CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-187

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-187**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**7/18/2007**
PRODUCT:	**Intelence® (Etravirine)**
INTENDED CLINICAL POPULATION:	**Treatment of HIV infection**
SPONSOR:	**Tibotec, Inc**
DOCUMENTS REVIEWED:	**Electronic**
REVIEW DIVISION:	**Division of Antiviral Products (HFD-530)**
PHARM/TOX REVIEWER:	**Kuei-Meng Wu, PhD**
PHARM/TOX SUPERVISOR:	**Hanan Ghantous, PhD, DABT**
DIVISION DIRECTOR:	**Debra Birnkrant, MD**
PROJECT MANAGER:	**Anne Marie Russell, Ph.D.**

Date of review submission to DFS: 1/15/2008

PHARMACOLOGIST'S REVIEW ON NDA 21-567 (Etravirine)

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Yes, the sponsor has provided sufficient nonclinical safety information on etravirine in support of approval for marketing in the U.S.

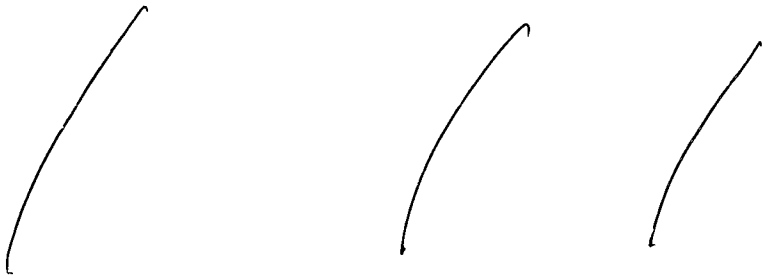
B. Recommendation for nonclinical studies

None

C. Recommendations on labeling

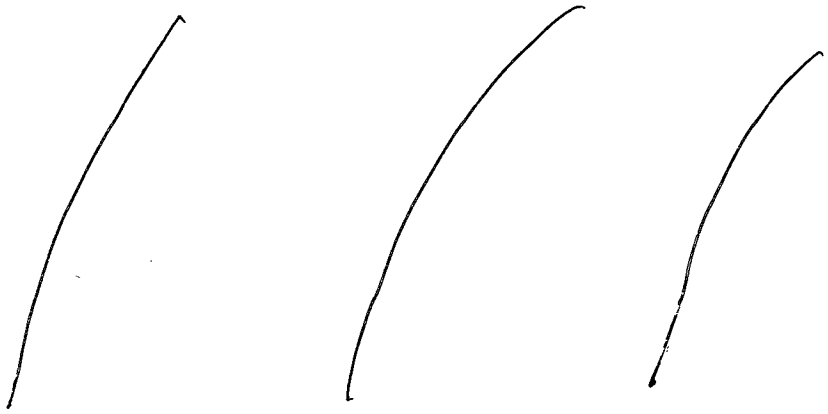
The nonclinical Pharmacology/Toxicology portion of the sponsor's drug product labeling is modified below:

8 Use in Specific Populations
8.1 Pregnancy



Pregnancy Category B
There are no adequate and well-controlled studies of INTELENCE use in pregnant women. In addition, no pharmacokinetic studies have been conducted in pregnant patients. Animal reproduction studies in rats and rabbits at systemic exposures equivalent to those at the maximum recommended human dose of 400 mg revealed no evidence of fetal harm. INTELENCE should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Nonclinical Toxicology (13.2)]

13 NONCLINICAL TOXICOLOGY



13.1 Carcinogenesis, mutagenesis, impairment of fertility

Carcinogenesis and Mutagenesis: Carcinogenicity studies of etravirine in rodents are ongoing. Etravirine tested negative in the *in vitro* Ames reverse mutation assay, *in vitro* chromosomal aberration assay in human lymphocyte, and *in vitro* clastogenicity mouse lymphoma assay, tested in the absence and presence of a metabolic activation system. Etravirine did not induce chromosomal damage in the *in vivo* micronucleus test in mice.

Impairment of Fertility: No effects on fertility and early embryonic development were observed when etravirine was tested in rats at maternal doses up to 500 mg/kg/day, resulting in systemic drug exposure up to the maximum recommended human dose (400 mg/day).

13.2 Animal Toxicology and/or Pharmacology

Reproductive Toxicology Studies: Developmental toxicity studies were performed in rabbits (at oral doses up to — mg/kg/day) and rats (at oral doses up to — mg/kg/day). In both species, no treatment-related embryo-fetal effects including malformations were observed. In addition, no treatment-related effects were observed in a separate pre- and postnatal study performed in rats at oral doses up to 500 mg/kg/day. The systemic drug exposures achieved in these animal studies were equivalent to those at the maximum recommended human dose.

II. SUMMARY OF NONCLINICAL FINDINGS

A. BRIEF OVERVIEW OF NONCLINICAL FINDINGS

The sponsor had employed conventional species of rats, mice and dogs as the surrogates for etravirine's toxicity profile exploration, in assisting human risk prediction during the pre-market drug development

maximally achievable or feasible. The liver is considered to be the primary target organ of toxicity across all species. Other toxicities such as thyroid toxicity in rats, and clotting abnormalities and the associated cardiac toxicities in mice are considered to be species-specific, because further investigations showed no such findings occurred in others, including humans. The choice of the rat as a human safety predicting species might have been compromised by the thyroid toxicity. However, there is no effort made to employ alternative animal species for toxicity exploration during the IND phase. In regard to genotoxicity and reproductive toxicity, the drug has been tested negative in a series of in vivo and in vitro studies. In summary, while additional toxicity testing on etravirine are still ongoing (e.g., carcinogenicity studies in both mice and rats), it is concluded that this NDA has provided sufficient nonclinical safety information in support of its approval for marketing in the U.S.

The nonclinical targets and profile of toxicity are discussed in more detail in the following:

TOXICITY TARGETS AND PROFILE

Major toxicity findings and key target organs/systems of toxicity were identified in a series of repeat-dose toxicity studies conducted in rodents, and dogs, as highlighted below. For safety margin estimation purpose, an average daily clinical exposure of 7.4 ug.h/ml ($AUC_{0\text{-}24h}$; abbreviated as AUC below) as provided by the sponsor is used here as a reference or comparator for risk assessment.

1. HEPATO-TOXICITY

The liver toxicity profile of etravirine in rats, dogs and mice is summarized below based on pivotal studies submitted. The toxicological implications, no-adverse-effect level (NOAEL), and the associated systemic drug exposure (AUC) of each study are provided as follows:

Hepatotoxicity profile in rodents include: liver organ weight increases or hepatocellular hypertrophy/vacuolation/necrosis and/or liver enzyme elevations (3-month mouse gavage study NOAEL=10 mg/kg AUC=1.1-1.4 ug.h/ml; 3-month mouse diet study [No NOAEL] low dose AUC=1.5-2.4 ug.h/ml; 1-month transgenic mouse study NOAEL=50 mg/kg AUC=1.2 ug.h/ml; 3-month rat gavage study NOAEL=70 mg/kg AUC=0.8-1.1 ug.h/ml; 3-month rat diet study [No NOAEL, low dose=330 mg/kg AUC=0.9-2.4 ug.h/ml; 6-month rat gavage study NOAEL=200 mg/kg AUC=1.6 ug.h/ml♀ [liver weight increases without significant liver enzyme elevations in rats]). The margin of safety (MOS) as compared with an averaged daily human exposure (7.4 ug.h/ml) is approximately 1/7-2/7 using the rat model.

The severity of hepatotoxicity in rodents increased with dose, with organ weight/liver enzyme elevations as high as 2.4-fold/9 fold in mice and to a lesser extent in rat (up to 20% increase in liver weight). In mice, the increases in organ weight and hypertrophy proceeded before enzyme elevations that emerged at higher dose or longer duration. Although liver weight increases were not accompanied with remarkable enzyme changes in rats, the hypertrophy show a pathological fatty vacuolation at the cellular level. Please also note that these findings in rodents were not accompanied by

	significant bilirubin increases. In the dog, liver enzyme elevations (ALP, ALT, up to 3-fold; reversible) were accompanied by bilirubin increases (2.9-fold in 1-month, 79% in 6-month study) and bile inspissation (i.e., thickening of bile) was reported. Histologically, microgranuloma (very small nodular inflammatory lesion containing grouped monocytes) occurred in the liver (1-month dog study NOAEL=160 mg/kg AUC= 54 ug.h/ml; 6-month dog study NOAEL=160 mg/kg AUC=50 ug.h/ml.) Liver toxicity occurred at much higher drug exposures in the dog than those in the mouse or rat. The MOS is estimated around 7 using the dog model. In humans, liver enzyme elevations (AST/ALT 2%-3%, grade 3-4) in clinical trials are listed as ADRs in the proposed drug's labeling.
2. RENAL TOXICITY	Focal renal tubular basophilia, and high urinary excretion on Na, Cl, Ca, total protein, higher plasma K were reported in the 3-month rat study (≥200 mg/kg for clinical chemistry, 600 mg/kg for histopathology, AUC=0.6-1 ug.h/ml). In the 6-month rat study, no renal findings (except an increased urine volume) were reported, probably due to poor drug exposures. A marginal increase in focal basophilic tubules was also reported in a 3-month precarcinogenicity study conducted in mice (females at 800 mg/kg). Renal toxicities were not observed in the dog. In humans, no significant abnormal renal function test results were reported in human trails.
3. THYROID TOXICITY	Follicular cell hypertrophy and hyperplasia were reported in the rat (3- and 6-month studies NOAEL=70 mg/kg AUC=0.21[M]-0.66[F] ug.h/ml). The increases in TSH, and decreases in T4 and T3 were also reported in the 3-month rat study. The toxicity appeared to be secondary to the enhanced hepatic clearance of T4 as T4-metabolizing enzymes in liver, including CYP3A, CYP2B and UDP- glucuronosyl transferase, were shown induced in additional mechanistic studies. No thyroid toxicity has been reported in any dog or mouse studies to this date. In humans, no changes in T4, T3 or TSH were observed in the Phase IIb trials. The sponsor indicated that T4/T3/TSH data analysis on Phase III C206 and C216 trials are ongoing.
4. PITUITARY TOXICITY:	Hypertrophic and vacuolated cells of anterior lobe were seen in male rats (3-month study, NOAEL=200 mg/kg AUC=0.7ug.h/ml, male). This finding is considered to be related to the feedback response (high TSH activities) induced by thyroid toxicity, occurred specifically in the rat (no pituitary findings in 6-month rat study due to poor drug exposures).

5. CARDIAC TOXICITY & CLOTTING ABNORMALITIES

In a 3-month diet study, hemorrhagic cardiomyopathy and hemothorax (degeneration/necrosis, myolysis, interstitial hemorrhages [subendocardial and/or subepicardial], fibroblast proliferation, pericarditis and hemosiderosis; atria and ventricles) occurred in male mice at ≥450 mg/kg (NOAEL=200 mg/kg AUC=2.35 ug.h/ml; MOS=0.3). In another gavage study (3-month), hemorrhages and multifocal necrosis/degeneration, pericarditis, myocarditis and endocarditis occurred in 1 male mouse at 800 mg/kg gavage (NOAEL=200 mg/kg; AUC=3.9 ug.h/ml).

Hemorrhagic cardiomyopathy appeared to be related to TMC125 induced clotting abnormalities (↑3.9X in PT/>100sec in APTT during moribund; ↑PT/APTT up to 72/56 and 34/51% in other males and females, respectively) in the mouse, and was correlated with elevated troponin levels, a biomarker of cardiac injury, as monitored during the mechanistic studies. Troponin was undetectable in those male mice that did not develop hemorrhagic heart and there was no clear evidence that troponin elevations preceded cardiac injury suggesting that troponin release was result of cardiomyopathy and that there was no evidence of direct cardiac injury by the drug.

Clotting abnormalities were also reported in a 3-month rat diet study, at a lesser extent and occurred primarily in males (↑PT/APTT up to 22/61 and -13/+16% for males and females, respectively), without any similar cardiac histopathology findings. The sponsor claimed that coagulopathy induced hemorrhagic cardiomyopathy is a mouse-specific toxicity, partly due to the animal's high intrinsic heart rate (500-700 bpm). However, how clotting disorders, which led to a cardiac-specific injury, occurred in male mice only could not be reasonably explained with certainty.

No clotting abnormalities or cardiac injury were reported in any dog study.
In clinical trials (e.g., Phase IIb C203 and C223) no significant changes in APTT/PT occurred and no such events were included in the proposed labeling.

6. HEMATO-TOXICITY

Increases in platelet counts were reported in mice (males, 20-30% in both 3-month diet and gavage studies), without thrombus formation detected. This finding may be related to coagulopathy and associated hemorrhagic cardiomyopathy in the male mice discussed above. Decreases in reticulocyte counts in mice were also reported in a 3-month gavage study (NOAEL=200mg/kg AUC=4.3ug.h/ml). Decreased reticulocyte counts was reported in a 1-month dog study (unremarkable in the 3-, 6- and 12-month studies) that had higher drug exposures (NOAEL=160mg/kg AUC=54ug.h/ml) than other dog studies. In the rat, hematotoxicity is unremarkable all studies, except one (3-month gavage) that showed increases in neutrophil (up to 89%) and monocytes (48%) and decrease in eosinophil counts (40%). Thymus/lymph nodes (popliteal) and bone marrow (sternum) atrophy reported in a one-month dog study (NOAEL=160mg 53 ug.h/ml) that was claimed to be due to body weight loss in the high dose animals.

In humans, ADRs on RBC, neutrophil and platelet counts were similar between drug-treated and placebo groups in DUET-1 and DUET-2 clinical trials, as reported in the proposed drug's labeling.

7. DERMATOLOGICAL TOXICITY

Erythema and alopecia were seen in dogs (NOAEL 20 mg/kg AUC=11 ug.h/ml, 3-month study; NOAEL 20 mg/kg AUC=13 ug.h/ml, 6-month study, not reversible). No skin-related toxicity findings were reported in other animal species.

In humans, skin rash and erythema were among the major AEs reported and are listed in the drug's labeling.

8. OTHER GENERAL TOXICITIES:

Other potential animal toxicities reported in this NDA included:
GI Toxicity: Dose-related emesis, decrease in body weight and food consumption in dogs (40 mg/kg and above, spray-dried) and decreased body weight in rats (600 mg/kg and above, spray-dried) were reported.

9. GENOTOXICITY:

Etravirine tested negative in bacterial reverse mutation assays, mouse lymphoma assays, chromosome aberration assays (in vitro; human lymphocytes; in vivo: mouse micronucleus test). The drug is thus considered to be not genotoxic to DNA or chromosomes.

10. TERATOLOGY, MATERNAL, EMBRYONIC AND FETAL TOXICITY

Reproductive toxicology of etravirine was investigated in rats and rabbits. The key findings are as follows.

Rat.
In fertility studies, etravirine at systemic exposure levels up to 5 ug.h/ml for males and 9 ug.h/ml for females (500 mg/kg), showed no remarkable effect on estrus cycle, mating/pregnancy rate, number of corpora lutea, implantations and live fetuses.
In embryo-fetal studies, etravirine at similar systemic exposure levels (8.3 ug.h/ml for pregnant females) did not produce significantly effects on gravid uterine weight, pregnancy rate, number of corpora lutea, number of live or lost implantations, fetal body weight/sex ratio or fetal organogenesis.

In a separate pre- and postnatal developmental toxicity study in rats, no significant findings on gestation, parturition or the numbers of pups born, the sex ratio, or pup survival were reported. Pup body weight was higher in drug-treated animals and the developmental landmarks occurred earlier in comparison with control pups. Clinical condition, sensory function/reflexes, behavior and reproductive performance in F1 pups were unremarkable (NOAEL=500 mg/kg, AUC=12.1[GD 7]-3.6[GD 17] ug.h/ml).

