279±21	281±24	284±31	281±22	284±26
7	317±24(10)	323±28	328±34(9)	325±35(9)	315±29(11)	320±38(11)
14	360±28	362±30	367±35	360±31	356±30	356±27
17	390±27	393±32	398±37	394±30	392±29	386±31
18	403±28	407±31	410±39	404±31	408±28	401±31
19	419±30	423±33	425±40	419±29	426±30	414±31
20	435±29	437±31	441±40	434±31	437±33	421±32
21	445±31	446±37	451±39	439±31	435±34	417±35
Body weight gain (g)						
Day 17-21	55±7	54±10	52±8	45±11	43±11*	30±15***
Day 0-21	161±18	167±22	169±25	154±19	154±18	132±23***
Lactation period						
No. of dams	11	12	10	12	9	4
Day 0	322±42	318±41	323±39	316±31	301±22	310±35
4	346±33	337±34(11)	345±36	336±27	311±36(9)	353(4)
7	357±23	352±29(11)	355±30	341±28(11)	337±23(9)	358(4)
11	372±21	366±25(11)	364±29	354±28(10)	351±22(9)	358(4)
14	371±17	368±30(11)	369±25	357±22(10)	350±28(9)	372(4)
18	363±15	355±25(11)	356±27	346±19(10)	344±28(9)	344(4)
21	340±14	335±21(11)	342±23	335±17(10)	330±18(9)	329(4)

Significantly different from 0.5% CMC group *P < 0.05, ***P < 0.001 (Dunnett's d-test)
( ) No. of dams

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Table 9 Findings in rat offspring

			0.5% CMC	0.5% CMC + Mevalonic acid lactone 500 mg/kg × 2	NK-104 10 mg/kg + Mevalonic acid lactone 500 mg/kg × 2	NK-104 30 mg/kg + Mevalonic acid lactone 500 mg/kg × 2	NK-104 10 mg/kg	NK-104 30 mg/kg
No. of live newborns		Total	155	166	138	168	133	38
		Per litter	14.1±3.0	13.8±2.7	13.8±2.4	14.0±1.9	13.3±4.9	9.5±6.4
No. of stillborns		Total	5	14	10	11	26	18
		Per litter	0.5±0.5	1.2±1.0	1.0±1.7	0.9±1.3	2.6±3.2	4.5±7.1
Male/Female			85/70	82/84	68/70	77/91	59/74	23/15
(Male %)			54.8	49.4	49.3	45.8	44.4	60.5
No. of live newborns with external anomalies (%)			0	0	0	1 (0.8)[a]	0	0
No. of offspring after birth	Day 1		154	157	138	150	115	37
	Day2		152	157	138	149	111	36
	Day3		151	157	138	149	110	28
	Day4		150	157	138	146	102	12
Viability index (%)[b]			97.2	90.3	100	87.3	77.3	25.0[##]
Body weight (g) in offspring	Day 0	Male	6.7±0.6	6.6±0.9	6.7±0.5	6.4±0.5	6.3±0.4	6.7±0.3
		Female	6.4±0.7	6.2±0.8	6.3±0.7	6.0±0.5	6.0±0.3	6.3±0.4
	Day 4	Male	10.6±1.6	10.1±2.0	10.7±1.6	9.8±1.1	8.6±1.6	11.1
		Female	10.3±1.8	9.6±2.0	10.2±1.8	9.5±1.0	8.3±1.6	10.8
Weaning index (%)[c]			98.9	100	100	77.1	87.5	100

[a] Anury
[b] (No. of live offspring on Day 4 after birth/No. of live newborns) × 100
[c] (No. of live offspring on Day 21after birth/No.of offspring after culling on Day 4 after birth) × 100
Significantly different from 0.5% CMC group ##P < 0.05 (Wilcoxon's rank test)

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Protocol No. 97028

Table 10 Body weight in rat offspring after culling on Day 4 after birth

		0.5% CMC	0.5% CMC + Mevalonic acid lactone 500 mg/kg × 2	NK-104 10 mg/kg + Mevalonic acid lactone 500 mg/kg × 2	NK-104 30 mg/kg + Mevalonic acid lactone 500 mg/kg × 2	NK-104 10 mg/kg	NK-104 30 mg/kg
No. of male offspring		45	44	39	40	28	4
	Day 7	18±3	17±3	18±2	16±3##	16±2##	18±3
	Day 14	38±4(##)	37±4	37±3	35±6#(##)	35±3*	35±9
	Day 21	62±7(##)	62±7	62±5	57±6#(##)	60±5	57±14
No. of female offspring		43	44	41	38	28	4
	Day 7	18±3	16±3	17±2	15±2##	16±2##	17±1
	Day 14	37±4	36±4	36±4	33±3***	35±3*	36±1
	Day 21	61±6	60±6	59±6	55±6***	58±7	58±2

Significantly different from 0.5% CMC group *P < 0.05, ## P < 0.01 (Dunnett's rank test)
Significantly different from 0.5% CMC group *P < 0.05, *** P < 0.001 (Dunnett's t-test)

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Metabolites:

NK-104 lactone was negative in a series of Ames reverse mutation assays, both with and without metabolic activation. NK104 was negative for chromosomal aberrations in CHL cells, both with and without metabolic activation.

