

Although ocular opacities were observed in a mouse study at 13-weeks at  $\geq 75$  mg/kg/day, it was not seen at the same dose following lifetime exposure in a 92 week carcinogenicity study. Lens concentrations of pitavastatin at 4 mg/day were 7 ng/g, which is below the concentration range in dogs of 123-616 ng/g that led to the formation of cataracts after daily oral dosing at 3 or 12-months. These data, in addition to the known association of statin exposure and cataracts in dogs, suggest that the dog lens is particularly hypersensitive to the distribution of HMG-CoA reductase inhibitors.

#### **Renal toxicities**

Renal toxicities were seen in male and female monkeys in 1 month and 6 month toxicology studies. Findings consisted of mild swelling of the proximal tubule epithelium with slight desquamation of tubular epithelium at  $\geq 6$  mg/kg/day ( $\geq 8.7$ -fold MRHD at a 4 mg/day dose) in conjunction with increased kidney weight. Lower doses showed this finding, but toxicities were recoverable by 2 months post-withdrawal. Creatinine and blood urea nitrogen or phenolsulphonephthalein clearance were unremarkable. Higher doses of pitavastatin at shorter duration resulted in further exacerbation of renal toxicities, which suggests dose-dependency. Metabolism studies suggest that monkeys, more so than any other test species, form a greater proportion of 8-hydroxypitavastatin (active metabolite). While humans have a potential to form 8-hydroxypitavastatin, it was not considered a major human metabolite and was not measured in clinical studies. This metabolite is observed in monkey urine and feces. As a polar compound, 8-hydroxypitavastatin would be expected to be excreted in the urine along with the dihydroxypitavastatin and/or glucuronidated and excreted in the bile. A mechanistic study investigated a link between 8-hydroxypitavastatin and renal toxicity. In this study rats were administered 8-hydroxypitavastatin at 200 mg/kg BID for 2 weeks and 400 mg/kg/day BID for 1 week. All rats were found dead or sacrificed moribund before the study was scheduled for termination. Degeneration of the renal tubules, increased blood urea nitrogen and creatinine were also observed, suggesting a correlation. Fertility studies in rabbit showed mortality of treated males and females at 1 mg/kg/day (AUC=15,670 ng·h/ml,  $C_{max}$ =1184). Gross pathology (renal histopathology not performed) indicated whitening of kidneys, which may indicate ischemia, occurred at 100-fold clinical exposure at a 4 mg/day dose based on AUC. An explanation for the increased sensitivity of the rabbit to renal toxicity was not provided.

#### **Thyroid toxicities**

Pitavastatin increased thyroid follicular adenocarcinomas in male rats given  $\geq 25$  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 thyroid stimulating hormone (TSH). Pitavastatin appears to induce  $T_4$  UDG-GT activity in liver which results in decreased plasma  $T_4$  concentrations due to increased metabolism. Decreased  $T_4$  leads to feedback-mediated increases in TSH release resulting in extensive follicular cell stimulation in the thyroid. This has been established for liver enzyme inducing agents, including statins. Follicular cell hypertrophy has been observed previously in subchronic toxicity testing. Also consistent with this hypothesis are potential mechanisms that address this finding. In short-term rat initiation/promotion studies designed to evaluate the follicular cell-promoting effect, this effect of pitavastatin

was eliminated upon concomitant administration of T<sub>4</sub>. This finding supports that idea that the thyroid follicular cell tumors observed at high doses are induced by increased plasma TSH concentrations due to negative feedback regulation due to induced hepatic T<sub>4</sub> metabolism. Rats are known to be particularly sensitive to this induced metabolism, because they lack T<sub>4</sub>-binding globulin, which results in increased turnover of T<sub>4</sub> relative to other thyroid hormone species.

### **Hepatic toxicities**

Hepatic effects have been seen in mice, rats and dogs. In a 1-month rat toxicity study, increases in transaminases (AST, ALT) were observed in females given 50 mg/kg/day (894-fold human exposure at 4 mg/day). Elevated transaminase levels were recoverable following drug withdrawal. In the chronic 6-month rat toxicity study no increases in transaminases were observed at the highest dose studied 10 mg/kg/day (56-fold human exposure at 4 mg/day dose). In the 3-month dog toxicity study an increase in ALT was seen at 3 mg/kg/day (24-fold human exposure at 4 mg/day dose). In the 12 month toxicity study both AST and ALT activity was increased in male dogs given 3 mg/kg/day (24-fold human exposure at 4 mg/day dose). Elevated transaminases were not detected upon recovery following drug withdrawal; however, hepatic histopathologies were not observed. In a 3-month dog toxicity study with pitavastatin at 10 mg/kg/day centrilobular dilatation of liver sinusoids were observed, which resolved during recovery. Increased centrilobular hepatocytes hypertrophy was observed in male mice given  $\geq 12$  mg/kg/day in the carcinogenicity study. A 3-month dog toxicity study with co-administration of mevalonic acid (100-150 mg/kg/day) and pitavastatin (5 mg/kg/day) resulted in an absence of transaminase elevations at doses up to 5 mg/kg/day (42-fold human exposure at 4 mg/day based on AUC). Studies in mice up to 225 mg/kg/day (150-fold human exposure based on AUC) did show the liver histopathology; however, pitavastatin does not appear to induce drug metabolizing enzymes, nor is it associated with the severe necrosis and cellular atypia and cholestasis observed in animals following treatment with other statins. Further support of a lessened biliary toxicity is seen in guinea pigs (3 mg/kg/day for 15 days) and hamsters (1 mg/kg/day for 14 days) where pitavastatin did not show any change in biliary lipids.

### **Skeletal muscle toxicities**

Degeneration and necrosis were observed in the 1- and 3-month rat toxicity study at 50 mg/kg/day (894-fold human exposure at the 4 mg/day dose). Myopathy was not observed in the chronic rat study at doses up to 10 mg/kg/day (56-fold MHRD based on AUC). Myopathy was observed in the mouse carcinogenicity study at the highest dose (75 mg/kg/day) and in male rats given 25 mg/kg/day in the carcinogenicity study. Doses associated with myopathy in the lifetime carcinogenicity studies in mice and rats represent exposure levels 26 and 295-fold MRHD, respectively, based on AUC.

### **Lung toxicities**

Pulmonary lesions were seen at 3 mg/kg/day (24-fold MRHD based on AUC) in the 3 month and 12-month dog toxicity studies. The Sponsor described these lesions as lipid, pneumonia-like, composed of foam and inflammatory cells. No other organ or species shows a similar lesion. This may be indicative of phospholipidosis, although

transmission electron microscopy was not performed and it is uncertain if the lesions represented foamy macrophages that are the hallmark of phospholipidosis. Assessment in dogs given pitavastatin co-administered with mevalonic acid did not repeat the pulmonary findings suggesting an association with HMG-CoA reductase inhibition.

#### **Forestomach toxicities**

In mice and rats hyperkeratosis and acanthosis (hyperplasia) were observed with pitavastatin treatment. The thickening of the forestomach was not seen with subcutaneous administration, suggestive of a local, rather than systemic, effect. The Sponsor proposes a species-specific mechanism to explain this common finding in rodents given statins. Specifically, HMG-CoA reductase inhibition in keratinocytes of the mucosal epithelium of the forestomach results in decreased mevalonic acid and suppressed cholesterol synthesis by keratinocytes. This leads to an abnormal lipid composition in the presence of the statin, a result of accumulation of lipids in the junctional gaps of the keratinocyte layer. The results are structural changes at the mucosal epithelial surface of the forestomach, which may lead to detachment of keratin at the forestomach surface. Superficial cell proliferation, including basal cells of the epithelium, is induced to compensate for the detached keratin resulting in thickening of the mucosal epithelium through hyperplasia. Mevalonic acid has been shown to reverse this effect on the forestomach with other statins.

#### **Testicular findings**

Testicular changes (decreased testes weight, atrophy/degeneration of seminiferous tubules, impaired spermatocyte maturation and increased multinucleated giant cells) have been reported in dogs treated with statins. Histological changes in dog reproductive organs were not observed in the 12-month toxicity study at doses up to 3 mg/kg/day (>24-fold human systemic exposure at 4 mg/day dose) including sperm counts.

#### **Metabolites and impurities**

The principal circulating metabolites of pitavastatin in humans include the lactone, which the Sponsor considers pharmacologically inactive at the target, and small amounts of 8-hydroxypitavastatin which has pharmacologic activity slightly greater than that of the parent pitavastatin based on *in vitro* HMG-CoA reductase inhibition assays. Studies performed with the lactone included single dose toxicology study in the dog were augmented by negative results in the *in vitro* genetic toxicology battery, a bacterial gene mutation and chromosomal aberration test. Repeat-dose studies with pitavastatin lactone at 0, 1, 3, 10 mg/kg/day and 10 mg/kg/day pitavastatin showed similar toxicology findings. The similarity in toxicity profile is not surprising; pitavastatin undergoes glucuronidation and subsequent hydrolysis to form pitavastatin lactone, and pitavastatin lactone can further hydrolyze to reform pitavastatin), *in vivo*. The NOAEL of the lactone was 1 mg/kg/day. Qualification of the 8-hydroxypitavastatin has been discussed under “renal toxicities”.

Specific studies have been conducted on the 5-ketone metabolite of pitavastatin (M-3 metabolite) and the process impurities <sup>(b) (4)</sup> [REDACTED]. Specifications for these impurities have been set for these compounds as

follows: (b) (4) (b) (4)

The total impurities specification for pitavastatin, excluding the (b) (4) The (b) (4) impurity was evaluated in single-dose oral toxicity study in mice where the toxicities observed were comparably to pitavastatin. Five studies were performed with a mixture of the (b) (4), including a single-dose study, a 1-month repeat-dose rat study, and an evaluation of potential genotoxicity using an *in vitro* battery (including Ames and chromosomal aberrations analyses) and an *in vivo* mouse micronucleus assay. The (b) (4) were considered to have a weaker toxicity potential than pitavastatin and a NOAEL of 50 mg/kg/day with 1-month dosing supports this. An equivocal positive response was seen in the chromosome aberration test at concentrations of  $\geq 150$   $\mu\text{g/ml}$ , which were associated with significant cytotoxicity. Other genetic toxicology tests in the completed battery were negative, so based on a weight of evidence approach, the (b) (4) were considered not to have appreciable genotoxic potential. Similar toxicity studies were conducted in the rat with the (b) (4). The (b) (4) showed less toxicity than pitavastatin and did not show a genotoxic potential in the bacterial gene mutation or micronucleus assays. The chromosomal aberration test in Chinese hamster lung cells showed an increase in aberrations at the highest concentration tested; slight increases in chromosomal aberrations were noted in the direct plate method (as opposed to preincubation method) within 24 hours (with metabolic activation) at 300  $\mu\text{g/ml}$ . The same concentration for 48 hours was too cytotoxic for evaluation. Weak increases were seen with preincubation, without metabolic activation at 750  $\mu\text{g/ml}$ , and with metabolic activation at 600-825  $\mu\text{g/ml}$ . Cytotoxicity was not measured in the preincubation protocol, but similar plates in parallel cultures indicated acceptable survival (50%) suggesting a valid study. This would suggest that the positive response occurred under conditions expected to cause significant cytotoxicity, which is very likely to be at significantly higher exposure levels than those achieved *in vivo* with pitavastatin.

Pitavastatin absorbs light in the 290-700 nm range with a minor peak at 328 and major peak at 245 nm. Specific studies to address phototoxicity were not performed. Tissue distribution studies in pigmented and non-pigmented rats did not indicate any particular affinity for skin or eyes in either strain. These studies, in addition to animal and clinical studies, indicate that the likelihood of pitavastatin provoking phototoxic reactions is small.

### Genetic Toxicology

Pitavastatin is not genotoxic, by weight of evidence. Pitavastatin was positive in a chromosomal aberration assay with metabolic activation in Chinese hamster lung (CHL) cells. The positive result was obtained at a concentration of pitavastatin that was close to that which caused 50% cytotoxicity with metabolic activation. Pitavastatin was negative for genotoxicity in a chromosomal aberration assay without metabolic activation in CHL cells. Pitavastatin was also negative in an Ames reverse mutation battery, *in vivo* mouse and rat micronucleus assays, an *in vivo/in vitro* single cell gel (Comet assay), and an *in vivo/in vitro* rat unscheduled DNA synthesis (UDS) assay.

### **Carcinogenicity**

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% resulting in the early termination of the females. Male survival at week 104 was 35%, 38%, 38%, 56%. 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  $\geq 25$  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<sub>4</sub> UDG-GT activity in male rat liver which results in decreased plasma T<sub>4</sub> based on increased metabolism. Decreased T<sub>4</sub> 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<sub>4</sub>-binding globulin, which results in increased turnover of T<sub>4</sub> 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 tumorigenicity 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.

#### **Reproductive Toxicity – Fertility**

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.

#### **Reproductive Toxicity – Pregnancy**

Pitavastatin crosses the placental barrier into the fetus. Rat fetus concentrations were  $\leq 36\%$  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 (82-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 ( $>25$ -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  $\geq 0.3$  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.

In peri-postnatal studies in pregnant rats given oral gavage doses of pitavastatin at 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 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 4-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.

#### Findings by species:

#### **Mice**

**13 Week oral gavage study:** In a 13 week repeat-dose toxicity study in CD-1 mice administered pitavastatin at 25, 75 and 225 mg/kg/day daily by oral gavage, there was centrilobular enlargement of hepatocytes at the 75 mg/kg/day dose in males and at the 225 mg/kg/day dose in both sexes. Corneal opacity was noted in both sexes at 75 and 225 mg/kg/day. There was no kidney toxicity evident at doses up to 225 mg/kg/day in either sex. There were findings of forestomach epithelial hyperplasia and hyperkeratosis at daily oral gavage doses  $\geq$  25 mg/kg/day pitavastatin in both male and female animals during a 13 week toxicity study. There was evidence of focal ulceration and erosion of the forestomach in mice of both sexes at the 225 mg/kg/day dose. The NOAEL was less than 25 mg/kg/day and not determined. The multiple of human exposure at this dose was <4.1, based on AUC<sub>0-24</sub> at 4 mg.

**4 Week oral gavage study:** In dose range-finding study in CD-1 mice administered pitavastatin at doses of 50 to 400 mg/kg/day by oral gavage for 4 weeks, forestomach thickening and hyperkeratosis was observed at all doses. Degeneration of hepatocytes was observed at 400 mg/kg/day in females only. Kidney tubular dilatation with epithelial necrosis and vacuolation was observed at 400 mg/kg/day in females only. The NOAEL was less than 50 mg/kg/day and not determined. The multiple of human exposure at this dose was <8.2, based on AUC<sub>0-24</sub> at 4 mg after repeat-dose administration for 3 weeks.

**4 Week oral gavage study:** In a separate dose range findings study, where CB6F1-Tg rasH2 mice were administered 70, 125, and 250 mg/kg/day by daily oral gavage, mice of both sexes showed forestomach hyperplasia, mononuclear/polymorphonuclear cell infiltration all dose levels. Findings were minimal at 70 mg/kg/day and severe at 250 mg/kg/day. The NOAEL was less than 70 mg/kg/day and not determined. The multiple of human exposure at this dose was <11.5, based on AUC<sub>0-24</sub> at 4 mg after repeat-dose administration for 3 weeks.

**Single-dose oral gavage study:** After single acute oral dosing of pitavastatin in mice, there were findings of forestomach thickening (2000 mg/kg in males, and 1000 mg/kg in females). There were liver nodules noted in males at 1000 mg/kg and discoloration of liver in females at 2000 mg/kg.

**Other notable findings:** There was no toxicity in thyroid tissues in any study in mice, including a 13 week oral gavage study where daily administrations of up to 225 mg/kg took place. There were no remarkable findings in epididymes of mouse at any dose or duration studied. There were no remarkable lung findings in mice in any study, at any dose, or any duration.

### Rats

**6 Month oral gavage study, 1 month recovery:** In Wistar rats administered pitavastatin by oral gavage at doses of 0.3, 1, 3, and 10 mg/kg/day for six months, forestomach thickening and hyperkeratosis was observed at 1 mg/kg/day, which were recoverable by one month. At 3 mg/kg/day and higher, edema of the submucosa and cellular infiltration of lamina propria of forestomach was observed. No kidney toxicity was noted in a 6 month chronic toxicology study in Wistar rats at doses up to 10 mg/kg/day. Forestomach findings were observed in a 6 month oral gavage chronic toxicity study in Wistar rats. Forestomach hyperkeratosis was found at 1 mg/kg/day and above in both sexes, while edema and cellular infiltration was seen in males and females at 3 mg/kg/day and greater doses. Forestomach findings were recoverable upon completion of a 35 day recovery period. Minute liver toxicity (small, granulomatous nodules and interlobular bile duct proliferation) was rarely observed (2/12 per sex) and only noted at the high dose, 10 mg/kg/day level in males and females. The NOAEL was 0.3 mg/kg/day in rats administered pitavastatin daily by oral gavage based on forestomach findings. The multiple of human exposure at this dose was 1.9, based on AUC<sub>0-24</sub> at 4 mg after repeat-dose administration for 3 weeks.

**3 Month oral gavage study:** Although no thyroid toxicity was detected during a 6 month chronic study in Wistar rats at doses up to 10 mg/kg/day, there were findings of increased thyroid weight at 30 mg/kg/day and thyroid follicular cell hypertrophy at 50 mg/kg/day, during in a shorter, 13 week study in Sprague Dawley rats of both sex. In a 13 week dose range-finding study in Sprague Dawley rats, there was forestomach hyperplasia and hyperkeratosis at 10 mg/kg/day and higher doses in both sexes. No kidney findings were observed in Sprague-Dawley rats in this 13-week study (up to 50 mg/kg/day). The NOAEL was <10 mg/kg/day in rats administered pitavastatin daily by oral gavage based on forestomach findings. The multiple of human exposure at this dose was <56, based on AUC<sub>0-24</sub> at 4 mg after repeat-dose administration for 3 weeks.

**1 Month oral gavage study:** In a one-month toxicity study in Wistar rats administered pitavastatin daily by oral gavage at 2, 10, 50, and 100 mg/kg/day with a two-week recovery period, degeneration of skeletal muscle (3/12 cases of mild to moderate degeneration/atrophy/necrosis in females only at 50 mg/kg/day only; no females lived in

100 mg/kg/day group), with no findings at recovery. Liver toxicity (mild congestion in 1/12 animals at 100 mg/kg/day) was observed in males only, with no findings at recovery. Mild to moderate forestomach findings were observed at 10 mg/kg/day and higher doses of pitavastatin, with no findings observed at recovery. In kidneys, tubular regeneration evident (recoverable) and calcium deposition (not recoverable at 2 weeks) was detected in females at 50 mg/kg/day. Forestomach hyperkeratosis and thickening of the striatum spinosum, with observable cellular debris, was noted at 10 mg/kg/day, but all animals recovered after 2 weeks without dosing. The NOAEL was 2 mg/kg/day in rats administered pitavastatin daily by oral gavage based on forestomach findings. The multiple of human exposure at this dose was 11.3, based on AUC<sub>0-24</sub> at 4 mg after repeat-dose administration for 3 weeks.

**2 Week oral gavage study:** In a 2 week study in Wistar rats, there was no kidney toxicity observed at doses up to 50 mg/kg/day. There were no findings in epididymes of rats at higher doses administered by oral gavage at two weeks. The NOAEL was 2 mg/kg/day in rats administered pitavastatin daily by oral gavage based on forestomach findings. The multiple of human exposure at this dose was 11.3, based on AUC<sub>0-24</sub> at 4 mg after repeat-dose administration for 3 weeks.

**2 Week intravenous study:** During a two-week daily dosing study by intravenous administration of doses up to 4 mg/kg/day, there were findings of nodules in epididymes with sperm granuloma and cellular debris at 2 and 4 mg/kg/day, but not at 1 mg/kg/day. No forestomach findings were noted during a 2-week, daily intravenous dosing in Wistar rats. The NOAEL was 1 mg/kg/day in rats administered pitavastatin daily by oral gavage based on forestomach findings. No toxicokinetic values were reported for this study.

**Single-dose oral gavage study:** In a single dose toxicity study in rats administered pitavastatin by oral gavage, target organs were identified as forestomach (gastric thinning, ulceration, hyperkeratosis, and hemorrhage) at doses of 500 mg/kg and greater in females, and 1000 mg/kg and greater in males. The acute lethal dose was greater than or equal to 500 mg/kg in male rats and greater than or equal to 250 mg/kg in females; no NOAEL was identified by acute dosing in rats. In Wistar rats, gastric thinning, ulceration, hyperkeratosis and hemorrhage was noted after single oral dosing of 500 mg/kg pitavastatin in males or 125 mg/kg in females. Furthermore, there was no acute kidney toxicity noted after single dose oral administration of up to 200 mg/kg. No liver toxicity was noted in rats at any concentration or duration, in chronic or acute dosing, in either sex.

**Other notable findings:** There were no adverse findings in the lens of rats noted in any study.

## Dogs

**12 Month oral capsule study, 2 month recovery:** Pitavastatin caused lens opacity in a 12 month toxicity studying dogs where pitavastatin was administered daily at the 0.3, 1,

and 3 mg/kg/day dose, with a two-month recovery period. Pitavastatin caused lens opacity, with interstitial edema, at 1 mg/kg/day and greater doses, which was not recoverable. No liver toxicity was observed at any dose or either sex. There was epididymal atrophy noted at the high dose of 3 mg/kg/day noted in gross findings, but no correlating histopathological lesion was noted. There were no findings of kidney toxicity at oral doses up to 3 mg/kg/day for 12 months. The NOAEL was established at 0.3 mg/kg/day, based on irreversible lens opacity. The multiple of human exposure at this dose was 2.8, based on AUC<sub>0-24</sub> at 4 mg after repeat-dose administration for 3 weeks.

**3 Month oral capsule study, with 7 week recovery:** In a 3-month toxicity study in Beagle dogs administered pitavastatin at doses of 1, 3, and 10 mg/kg/day by oral capsule, NK-104 caused lens opacity in a female dog (1/3) at 3 mg/kg daily oral administration for three months. Recovery was not confirmable. Findings of lens opacities also occurred, 10 mg/kg/day. Changes were irreversible upon at 7 weeks. Lung toxicity consisting of aggregated foci of foam cells and inflammatory cells was present in the lungs of males and females at 3 mg/kg/day (recoverable). In females only, there were findings of mammary gland lipogranuloma at 3 mg/kg/day (1/3 females) and 10 mg/kg/day (1/3 females), which was not observed in recovery (0/2). Dilatation of hepatic sinusoid was noted at 10 mg/kg/day, in males only (slight to mild, complete recovery). Epididymal atrophy was also noted in dogs administered the high dose of 10 mg/kg/day (not observed in recovery animals). There were no findings of kidney toxicity at doses up to 10 mg/kg/day for 3 months. A NOAEL was established at the 1 mg/kg dose of pitavastatin based on eye, lung and mammary findings. The multiple of human exposure at this dose was 8, based on AUC<sub>0-24</sub> at 4 mg after repeat-dose administration for 3 weeks.

**2 Week oral capsule study:** In a two-week dose range-finding study in Beagle dogs, no lens opacity was observed at doses up to 50 mg/kg/day. Mortality was observed at 15 and 50 mg/kg/day. There were findings of white materials in lung observed at dose 10 and 50 mg/kg/day (recall dosing is by oral capsule). There were no histopathological findings in livers of dogs at doses up to 50 mg/kg/day. A NOAEL was established at the 5 mg/kg dose of pitavastatin based on mortality, cardiac congestion, hemorrhage in decedents; thymic congestion possibly related to overt toxicity. The multiple of human exposure at this dose was 36.7, based on AUC<sub>0-24</sub> at 4 mg after repeat-dose administration for 3 weeks.

**2 Week intravenous study:** There were no findings of toxicologically significant effects at intravenous doses up to 2 mg/kg/day for 2 weeks, except for injection site inflammation and hemorrhage. No TK was reported for this study.

**Single-dose oral capsule study:** After a single acute dose of pitavastatin, the gastrointestinal tract liver and were target organs. In a single-dose study, no lens opacity was observed at doses up to 1000 mg/kg. No epididymal findings were noted. Acute oral dosing produced findings of increased liver enzymes in plasma of 1/3 dogs at 100 mg/kg. There were no findings of kidney toxicity acute oral doses up to 50 mg/kg. The maximum tolerated dose was <100 mg/kg; 1/3 male dogs died at the lowest dose tested.

**Other notable findings:** In Beagle dogs, there were no findings in thyroid at any dose, in either sex, or for any duration. Dogs (and humans) lack tissue analogous to the rodent forestomach.

### Monkeys

**6 Month oral gavage study, 2 month recovery:** In monkeys administered 0.5, 1, 3, and 6 mg/kg/day pitavastatin for 26 weeks by daily oral gavage with an 8 week recovery period, there was swelling of proximal convoluted tubule epithelium with very slight desquamation of tubular epithelium at 6 mg/kg/day and higher in males and females (changed in amendment to 6 mg/kg/day from 1 mg/kg/day for males, recoverable) and swelling of the nucleus of tubular epithelium at doses of 3 mg/kg/day in females and 6 mg/kg/day in males (recoverable). At doses equal to or higher than 1 mg/kg/day in monkeys, there was increased kidney weight. At a daily dose of 6 mg/kg/day there was decreased weight of prostate and testis in males (recoverable). There was no liver toxicity found in either sex at doses up to 6 mg/kg/day. There were findings of foamy cells in the alveolus of female monkeys administered 1 mg/kg/day or higher, but not at the 0.5 mg/kg/day dose. No lung findings were reported for male monkeys at any dose. Lung toxicity was recoverable at 8 weeks. The NOAEL was established at 1 mg/kg/day, based on foamy cells in the alveolus of the lung, mononuclear cell infiltration in the esophagus, and mineralization between the cortex and medulla of the adrenal gland. The multiple of human exposure at this dose was 2.9, based on  $AUC_{0-24}$  at 4 mg after repeat-dose administration for 3 weeks.

**1 Month oral gavage study:** In a one month monkey study where pitavastatin was administered daily by oral gavage, there were tubular lesions in kidney and signs of liver toxicity at 3 mg/kg/day and higher. Vacuolar swelling of hepatocytes, with neutrophilia and decreased glycogen was observed at 15 mg/kg/day in females only. Granuloma in hepatocytes was observed at 8 and 15 mg/kg/day in male monkeys in the 4 week study. There were findings of foamy cells in alveolus of males and females administered the high-dose of 15 mg/kg/day. The NOAEL was established at 3 mg/kg/day, based on kidney necrosis/regeneration, mononuclear cell infiltration in heart and lacrimal gland, atrophy of the white pulp of the spleen, acinar cell atrophy of pancreas, granuloma in hepatocytes, and adrenal hyperplasia with decreased fat in the cortex. The multiple of human exposure at this dose was <3.4, based on  $AUC_{0-24}$  at 4 mg after repeat-dose administration for 3 weeks.

**Single-dose oral gavage study:** In a single oral dose study in monkeys, no effects were noted. In Cynomolgus monkeys, there were no remarkable findings in epididymes at any dose or duration. There were no lung findings after a single dose of pitavastatin in dogs or monkeys. Monkeys (and humans) lack tissue analogous to the rodent forestomach. Thyroid was also not a target organ in cynomolgus monkeys. Pitavastatin did not affect lens of monkeys at any dose or duration studied. The NOAEL was 50 mg/kg. No MTD was identified

## 2.6.6.2 Single-dose toxicity

**Table 2.4.6: Single Dose Toxicity Studies with Pitavastatin**

Species	Route	Dose Levels (mg/kg)	MLD* (mg/kg)	Reference
Mouse	p.o.	0, 125, 250, 500, 1000, 2000	M: 1000 F: 1000 to 2000	[RFG2510]
Rat	p.o.	M – 0, 500, 1000, 2000 F – 0, 125, 250, 500, 1000, 2000	M: 1000 F: 500	[RG25001]
	p.o.	100, 200, 400, 600, 800	400	[RF9808]**
	i.v.	Preliminary Study: 5, 10, 20 Main Study: 0, 20	> 20	[387/002]
Dog	p.o.	0, 100, 300, 1000	100	[RFG2501]
Cynomolgus monkey	p.o.	10, 30, 50	> 50	[SBL17-33]

\*MLD: Minimum lethal dose; \*\*: Preliminary toxicity study prior to an unscheduled DNA synthesis (UDS) study [3638 (144-014)]

Decreased locomotor activity was observed in mice at doses of 250 mg/kg and above with piloerection, closed eyelids and conjunctival discharge at 2000 mg/kg. Necropsy revealed

(Sponsor, M2.4, Nonclinical overview, p25)

Recall that in conscious Beagle dogs in study FBM 06-4115, heart rate was slightly but significantly increased after administration of the highest dose, 10 mg/kg dose of NK-104 at 3 and 6 hours post-dose ( $p < 0.05$ ,  $p < 0.01$ ), respectively. All other parameters, including PR, QRS, and QT/QTc intervals measured pre-dose and 0.5, 1, 3, 6, 12, and 24 hours post-dose, were unremarkable.