Rabbits.
Etravirine caused maternal toxicity in rabbits (i.e., decreased body weight gain; reduced food consumption) (NOAEL 125 mg/kg: 6.1-7.6 ug.h/ml). No teratogenic findings were reported at the maximally feasible and maternally toxic doses (375 mg/kg, AUC=9.7-12.3[GD 6]-9.7[GD 18] ug.h/ml.)

Conclusions: No significant teratologic, maternal or fetal findings were reported from studies conducted on etravirine in pregnant rats or rabbits at the doses tested. There is limited margin of safety in regard to reproductive toxicity for etravirine, as based upon the exposure data (AUC) listed above, because of feasibility of dosing (ref. human AUC= 7.4 ug.h/ml). The sponsor proposed a Pregnancy Category B labeling without achieving sufficient overage of human exposure levels. However, the dosing in animals reached maximal feasible dose (MFD). According to relevant regulatory documents, results obtained at MFD maternal exposure are acceptable for labeling purpose.

11. CARCINOGENICITY

Studies are ongoing.

12. TOPICAL TOXICITIES

TMC125 was classified as a "mild" eye irritant, and the (TMC125 as a "very severe" eye irritant as studied by the bovine corneal opacity and permeability assays. The current final drug product used the spray-dried TMC125 form in the manufacturing processes.
TMC125 form) tested negative in other regular skin sensitization, irritation and phototoxicity assays.

13. ROLE OF DRUG METABOLITES IN TOXICITY EXPRESSION

Contributions of drug metabolites to the overall toxicity profile are comparably small and generally equivalent among species. Approximately 100% of the drug, in rats, mice or dogs, was eliminated in the feces and more than 80% was excreted as unchanged form (60% in mice). Further, renal elimination was limited (<1% excreted in urine and no unchanged drug was found in urine of the 3 species studied.). The minor contribution of metabolic elimination to the overall disposition of etravirine in rats, mice or dogs has been proposed by the sponsor in the IND. Human metabolic profile appears to be similar to that of animals (see Pharmacokineticist's Review). The percentages of etravirine metabolized in the mouse, rat, dog and human are listed in the table below:

Metabolic pathway	Mouse		Rat		Dog	Human
	Male	Female	Male	Female	Male	Male
Methyl hydroxylation (M8, M11[a], M12)	8.0	6.5	11.3	6.8	9.7	3.8-9.0
Aromatic hydroxylation (M10, M13, M14, M15)[b]	4.3	4.3	< 1.3	< 1.3	5.3	0-0.2
Hydroxylation + glucuronidation (M1,M6, M7[c], M9[c])	1.1	1.2	< 0.03	0.04	< 0.7	0.2-0.8
Unchanged TMC125	57.5	58.0	85.1	87.4	81.9	81.2-86.4

Total Percentage	70.9	70.0	97.7	95.5	97.6	85.2-96.2

[a] the location of the second oxidation site is not known; [b] at either the dimethylbenzonitrile (M10, M13) or the (cyanophenyl)amine (M14, M15) moiety; [c] The location of the oxidation in the glucuronidated metabolites M7 and M9 is not known.

14. SAFETY OF PRODUCT IMPURITIES AND INTERMEDIATES

All impurities and intermediates tested negative in genotoxicity assays, except — an intermediate) which was positive in the chromosome aberration test (hamster v79 cells) — tested negative in the AMES and the in vivo micronucleus assay. Please see CMC review in regarding to recommendations on controlling amount of drug intermediate — in the final drug product —

B. PHARMACOLOGIC ACTIVITY :

Please see Microbiologist's review.

C. NONCLINICAL SAFETY ISSUES RELEVANT TO CLINICAL USE:

See Executive Summary.

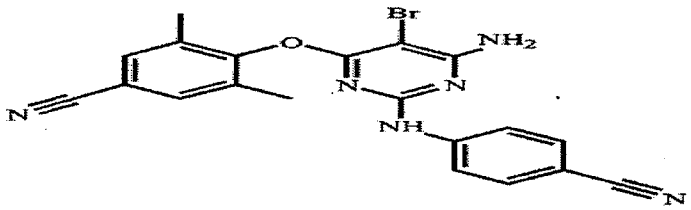
NOTE TO FOI REVIEWERS:

Please redact all text starting from this point (page 11) to end of page 101, and redact all text starting page 103 to end of this document (p. 105).

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 Introduction and Drug History

This NDA is originated from an ongoing IND 63,646 that was initially submitted on 9/3/98. The nonclinical program of this NDA consisted of animal toxicity studies conducted in rodents, dogs, and rabbits in support of the intended human uses. Portion of the toxicity studies have been reviewed under IND 63,646 (see Pharmacologist's Reviews on original IND and its amendments). This document reviews the up-to-date nonclinical pharmacology/toxicology study reports and provides summary and comments on overall nonclinical safety information and the proposed labeling on etravirine. Basic registration information related to this drug including the timeline and basic chemical is listed below:

NDA NUMBER:	**22-187**
SEQ./DATE/TYPE:	000/ July 18, 2007/Original NDA
INFO. TO SPONSOR	Yes (x) No ()
SPONSOR	Tibotec, Incorporation
MANUFACTURER	Same as above
DIVISION NAME:	DAVDP
HFD #:	HFD-530
COMPLETION	12/12/07
REVIEWER	Kuei-Meng Wu
TRADE NAME:	Intelence®
GENERIC NAME	Etravirine
CODE NAME	TMC 125; R165335 (final solid dosage form, R165335/F060)
CHEMICAL NAME	4-[[6-amino-5-bromo-2-[(4-cyanophenyl)amino]-4-pyrimidinyl]oxy]-3,5-dimethylbenzonitrile
FORMULA/MW	$C_{20}H_{15}BrN_6O$/435.28
STRUCTURE	
RELATED INDS	63,646
DRUG CLASS:	Antiviral

INDICATION: Treatment of HIV infection

FORMULATION: 100 mg oval tablet

Table 1: Composition of TMC125 100-mg Tablets (R165335/F060)

Component	Reference to Quality Standard	Function	Quantity per Unit (mg/tablet)
Spray Dried Powder	(R165335/F051)		
TMC125	Non-compendial	Active ingredient	100.0
Hypromellose (HPMC)[a]	USP		
Microcrystalline cellulose[b]	NF		
Colloidal silicon dioxide	NF		
Croscarmellose sodium	NF		
Magnesium stearate	NF		
Lactose monohydrate	NF		
Total Tablet Weight:			800

[a] Also referred to as 'HPMC' in the dossier

ROUTE: Oral; 200 mg bid (400 mg qd)

PROPOSED USE: HIV Infection

DISCLAIMER: Tabular and graphical information is from sponsor's submission unless stated otherwise.

LIST OF NONCLINICAL SAFETY STUDIES REVIEWED AND NOT REVIEWED

Animal toxicity studies conducted with etravirine include

- Single-dose toxicity studies in rats, mice and dogs
- Repeat-dose toxicity studies in rats, mice and dogs
- Pre-carcinogenicity rangefinding studies in rats and mice
- Ongoing carcinogenicity studies in rats and mice
- Reproductive toxicity studies in rats, and rabbits
- *In vitro* and *in vivo* mutagenicity assays
- Special toxicity studies

All nonclinical studies that were included in this NDA have been filed under IND 63,646. A detail list of nonclinical pharm/tox studies that are not reviewed is attached in Appendix.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

TMC125 (etravirine) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) for HIV-1 infection. The in vitro EC50 for wild type HIV-1 is 1.4 nM at cytotoxic concentration (CC_{50}) of > 100 uM. TMC125 showed in vitro antiviral activity against

recombinant clinical isolates, resistant to all currently approved NNRTIs, with EC_{50} below 10 nM for 76% of isolates.

Secondary pharmacology and safety pharmacology of TMC125 have been studied in acutely administered manner, as a single dose, in dogs, rats, and mice for basic pharmacology screening on cardiovascular/autonomic, respiratory, renal, CNS and GI functions. The drug did not, in general, exhibit unremarkable effects in the test systems used and parameters measured, including cardiac K channels and cardiac action potential characteristics, except a mild inhibitory effects on nicotinergic nerve-smooth muscle contraction, glycine receptor binding (both at uM concentrations).

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Please see Microbiologist's review.

Drug activity related to proposed indication: Please see Microbiologist's review.

2.6.2.3 Secondary pharmacodynamics

Please see Microbiologist's review.

2.6.2.4 Safety pharmacology

CNS EFFECTS:	Effects of TMC125 on overt behavior, reflexes and other body functions were studied after oral administration in rats. In a general observation test, TMC125 (80 mg/kg, p.o.), in a PEG400 formulation, did not affect overt behavior, palpebral opening, pupil diameter, body temperature, the pinna reflex, the cornea reflex, the tail withdrawal response to hot water, muscle tone, pentylenetetrazole-induced convulsions, respiration rate, the color of the ears, or mortality. Protection against castor oil-induced diarrhea was observed both after TMC125 and the solvent in 3 out of 5 tested rats. In motor activity cages, TMC125 did not affect motor activity (ED50: > 80 mg/kg, p.o.). TMC125 did not interact with methohexital-induced hypnosis (ED50: > 80 mg/kg, p.o.). Pronounced solvent-related effects may have masked effects on gastric emptying. TMC125 inhibited nicotine-induced contractions at 10^{-5} and 10^{-6} M but not at 10^{-7} M. Electrical field stimulation-induced contractions were not affected at concentrations up to 10^{-5} M. It is concluded that TMC125 has inhibitory effects on nicotinergic nerve-smooth muscle function, (at uM levels).
CARDIO-VASCULAR EFFECTS	At concentrations up to 10 uM, TMC125 has no relevant effect (<20% inhibition) on a membrane potassium current (hERG assay). In isolated papillary muscles of guinea pig hearts, TMC125 at concentrations of $1x10^{-8}$ M, $1x10^{-7}$ M, $3x10^{-7}$ M and $1x10^{-6}$ M has no or only minor effects on electrophysiological parameters such as the action

potential duration (5% increase of APD50 versus 10% increase with solvent; no effect on APD20 or APD90), the amplitude of the action potential (3% decrease versus 6% increase with solvent), the resting membrane potential, the maximal rate of rise of action potential and contractile force (no effect).
Compared to solvent (n=4), a single oral dose of 10 mg/kg of TMC125 (n=4) in PEG400 had no effect on the following cardiovascular parameters, monitored throughout a 240 min observation period after medication (no significant changes versus solvent): LVdp/dt max, cardiac output and the ECG (PQ, QRS, QT, QTc Bazett, QTc Fridericia, QTc Janssen). A slight tendency for an increase was noted on systolic blood pressure (not significant versus solvent), diastolic blood pressure ($p< 0.05$ versus solvent at 15 min only), LVdp/dt max/p (not significant versus solvent), LVdp/dt min ($p< 0.05$ versus solvent at 45 min only) and total systemic resistance (not significant versus solvent) during the first 2h after the administration of the compound (median peak effect: +17%, +10%, +14%, +23% and +13%, respectively). After the administration of the compound, a trend for increase in heart rate (median peak effect: + 26%; $p <0.05$ versus solvent at 105 min only), pressure rate product (median peak effect: +42%, $p <0.05$ versus solvent at 105 min only) and a trend for a decrease on relaxation time constant and stroke volume (median peak effect: -27% and -24%; not significant versus solvent) were observed. No changes in behavior were noted in 4 out of 4 dogs in the solvent treated group and in 3 out of 4 dogs in the group medicated with TMC125. Trembling was observed in one dog of the group medicated with TMC125. Median plasma levels of TMC125 were 51, 187 and 226 ng/ml at respectively 30, 60 and 240 min after the oral administration of the compound.
Compared to solvent (n=6), a single oral dose of 40 mg/kg of TMC125 (n=6) in PEG400 had no effect on the following cardiovascular parameters monitored throughout a 240 min observation period (no significant changes versus solvent): heart rate, systolic and diastolic blood pressure, pressure rate product, LVdp/dt max, LVdp/dt min, cardiac output, stroke volume, total systemic resistance and ECG (PQ, QRS, QT, QTc Bazett, QTc Fridericia, QTc Van de Water). A slight trend for an increase was observed on relaxation time constant during the post-medication period (median peak effect: +8%; $p<0.05$ versus solvent at 210 and 225 min only). No changes in behavior were noted in 5 out of 6 dogs in the solvent treated group and in 4 out of 6 dogs in the group medicated with TMC125. Licking, trembling and restlessness were observed in one dog and hiccup in another dog of the test article group. Trembling and urination were observed in one dog of the solvent group. Plasma levels of TMC125 (n=4) amounted to median values of 156, 302 and 229 mg/ml at respectively 30, 60 and 240 min after administration of the compound.
TMC125 spray dried — dosed was tested by gavage in male dogs and the NOAEL was 200 mg eq./kg. Increases on heart rate and aortic blood pressure were present approximately at 2 hours after dosing with 400 mg eq./kg b.w. (200 + 200 mg eq./kg given with a 1h interval). This was accompanied by some clinical observations as

restlessness and vomiting.

IN VITRO RECEPTOR BINDING PROFILE: At uM concentrations, TMC125 competed with [14 C]glycine for binding to the glycine-1 transporter (GlyT1); Ki=5.4 μM. Up to a concentration of 10 μM, TMC125 did not interact with any of the other receptors, ion channels or transporter binding sites investigated in the study.

SAFETY PHARMACOLOGY SUMMARY: Safety pharmacology studies showed that the drug generally did not exhibit significant neurological effects, except that it had certain inhibitory effects on nicotinergic nerve-smooth muscle, and bound to glycine receptors. These studies also showed that drug has no significant effects on cardiac K channels and cardiac action potential characteristics, but showed mild increases in blood pressure, heart rate and other cardiac dynamic at the 10 mg/kg but not the 20 mg/kg dose.

2.6.2.5 Pharmacodynamic drug interactions

Please see Microbiologist's review.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

ADME (absorption, distribution, metabolism and excretion) data on TMC125 were obtained from conducted in mice, rats and Beagle dogs, as those used in the toxicology studies. They are obtained to support the drug exposure in toxicity studies and to delineate PK profile of the drug in animal species.

2.6.4.2 METHODS: Plasma concentrations of TMC125 were determined using a validated LC-MS assay (lower detection limit: 5 ng/ml). The structures of metabolites (see graph at the end of this section) were determined by MS analysis by comparison to authentic standards.

2.6.4.3 ABSORPTION: Plasma kinetics after intravenous administration could not be studied due to the very poor solubility of the compound in water. As a consequence, no data on the absolute oral bioavailability are available. However, the sponsor estimated that the oral absorption of TMC125 in humans should be intermediate to low. For rats, after single oral administration of TMC125 at 20 mg/kg as a PEG400 solution, an oral bioavailability below 10% can be expected comparing the observed clearance after oral administration (Cl/f=68.7 l/h/kg) with the maximum hepatic clearance in the male rat. In preliminary non-GLP studies after single oral administration of TMC125 in rats at 20 mg/kg, only a limited exposure was observed (Cmax plasma levels of about 0.025 μg/ml, AUC8h of 0.168 μg.h/ml).
Higher exposures could best be obtained by using the [redacted] TMC125,

TMC125 r especially for studies in rodents. The formulation in PEG400 (TMC125 r G06) was chosen for formulation feasibility reasons and also because other toxicokinetic studies had been performed using a PEG400 formulation with the — TMC125.

2.6.4.4 DISTRIBUTION:

In rats, TMC125 was rapidly distributed to the tissues, with peak levels obtained within 3h. Generally, plasma and tissue concentrations of TMC125 declined at a similar rate. Tissue to plasma ratios (AUC8h) ranged from about 40 in liver and adrenal gland, over 10 to 15 in heart, lung and kidney, to about 5 in brain, spleen and muscle. There was no undue retention of the compound in any of the investigated tissues.
The *in vitro* plasma protein binding of TMC125 was very high for all species investigated, and amounted to 99.89% in man, 99.93% in dogs, 99.88% in male rats and 99.86% in female rats at a concentration of 1000 ng/ml. The fraction unbound ranged from 0.07% in dogs to 0.14% in female rats.