RFG2517 – Single dose toxicity study of NK-104 lactone in dogs by oral administration

SINGLE DOSE ORAL TOXICITY STUDY IN DOGS

100 and 300 mg/kg NK-104 lactone were orally administered at a single dose in two ma dogs each in a group and the acute toxicity of NK-104 lactone was examined.

1. No dead animal was observed in any groups during the observation period.
2. In general conditions, vomiting, decreases in spontaneous locomotion, discoloration of or mucous membrane, bloody stools, weight loss, and decreases in food and water consumptio were observed in one case in the 300 mg/kg group. These findings were improved or tende to be improved on Day 7. The other one case showed a slight weight loss by Day 4.
3. In urinalysis, decreases in chloride and chloride excretion were observed in one case in the 30 mg/kg group.
4. In blood biochemistry, decreases in potassium and chloride and increases in GPT activity wer observed in one case in the 300 mg/kg group.
5. In hematology and the ophthalmologic examination, no abnormalities were observed in an groups.
6. In the toxicokinetic examination, Cmax and AUC of NK-104 lactone increased in a dose dependent manner. These parameters increased at over the dose ratio, as compared between th low dose and the high dose groups. Exposure with NK-104 lactone in any groups was almos the same as that with NK-104 transformed from NK-104 lactone in vivo. Particularly, Cma of NK-104 in the 300 mg/kg group was over the Cmax of NK-104 at the dose that the simila findings occurred in the NK-104 studies.

These findings were qualitatively same as those observed with NK-104, showing no finding different from those observed with NK-104. The lethal dose was estimated to be around 30 mg/kg, since the findings almost the same as those in the lethal cases in the NK-104 studies wer observed in the 300 mg/kg group, even though no animal died in the group. The lethal dose o NK-104 has been estimated as 100 mg/kg or less [1] and the acute toxicity of NK-104 lactone wa less than that of NK-104.

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Vomiting decreased in food consumption, weight loss, decreases in potassium and chloride and increases in GPT suggesting that NK-104 lactone affected the GI tract and liver. Note only males (2/group) were used in the study. These findings were similar to those in lethal cases of NK-104. Exposures to the lactone approximate that of NK-104 transformed *in vivo*.

In the 100 mg/kg group, Cmax values of NK-104 lactone were 1.34 and 1.21 μg/ml and the AUC values were 7.94 and 6.27μg. hr/mL. Cmax values of NK-104 were 0.98 and 1.16 μg/ml and the AUC values were 7.12 and 5.52μg. hr/mL. Tmax values were 1 and 2 hours.

In the 300 mg/kg group, Cmax values of NK-104 lactone were 15.7 and 11.8 μg/ml and the AUC values were 209 and 91.2μg. hr/mL. Cmax values of NK-104 were 25.8 and 9.68μg/ml and the AUC values were 206 and 55.6μg. hr/mL. Tmax values were 4 and 6 hours.

From these results, the exposures (Cmax and AUC) with NK-104 lactone and NK-104 were almost the same among the animals in the 100 mg/kg group. While, in the 300 mg/kg group, the high exposures with NK-104 lactone and NK-104 were shown in the case 3M1, and the Cmax and the AUC of NK-104 lactone were 1.3 and 2.3 times high, respectively, and those of NK-104 were 2.7 and 3.7 times high, respectively, compared with the other animal.

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RFG2518 – One month oral toxicity NK-104 lactone in male dog

Doses of 1, 3, 10 mg/kg lactone and 10 mg/kg NK-104 were given. One animal in the 10 mg/kg lactone group died. Vomiting, decreased feces, decreased food consumption, weight loss and decreased activity were observed a few days before death. Shortly before death lateral position, emaciation, hypothermia and cyanosis were observed. Histopathology in this dog revealed atrophy and vacuolar degeneration of hepatocytes. Dose dependent decreases in cholesterol and phospholipids were found in the 1, 3, 10 mg/kg lactone surviving animals. A decrease in triglycerides was found in the 10 mg/kg groups given lactone and parent. Histopathology was not observed in the 1 mg/kg/day group. In the mid-dose, high-dose however, milky reticular structure was found in the lung. Aggregation of foamy cells and infiltration of inflammatory cells in the interstitium were found. In the high-dose a trace of hemorrhage was found in the intestine but not confirmed by histopathology.

Pitavastatin lactone is not associated with cataracts, but the lactone could be associated with lung toxicity in dogs.