Single-Dose Toxicology Studies – Summary					
Study Type Study # GLP Status	Species/strain Number/group Route of admin Dose volume Excipients	Doses (mg/kg)	Endpoints	Target organs	Key findings
Single-dose RFG2510 GLP	Mouse/CD-1 5M/5F Oral gavage 40 mL/kg 0.5% CMC	M/F:0,125, 250,500, 1000,2000	Mortality, clinical observations, body weight, gross pathology	Forestomach, liver, gastrointestinal tract, CNS	MTD ~500♂, 1000♀ Decreased locomotor activity 250♀, 500♂, decreased locomotor activity and crouching 500♀ Forestomach thickening, 1000♀, gastric bleeding 1000♂ Liver nodules 2000♂, discoloration 2000♀
Single-dose RG25001 GLP	Rat/Wistar 5M/5F Oral gavage 40 mL/kg 0.5% CMC	M:0,500, 1000,2000  F:0,125, 250,500, 1000,2000	Mortality, clinical observations, body weight, gross and histopathology	Forestomach	MTD<500♂, ~250♀ Forestomach ulceration, hyperkeratosis ≥500♂, ≥125♀ Stomach hemorrhage ≥500♂, ≥125♀ Liver congestion 2000♂
Single-dose (range finding) RF9808 Non-GLP	Rat/Sprague-Dawley 10M Oral gavage 40 mL/kg 0.5% CMC	M:0,100, 200,400, 600,800	Mortality, clinical observations, body weight, gross pathology	Thymus	MTD~200, based on moribundity Decreased locomotor activity, paralytic gait, decreased body weight gain, thymic atrophy≥400
Single-dose 387/002 GLP	Rat/Wistar (Pre) 2M/2F (Main) 5M/5F Intravenous 20 mL/kg 0.9% NaCl	(Pre)M/F: 0,5,10,20 (Main) M/F: 0,20	Mortality, clinical observations, body weight, gross pathology	N/A	MTD >20♂♀, based on no effects at these doses
Single-dose RFG2501 GLP	Dog/beagle 2M Oral	M:0,100, 300,1000	Clinical observations, vital signs, body weight, food/water consumption, urinalysis, hematology, ophthalmologic examination, gross and histopathology	Liver, gastrointestinal tract	MTD<100♂ Liver enzyme elevations in all survivors, reversible by D14 Liver enzyme elevations, hepatic congestion, intestinal congestion and hemorrhage, atrophy of hepatocytes, thickening of alveolar septum in lungs in decedents 1/2 at 100 and 1/2 at 1000
Single-dose SBL17-33 GLP	Monkey/Cyno 1M/1F Oral 5mL/kg 0.5% carmelose	M/F:0,10, 30,50	Clinical observations, food intake, body weight, plasma, gross and histopathology	N/A	MTD>50, based on no effects at these doses
Single-dose TK (SBL17-33) RFG2513 Non-GLP	Monkey/Cyno 1M/1F Oral 5mL/kg 0.5% carmelose	M/F:10, 30,50	Toxicokinetics	N/A	MTD>50, based on no effects at these doses

**RFG2510 – Single dose oral toxicity study of NK-104 in mice**

**Key study findings:**

- The NOAEL for males was 250 mg/kg due to decreased activity (3/5) in the first hour post-dose. The NOAEL for females was 125 mg/kg due to decreased activity (1/5) in the first 30 minutes post-dose.
- The MTD was 500 mg/kg in males and 1000 mg/kg in females based on mortality at higher doses

Crj:CD-1(ICR)(SPF) mice (n=5/sex/group) were administered a single dose of 125 to 2000 mg/kg NK-104 by oral gavage.

DOSE GROUP ASSIGNMENTS

Group	Drug	Dosage (mg/kg)	Dosage constant volume(ml/kg)	Concentration (w/v%)	No. of animals	
					Male	Female
1	0.5%CMC	0	40	0.0	5	5
2	NK-104	125	20	0.63	5	5
3	NK-104	250	20	1.3	5	5
4	NK-104	500	20	2.5	5	5
5	NK-104	1000	20	5.0	5	5
6	NK-104	2000	40	5.0	5	5

(Sponsor, M4, RFG2510, p11)

Mortality in Mice after Single Oral Administration of NK-104						
Group	C	1	2	3	4	5
Dose (mg/kg)	0	125	250	500	1000	2000
Male	0/5	0/5	0/5	0/5	1/5	4/5
Female	0/5	0/5	0/5	0/5	0/5	5/5

The NOAEL for males was 250 mg/kg due to decreased activity (3/5) in the first hour post-dose. The NOAEL for females was 125 mg/kg due to decreased activity (1/5) in the first 30 minutes post-dose. Decreased locomotor activity was also observed in safety pharmacology studies at  $\geq 3$  mg/kg doses. At doses greater than 125/250 mg/kg in males/females, toxicities included decreased locomotor activity, crouching, prone positioning, decreased body weight decrement, forestomach thickening, and gastrointestinal effects, including gastric bleeding. Liver findings included nodules (2/5) in the 2000 mg/kg dose group of males and discoloration (1/5) in the 2000 mg/kg dose group of females.

**RG25001 – Single dose toxicity study of NK-104 by oral administration in rats****Key Study Findings:**

- MTD was 500 mg/kg in males and 250 mg/kg in females, due to mortality
- Forestomach (ulceration, hyperkeratosis and hemorrhage) at all doses examined in both sexes, and liver (low incidence of congestion) at high doses in males, were identified as potential target organs for pitavastatin in rats after a single acute oral dose of  $\geq 500$  mg/kg in females and  $\geq 1000$  mg/kg in males.
- No skeletal muscle abnormalities were found in either dose group at any dose in any animal showing ataxic gait and paralytic gait, but skeletal muscle was not examined in all animals.
- NOAEL not observed in males ( $< 500$  mg/kg), but was 125 mg/kg in females, based on decreased body weight gain

**DOSE GROUP ASSIGNMENTS**

Group No.	Drug	Dosage (mg/kg)	Concentration (w/v%)	Number of animals	
				Male	Female
1	–	0	0	5	5
2	NK-104	500	1.25	5	5
3	NK-104	1000	2.5	5	5
4	NK-104	2000	5	5	5
5	–	0	0	-	5
6	NK-104	125	0.3125	-	5
7	NK-104	250	0.625	-	5

(Sponsor, M4, RG25001, p9)

In a single dose toxicity study in rats administered pitavastatin by oral gavage forestomach (ulceration, 3/4 males; hyperkeratosis, 3/4; hemorrhage, 1/4) in males at the 1000 mg/kg dose in males and forestomach (ulcer, 1/2; hyperkeratosis, 1/2) in females at the 500 mg/kg dose and liver (congestion, 1/3 males) was identified in males at 2000 mg/kg were identified as target organs. The acute lethal dose was between 500 and 1000 mg/kg in male rats and between 250 and 500 mg/kg in females; no NOAEL was identified by acute dosing in rats in this study (histopathology was not performed below 1000 mg/kg in males or below 500 mg/kg in females due to a lack of gross pathological findings).

**HISTOPATHOLOGY, MALES (SUBSET SHOWING GROSS PATHOLOGY)**

Dose		1000mg/kg					2000mg/kg							
No. of animals examined		4					3							
Organ	Finding	Severity	-	±	+	++	+++	*	-	±	+	++	+++	*
Liver	Congestion								0	0	1	0	0	2
Forestomach	Ulcer		1	1	1	0	0	1	2	0	0	0	0	1
	Hyperkeratosis		1	0	2	0	0	1	2	0	0	0	0	1
	Hemorrhage		3	0	0	0	0	1	1	1	0	0	0	1
Glandular stomach	Hemorrhage		1	2	0	0	0	1	1	1	0	0	0	1
Skeletal muscle			1	0	0	0	0	3	1	0	0	0	0	2

—: No change, ±: Slight, +: Mild, ++: Moderate, +++: Severe, \*: No tissue

(Sponsor, M4, RG25001, p9)

**HISTOPATHOLOGY, FEMALES (SUBSET SHOWING GROSS PATHOLOGY)**

Dose		500mg/kg					1000mg/kg					2000mg/kg								
No. of animals examined		2					2					1								
Organ	Finding	Severity	-	±	+	++	+++	*	-	±	+	++	+++	*	-	±	+	++	+++	*
Forestomach	Ulcer		1	0	1	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0
	Hyperkeratosis		1	0	1	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0
	Edema		2	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0
Glandular stomach	Hemorrhage		0	1	1	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0

—: No change, ±: Slight, +: Mild, ++: Moderate, +++: Severe, \*: No tissue

(Sponsor, M4, RG25001, p9)

**RF9808 – Single oral dose toxicity study of NK-104 in Crj:CD(SD) rats (UDS dose-finding study for genotox assay support)**

**Key study findings:**

- The MTD was determined to be 200 mg/kg for a single oral administration. There were no gross pathological findings at 200 mg/kg and below.
- A gross pathological finding of note in at 400, 600, and 800 mg/kg and above was atrophy of the thymus (2/10, 4/10/ 3/10, respectively).

This study was conducted for dose finding for the unscheduled DNA synthesis (UDS) assay, based on maximum tolerated dose (MTD). The dose finding study was carried out by oral administration in male Crj:CD(SD) rats (n=10/group). Doses administered were 100, 200, 400, 600, and 800 mg/kg. Animals were monitored for 7 days. Doses of 400 and above lead to moribundity and death. Clinical observations at 400 mg/kg included decreased motor activity and paralytic gait.

<b>Single Oral Administration of NK-104, Dose Finding Study - Moribundity and Mortality</b>		
<b>Dose</b>	<b>Moribundity</b>	<b>Mortality</b>
100	-	-
200	-	-
400	2	-
600	-	10
800	1	9

**387/002 – NK-104 - Single dose toxicity study by the intravenous route in the rat**

NK-104 was administered to Wistar rats by i.v. injection at 0 and 20 mg/kg (n=5/sex/group). No mortality or clinically significant observations were observed at 20 mg/kg NK-104 administered by i.v. injection. The NOAEL was 20 mg/kg by i.v. injection in Wistar rats.

**RFG2501 – Single dose toxicity study of NK-104 by oral administration in dogs**

The acute lethal dose was 100 mg/kg (1/3 male dogs at the lowest dose tested); no NOAEL (or MTD) was established after acute dosing in dogs. After a single acute dose of pitavastatin, the gastrointestinal tract liver and were target organs.

**SBL17-33 – Toxicity study of NK-104 on single oral administration in cynomolgus monkeys**

In a single oral dose study in monkeys, no effects were noted; no lethal dose was identified. Doses were 10, 30, and 50 mg/kg. The NOAEL was  $\geq 50$  mg/kg.

**RFG2513 – Plasma concentration during a single oral dose toxicological study of NK-104 in cynomolgus monkeys**

**Key Study Findings:**

- Exposure to pitavastatin and pitavastatin lactone (AUC) increased in a dose-related manner,  $T_{1/2}$  of approximately 3 hours for parent and lactone after a single oral dose of NK-104 in monkeys, and  $T_{max}$  was approximately 2 hours in monkeys.

One male and one female cynomolgus monkey were administered 10 (#1M-2F), 30 (#3M-4F) or 50 mg/kg (#5M-6F) pitavastatin for analysis of plasma at 0.5, 1, 2, 4, 8, and 24 hours post-dose. The lactone was present at approximately 13-28% of parent (at  $C_{max}$ ), and total exposure by AUC showed a similar ratio (15-30%).

PLASMA PHARMACOKINETICS OF NK-104 AND NK-104  
LACTONE AFTER ADMINISTRATION OF A SINGLE ORAL  
ADMINISTRATION IN MONKEYS

No.	Tmax (h)	Cmax (µg/mL)	AUC (µg.h/mL)	T1/2 (h)
<b>NK-104</b>				
1	0.5	1.14	2.89	4.1
2	2.0	0.54	1.88	1.5
3	2.0	3.38	16.84	3.0
4	2.0	5.43	17.31	3.0
5	2.0	9.85	33.44	2.9
6	2.0	27.70	76.33	2.7
<b>Lactone</b>				
1	0.5	0.34	1.00	2.4
2	2.0	0.15	0.67	1.5
3	4.0	0.45	3.19	3.9
4	2.0	0.98	3.45	1.6
5	2.0	2.20	8.40	3.1
6	2.0	3.85	11.68	2.8

(Sponsor, M4, RFG2513, p14)

2.6.6.3 Repeat-dose toxicity

**Table 2.4.7: Repeat Dose Studies with Pitavastatin**

Species	Route	Duration	Dose Levels (mg/kg/day)	Reference
Mouse	p.o.	15 or 28 days	15 Days: 300, 400 28 Days: 0, 50, 100, 200/150	[RF9514]
		13 weeks	0, 25, 75, 225	[KOW 14/952398]
Rat	p.o.	28 days	0, 3, 10, 30	[R92034]
		28 days*	0, 2, 10, 50, 100	[RG25002]
		13 weeks	0, 10, 30, 50	[KOW 12/942992]
		6 months*	0, 0.3, 1, 3, 10	[RFG2506]
	i.v. bolus	14 days	0, 1, 2, 4	[387/003]
Dog	p.o.	14 days	0, 5, 15, 50	[RF2502]
		3 months*	0, 1, 3, 10	[RFG2503]
		12 months*	0, 0.3, 1, 3	[RFG2504]
	i.v. bolus	14 days	0, 0.5, 1, 2	[387/001]
Monkey	p.o.	4 weeks	0, 3, 8, 15	[SBL17-34]
		26 weeks*	0, 0.5, 1, 3, 6	[SBL17-35]

\*: Studies including recovery animals

(Sponsor, M2.4, Nonclinical overview, p27)

Repeat-Dose Toxicology Studies – Summary					
Study Type Study # GLP Status	Species/strain Number/group Route of admin	Dose (mg/kg)	Endpoints	Target organs	Key findings
<b>Chronic Toxicology Studies in Rodents (with Supportive/TK studies)</b>					
6-Month (35-Day Recovery Period)  RFG2506  GLP	Rats/Wistar  12M /12F (8 recovery: C, HD)  Oral gavage  2ml/kg 0.5%CMC	M/F:0, 0.3,1, 3,10	Clinical observations, body weight, food intake, urinalysis, hematology, blood chemistry, ophthalmological evaluation, gross and histopathology, organ weight	Fore-stomach, pituitary, heart	<ul style="list-style-type: none"> <li>• ↑Total cholesterol(HD♀), at end of dosing; ↑GOT(HD♀)at recovery</li> <li>• Forestomach hyperkeratosis (LMD/HMD/HD♂♀); edema, cellular infiltration(HMD/HD♂♀), recovered</li> <li>• ↑AST (HD♀)</li> <li>• Pituitary Rathke’s pouch remnant, cyst (HD♂), recovered; (HD♀), at recovery</li> <li>• Heart mononuclear cell infiltration, myocardial vacuolation, degeneration (HD♀), recovered</li> <li>• NOAEL~0.3mkd</li> </ul>
<b>Sub-chronic Rodent Toxicity Studies (with Supportive/TK studies)</b>					
13-Week Dose-finding KOW12/942992  GLP	Rat/Sprague-Dawley 10M/10F  Oral	M/F:0, 10,30, 50	MTD, Clinical observations, food intake, body weight, efficiency of food utilization, blood chemistry (T <sub>3</sub> , T <sub>4</sub> , TSH only), TK, gross and histopathology, organ weights	Thyroid, fore-stomach,	<ul style="list-style-type: none"> <li>• MTD~30mg/kg</li> <li>• Excess mortality (HD♂♀)</li> <li>• ↓BWgain(MD/HD♂);↓FC(HD♂)</li> <li>• ↑T<sub>3</sub>(MD/HD♂);↓T<sub>4</sub>(MD/HD♀)</li> <li>• ↑Thyroid Wt (MD/HD♂♀); thyroid follicular cell hypertrophy(HD♂♀)</li> <li>• ↓Brain Wt (HD♀)</li> <li>• Forestomach hyperplasia, hyperkeratosis (LD/MD/HD♂♀)</li> <li>• NOAEL&lt;10mkd</li> </ul>
13-Week  KOW14/952398  GLP	Mouse/CD-1  (Main)10M/10F (TK)80M/80F  Oral	M:0.25, 75,225	Clinical observations, body weight, food consumption, food efficiency, water consumption, ophthalmology, blood chemistry, gross and histopathology, organ weights	Eyeball, fore-stomach, liver	<ul style="list-style-type: none"> <li>• MTD&lt;25(♂♀)</li> <li>• TK:MTD&lt;25(♂♀), no COD determined</li> <li>• ↓Body weight gain (MD/HD♂♀)</li> <li>• Corneal opacity(MD/HD♂♀)</li> <li>• Forestomach epithelial hyperplasia and hyperkeratosis(LD/MD/HD♂♀), focal ulceration and erosion, papillomatous hyperplasia (HD♂♀)</li> <li>• Centrilobular enlargement of</li> </ul>

					<ul style="list-style-type: none"> <li>hepatocytes(MD/HD♂;HD,F)</li> <li>Lethargy(HD♂♀)</li> <li>Increased heart, decreased ovarian weight (HD♀)</li> <li>Increased lung weight (LD/MD/HD♀), no correlating lesion by histopathology</li> <li>Liver, centrilobular enlargement of hepatocytes (MD/HD♂♀)</li> <li>Forestomach, ulceration, erosion, epithelial hyperplasia and hyperkeratosis(MD/HD♂♀)</li> <li>NOAEL &lt;25mkd</li> </ul>
<p>1-Month (2-week recovery)</p> <p>RF25002</p> <p>GLP</p>	<p>Rat/Wistar</p> <p>12M/12F</p> <p>Oral</p>	<p>M/F:0, 2,10</p> <p>50,100</p>	<p>Clinical observations, body weight, food intake, urinalysis, hematology, blood chemistry, ophthalmologic examination, gross and histopathology, organ weights</p>	<p>Fore-stomach, kidney (terminal)</p> <p>Multi-organ failure in (early decedents, HD,F)</p>	<ul style="list-style-type: none"> <li>↑Mortality(HD♀, all dead; COD unclear)</li> <li>↓BWgain(HMD♀;HD♂),recovered</li> <li>↓FC(HD♂♀),recovered</li> <li>Urine:↓Cl-,↑protein(HD♀), recovered</li> <li>↑WBC(HMD/HD♂),recovered</li> <li>↑Kidney wt(HD♂;HMD,♀); Kidney tubular regeneration(MHD♀), recovered; Ca<sup>2+</sup> deposition (MHD,♀), not recovered, not COD</li> <li>Forestomach hyperkeratosis, stratum spinosum thickening(LMD/HMD/HD♂), recovered</li> <li>Skeletal muscle degeneration, atrophy, necrosis, fibrosis, mononuclear cell infiltration (MD/HD♀), recovered</li> <li>NOAEL 2mkd</li> </ul>
<p>2-Week</p> <p>387/003</p> <p>GLP</p>	<p>Rat/Wistar</p> <p>13M/13F</p> <p>IV</p>	<p>M/F:</p> <p>0, 1, 2,4</p>	<p>Clinical observations, ophthalmologic evaluation, body weight, food and water consumption, hematology, clinical chemistry, urinalysis, TK, gross and histopathology, organ weight</p>	<p>Epididymes</p>	<ul style="list-style-type: none"> <li>MTD&gt;4mkd</li> <li>Epididymes, nodules (MD/HD♂); sperm granuloma, cellular debris</li> <li>NOAEL~1mkd</li> </ul>
<p>1-Month</p> <p>R92034</p> <p>Non-GLP</p>	<p>Rat/Wistar</p> <p>8M/8F</p> <p>Oral</p>	<p>M/F:</p> <p>0,3, 10,30</p>	<p>Clinical observations, body weight, food intake, urinalysis, hematology, blood chemistry, ophthalmologic examination, gross and histopathology, organ weights</p>	<p>Fore-stomach, kidney</p>	<ul style="list-style-type: none"> <li>↑CHE/↓Tgl(HD♂);↑A/G/GOT(HD♀); no correlating lesions</li> <li>↑Kidney wt(MD/HD♀); no correlating lesions</li> <li>Forestomach, hyperkeratosis epithelial hyperplasia(MD/HD♂♀)</li> <li>NOAEL 3mkd</li> </ul>
<p>1-Month (Dose-finding)</p> <p>RF9514</p> <p>Non-GLP</p>	<p>Mouse/CD-1</p> <p>8-10M/8-10F</p> <p>Oral</p>	<p>M/F:0,50, 100, (150), 200, 300, 400</p>	<p>MTD, clinical observations, body weight, food consumption, clinical chemistry, gross and histopathology</p>	<p>Liver, kidney, thymus, fore-stomach, eyeball</p>	<ul style="list-style-type: none"> <li>MTD~150(♂); 100(♀)</li> <li>Hepatocyte degeneration, swelling(HD♂♀)</li> <li>Tubular dilation, epithelial necrosis/vacuolation(HD♀)</li> <li>Forestomach thickening, hyperkeratosis(♂♀)</li> <li>Lens cortex vacuolation, fiber degradation(HD♀)</li> <li>NOAEL&lt;50mkd</li> </ul>
<p>1-Month (Dose-finding)</p> <p>(b) .138.01</p> <p>GLP</p>	<p>Mouse/CB6F1-rasH2</p> <p>10M/10F</p> <p>Oral gavage</p> <p>10 mL/kg 0.5%CMC</p>	<p>M/F:</p> <p>0,70, 125, 250</p>	<p>MTD, clinical observations, food consumption, body weight, ophthalmology, hematology, blood chemistry, gross and histopathology, organ weights</p>	<p>Fore-stomach, eyeball, prostate, liver</p>	<ul style="list-style-type: none"> <li>MTD~125♀, 250♂ based severe forestomach hyperplasia, mononuclear/polymorphonuclear cell infiltration</li> <li>NOAEL&lt;70 mkd, not determined based on mild forestomach hyperkeratosis at that dose</li> <li>1/10 (HD♀) showed cloudy eye</li> <li>Decreased prostate weight (HD♂)</li> <li>Increased liver weight (HD♂♀)</li> </ul>

Chronic Non-rodent Toxicology Studies (with Supporting/TK studies)					
26-week (8-Week recovery period)  SBL17-35  GLP (Kidney histo- pathology re- evaluated, severity down-graded)	Monkey/Cyno  4♂(+2C/HD recovery) 4♀(+2C/HD recovery)  Oral	♂♀:0, 0.5,1, 3,6	Clinical observations, ophthalmologic evaluation, body weight, food and water consumption, ECG, hematology, clinical chemistry, urinalysis, renal function, TK, gross and histopathology, organ weight	Kidney, adrenal, skeletal muscle, prostate, stomach, testes, seminal vesicles, thymus, bone marrow, lung, esophagus	<ul style="list-style-type: none"> <li>• ↓Triglycerides(HMD/HD♂)</li> <li>• ↓TChol(HMD/HD♀)</li> <li>• Kidney, swelling of proximal tubular epithelium (HD♂♀), recoverable</li> <li>• Prostate, immature(HMD/HD♂)</li> <li>• Adrenal mineralization between cortex/medulla(LMD/HMD♂,HD♀);mononuclear cell infiltration(HMD/HD♂)</li> <li>• Skeletal muscle, mononuclear cell infiltration(HD♂)</li> <li>• Seminal vesicles, immature(HD♂)</li> <li>• Thymic atrophy(HMD/HD♀)</li> <li>• Femoral bone marrow, brown pigment(LD/LMD/HD♀);germinal center development(LMD/HD♀)</li> <li>• Sternum bone marrow, brown pigment(LMD/HMD♀)</li> <li>• Lung, foamy cells in alveolus(LMD/HMD/HD♀), recoverable</li> <li>• Esophagus, mononuclear cell infiltration(LMD/HMD♀)</li> <li>• No liver toxicity</li> <li>• NOAEL~1mkd</li> </ul>
26-Week TK  RFG2515  Non-GLP	Monkey/Cyno 4♂(+2C/HD recovery) 4♀(+2C/HD recovery) Oral	♂♀:0, 0.5,1, 3,6	Toxicokinetics	N/A	<ul style="list-style-type: none"> <li>• N/A</li> </ul>
Histo- pathology SBL17-35 (peer review)  Non-GLP	Monkey/Cyno 4♂(+2C/HD recovery) 4♀(+2C/HD recovery) Oral	♂♀:0, 0.5,1, 3,6	Histopathology	Kidney	<ul style="list-style-type: none"> <li>• Altered interpretation of kidney findings</li> </ul>
12-Month  1-Month recovery  RFF2504  GLP	Dog/beagle  4♂ (+2C/HD recovery) 4♀ (+2C/HD recovery)  Oral capsule	♂♀:0, 0.3,1,3	Clinical observations, vital signs, body weight, food/water consumption, urinalysis, hematology, blood chemistry, ophthalmologic examination, ECG, BSP, auditory examination, gross and histopathology, organ weight, male sexual function, TK	Eyeball, epididymis, lung	<ul style="list-style-type: none"> <li>• PD effects:↓TChol/↓TG/phospholipids (HD♂),recovered; ↓TChol(HD♀),recovered; ↑α2GLB(HD♀),recovered</li> <li>• PD effects: Month3-12 ↑GOT(HD♂♀),recovered</li> <li>• Lens opacity, degeneration, interstitial edema(MD/HD♂♀), no recovery</li> <li>• Epididymal atrophy(HD♂),no correlating lesion</li> <li>• Lung inflammation, aggregated foci of foam cells and inflammatory cells, recovered(HD♂♀)</li> <li>• NOAEL~0.3mkd</li> </ul>
TK  AG2504  Non-GLP	Dog/beagle 4♂(+2C/HD recovery) 4♀(+2C/HD recovery) Oral	♂♀:0, 0.3,1,3	Toxicokinetics	N/A	<ul style="list-style-type: none"> <li>• N/A</li> </ul>
Sub-chronic Non-rodent Toxicology Studies (with Supporting/TK Studies)					
3-Month  RFG2503  GLP	Dog/beagle  3♂ (+2C/HD recovery) 3♀ (+2C/HD recovery)  Oral capsule  Lactose	♂♀:0, 1,3,10	Clinical observations, vital signs, body weight, food/water consumption, urinalysis, fecal examination, hematology, blood chemistry, ophthalmologic examination, ECG, BSP, auditory examination, gross and histopathology, organ weight	Eyeball, liver, mammary gland, epididymis, lung	<ul style="list-style-type: none"> <li>• ↓Body Wt(HD♂),during recovery period</li> <li>• ↓FC(HD♂),during recovery period</li> <li>• PD effects: ↓TChol/↓TG/ phospholipids (LD/MD/HD♂♀), recovered</li> <li>• Weeks4-12↑GOT/GPT(HD♂♀)</li> <li>• Lens opacity, degeneration, interstitial edema(HD♂♀), no recovery</li> <li>• Hepatic dilatation of sinusoid(HD♂)</li> <li>• Mammary gland lipogranuloma(MD/HD♂)</li> <li>• Epididymal atrophy(HD♂)</li> <li>• Lung inflammation, aggregated foci of foam cells and inflammatory cells, recovered(MD/HD♂♀)</li> <li>• NOAEL~1mkd</li> </ul>

4-Week SBL17-34 GLP	Monkey/Cyno 2♂ 2♀ Oral	♂♀:0, 3,8,15	Clinical observations, ophthalmologic evaluation, body weight, food consumption, ECG, hematology, clinical chemistry, urinalysis, TK, gross and histopathology, organ weight	Kidney, lung, stomach, thymus, kidney, heart, small intestine, large intestine, pancreas, liver, adrenal, skeletal muscle	<ul style="list-style-type: none"> <li>• MTD&gt;15mkd</li> <li>• ↓FC/↓Body Wt(HD♀)</li> <li>• Lung, alveolus, foamy cells in(HD♂♀)</li> <li>• Thymic atrophy(HD♂♀)</li> <li>• Stomach erosion, hyperplasia in pyloric part(HD♂♀)</li> <li>• Kidney, tubular epithelial desquamation, swelling, hyaline cast, hyaline droplets, mononuclear cell infiltration, (MD/HD♂♀); necrosis, regeneration(LD/MD/HD♂♀)</li> <li>• Lacrimal gland, interstitial mononuclear cell infiltration(LD/MD/HD♂)</li> <li>• Heart mononuclear cell infiltration(MD/HD♀)</li> <li>• Spleen, atrophy of white pulp(MD/HD♀)</li> <li>• Ileum, cecum, colon, rectum epithelial damage(HD♀)</li> <li>• Pancreas, acinar cell atrophy(MD/HD♀)</li> <li>• Liver, hepatocyte, vacuole, swelling, neutrophilia, ↓glycogen(HD♀); hepatocyte, granuloma (MD/HD♂)</li> <li>• Adrenal hyperplasia, decreased fat in cortex(MD/HD♀)</li> <li>• Skeletal muscle atrophy(HD♀)</li> <li>• Submandibular gland, interstitial mononuclear cell infiltration(HD♀)</li> <li>• Tonsil atrophy(HD♀)</li> <li>• NOAEL~&lt;3 mkd</li> </ul>
4-Week RFG2514 Non-GLP	Monkey/Cyno 2♂ 2♀ Oral	♂♀:0, 3,8,15	Toxicokinetics	N/A	<ul style="list-style-type: none"> <li>• N/A</li> </ul>
2-Week 387/001 GLP	Dog/beagle 3♂ IV	♂:0, 0.5,1,2	Clinical observations, ophthalmologic evaluation, body weight, food and water consumption, cardiovascular assessment, hematology, clinical chemistry, urinalysis, TK, gross and histopathology, organ weight	N/A	<ul style="list-style-type: none"> <li>• MTD&gt;2mkd</li> <li>• No lens opacity</li> <li>• ↓TChol(LD/MD/HD)</li> <li>• Injection site, hemorrhage and inflammation</li> <li>• NOAEL~2mkd</li> </ul>
TK AG25001 Non-GLP	Dog/beagle 3♂(+2C/HD recovery) 3♀(+2C/HD recovery) Oral	♂♀:1 3,10	Toxicokinetics	N/A	<ul style="list-style-type: none"> <li>• N/A</li> </ul>
2-Week (Dose finding) RF2502 Non-GLP	Dog/beagle 2♂ 2♀ Oral capsule	♂♀:0 5,15 50	Clinical observations, vital signs, body weight, food/water consumption, urinalysis, hematology, blood chemistry, ophthalmologic examination, ECG, gross and histopathology, organ weight, TK	Heart, thymus, lung,	<ul style="list-style-type: none"> <li>• MTD~5mkd</li> <li>• XS mortality(MD♂,HD♂♀all)</li> <li>• Increased respiratory rate(HD♂)</li> <li>• ↓Body Wt(MD/HD♂♀)</li> <li>• Cardiac congestion(MD/HD♂,HD♀) hemorrhage(HD♂♀decadents)</li> <li>• White materials in lungs</li> <li>• Possible PD effects on liver, gallbladder, intestine</li> <li>• Thymic congestion, edema, hemorrhage, related to multi-organ pathologies(MD/HD♂,HD♀)</li> <li>• NOAEL~5mkd</li> </ul>
TK A92005 Non-GLP	Dog/beagle 2♂/2♀ Oral	♂♀: 5,15 50	Toxicokinetics	N/A	<ul style="list-style-type: none"> <li>• N/A</li> </ul>

**RF9514 – One-month oral repeated dose toxicity study of NK-104 in mice**

Crj:CD-1 (SPF) mice were administered NK-104 at 0, 50, 100, 150, 200, 300, and 400 mg/kg/day by oral gavage for one month. This study was carried out with the purpose of dose range finding (MTD) for a preliminary (13-week) carcinogenicity study utilizing mice. The MTD was determined to be between 100 and 150 mg/kg NK-104 (28 days) based on hepatotoxicity, nephrotoxicity and an associated premature death in a female

(1/10) at the 200 mg/kg dose, Days 15-30 (150 mg/kg dose, Days 1-14) dose. Deaths occurred at the 300 mg/kg dose NK-104 in males (1/8) and females (2/8). There were no deaths in the 100 mg/kg dose, nor any deaths during administration of 150 mg/kg for the first 14 days.