2.6.4.5 METABOLISM:

In vitro incubations with liver subcellular fractions and hepatocytes demonstrated that the metabolism of TMC125 was slow in rat and very slow in dog and human subcellular liver fractions. In dog and rat hepatocytes, relatively faster metabolism was observed due to phase II metabolism. Oxidation at the pyrimidinyl moiety (M1, most probably an N-oxide) was a metabolic pathway in rat, dog and man. M1 was further glucuronidated in rat and dog hepatocytes (M5). Oxidation of the methyl at the dimethylbenzonitrile moiety (M2) was observed in all species. M2 was further glucuronidated in rat and dog hepatocytes (M6), and was further oxidized in rat and man (M3 and M7). Traces of glutathione conjugates and the oxidized and glucuronidated metabolite M4 (dogs) were also observed. M1, M2, M3, M6 and M7 were the major metabolites. Metabolites M1, M2, M3 and M7 were observed in human liver, and this metabolite pattern was also found in rat liver. M6, a major metabolite in rat and dog hepatocytes was not observed in human hepatocytes.

CYP3A4 (formation of M1, M2) and CYP2C (2C9, 2C18 and 2C19; (formation of M2, M3 and M7) are the major cytochrome P450 forms involved in the metabolism of TMC125 in human liver. CYP1A1, (formation of M2) could also be involved in the metabolism of TMC125. IC50 of ritonavir on the formation of metabolites M1 and M2 of TMC125 were 0.06 ug/ml and 3.67 ug/ml, respectively. In incubations with indinavir, the observed IC50 was 0.45 ug/ml for metabolite M1 and 4.68 ug/ml for metabolite M2. Based on these data an interaction between ritonavir or indinavir and the metabolism of TMC125 may be expected. In another study the interaction of 13 well known anti-HIV drugs with the cytochrome P450 mediated metabolism of TMC125 by pooled human liver microsomes has been studied. At concentrations equal to the average plasma levels *in vivo*, it is predicted that the cytochrome P450 mediated metabolism of TMC125 is only slightly inhibited (<26%) by saquinavir,

	efavirenz and abacavir, inhibited between 48% and 72% by indinavir, nelfinavir and amprenavir, and almost completely inhibited by ritonavir and delavirdine. The potential of TMC125 to inhibit specific CYP sub-enzymes was also studied. From these *in vitro* experiments it became clear that TMC125 mainly inhibits CYP2C9 (Ki 0.58 ± 0.09 uM). The IC50 for all other studies CYP enzymes were at least 10 times higher.
2.6.4.6 EXCRETION	TMC125 was predominantly excreted in feces, with 78% to 87% of the dose eliminated in mice, 84% to 93% in rats and 90% in dogs at 24 hours after dosing. The majority of TMC125 was eliminated as unchanged TMC125. The renal or biliary excretion of the drug was limited (0.1% to 0.6% of the dose) in all animal species.
2.6.4.7 PHARMACOKINETIC DRUG INTERACTIONS	AZT, 3TC, D4T, nevirapine and DDI did inhibit the CYP450-mediated metabolism of TMC125 (human liver cell assay) whereas ritonavir, indinavir, saquinavir, nelfinavir, amprenavir, delavirdine, efavirenz and abacavir were found to inhibit the metabolism of TMC125 (Ki = 0.2-120 uM). Ritonavir and indinavir showed to be strong inhibitors of the formation of M7 and M12 metabolites. (IC50 = 0.06 [M7], 3.67 [M12] µg/mL, respectively).

2.6.4.8 OTHER PK STUDIES

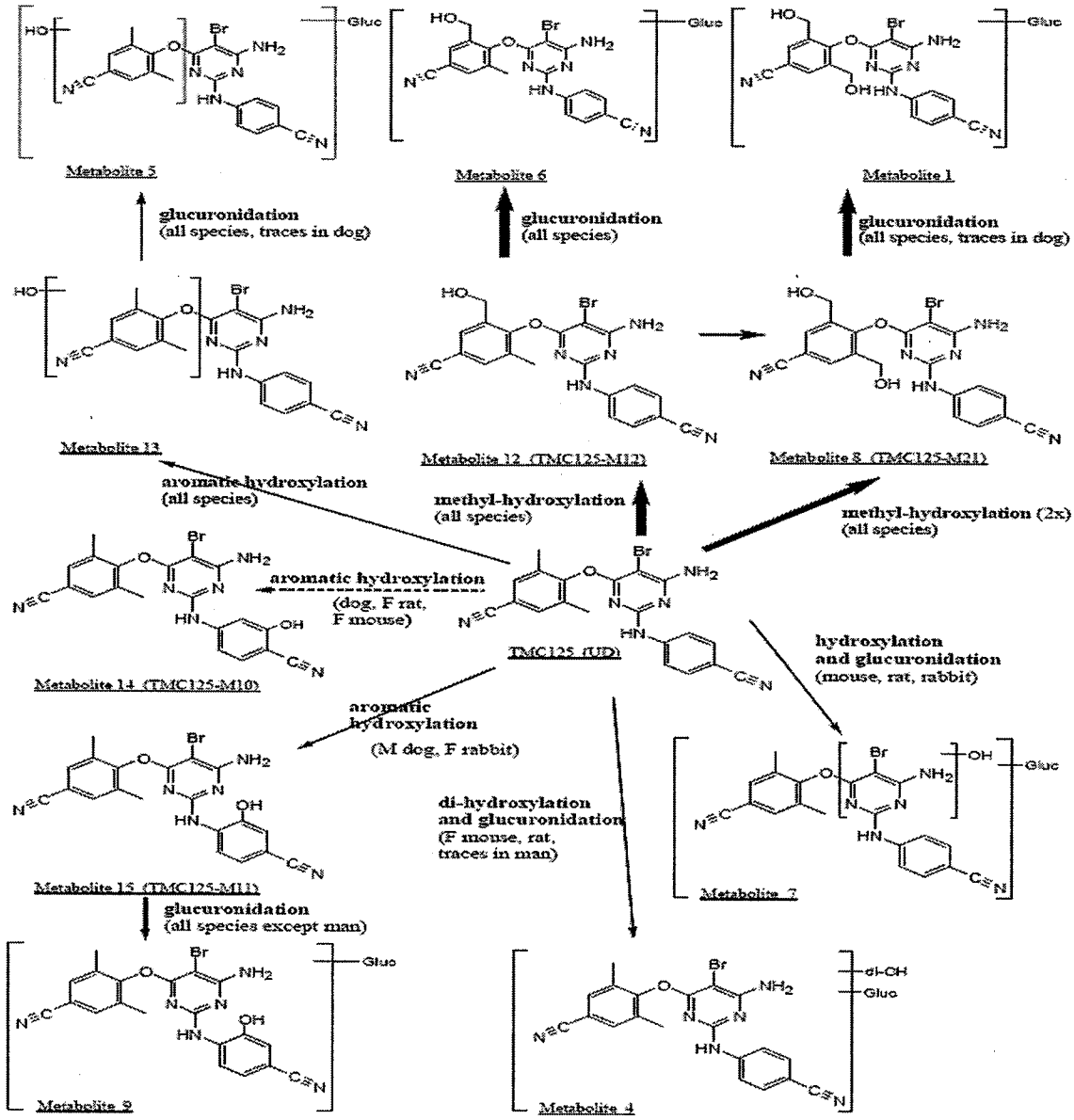
STUDY TITLE	SUMMARY AND COMMENTS
A Pilot study on the bioavailability and plasma pharmacokinetics following oral administration by dietary admixture for 7 days to male mice (NC185)	This study was part of preliminary research on the carcinogenicity range-finding study, which is currently ongoing. No remarkable toxicity findings on three formulations tested were reported in this study. The daily systemic exposure to TMC125 with the [redacted] form No. 1 ([redacted] active/polymer) at 200, 800 and 4000 mg/kg/day, as expressed by the AUC0-24h on day 7, amounted to 2812, 9826 and 25563 ng.h/mL, respectively. Dosing with the [redacted] form No. 2 ([redacted] active/polymer) and the crystalline [redacted] TMC125 (TMC125-[redacted]) at 800 mg/kg/day resulted in a daily systemic exposure (AUC0-24h) of 9695 and 2519 ng.h/mL, respectively. Both form no. 1 and 2 showed similar exposures and the [redacted] formulation yield <1/3 those of the [redacted] formulations.

A Pilot study on the bioavailability and plasma pharmacokinetics following oral administration by dietary admixture for 7 days to male mice (NC185).	This study was part of preliminary research on the carcinogenicity range-finding study, which is currently ongoing. No remarkable toxicity findings on three formulations tested were reported in this study. The daily systemic exposure to TMC125 with the —— form No. 1 ——active/polymer) at 200, 800 and 4000 mg/kg/day, as expressed by the AUC0-24h on day 7, amounted to 2812, 9826 and 25563 ng.h/mL, respectively. Dosing with the —— form No. 2 ——active/polymer) and the —— MC125 (TMC125 — at 800 mg/kg/day resulted in a daily systemic exposure (AUC0-24h) of 9695 and 2519 ng.h/mL, respectively. Both form no. 1 and 2 showed similar exposures and the —— formulation yield <1/3 those of the —— formulations.
Pilot study on the bioavailability and plasma pharmacokinetics following oral administration by dietary admixture for 7 days to male mice (NC186).	This study was similar to the previous study (NC185), except the doses were different. The AUC after dosing through the diet with the —— form No. 1 —— active/polymer) at 200, 600 and 2000 mg/kg/day, amounted to 851, 1769 and 5255 ng.h/mL, respectively. Dosing with the —— form No. 2 —— active/polymer) and the —— TMC125 (TMC125 — at 600 mg/kg/day resulted in AUC of 2542 and 670 ng.h/mL, respectively. Again, the —— formulation yield significantly less exposures than the —— formulations.
The absorption, metabolism and excretion of TMC125 in the male and female SPF CD-1 mouse after single oral administration of [14C]-TMC125 at 200 mg-eq./kg. (NC-234-PK)	This study provides ADME information of this drug on the mouse. The excretion was almost complete. It was 96 % and 101 % in male and female mice, respectively. Renal elimination was negligible (0.4 % of the radioactivity was excreted in urine.) About 30 % of plasma radioactivity exposure was accounted for by the unchanged drug and remaining percent was accounted for by TMC125 metabolites. Majority of the drug was eliminated as unchanged in feces (~ 60 % of the dose) within 0-48 h post dose in male and female mice. The contribution of metabolic elimination to the overall disposition of TMC125 was less appreciable. A number of metabolic pathways, viz., methyl hydroxylation as such and in combination with oxidation, aromatic hydroxylation, oxidation in combination with glucuronidation. M12, M13 and M8 were the major fecal metabolites accounting each of 2-5 % of the dose. In plasma, TMC125 was the major circulating radioactive compound and among the metabolites, methyl mono hydroxylated TMC125 (M12) was the prominent circulating metabolite observed at least up to 8 h post dose administration in male and female mice. There were no sex differences observed in the metabolism and disposition of TMC125 in mice.
Absorption, distribution, metabolism and	This study provides ADME information of this drug in the rat. After single oral dose administration of 60 mg/kg, the peak plasma concentration of TR (total radioactivity, Cmax) in females was 0.1013 µg-eq./g and was reached within 4 hours. The AUC_{4h}

excretion of 14C - TMC125 in the Wistar rat. (NC138)	of TR = 0.3307 µg-eq.h/g. Highest mean concentrations of TR of 432.0 µg-eq./g were observed in gastrointestinal contents at 4h after administration. In liver, lung, kidney and abdominal fat, highest mean concentrations ranged between 1.682 and 5.916 µg-eq./g. Low concentrations of TR were observed in spleen, seminal vesicles and heart tissue. Highest AUC-values were found in gastrointestinal tissue, abdominal fat and liver. After single administration of 14C-TC125 at a dose of 60 mg/kg, the amounts of radioactivity recovered in urine, feces and cage wash of male rats accounted for 5.1, 53.1 and 6.5% of the administered dose, respectively. In female rats, values of 1.4, 53.9 and 8.8 % were achieved for urine, feces and cage wash recovery, respectively. At a dose level of 600 mg/kg, the amounts of radioactivity recovered in urine, feces and cage wash accounted in male rats for 5.1, 49.6 and 4.5% of the dose, respectively, and in female rats for 3.1, 50.0 and 13.1% of the dose, respectively. As a result, the total recovery after single administration of 14CTMC125 is relatively low, and amounts to 64.6 and 59.2 % in male rats and 64.1 and 66.2 % in females at dose levels of 60 and 600 mg/kg, respectively. The sponsor suggested the cause for the low recovery was due to lack of homogeneity of the urine and feces samples in case any precipitation occurred during sample processing.
C14-TMC125:study of distribution in the pigmented rat following a single oral dose administration (NC-182)	This study (at 70 mg / — kg, by quantitative whole-body autoradiography) provide information of tissue distribution of the drug by autoradiography. The results show that highest concentrations of radioactivity (excluding the GI tract) over the study period were associated with the liver, adrenal, brown fat, kidney cortex, and pancreas. In addition, the preputial gland contained high levels of radioactivity at 4 and 24 hours. Lowest levels of radioactivity throughout the study period were associated with blood, plasma and bone. By 96 hours quantifiable levels of radioactivity were only present in the eye. At the final sampling time (336 hours), radioactivity was no longer quantifiable in any tissues.
The absorption, metabolism and excretion of tmc125 in the male and female Sprague-Dawley rat after single oral administration of 14c-tmc125 at 70 mg-eq./kg.	This study (NC201)provides ADME information of this drug on a different rat species (as compared with the above-mentioned species). The total radioactivity was rapidly excreted in feces such that by 24 h post dose 84 to 93 % of radioactivity was eliminated. The excretion was virtually complete by 96 h after dosing and renal elimination was negligible. The radioactivity of urine samples was very low and majority of the drug was eliminated as unchanged in feces (~ 85 % of the dose) within 0-48 h post dose in male and female rats. The contribution of metabolic elimination to the overall disposition of TMC125 was negligible. There were few metabolic pathways (e.g., methyl hydroxylation followed by glucuronidation and aromatic hydroxylation) were observed. Aliphatic methyl hydroxylation of TMC125 was the major metabolic pathway resulting in the formation of metabolite 8 and 12 and this pathway accounted for 7 to 11 % of the administered dose. There were no

gender differences in the metabolism and pharmacokinetics of TMC125 in rats. Overall, the metabolism of TMC125 was very limited in rats and unchanged drug eliminated via feces was the major route of excretion of TMC125 in Sprague-Dawley rats.

Figure 6-9: *In vitro* biotransformation pathways for TMC125 in male and female mouse (2 strains), male and female rat, male dog, female rabbit and man. Bold lines represent major biotransformation pathways, while the dotted line and solid lines represent pathways of minor and intermediate importance, respectively.



2.6.4.9 Discussion and Conclusions

Oral bioavailability of TMC124 in rats was poor in general (< 11%). In the dogs, oral bioavailability was 8-12% for TMC125 — and at 33-34% for spray-dried TMC125.

Plasma levels of TMC125 increased less than dose proportionally in mice, rats, rabbits and dogs after single oral administration. No significant gender differences in pharmacokinetics were observed in mice and dogs, whereas drug exposure was higher in female rats compared to male rats. In all species, the systemic exposure decreased after repeated dosing, partly due to induction of the liver enzymes involved in the metabolism of TMC125.

TMC125 is highly bound to plasma proteins, in man, dog and rat, (> 99%). In the rat, TMC125 distributed rapidly and extensively in tissues, especially in GI mucosa, liver and adrenal gland.

2.6.6 TOXICOLOGY

2.6.6.1 Overall Toxicology Summary

See Executive Summary.