1-Month Oral Toxicity in Male Dogs – Toxicokinetics					
TK Day 28		1 mg/kg lactone	3 mg/kg lactone	10 mg/kg lactone	10 mg/kg NK-104
NK-104 lactone	C_{max} (μg/mL)	0.273	0.789	2.205	1.403
	AUC (μg·h/mL)	1.21	4.69	13.18	10.77
NK-104	C_{max} (μg/mL)	0.066	0.166	1.429	9.873
	AUC (μg·h/mL)	0.3	1.03	6.97	21.71

3846 – Mutagenicity testing of NK-104 lactone in bacterial reverse mutation assays

Key study findings:

- NK-104 lactone was negative in a series of Ames reverse mutation assays, both with and without metabolic activation.

Volume #, and page #: M4, 3846

Conducting laboratory and location: (b) (4)

Date of study initiation: March 11, 1998
GLP compliance: Yes.
QA reports: yes (X) no ( )
Drug, lot number, and purity: YT-00213-193-B, 99.5% purity

Methods:
Strains/species/cell line: *S. typhimurium*, TA98, TA100, TA1535, TA1537, and *E. coli* WP2 uvrA.

Doses used in definitive study: See results tables.

Basis of dose selection:

Table 1. Summary data of dose-finding study with NK-104-lactone [direct method: -S9] Exp. No.3846 (144-019)

Compound	Dose (μg/plate)	Mean revertant colonies per plate				
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
DMSO[a]	0	97	12	25	27	9
Test substance	0.305	126	12	23	24	10
	1.22	107	14	23	17	16
	4.88	125	11	20	23	8
	19.5	108	9	26	24	12
	78.1	95[c]	11	26	15	8[c]
	313	80[c]	7[c]	18	18	6[c]
	1250[d]	70[c]	17[c]	31[c]	24[c]	7[c]
	5000[d]	59[c]	5[c]	17[c]	18[c]	1[c]
Positive control compound		AF-2	NaN_3	AF-2	AF-2	9-AA
Dose (μg/plate)		0.01	0.5	0.01	0.1	80
Mean revertant colonies per plate		514	439	157	662	604

AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide NaN_3: Sodium azide 9-AA: 9-Aminoacridine hydrochloride
[a]: Solvent control
[c]: Growth inhibition was observed
[d]: Visible precipitation was occurred

(Sponsor, M4, 3846, p36)

Table 2. Summary data of dose-finding study with NK-104-lactone [activation method: +S9] Exp. No.3846 (144-019)

Compound	Dose (μg/plate)	Mean revertant colonies per plate				
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
DMSO[a]	0	127	11	24	33	13
Test substance	0.305	135	14	23	32	12
	1.22	125	7	23	30	17
	4.88	135	8	16	31	13
	19.5	127	13	22	31	11
	78.1	131	18	23	30	8
	313	111[c]	9[c]	15[c]	32	6[c]
	1250[d]	96[c]	9[c]	15[c]	27[c]	5[c]
	5000[d]	51[c]	7[c]	15[c]	19[c]	2[c]
Positive control compound		2-AA	2-AA	2-AA	2-AA	2-AA
Dose (μg/plate)		1	2	10	0.5	2
Mean revertant colonies per plate		647	316	755	279	156

2-AA: 2-Aminoanthracene
[a]: Solvent control
[c]: Growth inhibition was observed
[d]: Visible precipitation was occurred

(Sponsor, M4, 3846, p37)

Negative controls: DMSO treated.

Positive controls:

Direct method	Strain	Positive control substance	Dose level
Salmonella typhimurium	TA100	AF-2	0.01 µg/plate
Salmonella typhimurium	TA98	AF-2	0.1 µg/plate
Salmonella typhimurium	TA1535	NaN_3	0.5 µg/plate
Salmonella typhimurium	TA1537	9-AA	80 µg/plate
Escherichia coli	WP2 *uvrA*	AF-2	0.01 µg/plate

Activation method	Strain	Positive control substance	Dose level
Salmonella typhimurium	TA100	2-AA	1 µg/plate
Salmonella typhimurium	TA98	2-AA	0.5 µg/plate
Salmonella typhimurium	TA1535	2-AA	2 µg/plate
Salmonella typhimurium	TA1537	2-AA	2 µg/plate
Escherichia coli	WP2 *uvrA*	2-AA	10 µg/plate

AF-2 : 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (b) (4) purity 98.0 – 102.0 %, Lot No. CAP0185)

NaN_3 : Sodium azide (b) (4) No. TPR1596)

9AA : 9-Aminoacridine hydrochloride (b) (4) purity 98.0 %, Lot No. AQ 08326HN)

2AA : 2-Aminoanthracene (b) (4) purity ≥ 90.0 %, Lot No.: DLH6052)

(Sponsor, M4, 3846, px)

Incubation and sampling times: Tests were carried out in duplicate at each concentration. Plates were pre-incubated for 20 minutes at 37 °C, and incubated after plating for 48 hours at 37 °C then counted by automated colony counter.