**ONE MONTH ORAL TOXICITY STUDY IN MICE. GROUP ASSIGNMENTS**

Group	Drug	Dosage (mg/kg)	Dosage constant volume (ml/kg)	Concentration (w/v%)	No. of animals		Period of treatment
					Male	Female	
1	0.5%CMC	0	10	0	10	10	28days
2	NK-104	50	10	0.5	10	10	28days
3	NK-104	100	10	1.0	10	10	28days
4	NK-104	200(150)*	10	2.0(1.5)	10	10	28days
5	NK-104	300	10	3.0	8	8	15days
6	NK-104	400	10	4.0	8	8	15days

\* : Mice in this group were dosed at 150 mg/kg/day for first 14 days and 200 mg/kg/day for following 14 days.

(Sponsor, M4, RF9514, p5)

(b) (4)

**138.01 - A four-week oral dose range-finding toxicity and toxicokinetics study of NK-104 in CB6F1-Tg rasH2 mice**

**FOUR-WEEK RANGE-FINDING STUDY FOR 26-WEEK rasH2 CARCINOGENICITY STUDY- GROUP ASSIGNMENTS**

Main Toxicity (Main Tox) Animals					
Treatment Group	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of animals (Study Animal Number)	
				Females	Males
1	0	0	10	10 (118-127)	10 (1-10)
2	70	7	10	10 (128-134, 227, 136, 137)	10 (11-20)
3	125	12.5	10	10 (138-147)	10 (21-30)
4	250	25	10	10 (148-157)	10 (31-40)
Toxicokinetics (TK) Satellite Animals					
Treatment Group	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of animals (Study Animal Number)	
				Females	Males
5	0	0	10	8 (158-183)	8 (41-48)
6	70	7	10	18 + 2 <sup>a</sup> (184-183)	18 + 2 <sup>a</sup> (47-68)
7	125	12.5	10	18 + 2 <sup>a</sup> (184-203)	18 + 2 <sup>a</sup> (67-86)
8	250	25	10	18 + 5 <sup>a</sup> (204-223, 225, 228, 228)	18 + 5 <sup>a</sup> (87-109)

Total dose volume (mL) was calculated based on the most recent body weight; control animals (Groups 1 and 5) were dosed with 0.5% CMC-Na solution.

<sup>a</sup> Additional two (Groups 6 and 7) or five (Group 8) animals were dosed at the respective dose levels and used for either Main Tox or TK groups as necessary. One female animal (no. 228) in Group 8 was used as Main Tox animal as an alternate of one Group 4 female (no. 149) which died as a result of dosing error on Study Day D8.

(Sponsor, M4, (b) (4) .138.01, p25)

N=10/sex/dose. Pitavastatin was administered at 0, 70, 125, and 250 mg/kg/day. There was 1/10 deaths and 1/10 incidences of moribundity in the 250 mg/kg dose group for

females. The MTD was 125 mg/kg for females and 250 mg/kg for males. 1/10 females showed cloudy eyes at 250 mg/kg. Prostate weights were decreased in males at 250 mg/kg by 15% ( $p < 0.05$ ). Liver weight was increased in males and females relative to body weights at 250 mg/kg by 17% and 14%, respectively ( $p < 0.05$ ). Hyperkeratosis of the forestomach was observed at all doses in both sexes, therefore no NOAEL was determined.

**KOW 14/952398 – NK-104 sub-acute toxicity to mice by repeated oral administration for 13 weeks**

**Key Study Findings:**

- The MTD was  $< 25$  mg/kg based on mortality and body weight changes at 25 mg/kg.
- Significantly higher mortality was observed in females at 225 mg/kg. The cause of death was not clear, presumably due to general toxicity of NK-104. Death due to gavage error could be excluded due to lack of histopathological finding in the trachea/lung/esophagus.
- Corneal opacity at 75 and 225 mg/kg/day doses in males and females
- Lethargy was noted at 225 mg/kg.
- Body weight gain was reduced by  $\geq 10\%$  in both treated males and females at the mid-dose and above.
- Increased heart weight (18%) and decreased ovarian weight (42%) was noted in female high dose group.
- **Increased lung weight (22 – 29%)** was noted in all female dose groups with no histopathological changes found.
- Increased testes weight was noted in mid and high dose groups.
- Histopathological changes were observed in liver (centrilobular enlargement of hepatocytes) and forestomach (ulceration, erosion, epithelial hyperplasia and hyperkeratosis at the low-dose and above in females and mid-dose and above in males
- Forestomach findings also included submucosal fibrosis, inflammatory cell infiltration, edema, and papillomatous hyperplasia at the high dose in males and females.
- $C_{max}$  and AUC increased greater than proportionally with dose. AUC ratios between mice treated with 75 mg/kg and human dose of 4 mg were 15 for males and 35 for females.

**Amendment #, Vol #, and Page #:** M4, 14/952398

**Conducting laboratory and location** <sup>(b) (4)</sup>

**Date of study initiation:** June 1995

**GLP Compliance:** Yes

**QA report:** Yes

**Methods:** Dose range-finding study for carcinogenicity study

**Dosing:**

- Species/strain: Mice, Crl:CD-1 (ICR) BR from <sup>(b) (4)</sup>

- #/sex/group or time point:

Dose Group Assignments					
Group	Dose (mg/kg)	Main		TK	
		Male	Female	Male	Female
Vehicle	0	10	10	-	-
LD	25	10	10	80	80
MD	75	10	10	76	76
HD	225	10	10	76	76

- Age: 6 weeks
- Body weight: male: 26-36 g, female: 20-28 g
- Route: oral gavage, 10 ml/kg

**Drug, lot number, and purity:** 104P-9401, 99.3% purity  
**Formulation/vehicle:** 0.5% hydroxypropyl methylcellulose

#### Observations and Times:

Endpoints	Time of observation
Clinical signs	Daily
Body weights	Weekly
Food consumption	Weekly
Water Consumption	<i>Ad libitum</i>
Ophthalmoscopy	Predose, weeks 2, 12
Hematology	At sacrifice
Clinical biochemistry	At sacrifice
Urinalysis	No data
Gross pathology	At sacrifice
Organ weight	At sacrifice
Histopathology	At sacrifice
Toxicokinetics	Days 1 and 90 (0.5, 1, 2, 4, 6, 8, 12 and 24 hrs post-dose).

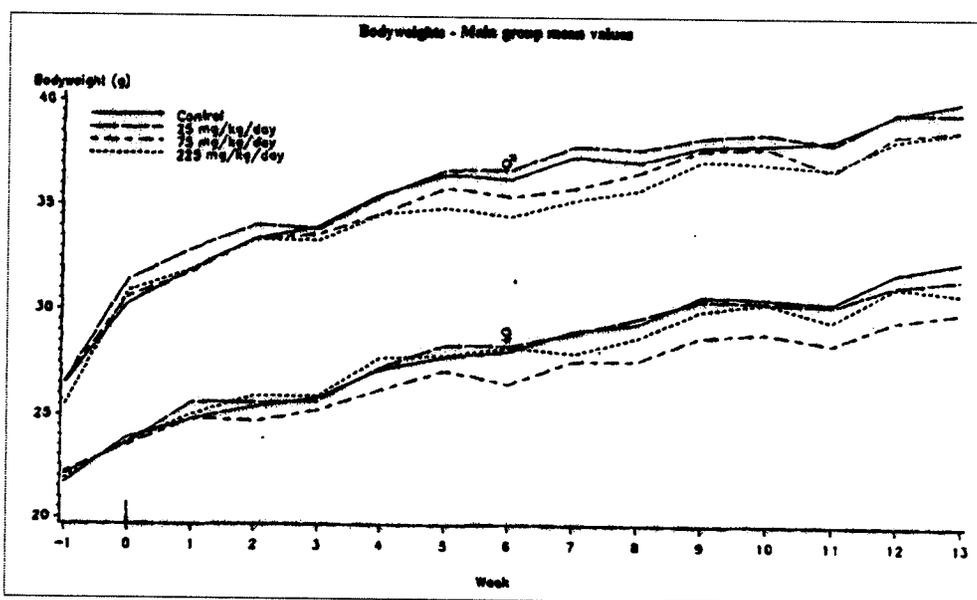
#### Results:

Clinical signs: Lethargy was noted in animals that found dead later.

Mortality: Females had a higher mortality at 225 mg/kg. Forestomach changes were seen in the decedents. However, histopathological examination of tissues revealed no consistent finding on assessment of factor contributory to death.

Mortality			
Group	Dose (mg/kg)	Male	Female
Vehicle	0	0/10	0/10
LD	25	1/90	2/90
MD	75	2/86	2/86
HD	225	2/86	7/86

Body weights: Lower body weight was noted in males at  $\geq 75$  mg/kg and in females at 75 mg/kg. Body weight gain was reduced by about 20% in treated males and 10-20% in treated females.



Body Weight Gain Decrements									
Dose(mg/kg)		Male				Female			
		0	25	75	225	0	25	75	225
Body weight gain (g/rat)	Week 0-13	9.5	7.8	7.9	7.6	8.4	7.7	6.4	7.3
Body weight gain (% control)	Week 0-13	100	82	83	80	100	92	76	87

Food consumption: Lower food consumption was noted in all males and in females at 75 mg/kg.

Food Consumption									
Dose (mg/kg)		Male				Female			
		0	25	75	225	0	25	75	225
Food consumption (total g/rat)	Week 1-13	552	525	489	507	495	498	458	497
% decrease compared to control	Week 0-13	-	5	11.5	8	-	0	7	0

Ophthalmologic Findings: At week 12, corneal opacities were noted in 2/44 males and 4/45 females at 75 mg/kg and in 6/44 males and 5/42 females at 225 mg/kg.

Biochemistry: Lower triglyceride levels were noted for females at  $\geq 75$  mg/kg.

Organ weight: Increased heart weight (18%,  $p < 0.05$ ) was noted in high dose females. Increased lung weight (22%,  $p < 0.05$  to 29%,  $p < 0.01$ ) was noted in all female dose groups. Decreased ovarian weight (42%,  $p < 0.05$ ) was noted in female high dose group. Increased testes weight was noted in high dose males (13%,  $p < 0.05$ ). Seminal vesicle weight was decreased (20%, n.s.s.).

Organ Weights (g)					
Dose(mg/kg)		0	25	75	225
Brain	♀	0.501	0.513	0.493	0.472*
Heart	♀	0.161	0.186	0.174	0.190*
Lung	♀	0.230	0.291*	0.281*	0.297**
Testes	♂	0.222	0.221	0.249	0.251*
Seminal vesicle	♂	0.356	0.346	0.316	0.284
Ovaries	♀	25.0	21.5	20.8	14.6*

\*:  $p < 0.05$ , \*\*:  $p < 0.01$ .

Gross pathology:

Gross Pathological Findings (Incidence)									
Dose(mg/kg)		Male				Female			
		0	25	75	225	0	25	75	225
Forestomach	Thickened	0/10	0/10	0/10	9/10	0/10	0/9	0/10	7/7
	White	0/10	0/10	0/10	9/10	0/10	0/9	0/10	7/7
	Roughened	0/10	0/10	0/10	9/10	0/10	0/9	0/10	7/7
	Invaginated	0/10	0/10	0/10	3/10	0/10	0/9	0/10	2/7

Histopathology:

Histopathology									
Dose(mg/kg)		Male				Female			
		0	25	75	225	0	25	75	225
Liver	Centrilobular hepatocyte enlargement	3/10	0/10	8/10	10/10	0/10	0/9	0/10	2/7
	Ulceration	0/10	0/10	1/10	3/10	0/10	0/9	0/10	1/7
Stomach	Erosion	0/10	0/10	0/10	1/10	0/10	0/9	0/10	1/7
	Epithelial hyperplasia and hyperkeratosis	0/10	0/10	7/10	10/10	0/10	3/9	9/10	7/7
	Submucosal fibrosis, inflammatory cells/ edema	0/10	0/10	0/10	7/10	0/10	0/9	0/10	3/7
	Papillomatous hyperplasia	0/10	0/10	0/10	4/10	0/10	0/9	0/10	1/7

There were no histopathological changes to account for the increase of lung weights noted at postmortem in treated females.

Toxicokinetics:  $C_{max}$  and  $AUC_{0-24}$  increased greater than proportionally with dose. AUC ratios of drug exposure in mice dosed with 75 mg/kg for 90 days compared to AUC in humans with the maximum recommended human dose of 4 mg were 15 for males and 35 for females (human AUC in men dosed with 4 mg for 14 days is 159 ng·hr/ml).

Selected Toxicokinetic Parameters								
NK-104 (mg/kg)	$C_{max}$ (ng/ml)				$AUC_{0-24}$ (ng·h/ml)			
	Males		Females		Males		Females	
	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90
25	417	448	818	699	508	528	963	723
75	4140	2062	5419	7348	4210	2402	6860	5621
225	11653	24060	12467	29306	18671	22486	20066	23274

(Sponsor, M4, KOW 14/952398, p374)

**R92034 - A 1-month oral toxicity preliminary study of NK-104 in rats**

This one-month study was carried out to evaluate tolerability of NK-104 in male and female Slc:Wistar rats. Doses and group assignments are outlined in the following table.

Group	Treatment	Dosage (mg/kg)	Concentration (w/v%)	Number of animals	
				Male	Female
1	0.5% CMC	0	0	8	8
2	NK-104	3	0.066	8	8
3	NK-104	10	0.22	8	8
4	NK-104	30	0.66	8	8

(Sponsor, M4, R92034, p5)

Cholinesterase (CHE) was increased 52% and triglycerides decreased 35% ( $p < 0.01$ ) in high dose male rats at 28 days. Glutamic oxaloacetic transaminase (GOT) was increased 22% ( $p < 0.01$ ) and albumin/globulin (A/G) ratio was increased 12% ( $p < 0.01$ ) in high dose female rats at 28 days. There was thickening of the forestomach in rats at 10 mg/kg (5/16) and 30 mg/kg (16/16) doses of NK-104. Female kidney weight was increased (absolute, 10%,  $p < 0.05$ ; and relative, 11%,  $p < 0.01$ ) in the high dose group. Female livers were increased absolute, 63%, n.s.s.; and relative 100%,  $p < 0.01$ ).

**RG25002 – One-month repeated dose toxicity study of NK-104 by oral administration in rats**

In a one month toxicity study in rats administered pitavastatin daily by oral gavage at 2, 10, 50 and 100 mg/kg/day, degeneration of skeletal muscle and liver toxicity was observed at 50 mg/kg/day and higher doses. Forestomach findings were observed at 10 mg/kg/day and higher doses of pitavastatin. At 50 and 100 mg/kg/day, females showed histopathological findings in skeletal muscle that included vacuolar degeneration of muscle cells, atrophy necrosis, fibrosis, and mononuclear cell infiltration. The NOAEL was determined to be 2 mg/kg/day. The MTD was 50 mg/kg/day due to mortality in high-dose, 100 mg/kg/day females.

**KOW 12/942992 – NK-104 13-week preliminary study to a rat carcinogenicity study by repeated oral administration****Key Study Findings:**

- Pitavastatin was administered by oral gavage daily for 13 weeks at doses of 10, 30, and 50 mg/kg/day.
- 50 mg/kg produced mortality in both sexes and exceeded the MTD.
- 30 mg/kg induced 11% body weight gain reduction and represented the MTD.
- Deaths occurred at 50 mg/kg in both sexes. The death appeared to be drug-related. Death due to gavage error could be excluded due to lack of histopathological finding in the esophagus/trachea/lung.
- Body weight gain decreased 11 and 13% in males at 30 and 50 mg/kg.
- Moderate to marked lesions were observed in the thyroid at 50 mg/kg and forestomach at  $\geq 10$  mg/kg.

- C<sub>max</sub> and AUC exhibited greater than proportional increases with dose without apparent gender difference.
- The NOAEL was <10 mg/kg/day due to hyperkeratosis of the forestomach at 10 mg/kg and greater doses.

**Amendment #, Vol #, and Page #:** M4, 12/942992, p1-442

**Conducting laboratory and location** <sup>(b) (4)</sup>

**Date of study initiation:** June, 1994

**GLP Compliance:** Yes

**QA report:** Yes

**Methods:** Dose ranging-finding study for carcinogenicity study

**Dosing:**

- Species/strain: Rats, Crl:CD(SD)BR from <sup>(b) (4)</sup>
- #/sex/group or time point:

Dose Group Assignments					
Group	Dose (mg/kg)	Main		TK	
		Male	Female	Male	Female
Vehicle	0	10	10		
LD	10	10	10	36	36
MD	30	10	10	36	36
HD	50	10	10	36	36

- Age: 6 weeks
- Body weight: male: 175-239 g, female: 137-199 g
- Route: oral gavage, 5 ml/kg

**Drug, lot number, and purity:** 104P-9202, 99.2% purity

**Formulation/vehicle:** 0.5% sodium carboxymethylcellulose

**Observations and Times:**

Endpoints	Time of Observation
Clinical signs	Daily
Body weights	Weekly
Food consumption	Weekly
Water Consumption	Ad libitum
Clinical biochemistry	At sacrifice
Gross pathology	At sacrifice
Organ weight	At sacrifice
Histopathology	At sacrifice (all groups)
Toxicokinetics	Day 1 and 90 (0, 0.5, 1,2,4,6,8,12, and 24 hrs post-dose).

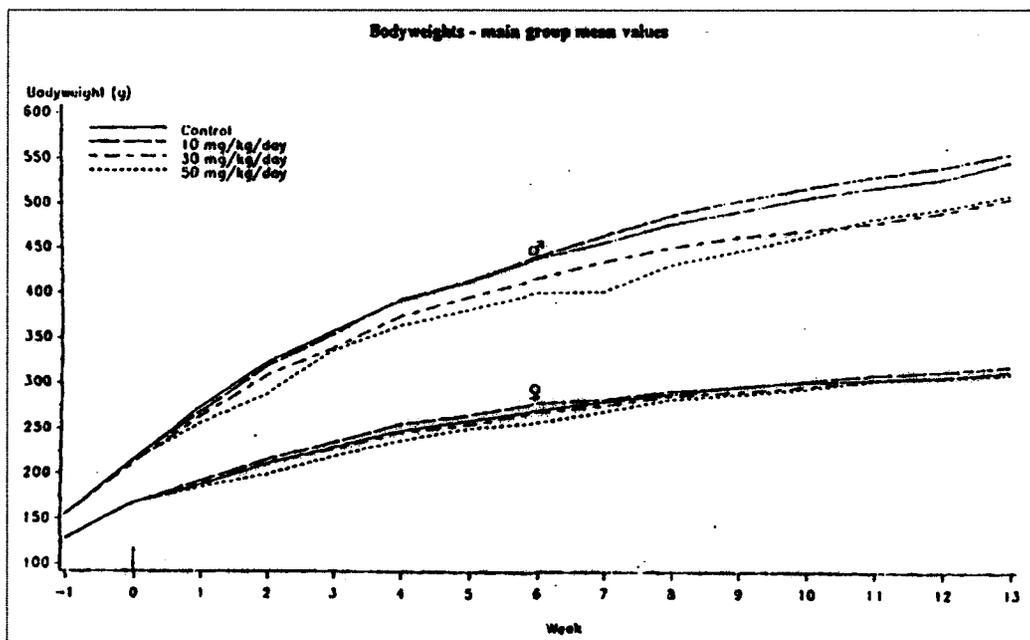
**Results:**

Clinical signs: Prior to death, most premature decedents exhibited hunched posture, piloerection, lethargy, emaciation, unsteady gait, walking on their toes, partially-closed eyes and were cold to touch. Salivation was noted in surviving animals at 50 mg/kg immediately after dosing.

Mortality: Higher mortality was noted at 50 mg/kg in both sexes. The death appeared to be drug-related. Death due to gavage error could be excluded due to lack of histopathological finding in the lung.

Mortality					
Group	Dose (mg/kg)	Main		TK	
		Male	Female	Male	Female
Vehicle	0	0/10	0/10	-	-
LD	10	0/10	0/10	0/36	1/36
MD	30	0/10	0/10	0/36	1/36
HD	50	5/10	4/10	20/36	16/36

Body weights: Body weight gain was decreased by 10% and 13% in males at 30 or 50 mg/kg, respectively. Body weight was not affected in females.



(Sponsor, M4, 12/942992, p31)

Body Weight Decrements									
Dose (mg/kg)		Male				Female			
		0	10	30	50	0	10	30	50
Body weight gain (g/rat)	Week 0-4	176	177	162	145*	81	88	79	69
	Week 4-8	85	93	77	63*	40	36	40	49
	Week 0-13	331	339	293	289	146	151	144	142
Body weight gain (% control)	Week 0-4	100	100	92	82	100	108	98	85
	Week 4-8	100	109	90	74	100	90	100	122
	Week 0-13	100	102	89	87	100	103	98	97

\*: p≤0.05

Food consumption: Lower food consumption was noted in males at 50 mg/kg.

Food Consumption (total g/rat)								
Dose(mg/kg)	Male				Female			
	0	10	30	50	0	10	30	50
Week 1-3	677	647	632	568*	434	453	458	413
% control	-	96	93	84	-	104	106	95
Week 1-13	3044	3019	2864	2959	2066	2099	2165	2201
% control	-	99	94	97	-	102	105	107

\*: p≤0.05

Serum chemistry: A dose-related increase in T<sub>3</sub> levels was noted in males. Mild decreases in T<sub>4</sub> levels were observed in mid and high dose females.

Clinical Chemistry								
Dose (mg/kg)	Male				Female			
	0	10	30	50	0	10	30	50
T <sub>3</sub> (ng/dl)	36	42	52**	56**	48	44	42	50
T <sub>4</sub> (ng/dl)	2.3	2.4	2.5	2.2	2.0	1.9	1.7*	1.8*
TSH (ng/dl)	2.8	2.9	2.9	2.4	2.3	2.3	2.3	2.5

\*: p≤0.05, \*\* p≤0.01

Organ weight: Higher thyroid weight and lower brain weights were noted in treated animals. Thyroid changes were corroborated at histopathological examination where thyroid follicular cell hypertrophy was observed.

Organ Weights								
Dose(mg/kg)	Male				Female			
	0	10	30	50	0	10	30	50
Brain (g)	2.14	2.16	2.08	2.02*	1.97	1.96	1.97	1.88*
Thyroid (mg)	23.8	25.0	30.4**	30.8**	15.7	18.7	22.4**	21.8**

\*: p≤0.05, \*\* p≤0.01

**Gross pathology:** Pathologic changes were observed in the forestomach and stomach of mid and high dose rats.

Gross Pathology									
	Dose(mg/kg)	Male				Female			
		0	10	30	50	0	10	30	50
Fore-stomach	Thickened	0/10	0/10	9/10	9/10	0/10	0/10	9/10	9/10
	Ridged appearance	0/10	0/10	10/10	5/5	0/10	0/10	8/10	6/6
	Roughened	0/10	2/10	0/10	1/10	0/10	3/10	0/10	2/10
	Sloughing of epithelium	0/10	0/10	2/10	6/10	0/10	0/10	4/10	5/10
Stomach (corpus mucosa)	Congestion	0/10	0/10	1/10	2/5	0/10	0/10	0/10	0/6

**Histopathology:** Higher incidence of histopathological changes was observed in the thyroid at high dose and stomach at  $\geq 30$  mg/kg. Lesions  $\geq$  moderate were only observed at  $\geq 30$  mg/kg.

Histopathology (Incidence and Severity)									
	Dose(mg/kg)	Male				Female			
		0	10	30	50	0	10	30	50
Thyroid	Follicular cell hypertrophy	2/10	2/10	4/10	9/10	0/10	0/10	0/10	6/10
	Trace	0	0	2	1	0	0	0	1
	Minimal	2	2	2	2	0	0	0	4
	Moderate	0	0	0	6	0	0	0	1
Forestomach	Epithelial hyperplasia	0/10	9/10	10/10	10/10	0/10	9/10	10/10	9/10
	Trace	0	4	0	0	0	1	0	0
	Minimal	0	5	0	0	0	7	0	1
	Moderate	0	0	10	10	0	1	10	7
	Marked	0	0	0	0	0	0	0	1
	Epithelial hyperkeratosis	0/10	9/10	10/10	10/10	0/10	8/10	10/10	9/10
	Trace	0	0	8	0	0	0	0	0
	Minimal	0	0	1	0	0	7	0	0
	Moderate	0	0	0	7	0	1	10	8
	Marked	0	0	0	3	0	0	0	1

**Toxicokinetics:** C<sub>max</sub> and AUC exhibited greater than proportional increases with dose without apparent gender difference. Dose ratio of 1:3:5 resulted in 1:6:13-20 fold increases in exposure. C<sub>max</sub> increased 1.5 – 2 folds from day 1 to day 90. AUC ratio between 30 mg/kg at day 90 and human dose of 4 mg (AUC 159 ng-hr/ml at day 14) was 360 for males or 320 for females.

Toxicokinetics of NK-104 in the Rat						
	NK-104 (mg/kg)					
	10		30		50	
	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90
<b>Males</b>						
C <sub>max</sub> (ng/ml)	2820	4652	14,491	29,352	49,547	81,252
T <sub>max</sub> (hr)	2.0	0.5	1.0	0.5	1.0	0.5
AUC (ng.hr/ml)	10,165	10,527	53,715	57,289	154,167	155,163
t <sub>1/2</sub> (h)	—	3.7	—	4.0	—	a
C <sub>min</sub> (ng/ml)	BLQ	5.9	11.7	20.1	BLQ	850.6
<b>Females</b>						
C <sub>max</sub> (ng/ml)	2717	5366	26,065	37,470	47,753	65,032
T <sub>max</sub> (hr)	0.5	0.5	1.0	0.5	1.0	0.5
AUC (ng.hr/ml)	10,135	6740	63,824	50,924	136,562	118,297
t <sub>1/2</sub> (h)	—	3.0	—	3.9	—	2.5
C <sub>min</sub> (ng/ml)	BLQ	BLQ	BLQ	18.1	8.1	15.8

BLQ = Below Limit of Quantization; a = could not be adequately estimated

(Sponsor, M4, 12/942992, p395-396)

**RFG2506 – Six-month consecutive oral toxicity study of NK-104 in rats [with a one-month recovery period]**

**Key Study Observations:**

NK-104 was orally administered to rats at doses of 0.3, 1, 3, and 10 mg/kg for 6 months and allowed to recover for 1 month

- 10 mg/kg: forestomach hyperkeratosis and acanthosis were observed in all animals. Submucosa edema and lamina propria cellular infiltration were observed in some animals. Elevated AST in females (55%). These changes were not observed at the end of the 1 month recovery period.
- 3 mg/kg: forestomach hyperkeratosis and acanthosis were observed in all animals. Submucosa edema and lamina propria cellular infiltration were observed in some animals.
- 1 mg/kg: forestomach hyperkeratosis and acanthosis were observed in some animals.
- 0.3 mg/kg: no treatment related changes were noted.
- NOAEL was 0.3 mg/kg (this dose represents a multiple of human exposure of ~1.6 at the human dose of 4 mg based on AUC).
- TK data were not collected.

**Amendment #, Vol #, and Page #: M4, RFG2506**

**Conducting laboratory and location:** Fuji Research Labs, Kowa Co., Shizuoka, Japan

**Date of study initiation:** August, 1993

**GLP Compliance:** Yes

**QA report:** Yes

**Methods:** chronic oral toxicity study

**Dosing:**

- Species/strain: Rats (Wistar)
- #/sex/group or time point:

<b>6 Month Oral Toxicology Study in Rats – Group Assignments</b>					
<b>Group</b>	<b>Dose mg/kg</b>	<b>6 month main</b>		<b>1 month recovery</b>	
		<b>M</b>	<b>F</b>	<b>M</b>	<b>F</b>
<b>Vehicle</b>	<b>0</b>	12	12	8	8
<b>LD</b>	<b>0.3</b>	12	12	-	-
<b>MD</b>	<b>1</b>	12	12	-	-
<b>MHD</b>	<b>3</b>	12	12	-	-
<b>HD</b>	<b>10</b>	12	12	8	8

- Age: 6 wks
- Body weight: male: 118-143 g, females: 104-124 g
- Route: oral gavage

**Drug, lot number, and purity:** 104P-9201, 99.3% purity

**Formulation/vehicle:** 0.5% CMC (carboxy-methylcellulose sodium)

**Observations and Times:**

<b>Endpoints</b>	<b>Time of Observation</b>
<b>Clinical signs</b>	Daily, but no data were recorded
<b>Body weights</b>	Pre-dose, weekly up to 3 month, monthly thereafter
<b>Food consumption</b>	Pre-dose, weekly
<b>Water Consumption</b>	None
<b>Ophthalmoscopy</b>	Day 170 of dosing, Day 23 of recovery
<b>Electrocardiography</b>	None
<b>Hematology</b>	End of dosing, end of recovery. RBC, WBC, platelet, hematocrit, Hb, MCV, MCH, MCHC, reticulocyte, leukocyte classification, PT, APTT, fibrinogen were examined.
<b>Clinical biochemistry</b>	End of dosing, end of recovery. Glucose, total protein, albumin, A/G Ratio, BUN, creatinine, Ca, ALT, AST, LDH, CPK, ALP, cholinesterase activity, triglyceride, total cholesterol, phospholipid, Na, K, Cl were examined.
<b>Urinalysis</b>	Days 91 and 177 during dosing; end of recovery. pH, protein, glucose, ketone bodies, occult blood, urobilinogen, bilirubin, and sediment were examined.
<b>Gross pathology</b>	At sacrifice
<b>Organ weight</b>	At sacrifice
<b>Histopathology</b>	At sacrifice. Only control and 10 mg/kg/day group were examined. See page 28.
<b>Toxicokinetics</b>	Data not collected.