2.6.6.2 Single-Dose Toxicity

Study title:	**ACUTE ORAL TOXICITY STUDY IN RATS**
Study no.:	TMC125-NC132
Laboratory:	—
Study initiation:	3/13/2001
GLP:	yes (x) no ( )
QA report:	yes (x) no ( )
Drug Lot/Purity:	AG1086/ —
Formulation/ vehicle:	TMC125: — Powder (w/w) in PEG400
Dosing:	0 (control), 500 and 1000 mg — TMC125 — kg/day
Species/strain:	Adult Wistar rats
#/sex/group:	5
Route	Oral gavage
Observations and times:	Body weight, survival, clinical signs, and macroscopic lesions were assessed periodically (≈1/day) and at the end of the study (day 15)
Results:	
Mortality	No mortality
Clinical signs:	No signs of toxicity

Toxicokinetics:	At the dose of 1000 mg — kg/day Cmax values of about 600 ng/ml and 950 ng/ml TMC125 were obtained in male and female animals, respectively. The systemic exposure, expressed as AUC8h, was about 2900 ng.h/ml and 4200 ng.h/ml for male and female animals, respectively, given 1000 mg — /kg doses. In male and female rats, maximum plasma concentrations as well as AUC8h values increased less than dose-proportionally between the two doses. Maximum plasma concentrations and AUC8h values were higher in females than in males

Study title:	**ACUTE ORAL TOXICITY STUDY IN MICE**
Study no.:	TMC125-NC131
Laboratory:	—
Study initiation:	3/12/2001
GLP:	yes (x) no ( )
QA report:	yes (x) no ( )
Lot #/% purity:	AG1086/ —
Formulation/ vehicle:	TMC125. — Powder (w/w) in PEG400
Methods:	
Dosing:	0 (control), 500 and 1000 mg — TMC125 — /kg/day
Species/strain:	Adult CD-1 mice
Route	Oral gavage
Observations and times:	Body weight, survival, clinical signs, and macroscopic lesions were assessed periodically and at the end of the study (day 15)
Results:	
Mortality:	No mortality
Clinical signs:	No signs of toxicity
Body weights:	A slight body weight loss was observed in two female mice given 1000 mg/kg on day 3.
Toxicokinetics:	At the dose of 1000 mg — kg/day, Cmax values of about 7700 ng/ml and 4500 ng/ml TMC125 were obtained in male and female animals, respectively. The systemic exposure, expressed as AUC8h, was about 28000 ng.h/ml and 24000 ng.h/ml for male and female animals, respectively at the 1000 mg — kg dose. TMC125 showed non-linear pharmacokinetics over the studied dose interval. With increasing doses, the maximum plasma concentration and AUC increases were greater than would be expected for a dose-proportional response

2.6.6.2 Repeat-Dose Toxicity

STUDY TITLE:	**TMC125 – 5-day repeated dose oral toxicity non-GLP study in the female rabbit.**
STUDY NO.:	TMC125-NC178
LABORATORY:	Johnson & Johnson Pharmaceutical Research & Development, Belgium
STUDY DATE:	7/2003
OBJECTIVES AND	This study served a preliminary test pilot for future reprotoxicity studies to be conducted

METHODS:	in the rabbit. It is designed to assess the systemic exposure and potential toxicity of TMC125 – vehicle=5 % (w/v) Vit E TPGS and – (w/v) aqueous Hydroxypropylmethylcellulose) when administered daily by oral gavage to 4 groups of female New Zealand White rabbits for a period of 5 consecutive days. The dose levels used were 750 or 1125 mg eq./kg/day or 750 mg eq./kg b.i.d. at dose volumes of 10, 15 or 2 x 10 ml/kg bodyweight. Mortality, clinical observations, body weight and food consumption were recorded for all dose levels. Blood samples for toxicokinetic analysis were collected on Day 4 from all rabbits of each group at 0, 0.5, 1, 2, 4, 6, 8, and 24 h after dosing. From the rabbits of the high dosage group and the vehicle Control an extra blood sample was taken from the rabbits at 4.5 and 5 h after the first dose administration (i.e. 0.5 and 1 h after the second dose). After completion of the dosing period the females were killed and a gross necropsy performed.
GLP:	yes () no (x)
QA REPORT:	yes () no (x)
RESULTS AND CONCLUSIONS	There were no unscheduled deaths, no effects of treatment with TMC125 – on bodyweight, food intake, clinical signs or gross pathology in females receiving 750, 1125 or 1500 (2 x 750) mg eq./kg/day. Mean plasma Cmax levels on Day 4 amounted to 1120 ng/ml at the 750 mg eq./kg/day dose level, to 2124 ng/ml at the 1125 mg eq./kg/day dose level and to 1380 (Cmax, 1) and 1835 (Cmax, 2) ng/ml at the 750 mg eq./kg/ bid dose. A rapid absorption of TMC125 was observed with Tmax within 0.5 and 4 h after dosing. The half-life of TMC125 between 8 and 24 h was estimated on average at 6 to 10 h. The mean total plasma exposure ($AUC_{0\text{-}24h}$) amounted on average to 9053 ng.h/ml (750 mg eq./kg/day), 20085 ng.h/ml (1125 mg eq./kg/day) and 20971 ng/ml (750 mg eq./kg bid). The exposure to TMC125, as expressed by plasma C_{max} and AUC increased slightly more than dose proportionally from 750 mg eq./kg/day to 1125 mg eq./kg/day ie, ~ 2 fold increase in exposure for a 1.5 fold increase in dose. Interestingly, the once daily dosing regimen at 1125 mg eq./kg resulted in the same AUC as the twice daily regimen at 750 mg eq./kg. The twice daily regimen at this dose did not improve the exposure to TMC125. The sponsor concluded that 1125 mg eq./kg /day is appropriate as the highest dose level for use in pregnant females to supplement the existing definitive reprotoxicity study in rabbits.

Study title:	**1-MONTH ORAL TOXICITY STUDY IN RATS**
Study no.:	TMC125-NC113
Laboratory:	———
Study initiation:	10/23/2000
GLP:	yes (x) no ( )
QA report:	yes (x) no ( )
Lot #, % purity:	TMC125 – VS256/———

Vehicle:	TMC125 — Powder (w/w) in PEG400, 5 ml/kg bw
Methods:	
Dosing:	0 (control), 20, 80 or 320 mg ——— kg body weight/day
Species/strain:	Wistar rats
Sex/group	10
TK groups:	5 additional animals per sex and per dose group (2 for the control group)
Route:	Oral gavage
Observations:	
Clinical signs:	once daily
Body weights:	Weekly
Food consumption:	Weekly
Ophthalmology:	pre-test and during week 4
Hematology:	at pre-test and at the end of the treatment period.
Clinical chemistry:	at pre-test and at the end of the treatment period
Urinalysis:	at pre-test and at the end of the treatment period.
Gross Pathology:	at termination
Organs weighed:	at termination
Histopathology:	at end of the study

Samples of the following tissues and organs were collected from all animals at necropsy and fixed in neutral phosphate buffered 4% formaldehyde solution:

Identification marks: not processed
Adrenal glands
Aorta
Brain
Caecum
Cervix
(Clitoral gland)
Colon
Duodenum
Female mammary gland area
Femur including joint
Heart
Ileum
Jejunum
Kidneys
(Larynx)
(Lacrimal gland, exorbital)
Liver
Lung, infused with formalin
Lymph nodes - mandibular, mesenteric
(Nasopharynx)
Oesophagus
Ovaries
Pancreas
Pituitary gland
(Preputial gland)
Prostate gland
Rectum
Salivary glands - mandibular, sublingual
Sciatic nerve
Seminal vesicles
Skeletal muscle
Skin
Spinal cord -cervical, midthoracic, lumbar
Spleen
Sternum with bone marrow
Stomach
Thymus
Thyroids including parathyroids
(Tongue)
Trachea
Urinary bladder
Uterus
Vagina
All gross lesions

Eyes, optic nerve and Harderian glands (fixed in Davidson's solution) **
Testes and Epididymides (fixed in Bouins fixative) **

** = transferred to formalin after fixation for at least 24 hours

Toxicokinetics:	Blood sampling for toxicokinetics was performed on day 1 and day 29
Results:	
Mortality:	In the satellite group, one control female rat at 320 mg/kg/day died after blood sampling on day 15, and one satellite female given 80 mg/kg/day died after blood sampling just before necropsy on day 30
Clinical signs:	Incidental observation of scabs, alopecia, head tilt, wounds and loss of a tail apex were noted in control animals and animals given 80 and 320 mg ——— /kg.
BW & Food	Unremarkable

consumption:

Ophthalmology: Unremarkable

ECG: Unremarkable

Hematology: Unremarkable

Clinical chemistry: At the end of the treatment period, an increase in calcium and inorganic phosphate was noted in blood plasma from females given 320 mg ——— kg/day. This change is considered to be of no toxicological relevance. The chloride concentration was increased in males and females, given 320 mg ——— kg/day. This increase is most probably due to the interference of measurement of bromide and chloride, related to the presence of bromide in the test substance.

Urinalysis: The urinary sodium concentration and total excretion of sodium and chloride were decreased in females groups given 80 and 320 mg ——— kg/day. Slightly decreased bilirubin in plasma from males and ASAT activity in females given 320 mg ——— kg/day were considered to be of no biological relevance.

Organ weights: Unremarkable

Pathology: Unremarkable

Histopathology: Unremarkable

Toxicokinetics: After single oral administration at the highest dose (320 mg ——— /kg TMC125, the NOAEL) Cmax values of 212 and 260 ng/ml were obtained in male and female animals, respectively. AUC values at the highest dose were approximately 1400 ng.h/ml in males (AUC24h) and 1200 ng.h/ml in females (AUC8h). Cmax and systemic exposure, expressed as AUC24h, did not increase after repeated dosing for a period of 29 days, as compared to the first dose. In general, there were no consistent differences between males and females for dose-normalized Cmax and AUC values. TMC125 showed non-linear pharmacokinetics over the studied dose interval. With increasing dose, the maximum plasma concentration and AUC increased less than would be expected for a dose-proportional response.

Multiple dose (1-month) – RAT (5 animals/sex/group; sampling from 3 animals/sex/time point) – Study TMC125-NC113

Dose (mg/kg/day)	Gender	Toxicokinetic parameters				
		C_{max} (ng/ml)	t_{max} (h)	C_{min} (ng/ml)	$t_{1/2}$ (h)	AUC_{tau} (ng.h/ml)
4-week oral dose - RAT[a] (PEG400 sol.-HBr-salt) (levels at day 1)		(mean)	(median)	(mean (± SD))	(mean)	
20 mg/kg/day	**male**	**69.80**	**1**	**< 5.00**	**13.09***	**281.7 [b]**
80 mg/kg/day		67.70	1	< 5.00	3.448*	410.1 [b]
320 mg/kg/day		211.7	1	13.50 ± 6.319	7.595	1378
20 mg/kg/day	female	64.53	1	< 5.00	3.263*	309.1 [b]
80 mg/kg/day		107.6	1	< 5.00	5.540*	589.8 [b]
320 mg/kg/day		260.3	1	< 5.00	3.414	1163 [b]
4-week oral dose - RAT (levels at day 29)						
20 mg/kg/day	male	62.80	1	< 5.00	2.640	334.9 [b]
80 mg/kg/day		52.30	1	6.833 ± 7.171	11.77*	439.9
320 mg/kg/day		201.0	1	21.39 ± 18.49	42.72*	1155
20 mg/kg/day	female	52.47	1	< 5.00	3.855*	262.8 [b]
80 mg/kg/day		90.77	1	16.52 ± 10.64	9.766*	1008
320 mg/kg/day		181.7	1	18.57 ± 4.944	10.98*	1516

*Accurate determination not possible
[b] AUC_{tau}

2-Week Repeated Dose Toxicity in the Dog

From these results, the NOAEL was considered to be 320 mg [illegible] kg/day.
This 2-week gavage study conducted in dogs, investigating the difference in toxicity profile between two batches of TMC125 [illegible] M6 [M60261002]; ZR [ZR293496PFA021]), one of which (batch ZR) contains two impurities that will be present at high levels in the clinical drug product [illegible] at [illegible] and [illegible] at [illegible]. The sponsor intended to qualify these two impurities by batch ZR293496PFA021 [illegible] at [illegible] and [illegible] at [illegible] used in this study. One control group and 4 treated groups were given, 0 (vehicle), 20 (M6), 240 (M6), 20 (ZR) and 240 (ZR) mg/kg/day.
The results showed no significant difference in toxicity profile including the target organ of toxicity (i.e., an enlarged spleen and a marked presence of erythrocytes in the red pulp) between the two batches. Based on this study, the sponsor had estimated an NOAEL (i.e., 20 mg/kg) and used it for the estimation of the margin of safety for each of the two impurities. The sponsor calculated the margins of safety of the two impurities (i.e., amounts of [illegible] at [illegible] and [illegible] at [illegible] were less than 1 for both impurities (i.e., [illegible]=0.27 [illegible]=0.14).

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (ug/mL)		AUC_{0-24h} (ug.h/mL)	
		M	F	M	F
20 (ZR)	Day 10	0.19	0.83	3.12	8.70
240 (ZR)	Day 10	1.17	1.12	18.9	18.4
20 (M6)	Day 10	1.07	0.37	10.8	4.39
240 (M6)	Day 10	1.02	2.48	18.5	27.3

Study title: **2-Week Repeated Dose Oral Toxicity Study in the Beagle Dog**

Study no.: TMC125-NC175

Laboratory: Johnson and Johnson Pharmaceutical Research & Development, Turnhoutseweg 30, 2340 Beerse, Belgium

Study initiation: 4/2003

GLP: yes (x) no ( )

QA report: yes (x) no ( )

Lot #, % purity: M60261002 [illegible] batch) (f = [illegible]
ZR293496PFA021 (J&J PRD batch)
(f = [illegible] (f = conversion factor)

Formulation/ vehicle: PEG400

Methods:

Dosing: The purpose of this study was to determine, assess and compare the potential toxicity of two batches of TMC125 [illegible] TMC125 [illegible] were administered once daily by the oral route (via gavage) to beagle dogs, for a period of two consecutive weeks at 20 and 240 mg eq./kg/day (see table below, * = J&JPRD batch; # = [illegible] batch)

Vehicle	LOW1	HIGH1	LOW2	HIGH2
0	20*	240*	20#	240#
0	10*	120*	10#	120#

Species/strain: Beagle dogs

#/sex/group	3
Route	by gavage for 2 weeks
Observations:	Mortality, clinical and eye observations, ECG and heart rate, body weight and weight gain, food consumption, hematology, serum analysis, coagulation, urinalysis, organ weights, gross pathology, and histopathology. The toxicokinetic parameters were also determined.
Results:	Dosing at 20 mg eq./kg b.w./day with either TMC125 – or TMC125— led to salivation, mucous and pale feces. In comparison to the dogs dosed with the vehicle, there seemed to be a tendency towards an increased incidence and severity in soft feces. A slight increase in serum chloride levels was observed in male and female dogs dosed at 20 mg/kg b.w./day with both batches. In female dogs, a marginal decrease in urinary pH was noted. In one male dosed with TMC125 – a pronounced increase in spleen weight was related to the observation of an enlarged spleen and a marked presence of erythrocytes in the red pulp. The latter was also seen in another male of the same group. When dogs were dosed at 240 mg eq./kg b.w./day with TMC125 – or TMC125 – following clinical observations were comparable between both TMC125 – dosed groups: soft feces, slight vomiting, salivation, mucous/pale feces. When compared to the 20 mg eq./kg b.w./day dosed groups an increased incidence of pale feces was observed. Additionally a slightly higher food consumption was noticed in males and females of both batches throughout the study. A slight to marginal increase in serum chloride levels was noted in dogs dosed with TMC125 – or TMC125#. A slight decrease in urinary pH was observed in females of both TMC125 – dosed groups. A moderate to pronounced increase in absolute and relative spleen weight was noted in one male and two females dosed with TMC125 –
Toxico-kinetics	At toxicokinetic examination no clear gender and batch related (J&J PRD vs. — differences in plasma profiles and exposure (Cmax, AUC) were observed on day 10 of this 2-week repeated dose oral toxicity study in the dog. However, as only two male and two female dogs were compared within each batch and because of the quite large interindividual differences these conclusions are not unequivocal. The exposure increased less than dose proportionally between 20 and 240 mg eq./kg, a 2 to 6 fold increase was observed for a 12-fold increase in dose.
Summary	In conclusion, the overall toxicological profile of TMC125 – (J&J PRD batch) and TMC125 – (— batch) are identical. The minor difference observed between the dogs dosed with the J&J PRD batch of TMC125 – or the — batch of TMC125 – do not represent a relevant toxicological difference. For both batches 20 mg eq./kg body weight can be considered the NOAEL. The sponsor calculated the margins of safety of the two impurities (i.e., amounts of

— at — and — at —, were less than 1 for both impurities (i.e., — =0.27, — =0.14).

