GLP:	yes (x) no ( )
QA report:	yes (x) no ( )
Lot #, purity:	AG1086/ —
Formulation/ vehicle:	TMC125 — PEG400
Methods:	
Dosing:	0 (control), 20, 40 and 80 mg — /kg/day, given in two equal doses. The second daily dose was given 7h after the first dose.
Species/strain:	Beagle dogs
sex/group	4
Route	Oral gavage for 3 months
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, tissue list identical to that in the 1-month dog study (see above)
Toxicokinetics:	Day 1, 30, 59, Week 13, Day 87
Results:	
Mortality	No mortality occurred.
Clinical signs:	From week 6 onwards, 2 animals in the high dose group (1 male and 1 female) as well as 1 female in the mid dose group displayed erythema and alopecia on limbs and abdomen and some piloerection. Sporadic occurrence of other clinical symptoms (retching, vomiting, salivation, lethargy, abnormal posture, labored respiration and rales) in all dose groups, including control, seemed to be related to gavage dosing of solutions with PEG400.
Body weights:	Body weight gain was lower in males in the low dose group and in females in the low and mid dose groups. However, this was considered to occur by chance.
Food consumption:	unremarkable

Ophthalmology: unremarkable

EKG: Some effects in ECG recording were noted (prolonged ST-interval in males in the mid dose group). Since the differences were small and not dose dependent, toxicological relevance of the findings was doubted.

Hematology: unremarkable

Clinical chemistry: Significantly higher chloride values were recorded for animals in the high dose group at week 13, and for females in the mid and high dose groups at week 6. Since chloride measurement is disturbed by bromide this is considered to be related to bromide present in the test article.

Urinalysis: unremarkable

Organ weights: Lower weights of the adrenal gland were noted for males at the mid and high doses, as well as higher liver weight in one female in the high dose group. However, these findings were not associated with morphological changes in histopathology

Gross pathology: unremarkable

Histopathology: unremarkable

Toxicokinetics: At the highest dose of 80 mg —— ,kg/day Cmax values of about 1000 ng/ml. TMC125 were obtained in male and female animals after single oral administration. Systemic exposure, expressed as AUC7h (= after morning dose), increased as much as 127% in female animals after repeated dosing for a period of 30 days. In males the increase was less pronounced. There were no consistent differences in pharmacokinetic parameters between male and female dogs. At day 87, AUC24h values were 12593, 21760, 24129 ng.h/ml in males at the 20, 40 and 80 mg/kg/day dose respectively. In females these values were 10211, 11633 and 15905 ng.h/ml, at the respective doses. 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. High concentrations of TMC125 in bile indicated that biliary excretion might be an important route of elimination for this compound. The sponsor concluded that the NOAEL was 80 mg —— kg/day, for this study.

Multiple dose (3-month) – DOG (4 animals/sex/group) – Study TMC125-NC116

Dose (mg/kg/day)	Gender	Toxicokinetic parameters					
		C_{max} (ng/ml)	t_{max} (h)	C_{max} (ng/ml)	$t_{1/2}$ (h)	AUC_{7h} (ng.h/ml)	AUC_{24h} (ng.h/ml)
3-month oral dose – DOG[2] (PEG400 sol. - (levels at day 1) ——		(mean ± SD)	(median)	(mean ± SD)		(mean ± SD)	
20 mg/kg/day	male	569.8 ± 132.5 [3]	2 [3]	273.0 ± 86.74 [3]	- [3]	2690 ± 536.7 [3]	- [3]
40 mg/kg/day		647.5 ± 167.3 [3]	2 [3]	329.3 ± 110.1 [3]	- [3]	3022 ± 717.7 [3]	- [3]
80 mg/kg/day		1015 ± 230.8 [3]	3 [3]	485.5 ± 101.6 [3]	- [3]	5302 ± 1022 [3]	- [3]
20 mg/kg/day	female	349.0 ± 245.6 [3]	2 [3]	187.6 ± 136.9 [3]	- [3]	1664 ± 1394 [3]	- [3]
40 mg/kg/day		450.0 ± 230.8 [3]	4 [3]	335.0 ± 179.8 [3]	- [3]	2326 ± 1223 [3]	- [3]
80 mg/kg/day		884.5 ± 270.7 [3]	2 [3]	497.8 ± 177.3 [3]	- [3]	4504 ± 1233 [3]	- [3]
3-month oral dose – DOG (levels at day 30)							
20 mg/kg/day	male	735.5 ± 81.18 [3]	2 [3]	529.3 ± 187.0 [3]	- [3]	4239 ± 631.0 [3]	- [3]
40 mg/kg/day		1034 ± 121.1 [3]	4 [3]	825.3 ± 297.5 [3]	- [3]	5973 ± 1150 [3]	- [3]
80 mg/kg/day		1383 ± 196.9 [3]	2 [3]	855.0 ± 335.8 [3]	- [3]	7599 ± 1617 [3]	- [3]
20 mg/kg/day	female	693.5 ± 90.38 [3]	3 [3]	443.0 ± 209.4 [3]	- [3]	3775 ± 862.8 [3]	- [3]
40 mg/kg/day		730.8 ± 112.8 [3]	2 [3]	571.3 ± 222.7 [3]	- [3]	4378 ± 944.9 [3]	- [3]
80 mg/kg/day		1146 ± 187.1 [3]	4 [3]	960.0 ± 327.6 [3]	- [3]	6877 ± 1202 [3]	- [3]

[2] Accurate determination not possible.
[3] Calculated after first dosing
[3] Parameter not calculated

Study title:	**A 6-MONTH ORAL TOXICITY STUDY IN RATS**
Study no.:	NC133
Laboratory:	[redacted]
Study initiation:	4/2/2001
GLP:	yes (x) no ()
QA report:	yes (x) no ()
Lot #, % purity:	00036 and AA6424-1-01/ [redacted]
Formulation/ vehicle:	TMC125 [redacted] Powder (w/w) in PEG400, 5 ml/kg
METHODS:	
Dosing:	0 (control), 70, 200 or 600 mg TMC125 [redacted] /kg body weight/day
Species/strain:	[redacted] WI(Glx/BRL/Han)BR rats
#/sex/group:	20
TK group:	9 animals per sex and per dose group
Route, volume:	Oral gavage, 5 ml/kg
Observations and times:	
Clinical signs:	once daily
Body weights:	Weekly
Food consumption:	Weekly
Ophthalmoscopy:	pre-test and during week 25
Hematology:	At pre-test, Week 13, and at the end of the treatment period.
Clinical chemistry:	at pre-test, Week 13 and at the end of the treatment period
Urinalysis:	At pre-test and at the end of the treatment period.
Bone Marrow Smears:	at termination
Gross pathology:	at termination
Organs weighed:	at termination
Histopathology:	Conducted at the end of the study, microscopically examined tissues were denoted as 'S' in the necropsy tissue list below:

Tissue list

adrenals (†) (S)
animal identification
aorta
bone marrow smear(femur) (a) (c)
brain (†) (S)
caecum (S)
colon (S)
duodenum (S)
eyes (b) (S)
femur with bone marrow and articular surface (S)
gross lesions (S)
Harderian glands (d)
head
heart (†) (S)
ileum (S)
jejunum (S)
kidney (†) (S)
Lacrimal glands (d)
larynx
liver (†) (S)
lungs with mainstem bronchi (S)
mammary (f) (S)
mandibular lymph nodes (S)
mesenteric lymph nodes (S)
muscle (quadriceps)
nasal turbinates (d)
nasopharynx (d)
oesophagus (S)
optic nerves (S)
ovaries (†) (S)
pancreas (S)
pituitary (†) (S)
prostate (†) (S)
rectum
salivary glands (S)
sciatic nerves (S)
seminal vesicles
skin (S)
spinal cord cervical (S)
spinal cord lumbar (S)
spinal cord thoracic (S)
spleen (†) (S)
sternum with bone marrow (S)
stomach (S)
testes + epididymides (e) (†) (S)
thymus (S)
thyroids + parathyroids (†) (S)
tongue
trachea (S)
tracheal bifurcation
urinary bladder (S)
uterus (S)
vagina
Zymbal glands (d)

fixative = 10% neutral buffered formalin except where indicated by:
a = methanol, b = Davidson's fluid e = Bouin's fixative and processed to block stage. Bone designated for histopathological examination was decalcified using Kristenson's fluid. c = see clinical pathology section. d = preserved with the head in situ. f = female only.

Toxico-kinetics: Blood sampling for toxicokinetics was performed on day 1 and weeks 13 and 26

RESULTS:

Mortality One animal 160 (female, 600 mg/kg/day) died on Day 119 (Week 17). The cause of demise was a pyogranulomatous skin lesion. There was no evidence of toxicity or treatment-related effects contributing to the animal's morbidity.

Clinical signs: Salivation and limb paddling were observed in both sexes immediately after dosing in all groups (including controls) from Day 6 of Week 2 through to completion of the dosing period.

Other post-dosing observations included reduced activity subdued behavior, raised tail, raised fur/piloerection and mouth rubbing. Reduced activity and subdued behavior were seen in all groups (including controls) during Weeks 2 to 3 of dosing. Raised fur/piloerection was observed in treated groups during Weeks 2 and 3. Raised tail was seen in male treated groups between Week 2 and 3. Raised tail and mouth rubbing were seen in all female treated groups from Week 2 through to completion of the dosing period. The majority of these observations were noted immediately after dosing, although during Weeks 2 and 3, some observations (reduced activity/subdued behavior and piloerection) were seen up to 4 hours after dosing.

An incidence of urogenital staining was observed among males of 600 mg/kg/day group from Week 19. The incidence was low with a maximum of seven animals affected in Week 24. One of two males was affected at 70 and 200 mg/kg/day. In addition, there was a 100% incidence of soft feces observed in males administered 200 and 600 mg/kg/day in Week 26.

Staining and thinning fur (head and dorsal) were noted in all groups, including controls. However, the incidence was greater among treated animals.

Body weights: For females at 200 and 600 mg/kg/day there was an increased weight gain of approximately 12% during the first 13 weeks of treatment ($p<0.05$). Between weeks 13

and 26, body weight gain for females at 200 and 600 mg/kg/day was slightly lower than the concurrent control weight gain over the same period ($p<0.0.3$). Overall weight gain after 26 weeks remained slightly higher than the concurrent control for females at 70, 200 and 600 mg/kg/day ($p<0.05$).

Food consumption: Unremarkable

Ophthalmology: Unremarkable

Hematology: Unremarkable

Clinical chemistry: At Weeks 13 and 26, there was a dose-related increase in plasma chloride levels, principally at 200 and 600 mg/kg/day for both sexes ($P<0.05$-0.01). The sponsor indicated that the apparent increase in chloride levels seen at all dose levels was considered to be due to the presence of bromide in the test article (there is evidence available from the manufacturer of the assay that the presence of bromide ions will interfere with the measurement of chloride ions in plasma and this is considered the most likely cause of the apparent increase in plasma chloride levels.)

For males at Week 13 and 26, there was an increase in total protein at 600 mg//kg/day ($p<0.01$ at Week 13, $P<0.05$ at Week 26)). For females at Week 13 and Week 26, the increase in total protein was present ($p<0.01$).

Urinalysis: At Week 12, urinary volume was increased for females in 200 and 600 mg/kg/day ($p<0.05$ at 200 mg/kg/day). At Week 25, urinary volume was increased for males and females at 600 mg/kg/day ($p<0.05$ for males, $p<0.01$ for females).

Organ weights: There was a very slight increase in thyroid weight for males at 600 mg/kg/day ($p<0.05$). The thyroid weight of females at 600 mg/kg/day was comparable with concurrent controls. There was a slight increase in liver weight for males and females at 600 mg/kg/day ($p<0.05$).

Necropsy: Unremarkable

Histopathology: There was an increase in the incidence and severity of thyroid follicular cell hypertrophy in mid- and high dose groups (see table below). Thyroid follicular hypertrophy was characterized by an increase in height of the epithelium lining the follicles, with an apparent decrease in the amount of colloid. The lesion was minimal to slight in severity and there was no evidence of epithelial stratification or papillary infolding.

Group incidence of selected microscopic findings – terminal kill

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (mg/kg/day)	0	70	200	600	0	70	200	600
Thyroid	No. examined:	20	20	20	20	20	20	20	19
follicular cell hypertrophy	Grade -	17	16	11	3	18	17	10	5
	1	2	3	6	8	2	3	7	8
	2	1	1	3	9	0	0	3	6

Key: "-" = finding not present, 1 = minimal, 2 = slight

The sponsor indicated that thyroid follicular hypertrophy is a response to the drug's stimulation to the thyroid. There are various pathogenesis mechanisms, causing decreased synthesis or increased excretion of thyroid hormones, which would affect the hypothalamic-pituitary-thyroid axis (no pituitary or hypothalamus abnormalities were

reported in this study). However, the precise pathogenesis of the lesion in this study is uncertain.

Toxicokinetics: A highest Cmax value of about 330 ng/ml was obtained in male rats after single oral administration of 600 mg/kg. At week 13 aid 26, Cmax values were on average about 130 ng/ml, in male rats. In females, the Cmax values were on average 273 ng/ml during the 26-week (see table below) of dosing.

In male and female rats, Cmax values increased less than dose proportionally between the 70- and 200- mg/kg dose levels, whereas between the 200- and the 600 mg/kg dose level, this parameter increased dose proportionally in males and females. Peak plasma concentrations and AUC values were higher in females than those in males.

Toxicokinetics:

Males

Parameter		Male rats	Male rats	Male rats
Dose	mg/kg	70	200	600
C_{max}	ng/ml	62.53	51.37	124.4
Dose-normalized C_{max}	ng/ml	0.8933	0.2569	0.2073
t_{max}	h	1.0	1.0	8.0
t_{last}	h	8.0	24.0	48.0
AUC_{last}	ng.h/ml	210.4	556.9	1891
Dose-norm. AUC_{last}	ng.h/ml	3.006	2.785	3.152
AUC_{24h}	ng.h/ml	-	-	1727
Dose-norm. AUC_{24h}	ng.h/ml	-	-	2.878
Accum. ratio		1.627	2.112	0.9619
λ_z	1/h	0.2774	0.07591	0.01376*
$t_{1/2}$	h	2.499	9.132	50.37*
Corr. Coeff.	r^2	0.9644	0.9975	1.000

*: Accurate determination not possible
Accum. ratio: AUC_{last} week 26/AUC_{∞} day 1
Accum. ratio: AUC_{last} week 26/AUC_{last} day 1

Females

Parameter		Female rats	Female rats	Female rats
Dose	mg/kg	70	200	600
C_{max}	ng/ml	55.63	140.3	275.3
Dose-normalized C_{max}	ng/ml	0.7947	0.7015	0.4588
t_{max}	h	1.0	4.0	4.0
t_{last}	h	24.0	48.0	48.0
AUC_{last}	ng.h/ml	656.0	1588	4023
Dose-norm. AUC_{last}	ng.h/ml	9.371	7.940	6.705
AUC_{24h}	ng.h/ml	-	1286	3562
Dose-norm. AUC_{24h}	ng.h/ml	-	6.430	5.937
Accum. ratio		2.405	1.724	2.068
λ_z	1/h	0.09747	NA	0.03490*
$t_{1/2}$	h	7.111	NA	19.86*
Corr. Coeff.	r^2	0.9997	NA	1.000

Conclusions: The sponsor concluded that the No Observed Adverse Effect Level (NOAEL) for TMC125 was 70 mg/kg/day in males (AUC_{last8h}=0.21ug.h/ml) and females ($AUC_{last24h}$=0.66ug.h/ml).