**Results:**

**Clinical signs:** No data, but claimed none occurred at all at for any dose in any group, or at any time point.

**Mortality:** None.

**Body weights:** No treatment related changes.

**Food consumption:** There were no treatment related changes in estimation of food consumption.

**Ophthalmology:** There was a white opacity observed in the eye of a female in the high-dose, 10 mg/kg/day NK-104 group, characterized as a calcium deposit upon histopathological examination. There was a similar finding in a male control animal at recovery, which was characterized as a cataract. These ophthalmological changes were not considered significant, because three females in the control group and three females in the high-dose NK-104 group showed calcium deposits upon recovery, an indication that this is probably a background finding in Wistar rats.

**Urinalysis:** There were no remarkable treatment-related changes detected upon urinalysis.

**Hematology:** There were no remarkable changes in hematology parameters at 6 months administration of NK-104 at doses up to 10 mg/kg/day. There were several statistically significant differences at the end of recovery, including increased percent lymphocytes, decreased % segmented neutrophils, and decreased prothrombin time. These findings were not observed during the treatment period, were present in females only, and were in the range of those observations noted at the terminal bleed for the main, six-month groups. Therefore, these differences, though statistically significant, are not considered biologically significant.

**Clinical chemistry:** Total cholesterol was higher in females at 10 mg/kg/day (13%,  $p < 0.001$ ). GOT increased in females at 10 mg/kg/day at the end of recovery period ( $\uparrow 55\%$ ,  $p < 0.01$ ). Chloride was decreased by a statistically significant ( $p < 0.01$ ), but not biologically significant, amount. There were trends for decreased triglycerides in males and females, but these findings were not statistically significant, and may be due to pharmacodynamic effects of the test article.

Clinical Chemistry											
	Sex Dose N	Males					Females				
		0	0.3	1	3	10	0	0.3	1	3	10
		12/8	12	12	12	12/8	12/8	12	12	12	12/8
Triglyceride	Main	83	78	85	90	75	58	55	45	46	42
	Rec	89				94	63				50
Total cholesterol	Main	69	65	64	64	66	95	96	96	104	113***
	Rec	69				61	98				95
Chloride	Main	110	108	109	109	109	110	109	109	109	109**
	Rec	109				109	109				110
GOT (AST)	Main	145	132	123	117	142	107	115	119	92	97
	Rec	236				192	106				164**

\*\* :  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$

**Organ weight:** Lung weight in males was increased in the 1 and 3 mg/kg/day dose groups, but not in the high-dose group. These differences were not large in absolute (3% and 5%, respectively) or relative (7 and 4%, respectively) terms. Lung weight changes were not considered biologically relevant.

Pancreas weights were decreased in a dose dependent manner in males and females, with a maximum difference between control and high-dose groups of 16 and 12% (absolute weight, respectively) and 14 and 13% (relative to body weight, for males and females, respectively). These findings were not statistically significant and were not noted in the one-month high-dose recovery groups of either sex. There were no correlating gross or histopathological lesions. This finding is not considered biologically significant.

**Gross pathology:** Forestomach thickening was observed at  $\geq 1$  mg/kg in both sexes at the end of dosing period, but not observed at the end of recovery period. Eye opacity was

not observed in the NK-104 group, but was observed in a male rat in the control recovery group. There was atrophy of the testes in a male rat of the high-dose group, but no such finding was observed in any animal of the recovery group.

In females, opacity of the right eye was observed in a high-dose female. No such finding was observed in any recovery group female. An adhesion on the diaphragm of the lung was noted in a 3 mg/kg/day female, but no lung findings were noted for the recovery group. This finding was not dose-related or frequent, and is not considered drug-related.

<b>6 Month Oral Toxicology Study in Rats – Gross pathology (Main study)</b>											
Dose(mg/kg)		Male					Female				
		0	0.3	1	3	10	0	0.3	1	3	10
N		12	12	12	12	12	12	12	12	12	12
Forestomach	Thickening of wall	-	-	1	7	12	-	-	2	8	12
Testes	Atrophy, left	-	-	-	-	1					
Eye	Opacity, right	-	-	-	-	-	-	-	-	-	1
Lung	Adhesion, diaphragm	-	-	-	-	-	-	-	-	1	-

Histopathology: Treatment related change was only observed in stomach. Forestomach hyperkeratosis, acanthosis were observed at  $\geq 1$  mg/kg in both sexes. Submucosa edema was observed in males at  $\geq 3$  mg/kg. Lamina propria cellular infiltration was noted at  $\geq 3$  mg/kg in both sexes.

6 Month Oral Toxicology Study in Rats – Histopathology (Main study)											
Dose(mg/kg) N		Male					Female				
		0 12	0.3 12	1 12	3 12	10 12	0 12	0.3 12	1 12	3 12	10 12
Liver	Interlobular bile duct proliferation	-				1±	1±				1±
	Small granulomatous nodule	-				2±	-				2±
Kidney	Any dose-related toxicity	-				-	-				-
Skeletal muscle	Any dose-related toxicity	-				-	-				-
Forestomach	Hyperkeratosis	-	-	6±	1± 7+ 4++	1± 5+ 6++	-	-	5± 1+	7+ 5++	4+ 8++
	Acanthosis	-	-	3±	1± 11+	6+ 6++	-	-	3±	2± 9+ 1+	8+ 4++
	Vacuolation of striatum spinosum	-	-	1±	-	-	-	-	-	-	-
	Submucosal cellular infiltration	-	-	-	-	1±	-	-	-	-	-
	Submucosal edema	-	-	-	1±	2±	-	-	-	-	-
	Cellular infiltration into mucosal lamina propria	-	-	-	1+	5± 2+	-	-	-	2±	3±
Testes	Atrophy of seminiferous tubule	3±				1++					
	Hypospermatogenesis	3±				1+++					
Thyroid	Follicular atrophy	-				-	-				1±
Epididymes	Decrease in sperm	-				1+++					
Duodenum	Ectopic pancreas	-				1+	1±				-
Eye	Cataract	-				-	-				-
	Calcium deposit in cornea	6±				5±	6±				4± 1+
Lung	Interstitial proliferation	-				-	-				1±
	Edema	-				-	-				1+
Pituitary	Rathke's pouch remnant	-				1+ 1*	-				-
	Cyst	1+				1*	5+				8+
Heart	Any dose-related toxicity	-				-	-				-
Thymus	Cyst	2*				-	-				1+ 1*

±: minute, +: mild, ++: moderate, +++: severe, \* no tissue

#### Recovery:

In male rats, histopathological findings in forestomach, liver, epididymes, and pituitary findings in males were absent at the end of the one-month recovery period. Findings in testes were partially recovered. Ectopic cyst in the duodenum was still present in one male in the recovery group.

In female rats, histopathological findings in forestomach, liver, thyroid, lung and thymus findings were recovered after one-month of recovery. Other toxicities observed during

histopathological examination were not dose-related, not severe, or were present in the control and high-dose groups, and were therefore not considered drug-related.

#### **387/003 - NK-104 - 2 week intravenous toxicity study in the rat**

This study was conducted for bioavailability assessment at steady state (i.e. comparison to oral pharmacokinetic parameters).

#### **2-WEEK I.V. TOXICITY STUDY IN RATS – DOSE GROUP ASSIGNMENTS**

Group/ Treatment	Dose level (mg/kg/day)	Dose volume (ml/kg/day)	Dose concentration (mg/ml)	Number of males (1)
1. Control	0	4	0	10
2. Low dose	1	4	0.25	10 (+ 3)
3. Intermediate dose	2	4	0.5	10 (+ 3)
4. High dose	4	4	1	10 (+ 3)

(Sponsor, M4, 387/003, p10)

The high dose was chosen because it was the maximum amount of NK-104 that could be dissolved in physiological saline. There was no mortality in this study. There were no significant clinical signs observed. There were no differences in body weight gains, food, or water consumption.

Rats experienced ↓% reticulocytes at 1, 2, and 4 mg/kg/day of up to 18%.

Nodules were present in epididymes of high-dose group (2/10), and mid-dose group (1/10), and this was correlated with sperm granuloma and cellular debris (Check rat numbers). There were some histopathological signs of kidney damage (lymphoid cell infiltration, 2/10) in the high-dose group versus controls (0/10).

#### **RF2502 - A 14-day oral toxicity study of NK-104 in dogs (preliminary study)**

This study was carried out in order to find appropriate doses for a 3-month toxicity study in dogs.

#### **14-DAY ORAL TOXICITY STUDY OF NK-104 – GROUP ASSIGNMENTS**

Group No.	Test article name	Dose <sup>a)</sup> mg/kg	Number of animals	
			Males	Females
1	Control	0	2	2
2	NK-104	2	2	2
3	NK-104	15	2	2
4	NK-104	50	2	2

(Sponsor, M4, RF2502, p5)

The 50 mg/kg dose was the MTD from a single-dose study in dogs, conducted previously, where there was a death at the 100 mg/kg dose, but no deaths at 250 mg/kg.

Single Oral Administration of NK-104, Dose Finding Study – Mortality (including Moribundity)		
Dose (mg/kg)	Males	Females
0	-	-
2	-	-
15	1/2	-
50	2/2	2/2

A92005 - Measuring plasma NK-104 levels (plasma samples from a 14-day toxicological study in dogs)

		C <sub>max</sub>			
NK-104 Dose		Day 1 ♂	Day 1 ♀	Day 9 ♂	Day 9 ♀
NK-104	5	2.9	3.3	2.5	4.0
	15	11.9	16.1	14.5*	16.1
	50	59	101	-	-
NK-104 lactone	5	0.2	0.2	0.5	0.3
	15	2.2	1.8	3.1*	1.5
	50	5.6	4.7	-	-

- both dogs died between days 4 and 7

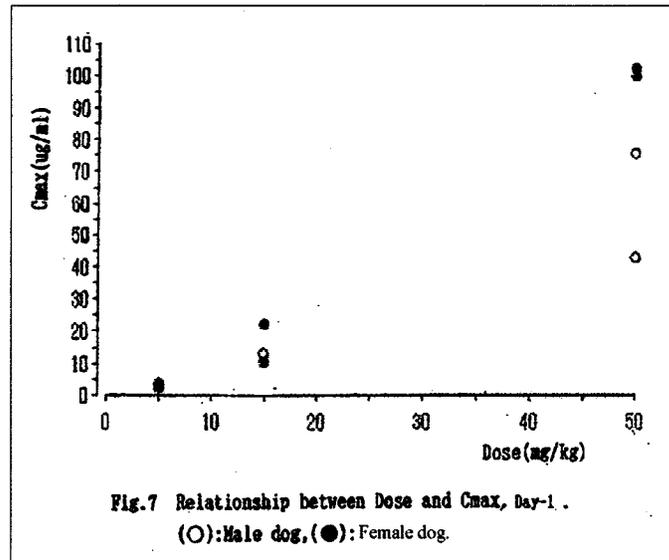


Fig.7 Relationship between Dose and C<sub>max</sub>, Day-1 .  
 (O):Male dog,(●): Female dog.

(Sponsor, M4, A92005, p17)

**RFG2503 – Three-month repeated dose toxicity study of NK-104 by oral administration in dogs [with a 7-week recovery period]**

**Key Study Findings:**

- 10 mg/kg: 1/3 female death, abnormal stools, vomiting, lower body weight in males, opacity of the lens in both sexes, increases in ALT and AST, increased lung weight, decreased ovary and uterus weight, histopathological changes were observed in lung (inflammation in 3/5 males and 3/5 females, hemorrhage in 1/5 female), liver (dilation of sinusoid in 3/5 males and 1/5 female, hemorrhage in 1/5 female), gallbladder (hemorrhage in 1/5 female), eyeball (degeneration of the lens in 4/5 males and 3/5 females, edema/degeneration in 3/5 males and 3/5 females, vacuolation in 2/5 males), epididymis (spermatic granuloma in 2/5 males), GI tract (hemorrhage in 1/5 female), and heart (eosinophilic degeneration and swelling of myocardium in 1/5 female).
- 3 mg/kg: abnormal stools, vomiting, opacity of the lens in 1/3 male and 1/3 female, increases in ALT and AST, decreased ovary and uterus weight, histopathological changes were observed in lung (inflammation in 1/3 male and 2/3 females), and eyeball (degeneration of the lens in 1/3 male). ). In females only, there were findings of mammary gland lipogranuloma at 3 mg/kg/day (1/3 females) and 10 mg/kg/day (1/3 females), which was not observed in recovery (0/2).
- 1 mg/kg: decreased ovary weight. No apparent drug-related changes.
- NOAEL was 1 mg/kg/day, based on degeneration of lens, increased liver enzymes, decrease ovary and uterine weight, mammary gland lipogranuloma. For the 4 mg human dose, this represents an exposure multiple of 7.3, based on AUC.

**Amendment #, Vol #, and Page #:** M4, RFG2503

**Conducting laboratory and location:** Fuji Research Labs, Kowa Co., Shizuoka, Japan

**Date of study initiation:** July, 1992

**GLP Compliance:** Yes

**QA report:** Yes

**Methods:** sub-chronic oral (capsules) toxicology study

**Dosing:**

- Species/strain: Beagle dogs
- #/sex/group or time point:

3 Month Oral Toxicology Study in Dogs – Group Assignments					
Group	Dose mg/kg	3-month main		7-week recovery	
		M	F	M	F
Vehicle	0	3	3	2	2
LD	1	3	3	-	-
MD	3	3	3	-	-
HD	10	3	3	2	2

- Age: 5 months
- Body weight: male: 7.4-9.1 kg, females: 7.1-9.1 kg
- Route: oral capsules

**Drug, lot number, and purity:** 104P-9202, 99.2% purity

**Formulation/vehicle:** lactose (gelatin capsules)

**Observations and Times:**

Endpoints	Time of Observation
Clinical signs	Daily
Body temperature, pulse rate, respiratory rate	Day 0, weeks 4, 8, 12 of dosing and weeks 4 and 7 of recovery period (prior, 1 and 3 hrs post dosing).
Body weights	Pre-dose, weekly
Food consumption	Pre-dose, weekly
Water Consumption	Pre-dose, weekly
Ophthalmoscopy	Pre-dose, weeks 4, 8 and 12 of dosing, weeks 2, 4, 7 of recovery period.
Auditory examination	Pre-dose, weeks 4, 8 and 12 of dosing, weeks 2, 4, 7 of recovery period.
Electrocardiography	Pre-dose, weeks 4, 8 and 12 of dosing, weeks 2, 4, 7 of recovery period.
BSP test	Pre-dose, weeks 12 of dosing, weeks 2, 4, 7 of recovery period. Blood stagnation rate of BSP was determined.
Hematology	Pre-dose, weeks 4, 8 and 12 of dosing, weeks 2, 4, 7 of recovery period. RBC, WBC, platelet, hematocrit, Hb, MCV, MCH, MCHC, reticulocyte, leukocyte classification, PT, APTT, fibrinogen were examined.
Clinical biochemistry	Pre-dose, weeks 4, 8 and 12 of dosing, weeks 2, 4, 7 of recovery period. Glucose, total protein, albumin, A/G Raito, BUN, creatinine, Ca, ALT, AST, LDH, CPK, ALP, GGT, cholinesterase activity, triglyceride, total cholesterol, phospholipid, Na, K, Cl were examined.
Urinalysis	Weeks 4, 8 and 12 during dosing, weeks 4 and 7 of recovery period. pH, protein, glucose, ketone bodies, occult blood, urobilinogen, bilirubin, and sediment were examined.
Fecal examination	Pre-dose, weeks 4, 8, and 12 of dosing, and weeks 4 and 7 of recovery period. Occult blood was tested.
Gross pathology	At sacrifice
Organ weight	At sacrifice
Histopathology	At sacrifice. Only control and 10 mg/kg/day group were examined.
Toxicokinetics	Report not attached.

**Results:**

Clinical signs: Food remains after being fed, abnormal stools (soft stools, bloody stools and/or diarrhea), and vomiting were observed in both control and treated animals, but appeared to be more frequently in treated animals. Abnormal stools and vomiting were not observed in females and less frequently in males at 10 mg/kg during recovery period.

Clinical Observations (Mean Percent)								
Weeks	Dose (mg/kg NK-104)							
	Males				Females			
<u>Dosing: 1-13</u>	<u>0</u>	<u>1</u>	<u>3</u>	<u>10</u>	<u>0</u>	<u>1</u>	<u>3</u>	<u>10</u>
Food remains	3.1	0.4	1.8	4.1	12.7	23.6	0.0	35.4
Abnormal stool <sup>b</sup>	1.5	4.4	13.6	26.3	0.4	10.9	3.3	8.5
Vomiting	0.9	0.4	2.2	3.5	1.1	1.4	1.8	3.0
<u>Recovery: 14-20</u>								
Food remains	24.5			22.4	49.0			91.8
Abnormal stool	0.0			5.1	0.0			0.0
Vomiting	0.0			1.0	1.0			0.0

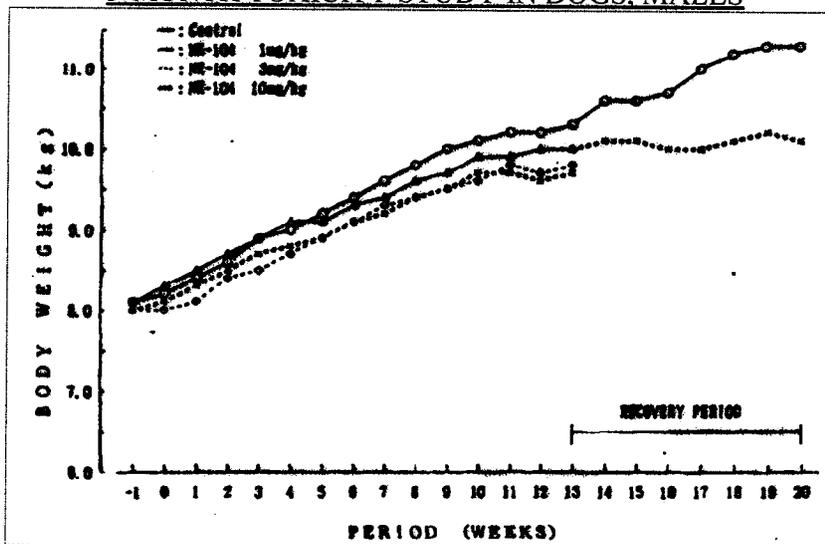
<sup>a</sup> Calculated: (Number of times observation made/potential number of times observation could be made) x 100. <sup>b</sup> Soft stool, bloody stool and/or diarrhea

(Sponsor, original report)

Mortality: One female dog at 10 mg/kg died on day 7. Histopathological changes include congestion of the liver and kidneys, swelling of the adrenal gland, swelling and edema of the pancreas, hemorrhage and edema of the thymus, hyperemia and hemorrhage of the gastrointestinal tract from the stomach to the rectum, swelling and hemorrhage of the mesenteric lymph node, hemorrhage of the submaxillary lymph node, and soil around anus.

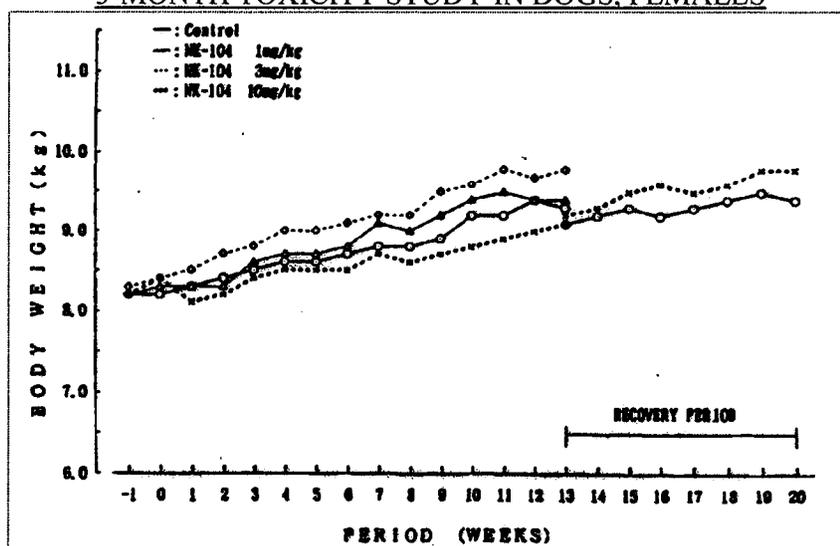
Body weights: Males at 10 mg/kg had a lower body weight than controls.

BODY WEIGHT MEASUREMENTS IN A 3-MONTH TOXICITY STUDY IN DOGS, MALES



(Sponsor, M4, RFG2503, p49)

**BODY WEIGHT MEASUREMENTS IN A  
3-MONTH TOXICITY STUDY IN DOGS, FEMALES**



(Sponsor, M4, RFG2503, p49)

Food and water consumption: No treatment related changes.

Body temperature, pulse rate, and respiration rate: No treatment related changes.

Ophthalmology: Opacity of the lens were observed in 1/3 male and 1/3 female at 3 mg/kg at week 12, 5/5 males and 4/4 females at 10 mg/kg at week 8. This change was progressive through the recovery period.

ECG examination: No apparent treatment related changes.

Recall that in conscious Beagle dogs in study FBM 06-4115, heart rate was slightly but significantly increased after administration of the highest dose, 10 mg/kg dose of NK-104 at 3 and 6 hours post-dose ( $p < 0.05$ ,  $p < 0.01$ ), respectively. All other parameters, including PR, QRS, and QT/QTc intervals measured pre-dose and 0.5, 1, 3, 6, 12, and 24 hours post-dose, were unremarkable. Increased heart rate occurred at 154-fold human exposure to NK-104 at the MRHD of 4 mg/day.

It is unclear how long after dosing the animals that the ECG was performed. Since  $T_{max}$  is  $\leq 2$  hours at 10 mg/kg in dogs.

BSP test: No apparent treatment related changes.

Auditory examination: No apparent treatment related changes.

Urinalysis: One female at 10 mg/kg exhibited a persistent moderate to marked positive result of protein since week 4.

Fecal examination: No apparent treatment related changes.

Hematology: No apparent treatment related changes.

Clinical chemistry: Significant increases in ALT and AST, decreases in triglyceride, total cholesterol, and phospholipid were observed at 10 mg/kg in males and females. These changes were recoverable.

**SERUM BIOCHEMISTRY PARAMETERS IN A  
3-MONTH ORAL TOXICITY STUDY IN DOGS**

Mean Value - Hour	Dose (mg/kg NK-106)							
	Males				Females			
	0	1	3	10	0	1	3	10
<b>SGOT (mU/ml)</b>								
-1	21.6	21.0	22.0	23.2	20.2	19.7	20.3	21.8
4	18.0	23.7*	20.3	29.8**	21.6	22.7	27.7	38.5*
8	19.6	24.0	24.7	30.4**	23.6	23.3	27.7	39.3*
12	20.8	25.0*	22.7	32.6**	24.0	27.7	28.3	40.3**
R2	20.5			21.0	17.0			29.0
R4	20.5			20.0	24.5			31.5
R7	21.0			21.5	20.5			23.5
<b>SGPT (mU/ml)</b>								
-1	29.4	28.0	32.7	29.2	34.4	30.0	35.3	31.0
4	28.0	34.7	36.3	50.4*	34.2	36.7	59.3	65.5
8	28.0	36.0	42.7*	59.2**	34.0	38.3	60.3*	89.3
12	30.2	35.0	41.0*	76.6*	31.4	35.7	59.3*	87.3
R2	29.0			35.0	38.5			46.0
R4	27.5			25.5	32.5			37.5
R7	26.0			32.0	29.0			39.0
<b>CPK (mU/ml)</b>								
-1	200	159	226	240	188	190	167	203
4	160	149	183	206*	144	134	153	225
8	139	149	155	183*	144	127	125	166
12	131	141	130	171**	101	108	103	143*
R2	98			131	89			113
R4	114			122	89			138
R7	103			97	91			110
<b>TRIG (mg/dl)</b>								
-1	43.6	37.0	36.7	35.2	40.2	44.0	34.3	44.8
4	44.2	26.3*	27.3*	19.6**	41.4	43.0	20.0**	24.8**
8	50.4	28.0*	27.0*	23.2**	38.2	31.7	24.3*	24.0*
12	50.4	25.3*	29.0*	24.6**	44.8	48.7	24.7*	42.5
R2	50.5			39.0	46.0			41.0
R4	47.0			38.5	46.0			51.5
R7	48.5			37.5	42.5			44.0
<b>TOT CHOL (mg/dl)</b>								
-1	165	159	161	171	148	155	132	147
4	166	107**	100**	89**	148	128	86**	82**
8	170	107**	105**	84**	149	125*	86**	69**
12	175	111**	109**	85**	171	133**	91**	72**
R2	157			162	173			134
R4	142			160	180			147
R7	152			151	187			141

R=Recovery Week; \* p<0.05 (compared to control group); \*\* p<0.01 (compared to control group)

(Sponsor, original report)

**SERUM BIOCHEMISTRY PARAMETERS IN A  
3-MONTH ORAL TOXICITY STUDY IN DOGS (CONT)**

Selected Serum Biochemistry Parameters (continued)								
Mean Value - Hour	Dose (mg/kg NK-104)							
	Males				Females			
	0	1	3	10	0	1	3	10
PHOS LIP (mg/dl)								
-1	302	278	291	306	259	265	241	264
4	273	176**	165**	141**	258	225	141**	127**
8	288	182**	180**	136**	263	218*	147**	115**
12	307	199**	200**	144**	313	258*	168**	146**
R2	296			285	312			246
R4	281			283	320			266
R7	308			265	306			259

R=Recovery Week; \* p<0.05 (compared to control group); \*\* p<0.01 (compared to control group)

(Sponsor, original report)

Organ weight: Lung weight increased 35% in males at 10 mg/kg. Uterus and ovary weight showed very significant decreases in all treated groups.

**ORGAN WEIGHTS IN A 3-MONTH ORAL TOXICITY STUDY IN DOGS**

Selected Mean Organ Weights													
Organ	Wt (g)	Dose (mg/kg NK-104)											
		Males						Females					
		0	1	3	10	R-0	R-10	0	1	3	10	R-0	R-10
Lungs													
Abs	71.5	72.3	70.2	96.8*	74.0	73.2	68.3	70.5	71.1	79.8	63.9	74.7	
Rel	0.69	0.73	0.72	1.01*	0.66	0.72	0.72	0.75	0.73	0.90	0.69	0.77	
Uterus													
Abs							16.71	7.62	10.26	1.84**	9.5	4.3	
Rel							0.176	0.082	0.108*	0.021**	0.09	0.044	
Ovaries													
Abs							2.94	1.15**	1.45**	0.63**	1.4	.79	
Rel							0.031	0.012**	0.015**	0.007**	0.01	0.006	

R-0 = Recovery Week 7, 0 mg/kg; R-10 = Recovery Week 7, 10 mg/kg; Abs = Absolute; Rel = Relative  
\* p<0.05 (compared to control group); \*\* p<0.01 (compared to control group); NA = Not Available

(Sponsor, original report)

Gross pathology: Opacity of lens was observed in 1/3 female at 3 mg/kg, and 5/5 males and 4/4 females at 10 mg/kg. In females only, there were findings of mammary gland lipogranuloma at 3 mg/kg/day (1/3 females) and 10 mg/kg/day (1/3 females), which was not observed in recovery (0/2).

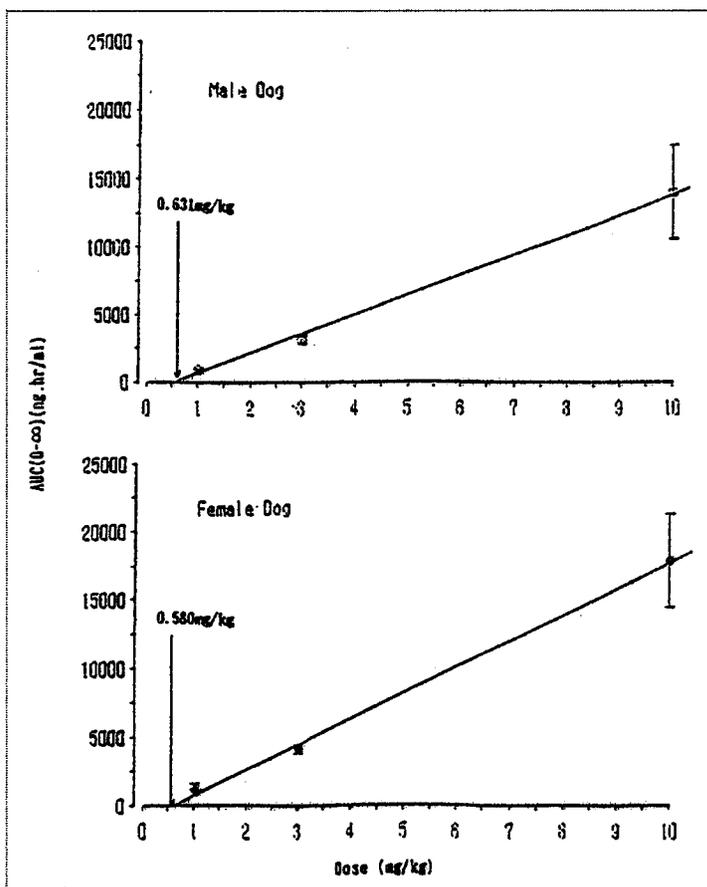
Histopathology: Histopathological changes were observed in lung (inflammation, hemorrhage at ≥ 3 mg/kg), liver (dilation of sinusoid, hemorrhage at 10 mg/kg), gallbladder (hemorrhage at 10 mg/kg), eyeball (degeneration of the lens, edema/degeneration, vacuolation at 10 mg/kg), epididymis (spermatic granuloma at 10 mg/kg), GI tract (hemorrhage at 10 mg/kg), and heart (eosinophilic degeneration and swelling of myocardium).