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC_{0-24h} (µg.h/mL)	
		M	F	M	F
20 (ZR)	Day 10	0.19	0.83	3.12	8.70
240 (ZR)	Day 10	1.17	1.12	18.9	18.4
20 (M6)	Day 10	1.07	0.37	10.8	4.39
240 (M6)	Day 10	1.02	2.48	18.5	27.3

Comments: In the original IND, the NOAELs for the dog were claimed to be 160, 80 and 80 mg/kg/day as estimated from the 1-, 3- and 6-month studies, respectively. This study will play a minor role in toxicity assessment and a new toxicity profile from dogs is available from submission 282. Please see review on submission 282 for details.

Study title: **2-Week Repeated Dose Oral Toxicity Non-GLP Study in the Mouse**
Study no.: TMC125-NC179
Laboratory: Johnson and Johnson Pharmaceutical Research & Development, Turnhoutseweg 30, 2340 Beerse, Belgium
Study initiation: 4/2003
GLP: yes () no (x)
QA report: yes () no (x)
Formulation/ vehicle: PEG400
Methods: In this study SPF Albino Swiss Mice were given a repeated oral dose of TMC125K by gavage at a dosage of 100, 300, 900 or 1200 mg eq./kg body weight/day for 14 consecutive days.

Dosing: 100, 300, 900 or 1200 mg eq./kg body weight/day
Species/strain: SPF Albino Swiss Mice
#/sex/group 3
Route by gavage for 2 weeks
Observations: Mortality, clinical observations, body weight and body weight gain, food consumption, hematology and serum analysis were measured. At the end of the in vivo phase, the mice were killed and a gross pathological examination, organ weights and histopathology were examined.
Results: Oral administration of TMC125— for 2 consecutive weeks at a dose up to 1200 mg eq./kg b.w./day to female mice, did not result in test article-related mortality.
No relevant changes in body weight, weight gain and food consumption were noted when male and female mice were dosed up to 1200 mg eq./kg b.w./day for two consecutive weeks.

At 100 mg eq./kg, a marginal decrease in albumin in males, a slight increase in urea nitrogen in females and in male and female mice a slight to moderate decrease in total

bilirubin, a slight to moderate increase in ALP and a marginal to slight increase in alanine ALT were reported. At post mortem examination, in females a minimal diffuse hepatocellular hypertrophy and a decrease in perilobular hydropic appearance was noted. In one female hydropic appearance/ vacuolation midzonal/perilobular was present.

At 300 mg eq./kg, a marginal decrease in total protein with a slight decrease in albumin and cholesterol, in females a slight increase in ALT. In male and female mice a slight to moderate decrease in total bilirubin and increase in AST were seen in males. At post mortem examination of male and female mice a slight increase in absolute and relative liver weight, a minimal diffuse hepatocellular hypertrophy and a marginal increase in single cell or focal necrosis in the liver were present. In females a decrease in perilobular hydropic appearance and a hydropic appearance/vacuolization was noted.

At 900 mg eq./kg, a slight increase in mean cell Hb was seen at in females. Increases in cholesterol in males, in ALT in females and in total bilirubin and AST in males and females were more pronounced when compared to the previous groups. Additionally in males a slight decrease in albumin and a moderate increase in ALP and in females a slight increase in calcium and urea nitrogen and a slight to moderate increase in cholesterol was reported. A moderate increase in absolute and relative liver weight was noted in male and female mice, a marginal increase in absolute and relative heart weight in male mice and a marginal to slight decrease in absolute pancreas weight in female mice. A slightly pale liver with more pronounced lobulation was seen in 1/5 male and female mice. Additionally in males a slight to moderately swollen liver was noted. Histological changes in the liver of males and females were more pronounced when compared to the previous groups. In males and females a decrease in perilobular hydropic appearance and a hydropic appearance/vacuolization was noted.

At 1200 mg/kg, anemia and a rough haircoat were noted during the second week of dosing in one male mouse. In males and females a slight increase in white blood cells and in females a slight increase in mean cell hemoglobin concentration, lymphocytes and monocytes were noted at hematological examination. At serum analysis following changes occurred: in males a slight to moderate decrease in total protein, glucose and cholesterol and in females a moderate increase in cholesterol, triglycerides and urea nitrogen were present. A slight increase in calcium, a marginal to moderate decrease in albumin, a pronounced decrease in total bilirubin, a moderate to pronounced increase in ALP, AST and ALT were noted in male and female mice. At post mortem examination the changes in liver weight in males and females were more pronounced and the changes in heart and pancreas weight in males and females respectively, were comparable to the previous group. Additionally to the decrease in absolute weight a slight decrease in relative pancreas weight was present in females. A slightly to moderately swollen liver and more pronounced lobulation was observed in male and female mice. Additionally in females a slightly to severely pale liver was noted. At histopathology the liver changes were comparable to the previous group. In males and females a decrease in perilobular hydropic appearance and hydropic appearance/vacuolization was noted.

Toxico-kinetics	Not performed
Summary	This study is performed as a pilot investigation for the future definitive range-finding mouse carcinogenicity study.

Study title:	**3-MONTH ORAL TOXICITY STUDY IN RATS**
Study no.:	TMC125-NC109
Laboratory:	
Study initiation:	4/2/2001
GLP:	yes (x) no ( )
QA report:	yes (x) no ( )
Lot #, % purity:	AG1086 and AA6426-1-01/
Formulation/ vehicle:	TMC125 Powder (w/w) in PEG400, 5 ml/kg bw
Methods:	
Dosing:	doses of 0 (control), 70, 200 or 600 mg TMC125 /kg body weight/day
Species/strain:	Wistar rats
#/sex/group:	10
TK group:	5 additional animals per sex and per dose group (2 for the control group)
Route, volume:	Oral gavage, 5 ml/kg
Observations	
Clinical signs:	once daily
Body weights:	weekly
Food consumption:	weekly
Ophthalmo-scopy:	pre-test and during week 4
Hematology:	at pre-test and at the end of the treatment period.
Clinical chemistry:	at pre-test and at the end of the treatment period
Urinalysis:	at pre-test and at the end of the treatment period.
Gross pathology:	at termination
Organs weighed:	at termination
Histo-pathology:	at end of the study, tissue list identical to 1-month rat study (see above)
TK:	Blood sampling for toxicokinetics was performed on day 1 and day 29
Results:	
Mortality	One control female, and one male and one female at 600 mg /kg/day died

	due to gavage accidents. One control female and one male at 70 mg/kg/day died from unknown causes.
Clinical signs:	Incidental observation of scabs, alopecia, head tilt, wounds and loss of a tail apex were noted in control animals and animals given 80 and 320 mg ——— /kg.
BW & Food Consumption	unremarkable
Ophthalmology:	unremarkable
ECG:	unremarkable
Hematology:	unremarkable
Clinical chemistry:	At 200 mg ——— kg/day: higher plasma potassium levels in males in week 6; higher plasma chloride levels in males and females in week 6 and 13; lower plasma bilirubin levels in males in weeks 6 and 13 and in females in week 13; lower plasma urea levels in males in weeks 6 and 13 and in females in week 6 and lower plasma ALAT levels in males in weeks 6 and 13. At 600 mg ——— kg/day: higher plasma potassium levels in males and females in weeks 6 and 13; higher plasma total protein levels in females in weeks 6 and 13 and in males in week 13; higher plasma chloride levels in males and females in weeks 6 and 13; lower plasma bilirubin levels in males and females in weeks 6 and 13; lower plasma urea levels in males and females in weeks 6 and 13 and lower plasma ALAT levels in males in weeks 6 and 13. The increase in chloride levels is most probably due to the interference of measurement of bromide and chloride, and thus related to the presence of bromide in the test substance. The observed decreases in plasma ALAT, urea and bilirubin levels may be secondary to adaptive responses of the liver. The findings at the dose of 70 mg/kg/day in urinary sodium excretion and plasma potassium levels were not consistent in time and not clearly dose related. Also, no supportive microscopic findings were present in the kidneys (see below).
Urinalysis:	At 200 mg ——— kg/day: higher urinary sodium excretion in males in week 6; higher urinary chloride excretion in males in weeks 6 and 13; At 600 mg ——— kg/day: higher urinary sodium concentration and higher urinary sodium excretion in males and females in weeks 6 and 13; higher urinary chloride concentration and higher urinary chloride excretion in males and females in weeks 6 and 13; higher urinary calcium concentration in males in week 13 and higher urinary calcium excretion in males in weeks 6 and 13
Organ weights:	At 600 mg ——— kg/day: higher liver weights
Necropsy:	Unremarkable
Histopathology:	At 200 mg ——— kg/day the following observations were made: an increased incidence of hypertrophy and/or hyperplasia of follicular thyroid cells in males and females; an increased incidence of focal renal tubular basophilia in males; At 600 mg ——— kg/day the following was noted: basophilic stippling of hepatocellular cytoplasm in females, an increase in severity of hypertrophic/vacuolated cells in the pars

Toxicokinetics: Maximum plasma concentrations were comparable between male and female rats on day 1, about 300 ng/ml. In males AUC8h was 1600 ng.h/ml. In females an AUC24h of 3160 ng.h/ml was observed on day 1. At week 13, AUC24h values (575.6, 694.3 and 1692 ng.h/ml in males and 1026, 1402 and 3870 ng.h/ml in females for the 70, 200 and 600 mg —— /kg doses respectively) were higher in females than in males. Similarly, Cmax values were higher in females than in males (35.37, 61.03 and 123.5 ng/ml in males and 93.73, 123.0 and 317.5 ng.h/ml in females for the 70, 200 and 600 mg —— /kg doses respectively) TMC125 showed non-linear pharmacokinetics over the studied dose interval. With increasing doses, the maximum plasma concentration and AUC increased less than would be expected for a dose-proportional response.

Multiple dose (3-month) – RAT (5 animals/sex/group; sampling from 3 animals/sex/time point) – Study TMC125-NC129

Dose (mg/kg/day)	Gender	Toxicokinetic parameters				
		C_{max} (ng/ml)	t_{max} (h)	C_{min} (ng/ml)	$t_{1/2}$ (h)	AUC_{24h} (ng.h/ml)
3-month oral dose - RAT[#] (PEG400 sol. — (levels at day 1)		(mean)	(median)	(mean (± SD))	(mean)	(mean)
70 mg/kg/day	male	68.43	4	<5.00	2.097*	403.6 [§]
200 mg/kg/day		148.1	1	<5.00	2.794*	835.3 [§]
600 mg/kg/day		284.0	4	<5.00	1.982*	1609 [§]
70 mg/kg/day	female	82.43	4	<5.00	5.262*	545.2 [§]
200 mg/kg/day		145.0	4	<5.00	3.690*	920.3 [§]
600 mg/kg/day		330.0	1	20.34 ± 24.84	5.499	3155
3-month oral dose - RAT (levels at week 13)						
70 mg/kg/day	male	35.37	1	10.06 ± 4.125	9.969*	575.6
200 mg/kg/day		61.03	1	13.30 ± 4.451	11.60*	694.3
600 mg/kg/day		123.5	8	13.75 ± 2.899	5.052*	1692
70 mg/kg/day	female	93.73	4	14.93 ± 4.840	10.56*	1026
200 mg/kg/day		123.0	1	30.67 ± 3.691	13.91*	1402
600 mg/kg/day		317.5	1	32.33 ± 27.99	16.60*	3870

[#] Accurate determination not possible.
[§] AUC_{tau}

The sponsor concluded that the NOAEL for TMC125 was 70 mg/kg/day in males and females for this 3-month study.

Study title: **1-MONTH ORAL TOXICITY STUDY IN DOGS**
Study no.: TMC125-NC107
Laboratory: ——
Study initiation: 8/8/2000
GLP: yes (x) no ( )
QA report: yes (x) no ( )
Lot #, % purity: AG1049/ —
Formulation/ vehicle: TMC125 — Powder (w/w) in PEG400
Methods:

Dosing:	0 (control), 40, 80 and 160 mg/kg/day, given in two equal doses of TMC125. The second daily dose was given 7h after the first dose. Each treatment group consisted of 4 male and 4 female dogs.
Species/strain:	Beagle dogs
#/sex/group:	4
Route, volume:	Oral gavage, 3 ml/kg bw
Observations:	
Clinical signs:	twice daily shortly after each dosing
Body weights:	Weekly
Food consumption:	Daily
Ophthalmology:	pre-test and during week 4
EKG:	pre-test and during week 4
Hematology:	at pre-test and at the end of the treatment period.
Clinical chemistry:	at pre-test and at the end of the treatment period
Urinalysis:	at pre-test and at the end of the treatment period.
Gross pathology:	at termination
Organs weighed:	at termination
Histopathology:	at termination Tattoo Skeletal muscle Sternum Heart Aorta Trachea Lung Spleen Lymph node (mandibular, mesenteric) Thymus Tongue Salivary gland (parotid, sublingual, submaxillary) Pancreas Liver Gall bladder Oesophagus Stomach Duodenum Jejunum Ileum Caecum Colon Rectum Kidneys Urinary Bladder Ureter Prostate gland Ovaries Uterus Cervix Vagina Skin +Mammary gland area, males and females (pelvic, left and right) Thyroids Parathyroid glands Adrenal glands Pituitary gland Brain (medulla, pons, cerebellum, cerebrum (cortex and hippocampus)) Spinal cord (cervical, thoracic, lumbar) Sciatic nerve Eyes and optic nerve :fixed in Davidson's solution** Testes and Epicidymides :fixed in Bouin's** ** = transferred to formalin after fixation for at least 24 hours.
Toxicokinetics:	Day 1, 7 and 28
Results: Mortality & Clinical signs:	One male animal (in the 80 mg/kg/day group) and one female (in the 160 mg/kg/day group) were sacrificed in extremis on day 9 and 5, respectively. They showed various toxic clinical signs (lethargy, labored respiration, irregular heartbeat, abnormal posture, tonic spasms, rales and retchings). Gross and histopathological examination of the lungs showed congestion and alveolar effusion. These clinical signs and pulmonary findings were attributed to gavage errors with large volumes of PEG400 given to these animals.

Subsequently the dose volume of vehicle was reduced from 3 ml/kg to 1 ml/kg from the second dose on day 9 onwards. No further deaths occurred. During the first 9 days of the study vomiting of test article and/or mucus was noted in all groups, including the control group.

Body weights: Several animals in the mid and high dose groups showed retarded body weight gain.

Food consumption: Unremarkable

Ophthalmology: Unremarkable

EKG: Unremarkable

Hematology: Unremarkable

Clinical chemistry: All surviving female animals in the high dose group had lower values for alkaline phosphatase activity at the pre-terminal sampling time. For animals killed in extremis several blood biochemistry values were abnormal.