Study title: **6-MONTH ORAL TOXICITY STUDY IN DOGS**

Study no.: TMC125-NC117

Laboratory: [redacted]

Study initiation:	11/21/2000
GLP:	yes (x) no ( )
QA report:	yes (x) no ( )
Lot #, % purity:	AG1086/ —
Formulation/ vehicle:	TMC125. — PEG400
Methods:	
Dosing:	0 (control), 20, 40 and 80 mg — kg/day, given in two equal doses. The second daily dose was given 7h after the first dose.
Species/strain:	Beagle dogs
#/sex/group	4
Route	by gavage for 6 months
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	Ileum
Skeletal muscle	Caecum
Sternum	Colon
Heart	Rectum
Aorta	Kidneys
Trachea	Urinary Bladder
Lung	Ureter
Spleen	Prostate gland
Lymph node (mandibular, mesenteric)	Ovaries
Thymus	Uterus
Tongue	Cervix
Pancreas	Vagina
Liver	Thyroids
Gall bladder	Parathyroid glands
Oesophagus	Adrenal glands
Stomach	Pituitary gland
Duodenum	Spinal cord (cervical, thoracic, lumbar)
Jejunum	Sciatic nerve

Brain (medulla, pons, cerebellum, cerebrum (cortex and hippocampus))
Skin +Mammary gland area, males and females (pelvic, left and right)
Salivary gland (parotid, sublingual, submaxillary)
Peyer's patches (jejunum, ileum) if detectable

Eyes and optic nerve :fixed in Davidson's solution**
Testes and Epididymides :fixed in Bouin's**
** = transferred to formalin after fixation for at least 24 hours.

Toxicokinetics:	Day 1, Week 13, Week 26,
Results:	
Mortality:	No mortality occurred.
Clinical signs:	One male treated at 40 mg/kg and one male treated at 80 mg/kg displayed general erythema and alopecia on limbs. These findings were reversible, since they were noted from week 5 until 25, except for the erythema in the male treated at 80 mg/kg, which persisted until termination. Although these types of skin changes are observed more often in Beagle dogs used in this type of study, a possible relationship to treatment with TMC125 cannot be fully excluded due to the distribution over the dose groups. During the course of the study, isolated occurrences of other different clinical signs were noted. However, the distribution of these signs showed no relationship with the test article. These findings are considered to be a result of the technical procedures of the study, twice daily dosing by oral gavage with PEG400 as the vehicle, which is supported by the fact that the incidence decreased after changing to once daily dosing (from day 73 onwards). The clinical signs are within the biological variation of this type of animal in this type of study.
Body weights:	unremarkable
Food consumption:	unremarkable
Ophthalmology:	unremarkable
EKG:	unremarkable
Hematology:	Unremarkable
Clinical chemistry:	At the end of treatment a statistically significant decreased value for inorganic phosphate was recorded for females treated at 80 mg [redacted] kg/day. Increased chloride values were recorded for males and females of the 40 mg/kg and 80 mg/kg groups from week 6 onwards. However, the effect was less prominent in the females and females of the 40 mg/kg group showed high pretest values for chloride. Since chloride measurement is disturbed by bromide this is considered to be related to bromide present in the test article. Increased relative spleen weights were recorded for males at termination at the 80 mg [redacted] kg/day dose, although no morphological change was identified by histopathology.
Urinalysis:	Unremarkable
Organ weights:	Unremarkable
Gross pathology:	Unremarkable
Histopathology:	Unremarkable

Toxicokinetics:

Multiple dose (6-month) – DOG – Study TMC125-NC117

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)
6-month oral dose – DOG[2] (PEG400 sol. – (levels at day 1)		(mean ± SD)	(median)	(mean ± SD)	(mean ± SD)	(mean ± SD)	(mean ± SD)
20 mg/kg/day	male	327.2 ± 202.9 [a]	1.5 [a]	229.3 ± 241.3 [b]	- [b]	1616 ± 1562 [b]	- [b]
40 mg/kg/day		828.0 ± 96.87 [a]	2 [a]	467.3 ± 110.6 [b]	- [b]	4288 ± 360.0 [b]	- [b]
80 mg/kg/day		1451 ± 354.6 [a]	2 [a]	780.3 ± 186.5 [b]	- [b]	7228 ± 1129 [b]	- [b]
20 mg/kg/day	female	348.1 ± 197.3 [a]	2 [a]	178.9 ± 151.0 [b]	- [b]	1695 ± 1021 [b]	- [b]
40 mg/kg/day		486.9 ± 261.6 [a]	2 [a]	371.2 ± 215.1 [b]	- [b]	2617 ± 1404 [b]	- [b]
80 mg/kg/day		712.3 ± 410.2 [a]	2 [a]	303.5 ± 110.9 [b]	- [b]	3129 ± 1920 [b]	- [b]
6-month oral dose – DOG (levels at day 86)							
20 mg/kg/day	male	984.3 ± 108.0	3	356.3 ± 74.60	16.79 ± 4.578*	5864 ± 1012	14240 ± 3280
40 mg/kg/day		1800 ± 125.7	4	299.8 ± 106.6	12.17 ± 4.657*	6506 ± 465.9	15174 ± 981.2
80 mg/kg/day		2030 ± 652.3	2	530.8 ± 195.0	12.15 ± 2.064*	11598 ± 3062	26666 ± 7304
20 mg/kg/day	female	887.3 ± 98.24	2	257.8 ± 39.56	25.28 ± 10.44*	5073 ± 589.8	11233 ± 1051
40 mg/kg/day		1857 ± 142.0	2	286.8 ± 53.03	15.01 ± 3.636	6027 ± 1051	13548 ± 2137
80 mg/kg/day		1950 ± 390.0	2	418.8 ± 113.3	13.81 ± 3.996*	10652 ± 2399	21966 ± 4464

*Accurate determination not possible.
[a] Calculated after first dosing; all other parameters calculated after once daily dosing
[b] Parameter not calculated

At the highest dose of 80 mg — kg/day, Cmax values of about 1450 ng/ml and 710 ng/ml TMC125 were obtained in male and female animals, respectively, after single oral administration at day 1. The systemic exposure, expressed as AUC7h, after repeated dosing for a period of 86 days, increased as much as 70 % in female animals. In males the increase was less pronounced. There were no consistent differences in the pharmacokinetic parameters between male and female dogs. AUC24h values at day 177 were 15730, 19763, 28629 ng.h/ml in males at the 20, 40 and 80 mg/kg/day dose respectively. In females these values were 10757, 9685 and 17174 ng.h/ml, at the respective doses.

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. High concentrations of TMC125 in bile indicated that biliary excretion might be an important route of elimination for this compound.

It was concluded that the NOEL was 20 mg — kg/day based on skin changes recorded at 40 and 80 mg — /kg/day, and the decreased inorganic phosphate value and increased relative spleen weight noted at 80 mg/kg/day. As the toxicological relevance of the observed skin changes is doubted and based on the absence of any functional disturbance or morphological changes in treated animals, a NOAEL for TMC125 of 80 mg — kg/day can be assumed.

Study title: **A 12-MONTH ORAL TOXICITY STUDY IN DOGS**
Study no.: TMC125-NC134
Laboratory: —
Study initiation: 10/2001

GLP: yes (x) no ()
QA report: yes (x) no ()
Lot #, % purity: M60261001; — M60261002: —
Formulation/ vehicle: TMC125 — powder (w/w suspension) in PEG400, 5 ml/kg

METHODS:
Dosing: 0 (control), 30, 80 or 240 mg TMC125 — kg body weight/day

From start of study up to and including week 5:

Batch Nr.	Dose level (mg — /kg/day)	Dosing route	Vehicle	Volume ml/kg	Animal numbers	
					males	females
M60261002	0	Oral	PEG400	1	1-4	23-26
M60261002	30	Oral	PEG400	1	5-8	27-30
M60261002	80	Oral	PEG400	1	9-12	31-34
M60261002	80	Oral	PEG400	1	13-15	35-37
M60261002	240	Oral	PEG400	1	16-19	38-41
M60261002	240	Oral	PEG400	1	20-22	42-44

From week 6 onwards:

Group	Dose level (mg — /kg/day)	Dosing route	Vehicle	Volume ml/kg	Animal numbers	
					males	females
M60261002	0	Oral	PEG400	1	1-4	23-26
M60261002	30	Oral	PEG400	1	5-8	27-30
M60261002	80	Oral	PEG400	1	9-12	31-34
M60261002	240	Oral	PEG400	1	16-19	38-41
M60261002	240	Oral	PEG400	1	20-22	42-44
M60261002	240*	Oral	PEG400	1	45-47	48-50

* As of week 6, recovery animals of Group 3 (animals 13-15 and 35-37) were reallocated to the high-dose group and dosed at 240 mg base/kg/day. Animal numbers were 45-47 and 48-50

Species/strain: Beagle dog
#/sex/group: 20
TK group: 9 animals per sex and per dose group
Route, volume: Oral gavage, 1 ml/kg
Observations and times:
Mortality 2/day
Clinical signs: once daily (1/week after 5 weeks)
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: pre-test and during week 26, 39, 52 and 65 (recovery animal only).
EKG Pre-test and during week 26, 39, 52 and 65 (recovery animal only
Hematology: Pre-test and during week 13, 26, 39, 52 and 65 (recovery animal only
Clinical Pre-test and during week 13, 26, 39, 52 and 65 (recovery animal only

chemistry:

Urinalysis: Pre-test and during week 13, 26, 39, 52 and 65 (recovery animal only

Bone Marrow Smears: at termination

Gross pathology: at termination (plus interim animal at group 4 at 13th week)

Organs weighed: at termination on:

Adrenals
Brain
Epididymides
Heart
Kidneys
Liver
Ovaries
Pituitary gland
Prostate
Spleen
Testes
Thymus
Thyroid with parathyroids
Uterus

Histo-pathology: Conducted at the end of the study, microscopically examined tissues were denoted as 'S' in the necropsy tissue list below:

Tattoo (not processed)
Adrenal glands
Aorta
Brain (medulla, pons, cerebellum, cerebrum (cortex and hippocampus))
Caecum
Cervix
Colon
Duodenum
Eyes with optic nerve and lacrimal gland*
Gall bladder
Heart including coronary arteries
Ileum
Jejunum
Kidneys
Liver
Lung
Lymph node (mandibular, mesenteric)
Oesophagus
Ovaries
Pancreas
Parathyroid glands
Peyer's patches (jejunum, ileum) if detectable
Pituitary gland
Prostate gland
Rectum
Salivary gland (parotid, sublingual, submaxillary)
Sciatic nerve
Skeletal muscle
Skin +Mammary gland area, males and females (pelvic, left and right)
Spinal cord (cervical, thoracic, lumbar)
Spleen
Sternum
Stomach
Testes and Epididymides **
Thymus
Thyroids
Tongue
Trachea
Urinary Bladder
Ureter
Uterus
Vagina
All gross lesions

* = fixed in Davidson's solution and transferred to formalin after fixation for at least 24 hours.
** = fixed in Bouin's fixative and transferred to formalin after fixation for at least 24 hours.

Toxico-kinetics: Blood sampling for toxicokinetics was performed on during week 13, 26, 39, 52 and 65 (recovery animal only)

RESULTS:

Mortality Two dogs died unexpectedly in weeks 16 / 17 of treatment. One male treated at 240 mg/kg/day (no. 17) was found dead on day 112 (week 16) and one female treated at 80 mg/kg/day (no. 31) was found dead on day 113 (week 17). Microscopic evaluation revealed inflammatory and necrotic lesions in the lungs and trachea of both animals. These deaths and lesions were considered by the sponsor to have resulted from accidental aspiration of gastric contents containing the test formulation..

Clinical signs: Feces with (many) white particles were noted in males and females treated at 240 mg/kg from week 2 onwards. This finding was not observed during the recovery period. Occurrences of vomiting, salivation, breathing difficulties, calm behavior, retching, rales, shaking of the head, vocalization, ventro-lateral recumbency, uncoordinated movements, abnormal or flat posture, coughing, pale gingiva and lethargy were recorded among individual animals of all groups. These findings were considered related to the method of dosing (oral gavage) and the vehicle (PEG400) used.
All other incidental findings (i.e. alopecia, scabs, wounds, general erythema, discoloration of faces, discharge from the eyes, discoloration of the eyes, swelling at various body sites, abnormal posture and/or gait, mucous faces with red particles, absence of faces, diarrhea, in heat, leanness, scrapings, nodule on the leg, faces

containing mucus) were considered by sponsor to be drug-unrelated.

Body weights: Unremarkable

Food consumption: Unremarkable

Ophthalmology: Unremarkable

EKG Unremarkable

Hematology: Unremarkable

Clinical chemistry: Blood biochemistry revealed lower albumin values for females treated at 240 mg/kg after 26, 39 and 52 weeks of treatment. One female of group 3 showed increased values of liver enzymes until the end of the study (this animal did not show any abnormalities in the histopathological evaluation.)

Urinalysis: The changes noted in urinalysis consisted mainly of increased volumes and electrolyte excretion. Based on the absence of any other findings that could support any disturbance of the electrolyte or water balance, the toxicological significance of these findings was doubted.

Organ weights: *Interim sacrifice after 13 weeks of treatment at 240 mg/kg:*
Based on historical control data, mean prostate weight was considered to be high and spleen weight was low in males.
At the end of treatment:
Increased absolute prostate weight was noted in males of groups treated at 80 and 240 mg/kg. Increased kidney weight was recorded in females of the 80 mg/kg group (abs + rel) and 240 mg/kg group (abs). No corroborative findings were present in the histopathological evaluation. These changes in organ weights were considered to be uncertain. Increased uterus and ovaries weights were recorded for group 3 animals after 52 weeks of treatment. These findings were considered by the sponsor to be related to the estrus cycle of the female dog rather than an effect of treatment.

After recovery:
Organ weight (ratios) obtained after recovery were considered to be within the normal range of biological variation for Beagle dogs.

Necropsy: An enlarged prostate was noted in a male treated at 240 mg/kg whilst another male in this group showed a reduced size of the prostate.
Findings observed during the scheduled necropsies consisted of (discolored) foci in duodenum, ileum, cecum, rectum, colon, kidneys, urinary bladder, spleen and heart; reduced size of the thymus and thyroid; enlargement of thyroid, ovaries, prostate and mandibular lymph node; discoloration of (parts of) different lymph nodes, duodenum, urinary bladder, kidneys and salivary glands; finely granulated contents of the gall bladder; cysts in thyroid and a thickened uterus.

Histopathology:

The animals of the interim sacrifice:
In the thymus of females only, a slight or moderate degree of medullary proliferation and a slight degree of lymphoid follicle formation was recorded. This finding was not present in animals at the end of 12 months of treatment and therefore considered unrelated to treatment.

The animals of main and recovery groups:
A range of inflammatory alterations were recorded in the lungs - mononuclear intra/-perialveolar inflammation, peri- vascular/bronchial inflammatory cell foci and alveolar inflammation, of both treated and control dogs at both sacrifices. They occurred at minor grades of severity, minimal or slight; in a few cases attaining moderate severity in treated groups. The features of the lesions, namely occasional presence of foreign material and foreign body giant cells, were suggestive of low grade aspiration of esophageal or oropharyngeal contents during the course of the study. They were noted in both treated and control animals and were still in evidence following the recovery period. Pulmonary aspiration was noted in the two unscheduled deaths.

Toxicokinetics:

The toxicokinetic evaluation (non-GLP) was performed as a separate study by [redacted] (see tabulated results below).

Cmax: At the highest dose at day 1, Cmax-values of 2777 (range [redacted] ng/ml were obtained in males and 2286 (range [redacted] ng/ml in females. In week 52, Cmax values were 2163 (range [redacted] ng/ml and 1771 (range [redacted] ng/ml in male and female animals, respectively.

AUC: At the highest dose level at day 1, AUC24h-values of 34761 (range 21763 - 47218) ng.h/ml were obtained in males and 26985 (range 12197 - 58755) ng.h/ml in females. In week 52, AUC24h-values were 35618 (range 23760 - 48145) ng.h/ml and 23249 (range 8740 -59157) ng.h/ml in male and female animals, respectively.

Time-Dependent AUC Changes: Systemic exposure, expressed as AUC24h, was constant over the study time interval, except at the 80 mg/kg dose level in females, where exposure tended to decrease over time from on average 17868 ng.h/ml on day 1 to on average 10232 and 10371 ng.h/ml during weeks 26 to 39, respectively. At week 52, mean values in females had increased to 19344 ng.h/ml, comparable to the data at the start of the study, but a high variability was observed.
In general, the systemic exposure in female dogs was lower in comparison with male dogs. A high inter-individual variability was observed, both in males and females over the treatment period.

Day 1 Toxicokinetics

Males

Parameter		Mean	±	SD	Mean	±	SD	Mean	±	SD
Dose	mg/kg	30			80			240		
C_{max}	ng/ml	903.8	±	424.0	2042	±	1971	2777	±	721.8
Dose-normalized C_{max}	ng/ml	30.13	±	14.13	25.52	±	24.63	11.57	±	3.009
t_{max}	h	3.0 (1.0-8.0)			2.0 (1.0-12.0)			4.0 (2.0-4.0)		
t_{last}	h	24.0 (24.0-24.0)			24.0 (24.0-24.0)			24.0 (24.0-24.0)		
AUC_{last}	ng.h/ml	9869	±	4574	22938	±	17364	34761	±	8936
Dose-norm. AUC_{last}	ng.h/ml	329.0	±	152.5	286.7	±	217.1	144.8	±	37.23
AUC_{∞}	ng.h/ml	12667*	±	6193	53895*	±	66108	45539*	±	12906
Dose-norm. AUC_{∞}	ng.h/ml	422.2*	±	206.4	673.7*	±	826 4	189.7*	±	53.76
$t_{1/2}$	h	10.95*	±	1.160	32.01*	±	54.09	11.04*	±	2.737

*: accurate determination not possible

t_{max}, t_{last}: median (min-max)

Females

Parameter		Mean	±	SD	Mean	±	SD	Mean	±	SD
Dose	mg/kg	30			80			240		
C_{max}	ng/ml	660.0	±	199.5	1576	±	521.2	2286	±	1374
Dose-normalized C_{max}	ng/ml	22.00	±	6.647	19.70	±	6.513	9.524	±	5.726
t_{max}	h	4.0 (2.0-12.0)			2.0 (1.0-8.0)			2.0 (2.0-12.0)		
t_{last}	h	24.0 (24.0-24.0)			24.0 (24.0-24.0)			24.0 (24.0-24.0)		
AUC_{last}	ng.h/ml	7142	±	1933	17868	±	2633	26985	±	15778
Dose-norm. AUC_{last}	ng.h/ml	238.1	±	64.43	223.4	±	32.92	112.4	±	65.74
AUC_{∞}	ng.h/ml	9433*	±	2048	32277*	±	27024	70936*	±	94145
Dose-norm. AUC_{∞}	ng.h/ml	314.4*	±	68.28	403.4*	±	337.7	295.6*	±	392.3
$t_{1/2}$	h	11.88*	±	1.939	19.23*	±	22.46	31.15*	±	50.06

*: accurate determination not possible

t_{max}, t_{last}: median (min-max)

Week 26

Males

Parameter		Mean	±	SD	Mean	±	SD	Mean	±	SD
Dose	mg/kg	30			80			240		
C_{max}	ng/ml	1255	±	144.3	1975	±	506.9	2192	±	702.4
Dose-normalized C_{max}	ng/ml	41.84	±	4.810	24.69	±	6.335	9.132	±	2.926
t_{max}	h	3.0 (2.0-4.0)			3.0 (2.0-4.0)			3.0 (2.0-4.0)		
t_{last}	h	24.0 (24.0-24.0)			24.0 (24.0-24.0)			24.0 (24.0-24.0)		
AUC_{last}	ng.h/ml	17082	±	3902	26672	±	5823	33390	±	13525
Dose-norm. AUC_{last}	ng.h/ml	569.4	±	130.0	333.4	±	72.77	139.1	±	56.37
Accum. ratio		1.575	±	0.7932	1.864	±	1.295	0.6257	±	0.2385
$t_{1/2}$	h	14.32*	±	3.474	13.88*	±	5.353	25.16*	±	21.32

*: accurate determination not possible

t_{max}, t_{last}: median (min-max)

Females

Parameter		Mean	±	SD	Mean	±	SD	Mean	±	SD
Dose	mg/kg	30			80			240		
C_{max}	ng/ml	772.3	±	56.05	764.3	±	316.2	1527	±	705.5
Dose-normalized C_{max}	ng/ml	25.74	±	1.870	9.554	±	3.952	6.360	±	2.938
t_{max}	h	3.0 (2.0-4.0)			2.0 (1.0-2.0)			4.0 (2.0-4.0)		
t_{last}	h	24.0 (24.0-24.0)			24.0 (24.0-24.0)			24.0 (24.0-24.0)		
AUC_{last}	ng.h/ml	9981	±	2623	10232	±	3254	21097	±	9391
Dose-norm. AUC_{last}	ng.h/ml	332.7	±	87.46	127.9	±	40.68	87.90	±	39.12
Accum. ratio		1.151	±	0.6153	0.4689	±	0.1306	0.7045	±	0.4662
$t_{1/2}$	h	16.52*	±	6.988	23.81*	±	17.88	11.21*	±	1.850

*: accurate determination not possible

t_{max}, t_{last}: median (min-max)

Week 52

Males

Parameter		Mean	±	SD	Mean	±	SD	Mean	±	SD
Dose	mg/kg	30			80			240		
C_{max}	ng/ml	963.8	±	159.9	2710	±	586.2	2163	±	614.7
Dose-normalized C_{max}	ng/ml	32.13	±	5.329	33.88	±	7.328	9.014	±	2.562
t_{max}	h	3.0 (2.0-4.0)			2.0 (2.0-2.0)			2.0 (2.0-12.0)		
t_{last}	h	24.0 (24.0-24.0)			24.0 (24.0-24.0)			24.0 (24.0-24.0)		
AUC_{last}	ng.h/ml	13460	±	4023	33901	±	11566	35618	±	9443
Dose-norm. AUC_{last}	ng.h/ml	448.7	±	134.1	423.8	±	144.6	148.4	±	39.34
Accum. ratio		1.185	±	0.4755	2.270	±	1.365	0.9245	±	0.2799
$t_{1/2}$	h	21.75*	±	7.694	29.74*	±	14.46	21.71*	±	5.743

*: accurate determination not possible
t_{max}, t_{last}: median (min-max)

Females

Parameter		Mean	±	SD	Mean	±	SD	Mean	±	SD
Dose	mg/kg	30			80			240		
C_{max}	ng/ml	812.0	±	121.9	1706	±	1247	1771	±	1320
Dose-normalized C_{max}	ng/ml	27.07	±	4.063	21.33	±	15.59	7.379	±	5.500
t_{max}	h	2.0 (1.0-2.0)			2.0 (2.0-8.0)			4.0 (1.0-12.0)		
t_{last}	h	24.0 (24.0-24.0)			24.0 (24.0-24.0)			24.0 (24.0-24.0)		
AUC_{last}	ng.h/ml	11893	±	2492	19344	±	11753	23249	±	16594
Dose-norm. AUC_{last}	ng.h/ml	396.5	±	83.04	241.8	±	147.0	96.87	±	69.14
Accum. ratio		1.356	±	0.6422	0.8605	±	0.4118	0.7112	±	0.2712
$t_{1/2}$	h	13.20*	±	5.259	13.20*	±	2.653	10.85*	±	4.318

*: accurate determination not possible
t_{max}, t_{last}: median (min-max)

Conclusions: The sponsor indicated that NOEL was 30 mg/kg based on the deaths of two animals (one at 80 mg/kg and one at 240 mg/kg) in weeks 16 and 17 of treatment, whereas NOAEL was determined to be 240 mg/kg. AUC24h values at the NOAEL of 240 mg /kg were 34761 (male) and 26085 (female) ng.h/ml on day 1 and 35618 (male) and 23249 (female) ng.h/ml in Week 52.

NON-GLP STUDY TITLE: **TMC125 (R165335): Single Dose Escalation Oral Toxicity Study Followed by 5-day Repeated Doses Oral Toxicity Study in the Beagle Dog (tolerance study)**

STUDY NO.: TMC125-NC224

LABORATORY: Johnson & Johnson Pharmaceutical Research & Development, Belgium

STUDY DATE: 1/2005

OBJECTIVES AND METHODS: The objective of this non-GLP study was to determine and assess the toxicity of TMC 125 (spray dried — when administered in a tablet or aqueous suspension. The tablet form was dosed once for each escalating dose po (n=1/sex) up to MFD, each dose being followed by 3-4 days observation/wash-out period. Six days (= wash-out period) after administration of the highest dose, the potential toxicity of TMC125 was further assessed by po qd (as a tablet), at MFD, for 5 days in beagle dogs. To increase exposure, a second repeated dose phase was conducted. After a wash-out period (4.5 months) and addition of 1 male and 1 female dog to the study, the toxicity of TMC125 was further assessed by po qd (as an aqueous suspension), at MFD. In this second repeated dose phase TMC125 was administered for 5 days to fasted dogs, followed by a 5-day wash-out period and a 5-day administration to fed dogs. The toxicokinetics of TMC125 were also studied.

GLP: yes () no (x)

QA REPORT: yes () no (x)

RESULTS AND 1. No mortality occurred during the study.

CONCLUSIONS

2. During the single dose escalation phase, single escalating doses of 40, 80, 120 and 160 mg TMC125 spray dried tablets/kg were given to one male and one female dog. No relevant clinical observations or relevant changes in ECG and heart rate, body weight, weight gain, hematological or serum parameters were noted during this SDE phase.
3. After the first repeated dose phase, a dose of 120 mg TMC125 spray dried tablets/kg given to one male and one female dog, did not result in relevant changes in ECG and heart rate, body weight or weight gain. Slight pale feces and occasional slight vomiting were noted in both dogs. In the female only, mucoid feces were seen. A slight increase in total bilirubin was noted in the male.
4. For the second repeated dose phase one male and one female dog were added to the study. A daily dose of 350 mg TMC 125 spray dried suspension/kg was given during two 5-day dosing periods (fasted and fed condition), with a 5-day wash-out in between. No relevant changes were noted in ECG, heart rate, body weight, weight gain or hematology during this 2nd RD phase. Soft feces and vomiting were noted during both 5-day dosing periods and mucoid and pale feces only during the fasted 5-day dosing period, at a higher frequency and/or severity when compared to the wash-out period. On the last day of the 5-day dosing period of dogs in fed condition, ataxia, stiff limbs, decubitus prostrate, spasms, pedaling movements and mydriasis were noted in one male dog, starting 1.5 hours after dosing, with normalization 4 hours after dosing. A slight increase in total bilirubin was noted in both males in fed condition. In females no relevant changes could be noted. Necropsy of one male and one female at the end of the 5-day repeated dose phase in fed condition did not reveal relevant macroscopic or microscopic changes.

In summary, daily oral administration of TMC125 (350 mg/kg) to one male and female dog during five consecutive days did not result in relevant histopathological findings. The relevance of the thymic atrophy in the male dog will be further evaluated in future studies.

5. During the SDE phase, Cmax were observed between 2-4 h after dosing of TMC125. After Tmax plasma concentrations declined slowly with estimated half-lives ranging between 22-29 h. Cmax were 1.09, 1.69, 1.35 and 1.84 ug/ml in the male dog and 0.903, 1.20, 1.54 and 2.43 ug/ml in the female dog at the 40, 80, 120 and 160 mg/kg b.w. dose level, respectively. Corresponding AUC values were 25.2, 31.2, 27.1 and 28.5 ug.h/ml in the male dog and 14.9, 14.4, 17.5 and 42.1 ug.h/ml in the female dog.

The highest increase in exposure was seen in the 350 mg/kg fed RD group, where the animals were gavaged before dosing. Food intake 30 minutes before dosing increased exposure to TMC125 on average 2-fold after single dosing and up to 3-fold after repeated dosing, compared to the administration of the 350 mg/kg suspension without food intake.

Methods:

Dosing: 0 (control), 40, 160 and 500 mg [redacted] kg/day, given in two equal doses. The second daily dose was given 5h after the first dose. TMC125 (spray dried [redacted], was formulated as an aqueous suspension in demineralised water at 3 concentrations of active form: 2, 8 and 25 mg eq./ml. The ingredients of the suspensions were: TMC125 spray dried and demineralised water

Species/strain: Beagle dogs

#/sex/group 3

Route by gavage for 1 months

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.

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

Tissue	Weighed	Fixed	Microscopic
animal identification (ear tattoo)		F	
adrenal glands	X	F	X
aorta		F	X
bone marrow (femur)[a]		F	X
bone marrow (sternum)[a]		F	X
bone, distal femur		F	X
bone, sternum		F	X
brain	X	F	X
cervix		F	X
epididymides		Bouin	X
esophagus		F	X
eyes		Mix	X
gall bladder		F	X
heart	X	F	X
kidneys	X	F	X
lachrymal glands[b]		F	X
large intestine (cecum, colon, rectum)		F	X
larynx		F	X
liver	X	F	X
lung	X	F	X
lymph node(s), mesenteric		F	X
lymph nodes, popliteal	X	F	X
mammary gland(s)		F	X

optic nerve(s)		Mix	X
ovaries	X	F	X
oviducts		F	X
pancreas		F	X
peripheral nerves, sciatic		F	X
Peyer's patch(es)[c]		F	X
pituitary gland	X	F	X
prostate	X	F	X
salivary gland (mandibular/parotid/sublingual)		F	X
skeletal muscle, quadriceps		F	X
skin		F	X
small intestine (duodenum, jejunum, ileum)		F	X
spinal cord (cervical, thoracic, lumbar)		F	X
spleen	X	F	X
stomach		F	X
testes	X	Bouin	X
thymus	X	F	X
thyroid glands with parathyroid gland(s)	X	F	X
tongue		F	X
trachea		F	X
ureter(s)		F	X
urinary bladder		F	X
uterus		F	X
vagina		F	X
all tissues showing gross lesions		F	X

a: bone marrow was not isolated separately; it remains within bone (evaluated on slide of distal femur and sternum); b: lachrymal glands within third eyelid
c: Peyer's patch(es) were not isolated separately; evaluated on slide of jejunum and/or ileum.

Toxicokinetics: Day 1 and Day 24.

Results:

Mortality: No mortality occurred.

Clinical signs: 160 mg eq./kg dose: Transient vomiting in all males and females mainly during the first week of dosing.
500 mg eq./kg dose: Excessive salivation in males and pale feces in all animals. In one female slight dehydration, a slight decreased activity, a purulent conjunctivitis with congested conjunctiva were noted in the last week of dosing. A moderately lower food consumption was noted in males and females from the first week of dosing onwards and was associated with a body weight loss, slightly more pronounced in females, and a moderately lower total food consumption.

Body weights and Food consumption:: In the high dose group, moderately lower food consumption was noted in males and females from the first week of dosing onwards and was associated with a body weight loss, slightly more pronounced in females, and a moderately lower total food consumption.

Ophthalmology: Unremarkable

EKG: Unremarkable

Hematology: In the high dose group, a marginal increase in RBC counts, Hb/Hct and a slight decrease in reticulocyte counts were noted in male and female dogs after two weeks of dosing.

Additionally after two weeks in females, a marginal and transient decrease in WBC was noted. After four weeks of dosing, the changes in RBC counts, Hb/Hct were more pronounced in males, but absent in females. In addition to the changes noted after two weeks, a slight decrease in WBC was noted in males.

Clinical chemistry: In the high dose group, after two and four weeks of dosing, a slight decrease in inorganic phosphate was noted in males and females, a slight increase in ALP and moderate increase in total bilirubin in 1 male and a slight increase in total bilirubin and decrease in ALP and AST in females. In two females, a slight decrease in calcium was noted after four weeks.

Urinalysis: A darker color of the urine was noted at urinalysis in males of mid- and high-dose groups.

Organ Weights and Gross Pathology: In the high dose group, slight dehydration and a red discoloration of the conjunctiva was noted in one female and slightly pale content in small and large intestines in another. A moderate decrease in thymic weight was present in males and females, whereas in females only a moderate decrease in weight of the popliteal lymph nodes was seen.

Histopathology: In the high dose group: Thymic involution, bone marrow atrophy and inspissation of bile in the gall bladder. Other changes which may be treatment-related in the high dose animals: Granulomas in the liver in one male and one female, and the mineralization of goblet cells in one female.

Toxicokinetics: After oral dosing of TMC125 at 20, 80 and 250 mg eq./kg b.i.d. (5-h dosing interval), peak plasma concentrations were reached between 1 and 5h ($T_{max,1}$) after the first daily dose and between 1 and 2 h ($T_{max,2}$) after administration of the second daily dose. After $T_{max,2}$, plasma levels declined mostly monophasically with half-lives ranging on average between 8.6 and 17 h over the entire dose range in both male and female dogs. Between 20 and 80 mg eq./kg b.w. b.i.d., a 4-fold dose increase resulted only in a 1.4 to 2.4-fold increase of the exposure. Between 80 and 250 mg eq./kg b.w. b.i.d., a 3.1-fold dose increase only resulted in a 0.8 to 1.5 fold increase of the exposure. The exposure after repeated dosing was somewhat lower than after a single dose, the accumulation index ranging between 0.95 and 0.49. From the present data it was unclear if the decrease in exposure after repeated dosing was dose dependent. No major differences in the exposure in male and female dogs was observed.

		Cmax,2 (µg/ml)		AUC0-24h (µg.h/ml)	
Dose	**Day**	**0**	**23**	**0**	**23**
20 b.i.d.	**Male**	3.17	2.47	39.0	37.3
	Female	2.92	2.08	37.4	31.7
80 b.i.d.	**Male**	5.08	3.70	71.7	51.9
	Female	7.06	4.11	87.9	55.7
250 b.i.d.	**Male**	7.56	3.64	85.7	42.2
	Female	8.27	5.60	105	75.1

Summary	In conclusion, the sponsor stated that based on the information above no NOEL level could be determined whereas 250 mg eq./kg bw twice daily was considered above the maximum tolerated dose.
Regulatory Conclusions:	The new formulation increased drug exposures in dogs by approximately 2-fold. With the apparent target organs presented in the study report, the sponsor claimed that toxicity findings were not significantly different from those by using the old formulation, and is requesting for a waiver of a longer term of canine toxicity bridging studies using the new formulation.
Regulatory Actions	Because target organs of toxicity (immune system, bone marrow, liver and gallbladder) began to emerge, at fairly low safety margins, from Study NC242, a longer duration of drug treatment appears to be necessary, in view of the lack of toxicity information available from this nonrodent species and the rodent toxicity information that was confounded by a seemingly species-specific and drug-induced thyroid disorder. It is recommended that the mid- (80 mg/kg bid) and high-dose (250 mg/kg bid) groups be placed in an additional bridging toxicity study for 6 months to fully explore the toxicity profile of TMC 125 in the dog (Submission 282).
Study title:	**6-MONTH ORAL TOXICITY STUDY IN DOGS (SPRAY-DRIED FORMULATION)**
Study no.:	TMC125-NC321
Laboratory:	Johnson and Johnson Pharmaceutical Research & Development, Turnhoutseweg 30, 2340 Beerse, Belgium
Study initiation:	4/2006
GLP:	yes (x) no ( )
QA report:	yes (x) no ( )
Lot #, % purity:	CA165335G1A281
Formulation/ vehicle:	The spray-dried formulation TMC125 + hydroxypropylmethylcellulose + microcrystalline cellulose [ratios: — or MCC, TMC125 and HPMC — respectively.] This corresponds to a drug load of — and a conversion factor of — from total test article (active+inactive) to active material — Hydroxypropyl Methylcellulose + — Microcrystalline cellulose —
Methods:	
Dosing:	Spray-dried TMC125 was administered bid, by oral gavage, for 6 months in beagle dogs. One control group and 2 treated groups were given, 0 (vehicle), 160 and 500 mg/kg/day, divided over 2 equal doses, 5 hours apart, in a dose volume of 10 mL/kg/dose (dosed in fed condition).
Species/strain:	Beagle dogs
#/sex/group	3
Route	by gavage bid for 6 months
Observations:	Mortality, clinical and eye observations, ECG and heart rate, body weight and weight gain, food consumption, hematology, coagulation, serum analysis (1, 3 and 6 months),

urinalysis (3 and 6 months), organ weights, gross pathology, and histopathology. The toxicokinetic parameters were also determined (Days 1, 30 and 181).

Histopathology: at termination

Tissue	Weighed	Fixed	Microscopic
animal identification (ear tattoo)		F	
adrenal glands	X	F	X
aorta		F	X
bone marrow (femur)		F	X
bone marrow (sternum)		F	X
bone, distal femur		F	X
bone, sternum		F	X
brain	X	F	X
cervix		F	X
epididymides		Bouin	X
esophagus		F	X
eyes		Mix	X
gall bladder		F	X
heart	X	F	X
kidneys	X	F	X
lachrymal glands		F	X
large intestine (cecum, colon, rectum)		F	X
larynx		F	X
liver	X	F	X
lung	X	F	X
lymph node(s), mesenteric		F	X
lymph nodes, popliteal	X	F	X
mammary gland(s)		F	X
optic nerve(s)		Mix	X
ovaries	X	F	X
oviducts		F	X
pancreas		F	X
peripheral nerves, sciatic		F	X
Peyer's patch(es)		F	X
pituitary gland	X	F	X
prostate	X	F	X
salivary gland (mandibular/parotid/sublingual)		F	X
skeletal muscle, quadriceps		F	X
skin		F	X
small intestine (duodenum, jejunum, ileum)		F	X
spinal cord (cervical, thoracic, lumbar)		F	X
spleen	X	F	X
stomach		F	X
testes	X	Bouin	X
thymus	X	F	X
thyroid glands with parathyroid gland(s)	X	F	X
tongue		F	X
trachea		F	X
ureter(s)		F	X
urinary bladder		F	X
uterus		F	X
vagina		F	X
all tissues showing gross lesions		F	X

Results: No mortality occurred.

Vomiting was noted in a dose-related frequency and severity (mainly after the second dose.) A decrease in food consumption (14%) in females at 500 mg/kg/day led to a decrease in body weight (11%) and weight gain (73%). There were no effects on

ophthalmoscopy or ECG related to TMC125. Hematological changes were unremarkable.

Total bilirubin (79%), ALP (3-fold) and alanine aminotransferase (ALT) (2.6-fold) were increased in males at 500 mg/kg/day while triglycerides were decreased (up to 50%) in males at both dose levels.

Microscopic examination showed microgranuloma in the liver in males only at both dose levels. Other minor inflammatory changes were seen in both sexes at both doses (see sponsor's table below). Bile inspissation in the gall bladder was present at the high dose only.

Dose (mg/kg/day) No. of animals	**0 (vehicle)**		**160**		**500**	
	M:3	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**
Died or killed prematurely	0	0	0	0	0	0
Mortality	0	0	0	0	0	0
Clinical observations						
- vomiting	0	0	2	1	3	3
Body weight (kg)a	8.1	6.3	1.05	1.08	1.0	0.89
Body weight gain (kg)a	1.6	1.1	1.44	1.36	0.94	0.27
Total Food consumption (kg)a	46.40	42.23	1.02	1.04	1.05	0.86
Ophthalmoscopy	-	-	-	-	-	-
ECG	-	-	-	-	-	-
Hematology	-	-	-	-	-	-
Serum chemistrya - triglycerides (mg/dL)	36	43	0.50	0.67	0.64	0.74
- total bilirubin (mg/dL)	0.14	0.13	1.14	0.85	1.79	1.08
- ALP (U/L)	109	93	1.19	1.89	3.02	1.89
- ALT (U/L)	34	60	1.24	0.48	2.56	0.57
Urinalysis	-	-	-	-	-	-
Organ weights	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-
Histopathology (no. examined)	**3**	**3**	**3**	**3**	**3**	**3**
Gall bladder						
- inspissated bile	0	0	0	0	2	1
grade 1	0	0	0	0	2	0
grade 3	0	0	0	0	0	1
Liver						
- microgranulomas	0	0	2	0	3	0
grade 2	0	0	2	0	1	0
grade 3	0	0	0	0	2	0
- chronic inflammation	0	2	3	3	3	3
grade 1	0	1	2	3	1	1
grade 2	0	1	1	0	2	2
- granulocytic infiltration	0	1	2	0	3	1
grade 1	0	1	2	0	2	1
grade 2	0	0	0	0	1	0

grade 1	0	0	1	0	0	0
grade 2	0	0	0	2	1	2

a for vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.ALP: alkaline phosphatase; ALT: alanine aminotransferase; F: female; M: male; - no treatment related changes

Toxicokinetics: The exposure after repeated dosing was lower than after a single dose. No major difference in the exposure in male and female dogs was observed.

Dose (mg/kg/day) No. of animals	0 (vehicle)		160		500	
	M:3	F:3	M:3	F:3	M:3	F:3
AUC0-∞ Day 1	---	---				
AUC0-24h Day 30			74.3	62.6	72.5a	70.2a
AUC0-24h Day 181			54.9	45.6	68.6	72.9

Summary In conclusion, the sponsor stated that based on the information above no NOEL level could be determined.

2.6.6.4 Genetic Toxicology

Study Title: ***Salmonella Typhimurium* Reverse Mutation & *E.Coli* Reverse Mutation Assay**
Study no: (TMC125-NC130)
Methods: TMC125 was tested in two independent experiments for the induction of reverse mutations in *Salmonella typhimurium* and *Escherichia coli*. Increasing concentrations were tested with the solubility of the compound being the limiting factor. Test conditions for the evaluation of TMC125 included both direct plate and metabolic activation assays. The latter occurring in the presence of liver S9-fraction from Aroclor-1254-induced rats.
Strains: *Salmonella typhimurium* tester strains TA1535, TA1537, TA102, TA98 and TA100 and *Escherichia coli* strain WP2uvrA
Dose selection criteria: Up to 333 µg/plate (with and without metabolic activation)
Results and Comments: No evidence of mutagenic activity was observed in any of the tester strains in either microbial system when tested up to the highest concentration of 333 µg/plate.

Study Title: ***In Vitro* Human Lymphocyte Chromosome Aberration Test**
Study no: TMC125-NC122
Methods: TMC125 was tested in the *in vitro* human lymphocyte metaphase assay for the detection of chromosomal aberrations. Mitogen-stimulated human lymphocytes were exposed to TMC125 up to the highest concentrations permitted by the compound's solubility with and without metabolic activation (Aroclor-1254-induced rat liver S9 fraction).
Cell line: Human lymphocyte
Comments: There was no evidence of increased numbers of chromosome damage in cells exposed to TMC125 up to the limits of solubility (100 µg/ml) in the first experiment (3h exposure and 24h fixation time with and without S9-mix). Based on the decreases in mitotic index, in the second experiment concentrations up to 10 µg/ml were tested (24h or 48h

exposure with the 24h or 48h fixation time in absence of S9), or up to 56 µg/ml with 3h exposure and 48h fixation time in the presence of S9. Also in this test no clastogenic potential of the compound was observed.

Study Title: ***In Vivo* Cytogenetic Study With Bone Marrow From Mice (Micronucleus)**
Study no: TMC125-NC123
Methods: TMC125. was evaluated at the limit dose of 2000 mg/kg (OECD Guideline for the testing of chemicals No. 474: Mammalian erythrocyte micronucleus test, adapted 21 July 1997) in the mouse micronucleus assay for the detection of chromosomal aberrations *in vivo*. Male mice were given TMC125 by oral gavage at doses up to 2000 mg/kg and were sacrificed after 24h and 48h.
Species Male mice
Results and Comments: There was no decrease in the ratio of polychromatic to normochromatic erythrocytes, which reflects a lack of toxic effects of TMC125 on erythropoiesis. There was no evidence of an increase in micronucleated polychromatic erythrocytes in the bone marrow of the animals treated with TMC125. Mean maximum plasma drug concentrations and AUC24h exposures at 2000 mg/kg amounted to 7847 ng/ml and 108276 ng.h/ml, respectively.

Conclusion: Overall, tmc125 was shown not to be mutagenic or clastogenic in this comprehensive battery of *in vitro* and *in vivo* tests.

OVERALL CONCLUSION No evidence of mutagenic activity up to the limit of solubility (with and without metabolic activation). No evidence of clastogenic potential. No increase in number of micronucleated cells.

Study Title: ***Salmonella Typhimurium* Reverse Mutation Assay On R293496**
Study no: TMC125-NC163; TMC125-NC163-TSR.pdf
Study Date: 7/2003
GLP: Yes
Sponsor: Johnson & Johnson Pharmaceutical Research & Development, a division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium
Lab: Global Nonclinical Development, Beerse site; Turnhoutseweg 30 B-2340 Beerse, Belgium
Drug Name R293496 Batch #: ZR293496PFA021; Vehicle: DMSO; There is no description in the report or in this submission about the identity of R293496. The CSO has requested the sponsor for a clarification of this issue.

Methods: R293496 was tested in two independent experiments for the induction of reverse mutations in *Salmonella typhimurium*. The test was carried out using standard operating procedures which are based on the most recent guidelines for performing this test.

Strains: *Salmonella typhimurium* tester strains TA1535, TA1537, TA102, TA98 and TA100.

Dose:	R293496 was used at seven concentrations in the absence and in the presence of S9-mix: 1.22, 4.88, 19.53, 78.13, 312.5, 1250 and 5000 µg/plate in the first study and 0.61, 2.44, 9.77, 39.06, 156.25, 625 and 2500 µg/plate in the second study.
Results:	R293496 was used at seven concentrations in the absence and in the presence of S9-mix: 1.22, 4.88, 19.53, 78.13, 312.5, 1250 and 5000 µg/plate in the first study and 0.61, 2.44, 9.77, 39.06, 156.25, 625 and 2500 µg/plate in the second study. In the absence and in the presence (20 and 50 µl S9 homogenate/plate) of a metabolic activation system, R293496 did not cause any biologically significant increase in the number of revertant colonies above the vehicle control incidence with all of the strains. With some strains in the absence of S9-mix, weak bacteriotoxic effects were observed, visualized by a decrease in the number of revertants at the top concentrations. A dose-related increase in precipitation of R293496 into the top agar was observed from the concentration of 156.25 ug/plate onwards. The vehicle and positive control counts of all strains fell within the required norms and the genotypes could be confirmed for all the strains validating the results from this study. It can be concluded that R293496 has no mutagenic properties towards the various *S. typhimurium* strains under our test conditions up to precipitating concentrations.
Conclusions:	No evidence of mutagenic activity was observed in any of the tester strains in either microbial system at the doses tested.

Study Title	***In Vitro* Bacterial Reverse Mutation Test with *Salmonella typhimurium* on R165335.**
Study no:	TMC125-NC163; TMC125-PRD Exp 6081-TSR.pdf
Study Date:	1/2000
GLP:	Yes
Sponsor:	Johnson & Johnson Pharmaceutical Research & Development, a division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium
Lab:	Global Nonclinical Development, Beerse site; Turnhoutseweg 30 B-2340 Beerse, Belgium
Drug Name	R165335 (TMC125) Batch #: ZR293496PFA021; Vehicle: DMSO. There is no description in the report or in this submission about the identity of R165335. The CSO has requested the sponsor for a clarification of this issue.
Methods:	R165335was tested in two independent experiments for the induction of reverse mutations in *Salmonella typhimurium*. The test was carried out using standard operating procedures which are based on the most recent guidelines for performing this test.
Strains:	*Salmonella typhimurium* tester strains TA1535, TA1537, TA102, TA98 and TA100.
Dose:	R293496 was used at seven concentrations in the absence and in the presence of S9-mix: 1.22, 4.88, 19.53, 78.13, 312.5, 1250 and 5000 µg/plate in the first study and 0.61, 2.44, 9.77, 39.06, 156.25, 625 and 2500 µg/plate in the second study.

—Report ' — Rmd588; Rcc 869101, April 2005.)	100, and the Escherichia coli strain WP2 uvrA. The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations: Pre-Experiment/Experiment I: 3, 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate Experiment II: 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate. The results showed no substantial increase in revertant colony numbers of any of the five tester strains following treatment with — at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). — is considered to be non-mutagenic in this Ames assay.
In Vitro Chromosome Aberration Test In Chinese Hamster V79 Cells With —	This study (Nc264 July, 2007 — Ccr Study Number 869102; Janssen Study Number: Rmd589)was performed to investigate the potential of new manufacturing intermediate impurity — to induce chromosome aberration in V79 cells of the Chinese hamster in vitro — was suspended (Pre-experiment) or dissolved (Main Experiment) in DMSO). In this study, in the absence of S9 mix, a dose-related and biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment with ' —. The highest concentration (12.5 µg/mL) scored for cytogenetic damage was statistically significant and clearly exceeded the sponsor's historical control data range. The sponsor concluded that under the experimental conditions reported, — induced structural chromosome aberrations in V79 cells (Chinese hamster cell line) in vitro. — is considered by the sponsor to be clastogenic in the chromosome aberration test in the absence of metabolic activation
Micronucleus Assay In Bone Marrow Cells Of The Mouse With ' — (NC325: — . Report — , RMD814: — 1018800, March 2007)	This study was performed to investigate the potential of — to induce micronuclei in polychromatic erythrocytes in the bone marrow of the mouse. Ten animals (5 males, 5 females) per test group were evaluated for the occurrence of micronuclei. Twenty four hours and 48 h after a single administration of — he bone marrow cells were collected for micronuclei analysis. The dose levels used were: 24 h preparation interval: 500, 1000, and 2000 mg/kg; 48 h preparation interval: 2000 mg/kg. Plasma levels at 2000 mg/kg were 58 and 74 ng/mL for males and females, respectively. In a separate study, AUC_{0-7h} at 2000 mg/kg were 192-222 ng.h/mL. There was no biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval after

Results:	In the main test, R165335 was used at seven concentrations: 5, 10, 25, 50, 100, 250 and 500 µg/plate. R165335 did not cause any biological significant increase in the number of revertant colonies above the solvent control incidence with all of the strains, either with or without the metabolic activation system, using 20 µl S9 homogenate per plate in the first study and 50 µl S9 homogenate per plate in the repeat study. Precipitation was observed from 250 µg/plate onwards with all strains. The solvent and positive control counts of all strains fell within the required norms and the genotypes could be confirmed for both strains validating the results from this study.
Conclusions:	No evidence of mutagenic activity was observed in any of the tester strains in either microbial system at the doses tested.

Manufacturing Intermediates	**SUMMARY AND COMMENTS**
Impurity — In Vitro Bacterial Reverse Mutation Test With Salmonella Typhimurium (NC226)	This study relates to an impurity of TMC125, called — Bacterial reverse gene mutation tests (Ames Test) were carried out, in triplicate, using five strains of Salmonella typhimurium, TA1535, TA1537, TA102, TA98 and TA100, in the absence and in the presence of a rat liver metabolic activation system (S9-mix) with — was used at seven concentrations in the absence and in the presence of S9- mix: 4.88, 9.77, 19.53, 39.06, 78.13, 156.25, 312.5 pg/plate in four separate studies. In the absence and in the presence (20 and 50 pl S9 homogenate/plate) of a metabolic activation system, — did not cause any biologically significant increase in the number of revertant colonies above the vehicle control incidence with the strains TA1535, TA102, TA98 and TA100. With strain TA1537, a biologically significant increase in the reversion rate was observed at one single concentration level in the second and third study. However, the sponsor claimed that these findings were considered to be fortuitous and not relevant as (i) no dose-related effect was observed in any of the studies, (ii) the biologically significant increase in reversion rate was found at a different concentration level in the second and third study, (iii) the increases are related to an increase in only one out of three agar plates, and (iv) the first and fourth study were clearly negative with strain TA1537. It can be concluded that — has no mutagenic properties towards the various S. *typhimurium* strains under our test conditions up to precipitating concentrations.
Salmonella Typhimurium & Escherichia Coli Reverse Mutation Assay With — NC263; —	This study was performed to investigate the potential of new manufacturing intermediate impurity — to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA

	administration of — with any dose level used (oral cyclophosphamide was used as positive control which showed a substantial increase of induced micronucleus frequency.) The sponsor concluded that — did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse, and that — is non-mutagenic in this micronucleus assay.
—: Screening Chromosome Aberration Test In Human Lymphocytes *In Vitro* — — *Report — Study NC339;* — 1827/090; September 2004)	This study was performed to investigate the potential of — to induce the potential of new manufacturing intermediate impurity — to induce chromosome aberration in human lymphocytes *in vitro*, a test system different from study NC264 described above. — did not induce biologically significant increases in the frequency of cells with chromosome aberrations in the absence or presence of a liver enzyme metabolizing system after 4(20) hours exposure or 24 hours continuous exposure. — was considered non-clastogenic to human lymphocytes *in vitro*.
Salmonella Typhimurium & Escherichia Coli Reverse Mutation Assay With —	This study ((NC381 — , Rmd487: — 846997; August 2003)) was performed to investigate the potential of new manufacturing intermediate impurity — to induce gene mutations in the AMES test (same methodology as NC263 above) and the results were negative.
In Vitro Chromosome Aberration Test In Chinese Hamster V79 Cells With —	This study ((NC385 — Report —, RMD488; —846998 June 2003.)) was performed to investigate the potential of new manufacturing intermediate impurity — to induce chromosome aberration in V79 cells of the Chinese hamster in vitro (same methodology as NC264 above) and the results were negative.
— Screening Chromosome Aberration Test In Human Lymphocytes *In Vitro*	This study ((— NC445; —1827/084, September 2004))was performed to investigate the potential of new manufacturing intermediate impurity — to induce chromosome aberration in human lymphocytes *in vitro*, (same methodology as NC339 above) and the results were negative.

2.6.6.5 Carcinogenicity

2-year rat and mouse carcinogenicity studies are ongoing.

2.6.6.6 Reproductive and Developmental Toxicology

Study title:	**FERTILITY AND EARLY EMBRYONIC TOXICITY STUDY IN WISTAR RATS**
Study no.:	STUDY NO. TMC125-NC125
Methods:	TMC125 — was given orally at 127, 253 and 506 mg — kg/day, corresponding to 150, 300 and 600 mg TMC125 — /kg/day.
Species/strain:	Wistar Rats
Doses employed:	127, 253 and 506 mg — kg/day, corresponding to 150, 300 and 600 mg

TMC125 — kg/day.

Route: Oral gavage

Study design: Male animals were given TMC125 from 4 weeks prior to mating until termination. Administration to female animals started 2 weeks before mating and continued until day 8 *post coitum* inclusive.

Number/sex/group: 24

Results: Reproduction parameters in male and female rats were unaffected by dosing with TMC125 — n all treatment groups. Based on these results the NOAEL was established at the highest dose of 506 mg — /kg/day.
Because no TK was performed. The sponsor applied exposure data from 3-month rat study (NC129) for a reference. The values were much lower than those obtained from study using spray dried formulation (see next study).

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
70	Day 1	0.07	0.08	0.46	0.55[b]
	Day 84	0.04	0.09	0.58	1.03
200	Day 1	0.15	0.15	0.82[b]	0.92[b]
	Day 84	0.06	0.12	0.69	1.40
600	Day 1	0.28	0.33	1.81	3.32
	Day 84	0.12	0.32	1.69	4.40[c]

[a] $AUC_{0-\infty}$ after single dose (Day 1) or AUC_{0-24h} after repeated dose; [b] AUC_{0-8h}; [c] AUC_{0-48h}

Study title: **Spray-Dried TMC125: Fertility and Early Embryonic Toxicity Study in Sprague-Dawley Rats**

Study no.: STUDY NO. TMC125-NC337; Date: July/2006; Site: J&J PRD

Methods: Spray-dried TMC125 (batch: CA165335G1A281) formulated as a suspension in water was administered 1/day for 4 weeks prior to mating, during mating and up to termination (males) or 2 weeks prior to mating, during mating and up to GD7 (GD0 was the day mating was confirmed) (females).
Observations: clinical signs, body weight and food consumption were made throughout the study; estrous cycles were recorded and the precoital interval was noted.
The females were sacrificed on Day 14 of pregnancy for evaluation (males were sacrificed after fertility rate in females was established.) Blood samples were collected on the first day of dosing (Day 0) and at the start of the mating period (Day 28 for males, Day 14 for females) during the 24 hours after dosing for TK analysis.

Species/strain: Sprague-Dawley Rats

Doses employed: 0 (vehicle: hydroxypropyl-methylcellulose + microcrystalline cellulose in water), 125, 250 or 500 mg/kg/day (20 ml/kg).

Route: Oral gavage

Study design: Male animals were given TMC125 from 4 weeks prior to mating until termination. Administration to female animals started 2 weeks before mating and continued until day 8 *post coitum* inclusive.

Number/sex/group: 24

Results: There were no mortalities associated with TMC125 (deaths due to gavage errors: 3 males in the vehicle group and 1 male in the 500 mg/kg/day group).

No adverse clinical signs were observed in males or females in any treated groups. No relevant effects on body weight performance, food consumption, estrous cycle, precoital interval, copulation index or the fertility index were reported at all dose levels.

No toxicologically relevant effects were observed on the number of corpora lutea of pregnancy, implantations, resorptions, the extent of pre- and post-implantation losses or the number of live embryos. It was concluded that there was no effect on male or female fertility up to 500 mg/kg/day. The NOAEL was considered to be 500 mg/kg/day.

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
125	Day 0	0.57	1.39	2.72	7.79
	Day 14/28[b]	0.38	1.12	1.53	5.45
250	Day 0	0.93	1.72	5.48	9.77
	Day 14/28[b]	0.57	1.21	2.77	5.02
500	Day 0	1.58	2.13	10.7	18.1
	Day 14/28[b]	0.76	1.44	4.54	9.09

[a] $AUC_{0-\infty}$ after single dose (Day 1) or AUC_{0-24h} after repeated dose; [b] Day 14 for females, Day 28 for males

Study title: **EMBRYO-FETAL DEVELOPMENTAL TOXICITY STUDY OF TMC125 IN FEMALE WISTAR RATS**

Study no.: NC123

Laboratory: [redacted]

GLP: Yes

QA: Yes

Initiation Date: 3/23/2001

Drug/Purity: TMC125 [redacted]

Batch No. AG1086

Vehicle/Solvent: PEG400

Study design: Hundred-four mated females were allocated to 4 groups of 26 females per group. From day 6 to day 16 post-coitum inclusive, females of the treatment groups received daily oral administration TMC125 [redacted]

Species/strain: Female Wistar Rats

Doses employed: 0, 250, 500 or 1000 mg/kg body weight/day during days 6 to 16 post-coital inclusive (females of the control group received daily oral administration of vehicle).

Route: Oral gavage

Toxicokinetics: blood sampling was performed on day 7 pre-dose, and day 16 pre-dose, 1, 2, 4, 8, and 24 hours after dosing (satellite females, 6/group)

Number/group: The control group consisted of 24 main and 2 satellite females, the 250 mg/kg and 1000

mg/kg dose groups consisted of 20 main and 6 satellite females, and the 500 mg/kg dose group consisted of 22 main and 4 satellite females.

Parameters Examined

Body weights and food consumption of females were determined at least once daily during pregnancy. On day 21 past-co/turn, all females were subjected to an examination post-mortem and external, thoracic and abdominal macroscopic findings were recorded. The ovaries and uterine horns were dissected and examined for the number of corpora lutea, the weight of the gravid uterus, the number of implantation site scars, the number and distribution of live/dead fetuses and embryofetal deaths, the weight of each live fetus and corresponding placenta, fetal sex and externally visible fetal macroscopic abnormalities. Alternate fetuses of each litter were preserved, sectioned and examined for skeletal or visceral malformations.

RESULTS

Maternal Findings:

No maternal or reproduction toxicity was observed with treatment up to 1000 mg/kg body weight/day.

Fetal Findings:

No teratogenicity finding was reported in this study.

Fetal toxicity of dams receiving 1000 mg/kg/day included anomalous thoracic vertebral centra and wavy/thickened/bent ribs. These fetal findings were not malformations and appeared to be variations in size and shape, instead of number or defects in ossification. Further, the values were not highlighted with any statistical significance by the sponsor, as methods such as Dunett, Steel or Fisher tests were mentioned for data analysis for this study. There were no treatment-related adverse effects upon morphological development of soft tissues in utero. No other signs of affected health of the dams were reported.

Toxicokinetics:

Mean peak plasma concentrations (Cmax) at gestational day 16 (see table below) were 512.3, 580 3 and 569.0 ng/ml for the 250-, 500- and 1000- mg/kg/day dosing groups, respectively. Mean AUC24h values were 3262, 7939 and 8268 ng.h/ml for the 250-, 500- and 1000- mg/kg/day dosing groups, respectively. The exposure of TMC125 in pregnant rats increased dose proportionally between the 250 and the 500 mg/kg/day groups, but increased less than dose proportionally from the 500- to 1000 mg/day group.

Parameter		250 mg base-eq./kg	500 mg base-eq./kg	1000 mg base-eq./kg
Dose	mg/kg	250	500	1000
C_{max}	ng/ml	512.3	580.3	569.0
Dose-normalized C_{max}	ng/ml	2.049	1.161	0.5690
t_{max}	h	2.0	4.0	8.0
t_{last}	h	24.0	24.0	24.0
AUC_{last}	ng.h/ml	3262	7939	8268
Dose-norm. AUC_{last}	ng.h/ml	13.05	15.88	8.268
λ_z	1/h	0.1114	0.1494*	0.1495*
$t_{1/2}$	h	6.224	4.639*	4.638*
Corr. Coeff.	r^2	0.9948	1.000	1.000

*: Accurate determination not possible

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)
250	Day 11 (GD16)	0.51	3.26
500	Day 11 (GD16)	0.58	7.94
1000	Day 11 (GD16)	0.57	8.27

GD: day of gestation

Conclusions: Based on the results in this embryotoxicity and teratogenicity study, the sponsor stated that the definitive fetal NOAEL was 500 mg/kg/day. The definitive maternal and reproductive NOAEL was established as being 1000 mg/kg body weight/day.

Study title: **TMC125: EMBRYO-FETAL DEVELOPMENTAL TOXICITY STUDY OF TMC125 IN FEMALE RABBITS**

Study no.: NC124

Laboratory: [redacted]

GLP: Yes

QA: Yes

Initiation Date: 7/1/2001

Drug/Purity: TMC125 [redacted]

Batch No. AA-6426-Batch-1-01 and 00036

Vehicle/Solvent: alpha-tocopheryl polyethylene glycol succinate + hydroxypropyl-methylcellulose

Study design and Parameters Examined TMC125 [redacted] formulated in TPGS/HPMC was administered once daily, by oral gavage, during the period of GD6 to GD18 (GD0 is the day mating was confirmed.
Regular observations: clinical signs, body weight and food consumption.
Blood/toxicokinetic sampling: Day 13 of dosing (GD18)
Dams: sacrificed on GD28; all animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy; uterus (with cervix and ovaries) examined:

Uterine data: the numbers of corpora lutea, implantations, live fetuses, fetal body weight and fetal sex distribution.

Live fetuses: examined externally and viscerally by fresh tissue examination (eviscerated, preserved, stained and examined skeletally for abnormalities.

Species/strain: Female New Zealand white rabbits (time-mated, sexually mature)

Doses employed: 0 (vehicle), 50, 200 and 750 mg/kg/day in a dose volume of 10 ml/kg.

Route: Oral gavage

Toxicokinetics: blood sampling was performed on day 7 pre-dose, and day 16 pre-dose, 1, 2, 4, 8, and 24 hours after dosing (satellite females, 6/group)

Number/group: 19 (due to an insufficient number of litters additional animals were added to provide dose groups of 25, 22, 22 and 29 animals, respectively.)

RESULTS No mortalities associated with TMC125 were reported (1 death in the vehicle group associated with the dosing procedure and 2 deaths at 750 mg/kg/day [cause unknown] were reported).

Maternal No other maternal or reproduction toxicity was reported.

Findings:

Fetal Findings: No teratogenicity finding was reported in this study. There was no effect on gross pathology, gravid uterine weight, pregnancy rate, number of corpora lutea, number of pre-implantation loss, post-implantation loss or live implantations, fetal body weight, sex ratio or fetal abnormalities. (see table below)

Noteworthy Findings

Daily dose (mg base eq./kg)	0 (vehicle)	50	200	750
No. of animals	F:25	F:22	F:22	F:29
Maternal Data:				
Died or killed prematurely (unrelated to treatment)	1	0	0	2
Clinical observations	-	-	-	-
Body weight (g)	-	-	-	-
Food consumption (g)	-	-	-	-
Gross pathology	-	-	-	-
No. inseminated	25	22	22	29
No. pregnant females	19	18	19	22
No. aborted or with total resorption of litter	1	1	1	4

F: female; GD: gestation day; NR: not reported; - no noteworthy findings

Noteworthy Findings (Continued)

Daily dose (mg base eq./kg)	0 (vehicle)	50	200	750
No. of animals	F:25	F:22	F:22	F:29
No. females with live fetuses	18	17	18	16
Mean no. corpora lutea	9	10	9	9
Mean no. implantations	7	8	7	8
Pre-implantation loss (% of corpora lutea)	21	17	23	16
Mean no. resorptions (% implantation sites)	4	7	4	3
Post-implantation loss (% of implantation sites)	4	7	4	3
Mean no. live fetuses	7	7	7	7
Litter data:				
No. litters evaluated	18	17	18	16
No. live fetuses	117	124	125	117
Mean male fetuses (% of fetuses)	52	55	38*	45
Mean fetal body weight (g)	36.4	35.3	33.9	36.7
Fetal abnormalities				
Soft tissue examination	-	-	-	-
Skeletal evaluation - ossification	-	-	-	-
Skeletal evaluation - morphological	-	-	-	-

* $p \leq 0.05$ (Fisher's exact test)

F: female; - no noteworthy findings

Toxicokinetics:

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)
50	Day 13 (GD18)	0.13	1.29
200	Day 13 (GD18)	0.24	2.24
750	Day 13 (GD18)	0.59	3.76

GD: day of gestation

Conclusions: Both maternal and fetal NOAELs were 750 mg/kg/day. There is no evidence that TMC125 is teratogenic in rabbits as revealed from this study. Please note that drug exposures achieved at 750 mg/kg were lower than those at 375 mg/kg by using the spray-dried formulation (see below).

Study title: **SPRAY-DRIED TMC125: EMBRYO-FETAL DEVELOPMENTAL TOXICITY STUDY OF TMC125 IN FEMALE RABBITS**

Study no.: NC150

Laboratory: [redacted]

GLP: Yes

QA: Yes

Initiation Date: 12/2004

Batch No. CA165335G1A011

Vehicle/Solvent: hydroxypropyl-methylcellulose + microcrystalline cellulose in purified water

Study design and Parameters Examined Spray-dried TMC125 [redacted]) formulated in water was administered 1/day by oral gavage during the period of GD6 to GD19 (GD0 was the day mating was confirmed).

Regular observations: clinical signs, body weight and food consumption.
The dams: sacrificed on GD28 (all animals sacrificed at the end of the study were subject to macroscopic examination at necropsy.)
Uterine data collected: numbers of corpora lutea, implantations, live fetuses, fetal body weight and fetal sex distribution.
All live fetuses were examined externally and viscerally (including the brain) by fresh tissue examination. The fetuses were then eviscerated, preserved, stained with Alizarin Red S, and examined skeletally for abnormalities.

Species/strain: Female New Zealand white rabbits (time-mated, sexually mature)

Doses employed: 0 (vehicle), 125, 250 and 375 mg/kg/day in a dose volume of 15 ml/kg.

Route: Oral gavage

Toxicokinetics: Day 1 (GD6) and Day 13 (GD18).

Number/group: 20

RESULTS

Maternal Findings: No mortalities associated with TMC125 were reported.
At 250 and 375 mg/kg/day body weight was decreased after the start of dosing until GD9 (up to 96 g of body weight loss versus 21 g in vehicle animals). At 375 mg/kg/day this resulted in a marginally reduced body weight gain over the entire treatment period. Food

consumption was similarly reduced until GD8 (up to 32%).

Fetal Findings: No teratogenicity finding was reported in this study. There was no effect on gross pathology, gravid uterine weight, pregnancy rate, number of corpora lutea, number of pre-implantation loss, post-implantation loss or live implantations, fetal body weight, sex ratio or fetal abnormalities (see table below).

Noteworthy Findings

Daily dose (mg/kg)	0 (vehicle)	125	250	375
No. of animals	F:20	F:20	F:20	F:20
Maternal data:				
Died or killed prematurely (accidental)	0	0	0	0
Clinical observations	-	-	-	-
Body weight gain GD6 - 9 (g)	-21	-23	-96***	-93***
Food consumption GD6 - 8 (g)	93	96	66*	63*
Gross pathology	-	-	-	-
No. pregnant females	20	17	17	20
No. aborted or with total resorption of litter	1	0	0	1

* $p \leq 0.05$; *** $p \leq 0.001$ (Analysis of variance and William's test)
F: female; GD: gestation day; - no noteworthy findings

Daily dose (mg/kg)	0 (vehicle)	125	250	375
No. of animals	F:20	F:20	F:20	F:20
No. females with live fetuses	19	17	17	19
Mean no. corpora lutea	12	11	11	10*
Mean no. implantations	10	9	10	9
Pre-implantation loss (% of corpora lutea)	13.7	15.8	10.3	8.4
Number of early embryo/fetal deaths	13	10	17	7
Number of late embryo/fetal deaths	35	23	16	15
Number of dead fetuses	0	1	0	0
Post-implantation loss (% of implantation sites)	33	23	17	13
Mean no. live fetuses/female	8	7	8	8
Mean % of implantations	77	77	83	87
Litter data:				
No. litters evaluated	19	17	17	19
No. live fetuses	143	117	132	149
Mean male fetuses (%)	45	48	48	50
Mean fetal body weight (g)	32.6	36.6	34.6	34.2
Fetal abnormalities				
Soft tissue examination	-	-	-	-
Skeletal evaluation - ossification	-	-	-	-
Skeletal evaluation - morphological	-	-	-	-

* $p \leq 0.05$ (Analysis of variance and William's test)
F: female; - no noteworthy findings

Toxicokinetics:

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)
125	Day 1 (GD6)	0.51	7.58
	Day 13 (GD18)	0.38	6.09
250	Day 1 (GD6)	0.63	11.6
	Day 13 (GD18)	0.41	5.27
375	Day 1 (GD6)	0.70	12.3
	Day 13 (GD18)	0.54	9.67

GD: day of gestation

Conclusions: The maternal NOAEL was considered to be 125 mg/kg/day, based on the effect on body weight and food consumption at the higher dose levels and the fetal NOAEL was considered to be 375 mg/kg/day based on the absence of any relevant findings. There is no evidence that TMC125 is teratogenic in rabbits up to maternally toxic dose levels.

Study title: **SPRAY-DRIED TMC125: DOSE RANGE FINDING STUDY ON EMBRYO-FETAL DEVELOPMENTAL TOXICITY OF TMC125 IN FEMALE RABBITS**

Study no.: NC235

Laboratory: [redacted]

GLP: Yes

QA: Yes

Initiation Date: 12/2004

Batch No. CA165335G1A011

Vehicle/Solvent: hydroxypropyl-methylcellulose + microcrystalline cellulose in purified water

Study design and Parameters Examined: Spray-dried TMC125 [redacted] active to polymer ratio) formulated in water was administered 1/day by oral gavage during the period of GD6 to GD19 (GD0 was the day mating was confirmed).

Regular observations: clinical signs, body weight and food consumption.
The dams: sacrificed on GD28 (all animals sacrificed at the end of the study were subject to macroscopic examination at necropsy.)
Uterine data collected: numbers of corpora lutea, implantations, live fetuses, fetal body weight and fetal sex distribution.
All live fetuses were examined externally and viscerally (including the brain) by fresh tissue examination. The fetuses were then eviscerated, preserved, stained with Alizarin Red S, and examined skeletally for abnormalities.

Species/strain: Female New Zealand white rabbits (time-mated, sexually mature)

Doses employed: 0 (vehicle), 125, 250 and 525 mg/kg/day in a dose volume of 15 ml/kg.

Route: Oral gavage

Toxicokinetics: Day 1 (GD6) and Day 13 (GD18) (n=3).

Number/group: 5

RESULTS No mortalities associated with TMC125 were reported. There were no relevant clinical signs or changes in body weight or food consumption. There was no effect on gross

250 or 500 mg/kg/day in a dose volume of 20 mL/kg.

Route: Oral

Study design: Male animals were given TMC125 from 4 weeks prior to mating until termination. Administration to female animals started 2 weeks before mating and continued until day 8 *post coitum* inclusive.

Number/sex/group: 24

Results: There were no mortalities associated with TMC125 (deaths due to gavage errors: 3 males in the vehicle group and 1 male in the 500 mg/kg/day group).

No adverse clinical signs were observed in males or females in any treated groups. No relevant effects on body weight performance, food consumption, estrous cycle, precoital interval, copulation index or the fertility index were reported at all dose levels.

No toxicologically relevant effects were observed on the number of corpora lutea of pregnancy, implantations, resorptions, the extent of pre- and post-implantation losses or the number of live embryos. It was concluded that there was no effect on male or female fertility up to 500 mg/kg/day. The NOAEL was considered to be 500 mg/kg/day.

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
125	Day 0	0.57	1.39	2.72	7.79
	Day 14/28[b]	0.38	1.12	1.53	5.45
250	Day 0	0.93	1.72	5.48	9.77
	Day 14/28[b]	0.57	1.21	2.77	5.02
500	Day 0	1.58	2.13	10.7	18.1
	Day 14/28[b]	0.76	1.44	4.54	9.09

[a] $AUC_{0-\infty}$ after single dose (Day 1) or AUC_{0-24h} after repeated dose; [b] Day 14 for females, Day 28 for males

Study title: **Range-Finding Study on Spray-dried TMC125: Prenatal and Postnatal Developmental Toxicity Study in Sprague-Dawley Rats**

Study no.: STUDY NO. TMC125-NC236; Date: 1/2005; Site: —

Batch No. CA165335G1A011

Methods: Spray-dried TMC125 (— —active to polymer ratio) formulated in water was administered once daily by oral gavage to time-mated female rats from Day 7 of gestation to Day 7 of lactation.

Regular observations: maternal clinical signs, body weight and food consumption.
TK: Day 1 (GD7) and Day 11 (GD17).
The females were allowed to litter, and nesting and nursing behavior were observed and any abnormalities recorded. Only dams that died or were sacrificed before the scheduled termination were subject to macroscopic examination. Pups were not examined macroscopically.

Species/strain: Sprague-Dawley Rats (n=6/group).
Doses employed: 0 (vehicle: hydroxypropyl-methylcellulose + microcrystalline cellulose in water), 125, 250 or 500 mg/kg/day in a dose volume of 20 ml/kg.
Route: Oral
Number/sex/group: 6. (satellite TK: 6/group)
Results: In the dams there were no mortalities associated with TMC125 (1 dam at 250 mg/kg/day died on Day 22; cause of death was attributed to difficulties at parturition; 3 accidental deaths associated with the dosing procedure: 1 main animal given 125 mg/kg/day and 2 satellite animals given 250 mg/kg/day.)

No relevant clinical signs or effects on body weight or food consumption of the dams and no effect of maternal treatment on parturition or the numbers of pups born, the proportion of male pups born, or pup survival, clinical condition or body weight were reported.

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
125	Day 1 (GD7)	0.89	6.59
	Day 11 (GD17)	1.02	7.22
250	Day 1 (GD7)	1.11	10.4
	Day 11 (GD17)	1.08	8.64
500	Day 1 (GD7)	1.62	23.6
	Day 11 (GD17)	0.99	12.8

[a] $AUC_{0-\infty}$ after single dose (GD7) or AUC_{0-24h} after repeated dose
GD: day of gestation

The maternal and developmental NOAEL was considered to be 500 mg/kg/day based on the absence of any effect. There were no apparent effects on the pups at this dose.

Study title: **Spray-Dried TMC125: Prenatal and Postnatal Developmental Toxicity Study in Sprague-Dawley Rats**
Study no.: STUDY NO. TMC125-NC145; Date: 1/2005; Site: —
Batch No. CA165335G1A011
Methods: Spray-dried TMC125 (— active to polymer ratio) formulated in water was administered once daily by oral gavage to time-mated female rats from Day 7 of gestation to Day 7 of lactation.
The first generation (F1) was allowed to mature untreated.
Regular observations: maternal clinical signs, body weight and food consumption.
Blood sampling: Day 1 (GD7) and Day 11 (GD17).

The females were allowed to litter and nesting and nursing behavior were observed. Parturition, litter size and numbers of each sex were recorded, as were clinical observations in pups and pup body weight.

vagina open) and sensory function/reflexes (static righting reflex, startle response and pupillary light reflex, tail flick test) and behavior (learning and memory and locomotor activity) and assessment of reproductive performance of offspring were recorded.

The F0 females were sacrificed at weaning of their litters. All principal F0 females and F1 offspring were examined macroscopically, including number of implantation scars in each uterine horn of F0 females (samples of tissues and organs were preserved but not examined microscopically).

Species/strain: Sprague-Dawley Rats (n=6/group).

Doses employed: 0 (vehicle: hydroxypropyl-methylcellulose + microcrystalline cellulose in water), 125, 250 or 500 mg/kg/day in a dose volume of 20 ml/kg.

Route: Oral gavage

Number/sex/group: 28. (satellite TK: 6/group)

Results: No mortalities associated with TMC125 were reported.
No relevant clinical signs or effects on body weight or food consumption of the dams, effect of maternal treatment on gestation, parturition or the numbers of pups born, the sex ratio, pup survival, or clinical condition were reported.

Pup body weight was higher in TMC125 treated animals than in controls (no dose-response effects). Developmental landmarks occurred earlier in comparison with the lighter control pups.
Clinical condition, sensory function/reflexes, behavior and reproductive performance of F1 pups were unaffected by maternal treatment with TMC125.
Macroscopic findings of dams or F1 offspring were unremarkable. The maternal NOAEL was considered to be 500 mg/kg/day based on the absence of any effect.

No apparent effects on the pups at this dose were reported. Offspring showed no delayed in development to adulthood on aspects of physical development, sensory functions, reflexes, behavior, sexual maturation or reproductive performance. The details of these finding are provided in the tables attached below.

The NOAEL for pup development after maternal treatment with TMC125 was considered to be 500 mg/kg/day (AUC and Cmax are provided below).

TMC125 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
125	Day 1(GD7)	1.03	6.15
	Day 11 (GD17)	0.65	5.29
250	Day 1(GD7)	1.30	9.42
	Day 11 (GD17)	0.85	7.60
500	Day 1(GD7)	1.66	12.1
	Day 11 (GD17)	0.64	3.63

[a] $AUC_{0-\infty}$ after single dose (GD7) or AUC_{0-24h} after repeated dose
GD: day of gestation

Dose (mg/kg/day)	0 (vehicle)	125	250	500
F0 Females: No. of Animals	F: 28	F: 28	F: 28	F: 28
No. pregnant	22	22	20	23
No. died or sacrificed moribund	0	0	0	0
No. with difficult parturition	2	0	0	1
No. with total litter loss	0	1	1	0
Clinical observations	-	-	-	-
Necropsy observations	-	-	-	-
Gestation body weight	-	-	-	-
Lactation body weight	-	-	-	-
Gestation food consumption	-	-	-	-
Lactation food consumption	-	-	-	-
Mean duration of gestation (days)	21.5	21.7	22.0**	21.9*
Mean no. implantations	12.6	13.0	13.1	12.1
Mean % post-implantation loss	6.7	9.8	8.0	6.2
Dose (mg/kg/day)	0 (vehicle)	125	250	500
F1 litters (preweaning to Day 5)				
Proportion of pups born live	98	99	98	99
Mean no. pups/litter	11.5	11.6	11.8	11.3
Postnatal survival to Day 5 (%)	95	97	96	98
Proportion of male pups pup sex ratios (% male)	-	-	-	-
Pup clinical signs	-	-	-	-
Pup necropsy observations	-	-	-	-
Pup body weights at Day 1 (g) - males	6.1	6.7*	6.6*	7.0**
Pup body weights at Day 1(g) - females	5.7	6.3*	6.2*	6.5**
Pup body weights at Day 22 (g) - males	57.5	59.3	61.4	61.4
Pup body weights at Day 22(g) - females	55.1	57.4	58.2	58.9
% male pups with ears open on Day 3	25	49	54*	70**
% female pups with ears open on Day 3	22	55*	57*	72**
% male pups - successful righting reflex Day 4	89	93	98*	92
% female pups - successful righting reflex Day 4	82	85	93	87
F1 animals (postweaning selected Day 22)				
Clinical observations	-	-	-	-
Necropsy observations	-	-	-	-
Body weight at Day 92 (g) - males	454.5	462.5	478.0	479.3
Body weight at Day 71 (g) - femaless	226.7	234.4	242.9	238.0
Eye opening (mean day) - males	15.5	15.2	15.1	14.9**
Eye opening (mean day) - females	15.4	15.1	15.1	14.9*
Pupillary reflex	-	-	-	-
Startle response	-	-	-	-
Preputial separation (Day of age)	-	-	-	-

* $p \leq 0.05$; ** $p \leq 0.01$ (Eyes open: Fisher Exact test; ears open: analysis of variance)
- no noteworthy findings

2.6.6.7 Local Tolerance

Both TMC125 — and TMC125 —were tested in a range of local tolerance studies. Skin sensitization and irritation were evaluated using in vivo models, whereas eye irritation and phototoxicity were evaluated using in vitro models, as follows:

1. EYE IRRITATION TEST on TMC125 — BOVINE CORNEAL OPACITY-PERMEABILITY ASSAY The bovine corneal opacity-permeability (BCOP) was used to

investigate the ocular irritation potential of TMC125 — by measuring the opacity and permeability after exposure of the corneal epithelium. TMC125 base was applied for 4 hours as a 20% w/w suspension in saline. TMC125 induced a small increase in opacity and no increase in permeability and was classified as a "mild" eye irritant.

2. EYE IRRITATION TEST on TMC125 — An identical test, as described above, was performed with TMC125 —, which induced a very strong increase in opacity and a small increase in permeability when applied as a 20% w/w suspension in saline. As a result of this reaction TMC125 — was classified as a "very severe" eye irritant.

3. PHOTOTOXICITY: BALB/C MOUSE FIBROBLAST ASSAY- The assay was used to investigate in vitro phototoxic and cytotoxic potential of TMC125 — This is measured by a reduction in Neutral Red uptake following exposure to UV light. Balb/c 3T3 fibroblast cells were treated with concentrations of TMC125 — from 0.002 to 6.42 mg/L (in Dulbecco's phosphate-buffered saline/DMSO) in the presence and absence of UVA light. No effect was observed and TMC125 — was considered to be non-phototoxic.

4. SKIN SENSITIZATION: LOCAL LYMPH NODE ASSAY (GLP) The test was done by measuring the amount of radiolabeled thymidine incorporated into the dividing cells in the lymph node after iv injection of thymidine. TMC125 — tested negative in this assay and was concluded that it did not cause skin sensitization or delayed hypersensitivity.

5. SKIN SENSITIZATION: DELAYED HYPERSENSITIVITY. The potential for TMC125 —to induce delayed contact hypersensitivity in guinea-pig was evaluated using the maximization test of Magnusson and Kligman. TMC125 did not induce delayed contact hypersensitivity in guinea pigs. TMC125 was classified as "nonsensitizing" to the skin.

6. SKIN IRRITATION: TMC125 — was evaluated in New Zealand white rabbits for skin irritation potential. No skin reactions, staining of skin or corrosive effects were observed and TMC125 was classified as "nonirritant" to the skin.

2.6.6.8 Special Toxicology Studies

STUDY TITLE:	**6-Week Repeated Dose Oral Dietary Mechanistic Non-GLP Toxicity Study of TMC125 Spray-Dried — in The Mouse**
STUDY NO.:	TMC125-NC240
LABORATORY:	Johnson and Johnson Pharmaceutical Research & Development, Turnhoutseweg 30, 2340 Beerse, Belgium
STUDY MONITOR:	Sponsor
OBJECTIVES:	The purpose of this non-GLP study was to mechanistically address the mortality caused by hemorrhagic cardiomyopathy that has been observed in a previous

study with dietary dosed TMC125 Spray-dried in male mice. More specifically, this study evaluated the role of vitamin K in the etiology of hemorrhagic cardiomyopathy caused by this drug.

STUDY INITIATION: 6/2004

GLP: yes () no (x)

METHODS/DOSING: Two groups of 20 male and 20 female mice were dietary dosed with TMC125 Spray-dried at 2320 mg eq./kg, a dose that was lethal in a previous dietary study in mice. One of these groups received daily subcutaneous injections of vitamin K1 as an antidote. Two vehicle groups received the main components of TMC125 Spray-dried formulation, one received vehicle alone and the other one was additionally injected with vitamin K1. The toxicokinetics of TMC125 Spray-dried in plasma and liver were studied in satellite TK mice.

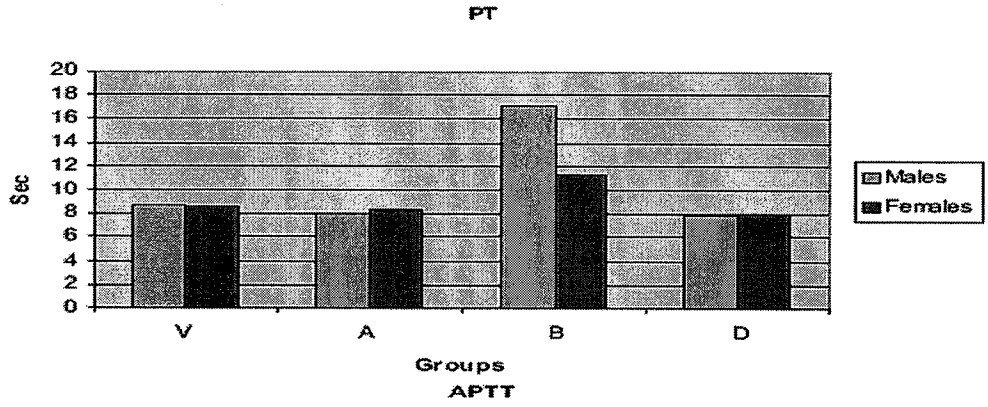
RESULTS: In the previous dietary study, mortality due to hemorrhagic cardiomyopathy was reported in a significant number of male animals by week 6.

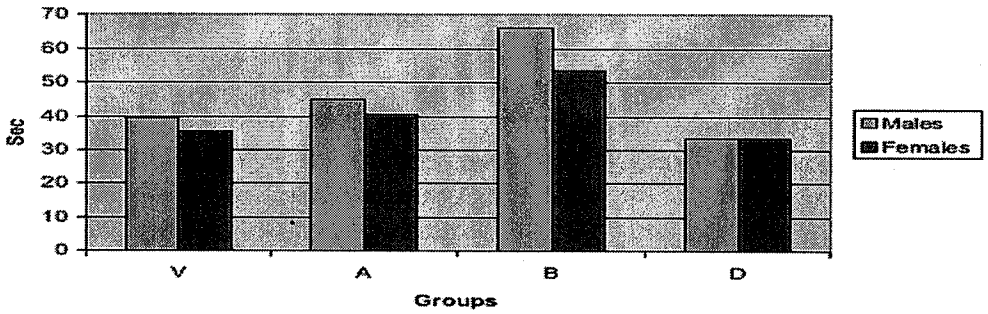
Although experimental conditions were identical to the previous dietary study, only one male animal, dosed with TMC125-alone, was sacrificed at day 16 of the study due to poor health condition. The animal displayed the hemorrhagic cardiomyopathy syndrome together with hemothorax and showed a pronounced prolongation in clotting times (APTT, PT) and decreases in clotting factors II, VII, VIII and XI. This was also the only animal that showed a high level of cardiac troponin in plasma.
Dosing with TMC125 resulted in slight to marked centrilobular to diffuse hepatocellular hypertrophy in all TMC125-dosed animals with hydropic appearance/vacuolation and minimal to marked single cell necrosis and (multi)focal necrosis. The necrosis was often associated with hemorrhages in different degrees of severity, but the incidence and severity of the TMC125-induced focal hemorrhagic necrosis was more pronounced in males dosed with TMC125-alone compared to males dosed with TMC125 with vitamin K1.
No microscopic cardiac lesions were seen in other animals and cardiac troponin levels were below or near the limit of detection.

The cause of the difference between the current and the previous study is unclear. In both TMC125-dosed male groups, a slightly lower body weight and weight gain were observed. In females, an increase in body weight and weight gain was seen in all groups versus vehicles, including the vehicle group dosed with vitamin K1.

Factor VIII (non-vitamin K-dependent) was increased in mice dosed with TMC125 and vitamin K1. The other non vitamin K-dependent clotting factor (XI) was decreased in groups dosed with TMC125. Decreases in this factor have also been reported in rodents with vitamin K deficiency. The decrease in factor

XI observed in the current study was partly counteracted by administration of vitamin K1. In general, animals dosed with TMC125-alone showed prolonged clotting times (APTT, PT) and decreases in the vitamin K-dependent clotting factors II and VII. This was counteracted by daily administration of Vitamin K1. These effects were less pronounced in females, which is in accordance with the findings described by Allen et al. In this study, plasma and liver exposure in females were somewhat lower than in males.





TOXICOKINETICS:

TMC125 plasma exposure, expressed as AUC24h, amounted to 8.3 and 9.0 ug.h/mL in males dosed with and without vitamin K1 at the end of the study, respectively, whereas corresponding values in females were 5.8 and 6.4 ug.h/mL. The AUC24h values in liver were 12 to 15 times higher. TMC125 liver exposure, expressed as AUC24h, amounted to 121 and 139 ug.h/mL in males dosed with and without vitamin K1 at the end of the study, respectively, whereas corresponding values in females were 71 and 96 ug.h/mL. Overall, plasma as well as liver concentrations in the different dosage groups showed a lot of interindividual variability. Therefore these should be interpreted with caution and only give a rough idea of the toxicokinetic exposure to TMC125 in the different dosage groups.

In conclusion, TMC125 has an effect on coagulation times and vitamin K-dependent clotting factors in mice. These effects are counteracted by administration of vitamin K and therefore it can be concluded that TMC125

affects coagulation parameters mediated by vitamin K in mice. Hemorrhagic cardiomyopathy and hemothorax, which was described by Allen et al as a result of vitamin K deficiency, was observed in one male mouse treated with TMC125-alone. Females were less affected by treatment. Clotting times, such as APTT and PT, can serve as a biomarker for this effect. The results of this study should be read in conjunction with a similar gavage mechanistic study (TMC125-NC241) (see below).

STUDY TITLE:	**1-Month Repeated Dose Oral (gavage) Mechanistic Non-GLP Toxicity Study of TMC125 – In The Mouse**
STUDY NO.:	TMC125-NC241
LABORATORY:	Johnson and Johnson Pharmaceutical Research & Development, Turnhoutseweg 30, 2340 Beerse, Belgium
STUDY MONITOR:	Sponsor
OBJECTIVES:	The purpose of this non-GLP study was to mechanistically address the mortality caused by hemorrhagic cardiomyopathy that has been observed in a previous study with dietary dosed TMC125 Spray-dried in male mice. This mechanistic gavage study with TMC125 – = TMC125 — was performed next to a similar dietary study to assess the impact of both dosing systems. More specifically, this study evaluated the role of vitamin K in the etiology of hemorrhagic cardiomyopathy caused by this drug.
STUDY INITIATION:	6/2004
GLP:	yes () no (x)
QA REPORT:	yes () no (x)
METHODS/DOSING:	Two groups of 20 male and 20 female mice were dosed with TMC125 – at 1000 mg eq./kg, a dose that was suspected to be associated with sporadic occurrence of hemorrhagic cardiomyopathy in previous studies. One of these groups received daily subcutaneous injections of vitamin K1 as an antidote. Two groups received the vehicle (PEG400), one alone and the other one with vitamin K1 injection. The toxicokinetics in plasma and liver of TMC125 – were studied in satellite TK mice.
RESULTS:	Dosing with TMC125 – resulted in minimal to moderate centrilobular to diffuse hepatocellular hypertrophy and hydropic appearance/vacuolation with single cell necrosis and minimal presence of focal necrosis in groups with and without vitamin K1 (A and B). No relevant histological differences were observed between groups A (TMC125 with vitamin K1) and B (TMC125 – -only). No animal developed the hemorrhagic cardiomyopathy syndrome in this study. No microscopic heart lesions were observed and cardiac troponin levels, a biomarker for cardiac injury, were below the limit of detection in all animals. Factor VIII (non-vitamin K-dependent) was increased in male and female mice dosed with TMC125 – and vitamin K1. The other non vitamin K-dependent clotting factor (XI) was decreased in the male group dosed with TMC125-alone. Decreases in this

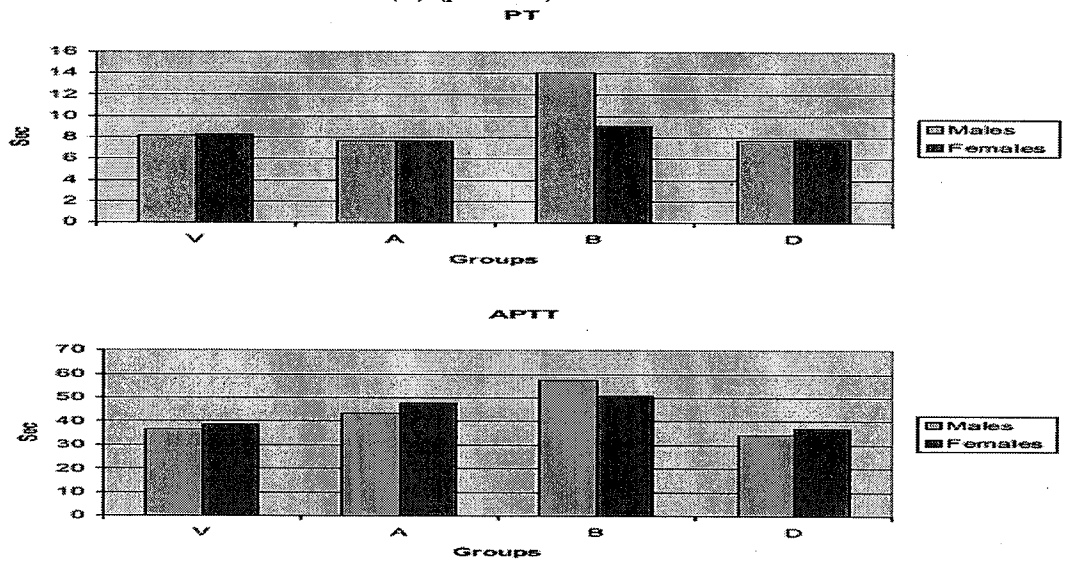
factor have also been reported in rodents with vitamin K deficiency. The decrease in factor XI observed in the current study was counteracted by administration of vitamin K1. In conclusion, TMC125 - dosed via gavage has an effect on coagulation times and vitamin K-dependent clotting factors, most pronounced in male mice. These effects are counteracted by administration of vitamin K. Females were almost unaffected by treatment.

Hemorrhagic cardiomyopathy and hemothorax, which was described by Allen et al as a result of vitamin K deficiency (and to which male mice are more sensitive), was not observed but the findings support the hypothesis that TMC125 affects coagulation parameters mediated by vitamin K in mice. Gavage dosing clearly has a lower impact on the clotting times compared to dosing via the diet and probably therefore did not result in the hemorrhagic cardiomyopathy syndrome. Obviously gavage dosing is more comparable to human dosing schedules than dietary dosing. Clotting times, such as APTT and PT, can serve as a biomarker for this effect.

Prothrombin Time (PT)
PT showed a pronounced prolongation in male mice dosed with TMC125 – without vitamin K1 (B) ($p<0.001$; +72% versus vehicle group V dosed with PEG 400). Both the male groups dosed with TMC125 - with vitamin K1 (A) and the vehicle group dosed with vitamin K1 (D), were comparable to vehicle group V, although both showed a marginal shortening ($p<0.01$ - $p<0.001$; -7% and -6% versus vehicle group V). Similar observations were made in females, although the prolongation in PT in the group dosed with TMC125 – without vitamin K1 (B) was much less pronounced ($p<0.001$; +10% versus vehicle group V) compared to males. A clear statistically significant difference was present between male and female groups dosed with TMC125 - with vitamin K1 (A), versus the groups dosed with TMC125 - without vitamin K1 (B) ($p<0.001$).

Activated Partial Thromboplastin Time (APTT)
APTT showed a pronounced prolongation in male mice dosed with TMC125 - without vitamin K1 (B) ($p<0.001$; +56% versus vehicle group V). Both the male groups dosed with TMC125 – with vitamin K1 (A) and the vehicle group dosed with vitamin K1 (D), were comparable to vehicle group V, although group A showed a minimal prolongation ($p<0.01$; +18% versus vehicle group V). Similar observations were made in females, although the prolongation in APTT in the group dosed with TMC125 - without vitamin K1 (B) was less pronounced ($p<0.001$; +31% versus vehicle group V) compared to males and therefore a smaller difference was present with the female group dosed with TMC125 – and vitamin K1. A clear statistically significant difference was present between the male group dosed with

TMC125 – with vitamin K1 (A), versus the male group dosed with TMC125K without vitamin K1 (B) ($p<0.001$).



TOXICOKINETICS: Overall, the plasma and liver exposure to TMC125, as expressed by Cmax and AUC, was fairly comparable between groups dosed with and without vitamin K1 as well as between the male and female mice of each dosage group. Only Cmax-values in liver seemed slightly lower (0.70-0.76 times) in females than in males within both dosage groups. Overall, plasma Cmax levels ranged between 1.5 and 1.7 ug/ml, while AUC0-24h-values ranged between 8.4 and 11.2 ug.h/ml. The Cmax- and AUC-values in liver were 10 to 16 times higher than in plasma, with Cmax ranging between 17.7 and 26.1 ug/g and AUC0-24h values between 113 and 130 ug.h/g. Thus, there was no difference in pharmacokinetics of TMC125 in plasma and liver between groups dosed with and without vitamin K1.

COMMENTS: These 2 non-GLP studies did not support the sponsor's claim that the drug-induced hemorrhage and cardiomyopathy was due to vitamin K deficiency (What had caused vitamin K deficiency? There is no data showing the drug binds vitamin K). The data merely showed that the drug caused prolongation of APTT and PT, and that the previously reported lethal hemorrhage and cardiomyopathy could not be reproduced in the current two studies.

NON-GLP STUDY TITLE: **In Vitro Phototoxicity Testing of R293496 (TMC125 –, by the Neutral Red Uptake Assay Using Balb/c 3T3 Mouse Fibroblasts (Study TOX7217; Tibotec Study Number TMC125-NC245) (29 AUGUST 2005)**

STUDY NO.: TMC125-NC241

LABORATORY: Johnson & Johnson Pharmaceutical Research & Development

STUDY MONITOR: Sponsor

OBJECTIVES: The purpose of this non-GLP study was to assess the in vitro phototoxic and cytotoxic potential of R293496 [TMC125 – , the [redacted] of R165335

	[TMC125])(Batch ID =ZR293496PFA051), as measured by a reduction in Neutral Red uptake in cultures of normal Balb/c 3T3 mouse fibroblasts following exposure to ultraviolet A (UVA) light. The study was sponsored by Tibotec Pharmaceuticals Ltd., a member of the J&J group of companies, Little island, CO. Cork, Ireland and performed by Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Raritan, NJ, from 22 June 2005 (initiation of range-finding experiment) to 28 July 2005 (end of main experiments).
STUDY INITIATION:	6/2005
GLP:	yes () no (x)
QA REPORT:	yes () no (x)
METHODS/DOSING:	Balb/c 3T3 fibroblast cells seeded into 96-well microtiter plates were treated with a range of concentrations of R293496. Doses tested ranged from 0.002 to 6.42 mg/L in the presence and absence of UVA light. A positive control (chlorpromazine) and vehicle control treatment were also included. One set of plates was exposed to 5 J/cm2 UVA light and a second set of plates was kept in the dark. Cytotoxicity was assessed by the Neutral Red uptake assay. The 50% inhibition concentration (IC_{50}), which is the concentration inducing a 50% reduction of the Neutral Red uptake values, was calculated for the positive control. The IC_{50} values for R293496 in the presence and absence of UVA light were not determined due to the absence of a cytotoxic effect at any tested concentrations. The photo irradiation factor (PIF) and mean photo effect (MPE) for the positive control and test article were calculated according to OECD draft guideline.
RESULTS AND CONCLUSIONS	The IC_{50} values from two main experiments for chlorpromazine (positive control) were within the proposed ranges in the presence and absence of UVA light. The PIF and MPE for chlorpromazine were 25.54 and 0.68 (Experiment 1), 25.01 and 0.67 (Experiment 2), respectively (values above five for PIF and above 0.15 for MPE indicate phototoxicity). The PIF and MPE for R293496 were 1 and 0.01 (Experiment 1), and 1 and -0.01 (Experiment 2), respectively. PIF and MPE values from both main experiments were less than 2 and 0.1, respectively. In conclusion, R293496 (TMC125 – is not phototoxic in vitro up to 6.42 mg/L, the highest concentration tested in the study.
STUDY TITLE:	**Immunotoxicity Study on TMC125 –**
STUDY NO.:	CIT/Study No. 26342 TSR/TMC125W Tibotec Pharmaceuticals Ltd., Tibotec Study Reference: TMC 125 - NC 180; Study Reference J&JF'RD Beerse: TOX6222
LABORATORY:	———— [TK: Johnson & Johnson Pharmaceutical Research & Development, Belgium]
DATE:	10/2003
OBJECTIVES:	The objective of this study was to evaluate the potential immunotoxicity of the test article, TMC125– following daily oral administration (gavage) to rats for 4 weeks.
GLP:	yes (x) no ()

QA REPORT: yes (x) no ()

METHODS/DOSING: Three treated groups of eight male and eight female Sprague-Dawley rats received the test article, TMC125 (Batch ZR293496PFA011), by gavage at the dose-level of 70,200 or 600 mg kg/day for 4 weeks. An additional group of eight males and eight females received the vehicle (PEG 400) under the same experimental conditions and acted as a control group. In addition, each test treated group included 6 satellite animals per sex used for plasma levels of the test article; blood samples were taken on day 26 at designated times (i.e. 0.5, l,2,4, 8 and 24 hours after dosing). The animals were checked daily for mortality and clinical signs. Body weight and food consumption were recorded once a week. Hematological investigations (blood and bone marrow) were performed on all principal animals at necropsy.

RESULTS AND CONCLUSIONS

Mortality

No unscheduled death occurred in principal animals during the study.

Clinical signs

Ptyalism was observed on some occasions in almost all test treated animals. Abdominal or loud breathing, chromorhinorrhea and/or dyspnea were observed transiently in a few animals given 600 mg kg/day.

Body weight

The slight decrease in body weight gain in the 600 mg eq./kg male dose group may have been related to treatment, though fortuity cannot be ruled out with certainty. Body weight gains of the 200 and 600 mg kg/day female dose groups showed moderate decreases as a consequence of treatment.

Toxicokinetics

TMC125 was fairly rapidly absorbed in all animals at the different dose levels, with Tmax occurring between 0.5 and 2 h after dosing. After peak time, plasma concentrations declined rapidly with estimated half-lives ranging from 2.1 to 5.8 h. Cmax-values amounted to 92.5, 1 50 and 25 ng/ml in male rats and to 1, 26, 205 and 580ng/ml in female rats at 70,200 and 600 mg kg/day, respectively. The total exposure (AUCO-24h) amounted to 574, 845 and 1535 ng.h/ml in male rats and to 586 (AUCO-8h), 1323 (AUCO-24h) and 5444 ng.h/ml (AUCO-24h) in female rats at 70,200 and 600 mg kg/day, respectively. Both Cmax- and AUC-values were consistently lower in male rats compared to the female rats, especially at the high dose level. At 70 and 200 mg kg/day, Cmax- and AUC-values in male rats amounted from 64 to 75% of the female values. At 600 mg kg/day, they only represented 28% (AUCO-24h) to 43% (Cmax) of the female values. When increasing from 70 mg kg/day to 200 mg kg/day, Cmax- and AUC-values increased less than dose-proportional in males and females (1.5-fold increase). When increasing the dose level from 200 mg kg/day to 600 mg kg/day, Cmax- and AUC-values increased less than dose-proportional in the male rats i.e. approximately 1.7-fold increase. In female rats, Cmax-values increased almost dose-proportional (2.8-fold increase) and AUCO-24h-values increased slightly more than dose-proportional (4.1 -fold increase).

Hematology
No differences of toxicological significance were noted between control animals and those treated with the test article.
Response to T-cell dependent antigen
The immune response of the treated groups as measured by IgM production was not affected by the treatment with the test article, whatever ELISA method was used for determination of the results.
Organ weights
There were no treatment-related effects on the lymphoid organ weights.
Macroscopic *post-mortem* examination
There were no treatment-related necropsy findings in the lymph nodes, spleen, thymus and intestinal Peyer's patches.
Microscopic examination
No relevant microscopic findings were seen in the spleen, the thymus or the lymph nodes. The microscopic evaluation of the Peyer's patches in the ileum showed that the treatment with the test article had no effect on these structures. Histopathological examination of the sternum and femur did not reveal any marked changes in bone marrow cellularity in animals treated with the test article.

In summary, no immunotoxicity, as evidenced by the results of a functional test (IgM antibody response to KLH), hematology investigations or examination of the principal lymphoid organs, was noted for etravirine.

2.6.6.9 Discussion and Conclusions

The sponsor had employed the conventional species of rats and dogs as their surrogates of etravirine's toxicity profile exploration. The choice of the rat as a species may have been compromised because the overall toxicity profile might have been confounded by the sponsor-claimed rat-specific thyroid toxicity. Further, the drug did not produce sufficient exposures in rats (AUC<7 ug.h/ml), regardless the formulations used, were comparable to those in humans (7-8 ug.h/ml). The low exposure levels may be due partly to hepatic enzyme autoinduction of etravirine.

The studies conducted in dogs provided adequate drug exposures, because bridging studies using a spray-dried formula did uncover more target organs of toxicity (e.g., 1-month and 6-month dog studies; AUC up to 60-70 ug.h/ml). Nevertheless, the studies had provided useful information on margin of safety for human trials and target organs of toxicity, some of which, were correlative to those seen in humans (e.g., hepatic, hematological and skin).

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The results of the nonclinical pharmacology/toxicology studies submitted by the sponsor adequately support the approval of etravirine.

Unresolved toxicology issues (if any): None

Recommendations:

This NDA in its present form has provided adequate nonclinical safety information in support of its approval. The sponsor has employed feasible levels of dosage and species of animals of both sexes in their studies and assay systems, granting no other alternative methods are currently available for a more preferred means of human risk prediction. The sponsor has fully explored the drug's toxicity and addressed issues regarding the modes and mechanisms of toxicity with good faith and efforts.

In summary, while the toxicity testing on etravirine is still ongoing (e.g., carcinogenicity studies in both mice and rats), it is concluded that this NDA has provided sufficient nonclinical safety information to allow for prediction of potential toxicity in humans with the judicious use of this drug.

Suggested labeling:

Please see the executive summary for the language to be used on the proposed label.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

3 Page(s) Withheld

1 Trade Secret / Confidential

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/s/

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