3 Month Oral Toxicology Study in Dogs – Histopathology									
Dose (mg/kg)		Male				Female			
		0	1	3	10	0	1	3	10
N		5	3	3	5	5	3	3	5
Lung	Foci of foam cells with inflammatory cell infiltration	-	-	1(1)*	2(3) 1(4)	-	-	2(1)	1(1) 1(2) 1(3)
	Focal suppurative pneumonia	-	-	-	-	-	-	-	1(4)
	congestion	-	-	-	-	-	-	-	1(4)
	Pericanal fresh hemorrhage and edema	-	-	-	-	-	-	-	1(4)
Liver	Centrilobular dilation of sinusoid	-	-	-	2(1) 1(2)	-	-	-	-
	Congestion	-	-	-	-	-	-	-	1(4)
	Diffuse dilatation of sinusoid and atrophy of hepatocytes	-	-	-	-	-	-	-	1(4)
	Fresh hemorrhage in the junction of the liver and gallbladder	-	-	-	-	-	-	-	1(4)
Gallbladder	Fresh hemorrhage in gallbladder wall	-	-	-	-	-	-	-	1(4)
Popliteal lymph node	Erythrocyte-phagocytes and hemorrhage in sinuses	1(1)	1(1) 1(2)	1(2)	2(1) 1(4)	3(1)	2(1)	1(1) 1(3)	2(1) 1(2)
Eyeball	Degeneration of the lens fiber	-	-	1(1)	2(2) 2(4)	-	-	-	1(2) 1(3) 1(4)
	Edema in interstitium accompanied with degeneration of the ciliary epidermis	-	0	0	1(3) 2(4)	-	-	-	1(1) 1(2) 1(3)
	Adhesion of the ciliary body and lens	-	0	0	1(1) 1(2)	-	-	-	1(2) 1(4)
	Vacuolation of the outer plexiform layer of the retina	-	-	0	2(1)	-	-	-	-
Epididymis	Unilateral spermatic granuloma	-	-	-	2(4)	-	-	-	-
Prostate	Cystic dilatation of the gland	1(1)	2(2)	0	1(2) 1(4)				
Pancreas	Edema in interstitium accompanied with congestion	-	-	-	-	-	-	-	1(4)
Stomach	Fresh hemorrhage in submucosa	-	-	-	-	-	-	-	1(2)
Duodenum	Small hemorrhagic foci in muscle layer	-	-	-	-	-	-	-	1(2)
	Hemorrhage in interstitium and congestion at an opening region of the bile duct	-	-	-	-	-	-	-	1(4)
	Exfoliation of cholionic epithelium	-	-	-	-	-	-	-	1(4)
Thymus	Congestion and edema in interstitium	-	-	-	-	-	-	-	1(4)
Adrenal gland	congestion	-	-	-	-	-	-	-	1(4)
Heart	Eosinophilic degeneration and swelling of myocardium	-	-	-	-	-	-	-	1(2)

Values in () represent severity of the findings. 1: slight, 2: mild, 3: moderate, 4: severe

**AG25001 - Three-month repeated oral dose toxicokinetic study of NK-104 in dogs [oral capsules]**

Kinetics were linear in male and female dogs on Day 1 of a 90-day toxicity study, where dogs were administered 1, 3 and 10 mg/kg NK-104 by oral capsule.



(Sponsor, M4, AG25001, p23)

$C_{max}$  tended to be lower over time upon repeated administration of NK-104 at 1, 3, and 10 mg/kg.  $AUC_{0-\infty}$  tended to increase after 35 and 90 days compared to Day 1 in males and females at each dose of NK-104, while  $T_{max}$  trended higher in females with repeated administration, but not in males. Total NK-104 exposure in females ( $AUC_{0-\infty}$ ) was higher than total exposure in males at each dose.

Toxicokinetics of NK-104 during Daily Administration of NK-104 to Dogs for 90 Days by Oral Capsule									
Dose (mg/kg)	Day	$C_{max}$		$AUC_{0-\infty}$		$T_{1/2}$		$T_{max}$	
		♂	♀	♂	♀	♂	♀	♂	♀
1	1	246	344	891	1141	3.9	2.7	1.7	1.0
	35	328	359	ND	ND	ND	ND	1.0	1.7
	90	239	302	ND	ND	ND	ND	1.7	1.7
3	1	1477	1671	3130	4196	2.8	3.1	1.0	1.0
	35	809	1212	ND	ND	ND	ND	1.7	1.0
	90	1346	1058	ND	ND	ND	ND	1.0	1.7
10	1	5179	8686	14185	18117	2.3	2.8	1.6	1.0
	35	5383	7027	ND	ND	ND	ND	1.2	1.0
	90	7637	4931	ND	ND	ND	ND	1.4	1.3

ND, not determined

Total exposure ( $AUC_{0-\infty}$ ) to NK-104 lactone was approximately 25-30% of total exposure to parent NK-104 in male dogs and 25-35% in female dogs. Maximum NK-104 lactone concentrations ( $C_{max}$ ) were approximately 12-19% of parent NK-104  $C_{max}$  in male dogs and 8-21% in female dogs.

Toxicokinetics of NK-104 Lactone during Daily Administration of NK-104 to Dogs for 90 Days by Oral Capsule									
Dose (mg/kg)	Day	$C_{max}$		$AUC_{0-\infty}$		$T_{1/2}$		$T_{max}$	
		♂	♀	♂	♀	♂	♀	♂	♀
1	1	38	51	263	406	3.9	4.0	3.3	1.3
	35	60	61	ND	ND	ND	ND	2.7	2.7
	90	38	53	ND	ND	ND	ND	4.0	2.7
3	1	171	208	922	1330	3.2	3.3	1.7	1.7
	35	151	224	ND	ND	ND	ND	1.7	2.0
	90	191	219	ND	ND	ND	ND	2.0	2.7
10	1	610	660	3515	4360	3.1	3.6	2.4	2.2
	35	824	771	ND	ND	ND	ND	3.2	2.5
	90	960	641	ND	ND	ND	ND	3.6	3.0

"nd" indicates not determined

**RFG2504 – Twelve-month consecutive oral toxicity study of NK-104 in dogs [in gelatin capsules, with a 9-week recovery period]**

**Key Study Findings:**

NK-104 was orally administered to dogs at doses of 0.3, 1 and 3 mg/kg for 12 months and allowed to recover for 2 months

- 3 mg/kg: opacity of the lens in 5/6 males and 6/6 females, increases in ALT, increased uterus weight, aggregated foci of foam cells and inflammatory cells in lung in both sexes, interstitial edema accompanied by degeneration of the ciliary epithelium in 3/6 males and 1/6 female, degeneration of the fibers in the lens of the eye in 4/6 males and 6/6 females. There was also a qualitative increase in protein in the urine.
- 1 mg/kg: opacity of lens (minute) in 1/4 male, increased uterine weight without correlating lesion.
- 0.3 mg/kg: no apparent treatment related changes.
- NOAEL was 0.3 mg/kg, due lens opacity (males) (females) at 1 mg/kg/day (this dose represents an exposure multiple of ~2.8 based on the AUC at the 4 mg human dose).

**Amendment #, Vol #, and Page #:** M4, RFG2504

**Conducting laboratory and location:** Fuji Research Labs, Kowa Co., Shizuoka, Japan

**Date of study initiation:** April, 1993

**GLP Compliance:** Yes

**QA report:** Yes

**Methods:** chronic oral (capsule) toxicology study

**Dosing:**

- Species/strain: Beagle dogs
- #/sex/group or time point:

12 Month Oral Toxicology Study in Dogs – Group Assignments					
Group	Dose mg/kg	12-month main		2-month recovery	
		M	F	M	F
Vehicle	0	4	4	2	2
LD	0.3	4	4	-	-
MD	1	4	4	-	-
HD	3	4	4	2	2

- Age: 5 months
- Body weight: male: 8.0-9.7 kg, females: 7.4-9.4 kg
- Route: oral gavage

**Drug, lot number, and purity:** 104P-9202, 99.2% purity

**Formulation/vehicle:** lactose in gelatin capsules

**Observations and Times:**

<b>Endpoints</b>	<b>Time of observation</b>
<b>Clinical signs</b>	Daily
<b>Body temperature, pulse rate, respiratory rate</b>	Day 0, months 1, 3, 6, 9, and 12 of dosing and months 1 and 2 of recovery period (prior, 1 hr post dosing).
<b>Body weights</b>	Pre-dose, weekly
<b>Food consumption</b>	Pre-dose, weekly
<b>Water Consumption</b>	Pre-dose, weekly
<b>Ophthalmoscopy</b>	Pre-dose, months 1, 3, 6, 9, and 12 of dosing and months 1 and 2 of recovery period.
<b>Auditory examination</b>	Pre-dose, months 1, 3, 6, 9, and 12 of dosing and months 1 and 2 of recovery period.
<b>Electrocardiography</b>	Pre-dose, months 1, 3, 6, 9, and 12 of dosing and months 1 and 2 of recovery period. Timing unspecified.
<b>Male sexual function test</b>	All male dogs at the control and 3 mg/kg groups were tested for the volume and number of sperm in the seminal fluid at month 6 and 12 of dosing period and month 2 of the recovery period.
<b>Hepatic function test (BSP test)</b>	Pre-dose, months 1, 3, 6, 9, and 12 of dosing and months 1 and 2 of recovery period. Blood retention rate of BSP was determined.
<b>Hematology</b>	Pre-dose, months 1, 3, 6, 9, and 12 of dosing and months 1 and 2 of recovery period. RBC, WBC, platelet, hematocrit, Hb, MCV, MCH, MCHC, reticulocyte, leukocyte classification, PT, APTT, fibrinogen were examined.
<b>Clinical biochemistry</b>	Pre-dose, months 1, 3, 6, 9, and 12 of dosing and months 1 and 2 of recovery period. Glucose, total protein, albumin, A/G Ratio, BUN, creatinine, Ca, ALT, AST, LDH, CPK, ALP, GGT, cholinesterase activity, triglyceride, total cholesterol, phospholipid, Na, K, Cl were examined.
<b>Urinalysis</b>	Pre-dose, months 1, 3, 6, 9, and 12 of dosing and months 1 and 2 of recovery period. pH, protein, glucose, ketone bodies, occult blood, urobilinogen, bilirubin, and sediment were examined.
<b>Gross pathology</b>	At sacrifice
<b>Organ weight</b>	At sacrifice
<b>Histopathology</b>	At sacrifice.
<b>Toxicokinetics</b>	Separate study report included.

**Results:**

**Clinical signs:** Higher incidence of abnormal stool (soft stool, blood stool and/or diarrhea) was observed in all treated groups in males (0.8, 4.7, 1.5, and 4.9% for 0, 0.3, 1, and 3 mg/kg group, respectively), however, lower incidence was observed in females (2.6, 1.9, 0.4 and 1.0% for 0, 0.3, 1, and 3 mg/kg group, respectively). No apparent difference was noted in the recovery period.

Mortality: No mortality occurred during the conduct of this study prior to terminal sacrifice.

Body weights: No apparent treatment related changes,

Food and water consumption: No apparent treatment related changes.

Body temperature, pulse rate, and respiration rate: No treatment related changes.

Ophthalmology: Opacity of the lens were observed in 1/4 male at 1 mg/kg at month 12, 5/6 males and 6/6 females at 3 mg/kg. The change in 3 mg/kg was still observed during the recovery period.

ECG examination: No apparent treatment related changes.

Recall that in conscious Beagle dogs in study FBM 06-4115, heart rate was slightly but significantly increased after administration of the highest dose, 10 mg/kg dose of NK-104 at 3 and 6 hours post-dose ( $p < 0.05$ ,  $p < 0.01$ ), respectively. All other parameters, including PR, QRS, and QT/QTc intervals measured pre-dose and 0.5, 1, 3, 6, 12, and 24 hours post-dose, were unremarkable. Increased heart rate occurred at 154-fold human exposure to NK-104 at the MRHD of 4 mg/day.

Hepatic function test (BSP test): No apparent treatment-related changes.

Auditory examination: No apparent treatment-related changes.

Male sexual function test: No apparent treatment-related changes in semen volume or sperm count.

Urinalysis: There was a qualitative dose-related increase in protein in the urine of high-dose females, which was apparent at Months 3, 6 and 12. Increased protein in the urine was absent during the recovery period. There were no other apparent treatment-related changes of biological significance. No instances of protein in the urine were described as severe.

**Hematology:** In males, there were dose-related percent increases in segmented neutrophils and percent decreases in lymphocytes at Month 1. Percent lymphocytes appeared to be decreased at Month 6, as well, but showed questionable dose-relatedness. In females, there were dose-related percent increases in segmented neutrophils at Month 3 and a decrease in stable neutrophils was noted at Month 9 ( $p < 0.05$  at the high dose). In females, decreased prothrombin time noted at Months 6, 12, and at the end of the recovery period. While statistically significant at the high dose ( $p < 0.05$ ), these changes were unlikely to be of biological importance. All other findings were either not dose-related, noted only at a single time-point, and/or were unlikely to be biologically significant. There were no other significant findings observed during recovery.

<b>12 Month Oral Toxicology Study in Dogs – Hematology</b>									
Dose(mg/kg)		Male				Female			
		0	0.3	1	3	0	0.3	1	3
Time (Mo)	N	6	4	4	6	6	4	4	6
<b>1</b>	Neutrophils segmented (%)	53	58	59	<b>61**</b>	55	58	57	55
	Lymphocytes (%)	36	31	32	<b>28*</b>	36	33	33	37
<b>3</b>	Prothrombin time (sec)	6.7	6.7	6.6	6.5	<b>6.7</b>	<b>6.5*</b>	6.7	6.4
<b>6</b>	Neutrophils segmented (%)	55	60	<b>64**</b>	61	<b>60</b>	<b>60</b>	<b>58</b>	<b>56*</b>
	Lymphocytes (%)	34	29	<b>27**</b>	30	29	33	34	34
<b>9</b>	Neutrophils stable (%)	0.8	1.1	0.8	0.6	<b>0.9</b>	<b>0.6</b>	<b>0.9</b>	<b>0.5*</b>
<b>12</b>	Neutrophils segmented (%)	53	<b>65**</b>	<b>62*</b>	59	57	56	54	55
	Prothrombin time (sec)	6.9	6.8	6.6	6.7	<b>7.3</b>	<b>7.1</b>	<b>7.4</b>	<b>7.0*</b>
<b>R1</b>	N/A	-	-	-	-	-	-	-	-
<b>R2</b>	Prothrombin time (sec)	-	-	-	-	<b>7.3</b>	-	-	<b>6.5*</b>

Findings that appeared to be dose-related are in bold

**Clinical chemistry:** Increases in ALT were noted at 3 mg/kg in both sexes. Triglyceride, total cholesterol, and phospholipid significantly decreased in males during dosing period, but, returned to control level during recovery period.

12 Month Oral Toxicology Study in Dogs – Clinical Chemistry (>20% different from control)							
Dose(mg/kg)	N	Male			Female		
		0.3	1	3	0.3	1	3
		4	4	6	4	4	6
Alpha-2-globulin	0	-	-	-	-	-	-
	1	-	-	-	↑*	-	↑*
	3	-	-	-	-	-	↑*
	6	-	-	-	-	-	↑*
	9	-	-	-	↑*	↑	↑*
	12	-	-	-	↑	↑	↑**
	R1			-			↑
	R2			-			↑
Lactate dehydrogenase	0	↑*	-	↑	↓	-	-
	1	-	-	-	↑	-	-
	3	↑	↑	↑*	-	-	-
	6	↑	-	↑*	-	-	-
	9	-	-	↑	-	-	-
	12	-	-	-	-	-	-
	R1			-			↓
	R2			↑			-
Aspartate transaminase	0	-	-	-	-	-	-
	1	-	-	↑	-	-	-
	3	-	-	↑**	-	-	-
	6	-	-	↑*	-	-	~↑*
	9	-	-	↑*	-	-	↑
	12	-	-	↑*	-	-	-
	R1			-			-
	R2			-			-
Alanine transaminase	0	-	-	-	-	-	-
	1	-	-	↑**	-	-	↑
	3	-	-	↑	↑	↑	↑**
	6	-	-	↑	-	-	↑*
	9	-	-	↑	-	-	↑
	12	-	-	↑	-	-	↑
	R1			↑**			-
	R2			↑			-
Alkaline phosphatase	0	-	-	-	-	-	-
	1	-	-	-	-	-	-
	3	-	-	↑	-	-	-
	6	-	-	↑	-	-	-
	9	-	-	↑	-	↑	↑
	12	-	-	↑	-	-	-
	R1			↑			-
	R2			↑			-
Creatinine	0	-	-	-	-	-	-
	1	-	-	-	-	-	~↑**
	3	-	-	-	-	-	-
	6	-	-	-	-	~↑*	~↑*
	9	-	-	-	-	~↑	-
	12	-	-	-	-	-	~↑*
	R1			-			-
	R2			-			-

\* indicates a statistically significant difference from controls, p<0.05

~ indicates <20% difference

Organ weights: The following findings were notable (n.s.s. but >20% different from controls): increased uterus weight at  $\geq 1$  mg/kg, increased spleen weight in males at 3 mg/kg/day, and decreased thyroid weight in males. These three findings did not appear recoverable at 13 weeks.

There were statistically significant increases in kidney weights in males and decreases in adrenal weights in females at 3 mg/kg/day ( $p < 0.05$ ). There was also a statistically significant decrease in epididymal weight at  $\geq 1$  mg/kg/day ( $p < 0.05$ ). These three findings were recovered at 13 weeks.

Increased prostate weight was observed in recovery animals at 3 mg/kg/day (>20%).

26 Week Oral Toxicity Study in Dogs – Organ Weights									
Dose (mg/kg)		Male				Female			
		0	0.3	1	3	0	0.3	1	3
Spleen	52wk	26.2	27.3	25.4	<u>35.1</u>	27.1	23.5	23.7	29.2
	Rel.	0.24	0.24	0.25	<u>0.31</u>	0.26	0.21	0.23	0.28
	R:9wk	24.0			<u>30.2</u>	30.8			<u>24.5</u>
	Rel.	0.22			<u>0.27</u>	0.30			<u>0.22</u>
Kidneys	52wk	48.9	53.9	53.7	58.4*	45.2	41.6	44.6	41.3
	Rel.	0.46	0.48	0.52	0.51	0.42	0.38	0.44	0.40
	R:9wk	55.0			50.9	43.6			46.4
	Rel.	0.50			0.47	0.43			0.43
Prostate	52wk	6.02	6.96	6.99	5.29				
	Rel.	0.06	0.06	<u>0.07</u>	0.05				
	R:9wk	6.32			<u>8.58</u>				
	Rel.	0.06			<u>0.08</u>				
Thyroids	52wk	0.9	0.8	0.8	<u>0.7</u>	0.8	0.7	0.7	0.7
	Rel.	0.008	0.007	0.008	<u>0.006</u>	0.007	0.006	0.007	0.007
	R:9wk	0.8			0.8	0.8			<u>0.6</u>
	Rel.	0.007			0.007	0.008			<u>0.006</u>
Uterus	52wk					6.2	<u>3.6</u>	<u>9.3</u>	<u>8.7</u>
	Rel.					0.059	<u>0.033</u>	<u>0.090</u>	<u>0.084</u>
	R:9wk					5.0			<u>10.9</u>
	Rel.					0.05			<u>0.10</u>
Adrenal glands	52wk	1.2	1.1	1.1	1.2	1.3	1.4	1.3	1.1*
	Rel.	0.011	0.010	0.011	0.011	0.012	0.011	0.012	0.011
	R:9wk	1.3			1.1	1.3			1.3
	Rel.	0.012			0.010	0.013			0.012
Epididymes	52wk	3.6	3.7	3.1	3.2				
	Rel.	0.033	0.031	0.031	0.028*				
	R:9wk	3.5			3.5				
	Rel.	0.032			0.032				

\*indicates statistically significant difference from control,  $p < 0.05$

underline indicates a >20% mean difference from control

Gross pathology:

White opacity of the lens were observed in 4/6 males and 6/6 females at 3 mg/kg. Atrophy of the lens was observed in 3/6 males and 5/6 females at 3 mg/kg.

Histopathology:

Notably there was no liver, kidney, stomach, thyroid, or skeletal muscle toxicity.

Administration of 3 mg/kg/day NK-104 produced aggregated foci of foam cells in lungs of both sexes; oral administration was by capsule, thus no possibilities of gavage-related findings. In the eyeball, interstitial edema accompanied by degeneration of the ciliary epithelium were observed in 3/6 males and 1/6 female at 3 mg/kg, degeneration of the fibers in the lens of the eye was observed in 4/6 males and 6/6 females at 3 mg/kg.

At 1 mg/kg/day, there was opacity of lens (minute) in 1/4 male, and increased uterus weight without a correlative lesion, while administration of 0.3 mg/kg was not associated with any treatment related changes.

The NOAEL was 0.3 mg/kg (this dose represents an exposure multiple of ~2.8 based on the AUC at the 4 mg human dose).

12 Month Oral Toxicology Study in Dogs – Histopathology (Main study)									
Dose(mg/kg)		Male				Female			
		0	0.3	1	3	0	0.3	1	3
N		4	4	4	4	4	4	4	4
Lung	Aggregated foci of foam cells and inflammatory cells	-	-	-	2(2)	-	-	-	3(2)
Duodenum	Dilation of tubular cavity	-	-	-	1(2)	-	-	-	-
Parathyroid gland	Cyst	1(3)	-	-	3(2)	-	1(1)	1(1)	-
Eyeball	Degeneration of the lens fiber	-	-	-	2(2) 1(3)	-	-	-	1(1) 2(2) 3(3)
	Interstitial edema accompanied with degeneration of the ciliary epithelium	-	-	-	2(1) 1(2)	-	1(1)	-	1(3)
Uterus	Hyperplasia of endometrium	-	-	-	-	-	-	1(2)	1(2)
Spleen	Congestion	1(2)	-	-	-	-	-	1(1)	1(3)
Kidney	Fibroid lymphangioma	-	-	-	-	-	-	-	1(2)
	Vacuolar degeneration of renal tubular epithelium	2(2)	1(2)	-	2(2)	3(2)	4(2)	1(1) 3(2)	4(2)
	Foam cell aggregation in glomerulus	1(2)	-	-	-	-	1(2)	-	-
	Calcium deposition of the collecting tubule	3(1)	1(1)	2(1)	2(1)	1(1)	2(1)	-	1(1)
Heart	Ectopic thyroid gland	-	-	-	-	-	-	-	1(2)
Cerebrum	Calcium deposition in vascular wall	-	-	-	-	-	-	-	1(2)
Skeletal muscle	Any finding	-	-	-	-	-	-	-	-
Thyroid	Round cell infiltration	1(3)	-	1(1)	-	-	1(2)	-	-
	Hyperplasia of C cells	1(1)	1(1)	-	-	1(1) 1(2)	1(1)	-	1(2)
Liver	Small Granuloma	-	-	-	-	-	1(2)	-	-
Stomach	Hyperplasia of lymphatic follicles	-	-	-	-	1(2)	-	-	-

Values in () represent severity of the findings. 1: slight, 2: mild, 3: moderate, 4: severe

12 Month Oral Toxicology Study in Dogs – Histopathology (Recovery animals)					
Dose(mg/kg)		Male		Female	
		0	3	0	3
		N	2	2	2
Lung	Aggregated foci of foam cells and inflammatory cells	-	-	-	-
Duodenum	Dilation of tubular cavity	-	1(1)	-	-
Parathyroid gland	Cyst	-	1(2)	1(2)	-
Eyeball	Degeneration of the lens fiber	-	1(3)	-	1(2) 1(3)
	Interstitial edema accompanied with degeneration of the ciliary epithelium	-	1(1)	-	-
Uterus	Hyperplasia of endometrium	-	-	-	-
Spleen	Congestion	-	1(2)	-	-
Kidney	Fibroid lymphangioma	-	-	-	-
	Vacuolar degeneration of renal tubular epithelium	-	-	1(2)	2(2)
	Foam cell aggregation in glomerulus	-	1(2)	-	-
	Calcium deposition of the collecting tubule	1(2)	2(1)	2(1)	2(1)
Heart	Ectopic thyroid gland	-	-	-	-
Cerebrum	Calcium deposition in vascular wall	-	-	-	-
Skeletal muscle	Any finding	-	-	-	-
Thyroid	Round cell infiltration	-	1(2)	-	-
	Hyperplasia of C cells	1(2)	-	-	-
Liver	Small Granuloma	-	-	-	-
Stomach	Hyperplasia of lymphatic follicles	-	-	-	-

Values in ( ) represent severity of the findings. 1: slight, 2: mild, 3: moderate, 4: severe

**AG25004 - Measurement of plasma and tissue concentrations of NK-104 in a 12-month repeated oral dose toxicity study of NK-104 in dogs**

AUC for NK-104 and NK-104 lactone increased in a slightly higher than dose-related manner for males administered 0.3, 1.0, and 3.0 mg/kg on Day 1 and Month 12. AUC for NK-104 and NK-104 lactone increased in a linear, dose-dependent manner for females administered 0.3, 1.0, and 3.0 mg/kg on Day 1 and Month 12. There was no evidence of accumulation of either NK-104 or NK-104 lactone in either sex of dogs over 1 year of daily administration of NK-104 by oral gavage.

**PLASMA TOXICOKINETICS OF NK-104 AND NK-104 LACTONE AFTER DAILY ADMINISTRATION OF 0.3, 1.0, AND 3.0 MG/KG NK-104 TO MALE AND FEMALE DOGS BY ORAL GAVAGE FOR ONE YEAR**

Sex	Drug	Sampling period	Mean pharmacokinetic parameter						Relative value <sup>a</sup>							
			0.3 mg/kg		1 mg/kg		3 mg/kg		0.3 mg/kg		1 mg/kg		3 mg/kg			
			C <sub>max</sub> (ng/ml)	AUC (ng.hr/ml)	C <sub>max</sub> (ng/ml)	AUC (ng.hr/ml)	C <sub>max</sub> (ug/ml)	AUC (ug.hr/ml)	C <sub>max</sub>	AUC (t)	C <sub>max</sub>	AUC (t)	C <sub>max</sub>	AUC (t)		
Male	NK-104	Day-1	78.1 (19.8)	280 (56)	493.4 (99.2)	1328 (142)	2.88 (0.44)	7.07 (0.92)	1.0	1.0	1.0	1.0	1.0	1.0		
		Month-3	93.7 (9.3)	313 (12)	295.1 (61.5)	907 (156)	1.80 (0.48)	5.02 (0.99)	1.2	1.1	0.6	0.7	0.6	0.7		
		Month-6	103.8 (17.8)	433 (33)	590.0 (203.6)	2001 (310)	3.01 (0.87)	9.29 (1.41)	1.3	1.6	1.2	1.5	1.0	1.3		
		Month-9	51.4 (6.7)	252 (28)	326.5 (28.8)	1143 (74)	1.21 (0.13)	4.12 (0.39)	0.7	0.9	0.7	0.9	0.4	0.6		
		Month-12	107.3 (30.2)	384 (83)	382.2 (9.2)	1360 (33)	2.89 (0.56)	7.75 (1.20)	1.4	1.4	0.8	1.0	1.0	1.1		
	Lactone	Day-1	10.8 (1.7)	78 (11)	50.8 (7.7)	271 (22)	0.25 (0.02)	1.35 (0.14)	1.0	1.0	1.0	1.0	1.0	1.0		
		Month-3	18.5 (1.1)	125 (8)	53.8 (5.7)	318 (21)	0.34 (0.05)	2.02 (0.27)	1.7	1.6	1.1	1.2	1.4	1.5		
		Month-6	18.6 (2.2)	139 (13)	86.2 (18.0)	558 (65)	0.32 (0.04)	2.19 (0.32)	1.7	1.8	1.7	2.1	1.3	1.6		
		Month-9	13.3 (0.3)	91 (5)	59.0 (5.3)	381 (30)	0.32 (0.03)	1.71 (0.15)	1.2	1.2	1.2	1.4	1.3	1.3		
		Month-12	16.0 (4.3)	105 (24)	58.5 (6.2)	341 (40)	0.31 (0.04)	1.70 (0.22)	1.5	1.3	1.2	1.3	1.2	1.3		
		Female	NK-104	Day-1	113.1 (12.0)	421 (40)	545.5 (50.3)	1463 (68)	2.19 (0.44)	5.35 (0.82)	1.0	1.0	1.0	1.0	1.0	1.0
				Month-3	75.5 (27.7)	249 (73)	402.4 (60.7)	1294 (182)	1.63 (0.43)	3.68 (0.90)	0.7	0.6	0.7	0.9	0.7	0.7
Month-6	75.5 (34.6)			309 (82)	545.5 (163.3)	1880 (434)	1.71 (0.27)	4.91 (0.44)	0.7	0.7	1.0	1.3	0.8	0.9		
Month-9	43.7 (16.2)			195 (68)	501.5 (87.0)	1477 (105)	1.15 (0.11)	3.60 (0.23)	0.4	0.5	0.9	1.0	0.5	0.7		
Month-12	60.4 (17.9)			288 (89)	324.5 (85.2)	1363 (244)	1.83 (0.49)	5.34 (1.31)	0.5	0.7	0.6	0.9	0.8	1.0		
Lactone	Day-1		13.5 (1.2)	87 (11)	53.8 (6.3)	331 (39)	0.24 (0.04)	1.28 (0.20)	1.0	1.0	1.0	1.0	1.0	1.0		
	Month-3		11.1 (3.3)	73 (22)	85.9 (22.8)	515 (109)	0.22 (0.04)	1.27 (0.29)	0.8	0.8	1.6	1.6	0.9	1.0		
	Month-6		13.1 (2.2)	99 (19)	86.8 (21.4)	586 (139)	0.20 (0.02)	1.32 (0.20)	1.0	1.1	1.6	1.8	0.8	1.0		
	Month-9		8.8 (1.2)	53 (12)	77.8 (12.0)	492 (63)	0.23 (0.03)	1.36 (0.17)	0.7	0.6	1.4	1.5	1.0	1.1		
	Month-12		10.6 (2.3)	70 (14)	78.6 (22.1)	468 (107)	0.21 (0.04)	1.15 (0.22)	0.8	0.8	1.5	1.4	0.9	0.9		

a. The value obtained in day-1 was taken as 1. Parenthesis represents the S.E. of 4-6 dogs

(Sponsor, M4, AG25004, p29)

NK-104 and NK-104 lactone were detected in the lens of dogs in the 3 mg/kg group, but NK-104 was below the lower limit of detection at lower doses. The percentage of NK-104 lactone in lens (0.6%) compared to parent NK-104 was approximately 18-fold lower (by C<sub>max</sub>) than the percent of lactone as a percent of parent NK-104 in dog plasma (approximately 11%) at the end of 12 months.