Urinalysis: unremarkable

Organ weights: unremarkable

Gross pathology: unremarkable

Histopathology: unremarkable

Toxicokinetics: Steady-state trough levels of TMC125 were achieved within 7 days of treatment. The systemic exposure, expressed as AUC7h, increased after repeated dosing for a period of 28 days with an overall increase of about 68 %. Exposure related pharmacokinetic parameters (AUC and Cmax) were consistently higher in males than in females, *i.e.* about 29 %. TMC125 showed non-linear pharmacokinetics over the studied dose interval. With increasing doses, the maximum plasma concentration and AUC increased less than would be expected for a dose-proportional response.

Multiple dose (1-month) – DOG (4 animals/sex/group) – Study TMC125-NC132

Dose (mg/kg/day)	Gender	Toxicokinetic parameters					
		C_{max} (ng/ml)	t_{max} (h)	C_{min} (ng/ml)	$t_{1/2}$ (h)	AUC_{7h} (ng.h/ml)	AUC_{24h} (ng.h/ml)
1-month oral dose - DOG[a] (PEG400 sol.) (levels at day 1)		(mean ± SD)	(median)	(mean ± SD)	(mean ± SD)	(mean ± SD)	(mean ± SD)
40 mg/kg/day	male	607.5 ± 38.28 [b]	4 [b]	455.0 ± 138.8	- [2]	3330 ± 432.1 [b]	-
80 mg/kg/day[3]		823.0 ± 62.98 [b]	4 [b]	646.0 ± 113.0	-	4294 ± 611.1 [b]	-
160 mg/kg/day		1029 ± 102.8 [b]	4 [b]	819.8 ± 130.7	-	5325 ± 344.0 [b]	-
40 mg/kg/day	female	475.5 ± 195.2 [b]	1.5 [b]	255.5 ± 130.8	-	2254 ± 1208 [b]	-
80 mg/kg/day		550.3 ± 434.3 [b]	2.5 [b]	422.8 ± 426.1	-	2742 ± 2435 [b]	-
160 mg/kg/day		682.8 ± 113.2 [b]	3 [b]	489.0 ± 51.70	-	3493 ± 360.4 [b]	-
1-month oral dose - DOG (levels at day 28)							
40 mg/kg/day	male	1187 ± 595.8	2	446.0 ± 92.02	11.76 ± 1.642*	6090 ± 1059 [b]	18641 ± 3153
80 mg/kg/day[3]		1273 ± 234.6	2	443.7 ± 17.93	10.56 ± 0.7101*	6919 ± 789.6 [b]	20368 ± 857.2
160 mg/kg/day		1493 ± 329.0	2	637.8 ± 200.2	14.18 ± 2.036*	8226 ± 1504 [b]	24440 ± 5299
40 mg/kg/day	female	812.0 ± 385.8	2	256.0 ± 81.82	9.688 ± 0.7324*	4348 ± 901.9 [b]	12710 ± 3057
80 mg/kg/day		921.8 ± 439.0	2	284.8 ± 148.7	10.26 ± 1.980*	4704 ± 2831 [b]	13758 ± 7131
160 mg/kg/day[3]		1230 ± 182.5	2	412.3 ± 52.54	12.39 ± 2.619*	6558 ± 381.1 [b]	18368 ± 2248

*Accurate determination not possible.
[a] Calculated after first dosing; all other parameters calculated after second dosing
[2] Parameter not calculated
[3] N=3

STUDY TITLE:	**3-MONTH ORAL GAVAGE TOXICITY STUDY IN THE SWISS MOUSE**
STUDY NO.:	TMC125-NC146
LABORATORY:	Johnson and Johnson Pharmaceutical Research & Development, a division of Janssen Pharmaceutica N.V.Turnhoutseweg 30 Belgium
OBJECTIVES:	This study was designed as a dose range finding study to assist in the selection of the doses for a subsequent carcinogenicity study in mice.
STUDY INITIATION:	8/26/2003
GLP:	yes (x) no ( )
QA REPORT:	yes (x) no ( )
LOT #, % PURITY:	ZR293496PFA011
FORMULATION/ VEHICLE:	TMC1[illegible]J65335) Powder (w/w) in PEG400; 20 & 80mg/ml; 10 ml/kg
METHODS:	
DOSING:	0, 10, 50, 200 and 800 mg/kg/day
SPECIES/STRAIN:	SPF Albino Swiss mice (CD1)
#/SEX/GROUP:	15
TK GROUP:	45/sex/group (no controls)
ROUTE, VOLUME:	Oral gavage, 10 ml/kg
OBSERVATIONS AND TIMES:	
CLINICAL SIGNS:	once daily
BODY WEIGHTS:	weekly
FOOD CONSUMPTION:	weekly
HEMATOLOGY:	at the end of the treatment period.
CLINICAL CHEMISTRY:	at the end of the treatment period
URINALYSIS:	at the end of the treatment period.
GROSS PATHOLOGY:	at termination
ORGANS WEIGHED:	at termination: lungs, spleen, liver, heart, kidneys, brain, thymus, adrenal glands and gonads (testes, ovaries).

HISTOPATHOLOGY:

- adrenal glands - aorta bone (stifle joints, sternum) bone marrow (femur, sternum) - brain -esophagus - exorbital lachrymal glands	- eyes with optic nerve(s) and Harderian glands - gall bladder - genital tract, female (ovaries, oviducts, uterus, cervix, vagina and clitoral gland) - genital tract,	- heart - kidneys - large intestine (cecum, colon, rectum) - larynx - liver - lung - lymph node(s) (mesenteric) - lymph nodes (submandibular) -	- peripheral nerves (sciatic nerves) - Peyer's patches - pituitary gland - salivary gland(s) (mandibular, sublingual and parotid) - skeletal muscle	- spinal cord (cervical, thoracic, - stomach - thymus - thyroid glands with parathyroid gland(s) -tongue - trachea - urinary bladder

- external ear with Zymbal gland	male (testes, epididyinides, prostate, seminal vesicles, coagulating glands and preputial gland)	mammary gland(s) - nose - pancreas lumbar) - spleen	(quadriceps femoris) - skin - small intestine (duodenum, jejunum, ileum)	- all tissues showing gross changes

TOXICO-KINETICS: Blood sampling for toxicokinetics was performed on day 1 and month 1 and 3

RESULTS:

MORTALITY Five animals died in the main study. Three of these were confirmed to be gavage accidents (1 vehicle male, 1 female at 50 and 1 female at 200 mg/kg/day). No cause of death was determined for 1 female at 50 mg/kg/day. One male at 800 mg/kg/day showed drug-related pericarditis, myocarditis and endocarditis with hemorrhages and multifocal necrosis/degeneration.

Five male and one female satellites also died during the study. Four males were at 800 mg/kg/day, while the other was at 50 mg/kg/day. The female satellite animal was dosed at 200 mg/kg. The sponsor speculated the causes of these deaths were related to gavage procedures.

CLINICAL SIGNS: Soft feces, noted at approximately 4 to 6 hours after dosing in all dosed groups and the vehicle group, was considered related to the PEG formulation.

BODY WEIGHTS: In males, all dosed groups showed a decreased body weight and weight gain, with body weight gain most affected (27%) at the 800 mg/kg/day dose.

TMC125 (mg/kg)	MALES					FEMALES				
	0	10	50	200	800	0	10	50	200	800
Mean body weight (g)	39.4	37.4*	37.0*	37.5	37.3	30.0	30.3	29.2	29.3	30.1
Change in body weight[1] (%)		-5	-6	-5	-5		+1	-3	-2	0
Mean body weight gain (g)	8.5	7.0	6.7*	7.2	6.2**	5.8	5.9	5.3	5.6	5.4
Change in body weight gain[1] (%)		-18	-21	-15	-27		+2	-9	-3	-7

[1] = compared to concurrent vehicles Statistical significance: *p<0.05 ** p<0.01

FOOD CONSUMPTION: unremarkable

HEMATOLOGY: A dose-related increase in thrombocytes was noted in males at 50 mg/kg or higher. At 800 mg/kg, a decrease in MCV in males and a decrease in number and percentage of reticulocytes, and an increase in Hb/MCH were noted.

TMC125 (mg/kg)	MALES					FEMALES				
	0	10	50	200	800	0	10	50	200	800
Thrombocytes($10^3/\mu L$)	1238	1362	1369*	1421**	1500**	1180	1190	1348	1264	1294
MCV (fl)	51.5	50.2	50.5	50.7	49.2**	51.5	51.5	50.9	51.0	50.5
MCHC(g/dL)	30.8	30.7	30.7	30.6	31.4	30.2	30.5	30.3	30.6	31.5***
Hemoglobin(g/dL)	14.0	14.2	13.7	13.7	13.8	13.8	14.2	14.2	14.3	14.5**
Reticulocytes($10^3/\mu L$)	248.2	251.2	240.7	257.5	216.1	284.7	269.5	244.3	276.5	239.4*

CLINICAL In serum, total bilirubin was decreased in males from 50 mg/kg/day (69%), while ALP

CHEMISTRY: was increased from the same dose (2-fold). Similar changes were seen in females at 800 mg/kg/day. AST (2.6-fold) and ALT (8.8-fold) were increased from 200 mg/kg/day in females, while ALT was increased at 800 mg/kg/day in males (65%). Triglycerides were increased at 800 mg/kg/day in both sexes (42%), while cholesterol (37%) and calcium (4%) were increased at the same dose (females).

TMC125 –	MALES					FEMALES				
(mg/kg)	0	10	50	200	800	0	10	50	200	800
Ca (mg/dL)	9.6	9.5	9.5	9.6	9.8	9.6	9.6	9.8	9.6	10.0*
Cholesterol (mg/dL)	122	121	126	109	110	91	89	104	83	125**
Triglycerides (mg/dL)	142	146	178	132	202	124	138	154	138	162
Total Bilirubin (mg/dL)	0.13	0.11	0.07**	0.07***	0.04***	0.10	0.09	0.11	0.09	0.07**
ALP(U/L)	62	69	79*	86	106*	69	95*	83	79	141
AST(U/L)	140	143	133	137	108	129	142	130	220	331**
ALT(U/L)	54	59	82	69	89*	40	65	58	193***	350***

*p<0.05; ** p<0.01; *** p<0.001

ORGAN WEIGHTS: The thymus weight was decreased (29%) in males at ≥10 mg/kg/day. The liver weight was increased (51%) at ≥50 mg/kg/day. The kidney weight was decreased (10%) in males at 800 mg/kg/day, whereas the adrenal weight (20%) was increased at the same dose level in males.

TMC125 –	MALES					FEMALES				
(mg/kg)	0	10	50	200	800	0	10	50	200	800
Liver (mg/100g)	5147	5551	5738**	5954**	7582***	5114	5479	5884***	6225***	7704***
Thymus (mg/100g)	103	84*	90	85	77**	147	123	120	129	120
Adrenals (mg/100g)	12	19*	15	14	16*	35	35	32	38	34
Kidneys (mg/100g)	1439	1465	1445	1456	1374	1180	1267	1150	1162	1151

GROSS PATHOLOGY: A pale liver was noted in one 200 mg/kg female (No. 453). A pale and swollen liver and a more pronounced lobulation of the liver were noted in male and female mice at 800 mg/kg.

HISTOPATHOLOGY: Adrenal glands: A marginal increase in cell swelling/ eosinophilia of the zona reticularis and inner zona fasciculata in males at 200 and 800 mg/kg. Swelling of the zona fasciculata cells was marginal in females at 200 mg and slight in females at 800 mg/kg.

Liver: A dose-related increase in hepatocellular hypertrophy occurred from 10 mg/kg onwards in females, from 50 mg/kg onwards in females after one month and in males after three months of dosing and from 200 mg/kg onwards in males after one month of dosing. Hepatocellular hydropic appearance/vacuolation was largely dose-related from 50 mg/kg onwards in females (after one and three months) and in males after three months, and from 200 mg/kg onwards in males after one month of dosing. These

changes were associated with eosinophilic cytoplasmic inclusion bodies in males from 200 mg/kg onwards after three months of dosing and with an increase in single cell necrosis from 50 mg/kg onwards in males after one and in both sexes after three months and from 200 mg/kg onwards in females after one month of dosing.
Kidneys: a marginal increase in focal basophilic tubules was reported in females at 800 mg/kg.

MALES TMC125[1] - mg /kg/day)		0	10	50	200	800
Adrenal glands Swollen/eosinophilic cortical cells (zona reticularis/inner zona fasciculata)	Incidence per group	0/10	0/10	1/10	2/10	3/10
Liver Hydropic appearance/vacuolation	Incidence per group	0/10	0/10	4/10	3/10	8/10***
Liver Hypertrophy (centrilobular)	Incidence per group	1/10	3/10	1/10	4/10	2/10
Liver Hypertrophy, diffuse	Incidence per group	0/10	0/10	1/10	4/10	10/10***
Liver Inclusion bodies, eosinophilic, multifocal (cytoplasm)	Incidence per group	0/10	1/10	0/10	2/10	6/10*
Liver Single cell necrosis, (multi)focal (with inflammatory cells)	Incidence per group	1/10	2/10	4/10	6/10	9/10**
FEMALES						
Adrenal glands Swollen cortical cells (zona fasciculata)	Incidence per group	0/10	1/10	1/10	2/10	5/10*
Kidneys Basophilic tubules, focal	Incidence per group	3/10	3/10	4/10	4/10	7/10
Liver Hydropic appearance/vacuolation	Incidence per group	0/10	0/10	5/10*	6/10*	10/10***
Liver Hypertrophy (centrilobular)	Incidence per group	0/10	3/10	3/10	3/10	0/10
Liver Hypertrophy, diffuse	Incidence per group	0/10	0/10	3/10	4/10	10/10***
Liver Single cell necrosis, (multi)focal (with inflammatory cells)	Incidence per group	2/10	3/10	5/10	5/10	9/10**

TOXICOKINETICS: The table below showed Cmax and AUC values after single dose (day 0) and repeated dosing (day 28 and 90) in males and females at 10, 50, 200 and 800 mg/kg/day. There were no major differences between male and female mice. The AUC and Cmax increased less than dose-proportionally.

		Cmax (ng/ml)			AUC (ng.h/ml)		
Dose	Day	0	28	90	0	28	90
10	Male	336	346	239	16191	1618	1443
	Female	227	296	222	12741	1478	1089
50	Male	603	504	557	4035	2439'	3138
	Female	673	394	366	4132	2764	1704
200	Male	1710	498	414	9731	3959	3926
	Female	1273	692	501	9377	3420	4598
800	Male	5207	1767	1500	43409	12236	7797
	Female	4597	2580	1480	41103	11292	10138

CONCLUSION: No NOAEL was determined in this 3-month oral gavage study. At the low dose of 10 mg/kg/day, slight drug-related toxic effects were noted (slight liver related changes in serum parameters and at histopathology).