**EYE TISSUE TOXICOKINETICS OF NK-104 AND NK-104 LACTONE AFTER DAILY ADMINISTRATION OF 0.3, 1.0, AND 3.0 MG/KG NK-104 TO MALE AND FEMALE DOGS BY ORAL GAVAGE FOR ONE YEAR**

Compound	Sex	No.	Conc. of eye tissue (ng/ml or g)											
			Group of 0.3 mg/kg			Group of 1 mg/kg			Group of 3 mg/kg					
			Aqueous	Lens	Vitreume	Aqueous	Lens	Vitreume	Aqueous	Lens	Vitreume			
NK-104	Male	1	n.d.	32.1	n.d.	1	n.d.	119.9	n.d.	1	0.54	122.5	n.d.	
		2	n.d.	42.8	n.d.	2	n.d.	183.1	n.d.	2	1.35	479.3	1.01	
		3	n.d.	36.3	n.d.	3	n.d.	135.8	n.d.	5	0.47	516.5	n.d.	
		4	n.d.	50.3	n.d.	4	n.d.	163.1	n.d.	6	0.76	437.8	1.59	
		Mean	-	0.00	40.4	0.00	-	0.00	150.5	0.00	-	0.78	389.0	0.65
	Female	1	0.33	22.3	n.d.	1	n.d.	146.6	0.48	2	0.86	150.3	1.17	
		2	n.d.	23.5	n.d.	2	n.d.	196.0	n.d.	3	0.44	205.8	6.61	
		3	n.d.	30.5	n.d.	3	0.22	131.6	n.d.	5	0.61	356.4	0.42	
		4	n.d.	25.0	n.d.	4	n.d.	121.5	n.d.	6	0.64	454.2	0.53	
		Mean	-	0.08	25.3	0.00	-	0.06	148.9	0.12	-	0.64	291.7	2.18
		S.E.	-	0.08	1.8	0.00	-	0.06	16.5	0.12	-	0.09	69.5	1.49
		NK-104 lactone	Male	1	n.d.	n.d.	n.d.	1	n.d.	n.d.	n.d.	1	n.d.	0.7
2	n.d.			n.d.	n.d.	2	n.d.	n.d.	n.d.	2	n.d.	4.7	n.d.	
3	n.d.			n.d.	n.d.	3	n.d.	n.d.	n.d.	5	n.d.	2.2	n.d.	
4	n.d.			n.d.	n.d.	4	n.d.	n.d.	n.d.	6	n.d.	1.9	n.d.	
Mean	-			0.00	0.0	0.00	-	0.00	0.0	0.00	-	0.00	2.4	0.00
Female	1		n.d.	n.d.	n.d.	1	n.d.	n.d.	n.d.	2	n.d.	n.d.	n.d.	
	2		n.d.	n.d.	n.d.	2	n.d.	0.7	n.d.	3	n.d.	4.2	n.d.	
	3		n.d.	n.d.	n.d.	3	n.d.	n.d.	n.d.	5	n.d.	2.8	n.d.	
	4		n.d.	n.d.	n.d.	4	n.d.	n.d.	n.d.	6	n.d.	n.d.	n.d.	
	Mean		-	0.00	0.0	0.00	-	0.00	0.2	0.00	-	0.00	1.8	0.00
	S.E.		-	0.00	0.0	0.00	-	0.00	0.2	0.00	-	0.00	1.1	0.00

(Sponsor, M4, AG25004, p30)

**387/001 - NK-104 – 2 week intravenous toxicity study in the beagle dog**

- No lens opacity was observed in dogs administered 0.5, 1, and 2 mg/kg/day by the intravenous route
- Total cholesterol was decreased at all doses
- The NOAEL was determined to be >2 mg/kg/day by intravenous administration due to no adverse and/or drug-related effects at that dose.

**SBL17-34 – Four week consecutive oral toxicity study of NK-104 in cynomolgus monkeys**

- In a one month monkey study where pitavastatin was administered daily by oral gavage, there were tubular lesions in kidney and signs of liver toxicity at 3 mg/kg/day and higher.
- A NOAEL was not identified in the one month study (<3 mg/kg/day).
- Decreased food consumption and decreased body weight in females administered 15 mg/kg/day.
- There were lung findings(alveolus, foamy cells) in animals of both sexes at the 15 mg/kg/day
- Ileum, cecum, colon, rectum showed epithelial damage in females administered 15 mg/kg/day.
- There was thymic atrophy in both sexes at the 15 mg/kg/day dose.
- Stomach erosion, hyperplasia in pyloric part was observed at the 15 mg/kg/day level.
- Kidney, tubular epithelial desquamation, swelling, hyaline cast, hyaline droplets, mononuclear cell infiltration, was noted at 7 mg/kg/day and above in both sexes; necrosis, regeneration was noted at all doses in both sexes (3 to 15 mg/kg/day)
- There were findings in lacrimal gland, interstitial mononuclear cell infiltration at all doses in males (3 to 15 mg/kg/day).
- Heart mononuclear cell infiltration was observed at 7 and 15 mg/kg/day in females only.
- Spleen, atrophy of white pulp was seen in females administered 7 and 15 mg/kg/day.
- Pancreas, acinar cell atrophy in females at 7 and 15 mg/kg/day.
- Liver, hepatocyte, vacuole, swelling, neutrophilia, ↓glycogen in high-dose, 15 mg/kg/day females; hepatocyte, granuloma in males at 7 and 15 mg/kg/day.
- Adrenal hyperplasia, decreased fat in cortex (MD/HD females)
- Skeletal muscle atrophy and submandibular gland, interstitial mononuclear cell infiltration, and tonsil atrophy were present in females administered 15 mg/kg/day.
- NOAEL <3mkg

Pivotal study already reviewed in detail.

**RFG2514 – Plasma drug levels in cynomolgus monkeys in a four-week repeated dose toxicity study of NK-104**

Monkeys in 2/sex/group were given oral doses of NK-104 for 4 weeks and plasma concentrations of parent and its lactone were measure. No accumulation was observed after repeated dosing.

**Table II. Plasma concentration and toxicokinetics parameter of NK-104 and its lactone form after oral administration of NK-104 to monkeys at the end of 29th day**

No.	Dose	Sex	Plasma Concentration (mg/ml)							T <sub>max</sub> (h)	C <sub>max</sub> (mg/ml)	AUC (mg.h/ml)	T <sub>1/2</sub> (h)
			Time (h)										
			0	0.5	1	2	4	8	24				
<b>NK-104</b>													
5	3	Male	0.003	0.009	0.156	0.049	0.030	0.022	0.006	1.0	0.156	0.594	3.0
6	3	Male	0.008	0.214	0.046	0.047	0.032	0.016	0.005	0.5	0.214	0.510	2.7
7	3	Female	0.000	0.102	0.049	0.038	0.021	0.021	0.000	0.5	0.102	0.418	3.9
8	3	Female	0.005	0.140	0.119	0.057	0.032	0.016	0.004	0.5	0.140	0.534	2.4
9	8	Male	0.016	0.445	0.338	0.143	0.105	0.071	0.006	0.5	0.445	1.778	3.0
10	8	Male	0.022	0.890	0.148	0.280	0.112	0.042	0.010	2.0	0.280	1.398	2.3
11	8	Female	0.008	0.220	0.161	0.287	0.298	0.214	0.223	4.0	0.298	5.501	8.4
12	8	Female	0.005	0.023	0.029	0.157	0.146	0.161	0.019	2.0	0.197	2.330	6.5
13	15	Male	0.018	0.214	0.464	0.863	0.289	0.095	0.020	2.0	0.863	3.731	2.0
14	15	Male	0.029	0.890	0.082	0.646	0.470	0.238	0.033	2.0	0.646	5.137	4.2
15	15	Female	0.042	37.130	37.693	13.276	2.269	0.471	0.069	1.0	37.693	78.828	1.1
16	15	Female	0.061	0.175	0.193	0.302	0.239	0.214	0.064	2.0	0.302	4.071	10.0
<b>NK-104 lactone form</b>													
5	3	Male	0.000	0.019	0.032	0.013	0.008	0.006	0.000	1.0	0.032	0.137	2.0
6	3	Male	0.000	0.060	0.010	0.010	0.007	0.003	0.000	0.5	0.060	0.104	0.7
7	3	Female	0.000	0.015	0.011	0.011	0.004	0.005	0.000	0.5	0.015	0.094	1.9
8	3	Female	0.000	0.035	0.027	0.017	0.009	0.005	0.002	0.5	0.035	0.156	1.8
9	8	Male	0.000	0.056	0.050	0.027	0.020	0.013	0.000	0.5	0.056	0.296	3.7
10	8	Male	0.003	0.005	0.012	0.031	0.018	0.007	0.000	2.0	0.031	0.183	2.8
11	8	Female	0.004	0.009	0.009	0.019	0.040	0.019	0.074	24.0	0.074	0.943	--
12	8	Female	0.000	0.003	0.003	0.027	0.047	0.048	0.006	8.0	0.048	0.928	4.6
13	15	Male	0.000	0.090	0.227	0.439	0.221	0.058	0.008	2.0	0.439	2.186	2.1
14	15	Male	0.003	0.011	0.012	0.121	0.083	0.054	0.005	2.0	0.121	1.026	5.3
15	15	Female	0.011	2.176	2.904	2.473	1.042	0.243	0.028	1.0	2.904	12.818	1.9
16	15	Female	0.003	0.012	0.012	0.021	0.016	0.018	0.003	2.0	0.021	0.315	5.1

(Sponsor, M4, RFG2514, p23)

**SBL17-35 – 26-week consecutive oral toxicity study and 8-week recovery study of NK-104 in cynomolgus monkeys**

**Key Study Findings:**

- 6 mg/kg: increased kidney weight in males and females and decreased testis, prostate, seminal vesicles, and epididymes weight in males, which was recoverable at 8 weeks.
- 6 mg/kg/day: slight swelling of the proximal convoluted tubule in high-dose males (3/4) and females (4/4), which was recoverable at 8 weeks (desquamation of proximal convoluted tubules in males at 1 mg/kg/day was described before findings were down-graded after peer review of histopathology).
- 3 mg/kg/day: Lung, foamy cells in alveolus, recovered. Esophagus, mononuclear cell infiltration, recovered. Adrenal gland, mineralization between cortex and medulla, recovered.
- 1 and 3mg/kg/day: increased kidney weight in males and females, recovered, without correlating histopathological lesions.
- 0.5 mg/kg: no apparent treatment related changes.
- Notably, there were no pathologic findings in the eye or gastrointestinal tract as is observed in dogs.
- NOAEL was 1 mg/kg (this dose represents ~2.9 multiple of human exposure by AUC), based on findings in lung, foamy cells in alveolus (recovered), esophagus, mononuclear cell infiltration (recovered), and adrenal gland, mineralization between cortex and medulla (recovered) at 3 mg/kg/day.

Amendment #, Vol #, and Page #: M4, SBL17-34

Conducting laboratory and location: <sup>(b) (4)</sup>

Date of study initiation: March 1996

**GLP Compliance:** Yes**QA report:** Yes**Methods:** chronic oral toxicology study**Dosing:**

- Species/strain: Cynomolgus monkeys
- #/sex/group or time point:

<b>26 Week Oral Toxicity Study in Monkeys – Group Assignments</b>					
<b>Group</b>	<b>Dose mg/kg</b>	<b>26-week main</b>		<b>8-week recovery</b>	
		<b>M</b>	<b>F</b>	<b>M</b>	<b>F</b>
<b>Vehicle</b>	<b>0</b>	4	4	2	2
<b>LD</b>	<b>0.5</b>	4	4	-	-
<b>MD</b>	<b>1.0</b>	4	4	-	-
<b>MHD</b>	<b>3.0</b>	-	-	-	-
<b>HD</b>	<b>6.0</b>	4	4	2	2

- Age: 3-5 years
- Body weight: male: 2.68-3.85 kg, females: 2.00-3.45 kg
- Route: oral gavage

**Drug, lot number, and purity:** 104P-9201, 99.3% purity**Formulation/vehicle:** 0.5% carmellose sodium solution

**Observations and Times:**

<b>Endpoints</b>	<b>Time of Observation</b>
<b>Clinical signs</b>	Daily
<b>Body weights</b>	Pre-dose, weekly
<b>Food consumption</b>	Pre-dose, weekly
<b>Water Consumption</b>	Pre-dose, weeks 4, 13, and 26 of dosing period and week 8 of recovery period.
<b>Ophthalmoscopy</b>	Pre-dose, weeks 3, 12 and 25 of dosing period and week 7 of recovery period.
<b>Electrocardiography</b>	Pre-dose, weeks 4, 13, and 26 of dosing period and week 8 of recovery period (5 to 8 hours after dosing).
<b>Renal function test</b>	Pre-dose, weeks 8, 16 and 23 of dosing and week 6 of recovery.
<b>Urinalysis</b>	Pre-dose, weeks 4, 13, and 26 of dosing period, week 4 and 8 of recovery period. pH, protein, glucose, ketone bodies, occult blood, urobilinogen, bilirubin, and sediment were examined.
<b>Hematology</b>	Pre-dose, weeks 4, 13, and 26 of dosing period, week 4 and 8 of recovery period. RBC, WBC, platelet, hematocrit, Hb, MCV, MCH, MCHC, reticulocyte, leukocyte classification, PT, APTT, fibrinogen were examined.
<b>Clinical biochemistry</b>	Pre-dose, weeks 4, 13, and 26 of dosing period, week 4 and 8 of recovery period. Glucose, total protein, albumin, A/G Ratio, BUN, creatinine, Ca, ALT, AST, LDH, CPK, ALP, GGT, cholinesterase activity, triglyceride, total cholesterol, phospholipid, Na, K, Cl were examined.
<b>Gross pathology</b>	At sacrifice
<b>Organ weight</b>	At sacrifice
<b>Histopathology</b>	At sacrifice.
<b>Toxicokinetics</b>	Report not attached.

**Results:**

Clinical signs: No apparent treatment related changes.

Mortality: None.

Body weights: No apparent treatment related changes.

Food and water consumption: No apparent treatment related changes.

Ophthalmology: No apparent treatment related changes.

ECG examination: No apparent treatment related changes, however the  $T_{max}$  in monkeys is ~2 hours, so the  $C_{max}$  had already been reached and the concentration would be declining.

Renal function test: No apparent treatment related changes.

Urinalysis: No apparent treatment related changes.

Hematology: No apparent treatment related changes.

Clinical chemistry: Slight increases of LDH and CPK were noted in treated groups, however, these changes appeared to be within the range of control groups.

26 Week Oral Toxicity Study in Monkeys – Clinical Chemistry											
Dose (mg/kg)		Male					Female				
		0	0.5	1	3	6	0	0.3	1	3	6
LDH	Pre	774	671	753	949	824	584	568	624	639	578
	4wk	1087	1042	1188	1338	976	602	575	778	712	793
	13wk	1080	1005	1399	1302	1204	832	732	1069	970	936
	26wk	1113	1114	1551	1215	1025	798	628	993	918	674
	R:4wk	809				656	440				514
	R:8wk	827				682	624				608
CPK	Pre	176	112	154	129	146	129	128	142	133	116
	4wk	222	126	150	170	216	137	148	128	157	213
	13wk	135	152	238	266	198	181	136	180	133	267
	26wk	167	206	194	191	294	190	105	134	208	192
	R:4wk	184				139	79				97
	R:8wk	477				121	214				149

Organ weight: Increased kidney weight was observed at  $\geq 1$  mg/kg (n=6/group/dose). Increased kidney weight was also present in control animals (n=2) at the end of 8 weeks of recovery in males (but not females). Decreased testis weight, prostate, epididymes, and seminal vesicles were observed at 6 mg/kg; findings appeared to be recoverable after 8 weeks.

26 Week Oral Toxicity Study in Monkeys – Organ Weights											
Dose (mg/kg)	Male					Female					
	0	0.5	1	3	6	0	0.3	1	3	6	
Testis	26wk	18.3	18.8	19.3	26.7	3.8					
	Rel.	4.0	4.2	4.3	5.6	1.0					
	R:8wk	14.2				21.5					
	Rel.	3.7				4.9					
Kidneys	26wk	16.4	17.1	19.8*	20.5*	18.4	10.0	12.8	14.8*	14.2*	15.7*
	Rel.	3.6	3.9	4.6*	4.6*	4.8*	3.7	4.1	4.8*	4.9*	5.0*
	R:8wk	19.5				17.6	13.3				13.6
	Rel.	4.9				3.9	4.2				4.5
Prostate	26wk	1.4	1.2	1.3	1.1	0.6					
	Rel.	0.3	0.3	0.3	0.2	0.2					
	R:8wk	0.6				0.9					
	Rel.	0.1				0.2					
Epididymis	26wk	3.1	3.2	3.3	4.2	1.3					
	Rel.	0.7	0.7	0.8	0.9	0.4					
	R:8wk	2.3				3.0					
	Rel.	0.6				0.7					
Seminal vesicle	26wk	11.1	5.6	7.1	8.2	1.9					
	Rel.	2.4	1.3	1.6	1.8	0.5					
	R:8wk	2.0				5.4					
	Rel.	0.5				1.2					

\*p&lt;0.05

Gross Pathology: There were no drug-related findings upon autopsy in male or female cynomolgus monkeys.

Histopathology: Microscopic findings included swelling of the proximal convoluted tubule (very slight severity) in high-dose, 6 mg/kg/day, males (3/4) and females (4/4), which was recoverable at 8 weeks. There was no incidence of this kidney finding outside of the high dose groups.

There was an increase in incidence (1/4, 2/4, 1/4) of foamy cells observed in the alveolus in females administered 1, 3, and 6 mg/kg/day lung, respectively. There was no incidence of this finding in females of the recovery groups. This finding may be attributable to oral gavage and not to the drug substance, per se.

In the adrenal gland, focal cell hypertrophy of zona fasciculata cells of female monkeys were observed at a frequency of 1/4, 2/4, and 1/4 for the 1, 3, and 6 mg/kg/day doses, respectively. This finding was observed in a control animal in the recovery group. Therefore, this finding is not considered drug-related.

It is notable that there was no significant liver, thyroid, or muscle toxicity observed.

26 Week Oral Toxicology Study in Monkeys – Histopathology of Terminal Group											
Organ	Dose(mg/kg) Finding N	Male					Female				
		0	0.5	1	3	6	0	0.5	1	3	6
		4	4	4	4	4	4	4	4	4	4
Liver	Periportal inflammatory cell infiltration(±)	-	1	-	-	-	-	-	-	-	-
	Hyaline droplet in hepatocyte(±)	-	1	-	-	-	-	-	-	-	-
	Periportal mononuclear cell infiltration(±)	3	4	2	4	4	3	3	2	4	4
Adrenal	Mineralization between cortex and medulla(±)	-	-	1	1	-	-	-	-	-	2
	Mononuclear cell infiltration in medulla(±)	-	-	-	3	1	-	1	1	-	1
	Focal cell hypertrophy of zona fasciculata cell	-	1	2	-	-	-	-	1	2	1
Kidney	Glomerulosclerosis(±)	-	1	-	-	1	-	-	-	-	-
	Swelling of proximal tubular epithelium(±)	-	-	-	-	3	-	-	-	-	4
Lung	Foamy cell in alveolus(±)	1	-	-	1	-	-	-	1	2	1
Stomach	Dilatation of gastric pit, fundus(±)	-	1	-	-	-	-	-	-	-	-
	Granuloma in submucosa, pylorus(±)	-	-	-	-	1	-	-	-	-	-
Skeletal Muscle	Mononuclear cell infiltration(±)	-	-	-	1	-	-	-	-	-	1
Esophagus	Mononuclear cell infiltration in muscle layer(±)	1	-	-	-	-	-	-	1	1	-
Pituitary	Cyst in pars distalis(±)	1	-	-	-	1	-	-	1	2	-
Thyroid	Ectopic thymus(P)	1	1	-	-	3	1	-	1	1	-
Thymus	Atrophy(±)	1	-	3	3	-	-	-	-	1	1
	Cyst(±)	2	1	2	2	2	-	-	-	2	2
Testis	Immature(P)	-	-	-	-	3					
Seminal vesicle	Immature(P)	-	-	-	-	1					
Ovary	Mineralization of oocyte(±, +,++)						3±	3+	1± 1++	3± 1++	2± 1+

(±) indicates “very slight”

(+) indicates “slight”

(++) indicates moderate

(P) indicates “present”

<b>26 Week Oral Toxicology Study in Monkeys – Histopathology of Recovery Group</b>					
		<b>Male</b>		<b>Female</b>	
<b>Organ</b>	<b>Dose(mg/kg) Finding N</b>	<b>0</b>	<b>6</b>	<b>0</b>	<b>6</b>
		<b>2</b>	<b>2</b>	<b>2</b>	<b>2</b>
<b>Liver</b>	Periportal inflammatory cell infiltration(±)	1	1	-	-
	Hyaline droplet in hepatocyte(±)	-	-	-	-
	Periportal mononuclear cell infiltration(±)	2	2	1	2
<b>Adrenal</b>	Mineralization between cortex and medulla(±)	-	-	-	-
	Mononuclear cell infiltration in medulla(±)	1	1	-	-
	Focal cell hypertrophy of zona fasciculata cell	-	-	1	-
<b>Kidney</b>	Glomerulosclerosis(±)	-	-	-	-
	Swelling of proximal tubular epithelium(±)	-	-	-	-
<b>Lung</b>	Foamy cell in alveolus(±)	-	-	-	-
<b>Stomach</b>	Dilatation of gastric pit, fundus(±)	-	-	-	-
	Granuloma in submucosa, pylorus(±)	-	-	-	-
<b>Skeletal Muscle</b>	Mononuclear cell infiltration(±)	-	-	-	-
<b>Esophagus</b>	Mononuclear cell infiltration in muscle layer(±)	-	-	-	-
<b>Pituitary</b>	Cyst in pars distalis(±)	-	-	-	-
<b>Thyroid</b>	Ectopic thymus(P)	1	1	-	1
<b>Thymus</b>	Atrophy(±)	-	-	1	-
	Cyst(±)	2	1	1	1
<b>Testis</b>	Immature(P)	1	-	-	-
<b>Seminal vesicle</b>	Immature(P)	-	-	-	-
<b>Ovary</b>	Mineralization of oocyte(±, +, ++)	-	-	-	1++

(±) indicates “very slight”

(P) indicates “present”

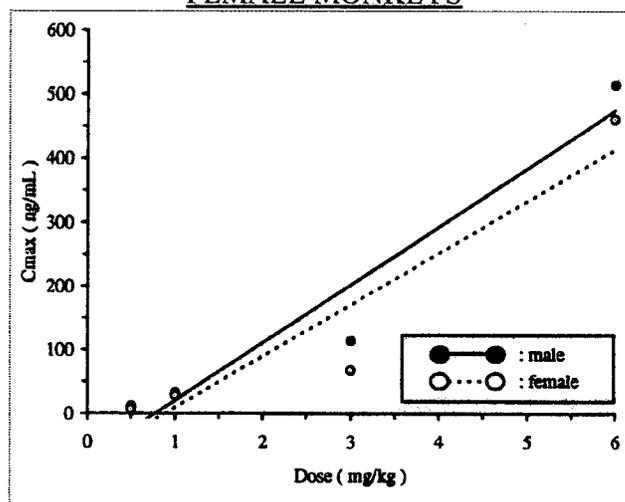
<b>Repeat Dose Toxicology Studies – Histopathology Inventory</b>				
<b>Study</b>	<b>RFG2506</b>	<b>RFG2503</b>	<b>RFG2504</b>	<b>SBL17-35</b>
<b>Species</b>	<b>Rat</b>	<b>Dog</b>	<b>Dog</b>	<b>Monkey</b>
Adrenals	X*	X*	X*	X*
Aorta	X	X	-	X
Bone Marrow smear	-	X	X	X
Bone (femur)	X	X	-	X
Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Cervix	-	-	-	-
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X	X	X	X*
Esophagus	X	X	X	X
Eye	X	X	X	X
Fallopian tube	-	-	-	-
Gall bladder	-	X	-	X
Gross lesions	-	-	-	-
Harderian gland	X	-	-	-
Heart	X*	X*	X*	X*
Hypophysis	-	-	-	-
Ileum	X	X	X	X
Injection site	-	-	-	-
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland	-	-	-	-
Larynx	-	-	-	-
Liver	X*	X*	X*	X*
Lungs	X*	X*	X*	X*
Lymph nodes, cervical	-	X	X	X
Lymph nodes mandibular	-	-	-	X*
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X	X	X	X
Nasal cavity	-	-	-	-
Optic nerves	-	X	X	X
Ovaries	X*	X*	X*	X*
Pancreas	X*	X*	X*	X*
Parathyroid	X	X	-	-
Peripheral nerve	-	-	-	-
Pharynx	-	-	-	-
Pituitary	X*	X*	X*	X*
Prostate	X*	X*	X*	X*
Rectum	X	X	-	X
Salivary gland	X*	X	-	-
Sciatic nerve	X	X	X	X
Seminal vesicles	X*	-	-	X*
Skeletal muscle	X	X	X	X
Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X*	X*	X*	X*
Sternum	X	X	X	X
Stomach	X	X	X	X
Testes	X*	X*	X*	X*
Thymus	X*	X*	X*	X*
Thyroid	X	X*	X*	X*
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X*
Uterus	X*	X*	X*	X*
Vagina	X	X	-	X
Zymbal gland	-	-	-	-

\* organ weight obtained

Toxicokinetics of NK-104 Lactone after Daily Administration of NK-104 to Cynomolgus Monkeys by Oral Gavage for 6 Months									
Day	Dose (mg/kg)	T <sub>max</sub> (h)		C <sub>max</sub> (ng/mL)		AUC (ng·hr/mL)		T <sub>1/2</sub> (h)	
		♂	♀	♂	♀	♂	♀	♂	♀
1	0.5	1.3	1.8	2	3	12	22	3.0	2.8
	1	0.6	1.1	11	10	55	41	3.3	3.8
	3	1.1	1.8	27	17	225	110	4.3	3.8
	6	1.0	1.3	112	94	473	397	2.7	2.1
28	0.5	0.6	2.3	1	1	10	4	10.1	1.9
	1	0.8	1.0	5	1	45	9	5.9	6.1
	3	2.5	1.4	16	15	213	94	5.2	3.8
	6	0.9	1.9	39	35	208	241	5.3	5.1
91	0.5	1.0	1.8	2	2	9	11	3.3	6.7
	1	0.9	1.1	3	5	22	19	7.9	2.9
	3	1.3	1.8	18	9	143	43	6.9	3.8
	6	1.1	1.3	36	24	205	183	3.7	5.0
182	0.5	2.6	1.0	21	3	20	17	11.0	6.4
	1	1.8	1.0	7	6	71	28	5.3	4.2
	3	1.3	1.0	21	14	194	96	3.5	4.2
	6	1.4	2.1	43	43	282	275	7.0	6.9

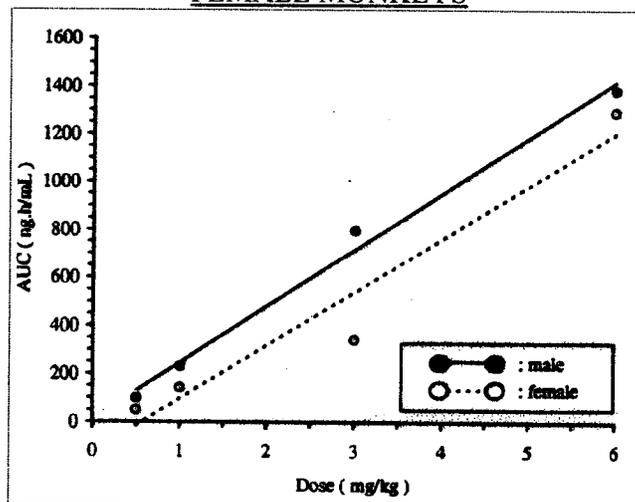
Increases in C<sub>max</sub> for NK-104 were higher than expected in male and female monkeys at the highest, 6 mg/kg dose based on the dose-concentration relationship at the 3 mg/kg and lower doses, while AUC for NK-104 was linear at all doses up to 6 mg/kg in male and female monkeys.