STUDY TITLE:	**3-MONTH DIET TOXICITY STUDY IN THE SWISS MOUSE**
STUDY NO.:	TMC125-NC195
LABORATORY:	[redacted]
STUDY MONITOR:	Johnson and Johnson Pharmaceutical Research & Development, Turnhoutseweg 30, 2340 Beerse, Belgium
OBJECTIVES:	This study was designed as a dose range finding study to assist in the selection of the dietary doses for a subsequent carcinogenicity study in mice.
STUDY INITIATION:	6/2004
GLP:	yes (x) no ( )
QA REPORT:	yes () no (x)
LOT #, % PURITY:	N/A
FORMULATION/ VEHICLE:	Spray-dried TMC125 ([redacted]) was added to the diet/ hydroxypropyl methylcellulose and microcrystalline cellulose in the diet (diet substitution factor for the top dose (active product + vehicle) = 5%).
METHODS/DOSING:	Male and female mice (30/sex/group) were dosed via dietary route at 0 (blank feed group), 00 (vehicle feed group), 450, 1620 and 2320 mg TMC125/kg for one or three months. Each group consisted of a first set of 10 animals/sex for the 1-month interim kill, and a second set of 20 animals/sex for the terminal kill. Due to the mortality observed in male mice at 1620 and 2320 mg/kg, dosing was terminated in both sexes at 2320 mg/kg on day 47 (week 7). The dose in the 1620 mg/kg dose group was lowered from day 50, onwards, to 800 mg/kg. A new low dose group, 200 mg/kg, was commenced from day 57, onwards. This group contained 10 main study animals/sex and 6 satellite animals/sex. These animals had previously been allocated to the second set of main study animals in the blank (for the main study animals) and vehicle feed groups (for the satellite animals).
SPECIES/STRAIN:	SPF Albino Swiss mice (CD1)
#/SEX/GROUP:	30
TK GROUP:	36/sex/group (18/control)
ROUTE, VOLUME:	Diet
OBSERVATIONS AND TIMES:	
CLINICAL SIGNS:	once daily
BODY WEIGHTS:	weekly
FOOD CONSUMPTION:	weekly
HEMATOLOGY:	at the end of the treatment period.
CLINICAL CHEMISTRY:	at the end of the treatment period
URINALYSIS:	at the end of the treatment period.
GROSS	at termination

PATHOLOGY:

ORGANS WEIGHED: at termination: lungs, spleen, liver, heart, kidneys, brain, thymus, adrenal glands and gonads (testes, ovaries).

HISTO-PATHOLOGY:

- adrenal glands - aorta bone (stifle joints, sternum) bone marrow (femur, sternum) - brain -esophagus - exorbital lachrymal glands - external ear with Zymbal gland	- eyes with optic nerve(s) and Harderian glands - gall bladder - genital tract, female (ovaries, oviducts, uterus, cervix, vagina and clitoral gland) - genital tract, male (testes, epididyinides, prostate, seminal vesicles, coagulating glands and preputial gland)	- heart - kidneys - large intestine (cecum, colon, rectum) - larynx - liver - lung - lymph node(s) (mesenteric) - lymph nodes (submandibular) - mammary gland(s) - nose - pancreas lumbar) - spleen	- peripheral nerves (sciatic nerves) - Peyer's patches - pituitary gland - salivary gland(s) (mandibular, sublingual and parotid) - skeletal muscle (quadriceps femoris) - skin - small intestine (duodenum, jejunum, ileum)	- spinal cord (cervical, thoracic, - stomach - thymus - thyroid glands with parathyroid gland(s) -tongue - trachea - urinary bladder - all tissues showing gross changes

TOXICO-KINETICS: Blood sampling for toxicokinetics was performed on day 7, 29 and day 90.

RESULTS:

MORTALITY In the 450 mg/kg dose group, 1 main study male and 1 satellite male were found dead towards the end of dosing. The first animal showed blackish liquid discharge from the ear.

In the 1620 mg/kg dosed main group, 2 males were found dead on day 33 while another male was found dead on day 39. Due to its bad condition, one male was sacrificed prematurely on day 37.

After lowering the dose in this group to 800 mg/kg, two more male animals were found dead on days 71 and 86. Three satellite male animals were found dead before the change of dose.

In the 2320 mg/kg dosed group, 3 males were sacrificed prematurely on days 19, 32 and 37. Eight males died prematurely between days 28 and 44. Seven satellite males were found dead or killed .

Dose (mg/kg)	Control	Vehicle	200#	450	1620/800@	2320°
Main animals	0/30	0/30	0/10	1/30	6/30	11/30
Satellites	-	0/18	0/6	1/36	3/36	7/36

- = no satellites included ° = terminated on day 47 #commenced on day 57 @dose lowered on day 50

CLINICAL SIGNS OF THE DEAD: All of the prematurely dead males showed hemorrhagic cardiomyopathy, which is characterized by myocardial degeneration/necrosis, myolysis, interstitial (sometimes associated with subendocardial and/or subepicardial) hemorrhages, fibroblast proliferation, pericarditis and hemosiderosis. The lesions affected both atria and ventricles. Some of these lesions were also observed in three males at 2320 mg/kg that were sacrificed terminally after one month and in four animals at 2320 mg/kg that survived up to week 7. In addition, in males at 1620 and 2320 mg/kg, hemorrhages

were observed in several organs, including the testes, liver, stomach mucosa, lungs and thymus. This was more frequently noted in the high than in the mid dose group and mostly one or more tissues were affected. In 9 preterminally dead males at 2320 mg/kg, reddish liquid content was found in the thoracic cavity, and in one preterminally dead male at 2320 mg/kg, reddish liquid content was present in the abdominal cavity at autopsy. The cardiac findings are considered to be the cause of death. No cardiac findings or hemorrhages have been observed in female mice. Similar cardiac lesions and hemothorax were observed in one high-dose male in the 3-month gavage study in mice. Most of the prematurely dead animals showed signs of a debilitated condition the day(s) before death.

BODY WEIGHTS: Inconsistent increases in weight gain were noted in males and females.

TMC125 (mg/kg)	MALES					FEMALES				
	0	00	200#	450	1620/ 800@	0	00	200#	450	1620/ 800@
Mean body weight (g)	36.4	35.8	37.9	36.5	37.0	29.0	28.9	28.1	28.9	29.7
Change in body weight[1] (%)		-2	+4	0	+2		0	-3	0	+2
Mean body weight gain (g)	4.2	4.5	0.3	5.2	4.8	4.5	4.1	0.7	4.9	5.3
Change in body weight gain[1] (%)		+7	+50	+23	+14		-9	+40	+9	+17

#Commenced on day 57 @dose lowered on day 50; [1] = compared to concurrent controls at end of dosing. For comparison in the 200 mg/kg group.

TMC125 (mg/kg)	MALES			FEMALES		
	0	00	2320°	0	00	2320°
Mean body weight (g)	36.0	34.5	33.1**	27.4	27.3	28.8*
Change in body weight[1] (%)		-4	-8		0	+5
Mean body weight gain (g)	3.8	3.2	1.6	2.9	2.5	4.5
Change in body weight gain[1] (%)		-16	-58		-14	+55

° = terminated on day 47; [1] = compared to concurrent controls in week 6 Statistical significance versus controls: *p<0.05; ** p<0.01

FOOD CONSUMPTION: When compared to controls, a higher food consumption (up to 36%) was noted in all drug groups, except at 200 mg/kg/day.

HEMATOLOGY: A higher platelet count (29%) was noted in males at 1620/800 mg/kg. In addition, lower white cell (60%) and lymphocyte (63%) counts were noted in vehicle males.

CLINICAL CHEMISTRY: In serum, increases were noted from 200 mg/kg, in urea (49%), ALT (4.1 fold), AST (1.6-fold) and ALP (2.6-fold). Total bilirubin was decreased (50%) from 200 mg/kg, in males. In males at 1620/800 mg/kg, creatinine decreased by 9% whereas triglycerides increased by 63%. Similar changes were noted after 4 weeks of dosing in urea (males only; 2.3-fold), ALP (5.5-fold), AST (2.2-fold) and ALT (3.7 fold) at 2320 mg/kg.

TMC125 (mg/kg)	MALES					FEMALES				
	0	00	200#	450	1620/ 800@	0	00	200#	450	1620/ 800@
Urea(mmol/L)	9.4	10.7	12.2	12.6	12.1*	9.2	9.2	11.0	13.3	13.7*
ALP (IU/L)	158	132	332*	409**	314**	221	197	226	254	350
AST(IU/L)	172	176	254	219	276	308	165	354	356	364
ALT(IU/L)	57	38	86*	84	136**	51	40	120**	206**	212**
Creatinine(µmol/L)	34	34	34	32	31*	35	35	34	34	33
Total bilirubin(µmol/L)	4	4	2*	2**	2**	3	3	2	2	2
Triglycerides(mmol/L)	0.91	0.75	0.95	0.92	1.48*	0.85	0.96	1.08	1.18	1.20

#Commenced on day 57 @dose lowered on day 50; [1] = compared to concurrent controls at end of dosing. Statistical significance versus controls at end of dosing: *p<0.05; ** p<0.01

TMC125 (mg/kg)	MALES			FEMALES		
	0	00	2320°	0	00	2320°
Urea(mmol/L)	9.3	9.0	21.2*	9.6	7.0	8.4
ALP (IU/L)	163	244	900*	221	245	483*
AST(IU/L)	184	140	403	234	195	427
ALT(IU/L)	80	46	203	62	47	231**

° terminated on day 47; values of week 4 presented; Statistical significance versus controls in week 4: *p<0.05 ** p<0.01

ORGAN WEIGHTS: A dose-related increase in liver weight was reported in all TMC125-dosed groups (up to 79% for relative weight), together with liver enlargement and sometimes accentuated lobular pattern at autopsy.

HISTOPATHOLOGY: Hepatocellular hypertrophy was observed in a dose-related manner in both sexes. This often was associated with vacuolated and/or foamy hepatocytes and hepatocellular necrosis, with higher incidence in the animals dosed at 1620/800 and 2320 mg/kg, and occasionally in animals dosed at 450 mg/kg. For histological changes in the heart, please refer to Clinical Signs Section.

At End of 3 Months					MALES
TMC125 - mg /kg/day)		**0**	**00**	**450**	**1620/800@**
Hepatocellular hypertrophy	Incidence per group	0/10	0/14	1/1	16/16
Vacuolated hepatocytes	Incidence per group	0/10	1/14	1/1	11/16
Hepatocellular degeneration/necrosis	Incidence per group	0/10	0/14	0/1	2/16
Hepatocellular foamy cytoplasm	Incidence per group	0/10	0/14	0/1	12/16
					FEMALES
Liver Hepatocellular hypertrophy	Incidence per group	0/10	0/14	2/2	20/20
Vacuolated hepatocytes	Incidence per group	1/10	3/14	0/2	6/20
Hepatocellular degeneration/necrosis	Incidence per group	0/10	0/14	0/2	1/20
Hepatocellular foamy cytoplasm	Incidence per group	0/10	0/14	0/2	4/20

@dose lowered on day 50

At End of 7 Weeks		MALES	FEMALES
TMC125 - mg /kg/day)		**2320**	**2320**
Pericarditis	Incidence per group	3/13	0/20
Heart degeneration/necrosis with inflammatory cells	Incidence per group	4/13	0/20
Heart Myolysis	Incidence per group	4/13	0/20
Heart Fibroblast proliferation	Incidence per group	3/13	0/20
Heart Interstitial hemorrhage	Incidence per group	4/13	0/20
Heart Hemosiderosis	Incidence per group	1/13	0/20
Hepatocellular hypertrophy	Incidence per group	13/13	20/20
Vacuolated hepatocytes	Incidence per group	10/13	17/20
Area of coagulative hepatocellular necrosis	Incidence per group	1/13	1/20
Focal coagulative hepatocellular necrosis	Incidence per group	3/13	2/20
Hepatocellular degeneration/necrosis	Incidence per group	3/13	2/20
Hepatocellular foamy cytoplasm	Incidence per group	6/13	16/20

In Preterminally Killed Or Sacrificed Males		MALES		
TMC125 - (mg kg/day)		**450**	**1620/800@**	**2320**
Pericarditis	Incidence per group	1/1	5/6	10/11
Heart degeneration/necrosis with inflammatory cells	Incidence per group	1/1	6/6	11/11
Heart Myolysis	Incidence per group	1/1	6/6	11/11
Heart Fibroblast proliferation	Incidence per group	1/1	6/6	11/11
Heart Interstitial hemorrhage	Incidence per group	1/1	6/6	11/11
Heart Hemosiderosis	Incidence per group	0/1	1/6	2/11
Hepatocellular hypertrophy	Incidence per group	0/1	6/6	11/11
Vacuolated hepatocytes	Incidence per group	0/1	4/6	8/11
Area of coagulative hepatocellular necrosis	Incidence per group	0/1	1/6	4/11

Focal coagulative hepatocellular necrosis	Incidence per group	1/1	4/6	3/11
Hepatocellular degeneration/necrosis	Incidence per group	0/1	1/6	3/11
Hepatocellular foamy cytoplasm	Incidence per group	0/1	3/6	5/11

@dose lowered on day 50

TOXICOKINETICS: C_{max} and AUC_{0-24h} increased less than dose-proportionally. There was no clear difference in C_{max} and AUC_{0-24h} between male and female animals. The C_{max} and AUC_{0-24h} values were lower after repeated dosing (probably due to autoinduction). AUC_{0-24h} after diet administration was 2-3 fold lower than those after gavage, at same dose levels (see table below).

Study	TK analysis	Dose (mg/kg)	C_{max} (ng/mL)		AUC_{0-24h} (ng.h/mL)	
			Male	Female	Male	Female
3-month Diet	Day 7	450	226	354	3199	4504
		1620	497	713	9761	12277
		2320	1293	1180	18905	16772
	Day 29	450	256	187	2950	2588
		1620	966	418	8791	7978
		2320	945	453	11519	7892
	Day 90	200	197	96.8	2353	1533
		450	126	195	1982	2586
		800	227	348	4544	3470
3-month Gavage	Day 0	10	336	227	1619°	1274°
		50	603	673	4035°	4132°
		200	1710	1273	9731°	7207°
		800	5207	4597	43239	40943
	Week 12	10	239	222	1443	1089°
		50	557	366	3138	1704°
		200	414	501	3926	4598
		800	1500	1480	7797	10138

°: AUC_{0-8h}

CONCLUSION: The 3-month dietary mice study showed lower systemic exposures than those seen in gavage study; and showed a similar toxicity profile to the latter. No APTT or PT testings were performed to support hemorrhagic cardiomyopathy/hemothorax findings. No NOAEL was determined because of hepatotoxicity findings at the low dose.

Study title: **3-MONTH DIET TOXICITY STUDY IN SPRAGUE DAWLEY RATS**

Study no.: TMC125-NC196

LABORATORY: ——

STUDY MONITOR: Johnson and Johnson Pharmaceutical Research & Development, Turnhoutseweg 30, 2340 Beerse, Belgium

Objectives: This study was designed as a dose range finding study to assist in the selection of the doses for a subsequent carcinogenicity study in rats.

Study initiation: 6/2004

GLP: yes (x) no ( )

QA report: yes () no (x)

Lot #, % purity: N/A

Formulation/ vehicle: Spray-dried TMC125 ——) was added to the diet/hydroxypropyl methylcellulose and microcrystalline cellulose in the diet (diet substitution factor for the top dose (active product + vehicle) = 5%).

Dosing: 0 (control), 330, 990 or 1300 mg TMC125 – (RI65335)/kg/day for 3 months
Species/strain: Sprague-Dawley rats
#/sex/group: 30
TK group: 6/sex/group (6/control)
Route, volume: Oral gavage, 5 ml/kg
Observations:
Clinical signs: once daily
Body weights: weekly
Food consumption: weekly
Ophthalmo-scopy: pre-test and during week 4
Hematology: at pre-test and at the end of the treatment period.
Clinical chemistry: at pre-test and at the end of the treatment period
Urinalysis: at pre-test and at the end of the treatment period.
Gross pathology: at termination
Organs weighed: at termination: lungs, spleen, liver, heart, kidneys, brain, thymus. adrenal glands, thyroid glands (including parathyroid glands), gonads (testes, ovaries).
Toxico-kinetics: Blood sampling for toxicokinetics was performed on day 1, 30 and 85.

Results:

Mortality None
Clinical signs: Unremarkable
Body weights: A decrease in body weight (8%) and weight gain (29%) was observed in male and female animals in both vehicle and TMC125-dosed groups.