C<sub>MAX</sub> ON DAY 1 IN MALE AND FEMALE MONKEYS



(Sponsor, M4, RFG2515, p31)

**AUC ON DAY 1 IN MALE AND FEMALE MONKEYS**



(Sponsor, M4, RFG2515, p31)

**2.6.6.4 Genetic toxicology**

Results of Genetic Toxicology Battery for Pitavastatin				
Study Number	Test	Species or Cell Line	Route	Pitavastatin Concentration or Dose
3140	Reverse mutation	<i>S. typhimurium</i> TA 98, TA100, TA 1535, TA1537 <i>E. coli</i> WP2uvrA	-	-S9: 10-313 µg/plate +S9: 156-5000 µg/plate
3141	Chromosomal aberration	CHL cells	-	-S9: 800-6200 µg/mL +S9: 313-2500 µg/mL
RG25003	Micronucleus	Mouse	Oral	250, 500, 1000 mg/kg/day
KW92117	Micronucleus	Rats	Oral	2, 10, 30(50) mg/kg/day
3638	UDS	Rat	Oral	5.2%
A819	Comet	Mouse	Oral	125, 250, 500 mg/kg

**3140 – Mutagenicity testing of NK-104 in bacterial reverse mutation assays**

**Key study findings:**

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

**Volume #, and page #:** M4, 3140, 1-22

**Conducting laboratory and location** <sup>(b) (4)</sup>

**Date of study initiation:** May 15, 1992

**GLP compliance:** Yes.

**QA reports:** yes (X) no ( )

**Drug, lot number, and purity:** 104P-9202, 99.2% purity

**Methods:**

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

Doses used in definitive study: Doses up to 313 µg/plate were used without metabolic activation, and doses up to 5000 µg/plate were used with metabolic activation.

Basis of dose selection: Doses were obtained after preliminary tests with 1.2, 4.9, 20, 78, 313, 1250, and 5000 µg/plate in all strains. Growth inhibition with metabolic activation was seen at 5000 µg/plate in *S. typhimurium* TA1537, however with TA1535 and TA98 growth was not considered to be inhibited at the maximum concentration of 5000 µg/plate. Growth inhibition without metabolic activation was observed at 313 µg/plate and greater concentrations in all strains.

With(+)or without(-) S9 Mix	Test substance concentration (µg/plate)	Number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1538	WP2uvrA	TA98	TA1537
S9 Mix (-)	Solventcontrol	136	15	18	25	10
	1.2	103	17	27	18	9
	4.9	131	12	19	30	11
	20	122	12	15	19	10
	78	126	15	14	23	9
	313	131*	18*	18*	18*	11*
	1250	106*	12*	24*	22*	4*
	5000	77*	17*	23*	12*	2*
S9 Mix (+)	Solventcontrol	121	15	33	28	14
	1.2	134	14	33	28	18
	4.9	116	7	34	22	20
	20	124	14	38	33	22
	78	128	17	38	23	19
	313	129	10	22	41	21
	1250	145	20	28	32	19
	5000	32	10	28	27	13*
Positive control not requiring S9 Mix	Name	AF-2 <sup>81</sup>	NaN <sub>3</sub> <sup>82</sup>	AF-2	AF-2	ICR-191 <sup>83</sup>
	Concentration (µg/plate)	0.01	0.5	0.01	0.1	1.0
	Number of colonies/plate	832	378	201	455	1525
Positive control requiring S9 Mix	Name	B[a]P <sup>84</sup>	2AA <sup>85</sup>	2AA	B[a]P	B[a]P
	Concentration (µg/plate)	5.0	2.0	10.0	5.0	5.0
	Number of colonies/plate	788	181	438	175	85

81:2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide    82:Sodium azide    83:Metagen ICR-191    84:Benzo[a]pyrene  
85:2-Aminoanthracene

\*:Growth inhibition of tested bacterium was observed.

(Sponsor, M4, 3140, p10)

Negative controls: DMSO treated.

Positive controls:

Strain	without S9 Mix (µg/plate)	with S9 Mix (µg/plate)
TA100	AF-2 (0.01)	B[a]P (5.0)
TA1535	N <sub>5</sub> N <sub>3</sub> (0.5)	2AA (2.0)
TA98	AF-2 (0.1)	B[a]P (5.0)
TA1537	ICR-191 (1.0)	B[a]P (5.0)
WP2 <i>uvrA</i>	AF-2 (0.01)	2AA (10.0)

AF-2	2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
(b) (4)	Lot No. SAJ0748; Special grade of JIS standard)
N <sub>5</sub> N <sub>3</sub>	Sodium azide
(b) (4)	Lot No. DCJ6398; Special grade of JIS standard)
ICR-191	Mutagen ICR-191
(b) (4)	Lot No. 52300; Purity greater than 95%)
2AA	2-Aminoanthracene
(b) (4)	Lot No. KPQ0892; Special grade of JIS standard)
B[a]P	Benzo[a]pyrene
(b) (4)	Lot No. AWK3751; Special grade of JIS standard)

Sodium azide (N<sub>5</sub>N<sub>3</sub>) was dissolved in distilled water (b) (4) Lot No. 9106VA), the other compounds were dissolved in DMSC (b) (4) ; Lot No. DSL5887).

(Sponsor, M4, 3140, p4)

Incubation and sampling times: Tests were carried out twice, in triplicate. Plates were incubated for 48 hours at 37 °C then counted by automated colony counter.

Results:

With(+)/or without(-) S9 Mix	Test substance concentration (µg/plate)	Number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2uvrA	TA98	TA1537
SBM1x (-)	Solvent control	119 125 167 (142±22.7)	12 17 19 (18±3.6)	32 30 30 (31±1.2)	21 25 11 (19±7.2)	8 8 7 (8±0.6)
	10	129 125 122 (125±3.6)	12 18 13 (14±3.2)	38 28 34 (33±8.6)	26 25 27 (26±1.0)	8 7 4 (8±2.1)
	20	103 140 97 (113±23.3)	13 17 20 (17±3.6)	29 22 27 (26±3.6)	25 23 21 (23±2.0)	7 8 9 (7±1.5)
	30	131 118 102 (117±14.5)	13 21 12 (15±4.8)	31 38 28 (29±2.8)	21 31 22 (25±5.5)	9 4 8 (7±2.8)
	70	111 138 109 (119±18.2)	17 19 15 (17±2.6)	37 34 37 (36±1.7)	30 24 28 (27±3.1)	12 8 9 (10±2.1)
	150	124 153 136 (137±14.8)	18 12 17 (15±2.8)	27 20 38 (32±5.7)	21 25 27 (24±3.1)	10 13 12 (12±1.6)
	313	128 <sup>a</sup> 99 <sup>a</sup> 125 <sup>a</sup> (117±16.9)	14 <sup>a</sup> 13 <sup>a</sup> 17 <sup>a</sup> (15±2.1)	21 <sup>a</sup> 24 <sup>a</sup> 31 <sup>a</sup> (25±5.1)	28 <sup>a</sup> 21 <sup>a</sup> 24 <sup>a</sup> (25±4.0)	15 <sup>a</sup> 11 <sup>a</sup> 7 <sup>a</sup> (11±4.0)
SBM1x (+)	Solvent control	110 125 128 (120±9.0)	14 11 16 (14±2.5)	38 25 32 (33±4.8)	40 38 30 (38±5.3)	17 19 20 (19±1.5)
	150	NT	24 20 17 (20±3.8)	NT	44 24 34 (34±10.0)	25 10 11 (15±8.4)
	313	110 118 110 (113±4.8)	19 19 11 (18±4.4)	48 28 43 (39±9.8)	38 43 33 (39±5.0)	21 12 18 (16±4.5)
	625	122 101 125 (116±13.1)	13 20 21 (18±4.4)	37 38 38 (38±0.8)	33 41 21 (35±5.3)	17 13 18 (15±2.1)
	1250	125 134 100 (119±17.3)	18 9 9 (11±4.0)	37 48 37 (39±3.5)	48 30 35 (37±8.2)	10 14 18 (14±4.0)
	2500	103 131 98 (110±18.5)	14 19 14 (14±6.8)	38 48 35 (38±5.0)	32 29 30 (29±3.1)	17 <sup>a</sup> 19 <sup>a</sup> 18 <sup>a</sup> (16±2.1)
	5000	84 85 88 (85±1.0)	18 11 12 (13±2.8)	35 25 29 (30±5.0)	28 18 32 (25±6.5)	10 <sup>a</sup> 8 <sup>a</sup> 8 <sup>a</sup> (9±1.2)
Positive control not requiring S9 Mix	Name	AF-2 <sup>11</sup>	N&N <sub>2</sub> <sup>12</sup>	AF-2	AF-2	ICR-181 <sup>13</sup>
	Concentration (µg/plate)	0.01	0.5	0.01	0.1	1.0
	Number of colonies/plate	894 871 920 (895±24.5)	388 342 380 (384±13.8)	298 288 178 (190±18.2)	498 423 514 (468±48.8)	1543 1488 1442 (1492±50.5)
Positive control requiring S9 Mix	Name	B[a]P <sup>14</sup>	2AA <sup>15</sup>	2AA	B[a]P	B[a]P
	Concentration (µg/plate)	5.0	2.0	10.0	5.0	5.0
	Number of colonies/plate	761 848 842 (814±54.7)	248 231 225 (231±13.4)	488 447 511 (482±48.5)	187 176 167 (176±10.1)	84 88 94 (88±5.8)

11:2-(2-Furyl)-3-(5-nitro-2-Furyl)acrylonitrile    12:Sodium azide    13:Metoson ICR-181    14:Benzo[*a*]pyrene  
 15:2-Aminanthracene

\*:Growth inhibition of tested bacterium was observed.    NT:Not tested

Notes:The average number of colonies in each concentration is shown in the ( ).

(Sponsor, M4, 3140, p11)

With(+)or without(-) S9 Mix	Test substance concentration (µg/plate)	Number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2uvrA	TA98	TA1537
S9 Mix (-)	Solventcontrol	158 138 164 (150±10.0)	11 18 21 (17± 5.1)	33 32 27 (31± 3.2)	19 17 20 (19± 1.5)	4 19 14 (12± 7.8)
	10	156 149 138 (148± 9.1)	17 16 17 (17± 0.8)	29 23 37 (31± 4.9)	25 15 25 (22± 5.8)	11 11 14 (12± 1.7)
	20	188 146 138 (151±15.1)	13 18 15 (15± 2.5)	34 25 28 (28± 4.9)	20 20 19 (20± 0.8)	12 6 14 (10± 4.7)
	39	166 128 145 (148±19.0)	8 16 9 (11± 4.4)	16 20 27 (21± 5.6)	17 19 18 (18± 1.0)	10 15 12 (12± 2.5)
	78	132 180 153 (148±14.8)	9 13 13 (12± 2.3)	33 23 29 (28± 5.0)	23 22 20 (22± 1.5)	11 12 16 (13± 2.6)
	156	166 122 135 (142±24.3)	11 11 11 (11± 0.0)	24 33 18 (25± 7.5)	21 23 22 (22± 1.0)	12 19 12 (14± 4.0)
	313	140* 150* 124* (138±13.1)	13* 9* 11* (11± 2.0)	21* 24* 24* (23± 1.7)	19* 25* 23* (24± 5.0)	18* 15* 9* (14± 4.8)
S9 Mix (+)	Solventcontrol	158 132 158 (149±15.0)	14 19 21 (18± 3.8)	23 18 18 (20± 2.9)	41 30 24 (32± 8.8)	21 15 21 (19± 3.5)
	156	NT	19 20 18 (19± 1.0)	NT	38 30 32 (34± 4.7)	20 28 27 (25± 4.4)
	313	143 158 146 (149± 8.5)	23 19 14 (19± 4.5)	26 19 20 (22± 3.8)	44 33 29 (35± 7.8)	23 19 25 (22± 3.1)
	625	151 158 128 (146±15.1)	22 9 15 (15± 6.6)	23 20 18 (22± 5.3)	43 40 28 (39±10.1)	14 23 17 (18± 4.6)
	1250	149 158 137 (147± 9.8)	14 13 14 (14± 0.8)	23 19 22 (21± 2.8)	28 43 22 (31±10.8)	21 17 29 (22± 6.1)
	2500	158 152 132 (147±13.8)	19 8 18 (14± 5.7)	18 23 18 (19± 3.8)	28 25 29 (27± 2.1)	18* 25* 19* (21± 3.8)
	5000	108 108 84 (100±14.2)	11 12 13 (12± 1.0)	29 19 15 (21± 7.2)	22 18 32 (22± 9.5)	8* 18* 10* (12± 5.3)
Positive control not requiring S9 Mix	Name	AF-2 <sup>11</sup>	NaN <sub>3</sub> <sup>12</sup>	AF-2	AF-2	ICR-191 <sup>13</sup>
	Concentration (µg/plate)	0.01	0.5	0.01	0.1	1.0
	Number of colonies/plate	847 874 858 (859±13.7)	390 396 384 (390± 4.0)	177 185 162 (168± 7.9)	440 430 411 (427±14.7)	1214 1457 1209 (1293±141.8)
Positive control requiring S9 Mix	Name	B[a]P <sup>14</sup>	2AA <sup>15</sup>	2AA	B[a]P	B[a]P
	Concentration (µg/plate)	5.0	2.0	10.0	5.0	5.0
	Number of colonies/plate	816 777 853 (815±38.0)	218 209 186 (204±15.7)	343 430 385 (386±43.5)	184 191 157 (181±20.8)	85 107 129 (107±22.0)

<sup>11</sup>:2-(2-Furyl)-9-(6-nitro-2-Furyl)acrylamide    <sup>12</sup>:Sodium azide    <sup>13</sup>:Mutagen ICR-191    <sup>14</sup>:Benzo[a]pyrene  
<sup>15</sup>:2-Aminoanthracene  
 \*:Growth inhibition of tested bacterium was observed.    NT:Not tested  
 Notes:The average number of colonies in each concentration is shown in the ( ).

(Sponsor, M4, 3140, p12)

Study validity: doubling or greater compared to the spontaneous reversion rate would indicate a positive result.

**3141 – Chromosomal aberration test in cultured mammalian cells on NK-104****Key findings:**

- Pitavastatin dissolved in 1% carboxymethylcellulose was positive for chromosomal aberration in CHL cells with metabolic activation (+S9) at a drug concentration that caused cytotoxicity (~50% growth inhibition).
- The concentration determined to be positive was 625 µg/mL; concentrations above 625 µg/mL were confounded by cytotoxicity.
- Pitavastatin dissolved in DMSO was negative for chromosomal aberrations in assays with and without S9; however, the test substance was not dissolved uniformly in DMSO.

**Volume #, and page #:** M4, 3141, p1-37

**Conducting laboratory and location** <sup>(b) (4)</sup>

**Date of study initiation:** May 15, 1992

**GLP compliance:** Yes.

**QA reports:** yes (X) no ( )

**Drug, lot number, and purity:** 104P-9202, 99.2% purity

**Methods:**

Strains/species/cell line: Chinese hamster lung (CHL cells) fibroblast cell line.

Doses used in definitive study:

<b>Chromosomal Aberration Assay in Mammalian CHL Cells – Main Study</b>						
<b>Chrom-Ab Test (Duplicate)</b>	<b>Solvent/ Positive Control</b>	<b>S9</b>	<b>Doses</b>	<b>Test Substance Exposure</b>	<b>Post-Exposure Incubation</b>	<b>Chromosomal Aberrations</b>
<b>Direct</b>	<b>DMSO/MitC</b>	<b>-</b>	<b>6.2, 3.1, 1.6, 0.8 µg/mL</b>	<b>24/48 hr</b>	<b>N/A</b>	<b>No</b>
<b>Metabolic activation</b>	<b>DMSO/DMN</b>	<b>±</b>	<b>3.9, 2.08, 1.04, 0.52, 0.26, 0.13, mg/mL</b>	<b>6 hr</b>	<b>18 hr</b>	<b>No</b>
<b>Metabolic activation</b>	<b>1% CMC-Na/DMN</b>	<b>±</b>	<b>2.5, 1.25, 0.938, 0.625, 0.468, 0.313 mg/mL</b>	<b>6 hr</b>	<b>18 hr</b>	<b>Yes (468.5 µg/mL, suspicious; 625 µg/mL, positive)</b>

Basis of dose selection:

<b>Chromosomal Aberration Assay in Mammalian CHL Cells – Preliminary Studies</b>						
<b>Growth Inhibition Test</b>	<b>Solvent/ Positive Control</b>	<b>S9</b>	<b>Doses</b>	<b>Test Substance Exposure</b>	<b>Post- Exposure Incubation</b>	<b>Cell Growth Inhibition</b>
<b>Direct/1</b>	DMSO/MitC	-	3.0, 1.5, 0.75, 0.375, 0.188, 0.094 mg/mL	48 hr	N/A	ND
<b>Direct/2</b>	DMSO/MitC	-	100, 50, 25, 12.5, 6.3, and 3.1 µg/mL	48 hr	N/A	3.1 µg/mL
<b>Metabolic activation/1</b>	DMSO/DMN	+	3.0, 1.5, 0.75, 0.375, 0.188, 0.094 mg/mL	6 hr	18 hr	520 µg/mL
<b>Metabolic activation/2</b>	DMSO/DMN	+	3.9, 3.0, 1.5, 0.75, 0.375, 0.188, 0.094 mg/mL	6 hr	18 hr	950 µg/mL
<b>Metabolic activation/1</b>	1% CMC- Na/DMN	+	5, 2.5, 1.25, 0.625, 0.313, 0.156 mg/mL	6 hr	18 hr	270 µg/mL
<b>Metabolic activation/2</b>	1% CMC- Na/DMN	+	5, 2.5, 1.25, 0.625, 0.313, 0.156 mg/mL	6 hr	18 hr	625 µg/mL

MitC: Mitomycin C

N/A: Not applicable.

ND: Not detected.

Negative controls: Untreated control, DMSO, 1% carboxymethylcellulose-sodium solution.

Positive controls: For the direct method, mitomycin C (MitC). For the metabolic activation method, N-nitrosodimethylamine (DMN).

Results:

S-9 Time #1 Mix (h)	Conc. (µg/ml)	Cells observed (%)	Polyploidy	Judge	Type of aberrations(%)							Judge		
					g	ctb	cte	csb	cse	others	TA	TAG		
-	24-0	N.T. #2	200	0.0	-	0.0	0.0	0.5	0.0	0.0	0.0	0.5	0.5	-
		S.C. #3	200	0.0	-	0.0	1.0	1.0	0.0	0.0	0.0	2.0	2.0	-
		0.8	200	0.0	-	0.5	0.5	0.0	0.0	0.0	0.0	0.5	1.0	-
		1.6	200	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		3.1	200	0.0	-	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5	-
		6.2	200	0.0	-	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.5	-
	P.C. #4	200	0.5	-	0.5	27.5	40.5	0.0	0.0	0.0	57.0	57.0	+	
-	48-0	N.T.	200	0.5	-	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.5	-
		S.C.	200	0.5	-	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.5	-
		0.8	200	0.5	-	1.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	-
		1.6	200	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		3.1	200	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		6.2	200	0.0	-	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.5	-
	P.C.	200	0.0	-	0.5	36.0	50.5	0.0	0.0	0.0	0.5	65.5	65.5	+

#1 Time : exposure time - recover time  
 #2 N.T. : Non treatment #3 S.C. : Solvent control #4 P.C. : Positive control

g : chromatid gap and chromosome gap    ctb : chromatid break  
 cte : chromatid exchange    csb : chromosome break  
 cse : chromosome exchange (dicentric chromosome, ring formation etc.)  
 others : fragmentation etc.  
 TA : Total aberrant cells excluding gap  
 TAG : Total aberrant cells including gap

Solvent Used : DMSO  
 Positive control : Nitomycin C 0.05 µg/ml

(Sponsor, M4, 3141, p23)

S-9 Time #1 Mix (h)	Conc. (ng/ml)	Cells observed (%)	Polyploidy	Judge	Type of aberrations(%)							Judge		
					g	ctb	cte	csb	cse	others	TA	TAG		
-	6-18	N.T. #2	200	0.0	-	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.5	-
		S.C. #3	200	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		0.016	200	0.0	-	0.0	0.0	0.5	0.0	0.0	0.0	0.5	0.5	-
		0.033	200	0.5	-	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.5	-
		0.065	200	0.0	-	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5	-
		0.13	200	0.5	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		0.26	200	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		0.52	200	1.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		1.04	200	1.0	-	0.5	0.5	0.0	0.0	0.0	0.0	0.5	1.0	-
			P.C. #4	200	1.0	-	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.5
+	6-18	N.T.	200	0.0	-	0.0	0.0	0.5	0.0	0.0	0.0	0.5	0.5	-
		S.C.	200	0.5	-	1.0	0.0	0.5	0.0	0.0	0.0	0.5	1.5	-
		0.016	200	0.5	-	0.0	0.5	0.0	0.5	0.0	0.0	1.0	1.0	-
		0.033	200	0.5	-	0.5	0.5	0.0	0.0	0.0	0.0	0.5	1.0	-
		0.065	200	0.5	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		0.13	200	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		0.26	200	0.0	-	0.0	0.5	0.5	0.0	0.0	0.0	0.5	0.5	-
		0.52	200	1.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		1.04	200	0.0	-	0.5	2.0	1.5	0.0	0.0	0.0	3.0	3.5	-
			P.C.	200	0.0	-	2.0	19.5	45.0	0.5	0.5	0.0	52.0	52.5

#1 Time : exposure time - recover time  
 #2 N.T. : Non treatment #3 S.C. : Solvent control #4 P.C. : Positive control

g : chromatid gap and chromosome gap    ctb : chromatid break  
 cte : chromatid exchange    csb : chromosome break  
 cse : chromosome exchange (dicentric chromosome, ring formation etc.)  
 others : fragmentation etc.  
 TA : Total aberrant cells excluding gap  
 TAG : Total aberrant cells including gap

Solvent Used : DMSO  
 Positive control : Dimethylnitrosamine 0.4ng/ml

(Sponsor, M4, 3141, p24)

S-9 Time #1 Mix (h)	Conc. (mg/ml)	Cells obsd.	Polyploidy cells (%)	Judge	Type of aberrations(%)								Judge	
					g	ctb	cte	csb	cse	others	TA	TAG		
-	8-18	N.T. #2	200	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		S.C. #3	200	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		0.52	200	0.0	-	0.5	0.5	0.0	0.0	0.0	0.0	0.5	1.0	-
		1.04	200	1.0	-	0.0	1.0	0.5	0.0	0.0	0.0	1.0	1.0	-
		2.08	200	1.0	-	0.0	0.5	0.5	0.0	0.0	0.0	1.0	1.0	-
		3.90	200	0.5	-	0.0	1.0	0.0	0.0	0.0	0.0	1.0	1.0	-
+	6-18	P.C. #4	200	0.0	-	0.0	1.0	0.0	0.0	0.0	0.0	1.0	1.0	-
		N.T.	200	0.0	-	0.0	1.0	1.0	0.0	0.0	0.0	2.0	2.0	-
		S.C.	200	0.0	-	0.0	0.5	0.5	0.0	0.0	0.0	0.5	0.5	-
		0.52	200	1.0	-	0.5	0.5	0.5	0.0	0.0	0.0	0.5	1.0	-
		1.04	200	2.0	-	0.0	1.0	0.0	0.0	0.0	0.0	1.0	1.0	-
		2.08	200	0.0	-	1.0	0.5	1.0	0.0	0.0	0.0	1.5	2.5	-
3.90	200	0.5	-	0.0	1.5	1.5	0.0	0.0	0.0	3.0	3.0	-		
P.C.	200	0.0	-	2.0	14.5	29.0	0.5	0.0	0.0	39.5	40.0	+		

#1 Time : exposure time - recover time  
 #2 N.T. : Non treatment #3 S.C.: Solvent control #4 P.C.: Positive control

g : chromatid gap and chromosome gap    ctb : chromatid break  
 cte : chromatid exchange    csb : chromosome break  
 cse : chromosome exchange (dicentric chromosome, ring formation etc.)  
 others : fragmentation etc.  
 TA : Total aberrant cells excluding gap  
 TAG : Total aberrant cells including gap

Solvent Used : DMSO  
 Positive control : Dimethylnitrosoamine 0.4mg/ml

(Sponsor, M4, 3141, p25)

S-9 Time #1 Mix (h)	Conc. (mg/ml)	Cells obsd.	Polyploidy cells (%)	Judge	Type of aberrations(%)								Judge	
					g	ctb	cte	csb	cse	others	TA	TAG		
-	8-18	N.T. #2	200	0.0	-	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.5	-
		S.C. #3	200	0.0	-	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.5	-
		0.313	200	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
		0.625	200	1.0	-	0.0	1.5	2.0	0.0	0.0	0.0	2.5	2.5	-
		1.25	30											TOX
		2.5	0											TOX
+	8-18	P.C. #4	200	0.5	-	0.0	0.0	0.5	0.0	0.0	0.0	0.5	0.5	-
		N.T.	200	0.0	-	0.5	0.0	0.5	0.0	0.0	0.0	0.5	1.0	-
		S.C.	200	0.0	-	0.5	0.0	0.5	0.0	0.0	0.0	0.5	1.0	-
		0.313	200	0.0	-	0.5	0.5	0.5	0.0	0.0	0.0	1.0	1.5	-
		0.625	200	0.5	-	1.0	9.0	9.0	0.0	0.0	0.0	12.5	13.5	+ *
		1.25	0											TOX
2.5	0											TOX		
P.C.	200	0.0	-	1.0	18.5	47.0	0.0	0.5	0.0	54.0	55.0	+		

#1 Time : exposure time - recover time  
 #2 N.T. : Non treatment #3 S.C.: Solvent control #4 P.C.: Positive control

g : chromatid gap and chromosome gap    ctb : chromatid break  
 cte : chromatid exchange    csb : chromosome break  
 cse : chromosome exchange (dicentric chromosome, ring formation etc.)  
 others : fragmentation etc.  
 TA : Total aberrant cells excluding gap  
 TAG : Total aberrant cells including gap

Solvent Used : 1% CMC-Na solution.  
 Positive control : Dimethylnitrosoamine 0.4mg/ml

TOX : Metaphase cells were observed less than 50 cells per culture by cytotoxicity.  
 \* : Significantly different from solvent control (p<0.05).

(Sponsor, M4, 3141, p26)

S-9 Time #1 Mix (h)	Conc. (µg/ml)	Cells observed	Polyploidy cells (%)	Judge	Type of aberrations(%)							TA	TAG	Judge
					g	ctb	cte	csb	cse	others				
-	N.T. #2	200	0.5	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
	S.C. #3	200	0.5	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
	488.5	200	0.0	-	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	-
	825	200	1.0	-	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.5	-
	938	200	2.0	-	0.0	3.5	5.5	0.0	0.0	0.0	0.0	7.0	7.0	± *
P.C. #4	200	0.0	-	0.0	0.5	0.0	0.5	0.0	0.0	0.0	1.0	1.0	-	
+	N.T.	200	0.5	-	0.0	0.0	1.0	0.0	0.0	0.0	1.0	1.0	-	
	S.C.	200	0.0	-	0.0	0.0	0.5	0.0	0.0	0.0	0.5	0.5	-	
	488.5	200	0.5	-	0.0	3.5	3.5	0.0	0.0	0.0	6.5	6.5	± *	
	825	53	1.9	-	0.0	9.4	9.4	0.0	0.0	1.9	18.9	18.9	+ **	
	938	0											TOX	
P.C.	200	0.0	-	0.5	35.5	54.0	0.5	0.0	0.0	71.5	71.5	+		

#1 Time : exposure time - recover time  
#2 N.T. : Non treatment #3 S.C.: Solvent control #4 P.C.: Positive control

g : chromatid gap and chromosome gap    ctb : chromatid break  
cte : chromatid exchange    csb : chromosome break  
cse : chromosome exchange (dicentric chromosome, ring formation etc.)  
others : fragmentation etc.  
TA : Total aberrant cells excluding gap  
TAG : Total aberrant cells including gap

Solvent Used : 1%CMC-Na solution.  
Positive control : Dimethylnitrosamine 0.4mg/ml

TOX : Metaphase cells were observed less than 50 cells per culture by cytotoxicity.  
\* : Significantly different from solvent control (p<0.05).  
\*\* : Significantly different from solvent control (p<0.001).

(Sponsor, M4, 3141, p27)

**Study validity:** A negative result was less than 5% aberrant cells. Suspicious was 5-10% aberrant cells. Positive was >10% aberrant cells. Less than 50 metaphase cells per culture was determined to be cytotoxic. The clastogenic response was detected near the cytotoxic threshold for pitavastatin dissolved in 1% carboxymethylcellulose in this assay.

There were no (false) positive results of structural aberrations or polyploidy among untreated and solvent-treated controls. There were no false negatives, as all the positive controls produced expected chromosomal aberrations. Metabolic activity was required by DMN for a positive response.

### 3638 - *In vivo/in vitro* unscheduled DNA synthesis (UDS) test of NK-104 with rat hepatocyte

#### Key findings:

- NK-104 was negative for DNA damage in a UDS assay at doses up to 200 mg/kg/day. Single dose, dose range-finding toxicity study RF9808 confirms this is a valid MTD in Sprague-Dawley rats.

Volume #, and page #: M4, 3638, p1-42

Conducting laboratory and location: (b) (4)

Date of study initiation: October 23, 1997

GLP compliance: Yes

QA reports: yes (X) no ( )

Drug, lot number, and purity: 104P-9603, 99.2% purity

**Methods:**Strains/species/cell line: Sprague-Dawley rats*In vivo phase:***UDS ASSAY – GROUP ASSIGNMENTS**

Test group	Test dose (mg/kg)	Number of administered animals and animal number	
		2-Hr posttreatment group	16-Hr posttreatment group
Vehicle control	0	4 [1001 - 1004]	4 [2001 - 2004]
NK-104	100	4 [1101 - 1104]	4 [2101 - 2104]
	200	4 [1201 - 1204]	4 [2201 - 2204]
Positive control	5**	4 [1301 - 1304]	-
	1000***	-	4 [2301 - 2304]

\*: Time from administration to hepatic perfusion  
 \*\*: DMN      \*\*\*: 2AAF

(Sponsor, M4, 3638, p16)

*In vitro phase:* At 2 or 16 hours after administration, primary cells were prepared from livers after perfusion. Cells viability was between 50-80%.

Uptake of labeled thymidine into hepatocytes was assessed as a measure of active DNA repair.

Doses used in definitive study: Doses of 100 and 200 mg/kg were administered 2 or 16 hours prior to harvesting of hepatocytes.Basis of dose selection: The dose was chosen on the basis of the previously determined maximum tolerated dose (MTD) in rats.Negative controls: Vehicle (0.5% carboxymethylcellulose-sodium in water)Positive controls: For 2 hour assay, DMN was used. For 16 hour assay, 2-acetylaminofluorine was used.Incubation and sampling times: Samples were incubated with test substance for 2 hours or 16 hours.

**Results:**

NK-104 was negative in an unscheduled DNA synthesis assay at doses up to 200 mg/kg. That is, all dose groups exhibited signs of DNA repair that were below the stated 20% threshold for positive findings (5.1% and 5.8% at 100 and 200 mg/kg NK-104, respectively).

**2 HOURS POST TREATMENT**

Compound	Dose (mg/kg)	Number of animals	Number of observed Cells	Nuclear grains Mean $\pm$ S. D.	Cytoplasmic grains Mean $\pm$ S. D.	Net nuclear grains Mean $\pm$ S. D.	Cells in repair a) Mean $\pm$ S. D.	Judgement
0.5% CMC-Na b)	0	3	450	6.3 $\pm$ 0.3	7.9 $\pm$ 1.0	-1.6 $\pm$ 0.7	2.2 $\pm$ 0.4	-
NK-104	100	3	450	7.9 $\pm$ 0.3	9.1 $\pm$ 0.9	-1.3 $\pm$ 0.7	5.1 $\pm$ 1.9	-
	200	3	450	7.3 $\pm$ 0.8	7.6 $\pm$ 0.6	-0.4 $\pm$ 0.6	5.8 $\pm$ 1.7	-
DNH c)	5	3	450	36.7 $\pm$ 2.5	6.3 $\pm$ 0.3	30.4 $\pm$ 2.5	100.0 $\pm$ 0.0	+

a) : UDS positive cells : Percent of cells having 5 or greater net nuclear grains  
b) : Vehicle control  
c) : Positive control (DNH : Diethylnitrosamine)

(Sponsor, M4, 3638, p27)

**16 HOURS POST TREATMENT**

Compound	Dose (mg/kg)	Number of animals	Number of observed Cells	Nuclear grains Mean $\pm$ S. D.	Cytoplasmic grains Mean $\pm$ S. D.	Net nuclear grains Mean $\pm$ S. D.	Cells in repair a) Mean $\pm$ S. D.	Judgement
0.5% CMC-Na b)	0	3	450	7.8 $\pm$ 0.5	8.4 $\pm$ 1.0	-0.7 $\pm$ 0.7	1.6 $\pm$ 0.4	-
NK-104	100	3	450	9.0 $\pm$ 1.1	9.2 $\pm$ 0.7	-0.2 $\pm$ 1.0	5.3 $\pm$ 2.3	-
	200	3	450	8.2 $\pm$ 0.3	8.3 $\pm$ 0.9	0.0 $\pm$ 0.7	6.4 $\pm$ 0.8	-
2AAF c)	100	3	450	37.9 $\pm$ 0.5	8.0 $\pm$ 0.4	29.8 $\pm$ 0.2	100.0 $\pm$ 0.0	+

a) : UDS positive cells : Percent of cells having 5 or greater net nuclear grains  
b) : Vehicle control  
c) : Positive control (2AAF : 2-Acetylaminofluorene)

(Sponsor, M4, 3638, p28)

**Study validity:** If the number of net nuclear grains in a treatment group was 5 or more and the incidence of repair cells was >20%, the result was judged as positive. All positive controls met these criteria; negative controls did not. Study is considered valid.

**RG25003 – Micronucleus test of NK-104 by single oral administration in mice**

**Key findings:** NK-104 was negative in a micronucleus assay after single-dose administration of up to 1000 mg/kg in mice.

**Volume #, and page #:** M4, RG25003, p1-53

**Conducting laboratory and location:** Kowa Company, Ltd., Tokyo, Japan

**Date of study initiation:** May 15, 1992

**GLP compliance:** Unknown.

**QA reports:** yes (X) no ( )

**Drug, lot number, and purity:** 104P-9202, 99.2% purity

**Methods:**Strains/species/cell line: CD-1(ICR)(SPF) miceDoses used in definitive study:**MAIN STUDY – GROUP ASSIGNMENTS**

Group	Time after administration	No. of animals	
		Male	Female
Negative control (0.5% CMC-Na)	24 hrs	6 (6)	6 (6)
NK-104	250 mg/kg 24 hrs	8 (6)	6 (6)
	500 mg/kg 24 hrs	8 (5) [3]	6 (6)
	1000 mg/kg 24 hrs	12 (6)	12 (6)
Positive control (Cyclophosphamide 50 mg/kg)	24 hrs	6 (6)	6 (6)

(Sponsor, M4, RG25003, p12)

Basis of dose selection: Dosing was based on dose-finding experiments that indicated that 1000 mg/kg was the maximum tolerated dose for a single oral administration of NK-104 in CD-1 mice. A preliminary micronucleus assay was performed at 500 and 1000 mg/kg and euthanasia of study animals at 24, 48, and 72 hours to confirm the MTD and establish the optimal time of sample preparation.

Negative controls: The negative control was 0.5% carboxymethylcellulose sodium salt in water.

Positive controls: The positive control was 50 mg/kg cyclophosphamide in water.

Incubation and sampling times: Bone was removed 24 hours after administration of control or test substances. Cells were washed out and prepared on glass slides. Cells were fixed, stained, and washed. The numbers of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes was counted.

**Results:**

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Body weights of control- and test-substance-exposed animals were unaffected. The carboxymethylcellulose negative controls and cyclophosphamide positive controls were within the historical range for frequencies of MNPCE and RCT with each compound. Counting of micronucleated polychromatic erythrocytes (MNPCE frequency) and reticulocytes in erythrocytes (RCT frequency) were split between observers and two fields were counted per sample.

Study outcome: MNPCE frequency and RCT frequency were calculated. Tables 12 and 13 below show that NK-104 did not increase MNPCE frequency or RCT frequency.

**Table 12 Results of the main micronucleus test in Crj: CD-1 male mice after single oral administration of NK-104**

Group	Time after administration	Animal number	No. of RCT/Total a	No. of MNPE/PCE b
Negative control (0.5% OMC-Na)	24hours	201M	575/1000	2/2000
		202M	577/1000	2/2000
		203M	608/1000	2/2000
		204M	491/1000	0/2000
		205M	572/1000	4/2000
		206M	526/1000	1/2000
		Total	3349/6000	11/12000
		% (Mean ± S. D.) (55.8 ± 4.2)	(0.09 ± 0.07)	
		%(Max., Min.) (60.8, 49.1)	(0.20, 0.00)	
NK-104 250mg/kg	24hours	207M	558/1000	4/2000
		208M	527/1000	1/2000
		209M	661/1000	2/2000
		210M	629/1000	5/2000
		211M	485/1000	3/2000
		212M	400/1000	3/2000
		Total	3280/6000	18/12000
		% (Mean ± S. D.) (54.3 ± 9.6)	(0.15 ± 0.07)	
		%(Max., Min.) (66.1, 40.0)	(0.25, 0.05)	
NK-104 500mg/kg	24hours	213M	573/1000	1/2000
		216M	569/1000	3/2000
		218M	514/1000	2/2000
		238M	503/1000	4/2000
		240M	561/1000	4/2000
		Total	2720/5000	14/10000
		% (Mean ± S. D.) (54.4 ± 3.3)	(0.14 ± 0.07)	
		%(Max., Min.) (57.3, 50.3)	(0.20, 0.05)	
NK-104 1000mg/kg	24hours	219M	536/1000	1/2000
		220M	504/1000	4/2000
		221M	530/1000	5/2000
		222M	501/1000	3/2000
		223M	493/1000	3/2000
		224M	512/1000	2/2000
Total	3076/6000	18/12000		
		% (Mean ± S. D.) (51.3 ± 1.7)*	(0.15 ± 0.07)	
		%(Max., Min.) (53.6, 49.3)	(0.25, 0.05)	
Positive control (CP)	24hours	225M	684/1000	65/2000
		226M	412/1000	62/2000
		227M	535/1000	66/2000
		228M	370/1000	41/2000
		229M	582/1000	58/2000
		230M	587/1000	47/2000
		Total	3150/6000	339/12000
		% (Mean ± S. D.) (52.5 ± 11.3)	(2.33 ± 0.51)#	
		%(Max., Min.) (66.4, 37.0)	(3.30, 2.05)	

a: Number of reticulocytes / total number of erythrocytes observed  
 b: Number of micronucleated polychromatic erythrocytes / total number of polychromatic erythrocytes observed  
 CP: Cyclophosphamide  
 Significant at P<0.05(#:Kastenbaum & Bowman's table, \*:Student's t-test)

(Sponsor, M4, RG25003, p29)

**Table 13 Results of the main micronucleus test in Crj: CD-1 female mice after single oral administration of NK-104**

Group	Time after administration	Animal number	No. of RCT/Total a	No. of MN/PCE/PCE b
Negative control (0.5% CMC-Na)	24hours	201 F	682/1000	2/2000
		202 F	631/1000	2/2000
		203 F	719/1000	2/2000
		204 F	618/1000	2/2000
		205 F	573/1000	0/2000
		206 F	661/1000	1/2000
		Total	3884/6000	9/12000
		%(Mean±S.D.) (64.7±5.1)	(0.08±0.04)	
		%(Max.,Min.) (71.9,57.3)	(0.10,0.00)	
NK-104 250mg/kg	24hours	207 F	692/1000	2/2000
		208 F	607/1000	4/2000
		209 F	687/1000	3/2000
		210 F	644/1000	6/2000
		211 F	651/1000	2/2000
		212 F	583/1000	0/2000
		Total	3864/6000	17/12000
		%(Mean±S.D.) (64.4±4.3)	(0.14±0.10)	
		%(Max.,Min.) (69.2,58.3)	(0.30,0.00)	
NK-104 500mg/kg	24hours	213 F	628/1000	3/2000
		214 F	613/1000	1/2000
		215 F	674/1000	6/2000
		216 F	711/1000	0/2000
		217 F	643/1000	6/2000
		218 F	572/1000	0/2000
		Total	3841/6000	16/12000
		%(Mean±S.D.) (64.0±4.8)	(0.13±0.14)	
		%(Max.,Min.) (71.1,57.2)	(0.30,0.00)	
NK-104 1000mg/kg	24hours	219 F	711/1000	2/2000
		220 F	667/1000	3/2000
		221 F	643/1000	2/2000
		222 F	588/1000	2/2000
		223 F	376/1000	2/2000
		224 F	570/1000	3/2000
		Total	3553/6000	14/12000
		%(Mean±S.D.) (59.3±11.8)	(0.12±0.03)	
		%(Max.,Min.) (71.1,37.6)	(0.15,0.10)	
Positive control (CP)	24hours	225 F	714/1000	21/2000
		226 F	575/1000	24/2000
		227 F	571/1000	43/2000
		228 F	602/1000	41/2000
		229 F	671/1000	45/2000
		230 F	594/1000	54/2000
		Total	3727/6000	228/12000
		%(Mean±S.D.) (62.1±5.8)	(1.90±0.64) #	
		%(Max.,Min.) (71.4,57.1)	(2.70,1.05)	

a: Number of reticulocytes / total number of erythrocytes observed  
 b: Number of micronucleated polychromatic erythrocytes / total number of polychromatic erythrocytes observed  
 CP: Cyclophosphamide  
 #: Significant at P<0.05 in Kastenbaum & Dowman's Table

(Sponsor, M4, RG25003, p30)

**KW92117 – Micronucleus test of NK-104 repeated oral administration in rats**

**Key findings:**

- NK-104 was negative in an *in vivo* micronucleus assay in male rats at doses up to 30/50 mg/kg/day after 93 consecutive daily dose administrations (Day 1-35 at 50 mg/kg/day, and 30 mg/kg/day thereafter).

**Volume #, and page #:** M4, KW92117, p1-12

**Conducting laboratory and location:**

**Date of study initiation:** December 25, 1992

**GLP compliance:** No (study from which animals were obtained was GLP with QA statements.

**QA reports:** yes ( ) no (X)

**Drug, lot number, and purity:** 104P-9202, 99.2%

**Methods:**

Strains/species/cell line: Crj:CD(SD) rats from KW92083, Segment I, males only.

Doses used in definitive study: 0, 2, 10, 50/30 mg/kg/day from Segment I rat study; Day 1-35 at 50 mg/kg/day, and 30 mg/kg/day thereafter.

Basis of dose selection: Dose levels were determined from 1-month repeated dose tox study in rats, where reduced body weight gain was observed in male rats at 100 mg/kg.

**MICRONUCLEUS ASSAY IN RATS – GROUP ASSIGNMENTS**

Group	Administered substance	Dose (mg/kg/day)	Number of used animals
Control group	0.5% CMC	–	6
Low dose group	NK-104	2	6
Middle dose group	NK-104	10	6
High dose group	NK-104	30 (50) <sup>a)</sup>	6

[<sup>a</sup>Day 1-35 at 50 mg/kg/day; Day 36-93 at 30 mg/kg/day.]

(Sponsor, M4, KW92117, p4)

Negative controls: Negative control animals were administered 0.05% carboxymethylcellulose as part of the preceding rat segment I study.

Positive controls: No positive control was utilized in this study. For *in vivo* studies, it is not necessary to include concurrent treatments with positive controls in every study, after a laboratory has established competence in the use of the assay (ICH S2(R1)Draft).

Incubation and sampling times: Male rats were administered NK-104 daily by oral gavage for 93 consecutive days. Samples (5 samples from bilateral femur at autopsy) were removed, bone tips cut, and cells washed out, centrifuged and resuspended in FBS. Supernatant was removed again and cells were smeared onto a glass slide and dried. Cells were fixed in methanol.

**Results:**

Study validity (comment on replicates, counting method, criteria for positive results, etc.): No positive control was utilized in this study, leading to an inability for this reviewer to rule out false negative results.

Study outcome: There was no increase in micronucleated polychromatic erythrocytes (MNPCEs) at doses up to 30/50 mg/kg/day after daily administration of NK-104 by oral gavage for 93 days (Day 1-35 at 50 mg/kg/day, and 30 mg/kg/day thereafter).

**MICRONUCLEUS ASSAY IN RATS – RESULTS**

Compound	Dose (mg/kg/day)	Number of animals	Number of PCEs <sup>a)</sup> observed	MNPCE <sup>b)</sup>		Number of erythrocytes <sup>c)</sup> observed	PCE/E (%) <sup>d)</sup>	
				Number of cells	% (Mean ± S.D.)		Mean ± S.D.	Min. – Max.
0.5% CMC	0	6	6000	5	0.08 ± 0.08	6000	26.42 ± 1.69	24.20 – 28.60
	2	6	6000	4	0.07 ± 0.08	6000	26.98 ± 1.21	25.30 – 28.00
NK-104	10	6	6000	6	0.10 ± 0.09	6000	27.20 ± 1.98	24.60 – 30.10
	30(50)	6	6000	7	0.12 ± 0.12	6000	26.40 ± 1.38	24.00 – 27.90

<sup>a)</sup> Polychromatic erythrocytes  
<sup>b)</sup> Micronucleated polychromatic erythrocytes  
<sup>c)</sup> Total erythrocytes (polychromatic and normochromatic erythrocytes)  
<sup>d)</sup> The ratio of polychromatic erythrocytes to total erythrocytes

(Sponsor, M4, KW92117, p4)

**A819 – Single cell gel (SCG) assay of NK-104 with mice (Comet Assay)****Key findings:**

- NK-104 was negative for genotoxicity in an *in vivo/in vitro* Comet Assay after twice administering NK-104 in mice at 500 mg/kg.

Volume #, and page #: M4, 3140, 1-22

Conducting laboratory and location <sup>(b) (4)</sup>

Date of study initiation: May 15, 1992

GLP compliance: Yes.

QA reports: yes (X) no ( )

Drug, lot number, and purity: NK-104, NK011N06M, 99.9% purity

**Methods:**

Strains/species/cell line: Slc:ICR[SPF] mouse

Doses used in definitive study:

**COMET ASSAY IN MICE– GROUP ASSIGNMENTS**

Group	Dosage (mg/kg)	Administration times	Number of animals and animal ID No.
Vehicle control	10 mL/kg	2	5 [1001 to 1005]
	125	2	5 [1101 to 1105]
Test substance	250	2	5 [1201 to 1205]
	500	2	5 [1301 to 1305]
Positive control*	300	1	5 [1401 to 1405]

(Sponsor, M4, A819, p21)

**Basis of dose selection:** Dose selection was based upon results of study RFG2510, in which the lethal dose of NK-104 in mice was determined to be 1000 mg/kg after administering a single oral dose. Decreases in locomotor activity were noted at 500 in males. Therefore the high dose was set at 500 mg/kg in male mice in this Comet assay.

**Negative controls:** Negative controls were administered 0.5% carboxymethylcellulose in water.

Positive controls: Positive control animals received 300 mg/kg ethyl methylsulfonate (EMS)

Incubation and sampling times: Mice were administered vehicle or test article one on two consecutive days by oral gavage. The positive control was administered once. Liver, lungs, spleen, thymus were removed and cleaned with buffer, fixed and tissues homogenized.

**Results:**

Study validity: 150 cells per organ were examined. The Sponsor utilized a CCD camera and a Comet assay analyzer <sup>(b) (4)</sup> and Comet scoring was obtained by % DNA in the tail and the Olive tail moment. Statistical test was Dunnett multiple comparison test (two-sided significant level of 0.05) between negative control and each dose group and an Aspen-Welch t-test was utilized to compare each negative control group to each positive control group. When the positive control group markedly increased with a statistically significant difference from the negative control in terms of at least liver and lung, the entire study was considered valid.

Study outcome: NK-104 was negative for genetic toxicity in an *in vivo* single cell gel (Comet) assay that utilized cells from liver, lung, spleen, and thymus of male mice treated for two days.

Table 1. Single cell gel electrophoresis (SCG) assay with liver cells obtained from NK-104-treated mice [Male mice dosed once a day, for two days]

Compound	Dose (mg/kg, p.o.)	Organ	Number of animals	Number of cells analyzed	Olive tail moment (Mean ± S.D.)	% tail DNA (Mean ± S.D.)
0.5 w/v% CMC-Na a)	0		5	750	0.406 ± 0.101	3.126 ± 0.720
NK-104	125		5	750	0.458 ± 0.148	3.344 ± 1.043
	250	Liver	5	750	0.601 ± 0.083 *	4.028 ± 0.638
	500		5	750	0.471 ± 0.066	3.698 ± 0.442
	EMS b)	300		5	750	4.009 ± 0.699 #

\* : Significant difference from negative control (Dunnett test); p≤0.05  
 # : Significant difference from negative control (Aspin-Welch t test); p≤0.025  
 a) : Negative control (0.5 w/v% Carboxymethyl cellulose sodium salt aqueous solution, 10 mL/kg)  
 b) : Positive control (Ethyl methanesulfonate, 10 mL/kg)

(Sponsor, M4, A819, p37)

Table 2. Single cell gel electrophoresis (SCG) assay with lung cells obtained from NK-104-treated mice [Male mice dosed once a day, for two days]

Compound	Dose (mg/kg, p.o.)	Organ	Number of animals	Number of cells analyzed	Olive tail moment (Mean ± S.D.)	% tail DNA (Mean ± S.D.)
0.5 w/v% CMC·Na a)	0		5	750	0.884 ± 0.151	5.365 ± 0.817
NK-104	125		5	750	0.958 ± 0.169	5.826 ± 0.549
	250	Lung	5	750	0.872 ± 0.044	5.742 ± 0.207
	500		5	750	0.791 ± 0.158	5.350 ± 0.807
BMS b)	300		5	750	4.153 ± 0.714 #	21.149 ± 2.037 #

# : Significant difference from negative control (Aspin-Welch t test):p<0.025  
a) : Negative control (0.5 w/v% Carboxymethyl cellulose sodium salt aqueous solution, 10 mL/kg)  
b) : Positive control (Ethyl methanesulfonate, 10 mL/kg)

(Sponsor, M4, A819, p38)

Table 3. Single cell gel electrophoresis (SCG) assay with spleen cells obtained from NK-104-treated mice [Male mice dosed once a day, for two days]

Compound	Dose (mg/kg, p.o.)	Organ	Number of animals	Number of cells analyzed	Olive tail moment (Mean ± S.D.)	% tail DNA (Mean ± S.D.)
0.5 w/v% CMC·Na a)	0		5	750	0.558 ± 0.055	5.565 ± 0.452
NK-104	125		5	750	0.516 ± 0.065	5.171 ± 0.340
	250	Spleen	5	750	0.531 ± 0.109	5.280 ± 0.735
	500		5	750	0.514 ± 0.036	5.245 ± 0.296
EMS b)	300		5	750	2.604 ± 0.603 #	15.821 ± 2.277 #

# : Significant difference from negative control (Aspin-Welch t test):p<0.025  
a) : Negative control (0.5 w/v% Carboxymethyl cellulose sodium salt aqueous solution, 10 mL/kg)  
b) : Positive control (Ethyl methanesulfonate, 10 mL/kg)

(Sponsor, M4, A819, p39)

Table 4. Single cell gel electrophoresis (SCG) assay with thymus cells obtained from NK-104-treated mice [Male mice dosed once a day, for two days]

Compound	Dose (mg/kg, p.o.)	Organ	Number of animals	Number of cells analyzed	Olive tail moment (Mean ± S.D.)	% tail DNA (Mean ± S.D.)
0.5 w/v% CMC·Na a)	0		5	750	0.408 ± 0.040	4.005 ± 0.465
NK-104	125		5	750	0.437 ± 0.088	4.253 ± 0.577
	250	Thymus	5	750	0.417 ± 0.071	4.017 ± 0.731
	500		5	750	0.500 ± 0.029	4.788 ± 0.170
EMS b)	300		5	750	2.087 ± 0.240 #	14.128 ± 1.442 #

# : Significant difference from negative control (Aspin-Welch t test):p<0.025  
a) : Negative control (0.5 w/v% Carboxymethyl cellulose sodium salt aqueous solution, 10 mL/kg)  
b) : Positive control (Ethyl methanesulfonate, 10 mL/kg)

(Sponsor, M4, A819, p40)

2.6.6.5 Carcinogenicity

**SPONSORS SUMMARY OF CARCINOGENICITY ASSESSMENT**

Table 14. NK-104 Carcinogenicity Studies

Study Number	Species	Route and Treatment Duration	Dose (mg/kg/day)	Key Findings
KOW 14/952398	Mouse	Oral 13 weeks	25, 75, 225	<ul style="list-style-type: none"> <li>Mortality and gastric lesions at 225 mg/kg. Selected dose levels for subsequent study &lt;225 mg/kg</li> </ul>
KOW 16/982522	Mouse	Oral 92 weeks	1, 12, 30, 75	<ul style="list-style-type: none"> <li>No carcinogenicity attributed to NK-104 ≤75 mg/kg in mice</li> </ul>
KOW 12/942992	Rat	Oral 13 weeks	10, 30, 50	<ul style="list-style-type: none"> <li>Thyroid follicular cell hypertrophy at 30 and 50 mg/kg; selected dose levels for subsequent study &lt;30 mg/kg</li> </ul>
KOW 13/971903	Rat	Oral Females: 92 weeks Males: 104 weeks	1, 5, 25	<ul style="list-style-type: none"> <li>Increased incidence of follicular tumors of thyroid (p&lt;0.001) in males treated with NK-104 25mg/kg/day</li> </ul>
RF9811	Rat	Oral 7, 14, and 28 days	25, 50, 75	<ul style="list-style-type: none"> <li>Mortality at 75 mg/kg</li> <li>Increases in T<sub>4</sub> in rats treated with 50 mg/kg ≥7 days</li> <li>Increases in TSH, thyroid weight, and T<sub>4</sub> UDP-GT in rats treated with 50 mg/kg ≥14 days</li> <li>Increases in T<sub>4</sub>, TSH, and T<sub>4</sub> UDP-GT in rats treated with 25 mg/kg, but at a lesser degree than at 50 mg/kg..</li> </ul>
RF9812	Rat	Oral 14 and 28 days	5	<ul style="list-style-type: none"> <li>No changes in T<sub>4</sub>, TSH, and thyroid weight at ≥14 days</li> <li>No changes in T<sub>4</sub> UDP-GT at 28 days</li> <li>No effect dose on thyroid gland 5 mg/kg/day</li> </ul>
G2523	Rat	Oral 15 weeks	Pretreatment with DHPN and then: 1, 5, 25, and 25 + T <sub>4</sub> 7.5µg, 25 + T <sub>4</sub> 15µg	<ul style="list-style-type: none"> <li>No promoting effect for thyroid carcinogenesis observed at dose levels 1 and 5 mg/kg</li> <li>Promoting effect (increase in follicular cell hyperplasia) for thyroid carcinogenesis observed at 25 mg/kg</li> <li>No promoting effect observed in rats treated with NK-104 25 mg/kg combined with T<sub>4</sub>.</li> </ul>

(Sponsor, M4)

<b>Dose-limiting Toxicities in Carcinogenicity Studies</b>			
<b>Duration/Type of study</b>	<b>Dose/pre-neoplastic findings</b>	<b>Dose/neoplastic findings</b>	<b>Multiple of human exposure at 4 mg HED</b>
<b>92-Week mouse</b>	75 mg/kg/day/Benign squamous cell papillomas in females (LOAEL)	75 mg/kg/day/No drug-related tumors at highest dose (NOAEL)	26x
<b>92-Week rat</b>	25 mg/kg/day/Benign squamous cell papillomas in males (LOAEL)	25 mg/kg/day/Thyroid follicular cell adenoma and carcinomas, forestomach carcinomas, males (LOAEL)	295x
<b>26-Week Tg rasH2</b>	30 mg/kg/day/Forestomach hyperplasia (LOAEL)	150 mg/kg/day/No drug-related tumors at highest dose (NOAEL)	19x and 97x, at 30 and 150 mg/kg/day
<b>26-Week Tg rasH2 + urethane</b>	30 mg/kg/day/Forestomach hyperplasia (LOAEL)	150 mg/kg/day/No drug-related tumors at highest dose (NOAEL)	19x and 97x, at 30 and 150 mg/kg/day

**KOW 16/982522 – NK-104 potential tumorigenic effects in repeated oral gavage administration to mice**

**Key study findings:**

- NK-104 was not carcinogenic in mice at doses up to 75 mg/kg for 92 weeks.
- No significant changes in clinical signs, body weight, food consumption, and ophthalmology.
- Higher mortality at 75 mg/kg during the first 6 months.
- Higher incidence of skeletal muscle myofiber atrophy and epithelial hyperkeratosis of forestomach in NK-104 treated animals.
- $C_{max}$  and  $C_{min}$  increased greater than proportionally with dose. Females appeared to have higher values than males.
- No significant increase in neoplastic lesions.

Adequacy of the carcinogenicity study and appropriateness of the test model: In the 13-week study, NK-104 caused animal death in females at 225 mg/kg. Body weight gain was reduced 20% in males at 75 mg/kg.

In the carcinogenicity study at doses of 1, 12, 30, and 75 mg/kg/day, the study was terminated at 92 weeks per study protocol. No significant change was noted in clinical signs, food consumption, and ophthalmology. Body weight gain was reduced in high dose groups (15% in females at 52 weeks and 13% in males at 92 weeks). Higher incidence of skeletal muscle myofiber atrophy and epithelial hyperkeratosis of forestomach was observed in treated animals.

Evaluation of tumor findings: The Committee felt that dose selections in the 92-week study were adequate in the female, but questionable in the male.

**RFG2515 - Measurement of the plasma concentrations of NK-104 in a 26-week repeated oral dose toxicity study of NK-104 in cynomolgus monkeys, followed by an 8-week recovery period**

Total exposure (AUC) to NK-104 in males was 1 to 3-fold higher than that observed in female monkeys at the same dose per body weight. Total exposure to NK-104 lactone was also 1 to 3-fold higher in males than in female monkeys at the same dose per body weight.

<b>Toxicokinetics of Unchanged NK-104 after Daily Administration of NK-104 to Cynomolgus Monkeys by Oral Gavage for 6 Months</b>									
Day	Dose (mg/kg)	$T_{max}$ (h)		$C_{max}$ (ng/mL)		AUC (ng-hr/mL)		$T_{1/2}$ (h)	
		♂	♀	♂	♀	♂	♀	♂	♀
1	0.5	1.1	1.4	12	7	98	51	5.2	16.1
	1	0.6	0.9	33	29	232	142	7.3	6.7
	3	0.8	1.0	114	68	796	341	5.0	6.4
	6	0.8	1.2	512	459	1383	1291	2.6	2.3
28	0.5	0.9	1.5	9	4	60	19	7.6	5.8
	1	2.9	1.6	20	11	233	73	7.7	5.0
	3	2.4	1.4	165	116	1313	489	4.9	3.1
	6	0.7	1.5	322	196	1573	1275	5.5	4.4
91	0.5	0.6	1.3	11	6	70	46	6.2	6.7
	1	0.9	2.8	23	19	164	114	6.7	6.4
	3	1.4	1.3	93	57	542	336	6.1	7.9
	6	0.9	1.5	187	139	932	893	3.8	4.1
182	0.5	1.9	0.6	8	7	89	51	8.4	16.4
	1	2.5	0.8	35	43	492	382	7.3	7.2
	3	1.1	0.6	110	66	704	459	2.9	3.3
	6	1.3	1.4	240	217	1468	1320	3.0	4.7