TMC125 (mg/kg)	MALES					FEMALES				
	0	00	330	990	1300	0	00	330	990	1300
Mean body weight (g)	538	531	543	499	512	301	277**	288	285	277*
Change in body weight[1] (%)		-1	0	-7	-5		-8	-4	-5	-8
Mean body weight gain (g)	226	211	215	193	201	90	74	76	72	64
Change in body weight gain[1] (%)		-7	-5	-15	-11		-18	-15	-20	-29

[1] = compared to concurrent controls Statistical significance versus controls: *= p<0.05 **= p<0.01

Food Consumption: Unremarkable

Hematology: Partial thrombin time (PT) and activated thromboplastin time (APTT) showed an increase at all dose levels in males (22% and 61%, respectively), whereas in females a decrease was seen in PT (13%) without a relevant effect on APTT at all dose levels. A decrease in PT was also noted in vehicle-dosed females. Fibrinogen was increased in males at 990 mg/kg (12%).

TMC125 (mg/kg)	MALES					FEMALES				
	0	00	330	990	1300	0	00	330	990	1300
PT(sec)	15.0	15.2	16.1*	17.6**	18.3**	15.8	15.0**	14.4**	13.7**	13.9**
APTT(sec)	19.9	21.0	26.0**	29.9**	32.0**	15.3	15.9	16.1	16.9**	16.2

Statistical significance versus controls: *= p<0.05 **= p<0.01

Clinical chemistry: Proteins were increased in males at ≥330 mg/kg, and in females at ≥mg/kg (6%). A similar change was noted in albumin (6%). Cholesterol was increased at ≥990 mg/kg in males and in females at the high dose (25%). Triglycerides were decreased in females at all dose levels (35%). ALT was marginally increased at ≥990 mg/kg in males (36%). Inorganic phosphate was decreased at the high dose in females (9%).

TMC125 (mg/kg)	MALES					FEMALES				
	0	00	330	990	1300	0	00	330	990	1300
Proteins(g/L)	67	67	70*	70**	70**	72	71	74	75*	76**
Albumin(g/L)	36	36	37*	37	38**	41	40	42	42	43
Cholesterol(mmol/L)	1.3	1.3	1.4	1.6*	1.6*	1.6	1.8	1.7	1.8	1.9**
Triglycerides(mmol/L)	0.55	0.39**	0.44	0.42*	0.48	0.46	0.40	0.27**	0.29**	0.30*
ALT(IU/L)	41	43	50	48*	56*	65	51	53	62	42
IP(mmol/L)	1.86	1.86	1.82	1.90	1.92	1.62	1.51	1.50	1.58	1.48

Urinalysis: Unremarkable.

Organ weights: An increase in thyroid weight was noted in males at the high dose and in females from 990 mg/kg.

Histopathology: Unremarkable except thyroid follicular cell hypertrophy in males and high dose females was mentioned.

Toxicokinetics: AUC_{0-24h} in both sexes increased less than dose-proportionally and, after repeated administration, were similar to those obtained after the first dose. The values were higher in females than in males, and did not yield higher gain in the systemic exposure when compared to oral gavage (see table below).

Study	TK analysis	Dose (mg/kg/day)	C_{max} (ng/mL)		AUC_{0-24h} (ng.h/mL)	
			Male	Female	Male	Female
Diet : 3-month study in Sprague Dawley rat	Day 7	330	69.5	140	907	2366
		990	140	439	1508	4732
		1300	193	686	2143	7122
	Day 84	330	61.9	174	1206	2947
		990	108	388	2091	6730
		1300	162	354	2955	5335
Gavage : 3-month in Sprague Dawley rats	Day 0	70	135	163	739	1137
		200	250	283	1438	1715
		600	436	1279	2503	5796
	Week 12	70	64	196	1002	1411
		200	124	457	739	2275
		600	256	801	2285	6815

°: AUC_{0-8h}; °°: AUC_{0-48h}; # After 4 weeks of dosing; Statistical significance:*= p<0.05; **= p<0.01; *** = p<0.001

CONCLUSION: This 3-month diet study in rat did not show as many target organs of toxicity as that explored in the gavage study (kidney, liver, adrenal and thyroid), probably because of poor systemic drug exposures.

STUDY TITLE: **3-MONTH ORAL GAVAGE TOXICITY STUDY IN SPRAGUE DAWLEY RATS**

STUDY NO.: TMC125-NC140

LABORATORY: Johnson and Johnson Pharmaceutical Research & Development, a division of Janssen Pharmaceutica N.V.Turnhoutseweg 30 Belgium

STUDY INITIATION:	This study was designed as a dose range finding study to assist in the selection of the doses for a subsequent carcinogenicity study in rats.
DATE OF STUDY:	8/26/2003
GLP:	yes (x) no ( )
QA REPORT:	yes (x) no ( )
LOT #, % PURITY:	ZR293496PFA011
FORMULATION/ VEHICLE:	TMC125 (RI65335) Powder (w/w) in PEG400 (14, 40, 120 mg/ml; 5 ml/kg)
METHODS:	
DOSING:	doses of 0 (control), 70, 200 or 600 mg TMC125 (RI65335)/kg
SPECIES/STRAIN:	Sprague-Dawley rats
#/SEX/GROUP:	20
TK GROUP:	6/sex/group (no control)
ROUTE, VOLUME:	Oral gavage, 5 ml/kg
OBSERVATIONS AND TIMES:	
CLINICAL SIGNS:	once daily
BODY WEIGHTS:	weekly
FOOD CONSUMPTION:	weekly
OPHTHALMO-SCOPY:	pre-test and during week 4
HEMATOLOGY:	at pre-test and at the end of the treatment period.
CLINICAL CHEMISTRY:	at pre-test and at the end of the treatment period
URINALYSIS:	at pre-test and at the end of the treatment period.
GROSS PATHOLOGY:	at termination
ORGANS WEIGHED:	at termination: lungs, spleen, liver, heart, kidneys, brain, thymus. adrenal glands, thyroid glands (including parathyroid glands), gonads (testes, ovaries).
HISTO-PATHOLOGY:	The following tissues were sectioned and stained with (H&E) and examined by light microscopy in all animals (interim kill and terminal kill): adrenal glands, kidneys (except interim killed males), liver, pituitary gland, thyroid glands (unilateral for almost all animals, bilateral for rats No. 20, 35, 110, 116) and all tissues showing gross changes in male and female rats. The following tissues were additionally examined histologically in terminally killed animals of vehicle and high dose groups. Aorta bone and bone marrow (stifle joint, sternum) brain -esophagus - eyes - genital tract, female (ovaries, oviducts, uterus, cervix and vagina) - genital tract, male (testes, epididymides, prostate, seminal vesicles, coagulating glands) - Harderian gland(s) - heart - large intestine (cecum, colon, rectum) - larynx - lungs - lymph nodes (mesenteric, popliteal-bilateral) - mammary gland - optic nerve(s) - pancreas - parathyroid gland(s) - peripheral nerves. sciatic nerves - Peyer's patche(s) - salivary gland (parotid, submandibular, sublingual) - skeletal muscle, quadriceps femoris - skin small intestine (duodenum. jejunum, ileum) spinal cord (cervical, thoracic, lumbar) spleen stomach (forestomach, glandular stomach) - thyuus - tongue - trachea - ureter(s) - urinary bladder
TK:	Blood sampling for toxicokinetics was performed on day 1, 30 and 85.

RESULTS: MORTALITY One low dose group male (No. 35) died pre-terminally in week 4 and one female vehicle rat (No. 116) died pre-terminally in week 10. The sponsor stated that they were related to gavage accidents.

CLINICAL SIGNS: Soft feces were reported in all dosed groups.

BODY WEIGHTS: In high dose males, a decrease in body weight and weight gain was present from the fifth week of dosing onwards with a decrease in weight gain of 13% at the end of the three month dosing period

TMC125 (mg/kg)	MALES				FEMALES			
	0	70	200	600	0	70	200	600
Mean body weight (g)	501	507	530	459*	285	289	280	300
Change in body weight[1] (%)		+1	+6	-8		+1	-2	+5
Mean body weight gain (g)	305	308	333	266*	136	139	132	145
Change in body weight gain[1] (%)		+1	+9	-13		+2	-3	+7

[1] = compared to concurrent vehicles. Statistical significance: *p<0.05

FOOD CONSUMPTION: A slight decrease in food consumption was noted during the last month in high dose males.

OPHTHALMOLOGY: unremarkable

HEMATOLOGY: No relevant changes on hematology were noted in males. In female rats, increases in Hb, Hct and neutrophils were noted after 1 and 3 months of dosing. Additionally after 3 months dosing a slight increase in monocytes was present.

TMC125 (mg/kg/day)	MALES				FEMALES			
	0	70	200	600	0	70	200	600
NEUTRO (10^3/µL)	1.37	1.72	1.98**	2.59	0.92	1.22	1.40*	1.27
MONO(10^3/µL)	0.45	0.45	0.50	0.54	0.25	0.35*	0.37*	0.32
EOS(10^3/µL)	0.20	0.17	0.21	0.12	0.13	0.14	0.11	0.10
Hb(g/dL)	16.3	16.4	16.4	16.3	14.5	15.1*	15.2**	15.3*
HCT(%)	48.1	48.0	48.3	48.3	42.3	44.4**	44.6**	44.8**
MCV (fl)	52.3	52.6	52.3	53.0	53.3	53.7	54.7*	54.9*
MCH (pg)	17.7	18.0	17.8	17.9	18.2	18.3	18.6*	18.7*

CLINICAL CHEMISTRY: An increase in calcium after 1 and 3 months and a decrease in total bilirubin after 1 month were observed. In females, a slight increase in albumin (and total protein) at 1 and 3 months and a slight decrease in glucose towards the end of dosing were seen. There were slight to moderate decreases in liver transaminases (ALP, AST, ALT) reported in males and females at all doses and sponsor speculated that it might be associated with hepatic enzyme induction and increases in liver weight.

TMC125 (mg/kg)	MALES				FEMALES			
	0	70	200	600	0	70	200	600
BIL(mg/dL)	0.12	0.11	0.09***	0.07***	0.12	0.12	0.09***	0.06***
Ca(mg/dL	11.5	11.8	12.0*	11.9	11.6	12.0*	12.3**	12.4***
Glucose(mg/dL)	93	86*	90	92	114	93***	97*	100**
Urea(mmol/L)	17.3	16.8	15.7	15.1*	17.4	18.7	17.0	15.4
Albumin(g/dL)	4.6	4.6	4.7	4.5	5.1	5.2	5.2	5.6**
TOP(g/L)	6.8	7.1	7.4***	7.4**	7.0	7.4*	7.6***	8.1***

URINALYSIS: Dose-related increases in occult blood and squamous epithelial cells were reported in urine in males of all dose groups. Similar findings occurred in the high dose females. Calcium oxalate crystals and increases in urine specific gravity in males in females were noted.

TMC125 (mg/kg)	MALES				FEMALES			
	0	70	200	600	0	70	200	600
Specific gravity	1.065	1.075	1.070	1.070	1.030	1.054*	1.046	1.054*
Proteins(score)	1.00	1.33	1.10	1.20	0.00	0.40	0.30	1.40**
Blood(score)	0.80	0.89	1.20	2.00*	0.89	0.30	0.60	0.90
Ca oxalate crystals (score)	0.00	0.22	0.40	0.20	0.00	0.00	0.10	0.20

Statistical significance: *= p<0.05 **= p<0.01

THYROID FUNCTIONS: Decreases in T4 and increases in TSH were reported in all dose groups. Alterations in T3 remained slight and varied between male and female rats.

TMC125 (mg/kg)	MALES				FEMALES			
	0	70	200	600	0	70	200	600
T_3(nmol/L)	0.85	0.74	0.63*	0.60**	0.89	0.84	0.88	0.99
T_4(nmol/L)	77	59*	49**	31***	46	32**	26**	31*
TSH(ng/mL)	6.3	6.5	6.1	9.0	4.2	4.5	4.6	8.7*

Statistical significance: *= p<0.05 **= p<0.01 ***= p<0.001

ORGAN WEIGHTS: Increases in thyroid (all doses), liver (all doses) and adrenal (high dose group) organ weight was noted in males. Increases in above three organ weights also occurred in the high dose females.

TMC125 (mg/kg)	MALES				FEMALES			
	0	70	200	600	0	70	200	600
Liver (mg/100g)	26367	28138*	27964*	30397***	26821	27046	28208	32989***
Adrenals (mg/100g)	123	124	127	154**	261	252	267	303
Thyroids[#](mg/100g)	48	61	63*	55	71	76	79	80

[#] After 4 weeks of dosing Statistical significance: *= p<0.05 **= p<0.01 *** = p<0.001

HISTOPATHOLOGY: Treatment-related histological changes were observed in the middle and high dose groups. Target organs of toxicity were as follows:

Adrenal glands: a minimal diffuse swelling of the adrenocortical zona fasciculata cells was observed in the high dose females.

Kidneys: a minimal increase in (multi)focal presence of basophilic cortical tubule and hyaline cast(s) was observed in the kidneys of the high dose females.

Liver: fatty-like vacuolization was increased in the high dose male and female groups and mid-dose females; centrilobular hypertrophy was noted in one male and one female of the high dose groups.

Thyroid gland: increase in small follicles (=microfollicles) and high thyroid follicular epithelium were observed in the middle and high dose males and females (less severe in mid-dose group).

The progression of above toxicity was supported by the findings at the interim kill: (1) minimal diffuse swelling of the adrenocortical zona fasciculata cells and marginal increase in renal hyaline cast(s) were already observed in the high dose females, (2) the hepatocellular changes were already present in the high dose females and marginally in the mid-dose females after one month of dosing, (3) a slight increase in small follicles (=microfollicles) and/or high thyroid follicular epithelium was already minimally present after 1 month in the middle dose and slightly in the high dose groups.

MALES	TMC125 – ng/kg/day)	0	70	200	600
Liver Hypertrophy (centrilobular)	Incidence per group	0/10	0/10	0/10	1/10
Liver Vacuolization (fatty-like), large	Incidence per group	1/10	0/10	2/10	3/10
Liver Vacuolization (fatty-like), small-midzonal	Incidence per group	0/10	0/10	1/10	3/10
Liver Vacuolization (fatty-like), small-perilobular	Incidence per group	1/10	0/10	0/10	3/10
Thyroid Glands High follicular epithelium	Incidence per group	7/10	6/10	10/10	10/10
Thyroid Glands Small follicles	Incidence per group	3/10	4/10	6/10	10/10**
FEMALES					
Adrenal glands Swollen zona fasciculata cells, diffuse	Incidence per group	0/10	0/10	0/10	6/10*
Kidneys Basophilic tubules, (multi)focal	Incidence per group	4/10	3/10	3/10	7/10
Kidneys Hyaline cast(s)	Incidence per group	2/10	2/10	2/10	4/10
Liver Hypertrophy (centrilobular)	Incidence per group	0/10	0/10	0/10	1/10
Liver Vacuolization (fatty-like), large	Incidence per group	0/10	2/10	2/10	5/10*
Liver Vacuolization (fatty-like), small-perilobular	Incidence per group	3/10	4/10	8/10	6/10
Thyroid High follicular epithelium	Incidence per group	2/10	2/10	2/9	9/10**
Thyroid Small follicles	Incidence per group	1/10	1/10	4/9	6/10

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; score 1 to 5: slight to severe.

TOXICOKINETICS: Exposure was higher in female than in male rats. This sex difference increased after repeated dosing and with higher doses. There was a less than dose-proportional increase in exposure. In females no clear changes in exposure were observed after repeated dosing.

Study	TK analysis	Dose /kg/day)	C_{max} (ng/mL)		AUC_{0-24h} (ng.h/mL)	
			Male	Female	Male	Female
Gavage : 3-month in Sprague Dawley rats	Day 0	70	135	163	739	1137
		200	250	283	1438	1715
		600	436	1279	2503	5796
	Week 12	70	64	196	1002	1411
		200	124	457	739	2275
		600	256	801	2285	6815

CONCLUSION: The 3-month gavage rat study showed target organs of toxicity including kidney, liver adrenal and thyroid. NOAEL was around 70 mg/kg.

Study title: **3-MONTH ORAL TOXICITY STUDY IN DOGS**
Study no.: TMC125-NC116
Laboratory: