CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202992Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 202992
Submission date: August 12, 2011
Drug: teriflunomide
Applicant: Sanofi Aventis US LLC
Indication: Relapsing-remitting Multiple sclerosis
Reviewing Division: Division of Neurology Products

Introductory Comments:
The pharmacology/toxicology reviewer and supervisor have determined that the nonclinical information is adequate to support approval of this NDA for the indication noted above.

Discussion:
Teriflunomide is the active metabolite of another approved drug, leflunomide, which was developed by the same applicant and is approved for treatment of rheumatoid arthritis.

As noted in the primary and secondary pharmacology/toxicology reviews, teriflunomide and leflunomide are teratogenic in rats and rabbits. Leflunomide is contraindicated in pregnant women. The potential of teriflunomide and leflunomide to cause fetal harm at the human exposures achieved with these drugs is thoroughly discussed in the primary and secondary pharmacology/toxicology reviews and a review by the Maternal Health Team. The effects of teriflunomide are consistent with a recommendation that it be contraindicated in pregnant women.

Carcinogenicity studies of teriflunomide were conducted in mice and rats. These studies were reviewed by the Executive Carcinogenicity Assessment Committee. The studies were found to be adequate and no drug-related neoplasms were noted.

Teriflunomide inhibits dihydroorotate dehydrogenase and consequently leads to the inhibition of pyrimidine synthesis. Therefore, "dihydroorotate dehydrogenase inhibitor" and "pyrimidine synthesis inhibitor" are both scientifically valid pharmacologic terms to describe teriflunomide. However, "pyrimidine synthesis inhibitor" may be more clinically meaningful than "dihydroorotate dehydrogenase inhibitor".

Conclusions:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that teriflunomide may be approved for the above indication from a nonclinical

perspective. No additional nonclinical studies are recommended. I discussed labeling with the pharmacology/toxicology supervisor and agree with the changes suggested.

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/s/

PAUL C BROWN
09/11/2012

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: July 20, 2012

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 202-992 (Aubagio, teriflunomide)

On August 12, 2011, Sanofi Aventis submitted NDA 202-992 for teriflunomide (HMR1726) "…for the treatment of patients with relapsing forms of multiple sclerosis (relapsing MS) (b) (4) " No deficiencies were identified and the NDA was filed (Agency letter dated October 25, 2011). Clinical development of teriflunomide for treatment of relapsing MS was conducted under IND 67476.

A complete battery of nonclinical studies were conducted in support of NDA 202-992 because higher plasma exposures for teriflunomide could be achieved in animals when teriflunomide was administered directly rather than as leflunomide and because apparent differences in toxicity or more severe toxicity were observed during development with teriflunomide. According to the sponsor, although early clinical trials were supported by nonclinical data for leflunomide, "…with the progression of development, the teriflunomide program became independent of its predecessor due to differences between the 2 compounds (distinct indications, differences in animal models, regulatory guidelines, and technology advances)." The nonclinical data were reviewed by Dr. Houghtling and, based on his review, he has concluded that the nonclinical data are adequate and sufficient to support approval of the NDA, with appropriate labeling; Dr. Houghtling recommends Pregnancy Category X for teriflunomide (*Pharmacology/Toxicology NDA Review and Evaluation, NDA 202-992, Rick Houghtling, Ph.D., 7/13/2012*).

Pharmacology

Teriflunomide is the active metabolite of leflunomide (Arava), an immunomodulatory agent approved for the treatment of rheumatoid arthritis. Following oral administration, leflunomide is rapidly and extensively metabolized to teriflunomide, which is responsible for all the *in vivo* activity of leflunomide. The mechanism(s) underlying the therapeutic

effects of teriflunomide in patients with MS are not completely understood. Numerous pharmacological activities have been proposed for teriflunomide (*cf.* Claussen MC, Korn T *Clin Immunol* 142:49-56, 2012; Warneke C *et al*. *Neuropsychiat Dis Treat* 5:333-340, 2009); however, the primary mechanism is thought to be inhibition of the 4th enzyme in *de novo* pyrimidine synthesis, dihydroorotate dehydrogenase (DHO-DH). By inhibiting DHO-DH, teriflunomide is thought to reduce activation and proliferation of rapidly dividing T- and B-lymphocytes (presumed to be involved in the pathology of MS) by depleting pyrimidine pools and, thus, DNA synthesis. Resting cells are not thought to be affected since they can overcome the inhibition of DHO-DH by relying on salvage pathways as a source of pyrimidines. As reported for leflunomide (*cf*. Ruckemann K *et al. J Biol Chem* 273(34):21682-21691, 1998), exogenous uridine was shown to reverse the antiproliferative effects of teriflunomide *in vitro*, confirming DHO-DH inhibition as a mechanism of action. Teriflunomide has been demonstrated to have uridine-independent activity (e.g., tyrosine kinase inhibition) but at *in vitro* concentrations reported to be "at least one order of magnitude higher than the concentrations necessary to block DHODH" (Claussen & Korn, 2012).

Inhibition of DHO-DH by teriflunomide was demonstrated *in vitro*, with marked interspecies differences in potency (IC_{50}'s of 3.5, 0.086, and 12.5 μM in mouse, rat, and human splenocytes, respectively; 12, 141, and 98 μM in dog, monkey, and human whole blood, respectively). However, an article published by the sponsor (Merrill JE *et al*. *J Neurol* 256:89-103, 2009) gives IC_{50} values for inhibition of DHO-DH by teriflunomide as 1.3 μM for human and 86 nM for rat; these data suggest less of a difference in *in vitro* potency between these two species.

The efficacy of teriflunomide as an MS therapeutic was assessed in various animal models of experimental autoimmune encephalomyelitis (EAE; SJL/J mouse, Lewis rat, Dark Agouti rat). In the SJL/J mouse, oral administration of teriflunomide one day after disease induction reduced disease symptoms at 20 but not 10 mg/kg. In the Lewis rat model of acute EAE, teriflunomide demonstrated efficacy when administered at an oral dose of 10 mg/kg at disease induction and at 3 and 10 mg/kg following symptom onset. In studies in the Dark Agouti rat, considered to exhibit pathology similar to humans with MS, teriflunomide demonstrated efficacy at oral doses of 3 and 10 mg/kg but not consistently at 1 mg/kg (Merrill *et al*., 2009). Merrill *et al*. (2009) identified 3 mg/kg as the "minimal effective dose." These oral doses are similar or higher than the maximum recommended daily dose (14 mg) in humans, based on body surface area (mg/m^2) comparisons; therefore, they do not reflect the substantial difference in potency suggested by the *in vitro* data.

PK/ADME

PK/ADME was assessed in the animal species used in the pivotal nonclinical studies (mouse, rat, rabbit, and dog) and human. The results of this assessment indicate that teriflunomide exhibits:

- High oral bioavailability in all animal species (100-102%) except for rabbit (63.8%).

- A volume of distribution less than total body water in all species (0.073-0.163 L/kg) following IV administration.
- Widespread tissue distribution of teriflunomide-related radioactivity in studies in several strains (Sprague-Dawley, Long-Evans, and Dark Agouti) of rat. Highest levels of radioactivity at 24 hrs post dose were detected in GI tract, liver, kidney, lung, and skin (particularly in skin of Long-Evans rat). The longest $t_{1/2}$ was detected in pigmented skin (radioactivity still detected at 672 hrs post dose). Distribution of drug-related radioactivity into the CNS was limited (1-4% of blood) in Sprague-Dawley rat and in intact and EAE Dark Agouti rats. Brain penetration was similar in intact and EAE Dark Agouti rats, but distribution into the cervical spinal cord was ≈3 times greater in EAE animals.
- High serum protein binding in all species (>96%).
- A relatively long $t_{1/2}$ in all animal species (18-37 hrs), except for rabbit (4-5 hrs), and in human (median $t_{1/2}$ of 19.4 days). Enterohepatic recirculation was demonstrated in rat, which is presumed to be responsible for the prolonged $t_{1/2}$. In humans, administration of cholestyramine or activated charcoal was demonstrated to substantially shorten the $t_{1/2}$ (to 2-3 days) (*cf. Clinical Pharmacology Review NDA 202-992 Veneeta Tandon, Ph.D., Joo-Yeon Lee, Ph.D., Jeffrey Kraft, Ph.D. 7/3/2012*).
- A similar *in vitro* and *in vivo* metabolic profile in animals and humans; *in vivo* teriflunomide was the major circulating drug-related compound, accounting for 85 to 99.4 and 100% of total circulating drug-related material in animals and humans, respectively.
- Teriflunomide was excreted in rat milk, with 23.3% of dose radioactivity (following a single oral 7.5-mg/kg dose of radiolabeled teriflunomide) being transferred to lactating pups.

General Toxicology

The pivotal repeat-dose oral toxicity studies were conducted in Wistar (3-month) or Sprague-Dawley (6-month) rat and Beagle dog (3- and 12-month). In addition, 1-month IV toxicity studies were conducted in Wistar rat (2 studies) and Beagle dog. A 3-month oral toxicity study in CD-1 mouse was submitted in support of dose-selection for the 2-year carcinogenicity study in CD-1 mouse. A brief discussion of the findings from the oral studies, and the non-neoplastic findings from the 2-year oral carcinogenicity studies in mouse and Sprague-Dawley rat, are discussed in this section.

Mouse: In the 3-month study, teriflunomide was administered at oral (gavage) doses of 0, 5, 25, 50, and 75 mg/kg/day. The two highest doses resulted in 100% mortality due primarily to adverse effects on lymphoid organs (necrosis/atrophy of the spleen, thymus, lymph nodes), the hematopoietic system (decreased rbc parameters, increased reticulocyte count, Heinz bodies; reduced hematopoietic cells of the bone marrow), and/or the GI tract (erosion/ulceration, glandular epithelial degeneration/regeneration). Similar findings, although of less severity, were observed at 25 mg/kg/day. Testicular (seminiferous tubule degeneration) and epididymal toxicity (cellular debris in lumen,

aspermia) were observed at 50 mg/kg/day; histopathology was not conducted at 75 mg/kg/day.

In the 2-year carcinogenicity study in CD-1 mouse, survival was decreased in males and females at the high dose. Deaths were attributed to increased ulceration and inflammation of the skin and/or GI tract. These findings, also observed in high-dose survivors, were considered secondary to the antiproliferative and/or immunosuppressive effects of teriflunomide, as were microscopic changes in other tissues (liver, heart, lymph nodes, bone marrow).

Rat: In the 3-month study, teriflunomide was administered at oral (gavage) doses of 0, 0.5, 1, and 4 mg/kg/day. Minimal effects, consisting of decreases in rbc count and hgb (high dose) and increases in relative liver weight (considered adaptive; mid and high doses), were observed. Similar doses were used in the 2-year carcinogenicity study (0, 0, 0, 0.5, 1.5, and 4 mg/kg/day). With the greater duration, drug-related effects were observed at all doses, consisting primarily of bone marrow hypocellularity (decreased hematopoietic cells) and effects on lymph nodes (decreased plasmacytosis) and spleen (decreased lymphocytes and increased pigment [hemosiderin] deposition).

Higher doses were used in the 6-month study (0, 0.3, 1.5/9, 3, and 6 mg/kg/day); the 1.5 mg/kg/day dose was increased to 9 mg/kg/day on Day 108 due to a lack of toxicity at 6 mg/kg/day. Two deaths (one spontaneous, one moribund sacrifice) occurred by Days 163-176, following the increase in dose. Both deaths were attributed to bone marrow and GI toxicity, as evidenced by marked decreases in hematopoietic cells in bone marrow and mucosal atrophy (loss of glandular epithelium and villous atrophy) of the GI tract. Additional findings in these two animals included absence of germinal centers in lymph nodes and lymphoid tissue, "marked to massive" atrophy of the thymus and spleen, bilateral atrophy of the corneal epithelium, pigment (hemosiderin) deposition in spleen, and "mild to moderate" centrilobular necrosis of the liver. Similar findings, although possibly of less severity or lower incidence, were detected in survivors at 3, 6, and/or 1.5/9 mg/kg/day. The low dose was identified as the NOAEL.

Dog: In the 3-month study (+4-week recovery), teriflunomide was administered at oral doses of 0, 0.8, 2.5, and 8.0 mg/kg/day. The high dose exceeded an MTD, as evidenced by death (Day 22) or moribund sacrifice (Day 15), leading to early termination of all surviving high-dose animals on Day 24. Early termination was due to the severe clinical signs evident at the high dose, which consisted primarily of erosions and/or ulceration of the oral cavity and bleeding of the gums; similar clinical signs were also observed at the mid dose. The sponsor characterized these changes as "more or less pronounced." CNS signs (tremors, ataxia, and/or convulsions) observed in two high-dose animals (1 M, 1 F) were thought to be secondary to submeningeal hemorrhage, although hemorrhage was detected only in the male. Marked anemia was evident in all high-dose and several mid-dose animals. The main target organs were bone marrow (absence of granulopoiesis, marked decrease in erythropoiesis), GI (characterized as "purulent and necrotizing and/or ulcerative inflammations of different degrees" in oral cavity [including tonsils], esophagus, stomach, and small and large intestine), and spleen (follicular atrophy). The

sponsor identified the low dose as the NOAEL, although a slight increase in alkaline phosphatase detected at this dose (and at the mid and high doses) was considered possibly drug-related.

Due to the severe toxicity observed in the 3-month study, lower doses were tested in the 1-year study (0, 0.2, 0.8, and 2/4 mg/kg/day). The initial high dose of 2 mg/kg/day was administered for 190 days. Due to the lack of observed toxicity, the high dose was increased to 4 mg/kg/day on Day 191. One high-dose female was sacrificed moribund on Day 248 (after 57 doses at 4 mg/kg/day), due to clinical signs ("poor doing") and clinical pathology and/or microscopic findings consistent with immunosuppression (inflammation of larynyx and pharynx and draining lymph nodes, thymic and lymph node atrophy) and anemia. (The anemia was attributed to blood loss; however, the data in dog and other species suggest the possibility of hemolytic anemia, as was observed in the 1-month IV study in dog.) All other animals survived to scheduled sacrifice. Although hematological changes (e.g., decreases in rbc count, hgb, and hct, and increases in methemoglobin) were also observed in high-dose survivors, the primary target organ was the pancreas. The study pathologist ((b) (4) D.V.M.) described the microscopic findings in the pancreas as follows:

> "Findings in the pancreas were observed in one male and one female at 0.8 mg/kg/day and all dogs at 2.0/4.0 mg/kg/day (including the unschedued *[sic]* death) and consisted of minimal to moderate, focal or multifocal acinar degeneration and individual acinar cell necrosis with loss of acini; acinar loss was associated with fibrosis and a minimal mononuclear inflammatory cell infiltrate."

Due to these findings, the sponsor retrospectively analyzed frozen serum samples for trypsin-like immunoreactivity (TLI), amylase, and lipase. No changes were detected in circulating amylase or lipase levels; however, TLI was reduced in high dose animals in an apparent dose- and duration-dependent manner. The control and high-dose data are summarized in the following table (TLI given in units of ng/dL):

SEX	GROUP	STUDY WEEK				
		-1	12	25	38	51
M	C	11.53±2.743	12.74±0.935	14.97±2.515	13.67±2.198	11.70±1.450
	HD	11.34±0.905	11.58±1.566	10.66±4.433	8.21±3.770	6.58±3.010*
F	C	13.05±3.458	14.71±3.302	15.82±3.633	17.32±5.187	37.13±43.932
	HD	12.89±7.408	12.00±2.427	10.31±3.424	8.14±3.778	7.31±4.162

The sponsor considered the decrease in TLI to be of "uncertain toxicological significance" because the microscopic changes were not considered to be sufficiently severe to explain the finding. The sponsor identified the low dose as the NOAEL. In a 1-year toxicity study of leflunomide in Beagle dog at oral doses of 0, 0.25, 0.8, and 2.5 mg/kg/day, no pancreatic effects were reported (*cf. Pharmacology Review NDA 20-905 Asoke Mukherjee, Ph.D., July 1, 1998*).

The plasma exposure at the low dose of teriflunomide (C_{max} = 1.4-1.5 µg/mL, $AUC_{(0\text{-}24\ hr)}$ = 20.2-26.6 µg*hr/mL) is substantially lower than that anticipated in humans at the

MRHD. At the high dose, associated with clear pancreatic toxicity, plasma exposure in dog (C_{max} = 58-69 µg/mL, $AUC_{(0\text{-}24\ hr)}$ = 1115-1313 µg*hr/mL) is similar to that anticipated in humans.

Since pancreatic toxicity in dog was identified during clinical development, clinical trials were modified to address this potential safety concern. For example, for certain clinical trials patients with "persistent elevations of serum amylase or lipase greater than 2-fold the ULN" were excluded (*cf. Clinical Review NDA 202992, Lourdes Villalba, M.D., Evelyn Mentari, M.D., July 12, 2012*) and monitoring (imaging, clinical pathology) was included. Based on review of the clinical safety data, Drs. Villalba and Mentari have concluded that a clinical signal for adverse effects on the pancreas has not been identified but suggest additional post-marketing follow-up.

Reproductive and developmental toxicity

A full battery of reproductive and developmental toxicity studies were conducted for teriflunomide.

Fertility and early embryonic development: Fertility and early embryonic development was assessed in males and female Sprague-Dawley rats in separate studies. In the study in males, teriflunomide was administered orally at doses of 0, 1, 3, and 10 mg/kg/day prior to and throughout the mating period. No effects on mating or fertility parameters were observed; however, a decrease in sperm count (cauda epididymis) was observed at the mid and high doses. (As noted previously, testicular and epididymal toxicity was observed in the 3-month study of teriflunomide in CD-1 mouse.)

In the study in females, teriflunomide was administered orally (0, 0.84, 2.6, and 8.6 mg/kg/day) to female Sprague-Dawley rats prior to and throughout the mating period and continuing through gestation day (GD) 6; females were sacrificed on GD 21 for assessment of reproductive parameters. A small, but dose-related, decrease was noted in the number of pregnant females: 26 (100%), 25 (96.2%), 24 (92.3%), and 23 (88.5%) in C, LD, MD, and HD females, respectively; the sponsor did not consider this a drug-related effect. Embryolethality was evident at the mid and high doses, with increases in early resorptions, total post-implantation loss, and the number of dams with total litter loss resulting in a decrease in the number of live fetuses at both doses. Malformations were detected in 2 fetuses from 2 litters at the low and mid doses (see table below). No malformations were detected in high-dose fetuses, but there were only 9 evaluable litters at that dose (compared to 26, 25, and 23 for C, LD, and MD, respectively).

SPECIES/ SEX	DOSE (mg/kg)	DAM	FETUS	MALFORMATIONS
male rat	0, 1, 3, 10	--	--	none
female rat	0	--	--	none
	0.84	253	6 (E)*	tail, short
		261	2 (E)	tail, short trunk, anus, atresia
	2.6	356	12 (E)	jaw, mandibular, micrognathia
		358	4 (E)	mouth/jaw, tongue, microglossia; cleft palate eyes, eye bulges; bilateral, absent
	8.6	--	--	none (only 9 evaluable litters)

*fetuses were examined for external malformations only.

Reduced fetal weight was observed at all doses in both males and females. A NOAEL was not identified in this study.

Embryofetal development: Embryofetal development (EFD) studies were conducted in Sprague-Dawley rat and Himalayan rabbit. Due to the malformations observed in these studies, the sponsor conducted an additional study (EFD-staged) in Sprague-Dawley in an attempt to identify the critical period(s) for inducing drug-related malformations.

Rat. In the EFD study in rat, teriflunomide was administered at oral doses of 0, 1, 3, and 10 mg/kg/day throughout the period of organogenesis (GD 6-17). Embryolethality was evident at the mid and high doses, with increases in postimplantation loss resulting in a slight decrease in the number of live fetuses at the mid dose and a marked decrease in the number of live fetuses at the high dose. At the high dose, there were only 6 (of 19) pregnant dams with live fetuses and a total of only 10 live fetuses. Fetal body weight and crown-to-rump length were both reduced in mid- and high-dose fetuses. Malformations were increased at the mid (3 fetuses/2 litters) and high doses (10 fetuses/6 litters) compared to control (2 fetuses/2 litters) or low dose (1 fetus/1 litter); at the high dose, all 6 litters contained at least one fetus with a malformation. From the data in individual fetuses (summarized in the following table; V or S denotes that the fetus was scheduled for viscera or skeletal examination), it is clear that most affected fetuses had more than one malformation.

DOSE (mg/kg)	DAM	FETUS	MALFORMATIONS
0	215	R07 (V)	testis, retention testis
	219	R05 (V)	diaphragm, hernia
1	230	R02 (V)	diaphragm, hernia
3	250	R03 (S)	diaphragm, hernia liver, partly displaced to thoracic cavity stomach, partly displaced to thoracic cavity
	251	L01 (S)	diaphragm, hernia liver, partly displaced to thoracic cavity stomach, displaced to thoracic cavity
		L07 (S)	Skull, mandibular, Rami fused; incisors of lower jaw, aplasia
10	264	L04 (S)	body, edematous jaw, mandibular, brachygnathia inferior hindpaw, tarsal region, bilateral, bent skull, basioccipital bone, dysplasia
	270	R09 (V)	eye, bilateral, anophthalmia
	271	L05 (S)	head, parietal bone, hematocyst eye, bilateral, anophthalmia skull, orbit, bilateral, reduced in size
		R05 (V)	brain, hydrocephalus internus eye, bilateral, anophthalmia
	273	L04 (S) (dead)	body, edematous eye, right, microphthalmia; left, aplasia lentis skull, orbit, bilateral, reduced in size
		L05 (V)	brain, hydrocephalus internus
		L07 (S)	eye, left microphthalmia skull, orbit, left, reduced in size
		L08 (V)	brain, hydrocephalus internus eye, bilateral, microphthalmia
	274	R01 (S)	eye, right, aplasia lentis; left, anophthalmia skull, exoccipital bone, right, fused with 1st cervical vertebra skull, orbit, bilateral, reduced in size
	276	L01 (S)	eye, right, aplasia lentis skull, mandibular, Rami fused skull, incisors of lower jaw, aplasia skull, orbit, right, reduced in size cervical vertebra, vertebra, anlage of only 4

fetuses/litters examined (C, LD, MD, HD): 110/17, 115/18, 110/19, 4/3 for visceral; 119/17, 123/18, 121/19, 6/6 for skeletal.

There were increases in a few findings at the low dose (distended ureter/kidney, growth retardation), in both the number of fetuses and litters; therefore, arguably, no NOAEL was identified in this study. (The sponsor considered the low dose to be an NOAEL.) TK analysis was not performed in this study; however, data from a TK bridging study provided plasma C_{max} and $AUC_{(0\text{-}24\ hr)}$ values at 1.0 mg/kg of 10.9 µg/mL and 110 µg*hr/mL, respectively.

In the EFD-staged dosing study in rat, teriflunomide was administered orally to Sprague-Dawley rats (20/group, except as noted) at an oral dose of 10 mg/kg/day during different days of gestation: 6-8, 9-11, 12-14, 15-17, or 6-17 (n = 10). A separate group (n = 10) received vehicle throughout the period of organogenesis, GD 6-17. The data clearly indicate the most vulnerable periods for teriflunomide-induced teratogenicity and embryolethality are GD 6-8 and 9-11, respectively. Dosing during only GD 9-11 resulted

in total litter loss (almost exclusively due to early resorptions) in all dams. Post-implantation loss (early resorptions) was also increased in dams dosed during GD 6-8 and 6-17; of these two groups, the effect was greater in the dams dosed throughout the period of organogenesis, presumably because dosing in this group encompassed the most vulnerable periods. However, it is difficult to reconcile the total embryolethality observed in dams dosed during GD 9-11 with the substantial number of surviving fetuses of dams dosed throughout organogenesis; the sponsor did not appear to address this issue.
The table below summarizes the number of fetuses with at least one malformation of each type; all fetuses were examined for external malformations; each fetus was examined either for visceral or skeletal findings.

DOSE* (mg/kg)	GD	TOTAL/ AFFECTED	EXAMINATION		
			EXTERNAL	VISCERAL	SKELETAL
0	6-17	total	121/10	62/10	59/10
		affected	0/0 **(0%/0%)**	1/1 **(2%/10%)**	1/1 **(2%/10%)**
10	6-8	total	179/20	87/20	92/20
		affected	0/0 **(0%/0%)**	2/2 **(2%/10%)**	6/4 **(6%/20%)**
	9-11	total	0/0	0/0	0/0
	12-14	total	225/20	114/20	111/20
		affected	0/0 **(0%/0%)**	0/0 **(0%/0%)**	1/1 **(1%/5%)**
	15-17	total	213/19	107/19	106/19
		affected	0/0 **(0%/0%)**	1/1 **(1%/5%)**	2/2 **(2%/11%)**
	6/17	total	51/9	24/9	27/9
		affected	26/9 **(51%/100%)**	22/9 **(92%/100%)**	27/9 **(100%/100%)**

*data given as fetuses/litters, absolute and percent of total.

Clearly, the teratogenic effect of teriflunomide was most evident in dams dosed throughout organogenesis (GD 6-17). In these dams, 9 of 20 (45%) had at least one fetus with at least one malformation, and most of the affected fetuses had multiple malformations. Increases in external, visceral, and skeletal malformations were observed in this group. External malformations consisted of gastroschisis, malrotated hindlimb, aglossia, protruding tongue, high-arched palate, agnathia, micrognathia, cleft lip, misshapen nose, absent eye bulge, anotia, malpositioned pinna, domed head, exencephaly, meningocele, microcephaly, and local edema. Visceral malformations consisted of anophthalmia, microphthalmia, cleft palate, misaligned palate rugae, misshapen palate rugae, dilated lateral and/or 3rd ventricle (generally reported as severe), dilated all ventricles, diaphragmatic hernia, interrupted aortic arch, retroesophageal aortic arch, right-sided aortic arch, double aorta, malpositioned posterior vena cava, malpositioned subclavian artery, and retroesophageal subclavian artery. Skeletal malformations were listed by the sponsor as follows: "vertebral defects (scoliosis, absent, misshapen and/or fused thoracic and/or cervical arches, misaligned thoracic centra, supernumerary vertebrae, lumbar or thoracic hemivertebrae), rib defects (supernumerary and intercostal ribs, fused or detached ribs), sternebrae defects (bifid or fused), shoulder girdle defects (misshapen scapula or spina scapula or spina scapula), skull defects (misshapen zygomatic, squamosal, premaxilla, palatine, nasal, maxilla, exoccipital, hole

in parietal, fused mandible, fused frontal and parietal, fused exoccipital and 1st cervical arch, and multiple cranial/facial abnormalities)."

In dams dosed with teriflunomide during GD 6-8, there was also an increase in visceral and skeletal malformations but to a less extent than in dams dosed during GD 6-17. No external malformations were detected in this group. Visceral malformations were detected in two fetuses, one exhibiting severe dilated lateral ventricles and the other ventricular septal defect. Skeletal malformations were listed by the sponsor as follows: "vertebral defects (absent lumbar or cervical arch, supernumerary thoracic vertebrae, fused thoracic or cervical arch, and misshapen thoracic arch), and rib defects (absent, intercostal, branched, fused, detached, and short rib)." All fetuses examined for skeletal findings exhibited multiple malformations.

There was a slight increase in malformations in dams dosed during GD 12-14 or 15-17. In dams dosed during GD 12-14, there was only a single fetus with one malformation (short rib). In those dosed during GD 15-17, there were 3 affected fetuses from 3 different litters; two fetuses exhibited one malformation (bilateral [partial] open eye, misshapen zogomatic bone) and one had a bent ulna and a bent radius.

Rabbit. In the EFD study in rabbit, teriflunomide was administered at oral doses of 0, 1, 3.5, and 12 mg/kg/day throughout the period of organogenesis (GD 6-18). Embryolethality was evident, with post-implantation loss (early resorptions) increased at the high dose, resulting in a decrease in the number of live fetuses at that dose. Fetal body weight and crown-to-rump length were reduced at the mid and high doses. Malformations were also increased primarily at the mid and high doses. The number of fetuses/litters examined (for external, visceral, and skeletal findings) and affected per group was as follows:

- Fetuses/litters examined:
  - control: 99/16; low dose: 137/19, mid dose: 123/18; high dose: 73/16.
- Affected fetuses/litters (% fetuses/% litters)
  - control: 7/5 (7%/31%); low dose: 8/7 (6%/37%); mid dose: 18/10 (15%/56%); high dose: 29/11 (40%/69%).

The majority of fetuses displayed numerous malformations, which included (but were not limited to) malformations of the forepaws (digits: malrotated, small, brachydactyly; phalanx anomalies) and forelimbs (misshapen, short, absent radius), fused sternebrae, cleft lip/palate, malformations of the trunk (short, umbilical hernia, gastroschisis), kinked tail, skull malformations, small orbit, malformations of the pelvic girdle (misshapen ileum, malpositioned or misshapen ischium, misshapen pubis), microphthalmia, small lens, microcardia, small lung, absent kidney, adrenal gland, and/or absent ureter.

Increases in "minor" visceral and skeletal morphological changes and delayed ossification of various skeletal structures were also observed in mid and high-dose fetuses. The low dose is identified as the NOAEL for embryofetal toxicity (including

teratogenicity) in rabbit. The plasma C_{max} and $AUC_{(0\text{-}24\ hr)}$ at this dose were 5.59 µg/mL and 59.8 µg*hr/mL, respectively.

Pre- and postnatal development: Doses for the pivotal study were selected based on results from preliminary dose-ranging study in which teriflunomide was administered to Sprague-Dawley rats (6/group) at oral doses of 0, 0.3, 0.6, and 1.0 mg/kg/day from GD 6 through lactation day (LD) 13. Severe toxicity (clinical signs [including external malformations] and/or body weight loss) and death (4.5 and 52.8%, respectively) resulted in termination of pups in the mid- and high-dose groups on LD 8-9. Selected pups were examined for skeletal findings. Clinical observations, observed at all doses in dose-related manner, were listed as follows by the sponsor: "malrotated digits on the forepaws/hindpaws, malrotated forepaws/hindpaws, white discoloration of forepaws/hindpaws, short digits of hindpaws, cool to touch, no milk in stomach, wavy tail, umbilical hernia, pale body, white discoloration of abdomen, back, or face." The skeletal findings were reported in these groups: "thickened radius, thickened ulna, incomplete ossification of cuneiform, incomplete ossification of cuboid, thickened fibula, unossified metatarsal epiphysis, short fibula, and misaligned caudal centra vertebra." An additional group was administered 1.0 mg/kg/day during GD 6-20 in order to assess postnatal effects of only prenatal teriflunomide exposure; no clinical signs were reported in these pups. It is difficult to understand the lack of clinical signs in this group since in the high-dose pups of dams dosed throughout gestation and lactation, clinical signs (and correlated skeletal findings in selected pups), clearly due to dosing during gestation, were evident.

In the pivotal study, teriflunomide was administered to Sprague-Dawley rats at oral doses of 0, 0.05, 0.10, and 0.30 mg/kg/day from GD 6 through LD 20. These doses are substantially lower than those tested in the fertility and EFD studies (and the preliminary pre/postnatal study) in rat. There were no drug-related effects on pre- or post-implantation loss or the number of live pups at birth or during lactation; body weight tended to be lower in high-dose pups (4-7%). Physical development (preputial separation, vaginal opening) was unaffected. Motor activity, startle reflex and habituation, and passive avoidance parameters were not consistently affected in either male or female pups. Drug-related clinical signs were noted primarily in pups of high-dose dams, and consisted of malrotated forepaws/hindpaws (30 affected pups from 12 litters), light brown discoloration of the entire body surface (9 affected pups from 3 litters), impaired coat growth (38 affected fetuses from 10 litters), eye opacity (2 affected pups from 2 litter), and eye discharge (2 affected pups [one also had eye opacity] from 2 litters). Pupillary reflex was absent in the pups with eye opacity and/or discharge.

In pups selected for assessment of reproductive function, there was a slight delay in time to mate in pups of mid- and high-dose dams but no differences in pregnancy or fertility parameters.

Based on these data, the mid-dose is the NOAEL for developmental toxicity, as assessed in this study. TK data are not available for this dose. Assuming linearity, and based on

TK data at 1.0 mg/kg, C_{max} and AUC at 0.1 mg/kg are estimated to be approximately 1 µg/mL and 11 µg*hr/mL, respectively.

Conclusion

Based on the reproductive and developmental studies conducted by the sponsor, teriflunomide was clearly embryolethal and teratogenic in both rat and rabbit and increased offspring mortality in rat at plasma exposures substantially lower than that anticipated in humans. In addition to the data on teriflunomide, the findings of reproductive and developmental toxicity studies of leflunomide are also relevant since, as noted previously, leflunomide is essentially a prodrug for teriflunomide. According to the approved labeling for Arava, leflunomide was also teratogenic in rat and rabbit dosed throughout the period of organogenesis at plasma exposures (AUC) less than or similar to, respectively, the plasma AUC anticipated in humans; leflunomide also increased offspring mortality in rats when dosed throughout gestation and continuing to the end of lactation at plasma AUCs substantially lower than the maximum plasma AUC anticipated in humans. Arava is designated a Pregnancy Category X for treatment of rheumatoid arthritis. (b) (4).

In published studies, leflunomide was reported to be teratogenic when administered orally to CD-1 mice at oral doses of 0, 10, 30, or 70 mg/kg/day during GD 6-15 (Fukushima R *et al*. *Reprod Toxicol* 24:310-316, 2007). Increases in post-implantation loss (resorptions) were observed at 30 and 70 mg/kg/day, with 100% post-implantation loss at the high dose; fetal body weight was reduced at 30 mg/kg/day. An increase in external, visceral, and skeletal malformations was observed at 30 mg/kg/day, with 100% of litters having fetuses with malformations and 77% of fetuses with malformations. The most common external malformations observed were exencephaly, cleft palate, open eye, and short and kinked tail; others reported included anasarca, misshapen or protruding head, misshapen face, microtia, exophthalmos, extrodactyly, brachydactyly, polydactyly, snydactyly, anal atresia, and short lower trunk. Multiple skeletal and visceral malformations were also reported. The authors reported a "high" frequency of malformations of the heart and great vessels ("persistent atrioventricular canal, membranous ventricular septum defect, transposed great vessels and persistent truncus arteriosus") and head ("misshapen cerebellum, cerebrum, rhinencephalon and absent rhinencephalon"). In a subsequent publication, Fukushima *et al*. (Fukushima R *et al*. *Toxicol Sci* 108(2):419-426, 2009) reported partial attenuation of leflunomide-induced teratogenicity in the CD-1 mouse when animals were treated with 2 or 4 doses of uridine (1000 mg/kg IP) at various time (0.5-24 hrs) following a single oral 70-mg/kg dose of leflunomide on GD 10, suggesting that inhibition of DHO-DH is at least partly responsible for the developmental toxicity (including teratogenicity) of leflunomide and, therefore, teriflunomide. However, since inhibition of DHO-DH is the mechanism by which teriflunomide is proposed to have therapeutic effects in MS patients, these data do not ameliorate concern regarding the teratogenic potential of teriflunomide.

A number of publications report pregnancy outcomes in pregnant women exposed to leflunomide. Neville and McNally (Neville CE, McNally J *Rheumatology* 46:1506, 2007

claimed to report the first case of congenital malformations (cerebral palsy with blindness in one eye), in the offspring of a women exposed to leflunomide for at least 8 months prior to becoming pregnant and continuing until at least 21 week of gestation. Chambers *et al*. (Chambers A *et al. Arthritis Rheum* 62(5):1494-1503, 2010) reviewed pregnancy outcomes in 64 women with rheumatoid arthritis (RA) exposed to leflunomide during the first trimester, compared to outcomes in 108 pregnant women with RA not exposed to leflunomide and in 78 healthy pregnant women. The authors note that 95.3% of leflunomide-exposed women underwent at least "one course of the cholestyramine washout procedure early in pregnancy immediately following discontinuation of leflunomide" and 18.8% reportedly receive more than 1 course (range: 2-6) of cholestyramine. Plasma data were available in 31 women, and indicated that teriflunomide levels <0.02 μg/mL were achieved at 5-19 weeks (mean ± SD = 10.7 ± 4.4 weeks) after conception. There were no significant differences in the rate of malformations among groups. The authors conclude that "…the data from this study suggest that if leflunomide is a human teratogen, the risks are not high, i.e., on the order of the ≥20% risks seen with some other category X medications such as isotretinoin and thalidomide." (Teriflunomide is a structurally related to thalidomide.) It is difficult to understand the basis for this conclusion, considering the very small amount of data and the fact that major of pregnant women exposed to leflunomide underwent the elimination procedure to rapidly lower plasma levels of teriflunomide. As the authors themselves note, "The findings of this study can be reassuring to women who inadvertently become pregnant while taking this medication and who undergo the recommended cholestyramine washout procedure."

A recent publication (Cassina M *et al*. *Arthritis Rheum* 64(7):2085-2094, 2012) provides pregnancy outcome data in 45 women, 16 of whom were exposed to leflunomide during pregnancy and 29 of whom were exposed only prior to conception. Spontaneous abortion occurred in only 2 women, both exposed prior to conception. Of the 15 live births, 2 exhibited major malformations: one (a twin) with aplasia cutis congenital and another with multiple malformations ("Pierre-Robin sequence, spina bifida occulta, patent ductus arteriosus, chondrodysplasia punctuate, and congenital heart block"); confounding factors were cited in both cases. The authors state that the data "…have not demonstrated an increase in the rate of major malformations or a specific pattern of major malformations…" in humans and that "These data provide additional reassurance that leflunomide is not a major human teratogen in women who inadvertently become pregnant while taking leflunomide *and who undergo the washout procedure*" (italics added). They do state, however, that the data are not conclusive.

There is also at least one published report of pregnancy outcomes in pregnant women with MS enrolled in clinical trials of teriflunomide (O'Connor PW *et al. NEJM* 365:1293-1303, 2011); however, the available pregnancy data for teriflunomide (as of June 1, 2011) have been reviewed by Drs. Villalba and Mentari (*cf. Clinical Review NDA 202992, Lourdes Villalba, M.D., Evelyn Mentari, M.D., 7/12/2012*). According to their review, 57 pregnancies were reported, involving a treated pregnant female (45) or a female partner of a treated male patient (12). Of the 10 females who delivered live offspring, 8 had undergone a rapid elimination procedure (cholestyramine or activated

charcoal); the actual extent of teriflunomide exposure during pregnancy was unclear. Of the 12 female pregnant partners of treated male patients, 8 delivered live offspring. No malformations were detected in any of the 18 newborns.

The sponsor states that the available human data suggest a lack of reproductive or developmental toxicity in pregnant women exposed to leflunomide or teriflunomide, and that the relevance of the reproductive and developmental finding in rats to humans "is uncertain" since, based on *in vitro* data, "…rats are 145 times more sensitive to the anti-proliferative effects of teriflunomide than humans." Other *in vitro* data suggest less of a difference between rat and human (Merrill JE *et al*., 2009). Although the rat appears to be somewhat more sensitive than the rabbit to the developmental effects of teriflunomide, the NOAEL in the rabbit provides no safety margin based on plasma exposure comparisons, and the difference in sensitivity (in terms of *in vitro* anti-proliferative effects) between rabbit and human is unknown. More importantly, the sensitivity of the developing organism to the anti-proliferative effects of teriflunomide is unknown. Based on a review of nonclinical and clinical data for leflunomide, Brent (Brent RL *Teratology* 63:106-112, 2001) concluded that "…since it cannot be determined whether the human embryo or fetus is more sensitive than the adult, these species difference and possible consequences on safety margins cannot be considered for estimating the reproductive risks of leflunomide in humans."

The sponsor indicated that species differences in potency were not taken into consideration in setting a "no-teratogenic risk" plasma level (b) (4), i.e., the trough level corresponding to $1/10^{th}$ the plasma AUC (59.8 µg*hr/mL) at the NOAEL in rabbit, considered by sponsor to be the more sensitive species. Women of childbearing potential who wish to become pregnant are to continue effective contraception until plasma levels of teriflunomide fall to or below (b) (4) Alternatively, plasma levels of teriflunomide may be reduced rapidly to that level through use of a "rapid elimination procedure" such as use of cholestyramine or activated charcoal, which has been demonstrated to reduce the $t_{1/2}$ of teriflunomide from approximately 19 to 2-3 days.

This "no-teratogenic risk" level is higher than that recommended in labeling for Arava. Labeling for Arava states that "It is possible that rapidly lowering the blood level of the active metabolite by instituting the drug elimination procedure…at the first delay of menses may decrease the risk to the fetus from ARAVA." That procedure involves administration of cholestyramine (8 grams 3 times daily) for 11 days, to achieve plasma levels to undetectable levels (<0.02 µg/mL). According to Brent (2001), a teriflunomide blood level of 0.03 µg/mL is 123 times that in rat and 136 times that in rabbit at the no-effect doses for teratogenicity and embryolethality. The sponsor's higher no-risk level would provide only a 10-fold margin compared to the trough level determined from plasma AUC at the NOAEL in the rabbit. A similar calculation based on the estimated plasma AUC in rat at the NOAEL in the pre- and postnatal study of teriflunomide would provide a "no-teratogenic risk" level of 0.05 µg/mL.

Teriflunomide is a multi-species teratogen (in rat and rabbit, and when given as leflunomide in mouse) that produces external, skeletal, and/or visceral malformations, in

the absence of maternal toxicity, in rat and rabbit at substantially lower plasma exposures than anticipated in humans at the maximum therapeutic dose. In addition, the mechanism through which teriflunomide is presumed to exert therapeutic effects in MS patients (i.e., inhibition of de novo pyrimidine synthesis, particularly in rapidly dividing tissues such as the developing organism) is the same mechanism considered responsible for its developmental toxicity (including teratogenic effects). Therefore, teriflunomide must be presumed to have the potential to increase the risk of adverse developmental effects (including teratogenicity) in humans when used at the recommended clinical doses. (b) (4) It would seem advisable to lower plasma levels in humans to or below the limit of detection, as recommended for Arava.

Genetic Toxicology

The sponsor conducted a standard battery of genetic toxicology studies for teriflunomide: *in vitro* Ames assay, *in vitro* chromosomal aberration assay in human lymphocytes, *in vivo* micronucleus assay in mouse. Additional genetic toxicology studies were also conducted in order to further investigate the positive responses obtained in the *in vitro* chromosomal aberration assay in human lymphocytes (Study MAF0073): *in vitro* HPRT assay in V79 cells, *in vivo* chromosomal aberration assay in Chinese hamsters, 14-day repeat dose oral chromosomal aberration assay in rat.

Teriflunomide was negative in the *in vitro* Ames and the HPRT assays and the *in vivo* micronucleus and chromosomal aberration assays. Teriflunomide (tested at concentrations up to 500 µg/mL) was, however, reproducibly positive in the *in vitro* chromosomal aberration assay in human lymphocytes in the absence of metabolic activation with 3-hr treatment, in a concentration-dependent manner and at concentrations not associated with excessive cytotoxicity. (Teriflunomide was negative in the absence of metabolic activation with 20-hr treatment; however, substantially lower concentrations were tested, i.e., ≤75 µg/mL). Teriflunomide was also positive in the presence of metabolic activation with 3-hr treatment.

The sponsor conducted additional studies to investigate the possibility that the positive responses obtained might be mediated by DHO-DH inhibition by teriflunomide, citing literature reporting that nucleotide pool imbalance and inhibition of DNA synthesis may damage DNA through an indirect mechanism. Addition of uridine (500 µM) decreased both the cytotoxicity and the number of cells with structural chromosomal aberrations; increasing the concentration of uridine (to 1000 µM) had no additional effect. However, significant increases in chromosomal aberrations were still observed in the presence of uridine, suggesting that the clastogenic effect of teriflunomide in this assay may only partially be mediated by DHO-DH inhibition.

Overall, these data suggest that teriflunomide may have clastogenic potential, and the findings should be described in labeling. (Several *in vivo* clastogenicity assays were conducted, but each evaluated only bone marrow.) However, concerns regarding

genotoxic potential are mitigated by the negative results of the 2-year carcinogenicity studies, although plasma exposures similar to that anticipated in humans at the MRHD could not be achieved in the rat.

According to the approved labeling for Arava, leflunomide was negative in *in vitro* (Ames, UDS, and HPRT gene mutation) and *in vivo* (micronucleus assay in mouse and cytogenic test in Chinese hamster bone marrow cells) assays. Labeling also discusses the results of genetic toxicology studies of the minor metabolite, 4-TFMA (b) (4) ; the sponsor has included these results in the proposed labeling for teriflunomide.

Carcinogenicity

Two-year oral (gavage) carcinogenicity studies of teriflunomide were conducted in CD-1 mouse (Study CAR0092) and Sprague-Dawley rat (Study CAR0093). (Non-neoplastic findings are discussed under the General Toxicology section.)

Mouse: Teriflunomide (in 2% potato starch) was tested in CD-1 mice (60/sex/group) at oral (gavage) doses of 0 (vehicle), 0 (vehicle), 0 (deionized water), 1, 4, and 12 mg/kg/day. Additional animals were used for toxicokinetic analysis. Excessive deaths in high-dose males resulted in cessation of dosing in this group during Week 96; survivors were maintained on study until scheduled sacrifice (Week 104). The mortality rate in high-dose females was also significantly increased; however, dosing was continued in survivors to scheduled sacrifice. The study was considered adequate and negative for drug-related tumors (*cf. Executive CAC Meeting Minutes, 2/23/2012*). Plasma data for teriflunomide and 4-TFMA (minor metabolite (b) (4)) are summarized in the following table (doses are in mg/kg/day; plasma data are in µg*hr/mL for teriflunomide and ng*hr/mL or ng/mL for 4-TFMA; n.c. = not able to calculate):

STUDY DAY	PARAMETER	MALES			FEMALES		
		1	4	12	1	4	12
TERIFLUNOMIDE							
29	$AUC_{(0-24)}$	162	1020	3600	184	828	3120
4-TFMA							
29	$AUC_{(0-24\ hr)}$	n.c.	n.c.	304	n.c.	23.1	301
	C_2	n.c.	n.c.	17.1	n.c.	1.30	18.3
	$C_{24\ hr}$	n.c.	n.c.	8.19	n.c.	n.c.	7.97
85	C_2	n.c.	2.82	18.4	n.c.	2.67	10.4
	$C_{24\ hr}$	n.c.	3.53	10.5	n.c.	1.39	7.81
176	C_2	n.c.	n.c.	6.99	n.c.	n.c.	8.73
	$C_{24\ hr}$	n.c.	n.c.	4.25	n.c.	n.c.	4.57

In humans, at the MRHD of 14 mg/day, the mean plasma $AUC_{(0-24\ hr\ SS)}$ was 1070 µg*hr/mL (median = 885 µg*hr/mL, 5^{th}-95^{th} percentiles = 353-2130 µg*hr/mL) (*cf. Clinical Pharmacology Review NDA 202-992 Veneeta Tandon, Ph.D., Joo-Yeon Lee, Ph.D., Jeffrey Kraft, Ph.D. 7/3/2012, Table, page 19*). Therefore, teriflunomide was negative in the 2-year study at plasma exposures up to approximately 3 times that anticipated in humans at the MRHD.

For NDA 20-905 (Arava; leflunomide), leflunomide was tested in a 2-year carcinogenicity study in CD-1 mice at oral (gavage) doses of 0, 0, 1.5, 5, and 15 mg/kg/day (vehicle: starch mucilage). The mortality rate was increased in high-dose males, but survivors were dosed up to scheduled sacrifice. According to approved labeling for Arava, drug-related findings in mouse consisted of an increase in the incidence of lymphoma in high-dose males (3, 5, 2, 4, and 12 at 0, 0, 1.5, 5, and 15 mg/kg/day, respectively; trend test p-value: 0.0017) and dose-related increases in the incidence of bronchoalveolar adenomas and carcinomas at all doses in females (3, 1, 7, 9, 15 at 0, 0, 1.5, 5, and 15 mg/kg/day, respectively; trend test p-value: 0.0025) (*cf. Statistical Review and Evaluation (Carcinogenicity Review) NDA #20-905, Baldeo K. Taneja, Ph.D. 6/22/98*). Plasma data for teriflunomide and 4-TFMA are summarized in the following table (doses are in mg/kg/day; plasma data are in µg*hr/mL for teriflunomide and ng/mL for 4-TFMA; n.d. = not detected):

STUDY DAY	PARAMETER	MALES			FEMALES		
		1.5	5	15	1.5	5	15
TERIFLUNOMIDE							
724	$AUC_{(0\text{-}24)}$	167.2	895.2	2688	143.9	698.4	2072
4-TFMA							
373	C_2	n.d.	24.4	108.6	n.d.	31.3	151
	$C_{24\ hr}$	n.d.	n.d.	37.4	n.d.	17.9	63.9

Rat: Teriflunomide (in 2% potato starch) was tested in Sprague-Dawley rats (60/sex/group) at oral (gavage) doses of 0 (vehicle), 0 (vehicle), 0 (deionized water), 0.5, 1.5, and 4 mg/kg/day. Additional animals were used for toxicokinetic analysis. Excessive deaths in mid- and high-dose males resulted in termination of high-dose males after approximately 96 weeks of dosing) and termination of all male groups after approximately 97 weeks of dosing. Mortality rate was unaffected in females. The study was considered adequate and negative for drug-related tumors (*cf. Executive CAC Meeting Minutes, 2/23/2012*). Plasma data for teriflunomide and 4-TFMA are summarized in the following table (doses are in mg/kg/day; plasma data are in µg*hr/mL for teriflunomide and ng*hr/mL or ng/mL for 4-TFMA; n.c. = not able to calculate):

STUDY DAY	PARAMETER	MALES			FEMALES		
		0.5	1.5	4	0.5	1.5	4
TERIFLUNOMIDE							
169/170	$AUC_{(0\text{-}24)}$	51.4	104	275	60.3	148	329
4-TFMA							
169/170	$AUC_{(0\text{-}24)}$	n.c	n.c	20.0	n.c	n.c	21.0
	C_{max}	n.c	n.c	1.69	n.c	n.c	1.97

Therefore, teriflunomide was negative in the 2-year study, but at plasma exposures less than that anticipated in humans at the MRHD. Plasma $AUC_{(0\text{-}24\ hr)}$ values at the highest dose tested was approximately 0.3 times the anticipated maximum human plasma AUC.

For NDA 20-905 (Arava; leflunomide), leflunomide was tested in a 2-year carcinogenicity study in Wistar rats at oral (gavage) doses of 0, 0, 0.5, 1.25, 3, and 6 mg/kg/day (vehicle: 2% starch mucilage). The mortality rate was increased in high-dose males and females. Due to excessive mortality, high-dose male survivors were sacrificed early (during Week 84); all groups were dosed until scheduled sacrifice. According to approved labeling for Arava, there were no tumor findings considered drug-related. Plasma data for teriflunomide and 4-TFMA are summarized in the following table (doses are in mg/kg/day; plasma data are in µg*hr/mL for teriflunomide and ng*hr/mL or ng/mL for 4-TFMA; n.c. = not able to calculate):

STUDY DAY	PARAMETER	MALES				FEMALES			
		0.5	1.25	3	6	0.5	1.25	3	6
TERIFLUNOMIDE									
380/381	AUC	4.43	9.36	23.4	40.9	9.75	11.9	26.3	45.6
4-TFMA									
365	AUC	n.c.	n.c.	29.6	58.5	n.c.	20.8	47.3	87.6
	C_{max}	n.c.	n.c.	8.62	18.1	n.c.	6.0	12.9	28.6

Impurities

Three impurities were identified as having genotoxic potential, (b) (4) [redacted] The structures of these impurities are provided below (from the sponsor's submission):

(b) (4) [redacted]

The sponsor has proposed a specification limit of (b) (4) consistent with a daily dose of ≤1.5 µg/day. Therefore, concerns regarding the presence of (b) (4) as an impurity of teriflunomide have been adequately addressed.

An impurity that exceeds the qualification threshold should, typically, be assessed in a repeat dose toxicity study (up to 90-days in duration for a chronic use therapy) and an embryo-fetal development study (for an indication that includes women of childbearing potential), as well as for genotoxic potential. In addition to the genotoxicity studies, the sponsor conducted only 1-month toxicity studies and no embryo-fetal development study. However, considering the extent of human experience and the overwhelming evidence of the developmental toxicity of teriflunomide, the studies conducted for (b) (4) are sufficient to support the proposed specification limit.

(b) (4): The sponsor notes that an individual specification limit for (b) (4) is not set "since the level is below (b) (4)." However, a level of (b) (4) would result in a daily dose of (b) (4) which is above the acceptable daily dose of a genotoxic impurity. An Ames test was conducted on (b) (4) to address concerns regarding genotoxic potential. This study was adequate and negative for genotoxicity. Therefore, the presence of (b) (4) at or below the level stated by the sponsor is acceptable.

Conclusions and Recommendations

I concur with Dr. Houghtling's conclusion that the nonclinical data submitted to NDA 202-992 support marketing approval. I also concur with Dr. Houghtling's conclusion that teriflunomide is teratogenic (in multiple species), and must be considered a potential human teratogen. However, a decision regarding the appropriate pregnancy category cannot be made based solely on nonclinical data; it is ultimately a clinical decision. Therefore, labeling recommendations will be provided in a separate memo, once this issue has been resolved.

In the case of inadvertent pregnancy, it is recommended that a rapid elimination procedure such as the one described in labeling for Arava be followed. (b) (4)

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/s/

LOIS M FREED
07/20/2012

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	202-992
Supporting document/s:	001
Applicant's letter date:	August 12, 2011
CDER stamp date:	August 12, 2011
Product:	Teriflunomide
Indication:	Relapsing-remitting Multiple sclerosis
Applicant:	Sanofi Aventis US LLC
Review Division:	Neurology
Reviewer:	Rick Houghtling, Ph.D.
Supervisor:	Lois M. Freed, Ph.D.
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Project Manager:	Hamet M. Toure, Pharm.D.

Disclaimer

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1 Executive Summary

1.1 Introduction

Teriflunomide (AUBAGIO) is a dihydroorotate dehydrogenase inhibitor that has been submitted as a treatment for relapsing-remitting multiple sclerosis. Teriflunomide is the active metabolite of an approved drug, leflunomide (ARAVA®), which was approved as a treatment for rheumatoid arthritis. In the present application, the sponsor has submitted a complete and adequate battery of nonclinical studies necessary to evaluate and determine the nonclinical safety profile of teriflunomide.

1.2 Brief Discussion of Nonclinical Findings

Teriflunomide is an immunosuppressive drug that inhibits dihydroorotate dehydrogenase, an enzyme required for *de novo* pyrimidine synthesis, which is utilized in proliferating cells. Thus, the toxicities associated with repeated teriflunomide exposure occur predominantly in organs and systems that actively proliferate, such as the bone marrow (hematopoietic system), lymph nodes (immune system), pancreas, and the developing fetus.

Repeated administration of teriflunomide in rodent and nonrodent species resulted in significant toxicities. The 6-month Sprague Dawley rat and one-year Beagle dog studies identified several primary target organs including, the bone marrow (rat and dog), immune system organs—in the rat, included lymph node, gastrointestinal associated lymphoid tissue (GALT), and spleen and in the dog, included the GALT and lymph node, and the pancreas (dog). In the reproductive and developmental toxicity battery, several notable findings were attributed to teriflunomide that may have significant consequences for the developing fetus. In the embryofetal development studies conducted in rat and rabbit, teriflunomide was identified as a teratogen resulting in several external, skeletal, and/or visceral malformations that were dose-related. Additionally, at the higher doses tested, teriflunomide was embryo-lethal.

The sponsor conducted two-year carcinogenicity bioassays in mouse and in rat; and, each was considered adequate. The doses evaluated were in agreement with those recommended by the Executive CAC. Although significant toxicities were noted in these studies, there were no neoplasms attributed to teriflunomide treatment in either mouse or rat.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical perspective, the application is approvable; the studies submitted and reviewed were adequate to assess the nonclinical safety profile of teriflunomide, its metabolite, and impurities.

1.3.2 Additional Non Clinical Recommendations

Based upon the significant reproductive toxicities associated with administration of teriflunomide during organogenesis, its association with increased embryo-lethality, increased post-implantation loss, and evidence of teratogenicity in two toxicological species at the MRHD based on AUC warrants that the pregnancy category (b) (4) should be (b) (4) X, and contraindicated in pregnant women. The rationale for this recommendation is that in animal studies, treatment with teriflunomide caused fetal harm; and, there are other safer forms of therapeutic modalities presently on the market.

1.3.3 Labeling

The following corrections to the sponsor-provided labeling are recommended based upon the data submitted for review.

Contraindications

Teriflunomide (AUBAGIO) may cause fetal harm when administered to a pregnant woman.

8. USE IN SPECIFIC POPULATIONS
8.1 Pregnancy

(b) (4)

(b) (4)

The above statement regarding genotoxicity should not be present in this section of the label.

Pregnancy Registry

(b) (4)

1 Page of Draft Labeling has been Withheld in Full Immediately Following this Page.

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number

- 163451-81-8

Trade Name

- AUBAGIO®

Generic Name

- Teriflunomide

Code Name

- HMR1726, A7771726

Chemical Name

- 2-Butenamide, 2-cyano-3-hydroxy-N-(4-trifluoromethyl)phenyl-(2Z)-
- (Z)-2-cyano-3-hydroxy-but-2-enoic acid-(4-trifluoromethyl-phenyl)amide

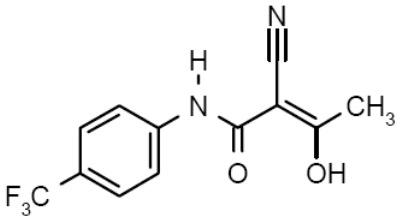
Molecular Formula/Molecular Weight

- $C_{12}H_9F_3N_2O_2$
- 270.21

Stereochemistry

- Teriflunomide is the (Z) diastereomer

Structure or Biochemical Description



Pharmacologic Class

- Pyrimidine synthesis inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND 67,476 Treatment of Multiple Sclerosis, DNP, Active

2.3 Drug Formulation

- Teriflunomide is formulated as an immediate release film-coated tablet in two dosage strengths, 7 and 14 mg. The composition of the 7 and 14 mg tablets is listed below in sponsor's Tables 1 and 2.

Table 1 - Composition of the 7 mg dosage strength

Components [a]	Composition		Function	Reference to standards [b]
	Percentage [%]	Per unit (1 film-coated tablet) [mg]		
Tablet core				
Teriflunomide	(b) (4)	7.0	Drug substance	In-house
Lactose monohydrate		(b) (4)		Ph. Eur., NF
Maize starch [Corn starch]				Ph. Eur., NF
Hydroxypropylcellulose				Ph. Eur., NF
Microcrystalline cellulose				Ph. Eur., NF
Sodium starch glycolate (b) (4) [Sodium starch glycolate]				Ph. Eur., NF
Magnesium stearate				Ph. Eur., NF
(b) (4)				
Film-coating				In-house [c]
Hypromellose [d]				Ph. Eur., USP
Titanium dioxide (b) (4) (b) (4)				Ph. Eur., USP
Talc [d]				Ph. Eur., USP
Macrogol [d] [Polyethylene glycol]				Ph. Eur., NF
Indigo carmine aluminium lake (b) (4) [FD&C Blue #2], (b) (4)				EC directive 2008/128, CFR 82.51 and 82.102
Ferric oxide [d] Iron oxide yellow (b) (4) (b) (4)				NF EC directive 2008/128, CFR 73.1200
(b) (4)				
Mass of film-coated tablet	100	155.0		
(b) (4)				

a Components are listed according to their pharmacopoeial names. If more than one monograph exists, other names are given in brackets, along with the compendial reference.

b Reference is made to the current edition of the Pharmacopoeia.

c (b) (4)

d

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Table 2 - Composition of the 14 mg dosage strength

Components [a]	Composition		Function	Reference to standards [b]
	Percentage [%]	Per unit (1 film-coated tablet) [mg]		
Tablet core				
Teriflunomide	(b) (4)	14.0	Drug substance	In-house
Lactose monohydrate		(b) (4)		Ph. Eur., NF
Maize starch [Corn starch]				Ph. Eur., NF
Hydroxypropylcellulose				Ph. Eur., NF
Microcrystalline cellulose				Ph. Eur., NF
Sodium starch glycolate (b) (4) [Sodium starch glycolate]				Ph. Eur., NF
Magnesium stearate				Ph. Eur., NF
(b) (4)				
Film-coating				In-house [c]
Hypromellose [d]				Ph. Eur., USP
Titanium dioxide (b) (4) (b) (4)				Ph. Eur., USP
Talc [d]				Ph. Eur., USP
Macrogol [d] [Polyethylene glycol]				Ph. Eur., NF
Indigo carmine aluminum lake (b) (4) [FD&C Blue #2], (b) (4)				EC directive 2008/128, CFR 82.51 and 82.102
(b) (4)				
Mass of film-coated tablet	100	155.0		
(b) (4)				

a Components are listed according to their pharmacopoeial names. If more than one monograph exists, other names are given in brackets, along with the compendial reference.
b Reference is made to the current edition of the Pharmacopoeia.
c (b) (4)
d

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2.4 Comments on Novel Excipients

- There are no novel excipients in the drug product.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance

- Three drug substance impurities/degradants of teriflunomide have been identified (b) (4)

Table 21 - Impurities (b) (4)

Chemical structure	Source of impurity
(b) (4)	

Drug Product

- No drug product film-coated tablet impurity/degradant was identified that exceeds that described for the drug substance.

2.6 Proposed Clinical Population and Dosing Regimen

- Multiple sclerosis patients, 14 mg tablet administered orally, once daily

2.7 Regulatory Background

3 Studies Submitted

3.1 Studies Reviewed

The following sponsor table lists the studies submitted and reviewed.

Table 2 - Repeat-dose toxicity studies using teriflunomide

Species	Study Reference	Type of study (duration/route)	Dosage (mg/kg/day)	Effect level	Plasma levels at Effect	
					AUC (µg.h/mL)	C_{max} (µg/mL)
Mouse	[2004-0511]	3 Months, PO	5, 25, 50 or 75	MTD[C]: <25 mg/kg/day	M: <4520 F: <4880	M: <234 F: <263
Rat	[013216], [016607], [016420]	1 Month, IV	3.2, 8, or 20	NOAEL not determined	ND	ND
	[013557], [015156], [016608]	1 Month, IV	0.25 or 1	NOAEL: 0.25 mg/kg/day	M: 3.45 F: 4.06	M: 1.07 F: 1.40
	[017716], [018057]	3 Months, PO	0.5, 1 or 4	NOAEL: 1 mg/kg/day	M: 71.4 F: 69.7	M: 4.7 F: 5.7
	[2003-1492]	6 Months, PO	0.3, 1.5/9 [a], 3 or 6	NOAEL: 0.3 mg/kg/day	M: 26.1 F: 29.4	M: 1.53 F: 1.75
Dog	[013400], [014960], [016612]	1 Month, IV	0.8, 2.5 or 8	NOAEL: 2.5 mg/kg/day	M: 423 F: 558	M: 28.8 F: 37.5
	[017737], [017737A1], [017737A2]	3 Months, PO	0.8, 2.5 or 8	NOAEL: 0.8 mg/kg/day	M: 107 F: 118	M: 6.4 F: 6.7
	[2003-1491]	12 Months, PO	0.2, 0.8 or 2/4 [b]	NOAEL: 0.2 mg/kg/day	M: 26.6 F: 20.2	M: 1.54 F: 1.39

Abbreviations: MTD = maximum tolerated dose; ND = Not determined; PO = per os (oral), IV = intravenous, NOAEL = No-observed-adverse-effect-level, M = Male, F = female, AUC = Area under the curve, C_{max} = maximum concentration
Note: Only plasma levels at the effect level are provided here.
Additional information: Values are rounded to 3 significant values or less.

[a] The 1.5 mg/kg/day dose was increased to 9 mg/kg/day after 107 days due to an apparent lack of toxicity at the 6 mg/kg/day dose.

[b] The 2 mg/kg/day dose was increased to 4 mg/kg/day after 191 days due to an apparent lack of toxicity at the 2 mg/kg/day dose.

[c] NOAEL was not within the objectives of the study. Study purpose was to determine the MTD.

Table 11 - Reproductive and developmental toxicity using teriflunomide

Species [study reference]	Type of Study	Dosage (mg/kg/day)	NOAEL
Rat [2003-1493]	Male fertility	0, 1, 3 or 10	Paternal NOAEL: 3 mg/kg/day Fertility/Reproductive performance NOAEL: 10 mg/kg/day
Rat [2003-1494]	Female fertility	0, 0.84, 2.6 or 8.6	Maternal NOAEL: 0.84 mg/kg/day Fertility NOAEL: 0.84 mg/kg/day Embryonic development NOAEL: < 0.84 mg/kg/day
Rat [F2002TOX0088]	Embryo-fetal development	0, 1, 3 or 10	Maternal NOAEL: 1 mg/kg/day Embryo-fetal development NOAEL: 1 mg/kg/day
Rat [DIV1364]	Toxicokinetics study in pregnant rats	0, 1, 3 or 10	N/A (not within the objectives of the study)
Rat [TER0631]	Embryo-fetal development (staged dosing)	0, 10	N/A (not within the objectives of the study)
Rabbit [TER0432]	Embryo-fetal development	0, 1, 3.5 or 12	Maternal NOAEL: 3.5 mg/kg/day Embryo-fetal development NOAEL: 1 mg/kg/day
Rat [DPP0029]	Exploratory pre- and postnatal developmental toxicity	0, 0.3, 0.6,1.0, or 1.0	N/A (not within the objectives of the study)
Rat [DPN0331]	Pre- and postnatal developmental toxicity	0, 0.05, 0.10, or 0.30	F0 pregnancy and parturition: 0.3 mg/kg/day F1 toxicity: 0.1 mg/kg/day

Abbreviations: NOAEL = No-observed-adverse-effect-level N/A: Not applicable

3.2 Studies Not Reviewed

The following study was not reviewed, Study No. 2004-1309, "HMR1726: Exploratory 5-Day Oral Toxicogenomics Study in Male Rats".

3.3 Previous Reviews Referenced

- IND 67,476 Teriflunomide for treatment of relapsing remitting multiple sclerosis Nonclinical Review of SDN #0126 and #0127 by David B. Hawver, Ph.D. 1/10/2008
- IND 67,476 Teriflunomide for treatment of relapsing remitting multiple sclerosis, Nonclinical Review of SDN#189 Rick A. Houghtling, Ph.D. 1/13/2009
- IND 67,476 Teriflunomide for treatment of relapsing remitting multiple sclerosis, Nonclinical Review of SDN#426 Rick A. Houghtling, Ph.D. 6/6/2011

4 Pharmacology

4.1 Primary Pharmacology

The sponsor submitted nine primary pharmacodynamic studies that are reviewed briefly, below.

1. Study No. PHM 1992-5905

Study Title: Test for inhibition of cellular proliferation by RS-61980-000 [A771726] using mitogen-stimulated cultures of murine splenic lymphocytes

(b) (4); Non-GLP, Initiated/Completed: 01/90; Report 01/92.

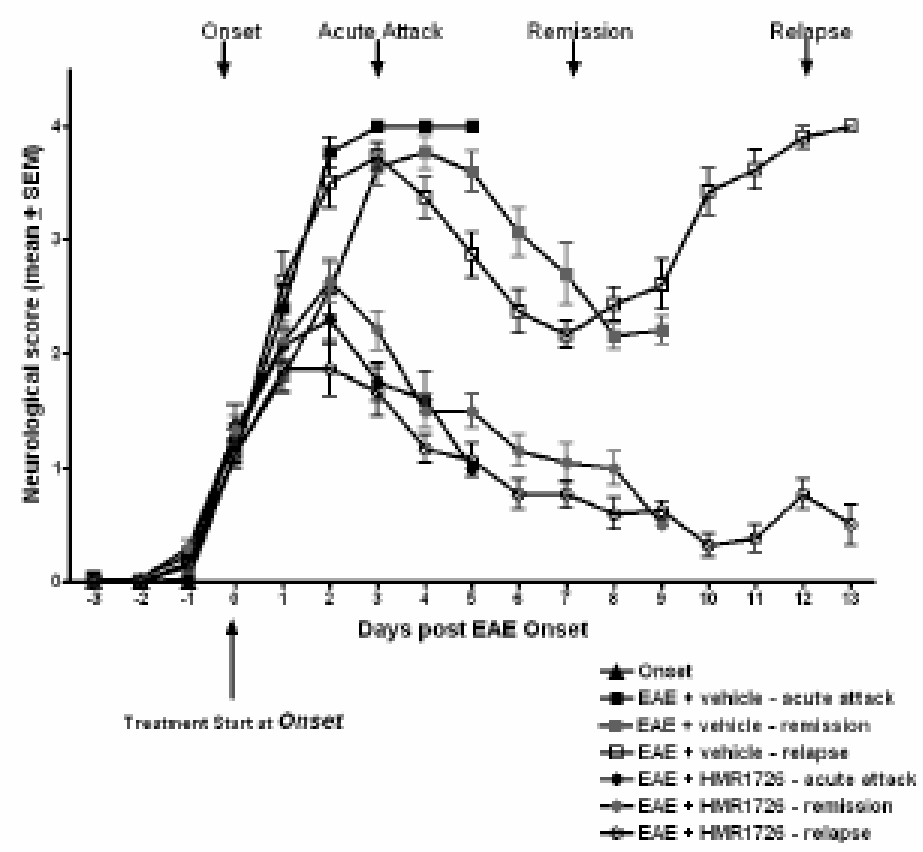
In this *in vitro* study, murine splenocytes were cultured in the presence of three different mitogens (lipopolysaccharide, phytohemagglutinin, and concanavalin A) and teriflunomide (0.1-100 µM). In the presence of an increasing concentration of teriflunomide, there was a decrease in the proliferative response to each of the tested mitogens. These data suggest that teriflunomide inhibits proliferation of splenic T and B cells.

2. Study No. IIVV0012

Study Title: The effects of therapeutic teriflunomide (HMR1726) on different immune cell populations in the DA rat EAE model

Sanofi-Aventis US, Inc., Bridgewater, NJ, USA; Initiated/Completed: Jan 2011/Feb 2011, Teriflunomide (batch no. 0500044553; 10 mg/mL formulated in a suspension of 6% carboxymethyl cellulose, 5% Tween 80, and deionized distilled water. DA rats (9-10 Weeks of age; 162-242 g) were administered teriflunomide (10 mg/kg) in a volume of 1 mL/kg by oral gavage. During various stages of EAE disease (i.e., disease onset, acute attack, remission, and relapse), splenic weight, immunocytes number, and distribution was determined in blood, cervical spinal cord, and spleen. Teriflunomide reduced the neurological symptoms observed in EAE-induced rats (see sponsor's Figure 1) at each disease stage; however, the greatest reduction occurred at relapse, with the lowest clinical scores observed during that stage. Disease-associated changes in spleen weight were attenuated in teriflunomide-treated animals at stages associated with acute attack, remission, and relapse. Disease-associated changes in immunocytes localized in whole blood, spleen, spinal cord, and PBMCs were identified.

Figure 1 - Effect of HMR1726 at 10 mg/kg on neurological scores in the DA rat model of EAE



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3. Study No. GVT0067

Study Title: Effect of (b) (4) on the proliferation of rat splenocytes

Sanofi-Aventis Research & Development, Bridgewater, NJ, USA; Initiated: 06/2007 Completed: 05/2008. Teriflunomide Batch no. W001

In this non-GLP study, the effect of teriflunomide on rat splenocyte proliferation in the presence of the mitogen, concanavalin A (ConA) was evaluated. Teriflunomide treatment (0.001 to 500 μM) of splenocytes cultures containing ConA resulted in the inhibition of proliferation in a concentration-dependent manner (IC_{50} = 109 nM). To determine if the mechanism of this inhibition by teriflunomide was mediated by its activity to inhibit de novo pyrimidine synthesis, uridine was added in culture. In the presence of uridine (30 μM), the IC_{50} value of teriflunomide shifted rightward, indicating that the inhibition of rat splenocytes proliferation was likely mediated by decreased de novo pyrimidine synthesis.

4. Study No. GVT0031

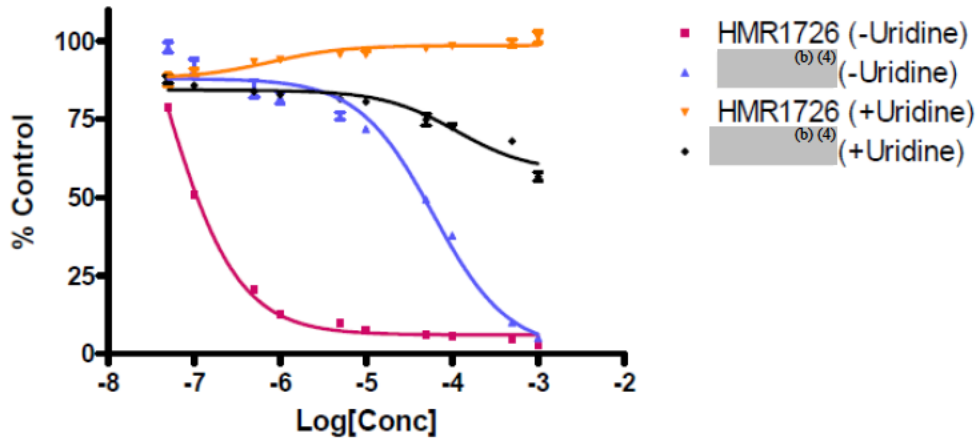
Study Title: Effect of [(b) (4)], an impurity in HMR1726, on the proliferation of rat spleen cells stimulated with concanavalin A

Sanofi-Aventis US, Inc., Bridgewater, NJ, USA; Initiated: June 2005; Completed: December 2005, [(b) (4)] (Batch No. C009), Teriflunomide (Batch No. W001)

In this non-GLP *in vitro* study, splenocytes were harvested from 11 Week old female SD rats and cultured with the mitogen ConA in the absence or presence of teriflunomide or [(b) (4)] (0.05-1000 μM). Splenocyte proliferation responses to ConA were reduced in a concentration-dependent manner by both teriflunomide and [(b) (4)]. The IC_{50} values were 102 nM for teriflunomide and 45.5 μM for [(b) (4)] suggesting teriflunomide was a more potent inhibitor of lymphocyte proliferation that the [(b) (4)] impurity. Exogenous addition of uridine to cultures rescued the teriflunomide-induced inhibition of splenocyte proliferation to ConA more so than it did in [(b) (4)] cells (see sponsor's Figure 3, below). These data suggest that the mechanism of both teriflunomide- and [(b) (4)] inhibition of splenocyte proliferation to ConA is mediated by its inhibition of dihydroorotate dehydrogenase (DHO-DH); however, the effect of [(b) (4)] is much less potent than that of teriflunomide.

Figure 3: Effects of uridine on splenocyte proliferation after HMR1726 and [(b) (4)] treatment.

Antiproliferative activities of [(b) (4)] and HMR 1726 mediated by DHODH activity was studied by the ability of exogenous uridine to restore cell proliferation. Shown below is a representative data from Experiment 3.



5. Study No. B2004res0061

Study Title: Effects of HMR1726D on Clinical Score in the SJL Mouse EAE Model: Oral dosing once daily from day 1 to 25 post-inoculation

Sanofi-Aventis US, Inc., Bridgewater, NJ USA; Initiated: 2004

In this non-GLP study, EAE was induced in SJL/J female mice (8 Weeks old) by immunizing mice with $PLP_{139-151}$ in complete Freund's adjuvant (CFA), heat-killed Mycobacterium Tuberculosis H37Ra, and pertussis toxin. Mice (28/group) were treated orally with vehicle, dexamethasone (5.6 mg/kg; positive control), or teriflunomide (10 or 20 mg/kg/day). As shown in sponsor's Table 1, teriflunomide = HMR1726D significantly increased the delay to disease onset and significantly reduced the maximal and cumulative clinical scores suggesting that teriflunomide may modify the course of EAE disease.

Table 1- EAE onset, maximal and cumulative scores

Group	N	Day of Disease Onset	Maximal Score (Mean±SEM)	Cumulative Score (Mean±SEM)
Vehicle	28	13.5	3.7 ± 0.2	27.4 ± 2.4
DEX (5.6 mg/kg)	28	N.A.	1.1 ± 0.3*	2.50 ± 0.8*
HMR1726 (10 mg/kg)	28	15.0	3.6 ± 0.2	27.2 ± 2.7
HMR1726 (20 mg/kg)	28	20.0*	2.5 ± 0.2*	13.3 ± 1.8*

Indicates that p-value is less than 0.05 in pair-wise comparison with vehicle

6. Study No. PHM1992-5901

Study Title: Test for Anti-Inflammatory or Immunomodulatory Activity of RS-61980-000 (A77 1726) Using Experimental Allergic Encephalomyelitis in the Rat

(b) (4); Non-GLP, Initiated: 05/86; Report 01/92. Female LEW/CrlBR rats (n=10/grp) were sensitized with an intradermal injection of syngeneic spinal cord homogenate emulsified in adjuvant resulting in the development of EAE (severe "hind end" paralysis) within 10-13 days following sensitization that recovered by days 18-20 after sensitization. Vehicle (0.9 % NaCl, 0.5% sodium carboxymethylcellulose, 0.4% polysorbate 80, 0.9% benzyl alcohol, 97.3% distilled water), teriflunomide (10 mg/kg/day), or dexamethasone (0.25 mg/kg/day; positive control) were administered on Day 0 following sensitization and daily for 17 days. As expected, vehicle-treated animals showed 100% paralysis by Day 18 and dexamethasone inhibited paralysis in all treated animals. Teriflunomide inhibited the development of paralysis in 9/10 treated rats suggesting it was almost as effective as dexamethasone at the dose tested (see sponsor's Table 1, below).

TABLE 1

THE PROTECTIVE EFFECT OF ORALLY ADMINISTERED RS-61980-000 ON THE DEVELOPMENT OF SYMPTOMS OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN THE RAT

Test Material	Daily Dose mg/kg	No. of Animals	Mean Body Weight g ± SE Day 15	Mean Body Weight g ± SE Day 18	Cumulative No. of Animals Paralyzed by Day 18	Percent Inhibition of Paralysis
Non-sensitized Animals						
Aqueous Vehicle	-	10	168 ± 3	171 ± 3	0	-
Sensitized Animals						
Aqueous Vehicle	-	10	139 ± 4	130 ± 3	10	-
Dexamethasone	0.25	10	129 ± 2[a]	121 ± 2[b]	0[b]	100
RS-61980-000	10	10	146 ± 2	138 ± 3[a]	1[b]	90

7. Study No. B2004RES0071

Study Title: Beneficial Effects of Teriflunomide in Experimental Allergic Encephalomyelitis

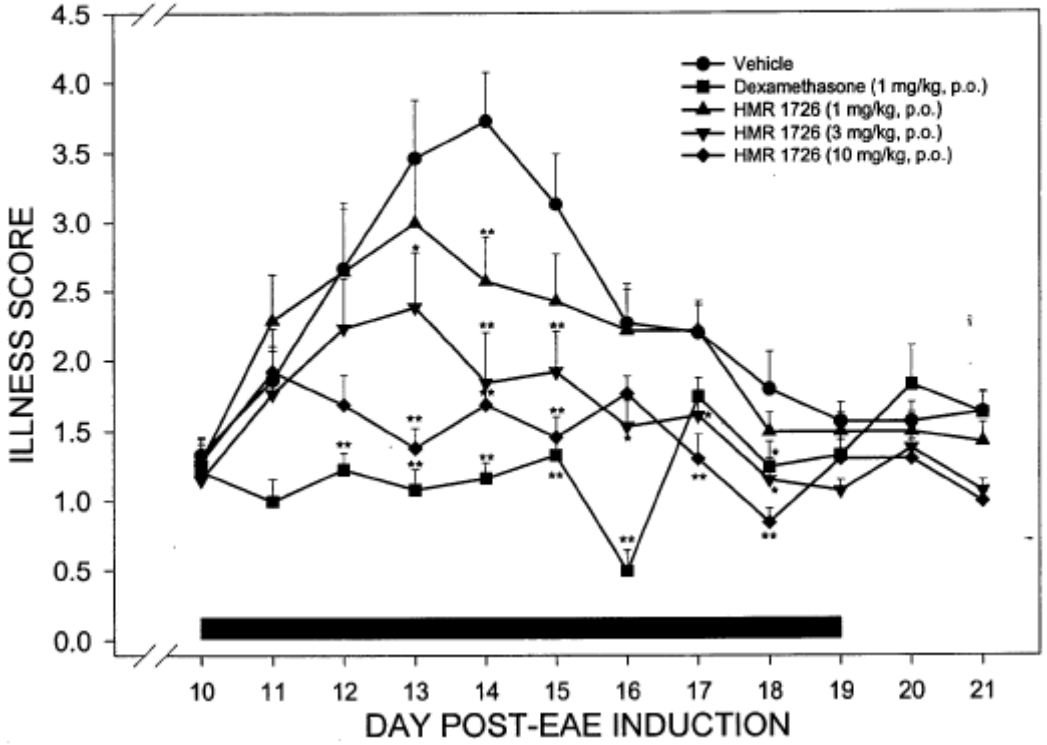
Sanofi-Aventis CNS and CD&HP, Bridgewater, NJ USA; Initiated: 2004

EAE was induced in female Lewis rats (n=10-15/group) by sensitization using whole guinea pig spinal cord homogenized in saline and complete Freund's adjuvant (supplemented with Mycobacterium tuberculosis H37 Ra) by intradermal injection into the hind footpad. Animals were observed daily commencing on day 10 post-EAE induction for clinical signs. Animals were dosed orally (gavage) beginning on Day 10 post-EAE with vehicle (0.2% carboxymethylcellulose), dexamethasone (1 mg/kg/day; positive control), or teriflunomide (1, 3, or 10 mg/kg/day) for either 10 or 21 days. In vehicle controls, EAE symptoms commenced on Day 11 with greatest paralysis observed on Day 14 that resolved by Day 18. Animal illness was scored using the following EAE behavioral scoring criteria (sponsor's Table 1) and the results were shown in sponsor's Figures 1 and 2 below.

Table 1. EAE Behavioral Scoring

STAGE 0	NORMAL
STAGE 1	Abnormal gate and tail atony
STAGE 2	Mild but definite weakness of one or both hind legs
STAGE 3	Severe weakness of one or both hind legs or mild ataxia
STAGE 4	Severe papraparesis and minimal hind leg movement
STAGE 5	No hind leg movement and paraplegia
STAGE 6	Moribund state with no spontaneous movement
STAGE 7	Death

Effect of 10-Day Treatment of Teriflunomide on Symptoms of EAE in Rat



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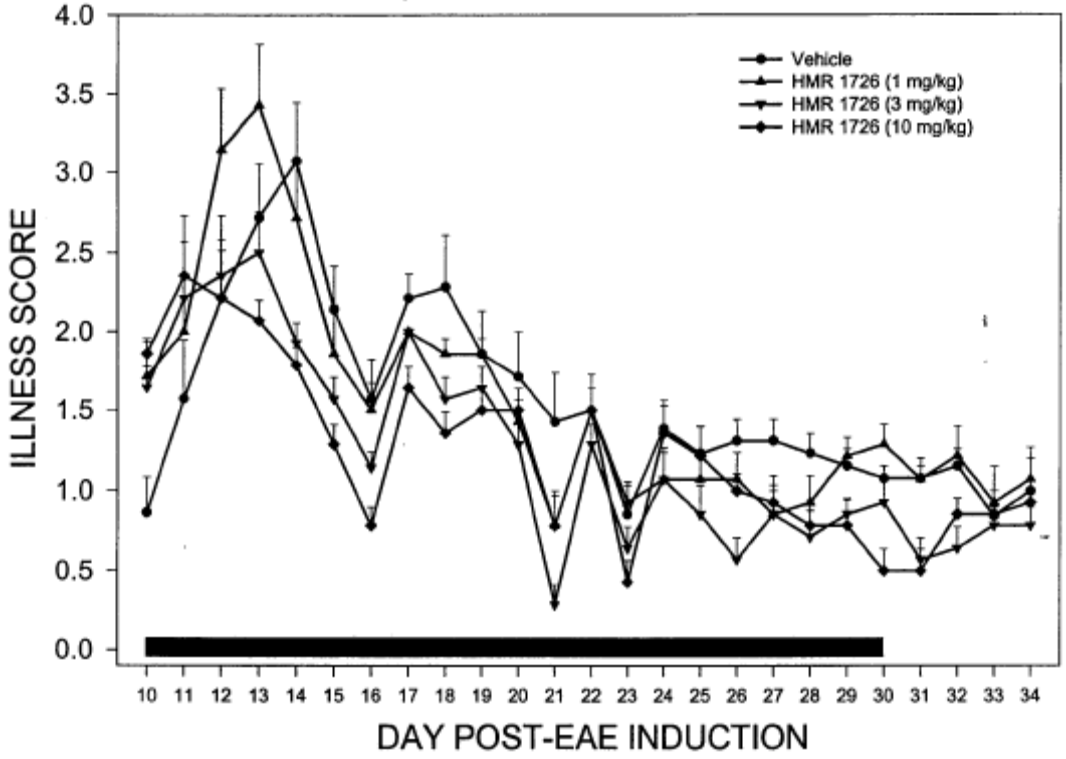
Effects of 21-Day Treatment of Teriflunomide on Symptoms of EAE in Rat



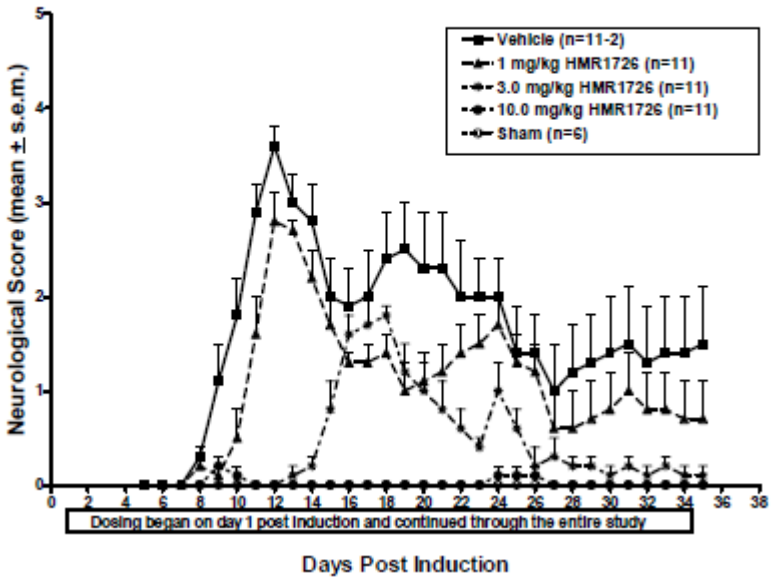
8. Study No. NVV0401

Study Title: The effects of HMR1726 on clinical signs, functional deficits, and pathological changes of experimental autoimmune encephalomyelitis in Dark Agouti rats

Sanofi-Aventis Research & Development, Bridgewater, NJ USA; Completed: November 2006

In this study, the sponsor examined the effect of teriflunomide treatment at different stages of EAE in DA rats. When teriflunomide was administered as a prophylactic treatment commencing on Day 1 post EAE induction, there was a delay of disease onset, noted (sponsor's Figure 2) as a rightward shift in the day to peak neurological score first evident at a dose of 3 mg/kg; teriflunomide at this dose also reduced the maximum clinical score. At a dose of 10 mg/kg, teriflunomide prevented onset of EAE over the course of the study.

Figure 2 - Experiment 1- Daily Effects of prophylactic treatment



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9. Study No. IIVT0017

Study Title: The effects of teriflunomide (HMR1726) on different immune cell populations in normal human blood

Sanofi-Aventis US, Inc., Bridgewater NJ, USA; Completed: 06/2011
Teriflunomide (Batch No. 0500044553)

In this non-GLP study, the sponsor evaluated the in vitro effect of teriflunomide on the proliferation, activation, and viability of lymphocyte populations of human peripheral

blood mononuclear cells isolated from healthy donors (n=4). Two concentrations of teriflunomide (25 and 100 μM, in 0.1% DMSO) were tested. T cell proliferation or activation by anti-CD3 antibody was evaluated in several T cell (CD3+) subpopulations (CD4+, CD8+, CXCR5+, CD45RO+); teriflunomide treatment at either 25 or 100 μM inhibited this activation and uridine treatment rescued the teriflunomide inhibition, as shown in sponsor's Tables 1, 3, 5, and 7. B cell and memory T cell proliferation and activation responses to CpG oligodeoxynucleotide and tetanus toxin. Teriflunomide inhibited B cell proliferation responses to CpG and this inhibition was largely rescued by addition of uridine; however, B cell activation was unaffected by teriflunomide (sponsor's Tables 9 and 11). Memory T cells (CD3+CD4+ CD45RO+) stimulated with tetanus toxin were strongly inhibited by teriflunomide at both concentrations; uridine rescued the inhibition of teriflunomide at 25 μM and partially rescued the teriflunomide inhibition at 100 μM (sponsor's Table 13). Activation of this cell population, as indicated by the loss of CCR7 expression (CD3+CD4+CD45RO+CCR7-), was unaffected by teriflunomide at either concentration (sponsor's Table 15).

Table 1 - Effect of HMR1726 on anti-CD3 antibody stimulated $CD3^+$ $CD4^+$ T cell proliferation

Treatment	Percent inhibition (%)				Mean	SE
	donor 002	donor 023	donor 229	donor 277		
Anti-CD3 Ab + 0 μM HMR1726 (positive control)	0.00	0.00	0.00	0.00	0.00	0.00
Anti-CD3 Ab + 0 μM HMR1726 + uridine	-0.13	0.00	0.21	0.00	0.02	0.07
Anti-CD3 Ab + 25 μM HMR1726	63.94	38.39	50.64	52.60	51.39	5.23
Anti-CD3 Ab + 25 μM HMR1726 + uridine	5.75	2.28	-0.83	0.43	1.91	1.43
Anti-CD3 Ab + 100 μM HMR1726	99.19	99.72	99.56	100.07	99.64	0.18
Anti-CD3 Ab + 100 μM HMR1726 + uridine	15.60	7.18	2.58	5.18	7.64	2.82

Table 3 - Effect of HMR1726 on anti-CD3 antibody stimulated $CD3^+$ $CD8^+$ T cell proliferation

Treatment	Percent inhibition (%)				Mean	SE
	donor 002	donor 023	donor 229	donor 277		
Anti-CD3 Ab + 0 μM HMR1726 (positive control)	0.00	0.00	0.00	0.00	0.00	0.00
Anti-CD3 Ab + 0 μM HMR1726 + uridine	0.57	3.51	-0.53	-0.21	0.83	0.92
Anti-CD3 Ab + 25 μM HMR1726	55.94	42.76	54.71	56.95	52.59	3.31
Anti-CD3 Ab + 25 μM HMR1726 + uridine	1.70	3.04	-1.90	0.64	0.87	1.04
Anti-CD3 Ab + 100 μM HMR1726	98.90	97.52	99.20	100.12	98.93	0.54
Anti-CD3 Ab + 100 μM HMR1726 + uridine	5.99	3.27	2.53	4.73	4.13	0.77

Table 5 - Effect of HMR1726 on anti-CD3 antibody stimulated $CD3^+$ $CXCR5^+$ T cell proliferation

Treatment	Percent inhibition (%)				Mean	SE
	donor 002	donor 023	donor 229	donor 277		
Anti-CD3 Ab + 0 µM HMR1726 (positive control)	0.00	0.00	0.00	0.00	0.00	0.00
Anti-CD3 Ab + 0 µM HMR1726 + uridine	-2.33	0.48	0.44	0.93	-0.12	0.74
Anti-CD3 Ab + 25 µM HMR1726	41.59	46.98	51.38	53.62	48.39	2.65
Anti-CD3 Ab + 25 µM HMR1726 + uridine	-1.64	1.69	-1.99	3.84	0.47	1.39
Anti-CD3 Ab + 100 µM HMR1726	96.63	100.01	98.98	100.14	98.94	0.81
Anti-CD3 Ab + 100 µM HMR1726 + uridine	-7.52	5.78	-1.99	3.61	-0.03	2.99

Table 7 - Effect of HMR1726 on the percent of $CD3^+$ $CD45RO^+$ activated T cells stimulated with anti-CD3 antibody

Treatment	Percent of $CD3^+$ $CD45RO^+$ T cells (activated T cells) (%)				Mean	SE
	donor 002	donor 023	donor 229	donor 277		
Negative control (cells alone)	21.30	54.10	28.90	39.00	35.83	7.09
Anti-CD3 Ab + 0 µM HMR1726 (positive control)	64.00	87.50	81.00	87.60	80.03	5.56
Anti-CD3 Ab + 0 µM HMR1726 + uridine	67.30	87.00	80.60	87.30	80.55	4.68
Anti-CD3 Ab + 25 µM HMR1726	52.40	79.50	57.00	73.90	65.70	6.52
Anti-CD3 Ab + 25 µM HMR1726 + uridine	64.20	89.10	82.50	90.00	81.45	5.99
Anti-CD3 Ab + 100 µM HMR1726	31.30	62.60	36.30	48.50	44.68	6.98
Anti-CD3 Ab + 100 µM HMR1726 + uridine	65.10	89.90	82.30	86.90	81.05	5.54

Table 9 - Effect of HMR1726 on CpG stimulated $CD19^+$ B cell proliferation

Treatment	Percent inhibition (%)				Mean	SE
	donor 002	donor 023	donor 229	donor 277		
CpG + 0 µM HMR1726 (positive control)	0.00	0.00	0.00	0.00	0.00	0.00
CpG + 0 µM HMR1726 + uridine	-3.69	-3.62	34.39	-9.46	4.40	10.05
CpG + 25 µM HMR1726	60.63	61.76	65.79	54.33	60.63	2.24
CpG + 25 µM HMR1726 + uridine	15.57	1.58	4.52	-5.61	4.01	4.33
CpG + 100 µM HMR1726	59.81	83.19	65.79	72.91	70.42	5.03
CpG + 100 µM HMR1726 + uridine	8.19	24.74	16.29	1.05	12.57	5.05

Table 11 - Effect of HMR1726 on the percent of $CD19^+$ $CD80^+$ activated B cells stimulated with CpG

Treatment	Percent of $CD19^+$ $CD80^+$ B cells (activated B cells) (%)				Mean	SE
	donor 002	donor 023	donor 229	donor 277		
Negative control (cells alone)	37.10	22.90	23.10	35.60	29.68	3.87
CpG + 0 µM HMR1726 (positive control)	85.00	85.00	88.70	94.50	88.30	2.24
CpG + 0 µM HMR1726 + uridine	83.50	83.10	87.30	95.60	87.38	2.90
CpG + 25 µM HMR1726	80.70	87.00	64.80	85.00	79.38	4.94
CpG + 25 µM HMR1726 + uridine	83.00	88.10	80.60	89.20	85.23	2.04
CpG + 100 µM HMR1726	81.00	83.60	84.90	92.20	85.43	2.42
CpG + 100 µM HMR1726 + uridine	80.80	86.80	83.10	94.20	86.23	2.95

Table 13 - Effect of HMR1726 on tetanus toxin stimulated $CD3^+$ $CD4^+$ T cell proliferation

Treatment	Percent inhibition (%)				Mean	SE
	donor 002	donor 023	donor 229	donor 277		
TT + 0 µM HMR1726 (positive control)	0.00	0.00	0.00	0.00	0.00	0.00
TT + 0 µM HMR1726 + uridine	-100.00	0.18	36.97	-9.41	-18.06	29.08
TT + 25 µM HMR1726	97.50	94.39	98.82	95.10	96.45	1.03
TT + 25 µM HMR1726 + uridine	6.72	-0.92	12.64	-115.84	-24.35	30.62
TT + 100 µM HMR1726	99.22	99.24	101.15	99.90	99.88	0.45
TT + 100 µM HMR1726 + uridine	56.88	20.15	41.23	65.35	45.90	9.93

a TT - tetanus toxin

Table 15 - Effect of HMR1726 on $CCR7^-$ $CD45RO^+$ activated T cells stimulated with tetanus toxin

Treatment	Percent of $CCR7^-$ $CD45^+$ T cells (activated $CD3^+$ $CD4^+$ T cells) (%)				Mean	SE
	donor 002	donor 023	donor 229	donor 277		
Negative control (cells alone)	11.00	22.20	13.50	22.90	17.40	3.02
TT + 0 µM HMR1726 (positive control)	16.30	58.10	23.00	31.30	32.18	9.17
TT + 0 µM HMR1726 + uridine	15.80	59.80	20.80	30.70	31.78	9.84
TT + 25 µM HMR1726	15.10	30.10	16.80	26.00	22.00	3.61
TT + 25 µM HMR1726 + uridine	15.60	59.20	22.70	31.80	32.33	9.55
TT + 100 µM HMR1726	14.20	33.80	16.80	27.50	23.08	4.59
TT + 100 µM HMR1726 + uridine	15.80	51.20	21.70	29.70	29.60	7.74

a TT - tetanus toxin

Table 17 - Effect of HMR1726 on viability of total lymphocytes stimulated with anti-CD3 antibody and CpG at 5 days and tetanus toxin at 7 days

Treatment	Percent of viable lymphocytes (%)				Mean	SE	p value
	donor 002	donor 023	donor 229	donor 277			versus positive control
Anti-CD3 alone (positive control)	97.80	96.30	92.80	97.80	96.18	1.18	-
Anti-CD3 + 25 µM HMR1726	96.10	96.10	96.40	97.90	96.63	0.43	0.7151
Anti-CD3 + 100 µM HMR1726	92.30	94.40	89.90	96.00	93.15	1.32	0.0392
CpG alone (positive control)	94.90	96.10	91.60	92.30	93.73	1.06	-
CpG + 25 µM HMR1726	94.60	95.20	89.40	91.80	92.75	1.34	0.1066
CpG + 100 µM HMR1726	93.30	93.50	90.70	93.40	92.73	0.68	0.2910
TT alone (positive control)	95.50	95.90	95.70	95.70	95.70	0.08	-
TT + 25 µM HMR1726	94.40	97.10	92.60	94.00	94.53	0.94	0.2809
TT + 100 µM HMR1726	90.50	97.80	86.80	90.40	91.38	2.31	0.1511

a p value by paired t-test
b TT - tetanus toxin

4.2 Secondary Pharmacology

The sponsor submitted one secondary pharmacology study (Study No A000002225) that assessed the binding of teriflunomide (Batch No. 821455-002) to a standard battery of receptors in an *in vitro* binding screen as well as in 18 different enzyme assays. In this study, teriflunomide (10 µM) did not bind to any of the receptors evaluated or affect any of the 18 different enzymes tested.

4.3 Safety Pharmacology

The sponsor submitted 8 safety pharmacology studies to assess effects of teriflunomide on the CNS, cardiovascular, respiratory, and urinary systems. Modified Irwin studies were conducted in mouse (Study No. F1999PHM0135) and in rat (Study No. 99-11331-PH) after a single oral dose of vehicle and teriflunomide (3, 10, and 30 mg/kg). In mouse (n= 6/group), no behavioral or vegetative signs were noted, with the exception of reduced feces in the HD group compared to control, LD, and MD animals at 6 hr postdose. No other behavioral or functional deficits were noted. In rat, teriflunomide treatment had no effect on behavior or body temperature, and displayed no evidence of convulsant or anticonvulsant properties at the doses tested; however, a significant decrease in locomotor activity was observed in the MD and HD groups when activity was recorded for 23 hr.

To assess putative cardiovascular effects of teriflunomide, two in vitro studies were performed, i.e., the hERG channel assay (Study No. PAT0171) and action potential evaluation in the isolated rabbit Purkinje cell assay (Study No. FIP0156). In the hERG, teriflunomide at concentrations of 10, 30, and 100 µM reduced the hERG channel currents on an average of 6.6, 11.1, and 4.9% respectively. The results of this study suggest that teriflunomide is not expected to prolong the Q-T interval. In the isolated

rabbit Purkinje cell assay, teriflunomide was evaluated at concentrations of 0.1, 1, 10, and 100 μM; no effect on the action potential or resting membrane potential was observed up to 10 μM. At the highest concentration tested, a shortening of the action potential duration was noted; however, there was no effect on resting membrane potential, APA, or Vmax. The effect on action potential duration did reverse following a 30-min washout period. In Study No. F1999PHM0136, blood pressure and heart rate were evaluated in anesthetized rats after intraduodenal administration of teriflunomide (2.5, 10, and 25 mg/kg); no effect on either blood pressure or heart rate was observed at the LD. In MD and HD groups, there were increases in systolic and diastolic pressures and heart rate that were similar in magnitude at both doses. In Study No. 99-11305, no significant effect of oral administration of teriflunomide (3 and 10 mg/kg) was recorded on arterial blood pressure, heart rate, or electrocardiogram parameters (PQ, RR, QT interval duration, QTc, and QRS durations) compared to vehicle control in 3M and 3F evaluated in a cross-over experimental design.

Teriflunomide (3 or 10 mg/kg) was administered as a single oral dose to evaluate its potential to alter respiratory parameters (respiratory rate, tidal volume, minute volume, peak inspiratory, and expiratory flows, inspiration and expiration times, and airway resistance) in conscious guinea pigs (Study No. 99-11204-PH). Neither dose of teriflunomide had any effect on the respiratory parameters. In Study No. 99-11289-PH, renal function was evaluated in saline-loaded rats after single oral administration of teriflunomide (1, 3, 10, 30 mg/kg) by assessing urinary volume, pH, ions, creatinine, urea, and total protein excretion over a 24-hr period. During the 24-hr period, in the first experiment testing doses of 3, 10, and 30 mg/kg, there was a 34% increase in urine volume at the HD; in the second experiment testing doses of 1, 3, and 10 mg/kg, there was an increase in urine volume of 21% and 25% at the MD and HD. The LD was a NOEL. The effect of teriflunomide on urinary ion, creatinine, and protein secretion is shown in sponsor's Table 6.

Table 6: Effect of HMR 1726 on urinary pH, ions, creatinine, urea and total protein excretion (5-24 hour period) in the rat *second experiment*

Group	Dose mg/kg p.o.		pH	Na^+	K^+	Cl^-	Ca^{++}	Mg^{++}	$PO4^{--}$	Creatinine	Urea	T. proteins	Na^+/K^+	$Cl^-/(Na^+ + K^+)$
				μmol/rat/period								mg/rat/period		
Vehicle	**0**		6.4	717.5	482.3	607.2	4.7	27.5	491.7	30.38	1971.3	1.27	1.53	0.51
		±	0.1	44.6	32.5	36.5	1.0	6.1	35.1	1.44	131.8	0.08	0.12	0.01
HMR 1726	**1**		6.3	869.5	528.3	725.1	4.8	30.3	507.8	32.13	2020.0	1.49	1.64	0.53
		±	0.1	61.9	26.2	31.8	0.6	4.0	22.5	1.37	106.9	0.09	0.09	0.02
				2%	*10%*	*19%*								
HMR 1726	**3**		6.5	838.4	547.7	688.9	5.6	24.0	488.0	30.34	1936.0	1.48	1.55	0.50
		±	0.1	44.8	31.9	38.0	1.2	4.6	24.8	1.63	81.9	0.07	0.07	0.02
				17%	*14%*	*13%*	*19%*		*-10%*					
HMR 1726	**10**		6.3	986.7**	612.1*	794.3**	7.2	34.9	520.8	29.21	1975.8	1.50	1.64	0.50
		±	0.1	52.3	36.0	34.0	1.4	5.0	25.5	0.99	74.6	0.07	0.08	0.01
				38%	*27%*	*31%*	*53%*	*27%*	*29%*					

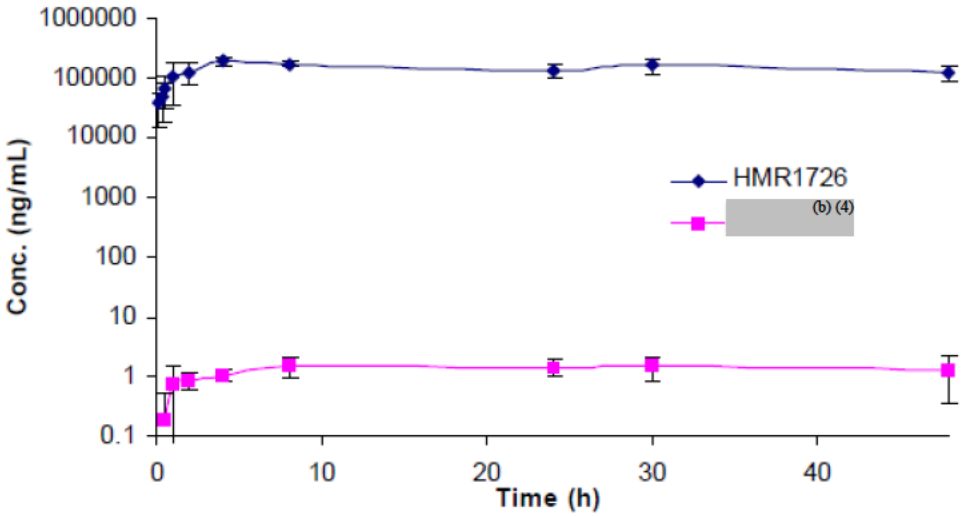
Data are expressed as mean ± sem
n = 10 rats/dose
** $p < 0.01$, * $p < 0.05$ according to Dunnett's test in comparison with vehicle

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The following sponsor-provided figure demonstrates the plasma concentration versus time profile of oral teriflunomide administration to rats.

Mean (± SD, n=3) plasma concentrations of teriflunomide (HMR1726) and (b) (4) in male rats after a single oral administration of teriflunomide at 100 mg/kg



The following sponsor's table summarizes the PK of teriflunomide administered to each of the species utilized in the toxicology studies.

Table 4 – Mean pharmacokinetic parameters of teriflunomide following single administration to male animals (n = 3)

Species	Route [a]	Dose (mg/kg)	C_{max} [b] (µg/mL)	t_{max} [c] (h)	AUC (µg.h/mL)	CL (mL/min/kg)	V_{ss} (L/kg)	$t_{1/2z}$ (h)	F (%)
Mouse	PO	7.5	36.9	1.00	1080	-	-	25.5	102
	IV	3	22.2	0.083	306	0.126	0.163	18.3	-
Rat	PO	7.5	48.4	6.00	959	-	-	-	102
	PO	100	200	30	11100	-	-	-	-
	IV	3	27.7	0.083	372	0.134	0.112	37.1	-
Rabbit	PO	7.5	25.9	4.00	354	-	-	4.69	63.8
	IV	3	42.2 [b]	0.083	216	0.228	0.073	4.20	-
Dog [d]	PO	7.5	58.9	1.00	1830	-	-	29.5	100
	IV	3	28.9 [b]	0.083	668	0.074	0.151	29.6	-

Values are rounded to 3 significant figures or less.

Abbreviations: PO = per os (oral); IV = intravenous; C_{max} = maximum plasma concentration observed; t_{max} = (first) time to reach C_{max}; AUC = area under the plasma concentration versus time curve extrapolated to infinity; CL = total body clearance; Vss = volume of distribution at steady-state; $t_{1/2z}$ = terminal half-life; F = bioavailability; h = hours.

a. IV bolus, except in rabbits and dogs (5-minute infusion);

b. For IV bolus, C_{max} is the concentration 5 min after the end of infusion;

c. median;

d. n=6.

Absorption

The sponsor conducted mass balance studies of teriflunomide in each of the species used in toxicology studies (mouse, rat, rabbit, and dog). The results from the rat, rabbit, and dog studies are summarized below.

Rat

Study No. 2005-0047
Pharmacokinetic, Radiokinetic, and ^{14}C Excretion/Mass Balance Study of ^{14}C-HMR1726 in Male Sprague Dawley Rats

In this non-GLP study, radiolabeled teriflunomide was administered intravenously (3 mg/kg) or orally (7.5 mg/kg) to SD male rats. Plasma concentration versus time profiles for both teriflunomide and its metabolite 4-TFMA (sponsor's Figure 1) were determined. The distribution of ^{14}C-labeled teriflunomide in red blood cells and plasma (sponsor's Table 7) and excretion profile (sponsor's Table 8) are shown below.

Figure 1. Concentrations of HMR1726, 4-TFMA Metabolite, and Radioactivity in Plasma of Male Sprague Dawley Rats (n = 3/time point) Given a Single 3-mg/kg Intravenous or 7.5-mg/kg Oral Dose of ^{14}C-HMR1726

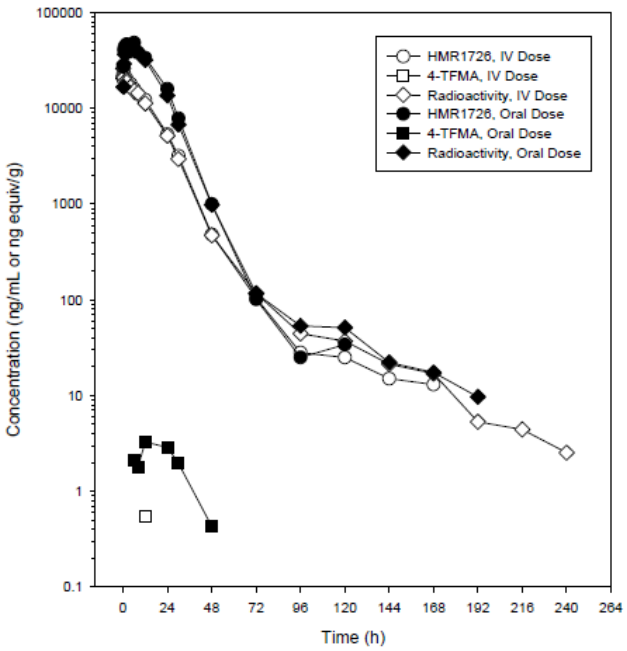


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Table 7. Red Blood Cell:Plasma Concentration Ratios for Radioactivity in Male Sprague Dawley Rats (n = 3/route/time point) Given a Single Intravenous or Oral Dose of ^{14}C-HMR1726

Time (h)	3-mg/kg Intravenous			7.5-mg/kg Oral		
	Mean	SD	%CV	Mean	SD	%CV
0.25	0.299	0.015	5	0.301	0.036	12
1	0.283	0.011	4	0.295	0.024	8
8	0.276	0.025	9	0.336	0.047	14
24	0.262	0.022	8	0.329	0.010	3

SD = standard deviation.
%CV = percent coefficient of variation.

Table 8. Cumulative (0–168 h) Excretion of Radioactivity in Male Sprague Dawley Rats (*n* = 6/dose route) Given a Single Intravenous or Oral Dose of ^{14}C-HMR1726

Matrix	Percent Dose (Mean ± SD)					
	3-mg/kg Intravenous			7.5-mg/kg Oral		
	Mean	SD	%CV	Mean	SD	%CV
Urine	39.36	10.16	26	51.08	6.41	13
Feces	42.74	12.25	29	28.46	4.67	16
Cage rinse/wash	10.60	3.14	30	8.92	3.24	36
Expired air	—	—	—	0.00	0.00	0
Carcass	1.69	0.51	30	1.42	0.30	21
Total	94.39	2.35	2	89.88	3.91	4

SD = standard deviation.
— indicates not applicable.

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Rabbit

Study No. 2005-0048

Pharmacokinetic, Radiokinetic, and ^{14}C Excretion/Mass Balance Study of ^{14}C-HMR1726 in Male New Zealand White Rabbits

In this non-GLP study, radiolabeled teriflunomide was administered intravenously (3 mg/kg) or orally (7.5 mg/kg) to male NZW rabbits. Plasma concentration versus time profiles for both teriflunomide (sponsor's Figure 1 and Table 3) was determined; its metabolite 4-TFMA was measured but its concentration was below the lower limit of detection (data not shown).

Figure 1. Mean (±SD) Concentrations of HMR1726 and Radioactivity in Plasma of Male Rabbits (*n* = 3/route) Given Single 3-mg/kg Intravenous or 7.5-mg/kg Oral Doses of ^{14}C-HMR1726

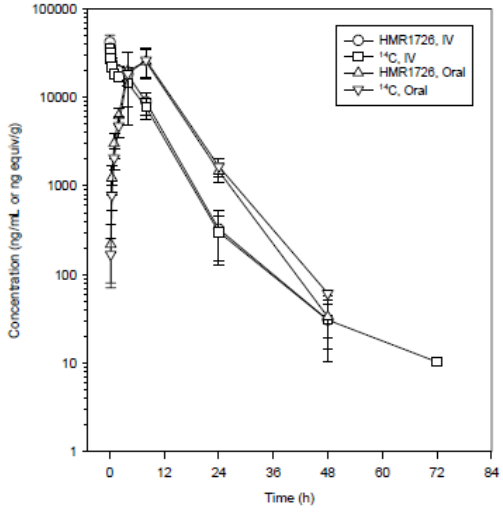

Table 3. Pharmacokinetic Parameter Values for HMR1726 in Plasma of Male Rabbits (*n* = 3/route) Given a Single Intravenous or Oral Dose of ^{14}C-HMR1726

Parameter (units)	3-mg/kg Intravenous			7.5-mg/kg Oral		
	Mean	SD	%CV	Mean	SD	%CV
C_{max} (ng/mL)	42200	8070	19	25900	8970	35
t_{max} (h)	0.11	0.05	47	5.33	2.31	43
AUC_{last} (ng•h/mL)	216000	24100	11	354000	110000	31
AUC_{INF} (ng•h/mL)	216000	24100	11	354000	110000	31
$t_{1/2}$ (h)	4.20	1.40	33	4.69	0.92	20
Cl (mL/min/kg)	0.228	0.026	11	—	—	—
Vss (L/kg)	0.073	0.001	1	—	—	—
Bioavailability (%)[a]	—	—	—	63.8	—	—

[a] Bioavailability was determined from the actual (measured) mean dose for each route of administration.
SD = standard deviation.
%CV = percent coefficient of variation.
C_{max} = maximum observed plasma concentration.
t_{max} = time of maximum observed plasma concentration.
AUC_{last} = area under the plasma concentration-time curve from time zero to the time of final quantifiable sample.
AUC_{INF} = area under the plasma concentration-time curve from time zero extrapolated to infinity.
$t_{1/2}$ = apparent terminal half-life.
Cl = total body clearance.
Vss = apparent steady-state volume of distribution.
— indicates not applicable.

The distribution of radioactivity in red blood cells and plasma (sponsor's Tables 5 and 6) and excretion profile (sponsor's Table 7) following administration of ^{14}C-labeled teriflunomide are shown below.

Table 5. Pharmacokinetic Parameter Values for Radioactivity in Plasma of Male Rabbits (*n* = 3/route) Given a Single Intravenous or Oral Dose of ^{14}C-HMR1726

Parameter (units)	3-mg/kg Intravenous			7.5-mg/kg Oral		
	Mean	SD	%CV	Mean	SD	%CV
C_{max} (ng equiv/g)	35540	3311	9	26540	9967	38
t_{max} (h)	0.08	0.00	0	6.67	2.31	35
AUC_{last} (ng equiv•h/g)	186100	28990	16	361600	112100	31
AUC_{INF} (ng equiv•h/g)	186400	29190	16	362100	112000	31
$t_{1/2}$ (h)	13.0	10.2	78	6.12	2.38	39

SD = standard deviation.
%CV = percent coefficient of variation.
C_{max} = maximum observed plasma concentration.
t_{max} = time of maximum observed plasma concentration.
AUC_{last} = area under the plasma concentration-time curve from time zero to the time of final quantifiable sample.
AUC_{INF} = area under the plasma concentration-time curve from time zero extrapolated to infinity.
$t_{1/2}$ = apparent terminal half-life.

Table 6. Red Blood Cell:Plasma Concentration Ratios for Radioactivity in Male Rabbits (*n* = 3/route) Given a Single Intravenous or Oral Dose of ^{14}C-HMR1726

Time (h)	3-mg/kg Intravenous			7.5-mg/kg Oral		
	Mean	SD	%CV	Mean	SD	%CV
0.25	0.169	0.015	9	0.166	0.023	14
1	0.182	0.029	16	0.165	0.016	10
8	0.172	0.069	40	0.161	0.019	12
24	0.297	0.076	26	0.239	0.061	25

SD = standard deviation.
%CV = percent coefficient of variation.

Table 7. Cumulative (0–336 h) Excretion of Radioactivity in Male Rabbits (*n* = 4/route) Given a Single Intravenous or Oral Dose of ^{14}C-HMR1726

Matrix	Percent Dose (Mean ± SD)					
	3-mg/kg Intravenous			7.5-mg/kg Oral		
	Mean	SD	%CV	Mean	SD	%CV
Urine	62.17	2.37	4	71.90	7.51	10
Feces	27.06	2.33	9	23.38	4.57	20
Cage rinse/wash	3.44	1.65	48	4.04	1.89	47
Total	92.97	2.70	3	99.33	1.80	2

SD = standard deviation.
%CV = percent coefficient of variation.

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Dog

Study No. 2005-0049

Pharmacokinetic, Radiokinetic, and ^{14}C Excretion/Mass Balance Study of ^{14}C-HMR1726 in Male Beagle Dogs

In this non-GLP study, radiolabeled teriflunomide was administered intravenously (3 mg/kg) or orally (7.5 mg/kg) to male Beagle dogs. Plasma concentration versus time profiles for both teriflunomide (sponsor's Figure 4) and its metabolite 4-TFMA (sponsor's Figure 4 and Table 4) were determined for each animal (#1-6). The sponsor did not provide summary data—only data for each individual dog was provided. The data presented for Dog#4, was selected as a representative example of these data.

Figure 4. Concentrations of HMR1726, 4-TFMA Metabolite, and Radioactivity in Plasma of a Beagle Dog (no. 4) Given a Single 3-mg/kg Intravenous and 7.5-mg/kg Oral Dose of ^{14}C-HMR1726

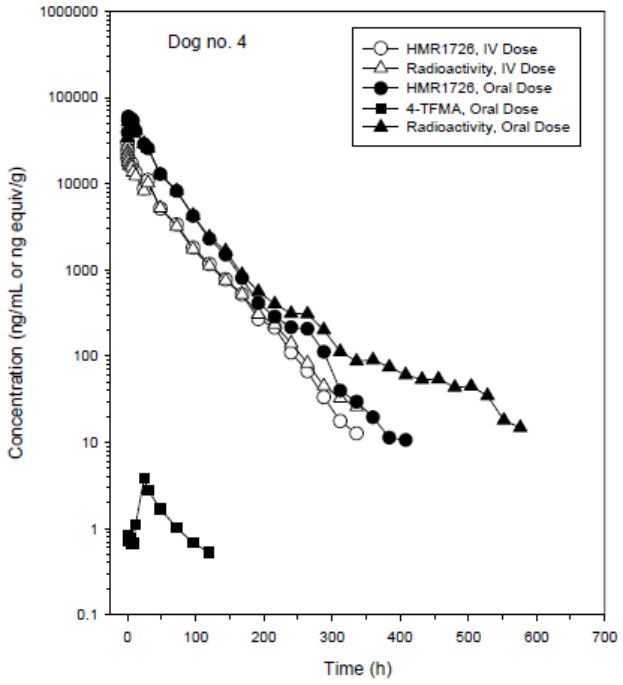

Table 4. Pharmacokinetic Parameter Values for 4-TFMA Metabolite in Plasma of Male Beagle Dogs (n = 6) Given a Single Intravenous and Oral Dose of ^{14}C-HMR1726

Parameter (units)	3-mg/kg Intravenous			7.5-mg/kg Oral		
	Mean	SD	%CV	Mean	SD	%CV
C_{max} (ng/mL)	ND	—	—	3.08	1.11	36
t_{max} (h)	ND	—	—	25	2	10
AUC_{last} (ng•h/mL)	ND	—	—	140	50	36
AUC_{INF} (ng•h/mL)	ND	—	—	191	43	23
$t_{1/2}$ (h)	ND	—	—	36.0	5.3	15

SD = standard deviation.
%CV = percent coefficient of variation.
ND = not determinable; concentrations of 4-TFMA were BQL in most samples collected after intravenous administration.
C_{max} = maximum observed plasma concentration.
t_{max} = time of maximum observed plasma concentration.
AUC_{last} = area under the plasma concentration-time curve from time zero to the time of final quantifiable sample.
AUC_{INF} = area under the plasma concentration-time curve from time zero extrapolated to infinity.
$t_{1/2}$ = apparent terminal half-life.
— indicates not applicable.

The distribution of radioactivity in red blood cells and plasma (sponsor's Tables 5 and 6) and excretion profile (sponsor's Table 7) following administration of ^{14}C-labeled teriflunomide are shown below.

Table 5. Pharmacokinetic Parameter Values for Radioactivity in Plasma of Male Beagle Dogs (n = 6) Given a Single Intravenous and Oral Dose of ^{14}C-HMR1726

Parameter (units)	3-mg/kg Intravenous			7.5-mg/kg Oral		
	Mean	SD	%CV	Mean	SD	%CV
C_{max} (ng equiv/g)	27420	2193	8	57090	4459	8
t_{max} (h)	0.10	0.04	39	1.8	1.3	72
AUC_{last} (ng equiv•h/g)	642800	109000	17	1873000	452100	24
AUC_{INF} (ng equiv•h/g)	644400	108500	17	1878000	449400	24
$t_{1/2}$ (h)	43.9	8.8	20	142	41	29

SD = standard deviation.
%CV = percent coefficient of variation.
C_{max} = maximum observed plasma concentration.
t_{max} = time of maximum observed plasma concentration.
AUC_{last} = area under the plasma concentration-time curve from time zero to the time of final quantifiable sample.
AUC_{INF} = area under the plasma concentration-time curve from time zero extrapolated to infinity.
$t_{1/2}$ = apparent terminal half-life.

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Table 6. Red Blood Cell:Plasma Concentration Ratios for Radioactivity in Male Beagle Dogs (n = 6) Given a Single Intravenous and Oral Dose of ^{14}C-HMR1726

Time (h)	3 mg/kg-Intravenous			7.5-mg/kg Oral		
	Mean	SD	%CV	Mean	SD	%CV
0.25	0.207	0.033	16	0.182	0.022	12
1	0.221	0.017	8	0.221	0.025	11
8	0.240	0.018	7	0.225	0.023	10
24	0.275	0.057	21	0.235	0.050	21

SD = standard deviation.
%CV = percent coefficient of variation.

Table 7. Cumulative Excretion of Radioactivity (as percent dose) in Male Beagle Dogs (n = 6) Given a Single Intravenous and Oral Dose of ^{14}C-HMR1726

Matrix	Percent Dose (Mean ± SD)					
	3-mg/kg Intravenous			7.5-mg/kg Oral		
	Mean	SD	%CV	Mean	SD	%CV
Urine	28.04	7.14	25	51.51	6.40	12
Feces	57.03	6.65	12	36.95	5.82	16
Cage rinse/wash	4.93	1.05	21	3.10	1.17	38
Total	90.01	1.48	2	91.56	2.80	3

SD = standard deviation.
%CV = percent coefficient of variation.

Distribution

Seven studies were completed to evaluate the plasma protein binding and tissue distribution of teriflunomide in the species used in toxicology studies, and plasma protein binding in human blood. Data from these studies are briefly reviewed below.

Teriflunomide was shown to be highly protein bound in each of the species tested. In Study No. LPR0959, binding of teriflunomide to mouse plasma proteins was 99.98%, and the binding was not saturated up to a concentration of 100 µg/mL. In a non-GLP in vitro study (Study No. HMR008477), radiolabeled teriflunomide (code name: [^{14}C] A771726) binding to human, rat, and dog plasma proteins was evaluated; teriflunomide was shown to be highly bound to plasma proteins—human (99.5-99.7%), rat (96-99.2%), and dog (97.2-98.8%). In Study No. HMR012565, teriflunomide ([^{14}C] A771726) was highly bound to monkey plasma protein (98.9-99.2%).

In Study No. DIS0471, the tissue distribution of orally administered [^{14}C] HWA1726 (teriflunomide; 7.5 mg/kg) was determined in rats—albino (Sprague Dawley) at 1-72 hr postdose and in pigmented (Long Evans) at 24, 336, and 672 hr postdose. The tissue distribution of radioactivity at 24 hr postdose was similar for both pigmented and albino rats. The highest levels of radioactivity in LE and SD rats at 24 hrs postdose were found in the gastrointestinal tract. The values for the LE rat are summarized—"cutaneous stomach" (24,400 ng-eq/g), stomach contents (6360 ng-eq/g), and liver (6120 ng-eq/g), kidney medulla (10,500 ng-eq/g) and cortex (5660 ng-eq/g), colon contents (10,300 ng-eq/g) and colon wall (5560 ng-eq/g). In the LE rat, radioactivity was concentrated in the skin (6330 ng-eq/g), seminal vesicle (5960 ng-eq/g), the uveal tract (1600 ng-eq/g) and eye (105 ng-eq/g). After 336 hr postdose, radioactivity was eliminated from most tissues; however, radioactivity remained in the skin (4790 ng-eq/g), glandular stomach (715 ng-eq/g), stomach contents (313 ng-eq/g), cutaneous stomach (142 ng-eq/g), and quantifiable levels were noted in the lung (191 ng-eq/g), spleen (148 ng-eq/g), blood (141 ng-eq/g), and liver (111 ng-eq/g). After 672 hr postdose, radioactivity was eliminated from all tissues except the skin (898 ng-eq/g) of pigmented rats, consistent with prolonged retention of teriflunomide in pigmented skin.

Two studies were conducted in Dark Agouti (DA) rats to assess tissue distribution in EAE-diseased animals after single or repeated oral administration of [^{14}C]-teriflunomide (10 mg/kg). In the first non-GLP study (DIS0553), teriflunomide was administered either as a single dose or for 15 consecutive days to male DA rats with or without EAE and tissue distribution was determined at 0.5, 1, 2, 6, or 24 hr postdose. The pattern of distribution was similar with single and repeat-dose administration. The second non-GLP study in male DA rats (DPK0205) was designed to determine CNS penetration of teriflunomide. The study design and data are shown in the sponsor's tables below. In both non-EAE (naïve) and EAE DA rats, the tissue distribution of single oral administration of [^{14}C]-teriflunomide was similar and extensive. In the CNS, the mean concentration of radioactivity observed in the brain and spinal cord ranged from 680-1110 ng-eq/g. It is noted that EAE rats had higher levels in the cervical spinal cord (3040 ng-eq/g) compared to that of naïve control (no EAE) cervical spinal cord (1110 ng-eq/g). These data suggest that greater penetration of teriflunomide is achieved in cervical spinal cord of EAE rats. After repeated oral treatment with teriflunomide (10 mg/kg) for 15 days, the distribution of [^{14}C]-teriflunomide (10 mg/kg) in naive and EAE rats was similar; the CNS (brain and spinal cord) concentrations ranged from 310 to 460 ng-eq/g.

8.3.2.1 Study Design

Group	Disease Induction	Treatment	Dose (mg/kg)	Number of animals ** Number/Time point	Max Total
N	Non-EAE	None	-	10	10
A	EAE	None	-	10	15
N'	EAE	HMR 1726, starting on day 11	10	10	15
A'	Non-EAE	HMR 1726, starting on day 12	10	10	10

**A total of 30 animals inoculated with rSCH to ensure 10 animals per timepoint because of mortality or lack of disease symptoms. The first 10 animals did not receive treatment until day of BBB study takedown. The animals that were left in both E and N groups began treatment with HMR 1726 at 10 mg/kg as follows: E group began treatment on day 11 and N group began treatment on day 12.

Table 9 - Mean (n=9) Concentrations of Total Radioactivity (µg-eq/g) in Organs and Tissues of Dark Agouti Rat

Group A (µg-eq/g)	RA1	RA2	RA3	RA4	RA5	RA6	RA7	RA8	RA9	Mean	SD
Blood	32.11	68.18	43.72	45.41	13.95	40.47	30.12	27.39	28.76	36.68	15.26
Brain	0.70	0.87	0.73	0.79	0.26	1.05	0.53	0.51	0.69	0.68	0.23
Cerebellum	0.64	1.22	0.89	1.10	0.30	1.12	0.85	0.72	0.96	0.87	0.28
Cervical spinal	2.28	1.91	0.90	0.99	0.27	1.28	0.68	0.78	0.88	1.11	0.63
Thoracic spinal	0.79	1.09	0.86	0.90	0.25	1.02	0.60	0.82	1.03	0.82	0.26

Group B (µg-eq/g)	RB1	RB2	RB3	RB4	RB5	RB6	RB7	RB8	RB9	Mean	SD
Blood	58.06	16.69	37.96	27.66	39.81	53.98	22.10	38.44	29.76	36.05	13.73
Brain	1.09	0.43	0.92	0.73	0.86	1.03	0.53	0.67	0.52	0.75	0.24
Cerebellum	1.19	0.55	1.04	0.77	0.91	1.24	0.74	0.82	0.68	0.88	0.23
Cervical spinal	5.26	2.01	3.97	1.80	1.57	5.15	2.02	3.35	2.26	3.04	1.45
Thoracic spinal	1.12	0.47	0.91	0.66	0.89	1.27	0.58	0.88	0.77	0.84	0.25

Group C (µg-eq/g)	RC1	RC2	RC3	RC4	RC5	RC6	RC7	RC8	RC9	Mean	SD
Blood	1.54	15.20	4.34	3.70	16.89	9.73	5.36	6.68	10.93	8.26	5.29
Brain	0.16	0.52	0.24	0.20	0.55	0.32	0.19	0.32	0.32	0.31	0.14
Cerebellum	0.19	0.67	0.26	0.28	0.62	0.34	0.25	0.33	0.42	0.37	0.17
Cervical spinal	0.13	0.58	0.30	0.31	0.80	0.41	0.40	0.50	0.39	0.43	0.19
Thoracic spinal	0.13	0.56	0.21	0.56	0.49	0.37	0.45	0.49	0.30	0.40	0.15

Group D (µg-eq/g)	RD1	RD2	RD3	RD4	RD5	RD6	RD7	RD8	RD9	Mean	SD
Blood	7.47	19.64	9.62	1.10	7.80	7.00	17.98	4.61	3.95	8.80	6.21
Brain	0.29	0.53	0.37	0.07	0.60	0.31	0.70	0.17	0.18	0.36	0.21
Cerebellum	0.33	0.63	0.44	0.15	0.82	0.42	0.81	0.27	0.25	0.46	0.24
Cervical spinal	0.39	0.62	0.38	0.08	0.67	0.44	0.90	0.26	0.14	0.43	0.26
Thoracic spinal	0.46	0.61	0.20	0.10	0.68	0.37	0.71	0.24	0.11	0.39	0.24

In a non-GLP/QA study (2002-0075), the steady state brain:plasma ratio of teriflunomide administered to SD rats during constant intravenous infusion was determined. Rats were administered an IV bolus of teriflunomide (5.0 mg/kg) followed immediately by constant IV infusion rate of 2.5 mL/kg/hr and 0.4 mg/kg/hr for 48 hrs; the plasma and brain concentrations achieved during this study are shown in the sponsor's

Tables 1 and 2 (below). These data show that the mean plasma concentration ranged from 25-45 μg/mL. The mean brain to plasma ratios of teriflunomide were 1.5% and 1.0% at 30 and 48 hr during constant IV infusion, respectively. The sponsor suggests that these data indicate there is poor to no penetration of teriflunomide in the brain and that the ratios achieved account for residual blood in the brain.

Table 1 – Plasma and Brain Concentrations of HMR 1726 During Constant IV Infusion in Sprague-Dawley Rats

Plasma Concentration (μg/mL)								
Time (hr)**	Rat# 1	Rat# 2	Rat# 3	Rat# 4	Rat# 5	Rat# 6	Mean	SD
8	41.092	37.284	26.774	34.031	41.045	36.199	36.071	5.330
24	50.224	6.161*	54.685	31.203	45.522	NS	45.409	10.183
30	38.863	26.498	6.357*	27.418	37.405	21.647	30.366	7.440
48	26.247	0.154*	26.258	NS	21.677	NS	24.727	2.642
Brain Concentration (μg/g)								
30	0.686	0.385	BLQ*	0.353	0.584	0.228	0.447	0.185
48	0.241	BLQ*	0.228	NS	0.263	NS	0.244	0.018

Rats were given an IV bolus dose of 5.0 mg/kg, immediately followed by a constant IV infusion at 2.5 mL/kg/hr and 0.4 mg/kg/hr.

NS, No sample.

*, outlier, different from the mean of all other values in the group by 4 fold or more; not included in the calculation of the mean value.

BLQ, below the limit of quantitation of 0.100 μg/g.

**, time from the start of IV infusion. Blood samples at 8 hr and 30 hr and brain samples at 30 hr during the IV infusion were collected from the first group of six rats. Blood samples at 24hr and 48 hr and brain samples at 48 hr during the IV infusion were collected from the second group of six rats

Table 2 – Brain/Plasma Ratios of HMR 1726 During Constant IV Infusion in Sprague-Dawley Rats

Time (hr)*	Mean Plasma Conc. (μg/mL)	Mean Brain Conc. (μg/g)	Brain/plasma ratio (%)
30	30.3662	0.447	1.5
48	24.727	0.244	1.0

*, time from the start of IV infusion.

Metabolism

The sponsor conducted in vivo metabolism studies in each species used in the toxicology studies. Review of each of these independent studies was performed and each study was conducted adequately; however, for the purpose of this review the discussion is limited to study MIS0061 as it provides a good summary of the results achieved in the individual studies. The results achieved for in vivo metabolism of [^{14}C]teriflunomide in mouse, rat, rabbit, dog, and human are summarized in sponsor's Table 3. In the sponsor's Table 3, the percents of the administered dose recovered in the urine, feces, cage wash, and carcass are provide for each toxicology species.

Table 3 – Excretion Summary – Percent of Administered Dose Recovered Following a Single Oral Dose of [^{14}C]-HMR1726 to Mouse, Rat, Rabbit, Dog, and Human

Excretion	Mouse (n = 6)	Rat (n = 6)	Rabbit (n = 4)	Dog (n = 6)	Human (n = 6)		
Collection Time (days)	14	14	14	28	0-21 days (BEX pooling)	22-28 days (Washout*)	Total
Urine	11.5±1.3	51.1±6.4	71.9±7.5	51.5±6.4	22.6±4.5	0.5±0.3	23.1±4.6
Feces	70.5±2.9	28.5±4.7	23.4±4.6	37.0±5.8	37.5±12.7	16.8±10.5	54.3±21.2
Cage Wash	5.8±1.3	8.9±3.2	4.0±1.9	3.1±1.2	NA	NA	NA
Carcass	0.0±0.0	1.4±0.3	NA	NA	NA	NA	NA
Total	87.8±1.0	89.9±3.9	99.3±1.8	91.6±2.8	60.1±10.4	17.3±10.7	77.4±19.2

* Cholestyramine was administered daily on Day 22 through Day 28.

In sponsor's Table 5 is a summary of teriflunomide and its metabolites that were detected in the urine and feces of mouse, rat, rabbit, dog, and human. In human, seven metabolites (M4, M5, M6, M8, M9, M10, and M14) and teriflunomide (parent) was observed in the urine and/or the feces. Of the identified human metabolites, coverage was achieved by at least one toxicology species. None of the identified metabolites was considered a major human metabolite; the minor metabolites that were identified in human were 4-TFMA and A813226.

Table 5 – Summary of HMR1726 and Its Metabolites Detected in Urine and Feces from Mouse, Rat, Rabbit, Dog, and Human

	Mouse (%dose)		Rat (%dose)		Rabbit (%dose)		Dog (%dose)		Human ((%dose in 21 days)	
ID	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
	9.67% (0-72h)	67.73% (0-144h)	50.24% (0-72h)	26.12% (0-72h)	70.51% (0-96h)	21.67% (0-96h)	50.68% (0-144h)	33.79% (0-216h)	22.6% (504h)	37.5% (504h)
HMR1726	0.28[a]	65.39	1.06	21.96	57.36[b]	19.73[c]	2.92[a,b]	26.14	1.3[a]	35.7
M1	0.28	ND	0.07	ND	ND	ND	ND	ND	0.0[d]	ND
M2	0.15	ND	ND	ND	ND	ND	ND	ND	ND	ND
M3	0.10	ND	0.71	ND	ND	ND	ND	ND	ND	ND
M4	0.95	ND	0.50	ND	0.54	ND	3.13	ND	1.3	ND
M5	6.69	0.77	43.05	0.43	4.48	0.31	38.25	0.86	18.1	0.4
M6	0.33	ND	ND	ND	ND	ND	ND	ND	0.3	ND
M7	[a]	ND	ND	ND	ND	[c]	[a,b]	ND	[a]	ND
M8	0.12	0.25	ND	ND	0.80	0.14	ND	ND	0.0[e]	0.3
M9	ND	ND	1.62	ND	ND	ND	ND	ND	0.4	ND
M10	ND	ND	ND	0.49	4.56	0.24	ND	2.17	0.7	1.1
M11	ND	ND	ND	0.09	ND	0.14	ND	0.24	ND	ND

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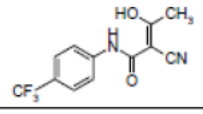
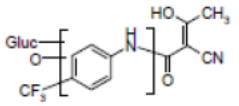
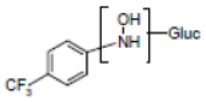
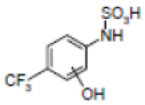
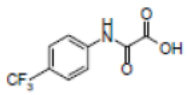
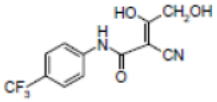
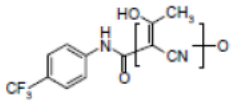
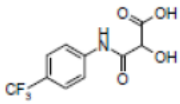
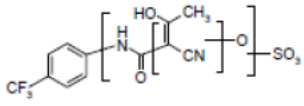
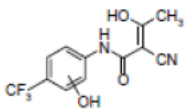
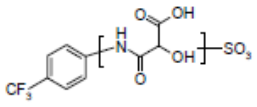
M12	ND	ND	ND	2.43	ND	ND	ND	0.18	ND	ND
M13	ND	ND	0.26	0.48	[b]	[c]	[a,b]	3.10	ND	ND
M14	ND	ND	ND	ND	ND	ND	0.37	ND	0.2	ND
TOTAL	9.34	66.41	47.28	25.87	67.73	20.56	44.71	32.47	22.6	37.5
Others	0.33	1.33	3.31	0.25	2.78	1.11	5.97	1.32	-	-

a. M7 was coeluted with HMR1726 in urine contributing to <0.3-3% of the administered dose.
b. M13 was a minor shoulder peak overlapping with HMR1726 based on HPLC radio-chromatograms and LC/MS.
c. M7 and M13 appeared to be minor metabolites based on LC/MS.
d. M1 was only detected in Subject M1001 at 0.1% of the dose.
e. M8 accounted for 0.2% of the dose in Subject M1002 and 0.2% of the dose in Subject M1005.

The structures of teriflunomide and its metabolites identified above are provided in sponsor's Table 6.

Table 6 – Summary of HMR1726 and its Metabolites Identified in Excreta from Mouse, Rat, Rabbit, Dog, and Human

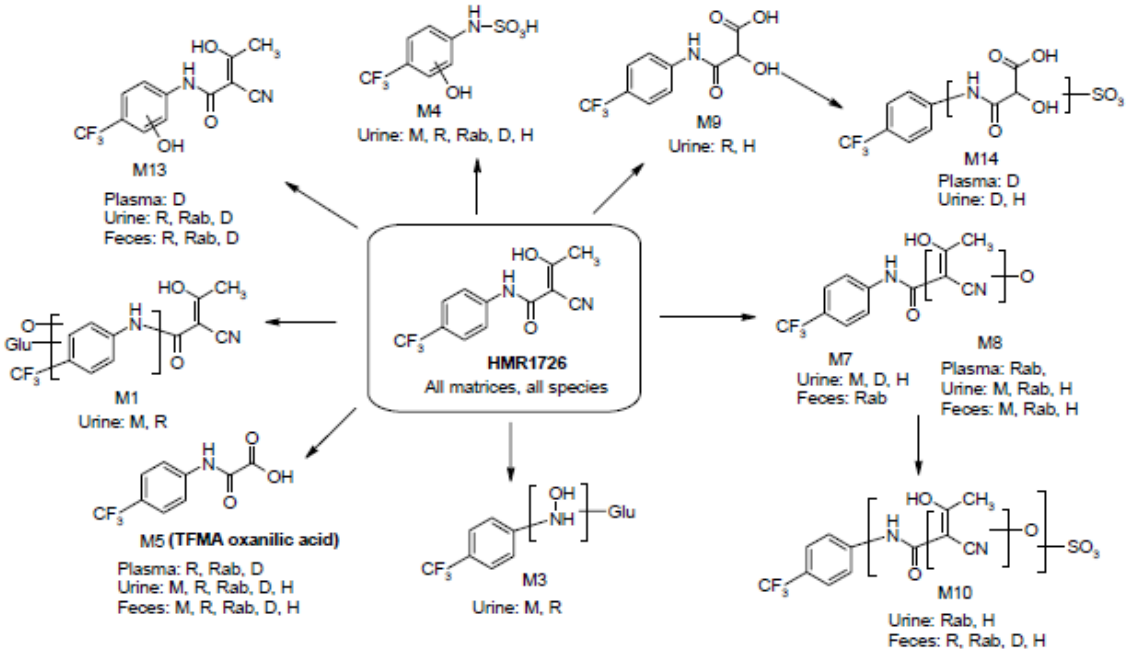
Compound	MW (Da)	Rt (min)	Structure Assignment	Mouse (%dose)	Rat (%dose)	Rabbit (%dose)	Dog (%dose)	Human (%dose in 21 days)
HMR1726	270	~45		65.67	23.02	77.09[c]	29.06[a,b]	35.7
M1	462	~15		0.28	0.07	ND	ND	[d]
M3	353	~25		0.10	0.71	ND	ND	ND
M4	257	~29		0.95	0.50	0.54	3.13	1.3
M5 (TFMA Oxanilic Acid)	233	~32		7.46	43.48	4.79	39.11	18.5
M7	286	~45		[a]	ND	[a]	[a,b]	1.3[a]
M8	286	~58		0.37	ND	0.94	ND	0.4
M9	263	~27		ND	1.62	ND	ND	0.4
M10	366	~34		ND	0.49	4.80	2.17	1.8
M13	286	~45		ND	0.72	[b]	3.10	ND

M14	343	~38		ND	ND	ND	0.37	0.3

a. M7 was coeluted with HMR1726 in urine contributing to <0.3-3.0% of the administered dose.
b. M13 was a minor shoulder peak overlapping with HMR1726 based on HPLC radio-chromatograms and LC/MS.
c. M7 and M13 appeared to be minor metabolites based on LC/MS.
d. M1 was only detected in Subject M1001 at 0.1% of dose.

Based on the results of the metabolism studies, the sponsor proposed the following metabolite pathways in the different toxicology species as well as human (Sponsor's Figure 4).

Figure 4 – Proposed Metabolite Pathways of HMR1726 in Mouse, Rat, Rabbit, Dog, and Human



M: Mouse, R: Rat, Rab: Rabbit, D: Dog, H: Human

Excretion

The sponsor conducted two excretion studies to measure the enterohepatic recirculation of [^{14}C]-teriflunomide in male SD rats and its excretion in milk in female rats. The enterohepatic study is reviewed briefly (below). The sponsor demonstrated the presence of [^{14}C]teriflunomide in SD rat milk (approximately 23.3% of total radioactivity) and its presence of measurable concentrations of teriflunomide in several tissues to include stomach contents, liver, blood, kidney, lung and CNS (see Pharmacology/Toxicology review of SDN#426, dated June 06, 2011).

Study Title: Enterohepatic Recirculation of [^{14}C]HMR1726 in Male Sprague-Dawley Rats

Study No.: AEB0452
In this non-GLP study, the excretion of [^{14}C]teriflunomide was determined in male SD rats. Donor rats were administered a single IV dose of [^{14}C]teriflunomide at a dose of 5 mg/kg; the bile collected from the donor group was intraduodenally administered to a second group of rats (recipient group) that was surgically prepared with an intraduodenal catheter. The results of this study are shown below (sponsor's table).

Mass balance and excretion following single administration of [^{14}C]HMR1726 to male Sprague Dawley rats

Group	Route	Dose (mg/kg)	Urine (0-48h) (%)	Feces (0-48h) (%)	Bile (0-48h) %	Cage Wash (0-48h) (%)
Donor	IV	5	26.4	25.4	12.3	3.72
Recipient	ID	-	19.5	25.3	19.9	2.66

values are rounded to 3 significant figures
IV = intravenous; ID = intraduodenal; h = hours;
- recipient rats each received [^{14}C]HMR1726-derived bile at a radioactive dose of *ca.* 1.2 µCi/rat.

There was a similar recovery (percent of dose) whether teriflunomide was administered IV or ID (67.8% vs. 67.4%, respectively). Following IV administration of teriflunomide, there was approximately 37.7% of the dose recovered in the bile and feces; this suggests that teriflunomide is secreted by the gastrointestinal tract. This was confirmed in the rats that received the intraduodenal administration of donor bile; the mean percent recovered dose in the urine and bile was approximately 40%.

The following table summarizes the excretion data for each of the species used in toxicology studies determined from several independent studies.
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Table 11 - Excretion/balance (mean % recovery of radioactive dose) following single ^{14}C-teriflunomide administration to animals

Species	Route	Dose (mg/kg)	Urine (0-t) (%)	Feces (0-t) (%)	Cage rinse/ wash (0-t) (%)	Carcass (0-t) (%)	Total (0-t) (%)	Study No. Tabulated summary
Mouse	PO	7.5	11.5	70.5	5.80	0	87.8	2005-0046 [TS 2.6.5.17]
	IV	3	9.63	76.8	5.74	0	92.2	
Rat	PO	7.5	51.1	28.5	8.92	1.42	89.9	2005-0047 [TS 2.6.5.18]
	IV	3	39.4	42.7	10.6	1.69	94.4	
Rabbit	PO	7.5	71.9	23.4	4.04	-	99.3	2005-0048 [TS 2.6.5.19]
	IV	3	62.2	27.1	3.44	-	93.0	
Dog	PO	7.5	51.5	37.0	3.10	-	91.6	2005-0049 [TS 2.6.5.20]
	IV	3	28.0	57.0	4.93	-	90.1	

t = 336 hours post-dose except 672 hours post oral dose in dogs.

Abbreviations: PO = per os (oral); IV = intravenous.

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose toxicity studies of teriflunomide were performed in mouse (Study Nos. 011156 and 011160) and rat (Study Nos. 011159 and 011149) for both oral (gavage) and intraperitoneal routes of administration. The estimated mean lethal dose values are provided in sponsor's Table 1.

Table 1 - Single-dose toxicity studies using teriflunomide

Species	Route	LD_{50}, mg/kg
Mouse	PO [011156]	100 to 200
	IP [011160]	100 to 160
Rat	IP [011159]	Approx. 100
	PO [011149]	100 to 200

Abbreviations: PO = per os (oral), IP = intraperitoneal, LD_{50} = median lethal dose

Mice (n=2/sex/group) were treated with teriflunomide (Batch No. Ot 24/2) at a oral dose of 100 or 200 mg/kg (vehicle= potato starch mucilage) and were observed for clinical signs for 21 days; body weight was measured once weekly. No clinical signs or change in body weights were observed in the LD group, whereas HD animals (both sexes) showed signs of reduced motility, bristling fur, squatting, transient trembling, crawling, prone position, palpable slits, colorless tears, and red-brown discolored feces after several days of administration as well as prior to death. Death of all HD animals occurred between Days 6 and 9 following teriflunomide treatment; in some of these

animals, dark red-brown or red discoloration of the bowel contents was noted. Intraperitoneal administration of teriflunomide (Batch No. Ot 24/2) at doses of 100 or 160 mg/kg to mice (n=2/sex/group) resulted in clinical signs in most dosed animals that included reduced motility, trembling gait, watery eyes, gasping, transient trembling, bristling fur, stilted gait, crawling (shaky), prone position, pronounced flank respiration, diarrhea, transient tremor, and red-brown feces. In one LDF, no clinical signs were noted throughout the entire observation period. One LDM had clinical signs on the morning following teriflunomide treatment and prior to death on Day 3; the remaining LD animals showed stilted gait on Days 6-8 post dose. All of the HD animals died within the first three days of the study; clinical signs included diarrhea within 30 min of teriflunomide treatment, reduced motility (within 1 hr postdose), crawling (shaky), and pronounced flank respiration (2-4 hr postdose) that lasted between 2 and 7 hr. One HD animal presented with transient tremor and prone position prior to death. In the LDM survivor, reduced body weight and body weight gain was observed during the first week; however, body weight gain was increased by Week 3.

Rats (Wistar, immature; n=1-2/sex/group) were treated orally with teriflunomide (Batch No. Ot 24/2) at a dose of 100 (2/sex), 200 (1/sex) or 500 mg/kg 1/sex (vehicle = potato starch mucilage). No adverse clinical signs or changes in body weight or body weight gain were observed in LD groups. Clinical signs were observed in both MD and HD groups and included reduced motility, squatting, or diarrhea prior to death. Deaths occurred within 3-5 days postdose. In premature descendants, some animals showed reddening of the gastric mucosa; isolated reddening of the entire intestinal mucosa was observed in one animal. Terminally sacrificed animals had no remarkable macroscopic organ findings. Intraperitoneal administration of teriflunomide (Batch No. Ot 24/2) at doses of 63 or 100 mg/kg to immature Wistar rats resulted in clinical signs at both doses. In the LD group, all animals had edema of the iris in both eyes, which commenced approximately 5 hr postdose and lasted through Day 2 but resolved by Day 3. In the HD group, several clinical signs were observed, including reduced motility, diarrhea, ataxic gait, and bristling fur that occurred within 2 to 9 days postdose. On Days 2 to 5, panting and stilted or trembling gait occurred sporadically. According to the study report, none of the premature decedents had macroscopic findings. In both HDM at terminal sacrifice, the liver was affected; fusions of partly swollen lobes of liver were observed, one HDM had a milky coating on the liver surface and the second HDM had a pinhead-sized deposit that was pale in the large lobe of the liver.

6.2 Repeat-Dose Toxicity

The sponsor conducted several repeat-dose toxicity studies of teriflunomide in mouse, rat, and dog (for a summary of the sponsor's findings, see sponsor's Table 2).

Table 2 - Repeat-dose toxicity studies using teriflunomide

Species	Study Reference	Type of study (duration/route)	Dosage (mg/kg/day)	Effect level	Plasma levels at Effect	
					AUC (µg.h/mL)	C_{max} (µg/mL)
Mouse	[2004-0511]	3 Months, PO	5, 25, 50 or 75	MTD[c]: <25 mg/kg/day	M: <4520 F: <4880	M: <234 F: <263
Rat	[013216], [016607], [016420]	1 Month, IV	3.2, 8, or 20	NOAEL not determined	ND	ND
	[013557], [015156], [016608]	1 Month, IV	0.25 or 1	NOAEL: 0.25 mg/kg/day	M: 3.45 F: 4.06	M: 1.07 F: 1.40
	[017716], [018057]	3 Months, PO	0.5, 1 or 4	NOAEL: 1 mg/kg/day	M: 71.4 F: 69.7	M: 4.7 F: 5.7
	[2003-1492]	6 Months, PO	0.3, 1.5/9 [a], 3 or 6	NOAEL: 0.3 mg/kg/day	M: 26.1 F: 29.4	M: 1.53 F: 1.75
Dog	[013400], [014960], [016612]	1 Month, IV	0.8, 2.5 or 8	NOAEL: 2.5 mg/kg/day	M: 423 F: 558	M: 28.8 F: 37.5
	[017737], [017737A1], [017737A2]	3 Months, PO	0.8, 2.5 or 8	NOAEL: 0.8 mg/kg/day	M: 107 F: 118	M: 6.4 F: 6.7
	[2003-1491]	12 Months, PO	0.2, 0.8 or 2/4 [b]	NOAEL: 0.2 mg/kg/day	M: 26.6 F: 20.2	M: 1.54 F: 1.39

Abbreviations: MTD = maximum tolerated dose; ND = Not determined; PO = per os (oral), IV = intravenous, NOAEL = No-observed-adverse-effect-level, M = Male, F = female, AUC = Area under the curve, C_{max} = maximum concentration
Note: Only plasma levels at the effect level are provided here.
Additional information: Values are rounded to 3 significant values or less.

[a] The 1.5 mg/kg/day dose was increased to 9 mg/kg/day after 107 days due to an apparent lack of toxicity at the 6 mg/kg/day dose.

[b] The 2 mg/kg/day dose was increased to 4 mg/kg/day after 191 days due to an apparent lack of toxicity at the 2 mg/kg/day dose.

[c] NOAEL was not within the objectives of the study. Study purpose was to determine the MTD.

The studies reviewed below include the 30-day repeat intravenous dose study of teriflunomide in rat as well as the pivotal chronic studies performed in rat and dog; discussion of the results of some of the shorter duration studies are included in the integrated summary.

Rat

Study title: A 771726 Testing for subchronic (1 month) intravenous toxicity in male and female Wistar rats

Study no.:	013216
Study report location:	EDR 4.2.3.2.1
Conducting laboratory and location:	Hoechst Aktiengesellschaft Frankfurt, Germany
Date of study initiation:	28 February 1994
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, Batch No. 1-4,

Key Study Findings

- No NOAEL established in this study; dose-dependent mortality occurred
- Target organs identified include: hematopoietic system, liver, lymph nodes, testes.
- Intercurrent deaths caused by presence of bacterial infection (Tyzzer's disease) resulting in lethal cerebral hemorrhages, pan myelopathy of the hematopoietic bone marrow, and damage of the germinal epithelium of the testes.

Methods

Doses:	0 (C), 3.2 (LD), 8 (MD), 20 (HD) mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Intravenous, tail vein
Dose volume:	2 mL/kg
Formulation/Vehicle:	Placebo solution
Species/Strain:	Rat/Wistar
Number/Sex/Group:	15/sex/group
Age:	6 Weeks
Weight:	M, 87-108 g; F, 91-118 g
Satellite groups:	5/sex/group for recovery; however, due to deaths this group was abandoned

Observations and Results

Mortality was examined daily.

- There was high mortality in a teriflunomide dose-dependent manner; males, 0/15, 5/15, 9/15, 15/15 and females, 0/15, 1/15, 3/15, 12/15 for C, LD, MD, HD, respectively.
- 1 MDM and 1HDF were sacrificed moribund.

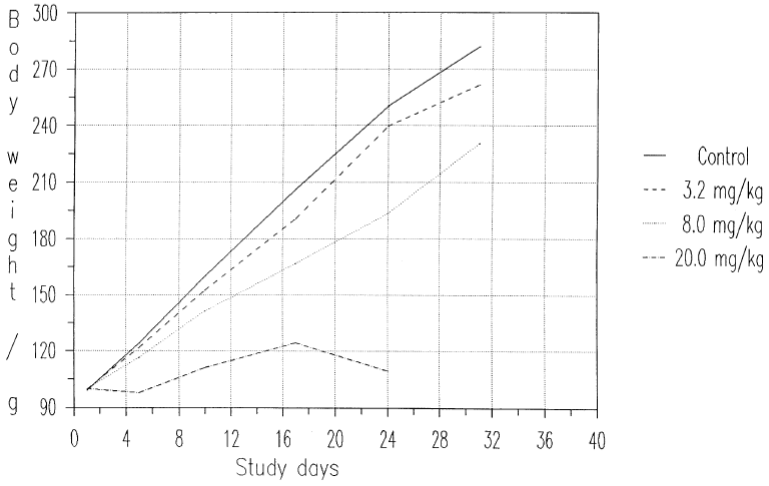
Clinical Signs were monitored daily and in a weekly exam for neurological disturbances, opacity of the refracting media of the eyes, damage to the oral mucosa and impairment of dental growth.

- In a dose-dependent manner, teriflunomide caused signs of intoxication including hypoactivity, bloody feces, pultaceous feces, prone position, general poor condition, poor nutritional state, bristling coat, stilted gait, and pale skin.
- No neurological disturbances, opacity of the eyes, damage to the oral mucosa, or impairment of dental growth were noted in teriflunomide-treated groups.

Body Weights were determined twice in the first week and then weekly throughout the study. The following figures from the sponsor demonstrate the mean results for this study. As illustrated below, significant decreases in body weight were observed in both sexes in MD and HD groups.

REPEATED DOSE (1 MON) I.V. TOXICITY STUDY OF A 77 1726

Body weight development of male rats (94.0100)



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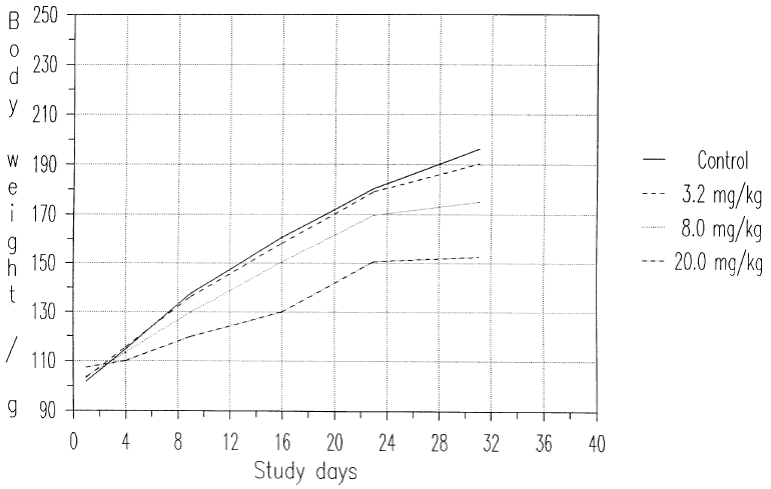
REPEATED DOSE (1 MON) I.V. TOXICITY STUDY OF A 77 1726

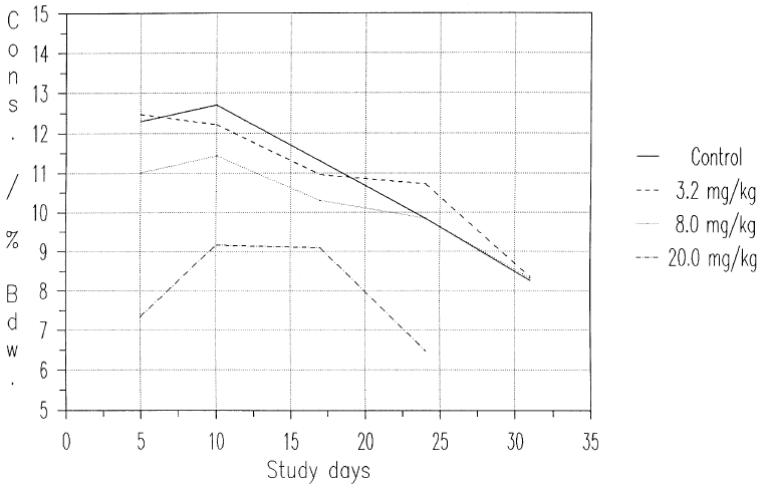
Body weight development of female rats (94.0100)



Food Consumption was determined twice in the first week and once weekly thereafter. The data in the sponsor's figures below represent the food consumed per 100 g body weight over a 24 hr period.

REPEATED DOSE (1 MON) I.V. TOXICITY STUDY OF A 77 1726

Food consumption of male rats (94.0100)



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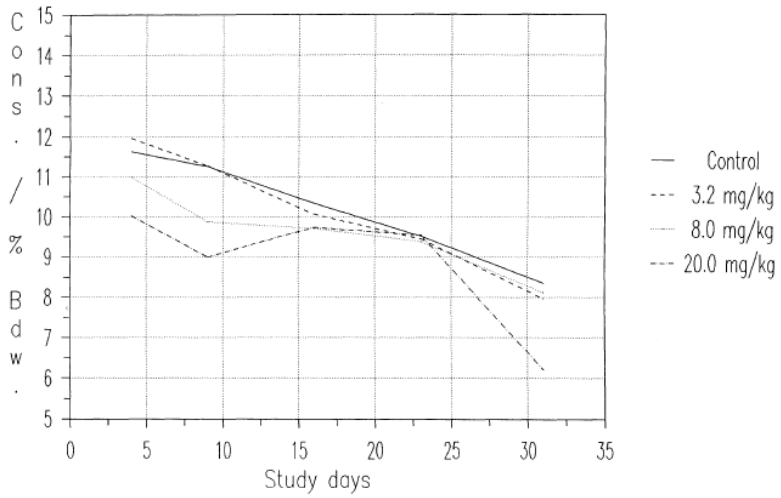
REPEATED DOSE (1 MON) I.V. TOXICITY STUDY OF A 77 1726

Food consumption of female rats (94.0100)



Hematology was performed on all surviving animals at the end of the study. The following parameters were evaluated: RBC count, Hgb, Hct, MCV, MCH, MCHC, Lymphocyte count, platelet count, Differential leukocyte count, RBC morphology, Heinz bodies, coagulation time, and Methemoglobin for MD and HD groups only.

- HDM did not survive to end of study so no hematological data are available; HDF, only 3 survivors.

- In MDM, decreases compared to mean CM were found for Hgb (-9.1%) [ss, p<0.05], Hct (-8.9%) [ss, p<0.05], absolute leukocyte count (-41.8%) [ss, p<0.05], platelet count (-30.8%) [ss, p<0.05], and increased coagulation time (+23.4%).
- In LDM, the following findings were reported: platelet count (-21.4%) [ss, p<0.05], increased coagulation time (+21.1%), and decreased mean leukocyte count (-25.4%) [ss, p<0.05].
- In 3 HDF survivors, several mean erythroid parameters were affected, with marked decreases compared to mean CF values, including: RBC count (-68.3%), Hgb (-62.7%), Hct (-58.1%); as well as increases in MCV (+31.1%) and MCH (+20%), marked increase in Reticulocyte count (+2717%), and the presence of Heinz bodies that were absent in the CF. Decreases in mean leukocyte count (-41.4%) and platelet count (-25.7%) and increases in mean coagulation time (+8.9%) and mean neutrophil count (+143%) were found.
- In MDF, similar findings in erythroid parameters as noted in HDF were observed, including reduced: RBC count (-13.8%) [ss, p<0.05], Hgb (-20.4%) [ss, p<0.05], Hct (-18.6%) [ss, p<0.05], as well as increased reticulocyte count (+200%). Decreases in mean leukocyte count (-39.6%) [ss, p<0.05] and platelet count (-30.8%) [ss, p<0.05], and increases in mean coagulation time (+24.6%) and neutrophils count (+128%) were found.
- In LDF, a slight reduction in mean leukocyte count (-13.8%) compared to CF was found.

Clinical Chemistry was performed on all surviving animals at the end of the study; the following parameters were evaluated: Na+, K+, Ca2+, Cl-, Pi, Uric acid, Bilirubin (total), Creatinine, Glucose, Urea, ASP, ALT, and AP.

- No values were collected for the HDM, due to death.
- In MDM, several notable changes were found, including decreases in Na^+ (-1.4%) [ss, p<0.05], Ca^{2+} (-4.5%) [ss, p<0.05], and total bilirubin (-29.7%) and increases in Cl^- (+2%) [ss, p<0.05], uric acid (+23.3%), creatinine (+18.6%), urea (+13.2%), AST, (+194%), and ALT (+149%).
- In LDM, a few changes were found, including a decrease in total bilirubin (-21.6%) and increases in uric acid (+16.7%), AST (+33.7%), and ALT (+22%).
- In 3 HDF survivors, several notable changes were found, including a decrease in inorganic phosphorus (-12.4%) and increases in K+ (+23.2%), Ca2+ (+5.4%), total bilirubin (+74%), serum glucose (+34.3%), uric acid (+113%), creatinine (+32.1%), urea (+40.8%), AST (+11.2%), and ALT (+7.9%).
- In MDF, several notable changes were found, including increases in K+ (+15%), Ca2+ (+2.1%), total bilirubin (+70%), serum glucose (+8.6%), uric acid (+47.4%), creatinine (+22.6%), urea (+43.5%), AST (+43%), and ALT (+57.9%).
- In LDF, a subset of the notable changes observed in MDF was observed, including increases in K+ (3.3%), uric acid (+10.5%), creatinine (+11.3%), urea (+10.3%), and AST (+12.1%).

Urinalysis was performed on Day 29 for all surviving animals; both food and water were withdrawn during the collection period. The following parameters were examined: Appearance, Color, pH, Hgb, Protein, Glucose, Bilirubin, and Sediment.

- Unremarkable in either sex at any tested dose.

Organ Weights (absolute and relative-to-body weight) were determined for the following organs: heart, lung, liver, kidneys, spleen, testes, ovaries, adrenals, pituitary, thyroid gland, and brain.

- A dose-related decrease in body weight was observed at the end of the study in MD (both sexes) and HDF; teriflunomide-related changes in body-weight corrected values are reported below.

Notable Teriflunomide-related changes in relative organ: body weights

Organ	Mean ± SD		% Control					
	C		LD		MD		HD	
	M	F	M	F	M	F	M	F
Liver	4.11 ± 0.24	4.24 ± 0.38	+13.1	+3.6	+17.4	+9.7		+10.5
Spleen	0.25 ± 0.02	0.27 ± 0.02	+14.2	+7.9	+15.8	+13.5		+42.1
Adrenals	0.017 ± 0.001		+5.2		+23.6			
Pituitary	0.003 ± 0.001		-3.4%		+10.3			
Heart	0.33 ± 0.02	0.36 ± 0.02	+2.7	+17	+7	+2.7		+34.1
Testes	1.12 ± 0.07		+4.4		+17.3			

Histopathology was performed on the following tissues or organs collected from all surviving animals:

Heart, lung, liver, kidneys, spleen, stomach, jejunum, colon, esophagus, duodenum, rectum, vagina, medulla oblongata, skin (with mammary gland), femur, urinary bladder, testes, epididymides, prostate gland, seminal vesicles, ovaries, uterus, thyroid gland, aorta, ileum, diaphragm, sciatic nerve, bone marrow, spinal cord (cervical), injection site, pancreas, adrenal glands, thymus, pituitary gland, brain, eye with optic nerve, sternum, trachea, salivary glands, cecum, skeletal muscle, tongue, knee-joint, and lymph nodes (cervical and iliac)

Adequate Battery Yes

Histological Findings

In the following reviewer-generated table, the incidence of notable teriflunomide-related microscopic findings is provided; this table includes findings from both premature decedents and survivors.

Organ / Finding	C		LD		MD		HD	
	M	F	M	F	M	F	M	F
Heart								
Myocyte, necrosis	0/15	0/15	5/15	2/15	4/15	2/13	2/12	2/12
Tyzzer bacteria	0/15	0/15	4/15	2/15	0/15	1/13	2/12	1/12
Liver								
Parenchymal, necrosis	0/15	0/15	5/15	3/15	9/15	6/13	11/12	7/12
Tyzzer bacteria	0/15	0/15	5/15	0/15	7/15	4/13	10/12	7/12
Fatty change	0/15	0/15	0/15	0/15	3/15	1/13	0/12	3/12
Bone Marrow								
Hematopoiesis, absent	0/15	0/15	1/11	0/13	0/8	0/8	4/6	0/2
Hematopoiesis, ↓	0/15	0/15	0/11	0/13	3/8	0/8	2/6	1/2
Sternum								
Hematopoiesis, ↓	0/15	0/15	1/14	1/15	10/15	3/13	12/12	9/12
Femur								
Hematopoiesis, ↓	0/15	0/15	2/15	1/15	10/15	3/13	12/12	10/12
Lymph Node, Cervical								
Sinus, histiocytosis	0/15	0/15	3/15	1/15	2/15	1/13	3/11	1/10
Congestion, partial	1/15	0/15	2/15	0/15	5/15	1/13	2/11	0/10
Follicles, hyperplastic	0/15	0/15	1/15	0/15	0/15	1/13	1/11	0/10
Tyzzer bacteria	0/15	0/15	0/15	0/15	0/15	0/13	1/11	0/10
Lymph Node, Iliac								
Sinus, histiocytosis	0/15	0/14	5/15	1/15	7/14	3/13	8/10	1/12
Spleen								
Pulpous hyperplasia	0/15	0/15	1/15	0/15	8/15	2/13	12/12	12/12
↑ Phagocytes	0/15	0/15	0/15	0/15	8/15	3/13	11/12	12/12
↑ Extramedullary hematopoiesis	0/15	0/15	2/15	2/15	1/15	4/13	0/12	0/12
Lungs								
Edema, congestion	0/15	0/15	1/15	0/15	5/15	1/13	1/12	0/12
Intravasal, round cells	0/15	0/15	0/15	0/15	0/15	0/13	5/12	0/12
Cerebrum								
Cortical hemorrhage	0/15	0/15	0/15	0/15	1/15	0/13	0/12	0/12
Cerebellum								
Hemorrhage, focal	0/15	0/15	0/15	0/15	1/15	0/13	0/12	0/12
Medulla Oblongata								
Hemorrhage, focal	0/15	0/15	0/15	0/15	1/15	0/13	0/12	1/8
Spinal Cord								
Hemorrhage, subdural	0/15	0/15	0/15	0/15	1/15	0/13	0/12	1/10
Testes								
Spermatogenesis, none	0/15		1/15		0/15		1/12	
Incipient, degeneration	0/15		0/15		3/15		5/12	
Tubular, necrosis	0/15		0/15		3/15		1/12	
Epididymides								
Oligospermia	0/15		1/15		3/15		2/12	
Aspermia	0/15		1/15		2/15		7/12	

Organ / Finding	C		LD		MD		HD	
	M	F	M	F	M	F	M	F
Intralumen detritis	0/15		0/15		1/15		8/12	
Granuloma, spermatic	0/15		0/15		0/15		1/12	
Prostate								
Immature	0/15		2/12		8/14		12/12	
Seminal vesicles								
Immature	0/15		2/12		7/13		11/11	
Uterus								
Juvenile status		0/15		0/15		0/13		3/12

In this study, a predominant finding was liver toxicity. Many of the animals that died prematurely had marked to severe liver necrosis that was often accompanied by the presence of Tyzzer bacteria. In survivors with liver toxicities, parenchymal necrosis was present; however, the severity was lessened. It is noteworthy that the presence of the Tyzzer bacteria was not necessarily lethal as it was often found in survivors and, at times, it was present in multiple organs (e.g., liver, heart, and/or lymph node), but it was absent in control animals.

- Control—no liver findings were present in either sex; Tyzzer bacteria were absent.
- LD—Males, 5/15 died on study (DOS). In 3 of 5 males, there was marked to severe parenchymal necrosis, slight to marked Tyzzer bacteria, and in one animal marked myonecrosis in the heart; 2/5 had no liver toxicity, but one had decreased hematopoiesis in the femur and the second had marked decreased hematopoiesis in both the sternum and femur. Two of 10 survivors had moderate parenchymal necrosis of the liver, slight to moderate Tyzzer bacteria, and in one animal, there was Tyzzer bacteria (slight) present in the heart where there was myonecrosis.
- LD—Females, 1/15 DOS (no liver toxicity present, markedly decreased hematopoiesis in both femur and sternum), 3/14 survivors had slight to moderate parenchymal necrosis of the liver and two had slight to moderate Tyzzer bacteria present and in both of these cases there was an associated finding in the heart of marked to massive myonecrosis.
- MD—Males, 9/15 DOS (7/9 had moderate or massive parenchymal necrosis of the liver; 2/9 had markedly or massively decreased hematopoiesis in bone marrow or sternum/femur and one also had a fatty liver as well as focal hemorrhage in the cerebellum and spinal cord). Two of the 6 survivors had liver parenchymal necrosis that was moderate to marked in severity; both had moderate Tyzzer bacterial presence.
- MD—Females, 3/15 DOS (1/3 had massive parenchymal necrosis of the liver, the remaining 2/3 animals were not examined). Of the 12 survivors, there were 5 animals that presented with parenchymal necrosis of the liver that ranged in severity from slight to marked. Of the 5 affected survivors, 2 had slight to minimal parenchymal necrosis of the liver without Tyzzer bacteria present, 1 had slight liver parenchymal necrosis and minimal Tyzzer bacteria present, and 2 had

moderate to marked liver parenchymal necrosis in the presence of moderate Tyzzer infection and slight to marked reduction in hematopoiesis of the sternum and femur.

- HD—Males, 15/15 DOS (3 did not undergo microscopic evaluation; 11/12 had a liver finding of parenchymal necrosis—in many affected animals, the severity was marked to massive. One was of minimal severity, but this animal had significant bone marrow suppression (i.e., hematopoiesis was absent from both femur and sternum). The remaining HDM had no liver findings; however, several focal hemorrhages were noted in the medulla oblongata and spinal cord as well as marked bone marrow suppression.
- HD—Females, 12/15 DOS (8/12 underwent microscopic evaluation; 6/8 had parenchymal necrosis ranging in severity from slight to massive, many of these had concomitant presence of Tyzzer bacteria and/or bone marrow suppression. Of the 3 survivors, one had moderate parenchymal necrosis of the liver and minimal Tyzzer bacteria, one had slight impairment of hematopoiesis in the femur, and in one animal liver and bone marrow were unaffected.

Toxicokinetics were determined by collection of blood 24 hrs after the last dose (Day 30). There was no teriflunomide detected in the control group, the concentrations of teriflunomide present in the dosed groups are provided in sponsor's tables, below.

Group 2: 3.2 mg kg^{-1}

Rat No. (Male)	A77 1726 Serum Concentration (µg ml^{-1})	Rat No. (Female)	A77 1726 Serum Concentration (µg ml^{-1})
31	2.66	46	1.13
32	1.23	47	1.90
33	1.92	48	3.55
34	2.73	50	1.28
39	0.95	51	0.93
40	1.91	52	1.84
41	1.45	53	0.87
42	1.33	54	0.77
43	6.13	55	1.22
44	1.53	56	1.32
		57	0.22
		58	1.58
		59	1.12
		60	0.54

TABLE 3: **Study: 94.0100**

Group 3: **8 mg kg^{-1}**

Rat No. (Male)	A77 1726 Serum Concentration (µg ml^{-1})	Rat No. (Female)	A77 1726 Serum Concentration (µg ml^{-1})
66	0.89	76	1.67
68	0.53	77	1.01
69	2.07	78	3.30
71	0.74	79	1.35
73	1.67	80	2.55
74	18.7	82	0.91
		84	0.82
		86	0.56
		87	0.66
		88	0.46
		90	0.90

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Group 4: **20 mg kg^{-1}**

Rat No. (Female)	A77 1726 Serum Concentration (µg ml^{-1})
110	0.33
112	1.76
117	ND*

Study title: 6-Month Oral Toxicity Study of HMR1726 in Rats

Study no.: 2003-1492
Study report location: EDR 4.2.3.2.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 02 March 2004
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Teriflunomide, Lot #W001, 99.1% purity

Key Study Findings

- Lymphoid organs and tissues (thymus, gut-associated lymphoid tissue, lymph nodes [mandibular and mesenteric], and spleen) were the primary targets identified in rat.
    - The NOAEL established for these findings was 0.3 mg/kg/day, which corresponds to a C_{max} of 1.53 µg/mL (male) and 1.75 µg/mL (female) and AUC of 26.1 µg.hr/mL (male) and 29.4 µg.hr/mL (female). It is noted that

the level of 4-TFMA present at the NOAEL was < lower level of quantitation (<0.5 ng/mL).
- Most of the adverse findings appeared to resolve following a 28-day recovery period; however, the increase in pigment in the spleen did not resolve.

Methods

Doses:	0 (C), 0.3 (LD), 1.5/9.0* (HD), 3.0 (MD1), 6.0 (MD2) mg/kg/day
	*On Study Day 107, the 1.5 mg/kg/day dose was increased to 9.0 mg/kg/day for the remaining 75 days, due to lack of toxicity at 6 mg/kg/day.
Frequency of dosing:	Once daily
Route of administration:	Oral (gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	2% potato mucilage
Species/Strain:	Rat/Sprague Dawley Crl:CD®(SD)
Number/Sex/Group:	15/sex/group
Age:	6 Weeks
Weight:	M, 159-194 g; F,
Satellite groups:	Control 3/sex/grp; Dosed groups 12/sex/grp Sampling Days 0, 27, 90, and 181
Unique study design:	4 Week Recovery grp (n=5/sex/grp) for 0, 3, and 6 mg/kg/day
Deviation from study protocol:	None

Observations and Results

Mortality was checked daily.

- There were 7 unscheduled deaths on study including 1 CM (#50568), 1 MD1M (#50496), 1 MD2F (#50750), and 4 HDM (#50465, 50499, 50530, and 50580).
  - Of these, only the 2 HDM deaths (#50465 found dead [Day 163] and #50530 moribund [Day 176]) were considered teriflunomide-related.
    - Both deaths were related to changes observed in the bone marrow, intestinal tract, and lymphoid tissue.
      - BM (femur and sternum): Marked decrease in hematopoietic cells.
        - Related secondary effects were mild hemorrhage in lung and mild to moderate necrosis in liver (centrilobular, single cell and/or focal)
      - Intestinal tract: Moderate to marked mucosal atrophy characterized by loss of glandular (crypt) epithelium and villous atrophy.
      - LN (GALT): Absence of germinal centers within lymphoid follicles.

- Thymus: atrophy, marked to severe.
- Spleen: increased pigment consistent with hemosiderin.

- The other deaths were consistent with dosing errors as noted by findings of moderate or marked hemorrhage in the lung and/or mediastinum suggesting gavage dosing trauma.
  - A teriflunomide-related finding was the absence of germinal centers in lymph nodes in these animals.

Clinical Signs were checked daily; once prior to initiation of dosing, daily at time of dosing, and within 1-2 hr postdose.

- In the 2 HDM premature decedents, clinical signs included hypoactivity, unkempt appearance, partial/complete eye closure, body and body extremities cool to touch and/or pale, decreased defecation/small feces, and red, yellow, or clear discharge around the eyes.
- In survivors, dose-related increases in hyperactivity were noted within 1 hr postdose in males and females; however, this sign was not present in the recovery groups of C, MD1, and MD2 of either sex.

	Hyperactivity—1 hr Postdose (Occurrence/Incidence)				
Sex	**C**	**LD**	**MD1**	**MD2**	**HD**
M	0/0	0/0	1/1	2/2	11/7
F	0/0	3/2	2/2	7/6	5/4

Body Weights were recorded weekly. Prior to necropsy of dosing and recovery groups, fasted animal body weights were recorded (main study survivors, only).

- In males, mean body weight was unaffected by teriflunomide.
- In females, mean body weight was decreased in both HD and MD2 groups in a time- and dose-dependent manner. Loss of mean body weight of more than 10% was observed in HDF at Week 18 and persisted to the end of dosing (maximum loss at Week 26 of -15%), and in MD2F at Week 21 and to the end of dosing (Week 26 = -11.7%). In the MD2F recovery group, there was no significant reduction in mean body weight at either Week 27 or 28.

Food Consumption was recorded weekly**.**

- In males, a significant decrease in food consumption at the HD occurred during Weeks 16 to 19; however, this did not appear to affect body weights.
- In females, there was a reduction in food consumption in HDF (-10 to -15%) compared to controls from Weeks 15-16 through the end of the study.

Ophthalmology was performed once prior to study initiation and during the 12th and 25th week of treatment using an indirect ophthalmoscope and a biomicroscope (slit lamp). Examinations were performed by (b) (4) D.V.M., M.S, D.A.C.V.O.

- Unremarkable, no treatment-related findings were present.

ECG was not performed in this study.

Hematology was evaluated at Weeks 12, 26, and 30 (recovery) for the standard battery of parameters including: RBC count, Hgb, Hct, MCH, MCHC, MCV, Total and Absolute Diff WBC count, Absolute Ct and percent Retic, Plt count, RBC morphology*, Heinz bodies*. (*Evaluations were performed in the control and HD and MD2 groups.) Coagulation parameters evaluated were PTT and APTT.

- In both sexes, teriflunomide treatment decreased RBC count, reduced Hgb, MCH, and MCHC, as well as increased absolute reticulocyte counts (see reviewer-generated table, below).

Teriflunomide responses at Week 26 (percent change from Control[1])								
RBC Parameter	C		MD1		MD2		HD	
	M	F	M	F	M	F	M	F
RBC Count	8.84	7.77	+0.6%	+1.0%	+0.4%	+1.1%	-9.6%	-4.5%
Hgb	15.6	14.7	-2.6%	↔	-4.5%**	+1.4%	-14.1%	-10.9%
MCH	17.6	19.0	-2.3%	-1.0%	-5.6%*	-4.7%	-4.0%*	-6.3%**
MCHC	33.0	33.8	-1.8%	-1.2%	-3.0%*	-3.2%*	-3.0%**	-4.1%**
Retic Absolute	154.3	137.3	+15.2%	+12.1%	+20%	+40.5%**	+43.7%**	+71.8%**

[1]Mean Control values provided; *[ss, p<0.05], **[ss, p<0.01] compared to mean Control
↔ No change from Control value

Clinical Chemistry was evaluated at Weeks 12, 26, and 30 (recovery) for the standard battery of parameters including: ASP, ALT, AP, Total Chol, Trig, Alb, Total Prot, Globulin, A/G ratio, Total Bili, Glu, Creatinine, Na+, K+, Cl-, Ca2+, BUN, and Pi.

- At MD1, MD2, and HD (both sexes), there was a significant reduction in globulins. Likewise, a significant reduction in total protein was observed at the MD1 (males), MD2 and HD (both sexes); these data are provided in the reviewer-generated table, below.
- The significant reduction of globulin and total protein observed, either recovered or was returning to baseline values at the end of the 28-Day recovery period.
-

	Group Mean		% Control							
	C		LD		MD1		MD2		HD	
	M	F	M	F	M	F	M	F	M	F
Week 12										
Globulin	2.7 ± 0.25	2.5 ± 0.19	-3.7	-12[b]	-11.1[b]	+28[b]	-14.8[b]	+36[b]	-11.1[b]	-24[b]

	Group Mean		% Control							
	C		LD		MD1		MD2		HD	
	M	F	M	F	M	F	M	F	M	F
Total Protein	7.0 ± 0.31	7.4 ± 0.4	100	-4	-5.7[b]	-6.8[b]	-8.6[b]	-12.2[b]	-4.3[a]	-4
A/G Ratio	1.59 ± 0.18	1.97 ± 0.26	+6.9	+15.7[a]	+11.3[b]	+44.7[b]	+12.6[b]	+54.3[b]	+10.1[a]	+44.2[b]
Week 26										
Globulin	3.1 ± 0.28	2.9 ± 0.18	-9.7[b]	-6.9[a]	-22.6[b]	-27.6[b]	-29[b]	-37.9[b]	-29[b]	-34.5[b]
Total Protein	7.5 ± 0.27	8.4 ± 0.55	-5.3[b]	-1.2	-10[b]	+4.8	-14.7[b]	-13.1[b]	-12[b]	-8.3[b]
A/G Ratio	1.41 ± 0.18	1.94 ± 0.27	+9.2	+9.8	+30.5[b]	+47.9[b]	+34.8[b]	+57.2[b]	+39[b]	+56.7[b]
Week 30 (Recovery)										
Globulin	3.0 ± 0.39	2.7 ± 0.17			-6.7	-3.7	-6.7	-14.8		
Total Protein	7.2 ± 0.36	8.3 ± 0.44			+1.4	+3.6	-1.4	-6		
A/G Ratio	1.45 ± 0.28	2.07 ± 0.26			+13.8	+12.1	+11.3	+19.8		

[a][ss, p<0.05]; [b][ss, p<0.01] from control

Urinalysis was performed on samples collected overnight from metabolism cages during study Weeks 12, 26, and 30 for the following parameters: Bili, Creatinine, Glu, Ketones, Osmol, pH, K+, Prot, Na+, Urobili, Color, Appearance, Volume, Occult blood, and microscopic examination of urine sediment.

- At MD1 (females), MD2 and HD (both sexes), there was a significant reduction in urine potassium and urine creatinine. After a 28-Day recovery period, the reduction in urine potassium and urine creatining in MD1 and MD2 females was lessened; however, in males there was a greater reduction observed (see reviewer-generated table, below).

	Group Mean±SD		% Control							
	C		LD		MD1		MD2		HD	
	M	F	M	F	M	F	M	F	M	F
Week 26										
Urine Potassium (mEq/L)	190.9 ± 81.3	203.4 ± 92.6	+6.2	-16.8	-1.8	-32.4[a]	-19.8	-51.4[b]	-37.3	-38.3[a]
Urine Creatinine (mg/dL)	144.4 ± 54.5	129.6 ± 64.7	+10	-21.7	+1.1	-29[a]	-14.5	-47.1[b]	-30.2	-39.7[b]
Week 30 (Recovery)										
Urine Potassium (mEq/L)	199.4 ± 108.5	189.1 ± 43.2			-29.8	-19.7	-9.7	-20.6		

	Group Mean±SD		% Control							
	C		LD		MD1		MD2		HD	
	M	F	M	F	M	F	M	F	M	F
Urine Creatinine (mg/dL)	227.0 ± 152.3	142.1 ± 28.1			-34.5	-19.8	-34.2	-29.1		

[a][ss, p<0.05]; [b][ss, p<0.01] from control

Organ Weights (absolute and relative weights) were recorded for terminally sacrificed animals. Animals that were euthanized moribund were weighed but excluded from summary statistics, and organs were not weighed for animals found dead on study. The following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, ovaries (with oviducts), pituitary gland, prostate (with seminal vesicles), spleen, testes, thymus, and thyroid gland (with parathyroids). Paired organs were weighed together.

- Absolute and relative thymus weights were reduced significantly at the MD1, MD2, and HD in both males and females; mean absolute and relative thymic weights were similar to or greater than C in both sexes following a 4-week drug-free period (see reviewer-generated table, below).

	Mean± SD		% Control							
	C		LD		MD1		MD2		HD	
	M	F	M	F	M	F	M	F	M	F
THYMUS										
26-Wk Absolute (g)	0.182 ± 0.059	0.182 ± 0.059	+0.01	-0.7	-24.1	-40.9	-46.3	-42.9	-57.6	-60.9
26-Wk Rel/Bwt (g/100g)	0.031 ± 0.009	0.056 ± 0.015	100	+5.4	-25.8	-33.9	-41.9	-33.9	-54.8	-53.6
Recovery Absolute (g)	0.169 ± 0.037	0.142 ± 0.042			-8.2	+25.7	+6.4	+37.9		
Recovery Rel/Bwt (g/100g)	0.030 ± 0.008	0.045 ± 0.016			-13.3	+15.6	+6.7	+44.4		

[a][ss, p<0.05]; [b][ss, p<0.01] from control

Histopathology: all main study and recovery animals were subjected to macroscopic examination and collection of a standard battery of tissues. Microscopic examination was performed on all slides from C, MD2, and HD groups, as well as all those found dead or euthanized in extremis and any gross lesion was examined microscopically. Tissues with potential treatment-related findings were evaluated in the recovery animals (0, 3, and 6 mg/kg/day).

Adequate Battery of tissues was evaluated and the pathology report is considered complete, as it was signed by (b) (4) D.V.M.

Peer Review was conducted by (b) (4) D.V.M, Ph.D.

Histological Findings

As shown in the reviewer-generated table, there were several teriflunomide-related changes observed in the main study animals. Most of the findings occurred primarily in immune-related tissues including the gut-associated lymphoid tissue (GALT) of the ileum, lymph nodes (LN, mandibular and mesenteric), and spleen in MD1, MD2, and HD groups. In the spleen, there was a high incidence of hemosiderin pigment present in females of control and treated groups; however, in males, teriflunomide increased the incidence of hemosiderin in the spleen. In males, MD and HD had liver necrosis that was focal or single cell.

Main Study

Incidence of Microscopic Pathology										
Tissue Finding	**C**		**LD**		**MD1**		**MD2**		**HD**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Immune-related findings										
Ileum—GALT Germinal centers—absent	0/15	0/15	1/15	1/15	8/15	10/15	10/15	11/15	10/11	13/15
Lymphoid atrophy	0/15	1/15	0/15	2/15	0/15	2/15	0/15	2/15	0/15	0/15
Lymph Node Mandibular Germinal centers—absent	2/15	0/15	2/15	0/15	12/15	5/15	15/15	12/15	10/11	15/15
Mesenteric Germinal centers—absent	6/15	3/15	7/15	1/15	10/15	8/15	14/15	8/15	10/11	13/15
Lymphoid hyperplasia	3/15	1/15	2/15	5/15	11/15	8/15	10/15	7/15	7/11	7/15
Atrophy	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	1/11	0/15
Spleen Lymphoid atrophy	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	1/11	0/15
↓ Mantle zone	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	3/11	0/15
Hemosiderin pigment	8/15	15/15	12/15	15/15	13/15	15/15	12/15	12/15	11/11	15/15
Thymus Lymphoid atrophy	1/15	1/15	0/15	1/15	3/15	15/15	8/15	12/15	9/11	15/15
Non-Immune-related finding										
Liver Necrosis, single cell	0/15	0/15	0/15	0/15	0/15	0/15	2/15	0/15	1/11	0/15
0/15		0/15	0/15	0/15	0/15	0/15	1/15	0/15	1/11	0/15

Shaded boxes indicate each finding noted in a separate animal; therefore, the total incidence of necrosis in liver of MD2 is 3/15 and of HD is 2/11 males.

Recovery

Incidence of Microscopic Pathology						
Tissue Finding	C		MD1		MD2	
	M	F	M	F	M	F
Immune-related findings						
Ileum—GALT Germinal center—hyperplasia	0/4	0/5	2/4	1/5	5/5	3/4
Lymph Node Mandibular Germinal center—absent	0/4	1/5	0/4	0/5	0/5	0/4
Germinal center—hyperplasia	0/4	0/5	0/4	5/5	0/5	2/4
Lymphoid hyperplasia	0/4	0/5	0/4	0/5	0/5	1/4
Mesenteric Germinal centers—absent	2/4	2/5	1/4	1/5	0/5	0/4
Spleen Hemosiderin pigment	4/4	5/5	3/4	3/5	5/5	4/4
Thymus Lymphoid hyperplasia	0/4	0/5	0/4	0/5	2/5	0/4
Non-Immune-related finding						
Liver Necrosis, single cell	0/1	NE	NE	NE	NE	NE

NE = Not Examined

Toxicokinetics

The toxicokinetics of teriflunomide for each of the treatment groups is shown in sponsor's table, below.

Table 5 - 6-Month Oral Toxicity Study in Rats: Toxicokinetics Summary

			Teriflunomide		4-TFMA	
Species, Dosing Day, Number	**Dose (mg/kg/day)**	**Sex**	**C_{max} (µg/mL)**	**AUC_{0-24h} (µg.h/mL)**	**C_{max} (ng/mL)**	**AUC_{0-24h} (ng.h/mL)**
Rat [2003-1492] Day 181 (6-month study) 3 rats per sex	0.3	M	1.53	26.1	*b*	*b*
		F	1.75	29.4	*b*	*b*
	1.5/9.0 [a]	M	40.3	487	6.79	105
		F	48.7	568	4.64	70.4
	3.0	M	19.8	240	2.56	41.5
		F	21.4	330	1.41	24.8
	6.0	M	33.3	342	4.40	70.9
		F	32.7	418	3.07	48.0

[a] The 1.5 mg/kg/day dose was increased to 9 mg/kg/day after 107 days due to an apparent lack of toxicity at the 6 mg/kg/day dose.

[b] Not analyzed since concentrations were below LLOQ (0.500 ng/mL) on Day 0

Abbreviations: M = Male, F = Female, C_{max} = maximum concentration, AUC = Area under the curve, 4-TFMA = 4-trifluoro-methylaniline

Additional information: Values are rounded to 3 significant values or less.

Dog

Study title: 12-Month Oral (Capsule) Toxicity Study of HMR1726 in Dogs

Study no.: 2003-1491

Study report location: EDR

Conducting laboratory and location: (b) (4)

Date of study initiation: 05 February 2004

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Teriflunomide, Lot # U001, 99.1-100.2% purity

Methods

Doses:	0 (C), 0.2 (LD), 0.8 (MD), 2.0/4.0* (HD) mg/kg/day *Dosed for 191 days at 2 mg/kg/day; 174 days at 4 mg/kg/day—dose was increased due to lack of toxicity
Frequency of dosing:	Once, daily
Route of administration:	Oral
Formulation/Vehicle:	Capsule
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4/sex/group
Age:	15-16 months
Weight:	M, 10.5-14.6 kg; F, 6.3-11.7 kg
Deviation from study protocol:	Raw data for ECG for study Weeks -2 and 12 were not retained; the sponsor did not consider this deviation to affect adversely the integrity of this study.

Observations and Results

Mortality was checked twice daily.

- 1 HDF (#0029) was euthanized *in extremis* on Study Day 248 (Weeks 35-36), approximately 57 days following dose increase from 2 to 4 mg/kg/day.
    - Prior to euthanasia, the following clinical signs were observed in this animal: increased body temperature and lethargy, inappetance (correlated with decreased food consumption), red material on cage floor, red mucoid feces, discharge around left eye, increased salivation and excessive drooling, ears cool to touch and pale, reddened ears or gums, decreased defecation and/or diarrhea.
    - Body weight was decreased by 1.9 kg over a period of 5 weeks prior to euthanasia.
    - Food consumption was 10% of what was offered in Weeks 35-36.
    - Bone marrow smear (femur) showed normal erythroid and myeloid precursors.
    - Hematology
        - At necropsy, RBC count, Hgb and Hct were all decreased 33-39%; both MCH and MCHC were reduced and there were mild increases in MCV and absolute reticulocytes.
        - Platelet count was decreased compared to Week 25 values (-43%), lymphocyte count was decreased by -30% and monocytes were markedly reduced by -73%.
        - Blood was evaluated for infectious organism in a non-GLP laboratory, and it was found to be negative.

- All other animals survived to scheduled termination.

Clinical Signs were performed once prior to initiation of dosing, and once daily during the dosing period (approximately 3 ± 0.5 hr postdose). The reviewer-generated table below summarizes the group incidence and number of occurrences; the following clinical signs were observed as noted briefly, below.

- At a detailed clinical exam, two clinical signs were noted: wet material around the mouth and cold to touch.
- At the time of dosing, additional clinical signs were noted. The cardiopulmonary signs, such as, ears—cool to touch, and clear discharge of the left and/or right eye, wet clear material around the mouth and/or ventral neck, reddened gums or pale gums.
- Approximately 3 hr postdose, behavioral/CNS signs included partial eye closure; clear discharge from eye, left and/or right, soft feces, wet material around the mouth and reddened gums.

Summary of clinical signs observed at detailed exam, at time of dosing or 3 hr postdose

Incidence of Clinical Signs Affected/group (#occurrences)								
Type / Clinical Sign	**C**		**LD**		**MD**		**HD**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Detailed Clinical Exam								
Oral/Dental								
Mouth, wet, clear discharge	1/4 (9)	0/4 (0)	2/4 (19)	1/4 (10)	4/4 (51)	1/4 (3)	3/4 (23)	4/4 (28)
Ventral neck, wet clear discharge	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	2/4 (10)
Cardiopulmonary	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Cold to touch	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(10)
At Dosing								
Cardiopulmonary	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Ears, cold to touch	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(6)
Eyes/Ears/Nose								
Eyes, discharge, clear, left	0/4 (0)	2/4 (3)	1/4 (1)	2/4 (11)	3/4 (13)	1/4 (69)	1/4 (58)	2/4 (66)
Eyes, discharge, clear, right	2/4 (2)	2/4 (9)	2/4 (19)	1/4 (1)	2/4 (7)	2/4 (86)	2/4 (67)	2/4 (3)
Oral/Dental								
Mouth, wet, clear discharge	2/4 (6)	1/4 (1)	1/4 (6)	1/4 (1)	4/4 (32)	1/4 (1)	3/4 (21)	4/4 (26)
Ventral neck, wet clear discharge	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	2/4 (6)	1/4 (1)	0/4 (0)	2/4 (10)
Gums, reddened	1/4 (2)	0/4 (0)	2/4 (16)	3/4 (44)	3/4 (22)	3/4 (5)	0/4 (0)	2/4 (10)
Gums, pale	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	1/4 (1)	0/4 (0)	2/4 (3)
Postdose (3 hr)								
Eyes/Ears/Nose	0/4 (0)	0/4 (0)	0/4 (0)	1/4 (2)	0/4 (0)	1/4 (8)	0/4 (0)	1/4 (14)

Incidence of Clinical Signs Affected/group (#occurrences)								
Type Clinical Sign	**C**		**LD**		**MD**		**HD**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Eye, discharge, left	1/4 (1)	1/4 (4)	2/4 (3)	1/4 (15)	0/4 (0)	1/4 (8)	0/4 (0)	1/4 (14)
Eye, discharge, right	3/4 (5)	2/4 (8)	4/4 (18)	1/4 (4)	2/4 (6)	2/4 (85)	0/4 (0)	1/4 (14)
Excreta Feces, soft	0/4 (0)	2/4 (6)	1/4 (5)	2/4 (10)	1/4 (5)	4/4 (10)	3/4 (6)	4/4 (13)
Oral/Dental Mouth, wet, clear discharge	3/4 (4)	1/4 (2)	2/4 (10)	2/4 (8)	4/4 (79)	1/4 (6)	4/4 (45)	4/4 (55)
Gums, reddened	0/4 (0)	1/4 (1)	2/4 (23)	3/4 (33)	3/4 (10)	2/4 (12)	1/4 (4)	3/4 (7)

Body Weights were recorded once weekly. On the day prior to necropsy, a non-fasted body weight was recorded and on day of necropsy, a fasted body weight was reported.

- In males, effect on group mean body weight was unremarkable at the end of the main study; however, mean body weight gain over the course of the study (Week 0 to 52) was reduced by 50% at the HD.

- In females, mean body weight at the HD was decreased >10% from Week 32 onward. Even after the euthanasia of HDF, #0029 between Weeks 35-36, the mean body weight of HDF was reduced by >10%. At Week 52, the mean body weight of HDF was -17.8%.

Food Consumption was recorded once weekly.

- Unremarkable, for most of the animals on study; however, HDF (#0029, as noted above) had reduced food consumption during Week 33 that was <10% of that offered.

Ophthalmology was performed once prior to study initiation and during Weeks 13, 26, 39, and 52 of treatment using an indirect ophthalmoscope and a biomicroscope (slit lamp). Examinations were performed by (b) (4), D.V.M., M.S, D.A.C.V.O.

- Unremarkable, there were no teriflunomide-related findings.

ECG was performed using a multi-lead electrocardiogram (leads I, II, III, aVR, aVL, aVF, rV2, V2, and V10) once prior to study initiation and again during study Weeks 12, 25, 38, and 51 within 2-4 hr postdose. The following parameters were evaluated by (b) (4) D.V.M., Ph.D., DACVIM, Veterinary Cardiologist.

- Unremarkable, there were no teriflunomide-related findings.

Hematology was evaluated prior to randomization (study Week -1) and on study Weeks 12, 25, 38, and 51 for the standard battery of parameters including: RBC count, Hgb, Hct, MCH, MCHC, MCV, methemoglobin@, Total and Absolute Diff WBC count,

Absolute Ct and percent Retic, Plt count, RBC morphology*, Heinz bodies*. Coagulation parameters evaluated were PTT and APTT. @Analyzed at Week 51 and presented in special chemistry tables. *Evaluations were performed in C and HD groups.

- In Week 38, a few notable parameters that reached statistical significance were found, including:
  - HDM
    - Increased mean reticulocyte count +108.6% [ss, p<0.01]
    - Decreased mean MCHC to -3.5% of control [ss, p<0.01]
    - Decreased mean lymphocyte count (absolute) by -20.8% of control [nss]
    - Basophils were absent
  - HDF
    - Lymphocyte count (absolute) was reduced (-37% [ss, p<0.01]).

- At end of study (Week 51), several erythrocyte parameters as well as other cell types were affected in HD survivors (see reviewer-generated table, below).

Incidence of Notable Hematological Parameters—Week 51

Parameter	Control (Mean±SD)		% Control					
	C		LD		MD		HD	
	M	F	M	F	M	F	M	F
RBC-related parameters								
RBC Count	7.13 ± 0.41	6.82 ± 0.58	-7.6	-5.1	-1.3	-1.9	-8.4	-17.2
Hgb	16.9 ± 1.3	15.9 ± 1.1	-7.1	+8.2	-2.4	-1.2	-10.6	-17.0
Hct (%)	49.6 ± 4	46.6 ± 3.7	-3.3	+2.8	-0.4	-1.1	-3.4	-15.4
MCV (fL)	69.5 ± 1.8	68.4 ± 2.2	+1.0	+0.9	+0.6	-0.6	+1.7	+1.7
MCH (pg)	23.7 ± 0.6	23.3 ± 0.6	+0.8	+0.8	-1.3	+1.3	-2.1	↔
MCHC (g/dL)	34 ± 0.2	34.1 ± 0.6	↔	+2.3	-1.5	+1.5	-3.5**	-1.8
Reticulocyte Absolute	21.75 ± 1.0	10.73 ± 3.1	-20.1	+189	-22.8	-7.3	+23.2	+116
Meth Hgb	1.7 ± 0.6	1.5 ± 1.0	-35.3	-53.3	+11.8	-6.7	+52.9	+113
Platelet Count (10^3/µl)	282 ± 34.5	317 ± 91.6	-2.8	↔	-21.3	+6	+42.6*	+57.4
WBC Count (10^3/µl)	6.43 ± 1.7	6.49 ± 1.0	+13.8	+11.6	+16.5	+22.3	+12.3	+41.8
Lymphocyte (10^3/µl)	1.70 ± 0.21	2.11 ± 0.37	+29.4	-2.4	+15.3	+10.9	-10.6	-16.1
Basophils (10^3/µl)	0.03 ± 0.02	0.03 ± 0.01	↔	+100	↔	+33.3	0.00	0.00
LUC	0.04 ±	0.06 ±	↔	↔	↔	+16.7	-50.0	-50.0

	Control (Mean±SD)		% Control					
Parameter	C		LD		MD		HD	
	M	F	M	F	M	F	M	F
(10^3/μl)	0.3	0.03						

↔ Denotes, no change in mean from control
* [ss, at p<0.05 compared to mean Control] ; ** [ss, at p<0.01 compared to mean Control]

Clinical Chemistry was evaluated prior to randomization (study Week -1) and on study Weeks 12, 25, 38, and 51 for the following parameters: ASP, ALT, AP, Total Chol, Trig, Alb, Total Prot, Globulin, A/G ratio, Total Bili, Glu, Creatinine, Na+, K+, Cl-, Ca2+, BUN, Pi, Amylase, Lipase, and TLI.

- In the HD group, steady decline in trypsin-like immunoreactivity (TLI) was found; however, no effects were found on either amylase or lipase.
  - Male, reduction compared to C mean was -28.8%, -39.9%, and -43.8% at Weeks 25, 38, and 51, respectively.
  - Female, reduction compared to C mean was -34.8%, -53.0%, and -80.3% at Weeks 25, 38, and 51, respectively.

Urinalysis was evaluated prior to randomization (study Week -1) and on study Weeks 12, 25, 38, and 51 for the standard battery of parameters including: bilirubin, creatinine, glucose, ketones, osmolality, pH, K+, protein, Na+, Urobilinogen, volume, occult blood, specific gravity, color, appearance, microscopic examination of urine sediment.

- Unremarkable, no teriflunomide-related findings were observed.

Gross Pathology was performed on all of the animals on study, and the following tissues were collected: adrenals, aorta, bone with bone marrow (femur - including articular surface and joint capsule of the distal end, sternum), bone marrow smear, brain (cerebrum level 1, cerebrum level 2, cerebellum with pons/medulla), cecum, colon, duodenum, epididymides, esophagus, eyes with optic nerves, gallbladder, heart, ileum, jejunum, kidneys, lacrimal glands, larynx, liver (caudate, left lateral and right portion of the median lobe), lung (including bronchi, fixed by inflation with fixative), lymph nodes (mesenteric, medial retropharyngeal), nasal cavity, ovaries, oviducts, pancreas, peripheral nerve (sciatic), pituitary, prostate, rectum, salivary glands, [mandibular (2), parotid (2), sublingual(2)], skeletal muscle (rectus femoris), skin with mammary gland, spinal cord (cervical), spleen, stomach, testes, thymus, thyroids/parathyroids (2), tongue, trachea, ureter, urinary bladder, uterus with cervix, vagina, and tissues with macroscopic findings.

Organ Weights were measured as absolute and relative to mean body weight. The following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver with gallbladder, ovaries, pituitary gland, prostate, spleen, testes, thymus, thyroids with parathyroids, and uterus.

- Increased absolute and relative weight of liver with gallbladder was found in both sexes in a dose-dependent manner (see reviewer-generated table, below).

- In HDF, increased absolute and relative heart, uterus, and ovary weights were observed.
- In HDM, decreased absolute and relative spleen and prostate weights were recorded.

Teriflunomide-dependent changes in organ weights (absolute and relative to body weight)

Absolute (A) Relative (R)	Male				Female			
	A = (g) R = g/100g	% Control			A = (g) R = g/100g	% Control		
	C	LD	MD	HD	C	LD	MD	HD
Liver w/ Gallbladder								
A	305.8 ± 23.3	+3.9	+18.6	+31	271.5 ± 44.7	↔	+14.2	+19.7
R	2.4 ± 0.1	+3.4	+15.2*	+36**	2.7 ± 0.5	-3.7	+10.4	+39.5**
Spleen					**Heart**			
A	100.6 ± 19.1			-38.9	73.5 ± 11	+13.2	+14.8	+19.6
R	0.8 ± 0.1			-36.5	0.7 ±0.1	+10	+14.3	+41.5
Prostate					**Uterus**			
A	10.4 ± 1.2			-28.4	9.0 ± 6.0	+4.4	+35.5	+37.2
R	0.08 ± 0.01			-26.5	0.09 ±0.07	+6.5	+37	+59.8
↔ Denotes, no change in mean from control * [ss, at p<0.05 compared to mean Control] ** [ss, at p<0.01 compared to mean Control]					**Ovary**			
					1.3 ±0.4			+22.7
					0.01± 0.004			+46.2

Histopathology was performed on all of the tissues collected for each dose group on study. The bone marrow smear of HDF (#0029) was examined due to clinical and anatomic pathology changes.

Adequate Battery of tissues was evaluated, and the pathology report was signed and dated; therefore, this pathology report is considered adequate for regulatory purposes.

Peer Review was performed by (b) (4) D.V.M., Ph.D.

Histological Findings

The relevant teriflunomide-related microscopic findings are shown below in the following reviewer-generated table.

Microscopic findings								
Organ	C		LD		MD		HD	
Finding	M	F	M	F	M	F	M	F
Bone Marrow								
Femur, hypocellularity	0/4	1/4	0/4	0/4	0/4	1/4	2/4	3/4
Sternum, hypocellularity	1/4	2/4	0/4	3/4	2/4	2/4	2/4	4/4
Sternum, prominent megakaryocyte	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Colon								
GALT, decreased	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Ileum								
GALT, decreased	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Esophagus								
Ulceration	0/4	0/4	0/4	0/4	2/4	0/4	0/4	1/4
Larynx								
Inflammation, chronic, active	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Lung								
Fibrosis	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4
Inflammation, not interstitial	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
Inflammation, interstitium	0/4	2/4	2/4	3/4	2/4	4/4	2/4	2/4
Lymph Node, mediastinal								
Sinus erythrocytosis	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
mesenteric								
Atrophy, diffuse, lymphoid	0/4	0/4	0/4	0/4	0/4	0/4	2/4	1/4
retropharyngeal								
Atrophy, diffuse, lymphoid	0/4	0/4	0/4	0/4	0/4	0/4	2/4	1/4
Inflammation, chronic	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Sinus, histiocytosis	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Spleen								
Congestion	0/4	4/4	4/4	3/4	2/4	2/4	1/4	2/4
Hemosiderin, pigment	2/4	2/4	4/4	3/4	2/4	2/4	4/4	4/4
Pancreas								
Degeneration/fibrosis	0/4	0/4	0/4	0/4	1/4	1/4	4/4	4/4
Duct, lymphocyte infiltration	0/4	0/4	1/4	0/4	0/4	1/4	1/4	1/4
Acinar, degranulation	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4
Thyroid								
Cyst (not ultimobranchial)	0/4	0/4	0/4	0/4	1/4	0/4	1/4	0/4
Testes								
Hypospermatogenesis	0/4		1/4		1/4		2/4	
Giant cell, multinucleate	1/4		1/4		2/4		2/4	

It is noted that the findings in the pancreas occurred at both the MD and HD; the findings at the MD were accompanied by minimal to mild mononuclear cell infiltrates, which was also observed in HDM (3/4). In HDF (2/4) was accompanied with multifocal degranulation of acinar cells. In the lung, interstitial inflammation was present in 2 CF, and in most animals treated with teriflunomide. At the HD, the interstitial inflammation was described as chronic in 1 HDM, and in 2HDF, the interstitium was fibrotic in the subpleural region (focal).

Toxicokinetics

In Table 7, the sponsor has summarized the toxicokinetic data derived from this study. At the NOAEL (0.2 mg/kg/day), the C_{max} and AUC values for male and female dogs in this study for teriflunomide were 1.54 μg/mL and 26.6 μg·hr/mL and 1.39 μg/mL and 20.2 μg·hr/mL, respectively. It is noted that at the established NOAEL, the amount of 4-TFMA present was below the level of quantitation (<0.5 ng/mL).

Table 7 - 12-Month Oral Toxicity Study in Dogs: Toxicokinetics Summary

			Teriflunomide		4-TFMA	
Species, Dosing Day, Number	Dose (mg/kg/day)	Sex	C_{max} (μg/mL)	AUC_{0-24h} (μg.h/mL)	C_{max} (ng/mL)	AUC_{0-24h} (ng.h/mL)
Dog [2003-1491]	0.2	M	1.54	26.6	BLQ	BLQ
Day 364 (12-month study)		F	1.39	20.2	BLQ	BLQ
	0.8	M	9.28	159	BLQ	BLQ
4 dogs per sex		F	10.5	166	BLQ	BLQ
	2/4 [a]	M	69.4	1313	3.76	81.0
		F	58.0	1115	2.08	45.8

Abbreviations: M = Male, F = Female, C_{max} = maximum concentration, AUC = Area under the curve, 4-TFMA = 4-trifluoro-methylaniline, BLQ = below the limit of quantification, LOQ for 4-TFMA = 0.500 ng/mL in foot note

Additional information: Values are rounded to 3 significant values or less.

[a] The 2 mg/kg/day dose was increased to 4 mg/kg/day after 191 doses due to an apparent lack of toxicity at the 2 mg/kg/day dose.

The complete TK data for teriflunomide are presented in sponsor's Table, below.

Mean TK Parameters of HMR1726 in Dog Plasma										
Dose (mg/kg/day)	Day	Gender	Mean Cmax (ug/mL)	SD (Mean Cmax)	Mean AUC_{0-24hr}	SD (Mean AUC_{0-24hr})	Mean Cmax/Dose	SD (Mean Cmax)/Dose	Mean AUC_{0-24hr} /Dose	SD (Mean AUC_{0-24hr}) /Dose
0.2	0	Female	0.630	0.221	7.26	1.42	3.15	1.10	36.3	7.08
		Male	0.816	0.322	8.90	1.82	4.08	1.61	44.5	9.09
	30	Female	1.30	0.514	21.3	9.56	6.49	2.57	106	47.8
		Male	1.42	0.145	23.9	1.9	7.08	0.727	119	9.49
	90	Female	1.14	0.515	16.7	9.18	5.72	2.57	83.4	45.9
		Male	1.72	0.333	25.3	5.30	8.61	1.67	126	26.5
	364	Female	1.39	0.244	20.2	4.20	6.93	1.22	101	21.0
		Male	1.54	0.754	26.6	14.4	7.68	3.77	133	71.8
0.8	0	Female	4.49	1.11	57.6	14.8	5.62	1.38	72.0	18.5
		Male	4.12	0.853	53.8	9.77	5.15	1.07	67.3	12.2
	30	Female	14.4	4.84	244	102	18.0	6.05	305	128
		Male	8.42	2.28	154	47.9	10.5	2.85	192	59.8
	90	Female	7.56	1.15	110	17.6	9.44	1.43	138	22.1
		Male	8.65	2.45	147	52.4	10.8	3.07	184	65.5
	364	Female	10.5	1.84	166	29.1	13.2	2.30	208	36.4
		Male	9.28	2.18	159	56.9	11.6	2.72	198	71.1
2/4	0	Female	12.9	0.794	203	14.8	6.43	0.397	101	7.39
		Male	11.9	1.69	209	13.1	5.93	0.845	104	6.56
	30	Female	34.9	11.2	693	255	17.5	5.60	347	127
		Male	35.4	7.80	671	141	17.7	3.90	336	70.6
	90	Female	36.3	11.5	699	257	18.2	5.74	349	128
		Male	34.2	8.59	639	204	17.1	4.29	320	102
	364	Female	58.0	4.01	1115	65	14.5	1.00	279	16.3
		Male	69.4	11.5	1313	191	17.3	2.87	328	47.6

7 Genetic Toxicology

The sponsor conducted a full battery of genotoxicity studies for teriflunomide as is illustrated in the sponsor's Table 8 that provides the concentrations, incubation times, and results of each of the studies conducted.

Table 8 - Genotoxicity study results - Teriflunomide

Test System [study reference]	Concentration or doses	Result
Ames test, with or without metabolic activation [017840]	up to 5000 µg/plate	negative
HPRT assay, with or without metabolic activation [2005-0144]	up to 1750 µg/mL	negative
In vitro chromosome aberration test in human lymphocytes with or without uridine supplementation [MAF0073]	20 h –S9: up to 150 µg/mL	negative
	3 h –S9: up to 500 µg/mL	positive at 350 µg/mL and above
	3 h +S9: up to 500 µg/mL	positive at 300 µg/mL and above
In vivo bone marrow micronucleus test in mice [017974]	10, 30, or 100 mg/kg/day, PO	negative
In vivo bone marrow chromosome aberration test in Chinese hamsters [F2001TOX0155]	20, 60, or 200 mg/kg, PO	negative
14-Day repeat dose oral mammalian bone marrow chromosome aberration test in rat [MAF0076]	1, 10, or 20 mg/kg/day, PO	negative

Abbreviations: HPRT = hypoxanthine-guanine-phosphoribosyl transferase, PO = per os (oral)

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: HMR 1726 Bacterial Reverse Mutation Test

Study no.:	017840
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	Hoechst Marion Roussel Deutschland GmbH Frankfurt, Germany
Date of study initiation:	November 18, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, U001, 99.5% purity

Methods

Strains:	TA98, TA100, TA1535, TA1537, WP2*uvrA*
Concentrations in definitive study:	16-5000 μg/plate
Basis of concentration selection:	Toxicity to bacterial lawn
Negative control:	Untreated and Solvent Controls
Positive control:	In absence of metabolic activation (-S9):

Strain	Positive Control
TA98	2-nitrofluorene
TA100, TA1535	Na azide
TA1537	9-aminocridine
WP2*uvrA*	MNNG

In presence of metabolic activation (+S9):
All strains used 2-aminoanthracene

Formulation/Vehicle: DMSO

Study Validity

As the sponsor conducted this assay, it has met the criteria for a valid study as the spontaneous mutant frequency and positive control responses for each strain were within the range of the historical controls for this laboratory.

Results

In the absence or presence of metabolic activation, teriflunomide did not increase mutant frequency in any of the tested bacterial strains.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: HMR1726: *IN VITRO* CHROMOSOME ABERRATION TEST IN CULTURED HUMAN PERIPHERAL BLOOD LYMPHOCYTES

Study no.:	MAF0073
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	Sanofi-aventis recherche et développement Vitry sur Seine, France
Date of study initiation:	April 04, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, Batch #0500024551, 99.8%

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	See sponsor's table below
Basis of concentration selection:	Cytotoxicity at approximately 50-60% of the mitotic index
Negative control:	Treatment medium containing DMSO (1%)
Positive control:	Without S9: Mitomycin C With S9: Cyclophosphamide
Formulation/Vehicle:	DMSO diluted in treatment medium
Incubation & sampling time:	3 and 20 hrs ±S9 (induced SD rat liver)

Assay number	Assay date	Donor	Assay description	Concentrations tested (µg/mL)
I	April 4	Female n°13	3-hour without S9 mix	50, 100, 200, 300, 350, 400, 450, 500
			20-hour without S9 mix	6.25, 9.375, 12.5, 18.75, 25, 37.5, 50, 75, 100, 150
II	April 25	Male n° 75	Repeat 3-hour without S9 mix	50, 100, 200, 250, 300, 350, 400, 450, 500
			3-hour with S9 mix	
III	May 16	Male n° 110	3-hour without S9 mix, without Uridine	100, 200, 300, 400, 500
			3-hour without S9 mix, with 500 µM Uridine during treatment	
			3-hour without S9 mix, with 500 µM Uridine during treatment up to harvest	
IV	June 27	Male n° 75	3-hour without S9 mix, without Uridine	100, 200, 250, 300, 325, 350, 375, 400, 425, 450, 500
			3-hour without S9 mix, with 1mM Uridine during treatment up to harvest	
V	Sept 05	Female n° 139	3-hour without S9 mix, without Uridine	200, 300, 400, 500
			3-hour without S9 mix, with 1mM Uridine during treatment up to harvest	

Study Validity

As conducted, this study met the criteria for a valid assay as the number of cells with spontaneous chromosomal aberrations was consistent with historical control data and the positive controls (mitomycin C and cyclophosphamide) induced a clear increase in the number of cells with chromosomal aberrations.

Results

In the 3-hour incubation of teriflunomide, in the absence of metabolic activation (±S9), a statistically significant increase in the number of chromosomal aberrations was found in study 1 and repeated in study 2. The results of these studies are provided in the sponsor's tables 1 and 2, below.

Table 1 - Cytotoxicity and chromosome aberration incidence: 3-hour treatment without metabolic activation
First assay

Compound	Replicate	Cells scored (a)	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Statistical significance	CYTOTOXICITY	
						Mitotic index (%)	Relative mitotic index (%)
Solvent control	A	100	2	0		6.3	100
	B	100	1	0		6.3	
	Totals	200	**3**	**0**		**6.3 (b)**	
HMR1726 100 µg/mL	A	100	2	1		5.6	87
	B	87	3	2		5.4	
	Totals	187	**5**	**3**	**NS**	**5.5 (b)**	
HMR1726 200 µg/mL	A	100	2	1		4.9	72
	B	100	2	2		4.2	
	Totals	200	**4**	**3**	**NS**	**4.6 (b)**	
HMR1726 300 µg/mL	A	100	2	2		6.3	96
	B	100	3	2		5.8	
	Totals	200	**5**	**4**	*	**6.1 (b)**	
HMR1726 350 µg/mL	A	100	5	4		6.8	97
	B	100	10	10		5.4	
	Totals	200	**15**	**14**	***	**6.1 (b)**	
HMR1726 400 µg/mL	A	100	12	11		5.8	86
	B	100	13	10		5.0	
	Totals	200	**25**	**21**	***	**5.4 (b)**	
HMR1726 450 µg/mL	A	100	6	6		3.7	57
	B	100	18	16		3.5	
	Totals	200	**24**	**22**	***	**3.6 (b)**	
HMR1726 500 µg/mL	A	89	18	15		2.8	40
	B	100	22	20		2.2	
	Totals	189	**40**	**35**	***	**2.5 (b)**	
MMC 0.3 µg/mL	A	100	20	17		-	-
	B	100	20	14			
	Totals	200	**40**	**31**	***		

See abbreviation list

Table 2 - Chromosome aberration description: 3-hour treatment without metabolic activation
First assay

Compound	Replicate	Cells (a)	Structural aberrations																Numerical aberrations		
			Chromosomal aberrations					Chromatid aberrations								Others		Total structural aberrations			
				breaks		exchanges			breaks			exchanges									
			csg	ace	d min	dic	csr	Ctg	ctb	min	su	tr	qr	ctr	cx	pu	>7	Abs +g	Abs -g	Hyper	Endo	Poly
Solvent control	A	100						2										2	0			1
	B	100						1										1	0		1	1
	Total	200						**3**										**3**	**0**		**1**	**2**
HMR1726 100 µg/mL	A	100		1				1										2	1	1		
	B	87						1	2									3	2		1	1
	Total	187		**1**				**2**		**2**								**5**	**3**	**1**	**1**	**1**
HMR1726 200 µg/mL	A	100						1			1							2	1		3	3
	B	100		1					1									2	2	3		2
	Total	200		**1**				**1**		**2**								**4**	**3**	**3**	**3**	**5**
HMR1726 300 µg/mL	A	100							2									2	2	1	1	1
	B	100						1	1			1						3	2			7
	Total	200						**1**		**3**			**1**					**5**	**4**	**1**	**1**	**8**
HMR1726 350 µg/mL	A	100			1			1	3				1					6	5			
	B	100		2				1	8			1	1					13	12			
	Total	200		**3**				**2**		**11**			**3**					**19**	**17**			
HMR1726 400 µg/mL	A	100		1				2	4			2	5					14	12			
	B	100		4				3	7				3					17	14	1		1
	Total	200		**5**				**5**		**11**			**10**					**31**	**26**	**1**		**1**
HMR1726 450 µg/mL	A	100	1	1					11			1					1	15	14			2
	B	100		6				3	19								3	31	28			
	Total	200	**1**	**7**				**3**		**30**			**1**				**4**	**46**	**42**			**2**
HMR1726 500 µg/mL	A	89		1				5	9				2				6	23	18			
	B	100		7				2	30			1	1				3	44	42			
	Total	189		**8**				**7**		**39**			**4**			**9**		**67**	**60**			
MMC 0.3 µg/mL	A	100		6				3	9			2	3					23	20			
	B	100		2				7	11				2					22	15	1		
	Total	200		**8**				**10**		**20**			**7**					**45**	**35**	**1**		

See abbreviation list

A 20-hr incubation of human lymphocytes (same female donor) with teriflunomide in the absence of S9 failed to result in an increase in chromosomal aberrations (see sponsor's tables 3 and 4); however, it is noted that the concentration of teriflunomide in this study was considerably lower.

Table 3 - Cytotoxicity and chromosome aberration incidence: 20-hour treatment without metabolic activation

Compound	Replicate	Cells scored (a)	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Statistical significance	CYTOTOXICITY Mitotic index (%)	CYTOTOXICITY Relative mitotic index (%)
Solvent control	A	100	0	0		6.7	
	B	100	0	0		6.7	100
	Totals	200	**0**	**0**		**6.7 (b)**	
HMR1726 18.75 µg/mL	A	100	0	0		5.5	
	B	100	2	1		5.7	84
	Totals	200	**2**	**1**	**NS**	**5.6 (b)**	
HMR1726 37.5 µg/mL	A	100	2	0		4.6	
	B	100	1	1		5.3	74
	Totals	200	**3**	**1**	**NS**	**5.0 (b)**	
HMR1726 50 µg/mL	A	100	0	0		5.1	
	B	100	1	1		4.1	69
	Totals	200	**1**	**1**	**NS**	**4.6 (b)**	
HMR1726 75 µg/mL	A	100	0	0		2.2	
	B	100	2	0		3.1	40
	Totals	200	**2**	**0**	**NS**	**2.7 (b)**	
MMC 0.2 µg/mL	A	100	32	26			
	B	100	27	22		-	-
	Totals	200	**59**	**48**	***		

Table 4 - Chromosome aberration description: 20-hour treatment without metabolic activation

Compound	Replicate	Cells (a)	Structural aberrations: Chromosomal aberrations: breaks csg	ace	d min	exchanges dic	csr	Chromatid aberrations: breaks ctg	Ctb	min	su	exchanges tr	qr	ctr	cx	Others pu	>7	Total structural aberrations Abs +g	Abs -g	Numerical aberrations Hyper	Endo	Poly
Solvent control	A	100																0	0			
	B	100																0	0			1
	Total	200																**0**	**0**			**1**
HMR1726 18.75 µg/mL	A	100																0	0	1		
	B	100						1	1									2	1			2
	Total	200						**1**		**1**								**2**	**1**	**1**		**2**
HMR1726 37.5 µg/mL	A	100	1					1										2	0			3
	B	100							1									1	1			
	Total	200	**1**					**1**		**1**								**3**	**1**			**3**
HMR1726 50 µg/mL	A	100																0	0			1
	B	100		1														1	1			2
	Total	200		**1**														**1**	**1**			**3**
HMR1726 75 µg/mL	A	100																0	0			
	B	100						2										2	0			
	Total	200						**2**										**2**	**0**			
MMC 0.2 µg/mL	A	100		7				11	12			2	8					40	29			
	B	100		2				6	14				9					31	25			1
	Total	200		**9**				**17**		**26**			**19**					**71**	**54**			**1**

See abbreviation list

In a repeat study using lymphocytes from a male donor, similar results were obtained after 3 hr incubation ±S9. These results are summarized in the following sponsor-provided Tables 5-8.

Table 5 - Cytotoxicity and chromosome aberration incidence: 3-hour treatment without metabolic activation
Repeat assay

Compound	Replicate	Cells scored (a)	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Statistical significance	CYTOTOXICITY Mitotic index (%)	CYTOTOXICITY Relative mitotic index (%)
Solvent control	A	100	0	0		10.4	100
	B	100	0	0		8.5	
	Totals	200	**0**	**0**		**9.5 (b)**	
HMR1726 200 µg/mL	A	100	3	0		5.0	56
	B	100	2	2		5.6	
	Totals	200	**5**	**2**	**NS**	**5.3 (b)**	
HMR1726 300 µg/mL	A	100	5	2		5.3	57
	B	100	5	4		5.5	
	Totals	200	**10**	**6**	**	**5.4 (b)**	
HMR1726 350 µg/mL	A	100	12	6		5.6	58
	B	100	6	4		5.3	
	Totals	200	**18**	**10**	***	**5.5 (b)**	
HMR1726 400 µg/mL	A	100	19	14		5.1	55
	B	100	14	11		5.3	
	Totals	200	**33**	**25**	***	**5.2 (b)**	
HMR1726 500 µg/mL	A	100	38	36		5.9	55
	B	100	39	36		4.5	
	Totals	200	**77**	**72**	***	**5.2 (b)**	
MMC 0.3 µg/mL	A	100	31	25		-	-
	B	100	23	17			
	Totals	200	**54**	**42**	***		

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Table 6 - Chromosome aberration description: 3-hour treatment without metabolic activation
Repeat assay

Compound	Replicate	Cells (a)	Structural aberrations: Chromosomal aberrations: breaks csg	ace	d min	exchanges dic	csr	Chromatid aberrations: breaks ctg	ctb	min	su	exchanges tr	qr	ctr	cx	Others pu	>7	Total structural aberrations Abs +g	Abs -g	Numerical aberrations Hyper	Endo	Poly
Solvent control	A	100																0	0			1
	B	100																0	0			
	Total	200																**0**	**0**			**1**
HMR1726 200 µg/mL	A	100	1					2										3	0		4	1
	B	100							2									2	2		1	
	Total	200	**1**					**2**		**2**								**5**	**2**		**5**	**1**
HMR1726 300 µg/mL	A	100						3	1				1					5	2		1	1
	B	100		1				2	2								1	6	4	4		
	Total	200		**1**				**5**		**3**			**1**			**1**		**11**	**6**	**4**	**1**	**1**
HMR1726 350 µg/mL	A	100	1					5	11									17	11			2
	B	100		1				2	4				1					8	6	1		1
	Total	200	**1**	**1**				**7**		**15**			**1**					**25**	**17**	**1**		**3**
HMR1726 400 µg/mL	A	100	1					4	10		1		2				1	19	14	1		1
	B	100	2	1				3	11		1	1	3					22	17	1		3
	Total	200	**3**	**1**				**7**		**23**			**6**			**1**		**41**	**31**	**2**		**4**
HMR1726 500 µg/mL	A	100	4	4				4	51				8			1	5	77	69			
	B	100	1	4		1		16	66			4	3		2		2	99	82			
	Total	200	**5**	**8**		**1**		**20**		**117**			**17**			**8**		**176**	**151**			
MMC 0.3 µg/mL	A	100	3	5				5	21				4					38	30			1
	B	100	2	3		1		5	13			1	2				1	28	21			
	Total	200	**5**	**8**		**1**		**10**		**34**			**7**			**1**		**66**	**51**			**1**

Table 7 - Cytotoxicity and chromosome aberration incidence: 3-hour treatment with metabolic activation

Compound	Replicate	Cells scored (a)	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Statistical significance	CYTOTOXICITY Mitotic index (%)	CYTOTOXICITY Relative mitotic index (%)
Solvent control	A	100	1	0		8.5	
	B	100	1	0		10.6	100
	Totals	200	**2**	**0**		**9.6 (b)**	
HMR1726 50 µg/mL	A	100	4	2		6.2	
	B	100	7	3		7.0	69
	Totals	200	**11**	**5**	*	**6.6 (b)**	
HMR1726 100 µg/mL	A	100	10	6		5.1	
	B	100	10	4		5.0	53
	Totals	200	**20**	**10**	***	**5.1 (b)**	
HMR1726 200 µg/mL	A	100	1	1		4.0	
	B	100	7	4		5.7	51
	Totals	200	**8**	**5**	*	**4.9 (b)**	
HMR1726 250 µg/mL	A	100	3	1		5.2	
	B	90	2	1		5.1	54
	Totals	190	**5**	**2**	NS	**5.2 (b)**	
HMR1726 300 µg/mL	A	64	4	3		5.2	
	B	96	10	9		4.4	50
	Totals	160	**14**	**12**	***	**4.8 (b)**	
HMR1726 350 µg/mL	A	100	18	16		4.6	
	B	87	17	13		5.1	51
	Totals	187	**35**	**29**	***	**4.9 (b)**	
HMR1726 400 µg/mL	A	36	7	7		4.1	
	B	100	22	19		6.3	54
	Totals	136	**29**	**26**	***	**5.2 (b)**	
CP 7.5 µg/mL	A	100	31	24		-	-
	B	100	26	24			
	Totals	200	**57**	**48**	***		

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Table 8 - Chromosome aberration description: 3-hour treatment with metabolic activation

Compound	Replicate	Cells (a)	Structural aberrations: Chromosomal aberrations: csg	breaks: ace	breaks: d min	exchanges: dic	exchanges: csr	Chromatid aberrations: ctg	breaks: ctb	breaks: min	breaks: su	exchanges: tr	exchanges: qr	exchanges: ctr	exchanges: cx	Others: pu	Others: >7	Total structural aberrations: Abs +g	Total structural aberrations: Abs -g	Numerical aberrations: Hyper	Numerical aberrations: Endo	Numerical aberrations: Poly
Solvent control	A	100	1															1	0	1		
	B	100						1										1	0			1
	Total	200	**1**					**1**										**2**	**0**	**1**		**1**
HMR1726 50 µg/mL	A	100	1	1				1	1									4	2			
	B	100	2					2	3									7	3			
	Total	200	**3**	**1**				**3**	**4**									**11**	**5**			
HMR1726 100 µg/mL	A	100		2				4	4									10	6			
	B	100		1				6	3									10	4	1	2	1
	Total	200		**3**				**10**	**7**									**20**	**10**	**1**	**2**	**1**
HMR1726 200 µg/mL	A	100		2														2	2		4	1
	B	100	1	2				2	1				1					7	4	2	2	1
	Total	200	**1**	**4**				**2**	**1**				**1**					**9**	**6**	**2**	**6**	**2**
HMR1726 250 µg/mL	A	100		1				2										3	1	3		
	B	90						1	1									2	1	2	1	
	Total	190		**1**				**3**	**1**									**5**	**2**	**5**	**1**	
HMR1726 300 µg/mL	A	64						1	2				1					4	3	2		3
	B	96		4				3	10									17	14	1		5
	Total	160		**4**				**4**	**12**				**1**					**21**	**17**	**3**		**8**
HMR1726 350 µg/mL	A	100	2	1				4	13	1		2	2					25	19			1
	B	87		2				4	10		1	3	4					24	20	2		2
	Total	187	**2**	**3**				**8**	**25**				**11**					**49**	**39**	**2**		**3**
HMR1726 400 µg/mL	A	36		2					6				1				1	10	10		1	2
	B	100	2	4				7	25		1	1	3					43	34	1		
	Total	136	**2**	**6**				**7**	**32**				**5**			**1**		**53**	**44**	**1**	**1**	**2**
CP 7.5 µg/mL	A	100	4	2		1		5	24			2	1					39	30			
	B	100	4	11				3	15			1	1					35	28			
	Total	200	**8**	**13**		**1**		**8**	**39**				**5**					**74**	**58**			

In a third assay, the sponsor evaluated whether enrichment of the culture medium with an exogenous pyrimidine (uridine, 500 µM) would reduce or prevent the increase in chromosomal aberrations observed after 3 hr treatment in the absence of S9. In the absence of uridine, the expected concentration-dependent increase in chromosomal aberrations was observed (sponsor's Tables 9 and 10); however, incubation in the presence of exogenous uridine shows a reduction but not prevention of chromosomal aberrations (sponsor's Tables 11 and 12). These data suggest that the increase in chromosomal aberrations at 3 hr may be mediated, in part, by the inhibition of DHO-DH by teriflunomide.

Table 9 - Cytotoxicity and chromosome aberration incidence: 3-hour treatment without metabolic activation without 500 µM Uridine

Compound	Replicate	Cells scored (a)	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Statistical significance	CYTOTOXICITY	
						Mitotic index (%)	Relative mitotic index (%)
Solvent control	A	100	2	0		9.0	
	B	100	2	1		11.0	100
	Totals	200	**4**	**1**		**10.0 (b)**	
HMR1726 100 µg/mL	A	100	3	3		5.2	
	B	100	2	2		6.8	60
	Totals	200	**5**	**5**	**NS**	**6.0 (b)**	
HMR1726 200 µg/mL	A	100	0	0		6.4	
	B	100	0	0		6.3	64
	Totals	200	**0**	**0**	**NS**	**6.4 (b)**	
HMR1726 300 µg/mL	A	(c)					
	B	100	7	5		7.2	72
	Totals	100	**7**	**5**	**	**7.2 (b)**	
HMR1726 400 µg/mL	A	100	28	26		5.8	
	B	100	14	14		6.2	60
	Totals	200	**42**	**40**	***	**6.0 (b)**	
HMR1726 500 µg/mL	A	100	34	33		3.5	
	B	100	32	30		4.3	39
	Totals	200	**66**	**63**	***	**3.9 (b)**	
MMC 0.3 µg/mL	A	100	21	16		-	-
	B	100	12	10			
	Totals	200	**33**	**26**	***		

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Table 10 - Chromosome aberration description: 3-hour treatment without metabolic activation without 500 µM Uridine

Compound	Replicate	Cells (a)	Structural aberrations																Numerical aberrations		
			Chromosomal aberrations					Chromatid aberrations								Others		Total structural aberrations				
				breaks		exchanges			breaks			exchanges									
			csg	ace	d min	dic	csr	ctg	ctb	min	su	tr	qr	ctr	cx	pu	>7	Abs +g	Abs -g	Hyper	Endo	Poly
Solvent control	A	100						2										2	0			
	B	100						1	1									2	1			
	Total	200						**3**		**1**								**4**	**1**			
HMR1726 100 µg/mL	A	100		2					1									3	3			
	B	100							2									2	2			2
	Total	200		**2**					**3**									**5**	**5**			2
HMR1726 200 µg/mL	A	100																0	0	1	1	
	B	100																0	0		2	3
	Total	200																**0**	**0**	**1**	**3**	3
HMR1726 300 µg/mL	A	(c)																				
	B	100		2				2	1			1	1					7	5	1		2
	Total	100		**2**				**2**		**1**			**2**					**7**	**5**	**1**		2
HMR1726 400 µg/mL	A	100		5				7	23			1	5				2	43	36			
	B	100		1				2	11				5					19	17	1		1
	Total	200		**6**				**9**		**34**			**11**			**2**		**62**	**53**	**1**		1
HMR1726 500 µg/mL	A	100		1				2	38			2	10	1	2		6	62	60			
	B	100	2	2				7	30			2	11		1		5	60	51			
	Total	200	**2**	**3**				**9**		**68**			**29**			**11**		**122**	**111**			
MMC 0.3 µg/mL	A	100	1	4				5	10	1			2					23	17	1		1
	B	100	1	2				1	6				2					12	10			
	Total	200	**2**	**6**				**6**		**17**			**4**					**35**	**27**	**1**		1

Table 11 - Cytotoxicity and chromosome aberration incidence: 3-hour treatment without metabolic activation with 500 µM Uridine during treatment

Compound	Replicate	Cells scored (a)	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Statistical significance	CYTOTOXICITY	
						Mitotic index (%)	Relative mitotic index (%)
Solvent control	A	100	0	0		10.5	100
	B	100	0	0		12.8	
	Totals	200	**0**	**0**		**11.7 (b)**	
HMR1726 100 µg/mL	A	100	1	1		8.8	79
	B	100	0	0		9.6	
	Totals	200	**1**	**1**	**NS**	**9.2 (b)**	
HMR1726 200 µg/mL	A	100	1	1		9.0	77
	B	100	5	4		8.9	
	Totals	200	**6**	**5**	*	**9.0 (b)**	
HMR1726 300 µg/mL	A	100	1	1		7.9	75
	B	100	5	5		9.6	
	Totals	200	**6**	**6**	**	**8.8 (b)**	
HMR1726 400 µg/mL	A	100	5	4		7.8	81
	B	100	19	19		11.0	
	Totals	200	**24**	**23**	***	**9.4 (b)**	
HMR1726 500 µg/mL	A	100	11	11		9.4	73
	B	100	17	17		7.6	
	Totals	200	**28**	**28**	***	**8.5 (b)**	
MMC 0.3 µg/mL	A	100	14	12		-	-
	B	100	15	14			
	Totals	200	**29**	**26**	***		

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Table 12 - Chromosome aberration description: 3-hour treatment without metabolic activation With 500 µM Uridine during treatment

Compound	Replicate	Cells (a)	Structural aberrations																Numerical aberrations		
			Chromosomal aberrations					Chromatid aberrations								Others		Total structural aberrations			
				breaks		exchanges			breaks			exchanges									
			csg	ace	d min	dic	csr	ctg	ctb	min	su	tr	qr	ctr	cx	pu	>7	Abs +g	Abs -g	Hyper	Endo	Poly
Solvent control	A	100																0	0			
	B	100																0	0			
	Total	200																**0**	**0**			
HMR1726 100 µg/mL	A	100							1									1	1	1		
	B	100																0	0		2	1
	Total	200							**1**									**1**	**1**	**1**	**2**	1
HMR1726 200 µg/mL	A	100		1														1	1	1		
	B	100						5	3				1					9	4	1		3
	Total	200		**1**				**5**	**3**				**1**					**10**	**5**	**2**		3
HMR1726 300 µg/mL	A	100							1									1	1	3		
	B	100							7			2						9	9	1	1	
	Total	200							**8**				**2**					**10**	**10**	**4**	**1**	
HMR1726 400 µg/mL	A	100						1	2				1				1	5	4	1		
	B	100		2				1	14		1	4	3		1	1		27	26	2	1	
	Total	200		**2**				**2**	**17**				**9**			**2**		**32**	**30**	**3**	**1**	
HMR1726 500 µg/mL	A	100							13			2	2					17	17			2
	B	100							34	1			5			1		41	41	1		1
	Total	200							**48**				**9**			**1**		**58**	**58**	1		3
MMC 0.3 µg/mL	A	100		3				2	7			1	2					15	13			
	B	100						4	11	1		1	2					19	15			
	Total	200		**3**				**6**	**19**				**6**					**34**	**28**			

To address the concern that the uridine concentration was not high enough to prevent the increase in chromosomal aberrations, a fourth assay was conducted at 1 mM; however, the increase in chromosomal aberrations remained. The same male donor as in assay # 2 was used for this study.

A final fifth assay was conducted to test the hypothesis that 1 mM exogenous uridine would reduce the chromosomal aberrations induced by teriflunomide after 3 hr incubation without S9. In this experiment, cells from a new female donor were used and a decrease was noted in the cytotoxicity as well as a reduction in the magnitude of the chromosomal aberrations induced. It is noted, however, chromosomal aberrations were achieved at teriflunomide concentrations of 400 and 500 µM (sponsor's Tables 19-22).

Table 19 - Cytotoxicity and chromosome aberration incidence: 3-hour treatment without metabolic activation
Without 1 mM Uridine – Repeat assay

Compound	Replicate	Cells scored (a)	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Statistical significance	CYTOTOXICITY	
						Mitotic index (%)	Relative mitotic index (%)
Solvent control	A	100	1	1		10.9	100
	B	100	2	1		8.8	
	Totals	200	**3**	**2**		**9.9 (b)**	
HMR1726 200 µg/mL	A	100	6	6		7.4	60
	B	100	9	6		4.4	
	Totals	200	**15**	**12**	**	**5.9 (b)**	
HMR1726 300 µg/mL	A	100	22	21		5.6	54
	B	100	13	11		5.0	
	Totals	200	**35**	**32**	***	**5.3 (b)**	
HMR1726 400 µg/mL	A	100	27	25		3.7	43
	B	100	26	23		4.7	
	Totals	200	**53**	**48**	***	**4.2 (b)**	
MMC 0.3 µg/mL	A	100	14	11		-	-
	B	100	28	21			
	Totals	200	**42**	**32**	***		

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Table 20 - Chromosome aberration description: 3-hour treatment without metabolic activation
Without 1 mM Uridine – Repeat assay

Compound	Replicate	Cells (a)	Structural aberrations															Numerical aberrations		
			Chromosomal aberrations					Chromatid aberrations							Others		Total structural aberrations				
				breaks		exchanges			breaks			exchanges									
			csg	ace	d min	dic	csr	ctg	ctb	min	su	tr	qr	ctr	cx	pu	>7	Abs +g	Abs -g	Hyper	Endo	Poly
Solvent control	A	100							1									1	1			
	B	100						1	1									2	1			
	Total	200						**1**		**2**								**3**	**2**			
HMR1726 200 µg/mL	A	100							6									6	6			1
	B	100		1				3	7			1						12	9	1	2	
	Total	200		**1**				**3**		**13**			**1**					**18**	**15**	**1**	**2**	1
HMR1726 300 µg/mL	A	100		2				1	24			3	3		1			34	33			
	B	100		5				2	8	1			5					21	19		1	
	Total	200		**7**				**3**		**33**			**12**					**55**	**52**		**1**	
HMR1726 400 µg/mL	A	100	1	3				1	27				3		1		3	39	37			
	B	100	1	2				3	26				7					39	35			
	Total	200	**2**	**5**				**4**		**53**			**11**			**3**		**78**	**72**			
MMC 0.3 µg/mL	A	100	1	1				3	5				5					15	11			
	B	100	1	2				7	15				6					31	23			
	Total	200	**2**	**3**				**10**		**20**			**11**					**46**	**34**			

Table 21 - Cytotoxicity and chromosome aberration incidence: 3-hour treatment without metabolic activation
With 1 mM Uridine during treatment up to harvest – Repeat assay

Compound	Replicate	Cells scored (a)	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Statistical significance	CYTOTOXICITY	
						Mitotic index (%)	Relative mitotic index (%)
Solvent control	A	100	0	0		10.7	100
	B	100	2	2		9.2	
	Totals	200	**2**	**2**		**10.0 (b)**	
HMR1726 200 µg/mL	A	100	1	1		5.8	64
	B	100	0	0		7.0	
	Totals	200	**1**	**1**	**NS**	**6.4 (b)**	
HMR1726 300 µg/mL	A	100	5	3		8.2	78
	B	100	11	11		7.3	
	Totals	200	**16**	**14**	***	**7.8 (b)**	
HMR1726 400 µg/mL	A	100	20	19		6.5	74
	B	100	16	15		8.2	
	Totals	200	**36**	**34**	***	**7.4 (b)**	
MMC 0.3 µg/mL	A	100	15	14		-	-
	B	100	22	18			
	Totals	200	**37**	**32**	***		

Table 22 - Chromosome aberration descrtption: 3-hour treatment without metabolic activation
With 1 mM Uridine during treatment up to harvest – Repeat assay

Compound	Replicate	Cells (a)	Structural aberrations																Numerical aberrations		
			Chromosomal aberrations					Chromatid aberrations								Others		Total structural aberrations				
				breaks		exchanges			breaks			exchanges										
			csg	ace	d min	dic	csr	ctg	ctb	min	su	tr	qr	ctr	cx	pu	>7	Abs +g	Abs -g	Hyper	Endo	Poly
Solvent control	A	100																0	0			
	B	100							2									2	2			
	Total	200							2									2	2			
HMR1726 200 µg/mL	A	100										1						1	1			1
	B	100																0	0	2	3	
	Total	200										1						1	1	2	3	1
HMR1726 300 µg/mL	A	100		1				2	3									6	4	1	4	
	B	100		1					10			1	1					13	13	1	1	
	Total	200		2				2	13			2						19	17	2	5	
HMR1726 400 µg/mL	A	100						3	21	1			3				1	29	26			
	B	100		4				2	11				5		1			23	21			
	Total	200		4				5	33				9			1		52	47			
MMC 0.3 µg/mL	A	100						1	9			2	3					15	14		1	
	B	100		6				5	10	1		1	3					26	21			
	Total	200		6				6	20				9					41	35		1	

Table 23 summarizes sponsor's historical data for chromosomal aberrations in human peripheral blood lymphocytes.

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Table 23 - Historical data

				Numbers of cells with aberrations excluding gaps (expressed per 100 scored metaphases)
3-hour treatment	Without S9 mix	Negative control	Number of observations	30
			Minimum	0
			Maximum	6
			Mean	2.10
			Standard deviation	1.67
		Positive control Mitomycin C 0.3 µg/mL	Number of observations	26
			Minimum	7
			Maximum	40
			Mean	19
			Standard deviation	7.47
	With S9 mix	Negative control	Number of observations	38
			Minimum	0
			Maximum	7
			Mean	1.82
			Standard deviation	1.67
		Positive control Cyclophosphamide 7.5 µg/mL	Number of observations	38
			Minimum	12
			Maximum	41
			Mean	23.32
			Standard deviation	7.35
20-hour treatment	Without S9 mix	Negative control	Number of observations	24
			Minimum	0
			Maximum	5
			Mean	1.67
			Standard deviation	1.37
		Positive control Mitomycin C 0.2 µg/mL	Number of observations	22
			Minimum	9
			Maximum	44
			Mean	29.87
			Standard deviation	9.58

Calculated on data obtained from March, 1998 to July, 2005 (historical data not audited).

The potential mutagenicity of teriflunomide was evaluated in an *in vitro* Chinese hamster V79 HPRT assay. As communicated to the sponsor in a Clinical Hold Letter dated June 17, 2004, the first two studies (Nos. F2001TOX0450 and F2000TOX0122) were

determined to be invalid due to noncompliance with GLP and ICH guidelines, respectively. In the third study, reviewed below, higher concentrations of teriflunomide were evaluated and the results were negative.

Study title: HMR1726 (b) (4) (Teriflunomide)

Study no.:	2005-0144
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	Aventis Pharma Deutschland GmbH Hattersheim, Germany
Date of study initiation:	January 27, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, Lot #W001, 100.2% purity

Methods

Cell line:	V79 Chinese hamster lung fibroblasts
Concentrations in definitive study:	Without S9: 62.5, 93.8, 125, 187.5, 250, 375, 500, 625, 750, 875, 1000 μg/mL With S9: 62.5, 93.8, 125, 187.5, 250, 375, 500, 625, 750, 875 μg/mL
Basis of concentration selection:	Without S9: Cellular cytotoxicity in test article treated cultures (1000 μg/mL) was 17.1%-21.8% of the solvent control With S9: Cellular cytotoxicity ranged 10-20% of the solvent control at a maximum test article concentration of 625-750 μg/mL
Negative control:	Untreated medium Medium containing DMSO (≤1%)
Positive control:	Without S9: Ethyl methane sulfonate (EMS) With S9: 7,12-dimethylbenz[a]anthracene (DMBA)
Formulation/Vehicle:	DMSO (≤1%) diluted in culture medium
Incubation & sampling time:	Induced SD rat liver S9 was used for metabolic activation 4 hr treatment

Study Validity

As conducted, this study is valid; the solvent control data were within the historical control range for spontaneous mutant frequency, positive controls induced a statistically significant increase that was within the range of the historical control, and the efficacy of plating for the solvent control was greater than 50%.

Results

In the absence or presence of metabolic activation, teriflunomide did not increase the number of HPRT mutant colonies or the mutant frequency in Chinese hamster V79 cells compared to solvent controls at any of the concentrations used in the absence or presence of metabolic activation.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

The sponsor evaluated the *in vivo* potential for teriflunomide to produce chromosomal aberrations in three rodent species—mouse, hamster, and rat.

Study title: HMR1726 Mammalian Erythrocyte Micronucleus Test in male and female NMRI mice

Study no:	017974
Study report location:	EDR 4.2.3.3.2
Conducting laboratory and location:	HMR Deutschland GmbH Frankfurt, Germany
Date of study initiation:	February 2, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, Lot #U001, 99.5% purity

Methods

Doses in definitive study:	0, 10, 30, 100 mg/kg/day
Frequency of dosing:	Once daily for 2 days
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	2% (w/v) starch mucilage
Species/Strain:	Mouse/HsdWin:NMRI
Number/Sex/Group:	5/sex/group
Basis of dose selection:	In a dose-range finding study, 100 mg/kg resulted in deaths in 3M and 1F; however, mortality occurred between Days 2-6 after second administration. Therefore, 100 mg/kg was utilized as the high dose in this study.
Negative control:	2% (w/v) starch mucilage
Positive control:	Cyclophosphamide (50 mg/kg); administered once on Day 2

Study Validity

This study was considered valid since a one-sided Wilcoxon-Test showed a significant increase in micronuclei in the positive control (p=0.05) for males and females combined.

Results

All dosed animals survived treatment; however, HD groups showed decreased motor activity following the second dose of teriflunomide. Upon necropsy, stomachs were described as "tight-filled" and the bone marrow suspensions of HD groups were dark-red in color.

In both sexes, the positive control (cyclophosphamide) resulted in a statistically significant increase in micronuclei. In contrast, none of the animals dosed with teriflunomide at 10, 30, or 100 mg/kg/day had increased micronuclei compared to the vehicle-treated controls.

These data suggest that teriflunomide is not a clastogen in the bone marrow of NMRI mice.

Study title: HMR 1726 Mammalian Bone Marrow Chromosome Aberration Test

Study no:	F2001tox0155
Study report location:	EDR 4.2.3.3.2
Conducting laboratory and location:	Aventis Pharma Deutschland GmbH Frankfurt, Germany
Date of study initiation:	March 12, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, Lot #C006 (b) (4), 100.3% purity

Methods

Doses in definitive study:	0, 20, 60, 200 mg/kg/day
Frequency of dosing:	Once daily for 2 days
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	2% (w/v) starch mucilage
Species/Strain:	Hamster/Chinese (b) (4)
Number/Sex/Group:	5/sex/group
Basis of dose selection:	In a preliminary dose range-finding study, 300 mg/kg resulted in deaths in 1M and 2F; the highest sub lethal dose was 200 mg/kg that was selected as the high dose in this study
Negative control:	2% (w/v) starch mucilage
Positive control:	Cyclophosphamide (50 mg/kg); administered once on Day 2

Study Validity

As conducted, this assay is a valid study; the negative control data were within the sponsor's historical control range and the positive control group increased significantly the mutation frequency compared to the negative control.

Results

There were 4 premature deaths of HD animals in this study, 3M and 1F. Clinical signs prior to death included hypoactivity, uncoordinated gait, prone position, panting, tonic-clonic convulsions, lateral position, trembling, and palpebral fissure closed. The animals that died on study were replaced; these replacement animals survived after treatment. No other clinical signs were noted in any other animals. There were no macroscopic related findings noted.

In this study, none of the teriflunomide-treated groups had a significant increase in the number of metaphases with aberrations excluding gaps compared to vehicle control groups. In contrast, the mean number of metaphases with aberrations excluding gaps in the cyclophosphamide-treated groups was increased 67.3-fold compared to vehicle control.

Taken together, under the conditions of this study, teriflunomide is not a clastogen in the bone marrow of Chinese hamsters.

Study title: HMR1726: 14-Day Repeat Dose Oral Bone Marrow Chromosome Aberration Assay in Rat

Study no:	MAF0076
Study report location:	EDR 4.2.3.3.2
Conducting laboratory and location:	Sanofi-Aventis Deutschland GmbH Hattersheim, Germany
Date of study initiation:	November 26, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, Lot# W002, 100.1% purity

Methods

Doses in definitive study:	0, 1 (LD), 10 (MD), 20 (HD) mg/kg/day
Frequency of dosing:	Once daily for 2 days
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	2% (w/v) starch mucilage
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	5/sex/group
Satellite groups:	Replacement animals of 1/sex/group for Control, LD or MD mg/kg teriflunomide; 3/sex/group for HD
Basis of dose selection:	Doses were based upon results of an exploratory 14-day oral toxicity study in which 2/6 rats died after oral administration of 30 mg/kg teriflunomide. At 20 mg/kg, the mitotic index in the bone marrow was 34% of control and was considered an MTD.
Negative control:	2% (w/v) mucilage starch
Positive control:	Cyclophosphamide (20 mg/kg, administered on Day 14)

Study Validity

As conducted, this study is considered valid as the spontaneous aberration frequency of the negative control was within the normal range of the historical control and cyclophosphamide treatment (positive control) significantly increased the aberration frequency.

Results

Animals were checked daily for mortality and clinical signs. One HDF was emaciated and found dead on Day 11; however, necropsy did not reveal any macroscopic findings. In HDF, clinical signs included decreased activity, uncoordinated gait, and piloerection. In males, teriflunomide decreased mean body weight compared to control in MD and HD groups by -11.5% and -18.6%, respectively. In females, mean body weight was reduced only in the HD group by -14.9% compared to control. At necropsy, most HD animals had a dark-colored spleen (most males and all females). Toxicokinetic data are provided in the sponsor's Tables 5 and 6.

Table 5 - Plasma concentrations of HMR1726 Day 1

Dose group[mg/kg/day]	AUC (0-24 h)	
	male [µg/mL x h]	female [µg/mLx h]
0	0	0
1	46.6	66
10	814	901
20	1590	1820

Table 6 - Plasma concentrations of HMR1726 Day 14

Dose group[mg/kg/day]	AUC (0-24 h)	
	male [µg/mL x h]	female [µg/mLx h]
0	0	0
1	41.4	71.7
10	411	474
20	626	1310

Determination of Cytotoxicity and Chromosomal Aberrations

In both sexes, a dose-dependent reduction in the mitotic index was achieved and the greatest reduction was observed in HD animals. In this study, the mean number of spontaneous aberrations was 0.2 ± 0.42, which is within the range of the historical control values (0.1-1.0). In teriflunomide-treated groups, none of the groups had mean aberration values (0.1, 0.5, and 0.3 for LD, MD, and HD, respectively) that exceeded or that was significantly increased from the mean negative control values. In contrast, animals treated with cyclophosphamide had a significantly increased mean number of aberrations excluding gaps of 11.9 compared to the negative control mean number of aberrations of 0.2.

In SD rat, repeated daily treatment (14 days) with teriflunomide did not increase the mean number of chromosomal aberrations in bone marrow cells. These data suggest that under the conditions of this study, teriflunomide is not clastogenic in bone marrow cells from rats.

8 Carcinogenicity

The sponsor conducted two carcinogenicity studies, one in mouse (CAR0092) and one in rat (CAR0093).

Study title: HMR1726 - Oral carcinogenicity study in mice

Study no.:	CAR0092
Study report location:	EDR
Conducting laboratory and location:	Sanofi-Aventis Deutschland GmbH* Frankfurt, GERMANY
Date of study initiation:	September 09, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	HMR-1726, Lot # W001, 100.1-100.6%
CAC concurrence:	Yes, Dec 19, 2007 Fax

Key Study Findings

Adequacy of the carcinogenicity and appropriateness of the test model: The mouse carcinogenicity bioassay was an adequate study. The doses of teriflunomide selected in this study produced clear toxicities, including decreased body weight and reduced survival at the HD. Dosing in the HDM was stopped prematurely during Week 95 due to the reduced survival rate, but the group was sacrificed at approximately Week 104. Cessation of dosing in the HDM group did not compromise the outcome of this bioassay.

Evaluation of tumor findings: Negative, M; Negative, F.

Methods

Doses:	0 (C), 1 (LD), 4 (MD), 12 (HD) mg/kg/day
Frequency of dosing:	Once a day
Dose volume:	10 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Control 1 and 2: 2% Potato starch Control 3: Deionized water
Basis of dose selection:	MTD
Species/Strain:	Crl:CD1(ICR)
Number/Sex/Group:	60/sex/grp
Age:	6-7 wks
Animal housing:	M: individually housed F: Group housed (maximum 3/cage)
Paradigm for dietary restriction:	Ad libitum
Dual control employed:	Yes, Control groups 1 and 2 (2% potato starch) Control group 3 (Deionized water)
Interim sacrifice:	
Satellite groups:	TK animals: Groups 1-3, 18/sex/grp Groups 4-6, 30/sex/grp
Deviation from study protocol:	None

Observations and Results

Mortality was recorded at least twice daily (once daily on weekend/holidays) and once on day of termination.

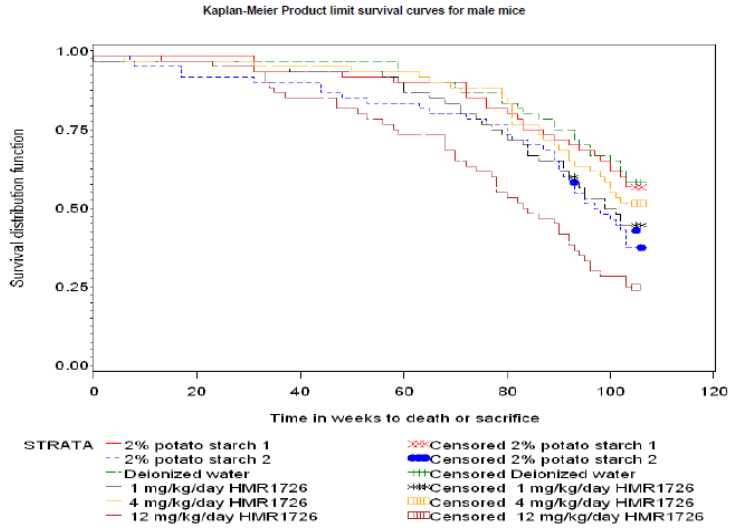
- In HD group (both sexes), there was a statistically significant decrease in survival (see sponsor's Text tables 4 and 5). In males, the mortality rate resulted in premature termination of dosing in the HD group during Week 96; the group was terminated on Day 729 (Week 104). The Kaplan-Meier Product limit survival curves are shown in the sponsor's Figures 3 and 4, below.

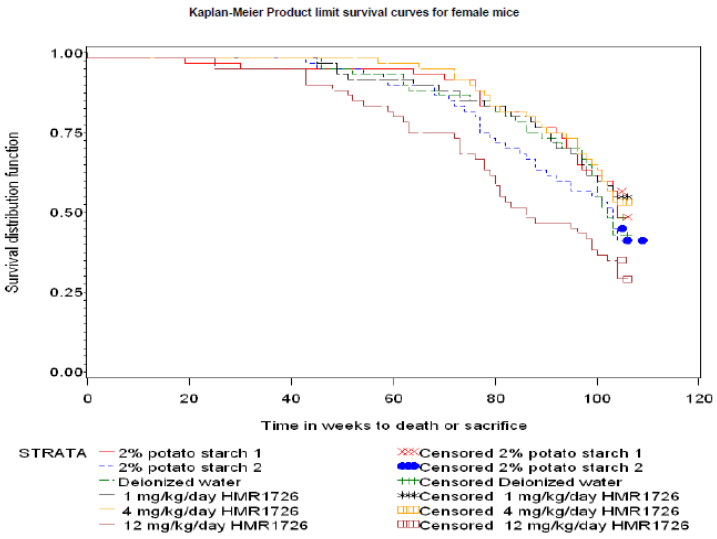
Text table 4 – Survival rates at 50, 80 and 104 weeks [%] in male mice

Group Dose (mg/kg/d)	1 0	2 0	3 0	4 1	5 4	6 12
Survival rates in [%]*						
Week 50	93	87	100	95	95	85 (-15)**
Week 80	82	77	87	73	85	55 (-37)**
Week 104 (before scheduled necropsy)	57	40	58	43	52	25 (-57)**
* [%]-values rounded ; ** = maximum difference to controls in [%]; rounded						

Text table 5 – Survival rates at 50, 80 and 104 weeks [%] in female mice

Group Dose (mg/kg/d)	1 0	2 0	3 0	4 1	5 4	6 12
Survival rates in [%]*						
Week 50	97	97	98	97	100	90 (-8)**
Week 80	83	73	83	85	87	62 (-25)**
Week 104 (before scheduled necropsy)	53	42	47	55	53	33 (-38)**
* [%]-values rounded ; ** = maximum Difference to controls in [%]; rounded						





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- In Text table 6 (sponsor's, below), the main causes of death are summarized; causes of death included ulcer and inflammation of the skin or of the gastrointestinal tract.

Text table 6 - Summary of main causes of death in male and female mice

mg/kg/d	0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group	1	2	3	4	5	6	1	2	3	4	5	6
Sex	M	M	M	M	M	M	F	F	F	F	F	F
Intercurrent death	26	35	25	33	29	45	28	34	32	27	28	40
Not evident	5	8	5	7	7	6	2	0	3	2	2	1
Skin: ulcer/ inflammation	7	9	6	10	11	19	4	4	2	4	4	9
GIT: ulcer/ inflammation	1	5	1	3	4	7	0	0	3	1	1	13
Malignant lymphoma	3	1	2	5	0	4	7	7	2	6	2	3
Sarcoma: histiocytic	1	0	0	0	1	1	4	6	5	3	6	3
CPN	1	2	1	1	2	1	4	6	5	4	1	5

[a] vehicle control group starch
[b] water control group

Clinical Signs were recorded every 4 weeks, commencing with Week 2, for the first 54 weeks of the study; thereafter, clinical signs were recorded every 2 weeks. Additional clinical signs were recorded outside the scheduled examination if adverse observations were noted.

- In Text table 7 (sponsor's), the clinical signs with the greatest incidences are summarized for all dose groups. In the HD groups, increased incidences of several of these clinical signs are noted; in particular, the incidences of soft feces and protruding anus and/or rectum are markedly increased in both sexes compared to the vehicle or water controls and LD and MD groups.

Text table 7 – Summary of main clinical signs

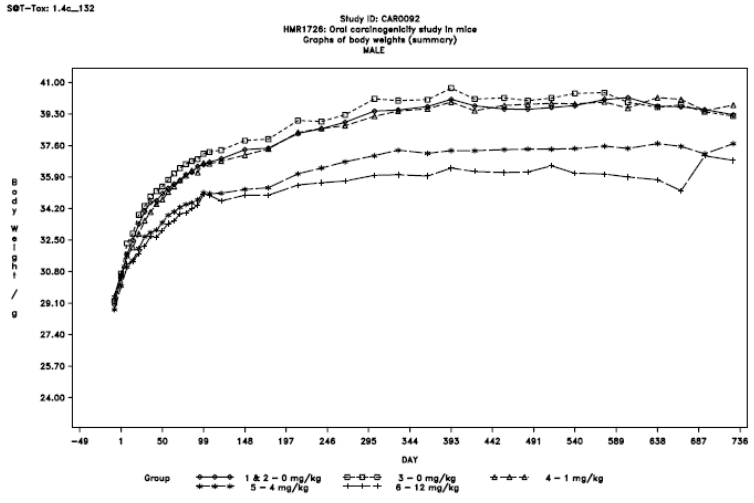
Daily Dose (mg/kg/d)	0* / 0**		1**		4**		12**	
Gender	M	F	M	F	M	F	M	F
Clinical Observations [number of animals with sign]								
Activity decreased [A]	33 / 13	31 / 14	13	15	12	13	15	22
Coat unkempt [A]	3 / 2	3 / 2	0	2	0	4	10	51
Cold to touch [A]	20 / 7	19 / 11	11	12	8	6	17	18
Feces soft [C]	0 / 0	0 / 0	1	0	0	0	16	45
Incoordination gait stilted; all limbs [A]	8 / 4	9 / 7	0	5	3	11	0	19
Paleness; whole body [A]	23 / 6	34 / 27	13	13	15	12	33	21
Protruding anus and/or rectum [C]	1 / 0	0 / 0	0	0	1	0	8	5
Scab; ear and/or forelimb right [T]	19 / 7	3 / 1	8	4	15	5	19	13
Scab; neck dorsal [T]	21 / 7	17 / 6	8	15	15	10	17	15
Sparse hair growth [A]	1 / 0	0 / 0	0	1	0	1	21	2
Humane euthanasia	25 / 10	31 / 19	11	15	18	17	19	16
Moribund euthanasia	11 / 5	10 / 3	9	6	6	2	10	13

* Pooled controls 1 & 2 = 120 control mice/sex; ** compared to 59 or 60 mice/sex in Group 3 (water control) and Groups 4 to 6 (treated);
[T] = secondary to treatment procedures; [A] = age-related findings influenced by test-article; [C] _ = test-article related;

Body Weights were measured on Day -7 (M) and Day -8 (F) during the pretest period; during the dosing period, body weights were measured commencing on Day 1 at weekly intervals for the first 16 weeks followed thereafter at monthly intervals. The effect of

teriflunomide on body weight in male and female mice, are shown in sponsor's Text tables 8 and 9.

- In CM, there was a significant difference in mean body weight between vehicle controls (Control-1 and Control-2) but only during study Days 1-148. The sponsor's figures below present the results compared to the pooled vehicle controls.
- In males, a significant reduction in body weight gain was observed at both the MD and the HD compared to the pooled vehicle controls. At the MD, there was a significant reduction in body weight gain from Day 1 to Day 694. At the HD, the significant reduction in body weight gain lasted until the end of the study.
- In females, there was a consistent significant reduction in body weight gain at the HD throughout the study.



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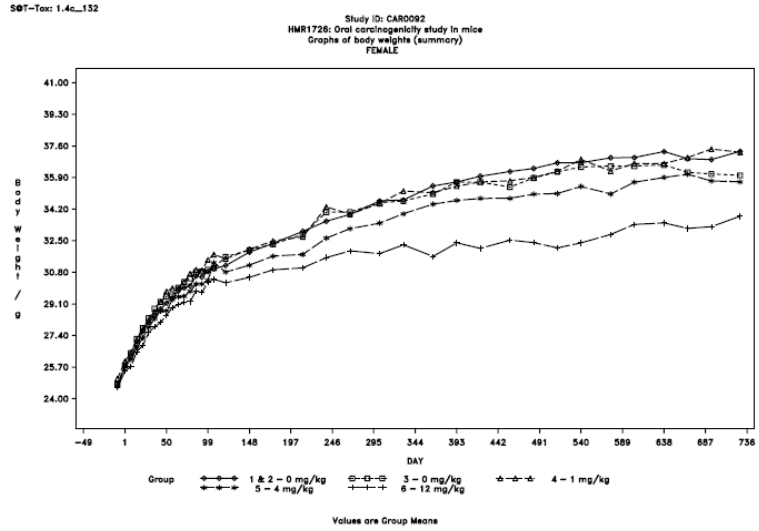

Text table 8 – Effects on body weight development in male mice

Daily dose (mg/kg/d); Males	0 °	0 (water)	1	4	12
Absolute Body Weight [g] & (%)a					
Day 29	33.91	+1	-1	-4	-5
Day 85	36.12	+2	0	-4	-5
Day 176	37.45	+1	0	-6	-7
Day 365	39.70	+1	0	-6	-9
Day 728	39.26	0	+1	-4	-6
Absolute body weight gain [g]; (%)a					
Day 1 - 29	3.55	+3	-17	-25	-58
Day 1 - 85	5.74	+5	-2	-21	-41
Day 1 - 176	7.03	+3	-3	-24	-40
Day 1 - 365	9.20	+2	-2	-22	-42
Day 1 - 728	8.92	-4	+7	-12	-24

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Text table 9 – Effects on body weight development in female mice

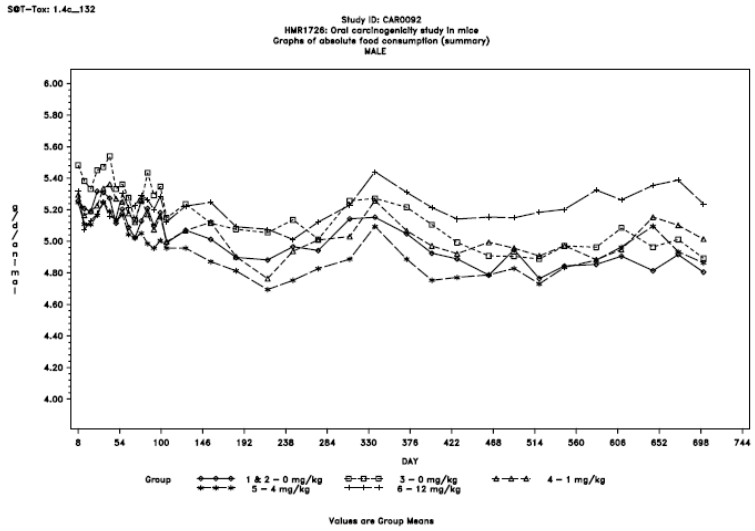
Daily dose (mg/kg/d); Males	0 °	0 (water)	1	4	12
Absolute Body Weight [g] & (%)a					
Day 29	28.13	+1	0	-1	-2
Day 85	30.61	+1	+1	-1	-3
Day 176	32.36	0	0	-2	-4
Day 365	35.45	-1	-1	-3	-11
Day 728	37.31	-3	0	-4	-9
Absolute body weight gain [g]; (%)a					
Day 1 - 29	2.34	+8	-5	-10	-12
Day 1 - 85	4.82	+4	+2	-8	-11
Day 1 - 176	6.57	-1	-2	-10	-17
Day 1 - 365	9.68	-4	-7	-10	-34
Day 1 - 728	11.54	-10	-3	-14	-27

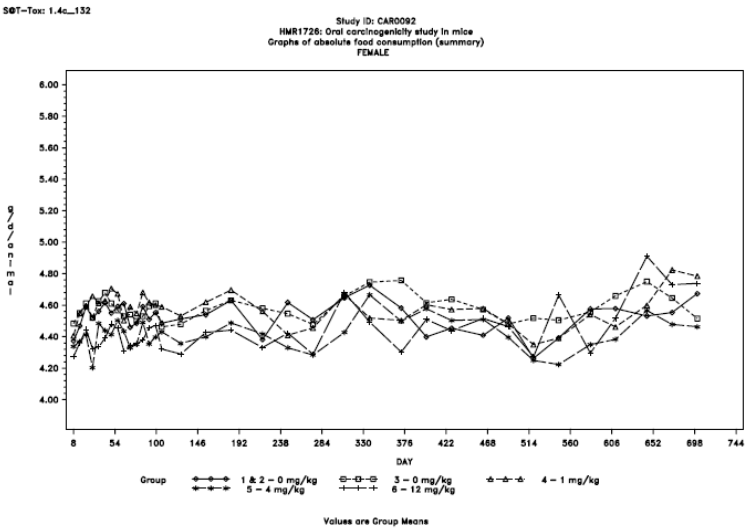
ontrol Groups 1 & 2 were pooled; a = for vehicle controls, group means (g) are shown. For treated groups (+ v
cent differences to pooled control Groups 1&2 are shown – grayish marked fields considered compound-relate

Food Consumption (g/animal/day) was measured during the dosing period at weekly intervals for the first 16 weeks of the study and then at monthly intervals thereafter.

- In males, there was a significant increase in food consumption in the HD group throughout the study compared to the pooled vehicle control group; however, this increase in food intake failed to prevent the decrease in mean body weight gain observed in this group.
- In females, there was no significant effect on food consumption.

(Sponsor's figues)





Gross Pathology

- In both sexes, an increased incidence of scabs/crust in the skin was found in the HD group (see sponsor's Text table 2); this finding correlated with a microscopic observation of ulcer/inflammation of the skin.
- Additionally at the HD, prolapse of the rectum was found in males (4/60) and females (5/60); this finding was not present in any other treatment group. The finding of prolapse of the rectum correlated with a microscopic observation of ulcer/inflammation of the large intestine.
    - 2/4 HDM with prolapse of the rectum also had ulceration of the gastrointestinal tract—one in the glandular stomach (#874) and one in the duodenum (#830).

- In the affected HDF, chronic inflammation (#944 and #964) or ulceration (#940, 958, and 965) was also present in the rectum.

Text table 2 - HMR1726-related macroscopic findings in male and female mice

mg/kg/d	0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group	1	2	3	4	5	6	1	2	3	4	5	6
Sex	M	M	M	M	M	M	F	F	F	F	F	F
Skin												
Scab/crust	10	17	9	11	14	24	4	3	4	11	5	15
Rectum												
Prolapse	-	-	-	-	-	4	-	-	-	-	-	5

[a] vehicle control group starch
[b] water control group

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Histopathology

As conducted, an adequate battery of tissues was evaluated by histopathology; a signed and dated pathology report was provided.

Peer Review was conducted by (b) (4) according to the sponsor, the pathology peer review statement was located in the test facility archives but was not provided in the final study report.

Neoplastic

In males, tumors (adenoma and/or adenocarcinoma) were identified in the small intestine (duodenum and/or jejunum). The sponsor reported these separately (Text Table 3) and combined for statistical analyses.

- No adenoma or adenocarcinoma was present in the vehicle (2% potato starch) control (Groups 1 and 2); however, a total of 4 findings occurred in the deionized water control (Group 3).
- In teriflunomide-treated male mice, there was a statistically significant trend for adenoma/adenocarcinoma in males treated with teriflunomide compared to pooled vehicle controls (Groups 1 and 2).
  - There was no non-neoplastic change in the gastrointestinal tract associated with the presence of adenoma/adenocarcinoma of the intestine.
- The sponsor did not consider these findings to represent teriflunomide-dependent findings since there was an occurrence of 1 adenocarcinoma and 3 adenomas present in the deionized water control.

Text table 3 - Neoplastic findings in the small intestine (duodenum/jejunum)

mg/kg/d		0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group		1	2	3	4	5	6	1	2	3	4	5	6
Sex		M	M	M	M	M	M	F	F	F	F	F	F
Duodenum	No. examined	50	51	53	54	56	56	57	54	58	54	58	55
	Adenocarcinoma	-	-	1	-	-	-	-	-	-	-	-	-
	Adenoma	-	-	2	-	2	1	1	-	1	1	-	-
Jejunum	No. examined	58	57	58	59	60	60	59	56	59	59	59	56
	Adenocarcinoma	-	-	-	-	-	1	-	-	-	-	-	-
	Adenoma	-	-	1	1	-	1	-	-	-	-	-	-

[a] vehicle control group starch
[b] water control group

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Table 3 - CAR0092 - Combined tumor incidence analysis - M Significant findings

Tumor by tissue/organ	Group Dose(mg/kg/day)	1 0	2 0	3 0	4 1	5 4	6 12
DUODENUM/JEJUNUM COMBINED	Examined tissues	59	57	59	60	60	60
Adenoma/ Adenocarcinoma	Non lethal tumors	0	0	3	1	2	3
	Lethal tumors	0	0	1	0	0	0
	Treated vs. Dual	**0.0056**		**0.0213**	0.1596	0.0866	**0.0075**
	Treated vs. Ctrl1	**0.0191**		0.0694	0.3473	0.2167	**0.0251**
	Treated vs. Ctrl2		**0.0236**	0.1284	0.3944	0.2541	**0.0308**

The p-values under the vehicle controls are from upper-tailed Peto trend tests.
The p-value under the water control (3) is from two-tailed Peto test to the vehicle control.
The p-values under each treated group are from upper-tailed Peto pairwise comparisons to the vehicle control.
Significant p-values are presented in bold at 2.5% level for trend tests and 5% level for pairwise comparisons.
: difference between the two vehicle controls is statistically significant at 5% level.
24 May 2011, 8:11

Non Neoplastic Findings

Several teriflunomide-related non-proliferative findings were identified.

- At the HD (both sexes), non-proliferative findings were identified in the skin, gastrointestinal tract, thymus, mesenteric lymph node, heart, liver, kidney, bone, and bone marrow. Amyloidosis was also observed in several organs including the stomach, intestine, pancreas, liver, kidney, spleen, lymph node, salivary glands, adrenal gland, and thyroid/parathyroid.
- In males, thymic atrophy was found in both MD and HD groups.

The teriflunomide-related findings are shown in sponsor's Text tables, below.

- At the HD (both sexes), an increased incidence of moderate ulcer was observed in the skin.

Text table 4 - HMR1726-related skin finding

mg/kg/d	0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group	1	2	3	4	5	6	1	2	3	4	5	6
Sex	M	M	M	M	M	M	F	F	F	F	F	F
No. examined	58	58	59	60	60	60	60	59	60	60	60	60
Ulcer	8	13	7	10	13	23	4	5	4	8	5	15
Grade 2	-	2	-	1	1	1	1	-	-	2	1	3
Grade 3	6	7	4	5	8	19	2	2	2	4	2	10
Grade 4	2	4	3	4	4	3	-	2	2	2	2	2
Grade 5	-	-	-	-	-	-	1	1	-	-	-	-

[a] vehicle control group starch
[b] water control group

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- At the HD (both sexes), there was an increased incidence of ulceration and chronic inflammation of the gastrointestinal tract (glandular stomach, duodenum, jejunum and cecum, colon and rectum).
    - In the duodenum, the incidence and severity of avillous hyperplasia was increased in females. The incidence of moderate (Grade 3) avillous hyperplasia in HDF was 59% compared to 40.8% in the pooled vehicle control group or 38% in the water control. Although the incidence was not significantly increased in LD and MD females, severe avillous hyperplasia was observed at all doses but not in any of the controls groups.
    - In the colon and rectum, there was an increased incidence of mild to moderate epithelial cell hyperplasia at the HD (both sexes). The sponsor considers the hyperplastic lesions as reactive to inflammation. The incidence of the hyperplastic lesions is similar to those showing evidence of chronic inflammation.

Text table 5 - HMR1726-related glandular stomach finding

mg/kg/d		0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group		1	2	3	4	5	6	1	2	3	4	5	6
Sex		M	M	M	M	M	M	F	F	F	F	F	F
Glandular stomach	No. examined	60	59	60	60	60	60	60	59	60	60	60	60
	Ulcer	0	0	0	0	0	2	0	0	1	0	0	4
	Grade 2	-	-	-	-	-	1	-	-	-	-	-	1
	Grade 3	-	-	-	-	-	1	-	-	-	-	-	3
	Grade 5	-	-	-	-	-	-	-	-	1	-	-	-

[a] vehicle control group starch
[b] water control group

Text table 6 - HMR1726-related findings in small intestine

mg/kg/d		0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group		1	2	3	4	5	6	1	2	3	4	5	6
Sex		M	M	M	M	M	M	F	F	F	F	F	F
Duodenum	No. examined	50	51	53	54	56	56	57	54	58	54	58	55
	Ulcer	0	1	1	0	0	3	2	4	6	4	2	7
	Grade 1	-	-	1	-	-	1	-	1	-	-	1	2
	Grade 2	-	1	-	-	-	1	1	3	5	4	1	2
	Grade 3	-	-	-	-	-	1	1	-	-	-	-	3
	Grade 5	-	-	-	-	-	-	-	-	1	-	-	-
	Chronic inflammation	0	0	0	0	2	3	0	0	1	1	2	1
	Grade 1	-	-	-	-	-	-	-	-	-	-	1	-
	Grade 2	-	-	-	-	1	2	-	-	1	1	1	1
	Grade 3	-	-	-	-	1	1	-	-	-	-	-	-
	Avillous hyperplasia	11	16	14	6	13	10	25	24	29	29	35	39
	Grade 1	1	6	7	-	3	-	6	5	6	3	7	2
	Grade 2	8	8	6	4	8	5	8	10	12	16	16	11
	Grade 3	2	2	1	2	2	5	11	9	11	8	11	23
	Grade 4	-	-	-	-	-	-	-	-	-	2	1	3
Jejunum	No. examined	58	57	58	59	60	60	59	56	59	59	59	56
	Ulcer	0	0	0	0	0	0	0	0	1	0	1	2
	Grade 2	-	-	-	-	-	-	-	-	-	-	-	1
	Grade 3	-	-	-	-	-	-	-	-	1	-	1	-
	Grade 4	-	-	-	-	-	-	-	-	-	-	-	1

[a] vehicle control group starch
[b] water control group

Text table 7 - HMR1726-related findings in large intestine

mg/kg/d		0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group		1	2	3	4	5	6	1	2	3	4	5	6
Sex		M	M	M	M	M	M	F	F	F	F	F	F
Cecum	No. examined	58	54	58	59	59	56	59	56	59	59	57	55
	Ulcer	0	0	0	0	0	1	0	1	0	2	0	6
	Grade 1	-	-	-	-	-	-	-	-	-	-	-	1
	Grade 2	-	-	-	-	-	-	-	-	-	1	-	1
	Grade 3	-	-	-	-	-	1	-	1	-	-	-	3
	Grade 4	-	-	-	-	-	-	-	-	.	1	-	1
Colon	No. examined	60	58	60	58	55	59	60	58	59	60	57	59
	Ulcer	0	0	1	0	1	3	0	0	0	0	1	3
	Grade 1	-	-	1	-	-	-	-	-	-	-	1	1
	Grade 2	-	-	-	-	-	2	-	-	-	-	-	1
	Grade 3	-	-	-	-	1	1	-	-	-	-	-	1
	Chronic Inflammation	2	1	0	1	1	2	1	0	0	0	2	11
	Grade 1	2	-	-	-	-	-	-	-	-	-	-	1
	Grade 2	-	-	-	-	-	1	1	-	-	-	1	2
	Grade 3	-	1	-	1	1	1	-	-	-	-	-	8
	Grade 4	-	-	-	-	-	-	-	-	-	-	1	-
	Epith. cell hyperplasia	2	3	2	0	1	0	0	0	0	1	4	8
	Grade 2	1	-	2	-	-	-	-	-	-	1	2	3
	Grade 3	1	3	-	-	1	-	-	-	-	-	2	5
Rectum	No. examined	56	57	55	58	55	56	57	57	59	56	58	60
	Ulcer	0	1	1	3	3	3	0	0	0	0	0	3
	Grade 1	-	-	-	-	-	1	-	-	-	-	-	-
	Grade 2	-	-	-	-	2	-	-	-	-	-	-	-
	Grade 3	-	1	1	3	1	1	-	-	-	-	-	3
	Grade 4	-	-	-	-	-	1	-	-	-	-	-	-
	Inflammation chronic	4	2	0	2	3	9	0	0	1	0	2	19
	Grade 1	2	1	-	-	-	-	-	-	1	-	-	-
	Grade 2	1	1	-	1	-	1	-	-	-	-	1	10
	Grade 3	1	-	-	1	3	8	-	-	-	-	1	9
	Epith. cell hyperplasia	1	5	0	0	0	8	0	0	0	0	2	13
	Grade 2	-	2	-	-	-	-	-	-	-	-	2	3
	Grade 3	1	3	-	-	-	8	-	-	-	-	-	10

[a] vehicle control group starch
[b] water control group

- In the mesenteric lymph node, there was an increased incidence of moderate lymphoid follicle hyperplasia at the HD in both sexes.

Text table 9 - HMR1726-related mesenteric lymph node finding

mg/kg/d	0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group	1	2	3	4	5	6	1	2	3	4	5	6
Sex	M	M	M	M	M	M	F	F	F	F	F	F
No. examined	45	46	47	51	42	53	48	53	50	46	47	51
Hyperplasia of lymphoid follicles	5	1	3	0	1	11	3	3	1	3	2	19
Grade 2	2	1	1	-	-	2	2	2	1	-	1	3
Grade 3	2	-	2	-	1	8	1	1	-	2	1	15
Grade 4	1	-	-	-	-	1	-	-	-	1	-	1

[a] vehicle control group starch
[b] water control group

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- In males, at the MD and the HD, there was an increased incidence of mild to moderate thymic atrophy. In contrast, no increase in thymic atrophy was found in females.

Text table 8 - HMR1726-related thymus finding

mg/kg/d	0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group	1	2	3	4	5	6	1	2	3	4	5	6
Sex	M	M	M	M	M	M	F	F	F	F	F	F
No. examined	48	40	51	46	47	43	54	56	53	54	47	40
Atrophy	3	3	3	3	18	18	1	3	1	-	2	2
Grade 2	2	-	2	1	10	8	1	2	1	-	1	1
Grade 3	1	3	1	2	8	10	-	1	-	-	1	1

[a] vehicle control group starch
[b] water control group

- In HDM, thrombi and bacterial colonies were found in the heart at a moderate severity. The sponsor considers this finding septic emboli resulting from the ulcer/inflammation of the skin or gastrointestinal tract that was observed in all affected mice.

Text table 10 - HMR1726-related septicemia finding in heart

mg/kg/d	0[a]	0[a]	0[b]	1	4	12
Group	1	2	3	4	5	6
Sex	M	M	M	M	M	M
No. examined	60	58	60	60	60	60
Thrombus/ bacteria	0	0	0	0	0	5
Grade 3	-	-	-	-	-	5

[a] vehicle control group starch
[b] water control group

- In HDF, there was an increased incidence of moderate granuloma, moderately increased mixed inflammatory cell infiltrate, and presence of extramedullary hematopoiesis in the liver.

Text table 11 - HMR1726-related liver findings

mg/kg/d		0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group		1	2	3	4	5	6	1	2	3	4	5	6
Sex		M	M	M	M	M	M	F	F	F	F	F	F
Liver	No. examined	60	59	60	60	60	60	60	59	60	60	60	60
	Granuloma	28	30	43	33	38	30	42	38	36	43	43	32
	Grade 1	15	21	28	20	14	13	20	17	20	19	18	7
	Grade 2	12	9	13	11	20	14	20	17	15	20	19	15
	Grade 3	1	-	2	2	4	3	2	4	1	3	6	**10**
	Grade 4	-	-	-	-	-	-	-	-	-	1	-	-
	Infiltrate: mixed inflammatory cell	23	28	32	19	25	32	34	31	39	35	40	**45**
	Grade 1	12	18	21	10	10	13	18	14	29	18	21	8
	Grade 2	10	10	9	8	13	16	16	16	8	17	16	**26**
	Grade 3	1	-	2	1	2	3	-	1	2	-	3	**11**
	Extramedullary hematopoiesis	3	13	4	8	5	13	8	8	9	6	10	**27**
	Grade 1	1	7	2	3	-	3	2	2	4	3	2	6
	Grade 2	-	4	2	4	3	5	6	4	3	3	6	**13**
	Grade 3	2	2	-	1	2	4	-	2	2	-	2	**8**
	Grade 4	-	-	-	-	-	1	-	-	-	-	-	-

[a] vehicle control group starch
[b] water control group

- In HDF compared to pooled Control (vehicle control), there was an increase in the incidence of granulopoiesis in the bone marrow of both the femur and sternum.

Text table 12 - HMR1726-related bone marrow finding

mg/kg/d		0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group		1	2	3	4	5	6	1	2	3	4	5	6
Sex		M	M	M	M	M	M	F	F	F	F	F	F
Femur	No. examined	59	58	60	60	60	59	60	59	60	58	60	58
Bone marrow	Increased granulopoiesis	4	10	4	7	5	9	1	3	9	0	1	**13**
	Grade 2	-	3	-	3	1	2	-	-	1	-	-	3
	Grade 3	4	6	2	4	3	7	1	3	8	-	1	8
	Grade 4	-	1	2	-	1	-	-	-	-	-	-	2
Sternum	No. examined	60	58	59	59	59	60	59	59	60	60	59	59
Bone marrow	Increased granulopoiesis	3	7	4	6	3	10	4	6	9	3	2	**11**
	Grade 2	1	3	-	1	2	1	1	2	3	2	1	1
	Grade 3	2	4	4	5	-	9	3	4	6	-	1	10
	Grade 4	-	-	-	-	1	-	-	-	-	1	-	-

[a] vehicle control group starch
[b] water control group

- In 7 HDF (#925, 935, 940, 947, 952, 959, and 976), an increased incidence of moderate to marked chronic progressive nephropathy was found that was also related to an increased incidence of mild to moderate renal osteodystrophy that was found in the sternum and femur.

Text table 13 - HMR1726-related kidney findings

mg/kg/d		0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group		**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Sex		**M**	**M**	**M**	**M**	**M**	**M**	**F**	**F**	**F**	**F**	**F**	**F**
Kidney	No. examined	60	59	60	60	60	60	60	59	60	60	60	60
	CPN	1	4	1	1	3	2	4	6	5	5	2	**11**
	Grade 3	1	3	1	1	1	1	1	1	1	4	1	7
	Grade 4	-	1	-	-	2	1	3	5	4	1	1	4

[a] vehicle control group starch
[b] water control group

Text table 14 - Kidney-related bone finding

mg/kg/d		0[a]	0[a]	0[b]	1	4	12
Group		**1**	**2**	**3**	**4**	**5**	**6**
Sex		**F**	**F**	**F**	**F**	**F**	**F**
Sternum	No. examined	59	59	60	60	59	59
	Fibrous osteodystrophy	**0**	**1**	**0**	**1**	**1**	**6**
	Grade 2	-	1	-	1	1	5
	Grade 3	-	-	-	-	-	1
Femur	No. examined	60	59	60	58	60	58
	Fibrous osteodystrophy	**0**	**2**	**2**	**3**	**1**	**5**
	Grade 1	-	-	-	-	-	1
	Grade 2	-	1	1	3	-	2
	Grade 3	-	1	1	-	1	2

[a] vehicle control group starch
[b] water control group

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- At the HD (both sexes), an increased incidence of mild to moderate amyloidosis was found.

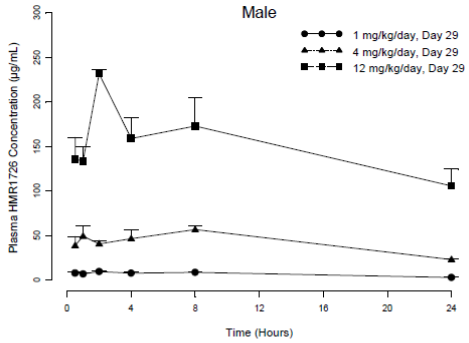
Text table 15 - HMR1726-related amyloidosis

mg/kg/d	0[a]	0[a]	0[b]	1	4	12	0[a]	0[a]	0[b]	1	4	12
Group	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Sex	**M**	**M**	**M**	**M**	**M**	**M**	**F**	**F**	**F**	**F**	**F**	**F**
No. examined	60	59	60	60	60	60	60	59	60	60	60	60
Amyloidosis	3	4	-	2	6	**19**	0	1	1	2	1	**28**
Grade 1	1	-	-	1	-	-	-	-	-	1	-	1
Grade 2	-	1	-	-	1	7	-	1	-	1	1	6
Grade 3	-	3	-	1	5	**10**	-	-	1	-	-	**21**
Grade 4	2	-	-	-	-	2	-	-	-	-	-	-

[a] vehicle control group starch
[b] water control group

Toxicokinetics

(Sponsor's figures and tables)



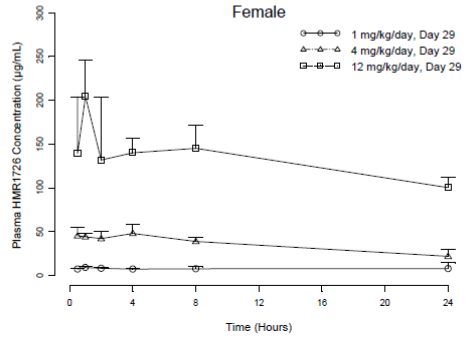


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Table 1 - Compiled table of toxicokinetic parameters

Analyte	Sex	HMR1726 Dose (mg/kg/day)	Day	t_{max} (h)	C_{max}[a]	AUC_{0-24}[b]	AUC_{0-24}/Dose[c]	R_{AUC} female/male
HMR1726	Male	1	29	2.0	9.74	162	162	NC
		4	29	8.0	56.8	1020	254	NC
		12	29	2.0	232	3600	300	NC
	Female	1	29	1.0	8.96	184	184	1.13
		4	29	4.0	47.8	828	207	0.814
		12	29	1.0	204	3120	260	0.867
4-TFMA	Male	1	29	NC	NC	NC	NC	NC
		4	29	8.0	2.02	NC	NC	NC
		12	29	2.0	17.1	304	25.3	NC
	Female	1	29	NC	NC	NC	NC	NC
		4	29	0.5	1.74	23.1	5.78	NC
		12	29	2.0	18.3	301	25.1	0.991

a. Units are µg/mL for HMR1726 and ng/mL for 4-TFMA
b. Units are µg.h/mL for HMR1726 and ng.h/mL for 4-TFMA
c. Units are µg.h.kg/mL.mg for HMR1726 and ng.h.kg/mL.mg for 4-TFMA
NC: Not able to calculate

Table 2 - Mean (SD) C_{2h} and C_{24h} on Days 29, 85 and 176

Day	Sex	HMR1726 Dose (mg/kg/day)	HMR1726 (µg/mL)		4-TFMA (ng/mL)	
			C_{2h}	C_{24h}	C_{2h}	C_{24h}
29	Male	1	9.74 (1.11)	3.09 (0.474)	<LLOQ (NC)	<LLOQ (NC)
		4	40.8 (3.27)	23.2 (0.839)	<LLOQ (NC)	<LLOQ (NC)
		12	232 (4.93)	106 (18.9)	17.1 (4.12)	8.19 (0.750)
	Female	1	8.12 (0.440)	7.70 (6.49)	<LLOQ (NC)	<LLOQ (NC)
		4	42.0 (8.52)	21.7 (8.18)	1.30 (0.163)	<LLOQ (NC)
		12	132 (71.7)	100 (11.8)	18.3 (1.47)	7.97 (1.96)
85	Male	1	5.92 (1.83)	3.11 (0.720)	<LLOQ (NC)	<LLOQ (NC)
		4	35.3 (NC)[a]	32.0 (1.40)	2.82 (NC)[a]	3.53 (2.05)
		12	148 (16.0)	97.5 (9.96)	18.4 (1.80)	10.5 (2.61)
	Female	1	6.20 (1.40)	4.52 (0.661)	<LLOQ (NC)	<LLOQ (NC)
		4	41.4 (4.65)	21.3 (14.2)	2.67 (0.759)	1.39 (1.22)
		12	109 (20.0)	87.5 (7.97)	10.4 (3.14)	7.81 (1.74)
176	Male	1	8.16 (0.883)	4.29 (0.419)	<LLOQ (NC)	<LLOQ (NC)
		4	61.9 (12.1)	44.2 (8.86)	<LLOQ (NC)[b]	<LLOQ (NC)
		12	149 (NC)[a]	107 (16.5)	6.99 (NC)[a]	4.25 (0.664)
	Female	1	7.34 (1.18)	3.28 (NC)[a]	<LLOQ (NC)	<LLOQ (NC)[a]
		4	37.2 (8.83)	26.3 (4.35)	<LLOQ (NC)	<LLOQ (NC)
		12	143 (10.2)	111 (16.4)	8.73 (0.671)	4.57 (1.43)

a. N = 2 due to unavailable blood sample from one animal (animal died at or before sampling time)
b. N = 2 due to insufficient sample for first analysis of 4-TFMA for one animal
LLOQ: 1 ng/mL for 4-TFMA
NC: Not able to calculate

Study title: HMR1726—Oral carcinogenicity study in rats

Study no.:	CAR0093
Study report location:	EDR 4.2.3.4.1
Conducting laboratory and location:	Sanofi-aventis US Inc., Bridgewater, NJ USA
Date of study initiation:	June 24, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	HMR1726, Lot #W001, 101.1%
CAC concurrence:	Yes, Dec 19, 2007 Fax

Key Study Findings

Adequacy of Carcinogenicity Study and Appropriateness of Test Models

The rat carcinogenicity bioassay was an adequate study. In this study, the doses of teriflunomide tested produced clear toxicities, including decreased survival of MDM and HDM and decreased mean body weight in HDF (>10%) compared to pooled vehicle control. Toxicokinetic analyses conducted at Weeks 29, 85, and 169/170 confirmed that plasma teriflunomide concentrations were approximately dose-proportional in both sexes.

Evaluation of Tumor Findings

The sponsor identified a statistically significant increase in the incidence of adenoma in the pituitary (pars distalis) of male rats treated with teriflunomide compared to the pooled vehicle (2% potato starch) control at the 2.5% level. An independent analysis of these data by [redacted] (b) (4) failed to confirm the statistical findings reported by the sponsor.

Methods

Doses:	Control 1: 2% potato starch Control 2: Deionized water Teriflunomide: 0.5 (LD), 1.5 (MD), and 4 (HD) mg/kg/day
Frequency of dosing:	once daily
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Vehicle: 2% potato start
Basis of dose selection:	Doses were selected based upon CAC recommendations from 19 Dec 2007 that based the doses on MTD considerations from the 6-month toxicity study in rats.
Species/Strain:	Rats/Crl:CD(SD)
Number/Sex/Group:	60/sex/grp
Age:	6-7 wks
Animal housing:	group housed 3/sex/cage
Paradigm for dietary restriction:	n/a
Dual control employed:	Yes: 2% potato starch, Grp 1 and 2 Deionized water, Grp 3
Interim sacrifice:	None
Satellite groups:	TK, 3/sex/grp Control Grp 1, 2, 3 12/sex/grp Teriflunomide
Deviation from study protocol:	None

Observations and Results

Mortality checked twice daily and once on termination day

- In males, a statistically significant decrease in survival was reported at the MD and the HD. HDM were sacrificed after approximately 92 Weeks of dosing and all surviving males in all dose groups were terminated after approximately 97 weeks of dosing.
- In females, survival was unaffected by teriflunomide treatment.
- In premature decedents of both sexes, the most common cause of mortality was pituitary gland adenomas (pars distalis) as summarized in the sponsor's Text Table 1.

Text Table 1 - Incidence of pituitary gland adenoma (pars distalis) in premature decedents

	Males						Females					
Group	1	2	3	4	5	6	1	2	3	4	5	6
HMR1726 mg/kg/day	0[a]	0[a]	0[b]	0.5	1.5	4.0	0[a]	0[a]	0[b]	0.5	1.5	4.0
No. unscheduled deaths	35	34	40	43	44	45	39	28	34	34	27	38
No. pituitary gland adenoma (pars distalis)	12	14	18	25	17	26	22	22	20	22	18	19
% pituitary gland adenoma (pars distalis)[c]	34	42	45	58	39	59	56	79	59	65	67	53

[a] vehicle control group (2% potato starch)

[b] deionized water control group

[c] % pituitary gland adenoma (pars distalis) per pituitary gland examined

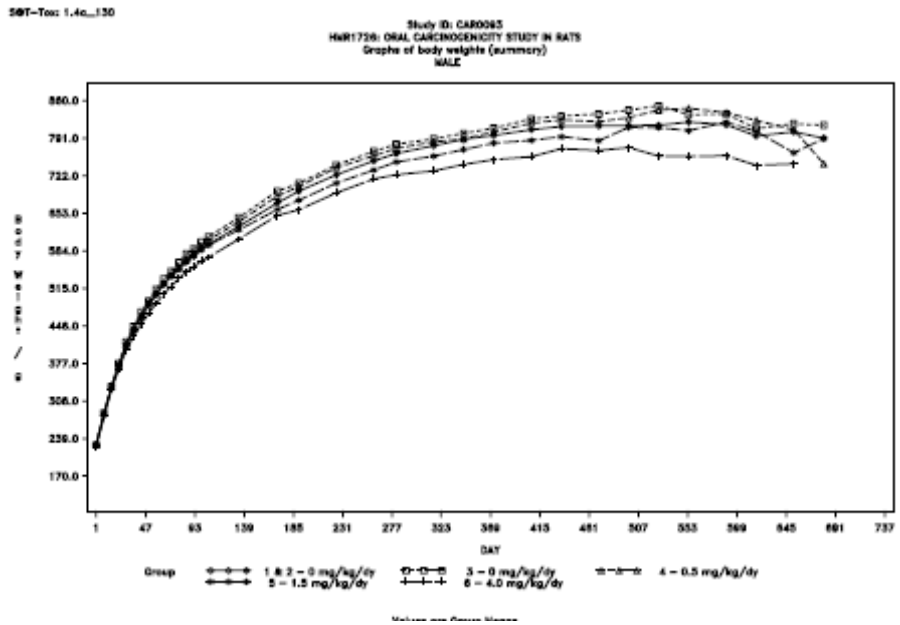
Clinical Signs were recorded monthly for the first 50 weeks of the study beginning in Week 2 and then twice per month thereafter.

- In both sexes, red or dark red ocular discharge occurred in either or both eyes more frequently in MD and HD groups.
- In HDM, additional findings included uncoordinated (unstable), paleness, and mild coat thinning (whole body) compared to the pooled vehicle control groups.

Body Weights

- In the control groups (both sexes), the mean body weight values were similar between the two vehicle control groups and between the pooled vehicle control and the water control groups.
- In males, mean body weight was decreased in the MD group during Days 85-190. At the HD, mean body weight was reduced compared to the pooled vehicle controls; however, this reduction in body weight was not significant.
- In females, mean body weight was reduced in LD animals on Days 1-85. At the MD and HD, mean body weights were reduced throughout the study. At the end of the study, the mean body weight was significantly reduced in the HD group (-14.5%) compared to the pooled vehicle control.

(Sponsor's figures)



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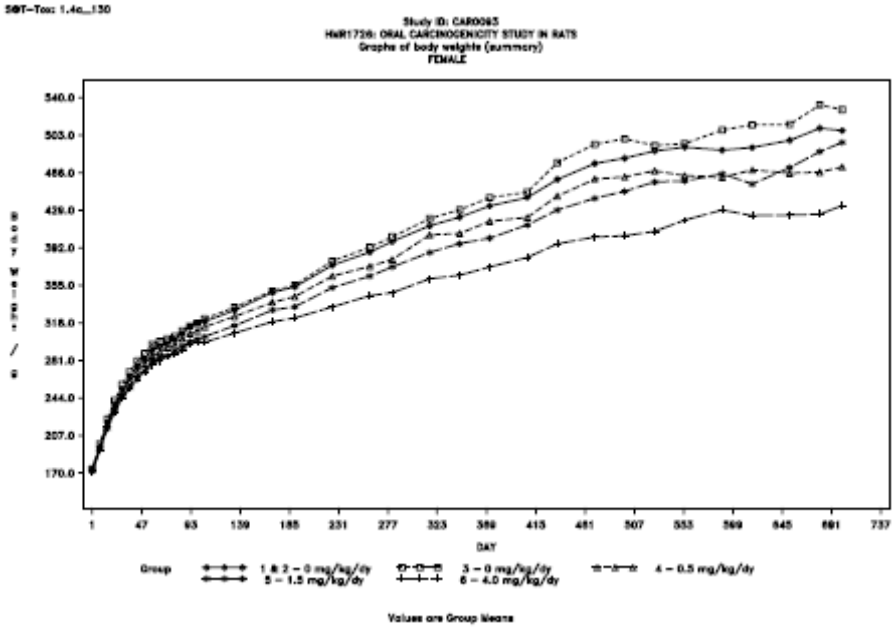
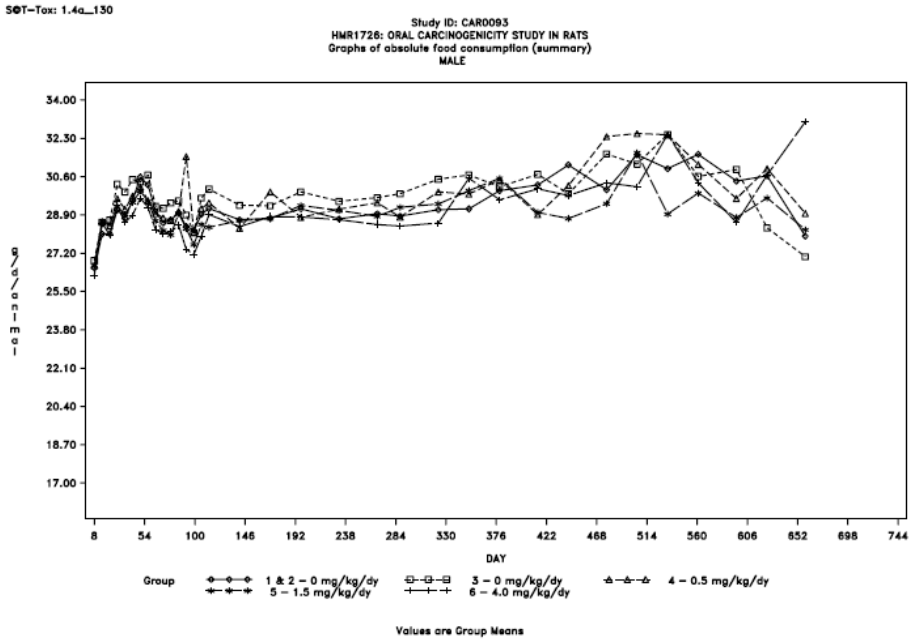


Food Consumption (g/animal/day) was measured during the dosing period at weekly intervals for the first 16 weeks of the study and then at monthly intervals thereafter (see sponsor's figures, below).

- In the pooled vehicle or water control groups, the mean food consumption was similar.
- In males, there was no significant difference in food consumption between teriflunomide treated groups and pooled vehicle control.
- In females, on Days 1 to 197, there was a statistically significant reduction in food consumption in all treatment groups compared to the pooled vehicle controls.



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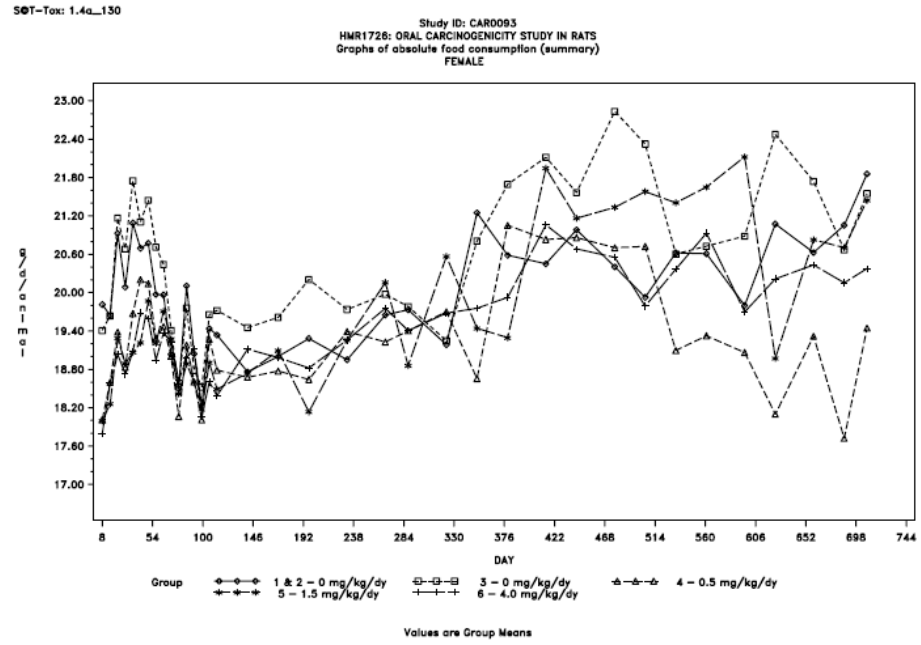

Histopathology

As conducted, an adequate battery of tissues was evaluated by histopathology; the sponsor provided a signed and dated pathology report.

Peer Review was performed by [redacted] (b) (4) DVM, DACVP. It is noted in the study report that the pathology peer review statement is located in the test facility archives.

Neoplastic

- The sponsor identified two neoplastic findings; the incidence of pituitary gland adenoma (pars distalis) and hyperplasia of the pars distalis is shown (sponsor's Text Table 2) and the incidence of thyroid gland C-cell adenoma and focal C-cell hyperplasia are provided in sponsor's Text Table 3. The sponsor did not consider either finding to be teriflunomide-related as these findings occur at a higher frequency in SD rats, occurrence in all treated groups with the absence of a clear dose-response.

Text Table 2 - Total incidence of pituitary gland adenoma (pars distalis) and hyperplasia of the pars distalis

	Males						Females					
Group	1	2	3	4	5	6	1	2	3	4	5	6
HMR1726 mg/kg/day	0[a]	0[a]	0[b]	0.5	1.5	4.0	0[a]	0[a]	0[b]	0.5	1.5	4.0
No. examined	60	59	60	60	60	59	60	60	60	60	60	58
Pituitary gland adenoma (pars distalis)	21	30	28	34	25	34	40	43	40	33	41	27
Hyperplasia: pars distalis	14	10	11	10	10	14	8	8	9	12	11	9

[a] vehicle control group (2% potato starch)

[b] deionized water control group

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Text Table 3 - Total incidence of thyroid gland C-cell adenoma and focal C-cell hyperplasia

	Males						Females					
Group	1	2	3	4	5	6	1	2	3	4	5	6
HMR1726 mg/kg/day	0[a]	0[a]	0[b]	0.5	1.5	4.0	0[a]	0[a]	0[b]	0.5	1.5	4.0
No. examined	59	60	60	60	58	60	60	60	59	59	60	59
C-cell adenoma	2	11	4	9	9	8	8	5	10	9	13	14
Focal C-cell hyperplasia	2	3	11	6	13	12	9	10	6	12	11	13

[a] vehicle control group (2% potato starch)

[b] deionized water control group

- An independent analysis of these data by (b) (4) did not find a statistical increase in pituitary pars distalis tumors in male rats and the C-cell adenoma in female rats as shown below.

Composite endpoint	*Quantity*	*Control*	*Low dose*	*Mid dose*	*High dose*
Pituitary pars distalis tumors	P-value of test of trend or comparison	.0646	.0706	.3494	.0488
	Number of animals reported with tumor	53	34	26	34
	Poly-3 adjusted incidence rate	59%	73%	63%	75%
	95% CI for poly-3 adjusted incidence rate (%)	(48,69.8)	(57.4,85.7)	(46.9,79.4)	(58.9,87.1)
	Poly-3 adjusted number of animals at risk	89.2	46.8	41.0	45.6

Composite endpoint	*Quantity*	*Water*	*Low dose*	*Mid dose*	*High dose*

Pituitary pars distalis tumors	P-value of test of trend or comparison	.2540	.3048	.6525	.2488
	Number of animals reported with tumor	28	34	26	34
	Poly-3 adjusted incidence rate	66%	73%	63%	75%
	95% CI for poly-3 adjusted incidence rate (%)	(49.1,80.4)	(57.4,85.7)	(46.9,79.4)	(58.9,87.1)

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Organ or tissue name	*Tumor name*	*Quantity*	*Control*	*Low dose*	*Mid dose*	*High dose*
THYROID GLAND	Adenoma: C-cell	P-value of test of trend or comparison	.0154	.2874	.0487	.0233
		Number of animals reported with tumor	13	9	13	14
		Poly-3 adjusted incidence rate	15%	20%	28%	31%
		95% CI for poly-3 adjusted incidence rate (%)	(7.92,23.7)	(9.36,34.6)	(15.6,43.5)	(17.7,46.6)
		Poly-3 adjusted number of animals at risk	89.0	45.5	46.3	45.6

Non Neoplastic

- In the lung, bronchioalveolar hyperplasia was found in both males and females. In both sexes, bronchioalveolar hyperplasia occurred in the vehicle and the water control groups as well as in teriflunomide-treated groups, as shown in the sponsor's Text Table 4.
  - In HDM, the incidence and severity was greater than either vehicle control (Groups 1 and 2) or the deinized water control (Group 3).
  - In females, there was no increase in incidence or severity of this hyperplasia in teriflunomide-treated groups compared to either the pooled vehicle control or water control groups.

Text Table 4 - Incidence and severity of bronchioloalveolar hyperplasia

	Males						Females					
Group	1	2	3	4	5	6	1	2	3	4	5	6
HMR1726 mg/kg/day	0[a]	0[a]	0[b]	0.5	1.5	4.0	0[a]	0[a]	0[b]	0.5	1.5	4.0
Lung No. examined	60	60	60	60	60	60	60	60	60	60	60	60
Hyperplasia: bronchioloalveolar												
No. affected	1	-	2	-	-	5	1	1	1	3	-	-
Grade 1	1	-	2	-	-	4	-	-	1	1	-	-
Grade 2	-	-	-	-	-	1	-	-	-	2	-	-
Grade 3	-	-	-	-	-	-	1	-	-	-	-	-
Grade 4	-	-	-	-	-	-	-	1	-	-	-	-

[a] vehicle control group (2% potato starch)

[b] deionized water control group

- none affected

- Hypocellularity was identified in the bone marrow, submandibular lymph node, and spleen of teriflunomide-treated animals. Compared to controls, teriflunomide-treated rats had an increased incidence and severity of hypocellularity; this finding was more pronounced in premature decedents than in scheduled deaths.

Text Table 5 - HMR1726-related bone marrow hypocellularity

	Males						Females					
Group	1	2	3	4	5	6	1	2	3	4	5	6
HMR1726 mg/kg/day	0[a]	0[a]	0[b]	0.5	1.5	4.0	0[a]	0[a]	0[b]	0.5	1.5	4.0
Premature Decedents												
Bone Marrow: Sternum No. examined	35	34	40	43	44	45	39	28	34	34	26	38
Hypocellularity: hematopoietic cells												
(% affected)	(11)	(18)	(13)	(21)	(30)	(44)	(13)	(25)	(18)	(44)	(39)	(58)
Grade 1	4	1	4	5	7	3	4	4	6	6	6	6
Grade 2	-	2	-	3	2	2	1	3	-	7	3	4
Grade 3	-	3	1	1	3	8	-	-	-	1	1	4
Grade 4	-	-	-	-	1	7	-	-	-	1	-	8
Bone Marrow: Femur No. examined	35	34	40	43	44	43	39	28	34	34	27	38
Hypocellularity: hematopoietic cells												
(% affected)	-	-	-	(5)	(7)	(33)	-	-	-	(6)	-	(29)
Grade 1	-	-	-	2	3	6	-	-	-	2	-	8
Grade 2	-	-	-	-	-	8	-	-	-	-	-	3
Scheduled Deaths												
Bone Marrow: Sternum No. examined	25	26	20	17	16	15	21	32	26	26	33	22
Hypocellularity: hematopoietic cells												
(% affected)	-	(15)	(20)	(6)	(13)	(7)	(10)	(22)	(19)	(8)	(39)	(9)
Grade 1	-	3	3	-	1	-	2	6	5	2	12	2
Grade 2	-	1	1	1	-	1	-	1	-	-	1	-
Grade 3	-	-	-	-	1	-	-	-	-	-	-	-
Bone Marrow: Femur No. examined	25	26	20	17	16	15	21	32	25	26	33	22
Hypocellularity: hematopoietic cells												
(% affected)	-	-	-	-	(6)	-	-	-	-	-	-	-
Grade 1	-	-	-	-	-	-	-	-	-	-	-	-
Grade 2	-	-	-	-	1	-	-	-	-	-	-	-

[a] vehicle control group (2% potato starch)

[b] deionized water control group

- none affected

Text Table 6 - HMR1726-related decreased plasmacytosis in submandibular lymph node

		Males						Females					
Group		1	2	3	4	5	6	1	2	3	4	5	6
HMR1726 mg/kg/day		0[a]	0[a]	0[b]	0.5	1.5	4.0	0[a]	0[a]	0[b]	0.5	1.5	4.0
Premature Decedents													
Lymph node: submandibular	No. examined	32	34	38	42	42	44	37	28	33	33	27	37
Plasmacytosis													
(% affected)		(78)	(77)	(87)	(91)	(71)	(46)	(81)	(86)	(94)	(70)	(59)	(30)
	Grade 1	23	24	26	36	30	20	21	20	26	23	16	9
	Grade 2	2	2	7	2	-	-	9	4	5	-	-	2
Scheduled Deaths													
Lymph node: submandibular	No. examined	25	26	20	17	16	15	21	32	25	26	33	22
Plasmacytosis													
(% affected)		(80)	(92)	(100)	(94)	(94)	(100)	(91)	(100)	(100)	(96)	(73)	(55)
	Grade 1	15	23	15	13	14	10	17	29	19	24	24	12
	Grade 2	5	1	5	3	1	5	2	3	6	1	-	-

[a] vehicle control group (2% potato starch)

[b] deionized water control group

- none affected

Text Table 7 - HMR1726-related decreased splenic lymphocytes in premature decedents

		Males						Females					
Group		1	2	3	4	5	6	1	2	3	4	5	6
HMR1726 mg/kg/day		0[a]	0[a]	0[b]	0.5	1.5	4.0	0[a]	0[a]	0[b]	0.5	1.5	4.0
Spleen	No. examined	35	34	40	43	44	45	39	28	34	34	26	38
Decreased lymphocytes													
(% affected)		(26)	(15)	(15)	(7)	(18)	(56)	(31)	(32)	(27)	(35)	(23)	(63)
	Grade 1	7	5	6	2	6	15	7	8	5	7	5	13
	Grade 2	2	-	-	1	-	5	4	1	2	3	-	9
	Grade 3	-	-	-	-	2	5	1	-	2	2	1	2

[a] vehicle control group (2% potato starch)

[b] deionized water control group

- none affected

Text Table 8 - HMR1726-related increased splenic pigment deposition in premature decedents

	Males						Females					
Group	1	2	3	4	5	6	1	2	3	4	5	6
HMR1726 mg/kg/day	0[a]	0[a]	0[b]	0.5	1.5	4.0	0[a]	0[a]	0[b]	0.5	1.5	4.0
Premature Decedents												
Spleen No. examined	35	34	40	43	44	45	39	28	34	34	26	38
Pigment												
(% affected)	(31)	(44)	(33)	(33)	(39)	(76)	(51)	(57)	(53)	(74)	(77)	(90)
Grade 1	11	12	13	11	15	24	18	13	14	17	13	26
Grade 2	-	1	-	3	2	8	2	3	4	8	7	8
Grade 3	-	2	-	-	-	2	-	-	-	-	-	-
Scheduled Deaths												
Spleen No. examined	25	26	20	17	16	15	21	32	26	26	33	22
Pigment												
(% affected)	(16)	(27)	(15)	(6)	(25)	(53)	(43)	(56)	(54)	(50)	(55)	(82)
Grade 1	4	7	3	1	2	8	9	16	14	13	18	18
Grade 2	-	-	-	-	1	-	-	2	-	-	-	-
Grade 3	-	-	-	-	1	-	-	-	-	-	-	-

[a] vehicle control group (2% potato starch)

[b] deionized water control group

- none affected

Toxicokinetics were measured for parent, HMR1726, and its metabolite, 4-TFMA, on Days 29, 85, and 169 (see sponsor's Text table 3) after repeated once-daily oral administration (n=3 rats/sampling time).

- In Control groups (both sexes), plasma teriflunomide and 4-TFMA exposures were below the lower limit of quantitation (LLOQ).
- Throughout this study, 4-TFMA was below the LLOQ at the LD and MD (both sexes). In contrast, 4-TFMA was measurable at the HD (both sexes); however, the metabolite to parent molar ratio for AUC_{0-24} was low (0.0000107 to 0.000393).
- On Days 29, 85, and 169, the increase in AUC_{0-24} was less than dose proportional increasing from 4.71- to 5.69-fold over the dose range of 0.5 to 4 mg/kg/day.
- Exposure of female rats was slightly greater than males on Days 29, 85, and 169 with the female:male AUC_{0-24} ratio ranging from 1.13 to 1.69. Likewise, 4-TFMA

exposure in female rats was also higher than male rats with the female:male AUC_{0-24} ratio ranging from 1.05 to 2.22 in HD group.

Text Table 3 - Toxicokinetic parameters of HMR1726 and 4-TFMA

Analyte	Sex	Dose (mg/kg/day)	Cmax [a]			AUC_{0-24} [b]		
			Day 29	Day 85	Day 169	Day 29	Day 85	Day 169/170
HMR1726	Male	0.5	2.55	3.23	2.93	37.4	52.9	51.4
		1.5	5.46	6.89	8.59	64.3	84.9	104
		4	17.5	20.1	20.2	213	253	275
	Female	0.5	3.37	4.12	4.22	45.0	61.1	60.3
		1.5	8.37	10.2	10.9	109	133	148
		4	19.6	22.8	27.0	244	287	329
4-TFMA	Male	0.5	NC	NC	NC	NC	NC	NC
		1.5	NC	NC	NC	NC	NC	NC
		4	2.08	2.31	1.69	27.5	30.4	20.0
	Female	0.5	NC	NC	NC	NC	NC	NC
		1.5	NC	1.60	NC	NC	NC	NC
		4	3.63	3.74	1.97	53.7	67.4	21.0

[a] Units are µg/mL for HMR1726 and ng/mL for 4-TFMA

[b] Units are µg.h/mL for HMR1726 and ng.h/mL for 4-TFMA

NC: Not able to calculate

9 Reproductive and Developmental Toxicology

The sponsor conducted a complete battery of reproductive and developmental toxicology studies. In the next three sections, the pivotal studies are reviewed in detail. In addition, the sponsor conducted a focused reproductive study to understand further the teratogenicity of teriflunomide; a brief review of this study is provided.

9.1 Fertility and Early Embryonic Development

The sponsor conducted two fertility and early embryonic development studies in rat—one in each sex.

9.1.1 Male rat

Study title: HMR1726: ORAL MALE FERTILITY STUDY IN RATS

Study no.:	2003-1493
Study report location:	EDR Section 4.2.3.5.1.
Conducting laboratory and location:	Aventis Pharma, Recherche-Developpement, Alfortville, France
Date of study initiation:	March 02, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	HMR-1726, Lot #W001, 99.1% purity

Methods

Doses:	0, 1, 3, 10 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg/day
Route of administration:	Oral (gavage)
Formulation/Vehicle:	2% potato starch
Species/Strain:	Rat/Crl: CD®(SD)IGS BR rat; virgin sexually mature
Number/Sex/Group:	26 M/group; Untreated 26 F/group except HDM had 25 due to premature sacrifice of 1 HDM
Satellite groups:	None
Study design:	Treatment: Males, 10 weeks prior to cohabitation, during cohabitation (21 day maximum), and after cohabitation through study Week 15; females were not treated.
Deviation from study protocol:	None

Observations and Results

Mortality was checked daily.

- One HDM (#412) was euthanized on Day 59. Prior to death, clinical signs included emaciation, decreased motor activity, and pale skin (Days 49-59). Hematology data were consistent with marked anemia (RBC = 1.13 $x10^{12}$/L, Hgb = 2.1 g/dL, and Hct = 6%). At necropsy, a pale liver was noted; however, no histopathology was performed.

- No deaths occurred in the LDM or MDM or in any of the untreated females.

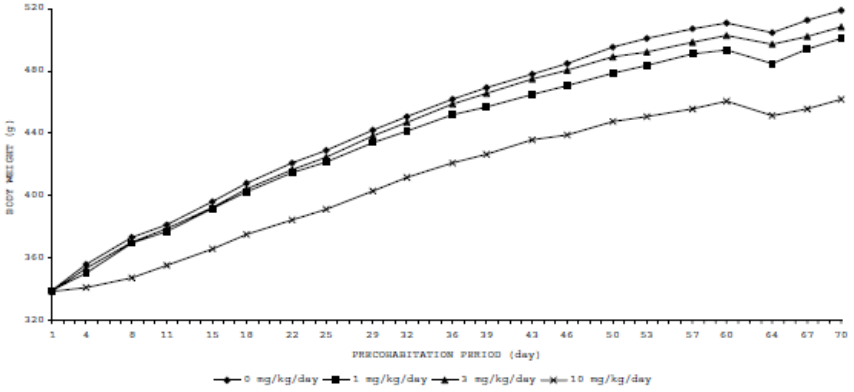
Clinical Signs were monitored three times daily for males during dosing and once daily in females.

Clinical signs present in terminal sacrifice animals were limited to HDM and included:

- Pale skin (#406) on Days 93-100
- Chromodacryorrhea (#401) on Days 2-48

Body Weight was measured twice weekly (from Days 1 to 98) during the study for males and on the day of necropsy. Females were weighed on Gestation Days 1, 7, 14, and 21.

- In HDM, there was a significant decrease in the mean terminal body weight (-11%) and mean body weight gain (see sponsor's Figures below).



Animals were fasted on Day 63 for the collection of blood samples.

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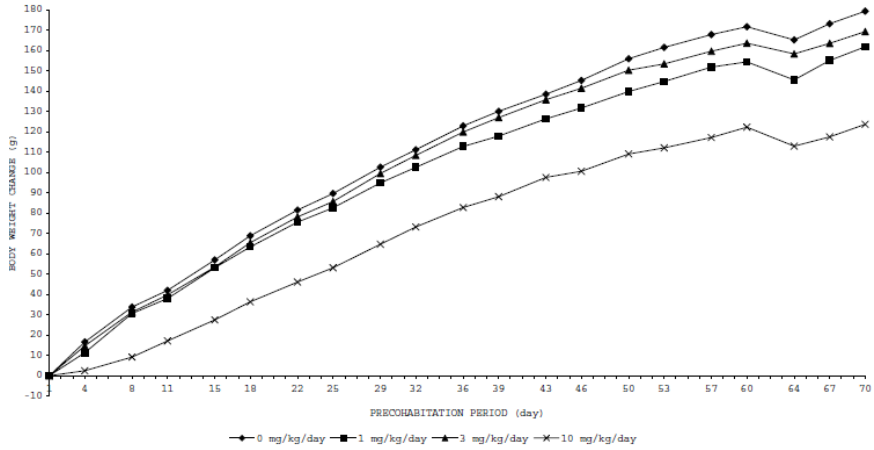

Animals were fasted on Day 63 for the collection of blood samples.

- In females, there was no difference among the group means for absolute terminal body weights with the gravid uterine weights subtracted or for body weight gain during the gestation period.

Food Consumption was measured in males only at weekly intervals, except during the cohabitation period. The following intervals were recorded: 1-8, 8-15, 15-22, 22-29, 29-36, 36-43, 43-50, 50-57, 57-64, 64-70 and 92-98.

- In HDM, mean food consumption was decreased throughout the study with the greatest decrease (-16%) found during Week 1.

Clinical Pathology was evaluated on Day 63 of the dosing phase for all the males on study, except for 1 HDM that was evaluated on Day 59 prior to euthanasia.

Hematology parameters evaluated included the following: RBC count, Hgb, Hct, MCH, MCHC, MCV, total and absolute WBC count, Retic (absolute and %), Plt count.

- A reduced red blood cell mass was found in HDM as indicated by a decreased mean RBC count, Hgb, and Hct of -6%, -11%, and -7%, respectively.
- Mean lymphocyte counts were decreased in MD and HD males by -17% and -37%, respectively, compared to CM. In HDM, the mean WBC count was also decreased (-24%) compared to CM.
- Mean neutrophil counts were increased in MD and HD males by +40% and +63% compared to CM.

Serum chemistry parameters evaluated in the HDM (#412) euthanized on Day 59 included: ASP, ALT, AP, total Chol, Trig, Alb, TP, Glob, A/G ratio, total Bili, Glu, Creatinine, Na^+, K^+, Cl^-,

- In this animal, there were elevations of AST and ALT values to 630 U/L and 740 U/L, respectively increased urea and creatinine, and decreased sodium, chloride, and total protein (reflecting lower globulin and albumin levels).

Dosing Solution Analysis

On the first day of dosing, the concentration of the LD and MD solutions were within 15% of nominal; however, the HD solution was lower (81% of nominal). All other samples evaluated were within the 15% of nominal and were considered acceptable.

Necropsy was completed on all surviving animals (Day 98 for males and GD 21 for females) as well as any unscheduled deaths. From all males, the right testes and epididymides were collected. From a CM (#106) and two HDM (#406 and #412), a selected set of tissues (brain, bone (sternum and/or femur), heart, kidneys, liver, lungs and spleen) were collected. The uterus from one female (#467) was collected due to the presence of a macroscopic finding.

Weights of the testes and epididymides (absolute and relative to body weight) were measured in all males; the absolute weight of the gravid uterus was determined for mated females.

- There was no difference in the mean absolute weight of either the testes or epididymides between control and teriflunomide-treated males.
- Consistent with the significant decrease in mean HDM body weight, a statistically significant increase was reported in the mean relative weights of HD testes (+13.2%) and epididymides (+9.9%).
- The mean gravid uterus weight on Day 21 in females mated with MDM and HDM was increased by +10% and +12%, respectively compared to those mated with CM [*ss, $p<0.05$].

Histopathology was performed on a limited number of tissues that included the testes and the epididymides from all animals in the control and dosed groups, as well as from the HDM (#412) euthanized on Day 59; however, microscopic evaluation of the testes and epididymides was limited to the C and HD groups. A signed pathologist's report was provided by [redacted] (b) (4) D.V.M.; and a peer review was performed by [redacted] (b) (4), D.V.M., Ph.D.

There were no teriflunomide-related microscopic findings in the testes or epididymides.

Fertility Parameters

Males (treated): Sperm motility, epididymal sperm count, and testicular sperm count were determined for all males (see sponsor's table, below).

- The mean absolute number of sperm in the cauda epididymis was decreased in teriflunomide-treated males of MD (-8.9%) and HD (-12.5%) groups; this decrease in both MD and HD groups was statistically significant.
- The relative number of sperm in the cauda epididymis normalized to its weight was decreased in the HD group (-9.9%) [ss, $p<0.05$].
- The mean percent of motile epididymis sperm was similar among treated and control males, as was the mean number of sperm in the testis (absolute or normalized to testis weight).

SUMMARY OF MALE SPERM EVALUATION DATA

Dose level (mg/kg/day)		Sperm per Cauda Epididymis x10^6	Sperm per g Cauda Epididymis x10^6	Percent Motile Epididymis Sperm	Sperm per Testis x10^6	Sperm per g Testis x10^6
0	Mean	322.12	1069.79	94.46	282.60	183.39
	STD	55.15	147.64	6.58	37.90	23.63
	N	26.00	26.00	26.00	26.00	26.00
1	Mean	308.30 NS	992.45	94.77	281.23	182.01
	STD	69.07	197.53	4.37	34.03	18.93
	N	26.00	26.00	26.00	26.00	26.00
3	Mean	293.51 *	1011.44 NS	95.77	275.03	182.29
	STD	63.40	159.01	4.18	49.87	23.90
	N	26.00	26.00	26.00	26.00	26.00
10	Mean	281.79 *	963.75 *	94.40 NS	278.95 NS	179.25 NS
	STD	50.71	181.45	5.87	27.00	14.91
	N	25.00	25.00	25.00	25.00	25.00

Females (untreated): Caesarean sections were performed on GD21 on all mated females to determine pregnancy status and number of corpora lutea. The number and position of implantation sites and implant status (early or late resorption, live or dead fetus) were determined.

- The pregnancy rate was similar in all groups (Control 100%, LD 96%, MD 100%, and HD 96%).

- The mean number of corpora lutea and pre-implantation loss were similar in all groups.
- A dose-related increase in both the mean number of implant sites and absolute number of live fetuses was observed in females mated with teriflunomide-treated males. There were increases in the number of implant sites in females mated to HDM and of absolute number of live fetuses in females mated to MDM and HDM [ss, $p<0.05$].

F1 Fetal Observations: All live fetuses were identified, weighed, and examined for sex and external abnormalities.

- There was no test article-dependent increase in external malformations.

9.1.2 Female rat

Study title: HMR1726: Oral Female Fertility Study in Rats

Study no.:	2003-1494
Study report location:	EDR 4.2.3.5.1
Conducting laboratory and location:	Aventis Pharma Vitry sur Seine, France
Date of study initiation:	January 26, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, Lot #W001, 99.1% purity

Key findings

- Maternal NOAEL was 0.84 mg/kg/day
- Female fertility NOAEL was 8.6 mg/kg/day
- Embryonic development NOAEL was <0.84 mg/kg/day

Methods

Doses:	0 (C), 0.84 (LD), 2.6 (MD), 8.6 (HD) mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg/day
Route of administration:	Oral (gavage)
Formulation/Vehicle:	2% potato starch mucilage (aqueous suspension)
Species/Strain:	Rat/SD Crl:CD®(SD)IGS BR
Number/Sex/Group:	26/sex/group
Satellite groups:	None
Study design:	Females treated 14 days prior to cohabitation, during cohabitation through GD 6. Females terminated on GD21; Males terminated on Study Day 49.
Deviation from study protocol:	The following deviations from study protocol are listed, below: 1) Body weight for female (#457) was not recorded on GD1 2) Food consumption was not determined for female (#151) on GD1-7 and GD1-14 or for female (#457) on GD1-7 Neither of the two deviations is considered by the sponsor to have jeopardized the conduct of the study.

Observations and Results

Mortality animals were monitored once daily for mortality.

- All animals survived to terminal sacrifice.

Clinical Signs were examined once daily, except during the dosing period in which females were examined twice daily.

- There were no teriflunomide-related clinical signs in either sex.

Body Weight was measured twice weekly in females during precohabitation, cohabitation periods, and on GD 1, 3, 7, 10, 14, 17, and 21.

- Compared to CF, reduced body weight gains were observed on precohabitation Days 1-14 by -34.5% and -221% for MD and HD, respectively.

Food Consumption was determined for females twice weekly during the precohabitation period and at the following intervals during gestation: GD 1-7, 7-14, and 14-21.

- During the precohabitation period, HDF had reduced absolute mean food consumption on Days 1-7 and 7-13 of -8.3% [ss, $p<0.05$] and -9% [ss, $p<0.05$], respectively, compared to CF.

- During gestation, food consumption was not affected by teriflunomide.

Clinical Pathology was assessed on the last day of precohabitation in females after an overnight fast for the following hematology parameters: RBC count, Hgb, Hct, MCH, MCHC, MCV, total and absolute WBC count, Retic (absolute and %), and Plt count.

- In MDF and HDF, there were several changes in hematological parameters, compared to CF.

Hematology Parameter	Control	% Control		
		LD	MD	HD
RBC	7.8 ± 0.4	+0.8	-3.1*	-10.6*
Hgb	14.9 ± 0.6	+0.5	-4*	-14*
Hct	41.3 ± 1.9	+1.1	-3.8*	-12.6*
MCV	53.2 ± 1.4	+0.2	-0.8	-2.3*
MCH	19.2 ± 0.5	-0.4	-1.0	-4.2*
MCHC	361.4 ± 7.1	-0.5	-0.2	-1.6*
Retic	0.20 ± 0.04	-2.8	+4.3	+42*
WBC	7.8 ± 2.5	-12.3	-26*	-23*
Neutrophils	1.2 ± 0.5	-15.6	-22.9*	-36.4*
Lymphocytes	6.0 ± 2.1	-12.7	-26*	-18.8*
Monocytes	0.25 ± 0.11	+11.8	-22*	-39.8*
Eosinophils	0.15 ± 0.10	+1.3	-42.1*	-47.4*
Basophils	0.02 ± 0.01	-43.8*	-43.8*	-37.5*
LU Cells	0.12 ± 0.05	-20.5*	-41*	-46.7*

*[ss, p<0.05]; **[ss, p<0.01] compared to Control.

Dosing Solution Analysis

The targeted dosing of teriflunomide for this study was 1, 3, and 10 mg/kg/day; however, the mean overall concentration achieved throughout this study was less than anticipated. The following sponsor provided table show the results of analyses of the test article formulated throughout this study. As a result, the actual doses of teriflunomide achieved in this study were 0.84, 2.6, and 8.6 mg/kg/day.

Calculation of actual concentrations and dose levels

Dose level (mg/kg/day)	**1**	**3**	**10**
Concentration (mg/mL)	**0.2**	**0.6**	**2**
Sample date:	**% Theoretical HMR1726 Content**		
FIRST HALF OF EACH GROUP			
27-Jan-04	**39**	**41**	**43**
06-Feb-04	98	101	96
09-Feb-04	101	94	94
13-Feb-04	89	90	92
17-Feb-04	86	**83**	91
Mean	***82.6***	***81.8***	***83.2***
SECOND HALF OF EACH GROUP			
06-Feb-04	98	101	96
09-Feb-04	101	94	94
13-Feb-04	89	90	92
17-Feb-04	86	**83**	91
21-Feb-04	**75**	89	93
25-Feb-04	**76**	**83**	**75**
26-Feb-04	**74**	**82**	86
Mean	***85.6***	***88.9***	***89.6***
OVERALL GROUP			
Overall Group mean (%)	**84.1**	**85.3**	**86.4**
Overall Concentration (mg/mL)	**0.17**	**0.5**	**1.7**
Overall Dose level (mg/kg/day)	**0.84**	**2.6**	**8.6**

Necropsy was performed on all females on GD21; males associated with pregnant females were euthanized and discarded. Males associated with nonpregnant females were necropsied for macroscopic observation on SD49.

- There were no treatment-related macroscopic findings.

Uterus Weight (absolute gravid) was determined in all pregnant females with at least one live fetus.

- At the MD and HD, mean absolute gravid uterine weights were reduced by -20% and -68%, respectively, due to decreases in the number of fetuses per dam and in fetal weights.

Fertility Parameters

Reproductive performance was determined by examination of vaginal smears for evidence of mating and estrus cycle evaluation.

No effect of teriflunomide was noted on the delay before mating, mating rate, estrus cycle, or pregnancy rate. It is noted, however, that there was a dose-related decrease in the number of pregnant females. Likewise, the ratio of pregnant to mated or pregnant to cohabited were dose-dependently decreased, although the effects were not

statistically significant. At the HD, there was an 11.5% reduction in the number of pregnant to mated.

Study ID: 20031494
HMR1726: Oral female fertility study in rats

Summary of Male and Female Fertility and Mating Performance

Dose level (mg/kg/day)		0	1	3	10	
Cohabited Pairs [a]	N	26	26	26	26	
Mated Females	N	26	26	26	26	
Pregnant Females	N	26	25	24	23	
Delay before mating (days):						
Excluding not mated	MEAN	4.0	2.9	3.3	2.8	NS
	STD	3.9	0.8	2.5	1.6	
	N	26	26	26	26	
Mated/Cohabited	%	100.0	100.0	NT	100.0	NT
Pregnant/Mated	%	100.0	96.2	92.3	88.5	NS
Pregnant/Cohabited	%	100.0	96.2	92.3	88.5	NS

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Caesarean section examinations

Uterine examination was performed to determine the pregnancy status and number of corpora lutea for each mated female. The number and position of implantation sites were determined. Macroscopic evaluation of the placenta was performed. Uteri from nonpregnant females were examined for implantations.

- The mean number of corpora lutea in the teriflunomide-treated groups were lower in a dose-related manner (LD, -1.2%; MD, -4.1%; and HD -9.4%).
- There was a lower absolute pre-implantation loss in teriflunomide-treated groups that was ss [$p<0.05$] at all doses; however, there was increased total postimplantation loss in both MD (1/24) and HD (14/23) groups.
    - Dose-dependent increases in mean early resorptions occurred in teriflunomide-treated dams (+18.2%, +245%, and +900% for LD, MD, and HD groups, respectively).
    - Teriflunomide did not affect the mean number of late resorptions and did not increase the number of dead fetuses at any dose level.

Study ID: 20031494
HMR1726: Oral female fertility study in rats

Summary of Female Cesarean Section Data (Excluding Females with Total Litter Loss)

Dose mg/kg/day		Corpora Lutea	-Preimplantation- -Loss- Absolute	%Corpora Lutea	Implant Sites	----------Postimplantation Loss---------- ----------Absolute---------- ---Resorptions--- Early	Late	Dead Fetuses	Total (i)	%Implan- tations	---Live Fetuses-- Absolute (i)	%Implan- tations
Group 1	MEAN	17.0	3.1	17.4	14.0	1.1	0.0	0.0	1.12	9.9	12.8	90.1
0	STD	2.5	3.6	20.4	3.3	1.2	0.2	0.0	1.21	13.5	3.7	13.5
	MEDIAN	16.5	1.5	9.0	15.0	1.0	0.0	0.0	1.00	6.7	14.0	93.3
	N	26	26	26	26	26	26	26	26	26	26	26
Group 2	MEAN	16.8	1.6 *	8.8	15.2	1.3	0.0	0.0	1.32 NS	8.6	13.9 NS	91.4
1	STD	1.9	1.8	9.5	1.4	1.4	0.2	0.0	1.41	9.2	1.8	9.2
	MEDIAN	17.0	1.0	6.7	16.0	1.0	0.0	0.0	1.00	6.3	14.0	93.8
	N	25	25	25	25	25	25	25	25	25	25	25
Group 3	MEAN	16.3	1.7 *	9.1	14.7	3.8	0.0	0.0	3.87 *	26.6	10.8 *	73.4
3	STD	2.7	2.5	11.6	2.2	2.6	0.2	0.0	2.51	16.8	3.0	16.8
	MEDIAN	17.0	1.0	6.3	15.0	4.0	0.0	0.0	4.00	25.0	12.0	75.0
	N	23	23	23	23	23	23	23	23	23	23	23
Group 4	MEAN	15.4 NS	0.7 *	4.3	14.8 NS	11.0	0.1	0.0	11.11 *	75.7	3.7 *	24.3
10	STD	1.6	0.7	4.4	1.6	2.8	0.3	0.0	2.98	19.4	3.2	19.4
	MEDIAN	16.0	1.0	5.9	15.0	11.0	0.0	0.0	11.00	84.6	2.0	15.4
	N	9	9	9	9	9	9	9	9	9	9	9

Fetal parameters were collected for all live fetuses; all live fetuses were identified, weighed, and examined for sex and external abnormalities.

- There was a dose-related decrease in the mean total number of live fetuses in the MD and HD groups (-15.6% and -71.1%, respectively).

- Of the survivors, a majority were male in both MD and HD groups [ss, $p<0.05$].

Study ID: 20031494
HMR1726: Oral female fertility study in rats

Summary of Female Cesarean Section Data (Excluding Females with Total Litter Loss)

Dose mg/kg/day		-------Live Fetuses------- Total (i)	Percent Males	----Mean Live Fetal Wt (g)---- Male	Female	Total
Group 1	MEAN	12.8	47.4	5.40	5.13	5.26
0	STD	3.7	16.0	0.35	0.33	0.32
	MEDIAN	14.0	46.2	5.47	5.19	5.26
	N	26	26	25	26	26
Group 2	MEAN	13.9 NS	53.2 NS	5.07 *	4.71 *	4.91 *
1	STD	1.8	14.0	0.42	0.43	0.41
	MEDIAN	14.0	53.3	5.19	4.84	5.08
	N	25	25	25	25	25
Group 3	MEAN	10.8 *	59.6 *	4.89 *	4.35 *	4.66 *
3	STD	3.0	11.4	0.29	0.44	0.29
	MEDIAN	12.0	58.3	4.91	4.46	4.71
	N	23	23	23	23	23
Group 4	MEAN	3.7 *	91.9 *	4.60 *	4.36 *	4.58 *
10	STD	3.2	16.5	0.67	0.70	0.65
	MEDIAN	2.0	100.0	4.80	4.19	4.80
	N	9	9	9	3	9

- In the sponsor's table (below), the malformations observed externally were summarized. These findings occurred in LD and MD groups, but were not present in the HD group. Lack of findings in HD group may be attributed to the markedly reduced number of live fetuses in this group.

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HMR1726 (DSE 2003-1494)

SUMMARY OF FETAL EXTERNAL OBSERVATIONS

DOSE LEVEL (MG/KG/DAY)		0			1			3			10		
	CLASS	F	L	%LM	F	L	%LM	F	L	%LM	F	L	%LM
Number Examined		334	26		348	25		249	23		33	9	
Number without Findings		334	26		346	23		247	21		33	9	
EYES													
Eye bulge, bilateral, absent	MALF	0	0	0	0	0	0	1	1	0.4	0	0	0
MOUTH/JAW													
Mandibular, micrognathia	MALF	0	0	0	0	0	0	1	1	0.4	0	0	0
Tongue, microglossia	MALF	0	0	0	0	0	0	1	1	0.4	0	0	0
Palate, cleft	MALF	0	0	0	0	0	0	1	1	0.4	0	0	0
TAIL													
Tail, short	MALF	0	0	0	2	2	0.6	0	0	0	0	0	0
TRUNK													
Anus, atresia	MALF	0	0	0	1	1	0.3	0	0	0	0	0	0

In the reviewer-generated table (below) is a detailed description of the presence of malformation in the fetuses.

DOSE GROUP	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
LD	253	6	F	Tail, short
LD	261	2	M	Tail, short; Anus, atresia
MD	358	4	F	Tongue, microglossia; Palate, cleft; Eye bulge, bilateral, absent
MD	356	12	M	Mandibular, micrognathia

9.2 Embryonic Fetal Development

9.2.1 EFD in Rat

Study title: HMR1726-Oral Embryo-Fetal Toxicity Study in Rats

Study no.:	F2002TOX0088
Study report location:	EDR 4.2.3.5.2.1
Conducting laboratory and location:	Aventis Pharma Deutschland GmbH Frankfurt, Germany
Date of study initiation:	October 23, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, C006, 98.5-101.5%

Key Study Findings

- Teriflunomide is a teratogen in rat based upon findings at MD and HD.
- NOAEL for both maternal and developmental effects was 1 mg/kg.

Methods

Doses:	0 (C), 1 (LD), 3 (MD), 10 (HD) mg/kg/day
Frequency of dosing:	Once daily

Dose volume:	5 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	2% starch mucilage suspension
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	20 mated/females/grp
Satellite groups:	None
Study design:	Mated female rats were dosed once daily during Gestation Days (GD) 6-17; termination GD20
Deviation from study protocol:	None

Observations and Results

Mortality was monitored daily.

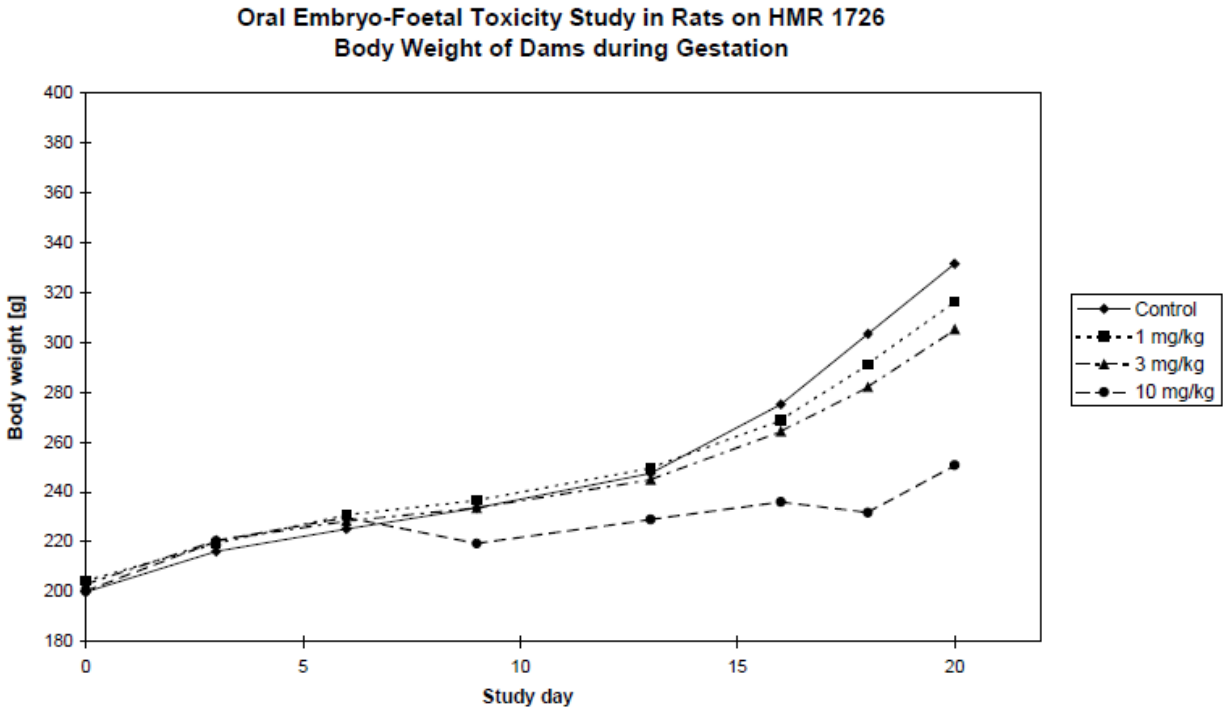
- There were no deaths reported.

Clinical Signs was monitored twice daily on weekdays and once on weekends and holidays.

- There were no clinical signs reported.

Body Weight was determined on the following gestation days: 0, 3, 6, 9, 13, 16, 18, and 20.

- As shown in the sponsor's figure (below), there was a significant decrease in mean body weight of HD dams (GD9-GD20).
- Terminal body weight was decreased in all dose groups by -4.6% (LD), -7.9% (MD), and -24.3% (HD); all dose groups were significantly different from the C group.



- There was a dose-related decrease in body weight gain compared to control for each of the following intervals: GD6-9, GD9-13, GD13-16, GD16-18, and GD18-20. The following reviewer-generated table summarizes these results as a percent of the control value for each respective interval.

INTERVAL (GD)	MEAN BODY WEIGHT GAIN (%CONTROL)		
	LD	MD	HD
0-3	-6.2	+9.3	+25.5
3-6	+27.5	-15.4	+4.4
6-9	-32.1	-36.9	-225
9-13	-6.5	-18	-30.2
13-16	-30.3	-30.3	-74.7
16-18	-20.3	-37	-115
18-20	-10.6	-17.7	-31.9

Food Consumption was determined at the following intervals during gestation: 0-3, 3-6, 6-9, 9-13, 13-16, 16-18, and 18-20.

- The following reviewer-generated table summarizes daily food consumption at the same intervals as shown above for body weight gain; these data are represented as a percent of the control value for each respective interval.
- At the HD, the greatest reduction in daily food consumption/100 g body weight was observed during the same intervals (GD6-9 and GD16-18) as the greatest reduction in body weight gain.

INTERVAL (GD)	MEAN FOOD CONSUMPTION (%CONTROL)		
	LD	MD	HD
0-3	+0	+3.6	+7.2
3-6	+1.3	+0	+0
6-9	+2.6	-5.1	-26.9*
9-13	-6.3	-3.8	-16.4*
13-16	-12*	-8.4*	-10.8*
16-18	-11*	-8.5*	-19.5*
18-20	-5.6	-2.8	+19.7*

*[ss] compared to Control

Dosing Solution Analysis

As noted in the sponsor's Table 2, the analysis of dosing suspensions showed teriflunomide content within acceptable limits of 92-100% of nominal.

Table 2: Results of dose suspension analysis

Dose level (mg/kg/day)	0	1	3	10
Concentration (mg/ml)	0	0.2	0.6	2
	Analysed content HMR 1726 (mean %)			
23-Oct-2001	n.d.	92	92	100

n.d. = not detected

Necropsy

Cesarean Section Data

- Intrauterine deaths were increased at the MD (1/20) and at the HD (13/19).

- At the HD, there was a reduced absolute number of corpora lutea (-64.4%) and number of implantations (-63.8%). On a per litter basis, however, the mean number corpora lutea was similar for the C and HD groups (see sponsor's table, below).
- Post-implantation loss at the MD was attributed to early resorptions and at the HD, it was mainly attributed to early resorptions as well as one death at term.

SURVEY OF RESULTS DURING GESTATION AND AT CAESARIAN SECTION

			GROUP 1 0 mg/kg	GROUP 2 1 mg/kg	GROUP 3 3 mg/kg	GROUP 4 10 mg/kg
NUMBER OF						
- PREGNANCIES	NE	TOTAL	17	18	20	19
- INTERCURRENT DEATH	NE	TOTAL	0	0	0	0
- FEMALES WITH ABORTION	NE	TOTAL	0	0	0	0
- FEMALES WITH PREMATURE DELIVERY	NE	TOTAL	0	0	0	0
- FEMALES AT TERM WITH INTRAUT. DEATHS ONLY	NE	TOTAL	0	0	1	13
- FEMALES AT TERM WITH LIVE FOETUSES	NE	TOTAL	17	18	19	6
- CORPORA LUTEA		TOTAL	250	257	289	89
		MEAN	14.7	14.3	15.2	14.8
		S.D.	1.8	1.6	2.1	1.7
- IMPLANTATIONS		TOTAL	240	248	283	87
		MEAN	14.1	13.8	14.9	14.5
		S.D.	1.9	1.9	2.1	2.1

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- There was a marked reduction in the total number of live fetuses at the HD (-95.6%); this effect was statistically significant.
- On a per litter basis, the mean number of live fetuses was reduced at both the MD (-13.8%) and the HD (-87.7%).

INDIVIDUAL ANALYSIS DATE: 29 Apr 2002

AVENTIS PHARMA Deutschland GmbH

STUDY: EMBRYOTOXICITY STUDY PREPARATION: HMR 1726 STUDY NO. 2001-1238
ANIMAL: Sprague Dawley Rat

SURVEY OF RESULTS DURING GESTATION AND AT CAESARIAN SECTION

			GROUP 1 0 mg/kg	GROUP 2 1 mg/kg	GROUP 3 3 mg/kg	GROUP 4 10 mg/kg
PRE-IMPLANTATION LOSS %	NE	MEAN	4.08	3.60	2.04	2.38
POST-IMPLANTATION LOSS %	NE	MEAN	4.68	4.12	17.86	88.26
- EARLY INTRAUTERINE DEATHS	NE	TOTAL	10	10	52	76
		MEAN	0.59	0.56	2.74	12.67
		S.D.	0.87	0.62	3.36	2.42
% OF IMPLANTATIONS		MEAN	4.26	4.12	17.86 +	87.14 +
- DEAD FOETUSES	NE	TOTAL	1	0	0	1
		MEAN	0.06	0.00	0.00	0.17
		S.D.	0.24	0.00	0.00	0.41
% OF IMPLANTATIONS		MEAN	0.42	0.00	0.00	1.11
- TOTAL INTRAUTERINE DEATHS	NE	TOTAL	11	10	52	77
		MEAN	0.65	0.56	2.74	12.83
		S.D.	0.86	0.62	3.36	2.32
- LIVE FOETUSES		TOTAL	229	238	231	10 -
		MEAN	13.5	13.2	12.2	1.7
		S.D.	2.1	2.0	3.4	0.8

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- Fetal body weight, crown/rump length, and placental weight were decreased compared at the MD and at the HD.

STUDY: EMBRYOTOXICITY STUDY PREPARATION: HMR 1726 STUDY NO. 2001-1238
ANIMAL: Sprague Dawley Rat

SURVEY OF RESULTS IN LIVE FOETUSES AT CAESARIAN SECTION

			GROUP 1 0 mg/kg	GROUP 2 1 mg/kg	GROUP 3 3 mg/kg	GROUP 4 10 mg/kg
NUMBER OF FOETUSES		TOTAL	229	238	231	10 -
		MEAN	13.5	13.2	12.2	1.7
		S.D.	2.1	2.0	3.4	0.8
% OF IMPLANTATIONS		MEAN	95.32	95.88	82.14 -	11.74 -
MALES (%)	NE		56.8	50.0	50.6	20.0
BODY WEIGHT (G)		MEAN	3.5	3.4	3.1 -	2.0 -
		S.D.	0.3	0.3	0.3	0.3
CROWN/RUMP LENGTH (MM)		MEAN	36.8	36.7	34.8 -	27.6 -
		S.D.	1.6	1.5	1.8	3.6
PLACENTAL WEIGHT (G)		MEAN	0.53	0.49	0.41 -	0.30 -
		S.D.	0.08	0.03	0.05	0.09

Offspring (Malformations, Variations, etc.)

In this study, several malformations and variations were identified particularly at the MD and HD in a dose-related manner. The following reviewer-generated table summarizes these data. The historical control database provided by the sponsor included 30 studies and evaluated 4184 fetuses from 604 litters.

Major developmental defects observed in rat fetuses from multiple litters

	Dose Group				
Finding	**C**	**LD**	**MD**	**HD**	**Historical Control (%)**
Total # Fetuses	119	123	121	6	
Total # Litters	17	18	19	6	
External/Visceral Defects at Autopsy	# fetuses (%) # litters (%)	# fetuses (%) # litters (%)	# fetuses (%) # litters (%)	# fetuses (%) # litters (%)	
Fetus—Retarded	0 (0) 0 (0)	2 (1.6) 2 (11.1)	7 (5.8)* 6 (31.6)	6 (100) 6 (100)	0-2.3%
Eye—Aplastic lentis, right	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	2 (33.3) 2 (33.3)	0-0.0%
—Anopthalmia, left or bilateral	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	2 (33.3) 2 (33.3)	0-0.0%
Skeletal Defects					
Skull—Retarded	5 (4.2)	7 (5.7)	4 (3.3)	4 (66.7)*	0-8.8%
—Orbit, reduced size, unilateral or bilateral	0 (0)	0 (0)	0 (0)	4 (66.7)*	0-0.6%

Drug-related minor defects, variations, and retardations observed in rat fetuses from multiple litters

	Dose Group				
Finding	**C**	**LD**	**MD**	**HD**	**Historical Control (%)**
Total # Fetuses	119	123	121	6	
Total # Litters	17	18	19	6	
Skeletal Defects [# fetuses (%); # litters (%)]					
Cervical vertebral arch—Aplasia, completely ventrad or dorsad; Dysplasia; Fragmented; Fused—completely dorsad, mediad or ventrad—uni or bilateral	0 (0) 0 (0)	0 (0) 0 (0)	12 (9.9) 3 (15.8)	4 (66.7) 4 (66.7)	0-0.6%
Thoracic vertebral arch—Aplasia, completely or dorsad; Fused; Dysplasia—uni or bilateral	0 (0) 0 (0)	0 (0) 0 (0)	1 (0.8) 1 (5.3)	4 (66.7) 4 (66.7)	0-0.5%
Thoracic vertebral centra—Aplasia; Dislocated; Dysplasia; Fragmented	1 (0.8) 1 (5.9)	0 (0) 0 (0)	1 (0.8) 1 (5.3)	4 (66.7) 4 (66.7)	0-1.5%
—Retarded, non- or weakly ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	5 (83.3) 5 (83.3)	0-0.3%
Lumbar vertebral centra—Retarded, weakly ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	2 (33.3) 2 (33.3)	0-0.3%
Sternebra—Dysplasia; Fragmented; Fused; Longitudinally displaced	1 (0.8) 1 (5.9)	0 (0) 0 (0)	4 (3.3) 2 (10.5)	4 (66.7) 4 (66.7)	0-1.4%
—Retarded, Non- or weakly ossified	24 (20.2) 9 (52.9)	22 (17.9) 11 (61.1)	33 (27.3) 13 (68.4)	6 (100) 6 (100)	9.4-33.5%
Rib—Shortened; Aplasia; Dysplasia; Fused—completely or proximal part—uni or bilateral	0 (0) 0 (0)	0 (0) 0 (0)	1 (0.8) 1 (5.3)	4 (66.7) 4 (66.7)	0-0.4%
—Extra, rib—At 1st lumbar vertebra—short or long	3 (2.5) 3 (17.6)	6 (4.9) 4 (22.2)	69 (57) 18 (94.7)	4 (66.7) 4 (66.7)	4.3-20.4%
Pectoral Girdle—Clavicula—Dysplasia; Bilateral	0 (0) 0 (0)	0 (0) 0 (0)	1 (0.8) 1 (5.3)	3 (50) 3 (50)	0-0.3%
Forepaw—Retarded, Metacarpal 5, non-ossified, bilateral	7 (5.9) 6 (35.3)	4 (3.3) 4 (22.2)	13 (10.7) 10 (52.6)	5 (83.3)* 5 (83.3)	3.2-29.4%

Finding	Dose Group C	LD	MD	HD	Historical Control (%)
Total # Fetuses	119	123	121	6	
Total # Litters	17	18	19	6	
Skeletal Defects [# fetuses (%); # litters (%)]					
—Retarded, 1st-5th toe phalanx III, non-ossified, bilateral	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	2 (33.3) 2 (33.3)	0-1.6%
Hindpaw—Retarded, Metatarsal 5, non-ossified, bilateral	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	2 (33.3) 2 (33.3)	0-5.0%
—Retarded, 1st-5th toe phalanx III, non-ossified, bilateral	2 (1.7) 1 (5.9)	1 (0.8) 1 (5.6)	5 (4.1) 4 (21.1)	4 (66.7)* 4 (66.7)	0-2.3%

External/visceral defects observed at body cross-section examination

Finding	Dose Group C	LD	MD	HD	Historical Control (%)
Total # Fetuses	110	115	110	4	
Total # Litters	17	18	19	3	
External/Visceral Defects [# fetuses (%); # litters (%)]					
Major Defects					
Brain—Hydrocephalus internus	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (75) 2 (66.7)	0.0 – 0.0
Eye—Anophthalmia, bilateral	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	2 (50) 2 (66.7)	0.0 – 0.0
—Microphthalmia	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	1 (25) 1 (33.3)	0.0 – 0.0
Retardation					
Fetus—Retarded	3 (2.7) 1 (5.9)	0 (0) 0 (0)	4 (3.6) 4 (21.1)	3 (75) 2 (66.7)	0.0 - 1.5
Minor Defects					
Kidney—Pelvis, distended, right or bilateral	1 (0.9) 1 (5.9)	3 (2.6) 3 (16.7)	3 (2.7) 2 (10.5)	1 (25) 1 (33.3)	0.0 – 4.8
Ureter—Distended, right or bilateral	1 (0.9) 1 (5.9)	2 (1.7) 2 (11.1)	3 (2.7) 2 (10.5)	1 (25) 1 (33.3)	0.0 - 2.4

On an individual fetus basis, the malformations (major defects) observed were summarized on a per animal basis in the reviewer-generated table, below.

DOSE GROUP	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
HD	264	L04	F	Major defect due to multiple findings External/Visceral,EXTERNAL,Foetus,Retarded,Retardation External/Visceral,BODY,Fetus,Edematous External/Visceral,JAW,Mandibula,Brachygnathia inferior External/Visceral,HINDPAW,Tarsal region, Bilateral, Bent, laterad
HD	270	R08	F	EXTERNAL,Fetus,Retarded,Retardation Bouin, EYE,Eye,Bilateral,Anophthalmia
HD	271	L05	M	Major defect due to multiple findings External/Visceral,EXTERNAL,Fetus,Retarded,Retardation External/Visceral,HEAD,Parietal bone,Haematocyst External/Visceral,EYE,Eye,Bilateral,Anophthalmia Skeletal,SKULL,Orbit,Bilateral,Reduced in size
HD	271	R05	M	Bouin,BRAIN,Brain,Hydrocephalus internus, Bouin,EYE,Eye,Bilateral,Anophthalmia
HD	273	L05	F	Bouin,EXTERNAL,Fetus,Retarded,Retardation Bouin,BRAIN,Brain,Hydrocephalus internus
HD	273	L07	F	External/Visceral,EXTERNAL,Foetus,Retarded,Retardation External/Visceral,EYE,Eye,Left,Microphthalmia Skeletal,SKULL,Orbit,Left,Reduced in size
HD	273	L08	F	Bouin,EXTERNAL,Fetus,Retarded,Retardation Bouin,BRAIN,Brain,Hydrocephalus internus Bouin,EYE,Eye,Bilateral,Microphthalmia
HD	274	R01	F	Major defect due to multiple findings. External/Visceral,EXTERNAL,Foetus,Retarded,Retardation External/Visceral,EYE,Eye,Right,Aplasia lentis External/Visceral,EYE,Eye,Left,Anophthalmia Skeletal,SKULL,Exoccipital bone,Right,Fused with 1st cervical vertebra Skeletal,SKULL,Orbit,Bilateral,Reduced in size
HD	276	L01	F	Major defect due to multiple findings. External/Visceral,EXTERNAL,Fetus,Retarded,Retardation External/Visceral,EYE,Eye,Right,Aplasia lentis Skeletal,SKULL,Supraoccipital bone,Slight ossification,Retardation Skeletal,SKULL,Mandibula,Rami fused Skeletal,SKULL,Incisors of lower jaw,Aplasia Skeletal,SKULL,Orbit,Right,Reduced in size Skeletal,CERVICAL VERTEBRA,Vertebra,Anlage of only 4

Toxicokinetics was not performed in this study; however, a separate study (DIV1364) was performed in pregnant SD rats (11 weeks old) that were administered teriflunomide, once daily, by oral gavage for 7 days (GD6-12). The results of this study are provided in sponsor's Text table 1, and the mean plasma TK parameters for both teriflunomide and its metabolite 4-TFMA were determined.

Text table 1 - Mean plasma toxicokinetic parameters of HMR1726 and its metabolite 4-TFMA

Analyte	Dose (mg/kg/day)	Sex	C_{max}[a] (µg/mL or ng/mL)	t_{max} (h)	AUC_{0-24}[b] (µg.h/mL or ng. h/mL)
HMR1726	1	Female	10.9	2	110
	3		36.2	1	298
	10		40.4	8	635
4-TFMA	1	Female	NC	NC	NC
	3		NC	NC	NC
	10		3.16	0.5	44.9

a. Units are µg/mL for HMR1726 and ng/mL for 4-TFMA

b. Units are µg.h/mL for HMR1726 and ng.h/mL for 4-TFMA

NC: Not able to calculate as plasma concentrations of 4-TFMA were not quantifiable in any pregnant females at 1 mg/kg/day and in most of females at 3 mg/kg/day.

Exposure values are rounded to 3 significant figures

Data from non pregnant female(s) at 1 and 3 mg/kg/day were excluded from all toxicokinetics analyses.

9.2.2. EFD staged dosing in Rat

Study title: HMR1726-Oral Embryo-fetal Toxicity Study in Rats—Staged Dosing

Study no.:	TER0631
Study report location:	EDR 4.2.3.5.2.1
Conducting laboratory and location:	Sanofi-Aventis US, Inc. Bridgewater, NJ USA
Date of study initiation:	September 07, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, Lot #W001, 100.5% purity

Methods

Doses:	0 (C) or 10 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	2% potato starch mucilage
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	C 10F; Dosed 20F
Satellite groups:	None
Study design:	See sponsor's table, below
Deviation from study protocol:	None

Dosing days, dosages, and number of animals per group

Group	Dosage (mg/kg/day)	Number of Mated Females	Animal Numbers	Days of Dosing (GD)
1[a]	0	10	1-10	6 to 17
2	10	20	11-30	6 to 8
3	10	20	31-50	9 to 11
4	10	20	51-70	12 to 14
5	10	20	71-90	15 to 17
6	10	10	91-100	6 to 17

[a]Control (vehicle): 2% potato starch mucilage

Observations and Results

Mortality was checked once daily on day of arrival and on termination day; all other times, evaluated twice daily.

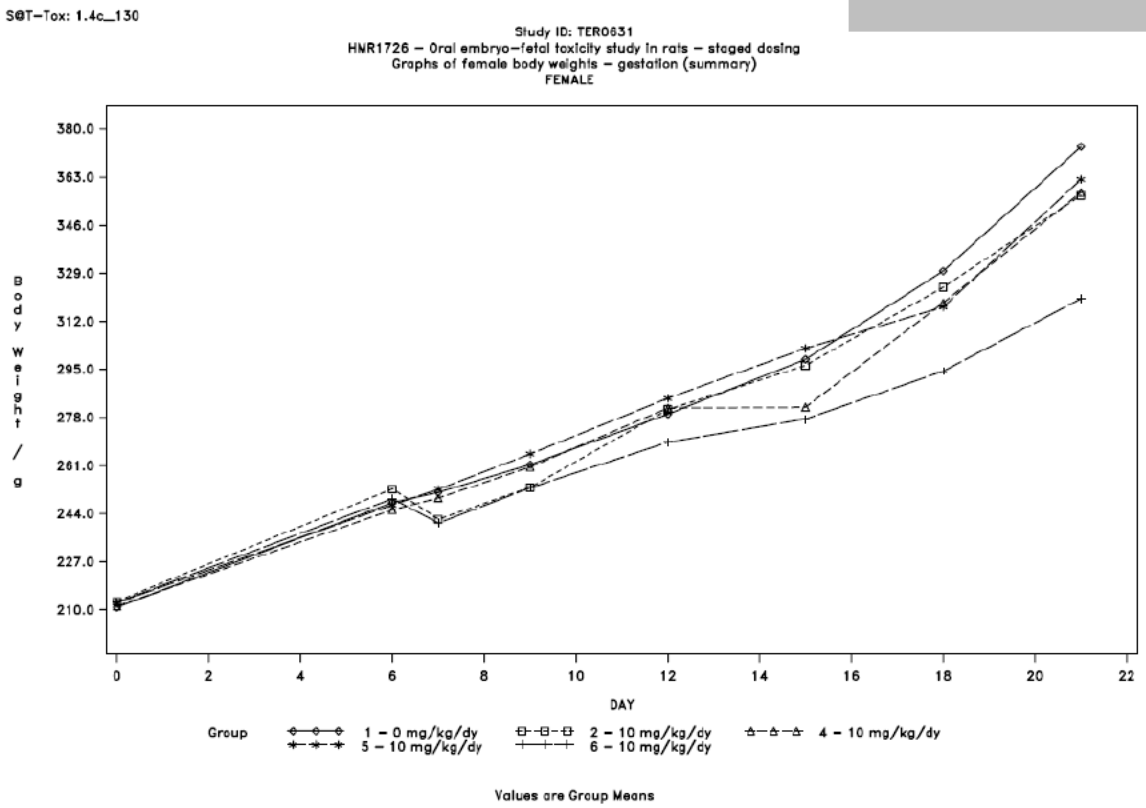
- One dam from Group 6 (#094) was terminated for humane reasons on GD15 due to adverse clinical signs that included: discolored feces (GD9-11), reduced skin elasticity (GD13-15), unkempt coat and piloerection (GD14-15), reduced feces (GD15), and body weight loss of -24.5% (GD6-15).

Clinical Signs were recorded once daily for all rats during GD6-21; however, on specified dosing days clinical signs were performed once predose and approximately 1 hr postdose.

- Aside from the findings noted for Dam #094, there were no remarkable findings.

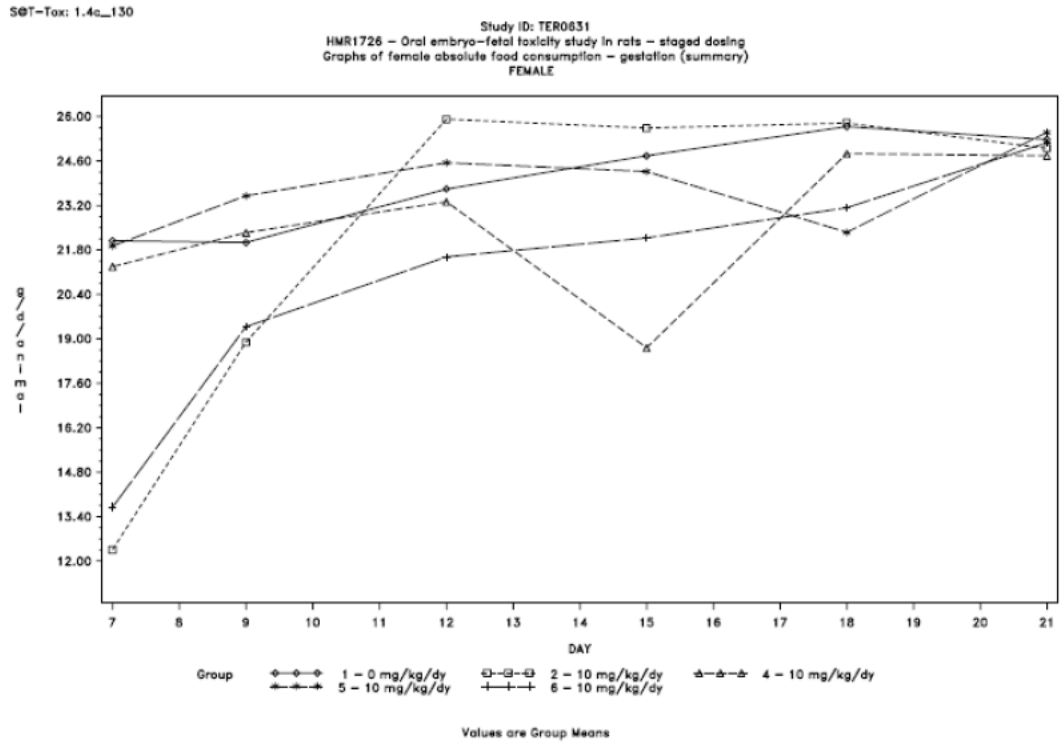
Body Weight was recorded on GD 0, 6, 7, 9, 12, 15, 19, and 21.

- There was a decrease in mean body weight (Grp 2, -10.6 g) or mean body weight gain (Grp 3, -97.2% during GD9-12; Grp 4, -97.9% during GD12-15; Grp 5, -53.2% during GD15-18). In non-dosing intervals, the body weight gain approached that of the control (Grp 1). The substantial loss of body weight in Grp 3, correlates with the total litter loss observed at cesarean section.
    - These data are illustrated in the sponsor's figure, below.



Food Consumption was measured on GD7, 9, 12, 15, 18, and 21.

- The food consumption of the dams treated with teriflunomide at different stages of gestational development is summarized in the sponsor's figure, below.



Toxicokinetics

- Plasma concentrations of teriflunomide were not determined in this study.

Dosing Solution Analysis

- Analysis of the dosing solutions determined that the concentration of teriflunomide achieved was within 85-115% of nominal and thus was acceptable.

Necropsy

Cesarean section

The results from the cesarean sections are presented in the sponsor's table, below; this dataset includes Group 3 (dosed GD9-11), in which there was complete post-implantation loss via early resorption (20/20) and thus teriflunomide treatment was embryo-lethal when animals were dosed with teriflunomide on GD9-11. There was also an increase in post-implantation loss via early resorption in Groups 2 and 6 in which teriflunomide was dosed from GD6-8 and GD6-17, respectively. The sponsor noted that this is a time in development when the heart and neural tube are forming and thus disruption of these developmental milestones could be lethal. Although the sponsor considers this lethality plausible as the rationale for the loss of Group 3, it does not explain why the profound embryo-lethality was not complete in Group 6 (dosed GD 6-17).

SWT-Tox: 1.4c_130

Study ID: TER0631
HMR1726 - Oral embryo-fetal toxicity study in rats - staged dosing

Summary of female cesarean section data (including females with total litter loss)

Dose mg/kg/dy		Corpora Lutea	-Preimplantation- -Loss- Absolute	%Corpora Lutea	Implant Sites	Postimplantation Loss Absolute ---Resorptions--- Early	Late	Dead Fetuses	Total	%Implan-tations	Live Fetuses Absolute	%Implan-tations
Group 1	MEAN	13.2	0.5	3.8	12.7	0.6	0.0	0.0	0.60	4.7	12.1	95.3
0	STD	1.8	0.5	4.1	1.8	0.7	0.0	0.0	0.70	5.5	1.9	5.5
	MEDIAN	13.5	0.5	3.3	13.0	0.5	0.0	0.0	0.50	3.6	11.5	96.4
	N	10	10	10	10	10	10	10	10	10	10	10
Group 2	MEAN	13.6	1.7	10.8	11.9	2.9	0.1	0.0	2.95	25.3	9.0	74.7
10	STD	2.1	2.3	14.1	1.2	2.0	0.3	0.0	2.19	19.8	2.6	19.8
	MEDIAN	13.0	0.5	3.6	12.0	3.0	0.0	0.0	3.00	25.0	9.0	75.0
	N	20	20	20	20	20	20	20	20	20	20	20
Group 3	MEAN	12.6	0.4	3.0	12.2	12.2	0.0	0.0	12.15	100.0	0.0	0.0
10	STD	1.9	0.6	4.5	1.8	1.8	0.0	0.0	1.79	0.0	0.0	0.0
	MEDIAN	13.0	0.0	0.0	12.0	12.0	0.0	0.0	12.00	100.0	0.0	0.0
	N	20	20	20	20	20	20	20	20	20	20	20
Group 4	MEAN	14.6	2.6	14.0	12.1	0.8	0.0	0.0	0.80	6.9	11.3	93.1
10	STD	4.1	4.1	19.0	2.3	1.7	0.0	0.0	1.67	13.7	2.9	13.7
	MEDIAN	14.0	1.0	7.4	12.5	0.0	0.0	0.0	0.00	0.0	12.0	100.0
	N	20	20	20	20	20	20	20	20	20	20	20
Group 5	MEAN	13.2	1.5	10.4	11.7	0.5	0.0	0.0	0.53	3.6	11.2	96.4
10	STD	2.6	3.3	20.3	3.5	0.8	0.0	0.0	0.84	5.5	3.1	5.5
	MEDIAN	13.0	0.0	0.0	12.0	0.0	0.0	0.0	0.00	0.0	12.0	100.0
	N	19	19	19	19	19	19	19	19	19	19	19
Group 6	MEAN	12.3	0.7	5.9	11.7	4.9	1.0	0.1	6.00	51.8	5.7	48.2
10	STD	1.2	0.9	7.9	1.9	3.2	0.9	0.3	2.78	22.0	2.7	22.0
	MEDIAN	13.0	0.0	0.0	12.0	4.0	1.0	0.0	6.00	53.8	6.0	46.2
	N	9	9	9	9	9	9	9	9	9	9	9

HMR1726 - Oral embryo-fetal toxicity study in rats - staged dosing

Summary of female cesarean section data (excluding females with total litter loss)

Dose mg/kg/dy		Live Fetuses: Total	Live Fetuses: Percent Males	Mean Live Fetal Wt (g): Male	Mean Live Fetal Wt (g): Female	Mean Live Fetal Wt (g): Total
Group 1 0	MEAN	12.1	47.1	5.92	5.49	5.68
	STD	1.9	12.4	0.33	0.24	0.26
	MEDIAN	11.5	45.5	6.01	5.58	5.77
	N	10	10	10	10	10
Group 2 10	MEAN	9.0	43.4	5.22	4.94	5.08
	STD	2.6	18.6	0.74	0.43	0.46
	MEDIAN	9.0	40.0	5.25	4.91	5.01
	N	20	20	20	20	20
Group 3 10	MEAN					
	STD					
	MEDIAN					
	N					
Group 4 10	MEAN	11.3	49.1	5.61	5.26	5.43
	STD	2.9	14.7	0.41	0.37	0.39
	MEDIAN	12.0	50.0	5.65	5.23	5.45
	N	20	20	20	20	20
Group 5 10	MEAN	11.2	50.3	5.39	5.21	5.31
	STD	3.1	11.7	0.49	0.38	0.43
	MEDIAN	12.0	50.0	5.34	5.23	5.30
	N	19	19	19	19	19
Group 6 10	MEAN	5.7	49.1	3.82	4.02	3.91
	STD	2.7	19.2	0.49	0.37	0.36
	MEDIAN	6.0	50.0	3.95	3.99	3.97
	N	9	9	9	9	9

Offspring (Malformations, Variations, etc.)

Most of the fetal malformations identified from the external, the visceral, and the skeletal examinations were from dams dosed with teriflunomide during GD6-17 (Group 6); however, there were a few skeletal malformations that occurred when dams were dosed at other intervals during gestation (see the reviewer-generated table, below).

Teriflunomide-related malformations observed in multiple litters from dams treated at different gestational intervals.

	Control	Teriflunomide (10 mg/kg/day)				
Group:	1	2	3	4	5	6
Dosing Interval GD:	6-17	6-8	9-11	12-14	15-17	6-17
External Examination [# fetuses (%); # litters (%)]						
Total # Fetuses	121	179	0	225	213	51
Total # Litters	10	20	0	20	19	9
Eye Bulge—Absent	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	22 (43.1) 8 (88.9)
Cranium—Domed	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	5 (9.8) 3 (33.3)
Trunk, Thorax—Gastroschisis	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	11 (21.6) 4 (44.4)
Mouth, Jaw—Aglossia	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (5.9) 3 (33.3)

	Control	Teriflunomide (10 mg/kg/day)				
Group:	1	2	3	4	5	6
Dosing Interval GD:	6-17	6-8	9-11	12-14	15-17	6-17
External Examination [# fetuses (%); # litters (%)]						
Total # Fetuses	121	179	0	225	213	51
Total # Litters	10	20	0	20	19	9
Mandibular--Micrognathia	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	4 (7.8) 4 (44.4)
Visceral Examination [# fetuses (%); # litters (%)]						
Total # Fetuses	62	87	0	114	107	24
Total # Litters	10	20	0	20	19	9
Eye—Anopthalmia	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	4 (7.8) 4 (44.4)
—Micropthalmia	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	8 (33.3) 6 (66.7)
Diaphragm—Hernia	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	7 (29.2) 5 (55,6)
Brain—Lateral ventricle, dilated, severe	0 (0) 0 (0)	1 (1.1) 1 (5)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	10 (41.7) 7 (77.8)
Skeletal Examination [# fetuses (%); # litters (%)]						
Total # Fetuses	59	92	0	111	106	27
Total # Litters	10	20	0	20	19	9
Thoracic Arch—Absent	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	4 (14.3) 3 (33.3)
4th and 5th—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
5th—Misshapen	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
5th and 6th—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
Thoracic Vertebrae 3rd and 4th Arch—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	8 (29.6) 5 (55.6)
1st and 2nd Arch—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	4 (14.8) 3 (33.3)
Cervical Vertebrae Arch—Absent	0 (0) 0 (0)	1 (1.1) 1 (5)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	14 (51.9) 6 (66.7)
5th and 6th Arch—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	5 (18.5) 5 (55.6)
1st and 2nd Arch—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
Ribs 8th and 9th—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
5th and 6th—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	4 (14.8) 4 (44.4)

	Control	Teriflunomide (10 mg/kg/day)				
Group:	1	2	3	4	5	6
Dosing Interval GD:	6-17	6-8	9-11	12-14	15-17	6-17
Skeletal Examination [# fetuses (%); # litters (%)]						
Total # Fetuses	59	92	0	111	106	27
Total # Litters	10	20	0	20	19	9
2nd and 3rd—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	5 (18.4) 4 (44.4)
1st and 2nd—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	4 (14.8) 4 (44.4)
Sternebrae 6th—BIFID	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	5 (18.5) 3 (33.3)
4th—BIFID	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	4 (14.8) 3 (33.3)
3rd—BIFID	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
1st and 2nd—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
Skull Maxilla—Misshapen	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
Mandible—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	11 (40.7) 5 (55.6)
Exoccipital and 1st Cervical vertebrae—Fused	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	16 (59.3) 7 (77.8)

The following reviewer-generated table summarizes the malformations observed in the individual fetuses examined.

GROUP	DOSING INTERVAL	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
2	GD 6-8	018	L04	M	Fixed Head, Brain, Lateral ventricles, Bilateral, Dilated, severe
2	GD 6-8	026	R06	M	Skeletal, Ribs, 3rd & 4th rib, Right, Fused, Proximal part Skeletal, Thoracic vertebrae, 3rd & 4th thoracic arch, Right, Fused, Partial
2	GD 6-8	029	L05	F	Skeletal, Cervical vertebrae, 1st & 2nd cervical arch, Bilateral, Fused, Partial Skeletal, Cervical vertebrae, Cervical arch, Bilateral, Absent, Only 5 present
2	GD 6-8	030	R03	M	Skeletal, Ribs, Between 10th & 11th rib, Right, Intercostal Skeletal, Ribs, 9th & 10th rib, Left, Fused, Proximal part Skeletal, Ribs, 9th rib, Left, Branched, Distal part

GROUP	DOSING INTERVAL	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
6	GD 6-17	091	L01	M	External, Mouth/Jaw, Tongue, Aglossia External, Head, Cranium, Domed head External, Mouth/Jaw, Mandibular, Micrognathia External, Ears, Ear, Bilateral, Malpositioned pinna, Low set Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused Skeletal, Skull, Mandible, Bilateral, Fused Skeletal, Skull, Maxilla, Bilateral, Misshapen Skeletal, Ribs, 1st & 2nd rib, Right, Fused, Proximal part Skeletal, Thoracic vertebrae, 1st & 2nd thoracic arch, Right, Fused, Partial Skeletal, Thoracic vertebrae, 3rd & 4th thoracic arch, Bilateral, Fused, Partial
6	GD 6-17	091	L05	F	Skeletal, Thoracic vertebrae, Thoracic vertebra, Supernumerary Skeletal, Thoracic vertebrae, 1st & 2nd thoracic arch, Right, Fused, Partial Skeletal, Ribs, 4th & 5th rib, Right, Fused, Medial part, Skeletal, Ribs, 10th & 11th rib, Left, Fused, Medial part
6	GD 6-17	091	R01	M	External, Trunk, Thorax/Abdomen, Gastroschisis
6	GD 6-17	092	L01	F	Skeletal, Skull, Exoccipital & 1st cervical vertebra, Right, Fused Skeletal, Cervical vertebrae, Cervical arch, Right, Absent, Only 6 present Skeletal, Cervical vertebrae, Cervical arch, Left, Absent, Only 4 present
6	GD 6-17	092	L02	F	Fixed Head, Eyes, Eye, Left, Microphthalmia
6	GD 6-17	092	L03	M	Skeletal, Thoracic vertebrae, 5th thoracic arch, Left, Absent Skeletal, Sternebrae, 5th sternebra, Bifid Skeletal, Sternebrae, 6th sternebra, Bifid Skeletal, Cervical vertebrae, 6th cervical arch, Right, Misshapen Skeletal, Cervical vertebrae, 4th cervical arch, Left, Misshapen Skeletal, Cervical vertebrae, 5th cervical arch, Left, Misshapen Skeletal, Cervical vertebrae, 1st & 2nd cervical arch, Right, Fused, Partial Skeletal, Cervical vertebrae, 4th & 5th cervical arch, Right, Fused, Partial Skeletal, Cervical vertebrae, 1st & 2nd cervical arch, Left, Fused, Partial

GROUP	DOSING INTERVAL	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
6	GD 6-17	092	L05	F	External, Eyes, Eye bulge, Bilateral, Absent External, Trunk, Thorax/Abdomen, Gastroschisis, External, Mouth/Jaw, Palate, High-arched palate External, Mouth/Jaw, Mandibular, Micrognathia External, Face/Nose, Nose, Misshapen External, Mouth/Jaw, Lip, Cleft, Upper Lip Skeletal, Sternebrae, 6th sternebra, Bifid Skeletal, Sternebrae, 1st & 2nd sternebrae, Fused Skeletal, Skull, Frontal & Parietal, Fused Skeletal, Skull, Exoccipital & 1st cervical vertebra, Right, Fused Skeletal, Cervical & Thoracic vertebrae, 7th cervical arch & 1st thoracic arch, Bilateral, Fused Skeletal, Skull, Mandible, Bilateral, Fused Skeletal, Cervical vertebrae, 1st cervical arch, Left, Misshapen Skeletal, Cervical vertebrae, 2nd cervical arch, Left, Misshapen Skeletal, Skull, Maxilla, Left, Misshapen Skeletal, Skull, Nasal, Left, Misshapen Skeletal, Skull, Premaxilla, Left, Misshapen Skeletal, Skull, Squamosal, Left, Misshapen Skeletal, Skull, Zygomatic, Left, Misshapen Skeletal, Skull, Exoccipital, Bilateral, Misshapen Skeletal, Skull, Palatine, Bilateral, Misshapen Skeletal, Ribs, 1st & 2nd rib, Right, Fused, Proximal part Skeletal, Thoracic vertebrae, 1st & 2nd thoracic arch, Right, Fused, Partial Skeletal, Thoracic vertebrae, 8th & 9th thoracic arch, Left, Fused, Partial Skeletal, Thoracic vertebrae, Thoracic arch, Left, Absent, Only 12 present Skeletal, Ribs, 3rd & 4th rib, Right, Fused, Complete Skeletal, Ribs, 5th & 6th rib, Right, Fused, Complete Skeletal, Ribs, 7th & 8th rib, Right, Fused, Complete Skeletal, Thoracic vertebrae, 3rd & 4th thoracic arch, Right, Fused, Complete Skeletal, Thoracic vertebrae, 7th & 8th thoracic arch, Right, Fused, Complete Skeletal, Cervical vertebrae, 3rd & 4th cervical arch, Left, Fused, Complete Skeletal, Ribs, 1st & 2nd rib, Left, Fused, Complete Skeletal, Ribs, 3rd & 4th rib, Left, Fused, Complete Skeletal, Ribs, 5th & 6th rib, Left, Fused, Complete Skeletal, Ribs, 8th & 9th rib, Left, Fused, Complete Skeletal, Thoracic vertebrae, 5th & 6th thoracic arch, Left, Fused, Complete

GROUP	DOSING INTERVAL	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
6	GD 6-17	092	L06	M	External, Eyes, Eye bulge, Bilateral, Absent External, Trunk, Thorax/Abdomen, Gastroschisis External, Limbs, Hindlimb, Left, Malrotated Skeletal, Skull, Exoccipital & 1st cervical vertebra, Right, Fused Skeletal, Skull, Exoccipital & 1st cervical vertebra, Left, Fused Skeletal, Skull, Mandible, Bilateral, Fused Skeletal, Cervical vertebrae, 5th cervical arch, Right, Misshapen Skeletal, Cervical vertebrae, 6th cervical arch, Right, Misshapen Skeletal, Thoracic vertebrae, 9th thoracic arch, Left, Misshapen Skeletal, Thoracic vertebrae, 2nd thoracic arch, Bilateral,Misshapen Skeletal, Thoracic vertebrae, 3rd thoracic arch, Bilateral, Misshapen Skeletal, Thoracic vertebrae, 4th thoracic arch, Bilateral, Misshapen Skeletal, Thoracic vertebrae, 5th horacic arch, Bilateral, Misshapen Skeletal, Thoracic vertebrae, 6th thoracic arch, Bilateral, Misshapen Skeletal, Thoracic vertebrae, 7th thoracic arch, Bilateral, Misshapen Skeletal, Thoracic vertebrae, 8th horacic arch, Bilateral, Misshapen Skeletal, Vertebral column, Vertebrae, Scoliosis, Skeletal, Thoracic vertebrae, 9th & 10th thoracic arch, Right, Fused, Partial Skeletal, Cervical vertebrae, Cervical arch, Left, Absent, Only 6 present Skeletal, Ribs, 2nd & 3rd rib, Right, Fused, Medial part Skeletal, Ribs, 2nd & 3rd rib, Left, Fused, Medial part Skeletal, Ribs, At 6th cervical arch, Left, Supernumerary, Full Skeletal, Ribs, 7th & 8th rib, Right, Fused, Complete
6	GD 6-17	092	R03	M	Fixed Head, Brain, Lateral ventricles, Bilateral, Dilated, severe Fixed Head, Eyes, Eye, Bilateral, Microphthalmia
6	GD 6-17	092	R04	F	Skeletal, Skull, Mandible, Bilateral, Fused Skeletal, Cervical & Thoracic vertebrae, Between 7th cervical arch & 1st thoracic arch, Bilateral, Supernumerary Skeletal, Cervical vertebrae, 5th & 6th cervical arch, Right, Fused, Partial Skeletal, Ribs, At supernumerary cervical arch, Left, Supernumerary, Full

GROUP	DOSING INTERVAL	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
6	GD 6-17	093	L01	M	External, Eyes, Eye bulge, Bilateral, Absent External, Mouth/Jaw, Tongue, Aglossia External, Mouth/Jaw, Mandibular, Agnathia External, Ears, Ear, Left, Anotia External, Head, Cranium, Exencephaly External, Trunk, Thorax/Abdomen, Gastroschisis External, Limbs, Hindlimb, Bilateral, Malrotated External, Head, Cranium, Microcephaly External, Ears, Ear, Right, Malpositioned pinna, Low set Skeletal, Skull, Exoccipital & 1st cervical vertebra, Right, Fused Skeletal, Shoulder girdle, Spina Scapula, Bilateral, Misshapen Skeletal, Skull, General, Multiple cranial/facial abnormalities Skeletal, Thoracic vertebrae, 3rd & 4th thoracic arch, Right, Fused, Partial Skeletal, Thoracic vertebrae, 2nd & 3rd thoracic arch, Left, Fused, Partial Skeletal, Cervical vertebrae, 3rd & 4th cervical arch, Bilateral, Fused, Partial Skeletal, Cervical vertebrae, Cervical arch, Right, Absent, Only 5 present Skeletal, Cervical vertebrae, Cervical arch, Left, Absent, Only 4 present Skeletal, Thoracic vertebrae, Thoracic arch, Left, Absent, Only 12 present Skeletal, Thoracic vertebrae, Thoracic arch, Right, Absent, Only 11 present, Skeletal, Ribs, 5th & 6th rib, Right, Fused, Medial part Skeletal, Ribs, 7th & 8th rib, Left, Fused, Complete Skeletal, Ribs, 9th & 10th rib, Left, Fused, Complete Skeletal, Ribs, 3rd & 4th rib, Bilateral, Fused, Complete

GROUP	DOSING INTERVAL	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
6	GD 6-17	093	L04	F	External, Eyes, Eye bulge, Bilateral, Absent External, Trunk, Thorax/Abdomen, Gastroschisis External, Head, Cranium, Meningocele Skeletal, Skull, Exoccipital & 1st cervical vertebra, Bilateral, Fused Skeletal, Thoracic vertebrae, 3rd thoracic centrum, Misaligned Skeletal, Thoracic vertebrae, 4th thoracic centrum, Misaligned Skeletal, Thoracic vertebrae, 5th thoracic centrum, Misaligned Skeletal, Thoracic vertebrae, 6th thoracic centrum, Misaligned Skeletal, Skull, Premaxilla, Bilateral, Misshapen Skeletal, Ribs, 2nd & 3rd rib, Left, Fused, Proximal part Skeletal, Cervical vertebrae, 4th & 5th cervical arch, Right, Fused, Partial Skeletal, Thoracic vertebrae, 3rd & 4th thoracic arch, Right, Fused, Partial Skeletal, Cervical vertebrae, 3rd & 4th cervical arch, Left, Fused, Partial Skeletal, Thoracic vertebrae, 6th & 7th thoracic arch, Left, Fused, Partial Skeletal, Cervical vertebrae, Cervical arch, Left, Absent, Only 5 present Skeletal, Thoracic vertebrae, Thoracic arch, Right, Absent, Only 12 present Skeletal, Ribs, 3rd & 4th rib, Right, Fused, Distal part Skeletal, Ribs, 9th & 10th rib, Left, Fused, Distal part Skeletal, Ribs, 4th & 5th rib, Left, Fused, Complete Skeletal, Ribs, 7th & 8th rib, Left, Fused, Complete Skeletal, Thoracic vertebrae, 2nd & 3rd thoracic arch, Left, Fused, Complete
6	GD 6-17	093	L06	F	Fixed Head, Eyes, Eye, Left, Anophthalmia External, Eyes, Eye bulge, Left, Absent External, Trunk, Thorax/Abdomen, Gastroschisis External, Limbs, Hindlimb, Right, Malrotated Fresh Visceral, Arteries, Subclavian artery, Right, Retroesophageal
6	GD 6-17	093	L07	F	Fixed Head, Eyes, Eye, Bilateral, Anophthalmia External, Eyes, Eye bulge, Bilateral, Absent External, Trunk, Thorax/Abdomen, Gastroschisis Fresh Visceral, Arteries, Subclavian artery, Right, Retroesophageal

GROUP	DOSING INTERVAL	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
6	GD 6-17	093	R01	F	External, Eyes, Eye bulge, Bilateral, Absent External, Trunk, Thorax/Abdomen, Gastroschisis External, Limbs, Hindlimb, Bilateral, Malrotated Skeletal, Sternebrae, 2nd sternebra, Bifid Skeletal, Sternebrae, 3rd sternebra, Bifid Skeletal, Sternebrae, 4th sternebra, Bifid Skeletal, Skull, Frontal & Parietal, Fused Skeletal, Skull, Exoccipital & 1st cervical vertebra, Bilateral, Fused Skeletal, Skull, Mandible, Bilateral, Fused Skeletal, Skull, Parietal, Left, Hole, large Skeletal, Skull, Maxilla, Bilateral, Misshapen Skeletal, Skull, Premaxilla, Bilateral, Misshapen Skeletal, Ribs, 8th & 9th rib, Right, Fused, Proximal part Skeletal, Cervical vertebrae, 3rd & 4th cervical arch, Right, Fused, Partial Skeletal, Thoracic vertebrae, 1st & 2nd thoracic arch, Right, Fused, Partial Skeletal, Cervical vertebrae, 5th & 6th cervical arch, Left, Fused, Partial Skeletal, Thoracic vertebrae, Thoracic vertebra, Absent, Only 12 present Skeletal, Ribs, 2nd & 3rd rib, Left, Fused, Medial part, Skeletal, Ribs, 2nd & 3rd rib, Right, Fused, Distal part, Skeletal, Ribs, 6th & 7th rib, Left, Fused, Distal part, Skeletal, Ribs, 4th & 5th rib, Right, Fused, Complete, Skeletal, Thoracic vertebrae, 4th & 5th thoracic arch, Right, Fused, Complete Skeletal, Cervical vertebrae, 2nd & 3rd cervical arch, Left, Fused, Complete Skeletal, Thoracic vertebrae, 3rd & 4th thoracic arch, Left, Fused, Complete Skeletal, Thoracic vertebrae, 5th & 6th thoracic arch, Left, Fused, Complete
6	GD 6-17	093	R02	F	Fixed Head, Eyes, Eye, Bilateral, Anophthalmia Fixed Head, Brain, Lateral ventricles, Bilateral, Dilated, severe External, Eyes, Eye bulge, Bilateral, Absent External, Trunk, Thorax/Abdomen, Gastroschisis Fresh Visceral, Diaphragm, Diaphragm, Hernia
6	GD 6-17	093	R04	F	Fixed Head, Eyes, Eye, Right, Anophthalmia Fixed Head, Brain, All ventricles, Dilated Fixed Head, Eyes, Eye, Left, Microphthalmia External, Eyes, Eye bulge, Bilateral, Absent External, Trunk, Thorax/Abdomen, Gastroschisis

GROUP	DOSING INTERVAL	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
6	GD 6-17	093	R05	F	External, Eyes, Eye bulge, Bilateral, Absent Skeletal, Sternebrae, 4th sternebra, Bifid Skeletal, Skull, Exoccipital & 1st cervical vertebra, Right, Fused Skeletal, Cervical vertebrae, 4th & 5th cervical arch, Right, Fused, Partial Skeletal, Cervical vertebrae, 6th & 7th cervical arch, Left, Fused, Partial, Skeletal, Cervical vertebrae, Cervical arch, eft, Absent, Only 6 present Skeletal, Ribs, 4th & 5th rib, Left, Fused, Distal part
6	GD 6-17	095	L02	M	Skeletal, Skull, Exoccipital & 1st cervical vertebra, Bilateral, Fused Skeletal, Ribs, Between 5th & 6th rib, Left, Intercostal Skeletal, Thoracic vertebrae, Thoracic vertebra, Thoracic Hemivertebra Skeletal, Ribs, 9th & 10th rib, Left, Fused, Distal part
6	GD 6-17	095	L03	F	Fixed Head, Brain, 3rd ventricle, Dilated, severe Fixed Head, Brain, Lateral ventricles, Bilateral, Dilated, severe
6	GD 6-17	095	L04	F	Skeletal, Skull, Mandible, Bilateral, Fused
6	GD 6-17	095	R01	F	Fixed Head, Mouth/Jaw, Palate, Cleft Fixed Head, Eyes, Eye, Left, Microphthalmia External, Eyes, Eye bulge, Left, Absent External, Mouth/Jaw, Tongue, Aglossia External, Mouth/Jaw, Mandibular, Micrognathia
6	GD 6-17	095	R03	M	External, Eyes, Eye bulge, Bilateral, Absent External, Trunk, Thorax/Abdomen, Gastroschisis External, Head, Cranium, Meningocele Skeletal, Cervical vertebrae, 2nd cervical arch, Left, Fused Skeletal, Cervical vertebrae, 4th cervical arch, Bilateral, Fused Skeletal, Skull, Mandible, Bilateral, Fused Skeletal, Cervical vertebrae, 3rd cervical arch, Right, Misshapen Skeletal, Cervical vertebrae, 4th cervical arch, Right, Misshapen Skeletal, Shoulder girdle, Scapula, Bilateral, Misshapen Skeletal, Thoracic vertebrae, 3rd & 4th thoracic arch, Right, Fused, Partial Skeletal, Cervical vertebrae, Cervical arch, Bilateral, Absent, Only 4 present Skeletal, Ribs, 4th & 5th rib, Right, Fused, Medial part Skeletal, Ribs, 8th & 9th rib, Left, Fused, Medial part Skeletal, Ribs, 10th & 11th rib, Left, Fused, Medial part Skeletal, Ribs, 1st & 2nd rib, Right, Fused, Distal part

GROUP	DOSING INTERVAL	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
6	GD 6-17	095	R04	F	Fixed Head, Brain, 3rd ventricle, Dilated, severe Fixed Head, Brain, Lateral ventricles, Bilateral, Dilated, severe External, Head, Cranium, Domed head Fresh Visceral, Great vessels, Aortic arch, Interrupted Fresh Visceral, Great vessels, Aortic arch, Retroesophageal Fresh Visceral, Great vessels, Aortic arch, Right sided
6	GD 6-17	095	R06	M	External, Head, Cranium, Domed head Skeletal, Skull, Mandible, Bilateral, Fused
6	GD 6-17	095	R07	F	Fixed Head, Brain, Lateral ventricles, Bilateral, Dilated, severe Fixed Head, Eyes, Eye, Right, Microphthalmia External, Eyes, Eye bulge, Right, Absent External, Head, Cranium, Domed head
6	GD 6-17	096	R01	M	Skeletal, Sternebrae, 2nd sternebra, Bifid Skeletal, Sternebrae, 3rd sternebra, Bifid Skeletal, Sternebrae, 4th sternebra, Bifid Skeletal, Skull, Exoccipital & 1st cervical vertebra, Right, Fused Skeletal, Cervical vertebrae, 1st & 2nd cervical arch, Left, Fused, Partial
6	GD 6-17	096	R04	F	Skeletal, Skull, Exoccipital & 1st cervical vertebra, Right, Fused Skeletal, Skull, Exoccipital & 1st cervical vertebra, Left, Fused Skeletal, Cervical vertebrae, Cervical arch, Left, Absent,Only 6 present
6	GD 6-17	096	R05	M	Fresh Visceral, Diaphragm, Diaphragm, Hernia
6	GD 6-17	096	R06	F	Skeletal, Skull, Exoccipital & 1st cervical vertebra, Right, Fused Skeletal, Cervical vertebrae, 1st cervical arch, Left, Misshapen Skeletal, Cervical vertebrae, Cervical arch, Right, Absent, Only 6 present
6	GD 6-17	096	R07	M	Fixed Head, Eyes, Eye, Right, Anophthalmia Fixed Head, Brain, Lateral ventricles, Bilateral, Dilated, severe Fixed Head, Eyes, Eye, Left, Microphthalmia External, Eyes, Eye bulge, Bilateral, Absent Fresh Visceral, Diaphragm, Diaphragm, Hernia
6	GD 6-17	096	R08	F	Skeletal, Skull, Exoccipital & 1st cervical vertebra, Right, Fused Skeletal, Lumbar vertebrae, Lumbar vertebrae, Lumbar hemivertebra Skeletal, Cervical vertebrae, 1st cervical arch, Left, Misshapen Skeletal, Cervical vertebrae, Cervical arch, Right, Absent, Only 6 present
6	GD 6-17	097	L02	M	Fixed Head, Brain, Lateral ventricles, Bilateral, Dilated, severe Fresh Visceral, Diaphragm, Diaphragm, Hernia Fresh Visceral, Veins, Posterior vena cava, Malpositioned

GROUP	DOSING INTERVAL	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
6	GD 6-17	097	R02	F	Skeletal, Ribs, 1st rib, Left, Detached Skeletal, Ribs, 5th rib, Left, Detached Skeletal, Skull, Exoccipital & 1st cervical vertebra, Right, Fused Skeletal, Thoracic vertebrae, 5th thoracic arch, Left, Misshapen Skeletal, Cervical vertebrae, 5th & 6th cervical arch, Right, Fused, Partial Skeletal, Thoracic vertebrae, 6th & 7th thoracic arch, Left, Fused, Partial Skeletal, Ribs, 4th & 5th rib, Right, Fused, Distal part
6	GD 6-17	097	R03	M	Fixed Head, Mouth/Jaw, Palate, Cleft Fixed Head, Eyes, Eye, Bilateral, Microphthalmia External, Eyes, Eye bulge, Bilateral, Absent External, Head, Cranium, Exencephaly External, Mouth/Jaw, Tongue, Protruding tongue Fresh Visceral, Diaphragm, Diaphragm, Hernia
6	GD 6-17	098	L02	F	Skeletal, Sternebrae, 1st sternebra, Bifid Skeletal, Sternebrae, 6th sternebra, Bifid Skeletal, Skull, Mandible, Bilateral, Fused Skeletal, Thoracic vertebrae, 3rd thoracic arch, Left, Misshapen Skeletal, Cervical vertebrae, 5th & 6th cervical arch, Bilateral, Fused, Partial Skeletal, Cervical vertebrae, Cervical arch, Right, Absent, Only 5 present Skeletal, Ribs, 3rd & 4th rib, Right, Fused, Medial part Skeletal, Ribs, 3rd & 4th rib, Left, Fused, Distal part Skeletal, Thoracic vertebrae, 3rd & 4th thoracic arch, Right, Fused, Complete
6	GD 6-17	098	L04	M	Skeletal, Sternebrae, 3rd sternebra, Bifid Skeletal, Sternebrae, 4th sternebra, Bifid Skeletal, Sternebrae, 6th sternebra, Bifid Skeletal, Cervical vertebrae, Cervical arch, Right, Absent, Only 6 present Skeletal, Cervical vertebrae, Cervical arch, Left, Absent, Only 5 present
6	GD 6-17	098	L05	M	External, Eyes, Eye bulge, Left, Absent Fresh Visceral, Great vessels, Aorta, Double
6	GD 6-17	098	R03	F	Skeletal, Skull, Mandible, Bilateral, Fused Skeletal, Thoracic vertebrae, 3rd & 4th thoracic arch, Right, Fused, Partial Skeletal, Cervical vertebrae, 1st & 2nd cervical arch, Bilateral, Fused, Partial Skeletal, Cervical vertebrae, Cervical arch, Bilateral, Absent, Only 6 present

GROUP	DOSING INTERVAL	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
6	GD 6-17	098	R04	F	External, Eyes, Eye bulge, Bilateral, Absent External, Head, Cranium, Domed head External, Mouth/Jaw, Mandibular, Micrognathia Skeletal, Skull, Mandible, Bilateral, Fused Skeletal, Thoracic vertebrae, 5th thoracic arch, Left, Misshapen Skeletal, Thoracic vertebrae, 4th & 5th thoracic arch, Right, Fused, Partial Skeletal, Cervical vertebrae, Cervical arch, Bilateral, Absent, Only 6 present Skeletal, Ribs, 2nd & 3rd rib, Right, Fused, Medial part
6	GD 6-17	098	R05	F	Fixed Head, Eyes, Eye, Bilateral, Anophthalmia External, Eyes, Eye bulge, Bilateral, Absent
6	GD 6-17	098	R06	M	Fixed Head, Brain, Lateral ventricles, Bilateral, Dilated, severe
6	GD 6-17	098	R07	M	Fixed Head, Brain, Lateral ventricles, Bilateral, Dilated, severe External, Eyes, Eye bulge, Bilateral, Absent Fresh Visceral, Diaphragm, Diaphragm, Hernia Fresh Visceral, Arteries, Subclavian artery, Right, Retroesophageal
6	GD 6-17	099	L04	M	Skeletal, Skull, Exoccipital & 1st cervical vertebra, Right, Fused Skeletal, Cervical vertebrae, 6th & 7th cervical arch, Right, Fused, Partial
6	GD 6-17	099	L06	M	Fixed Head, Eyes, Eye, Left, Anophthalmia Fixed Head, Brain, All ventricles, Dilated External, Eyes, Eye bulge, Left, Absent
6	GD 6-17	099	L07	M	External, Eyes, Eye bulge, Right, Absent Skeletal, Sternebrae, 6th sternebra, Bifid Skeletal, Ribs, 4th rib, Right, Detached Skeletal, Sternebrae, 1st & 2nd sternebrae, Fused Skeletal, Skull, Exoccipital & 1st cervical vertebra, Right, Fused Skeletal, Cervical vertebrae, 5th & 6th cervical arch, Right, Fused, Partial Skeletal, Thoracic vertebrae, 2nd & 3rd thoracic arch, Right, Fused, Partial Skeletal, Thoracic vertebrae, 4th & 5th thoracic arch, Right, Fused, Partial Skeletal, Cervical vertebrae, 2nd & 3rd cervical arch, Left, Fused, Partial Skeletal, Cervical vertebrae, Cervical arch, Left, Absent, Only 6 present Skeletal, Thoracic vertebrae, Thoracic arch, Right, Absent, Only 12 present Skeletal, Ribs, 5th & 6th rib, Right, Fused, Medial part Skeletal, Ribs, 2nd & 3rd rib, Right, Fused, Medial & distal part Skeletal, Ribs, 1st & 2nd rib, Left, Fused, Distal part

GROUP	DOSING INTERVAL	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
6	GD 6-17	100	R01	M	Skeletal, Sternebrae, 1st & 2nd sternebrae, Fused Skeletal, Skull, Exoccipital & 1st cervical vertebra, Bilateral, Fused Skeletal, Ribs, 5th & 6th rib, Left, Fused, Proximal part, Skeletal, Thoracic vertebrae, 5th & 6th thoracic arch, Left, Fused, Partial
6	GD 6-17	100	R02	F	Fixed Head, Brain, Lateral ventricles, Bilateral, Dilated, severe Fixed Head, Eyes, Eye, Left, Microphthalmia External, Eyes, Eye bulge, Left, Absent Fresh Visceral, Diaphragm, Diaphragm, Hernia Fresh Visceral, Great vessels, Aortic arch, Interrupted Fresh Visceral, Great vessels, Aortic arch, Right sided Fresh Visceral, Arteries, Subclavian artery, Left, Malpositioned, Subclavian arises from the pulmonary artery

In addition to the malformations, there were several variations, minor abnormalities, and developmental delays (ossification abnormalities) identified; the teriflunomide-dependent effects are summarized in the reviewer-generated table, below.

Teriflunomide-related minor abnormalities, variations, developmental delays (ossification abnormalities) observed in multiple litters from dams treated at different gestational intervals.

	Control	Teriflunomide (10 mg/kg/day)				
Group:	1	2	3	4	5	6
Dosing Interval GD:	6-17	6-8	9-11	12-14	15-17	6-17
Visceral Examination [# fetuses (%); # litters (%)]						
Total # Fetuses	62	87	0	114	107	24
Total # Litters	10	20	0	20	19	9
Arteries Innominate—Absent	0 (0) 0 (0)	3 (3.4) 3 (15)	0 (0) 0 (0)	0 (0) 0 (0)	2 (1.9) 2 (10.5)	4 (16.7) 3 (33.3)
Brain 3rd Ventricle—Dilated	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	5 (20.8) 5 (55.6)
Skeletal Examination [# fetuses (%); # litters (%)]						
Total # Fetuses	59	92	0	111	106	27
Total # Litters	10	20	0	20	19	9
Lumbar count Variation—27 Presacral vertebrae	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	8 (29.6) 7 (77.8)
Thoracic vertebrae 9th Centrum—Incompletely ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)

	Control	Teriflunomide (10 mg/kg/day)				
Group:	1	2	3	4	5	6
Dosing Interval GD:	6-17	6-8	9-11	12-14	15-17	6-17
Skeletal Examination [# fetuses (%); # litters (%)]						
Total # Fetuses	59	92	0	111	106	27
Total # Litters	10	20	0	20	19	9
8th Centrum— Bipartite ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	4 (14.8) 3 (33.3)
7th Centrum— Incompletely ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
6th Centrum— Unilaterally ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	6 (22.2) 4 (44.4)
4th Centrum— Unilaterally ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
13th Centrum— Incompletely ossified	0 (0) 0 (0)	1 (1.1) 1 (5)	0 (0) 0 (0)	1 (0.9) 1 (5)	3 (2.8) 3 (15.8)	3 (11.1) 2 (22.2)
5th Centrum— Bipartite ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
5th Centrum— Incompletely ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	5 (18.5) 3 (33.3)
3rd Centrum— Unilaterally ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
12th Centrum— Incompletely ossified	3 (5.1) 3 (30)	2 (2.2) 2 (10)	0 (0) 0 (0)	7 (6.3) 7 (35)	9 (8.5) 8 (42.1)	3 (11.1) 2 (22.2)
11th Centrum— Bipartite ossified	0 (0) 0 (0)	1 (1.1) 1 (5)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	4 (14.8) 3 (33.3)
11th Centrum— Incompletely ossified	1 (1.7) 1 (10)	1 (1.1) 1 (5)	0 (0) 0 (0)	13 (11.1) 7 (35)	13 (12.3) 10 (52.6)	3 (11.1) 3 (33.3)
11th Centrum— Dumbbell ossified	1 (1.7) 1 (10)	1 (1.1) 1 (5)	0 (0) 0 (0)	3 (2.7) 3 (15)	1 (0.9) 1 (5.3)	2 (7.4) 2 (22.2)
10th Centrum— Bipartite ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	5 (18.5) 5 (55.6)
10th Centrum— Incompletely ossified	0 (0) 0 (0)	3 (3.3) 1 (5)	0 (0) 0 (0)	3 (2.7) 2 (10)	3 (2.8) 3 (15.8)	3 (11.1) 2 (22.2)
Cervical 4th Arch— Incompletely ossified	1 (1.7) 1 (10)	2 (2.2) 2 (10)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	3 (11.1) 3 (33.3)
Ribs 14th Supernumerary	10 (16.9) 6 (60)	23 (25) 15 (75)	0 (0) 0 (0)	18 (16.2) 11 (55)	17 (16) 8 (42.1)	17 (63) 8 (88.9)
13th –Wavy	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	4 (3.8) 3 (15.8)	0 (0) 0 (0)

	Control	Teriflunomide (10 mg/kg/day)				
Group:	1	2	3	4	5	6
Dosing Interval GD:	6-17	6-8	9-11	12-14	15-17	6-17
Skeletal Examination [# fetuses (%); # litters (%)]						
Total # Fetuses	59	92	0	111	106	27
Total # Litters	10	20	0	20	19	9
12th –Wavy	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	6 (5.7) 3 (15.8)	0 (0) 0 (0)
Sternebrae 6th—Incompletely ossified	0 (0) 0 (0)	3 (3.3) 3 (15)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	4 (14.8) 3 (33.3)
5th—Incompletely ossified	7 (11.9) 6 (60)	17 (18.5) 10 (50)	0 (0) 0 (0)	21 (18.9) 13 (65)	12 (11.3) 8 (42.1)	11 (40.7) 7 (77.8)
5th—Unossified	0 (0) 0 (0)	3 (3.3) 3 (15)	0 (0) 0 (0)	0 (0) 0 (0)	1 (0.9) 1 (5.3)	10 (37) 7 (77.8)
3rd—Incompletely ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	6 (22.2) 3 (33.3)
2nd—Incompletely ossified	0 (0) 0 (0)	4 (4.3) 4 (20)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	7 (25.9) 4 (44.4)
1st—Incompletely ossified	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	5 (18.5) 4 (44.4)
Skull Zygomatic—Incompletely ossified	2 (3.4) 2 (20)	4 (4.3) 1 (5)	0 (0) 0 (0)	3 (2.7) 2 (10)	8 (7.5) 5 (26.3)	1 (3.7) 1 (11.1)
Suproccipital—Incompletely ossified	1 (1.7) 1 (10)	2 (2.2) 2 (10)	0 (0) 0 (0)	2 (1.8) 2 (10)	2 (1.8) 2 (10.6)	9 (33.3) 6 (66.7)
Squamosal—Incompletely ossified	1 (1.7) 1 (10)	3 (3.3) 1 (5)	0 (0) 0 (0)	3 (2.7) 2 (10)	1 (0.9) 1 (5.3)	5 (18.5) 3 (33.3)
Parietal—Incompletely ossified	2 (3.4) 2 (20)	4 (4.3) 2 (10)	0 (0) 0 (0)	4 (3.6) 4 (20)	1 (0.9) 1 (5.3)	12 (44.4) 7 (77.8)
Maxilla—Incompletely ossified	2 (3.4) 2 (20)	5 (5.4) 3 (15)	0 (0) 0 (0)	6 (5.4) 4 (20)	4 (3.8) 2 (10.5)	3 (11.1) 3 (33.3)
Interparietal—Incompletely ossified	2 (3.4) 2 (20)	2 (2.2) 1 (5)	0 (0) 0 (0)	2 (1.8) 2 (10)	3 (2.8) 3 (15.8)	11 (40.7) 5 (55.6)
Frontal—Incompletely ossified	2 (3.4) 2 (20)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	1 (0.9) 1 (5.3)	8 (29.6) 5 (55.6)

9.2.3. EFD study in Rabbit

Study title: HMR1726-Oral Embryo-Fetal Toxicity Study in Rabbits

Study no.:	TER0432
Study report location:	EDR 4.2.3.5.2.1
Conducting laboratory and location:	Sanofi-Aventis Deutschland GmBH Frankfurt, Germany
Date of initiation:	Jan 25, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, 0500024551, 99.8% purity

Methods

Doses:	0 (C), 1 (LD), 3.5 (MD), 12 (HD) mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	Gavage
Formulation/Vehicle:	Oral/2% potato starch mucilage
Species/Strain:	Rabbit/Chbb: HM(SPF) Himalayan
Number/Sex/Group:	20 Mated F/group
Satellite groups:	TK group, Mated F, C=2; LD, MD, HD 6/group
Study design:	Animals were dosed once daily during Gestation Days 6-18
Deviation from study protocol:	None

Observations and Results

Mortality was examined twice daily on weekdays and once daily on weekends for abortion and/or death of the doe.

- There were no unscheduled deaths.

Clinical Signs were evaluated twice daily on weekdays and once daily on weekends.

- No treatment-related findings.

Body Weight was measured for main study does on Gestation Days 0, 3, 6, 10, 13, 16, 19, 23, 26, and 29. For the TK group, body weight was measured on Gestation Days 0, 3, 6, 10, and 12.

- At the HD, there was a mean body weight loss (60 g), whereas, mean body weight of the C group increased by 88 g during the dosing period. Thus, at the HD, there was a significant body weight loss throughout this study. These data are demonstrated in the sponsor's figure (below).

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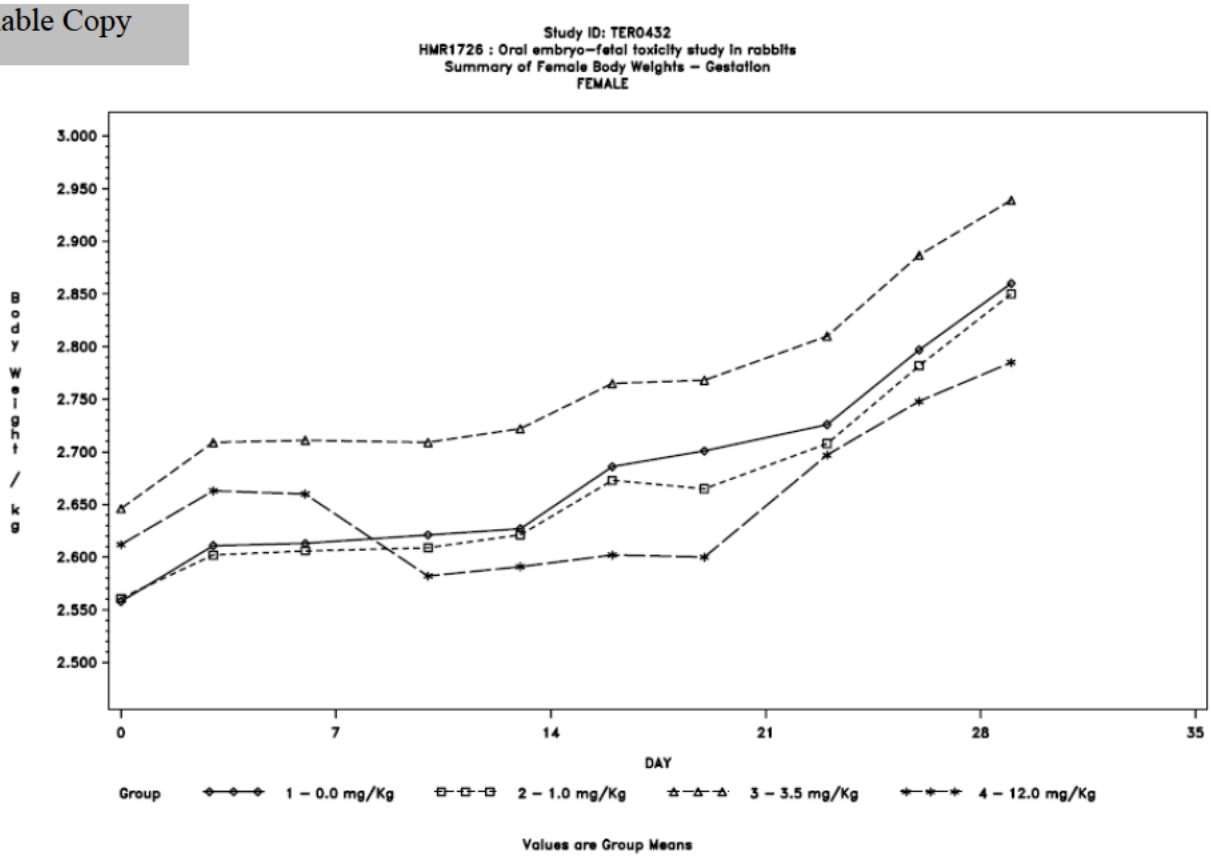

Food Consumption was measured for main study does at the following Gestation Day intervals: 0-3, 3-6, 6-10, 10-13, 13-16, 16-19, 19-23, 23-26, and 26-29. The mean group food consumption is provided in sponsor's Table, below.

- Reduced food consumption occurred throughout the study in HD does; this effect was statistically significant at each interval measured.
- During GD 6-10, reduced food consumption occurred in MD does; this effect was statistically significant.

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Study ID: TER0432
HMR1726 : Oral embryo-fetal toxicity study in rabbits

Summary of Female Relative Food Consumption - Gestation

Dose mg/Kg		Days 0-3	Days 3-6	Days 6-10	Days 10-13	Days 13-16	Days 16-19
Group 1 0.0	MEAN	3.18	3.12	3.16	2.80	2.43	2.96
	STD	0.60	0.64	0.49	0.90	0.85	0.67
	MEDIAN	3.20	3.16	3.17	3.13	2.58	3.07
	N	16	16	16	16	16	16
Group 2 1.0	MEAN	3.38	3.21	3.16 NS	2.79	2.32	2.44
	STD	0.49	0.47	0.49	0.79	0.77	0.88
	MEDIAN	3.42	3.27	3.26	3.01	2.44	2.57
	N	19	19	19	18	19	19
Group 3 3.5	MEAN	3.36	3.14	2.87 *	2.74 NS	2.13	2.69 NS
	STD	1.01	0.52	0.49	0.62	0.94	1.02
	MEDIAN	3.29	3.15	2.78	2.87	2.16	2.88
	N	18	18	18	18	18	18
Group 4 12.0	MEAN	3.15 NS	3.15 NS	1.87 *	2.34 *	2.01 NS	1.99 *
	STD	0.82	0.47	0.68	0.73	1.19	1.19
	MEDIAN	3.15	3.15	1.95	2.42	1.97	2.09
	N	16	16	16	15	16	16

Toxicokinetics of teriflunomide (in plasma) were determined on GD 12 and 13 at the following times after dosing: 0.5, 1, 2, 4, 8, and 24 hours (control group 0.5, 2, and 8 hours) after dosing (sponsor's Table 14, below).

Table 14 - Summary of teriflunomide toxicokinetics – Embryofetal toxicity study in rabbits

Species, Dosing day, Number	Dose (mg/kg/day) PO	Sex	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)
Pregnant Rabbits	1	F	5.59	59.8
Gestation Day 12 (dosing day)	3.5	F	27.9	431
3 rabbits per timepoint per group	12	F	221	4810

Abbreviations: PO = Per os (oral), F = Female, C_{max} = Maximum concentration, AUC = area under the curve
Additional information: Values are rounded to 3 significant values or less.

Dosing Solution Analysis (sponsor's table)

Results of concentration analyses

Dose level (mg/kg/day)	0	1.0	3.5	12.0
Concentration (mg/mL)	0	0.2	0.7	2.4
Sample date	% Theoretical HMR1726 Content			
01-Feb-2006	n.d.	105	106	100

n.d. = not detected
All HMR1726 formulation samples were suspensions.

Necropsy was performed on all does.

- One MD doe (#170) had two cysts present in the liver; however, this was not observed in any other dose group.

Cesarean Section Data

The pregnancy results are given in the sponsor's table below.

Study ID: TER0432
HMR1726 : Oral embryo-fetal toxicity study in rabbits

Summary of Female Mortality and Pregnancy data

Dose level (mg/Kg)	0	1	3.5	12
Mated Females	19	20	20	20
% Pregnant (total)	89.5	95.0	95.0	80.0
Unscheduled Deaths				
Pregnant (total)	0	0	0	0
Intercurrent Death	0	0	0	0
Nonpregnant	0	0	0	0
Surviving Females				
Pregnant (total)	17	19	19	16
With Live Fetuses	16	19	18	16
With Total Postimplantation Loss	1	0	1	0
Nonpregnant	2	1	1	4

In the following table, the sponsor summarizes the results of the cesarean section data. There was a dose-related increase in total postimplantation loss in teriflunomide-treated groups that was statistically significant at the HD, this is further reflected in the decreased number of live fetuses in the HD group [*ss, $p<0.05$]. Consistent with these findings, the mean gravid uterine weight of does at the HD was significantly decreased.

AvenTox: 3.1a_305

Study ID: TER0432
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Summary of Female Cesarean Section Data (Excluding Females with Total Litter Loss)

Dose mg/Kg		Corpora Lutea	-Preimplantation- -Loss- Absolute (c)	%Corpora Lutea	Implant Sites (c)	Postimplantation Loss Absolute ---Resorptions--- Early	Late	Dead Fetuses	Total (i)	%Implan- tations	Live Fetuses Absolute (i)	%Implan- tations
Group 1	MEAN	7.4	0.8	10.5	6.6	0.4	0.0	0.0	0.44	5.9	6.2	94.1
0.0	STD	1.1	1.3	17.7	1.8	0.8	0.0	0.0	0.81	11.1	1.8	11.1
	MEDIAN	7.0	0.0	0.0	7.0	0.0	0.0	0.0	0.00	0.0	6.0	100.0
	N	16	16	16	16	16	16	16	16	16	16	16
Group 2	MEAN	8.2	0.3	3.5	7.9	0.7	0.0	0.0	0.68	8.9	7.2	91.1
1.0	STD	1.2	0.6	6.3	1.1	0.9	0.0	0.0	0.95	12.4	1.5	12.4
	MEDIAN	8.0	0.0	0.0	8.0	0.0	0.0	0.0	0.00	0.0	7.0	100.0
	N	19	19	19	19	19	19	19	19	19	19	19
Group 3	MEAN	8.3	0.5	5.8	7.8	0.9	0.1	0.0	0.94 NS	14.4	6.8 NS	85.6
3.5	STD	1.4	0.9	10.5	1.4	1.3	0.2	0.0	1.35	22.6	2.3	22.6
	MEDIAN	8.5	0.0	0.0	8.0	0.5	0.0	0.0	0.50	5.0	8.0	95.0
	N	18	18	18	18	18	18	18	18	18	18	18
Group 4	MEAN	7.9 NS	0.9 NS	12.0	7.1 NS	2.3	0.1	0.2	2.50 *	35.9	4.6 *	64.1
12.0	STD	1.0	1.3	17.2	1.9	2.5	0.3	0.4	2.45	30.6	2.8	30.6
	MEDIAN	8.0	0.0	0.0	7.0	1.0	0.0	0.0	2.00	26.8	4.0	73.2
	N	16	16	16	16	16	16	16	16	16	16	16

AvenTox: 3.1a_305

Study ID: TER0432
HMR1726 : Oral embryo-fetal toxicity study in rabbits

Summary of Female Cesarean Section Data (Excluding Females with Total Litter Loss)

Dose mg/Kg		Live Fetuses Total (i)	Percent Males	Crown-Rump Length (mm) Male	Female	Total	Mean Live Fetal Wt (g) Male (f)	Female (f)	Total (f)	Placental Wt (g)
Group 1	MEAN	6.2	42.5	98.1	97.9	98.3	42.18	41.58	42.04	5.63
0.0	STD	1.8	20.0	3.7	3.9	3.2	3.37	3.13	2.74	0.42
	MEDIAN	6.0	42.2	98.0	98.5	99.0	41.55	42.07	42.16	5.73
	N	16	16	15	16	16	15	16	16	16
Group 2	MEAN	7.2	50.4	98.5	97.8	98.1	41.16	40.92	41.01	5.19 *
1.0	STD	1.5	17.8	3.3	3.4	3.2	3.27	3.47	3.14	0.62
	MEDIAN	7.0	50.0	97.8	97.2	97.1	40.07	41.65	41.06	5.22
	N	19	19	19	19	19	19	19	19	19
Group 3	MEAN	6.8 NS	42.8	97.6	97.1	97.5 NS	40.93 NS	40.43 NS	40.90 NS	5.22 *
3.5	STD	2.3	20.8	3.1	3.2	3.0	3.49	3.87	3.50	0.75
	MEDIAN	8.0	43.7	97.5	97.3	97.4	41.44	40.36	41.30	5.12
	N	18	18	16	18	18	16	18	18	18
Group 4	MEAN	4.6 *	53.3 NS	90.1	89.7	90.9 *	35.74 *	32.44 *	35.78 *	4.90 *
12.0	STD	2.8	32.5	8.6	7.4	7.3	10.91	8.39	9.72	1.59
	MEDIAN	4.0	59.0	88.0	91.6	89.6	35.70	35.59	34.97	4.52
	N	16	16	13	14	16	13	14	16	16

AvenTox: 3.1a_305

Study ID: TER0432
HMR1726 : Oral embryo-fetal toxicity study in rabbits

Summary of Uterus Weights

Dose mg/Kg		Terminal Body Weight (Gestation Day 29)	Uterus Weight	Terminal Body Weight minus Uterus Weight
Group 1 0.0	MEAN	2.860	371.25	2.489
	STD	0.139	88.88	0.133
	N	16	16	16
Group 2 1.0	MEAN	2.850	416.69	2.433
	STD	0.256	67.72	0.244
	N	19	19	19
Group 3 3.5	MEAN	2.939	389.54 NS	2.550
	STD	0.145	115.76	0.124
	N	18	18	18
Group 4 12.0	MEAN	2.785 NS	236.76 *	2.548 NS
	STD	0.164	122.98	0.169
	N	16	16	16

Offspring

Analysis of litters of live fetuses demonstrates teriflunomide-related developmental defects and dose-related effects on mean placental weights.

- At the HD, the number of live fetuses was reduced by -26% [*ss], the crown/rump length was shorter for males and females combined by -7.5%, and the live fetal weights were reduced for males by -15.3% [*ss], females by -22% [*ss], and total (M+F) by -14.9% [*ss] compared to control.
- At the MD, the mean placental weight was decreased by -7.3%.

The following reviewer-generated table summarizes the notable malformations, variations, and retardations found in the live fetuses. Clearly, a majority of these findings occurred in the HD group.

Drug-related major/minor developmental defects or delays (ossification abnormalities) observed in live rabbit fetuses/litters

	Dose Group				
Finding	**C**	**LD**	**MD**	**HD**	**Historical Control (%)**
External/Visceral Defects [# fetuses (%); # litters (%)]					
Total # Fetuses	99	137	123	73	
Total # Litters	16	19	18	16	
Fetus—Stunted (<10 g) malformation	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	1 (1.4) 1 (6.3)	0-0%
—Retarded (<20 g) minor	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	8 (11) 5 (31.3)	0-0.8%
Forepaw—hyperflexion, minor	7 (7.1) 3 (18.8)	2 (1.4) 2 (10.5)	2 (1.6) 2 (11.1)	12 (16.4) 4 (25)	0-4.4%
Carotid artery—branch, malpositioned, minor	6 (6.1) 5 (31.1)	8 (5.8) 7 (36.8)	11 (8.9) 6 (33.3)	23 (31.5) 12 (75)	
Lung—Fused or absent lobes, minor	17 (17.2) 8 (50)	14 (10.2) 8 (42.1)	23 (18.7) 10 (55.6)	27 (37) 12 (75)	0.4-12.9%
Skeletal Defects [# fetuses (%); # litters (%)]					
Total # Fetuses	99	137	123	73	
Total # Litters	16	19	18	16	
Sternebrae—Anomalies, malformation	7 (7.1) 5 (31.1)	5 (3.6) 5 (26.3)	13 (10.6) 6 (33.3)	20 (27.4) 11 (68.8)	0-9.1%
—Unossified or Incompletely ossified	27 (27.3) 11 (68.8)	40 (29.2) 12 (63.2)	50 (40.7) 16 (88.9)	19 (26) 8 (50)	
Ribs—13th thoracic, supernumerary, variation	0 (0) 0 (0)	0 (0) 0 (0)	1 (0.8) 1 (5.6)	6 (8.2) 3 (18.8)	0-4.5%
Caudal vertebrae centra—Anomalies unossified, retarded	0 (0) 0 (0)	0 (0) 0 (0)	3 (2.4) 3 (16.7)	2 (2.7) 2 (12.5)	0-0.4%
— <13 caudal vertebral centra ossified	19 (19.2) 10 (62.5)	22 (16.1) 11 (57.9)	26 (22.1) 12 (66.7)	48 (65.8) 14 (87.5)	10.5-32.9%
Pubis—unossified, retarded	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	5 (6.8) 2 (12.5)	0-0.4%
Skull—Abnormalities, malformation	0 (0) 0 (0)	0 (0) 0 (0)	1 (0.8) 1 (5.6)	3 (4.1) 2 (12.5)	0-0.9%

The malformations observed in individual fetuses are presented in the following reviewer-generated table.

GROUP	DOSE	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
1	C	124	L02	F	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused
1	C	124	L04	F	Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused
1	C	126	R01	M	Skeletal, Sternebrae, 4th & 5th sternebrae, Fused

GROUP	DOSE	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
1	C	127	R03	M	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
1	C	131	L02	F	Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
1	C	131	R02	F	Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
1	C	140	L04	F	Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused
2	LD	141	R01	M	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused, Skeletal, Sternebrae, 4th & 5th sternebrae, Fused, Skeletal, Ribs, Between 4th & 5th rib, Right, Intercostal
2	LD	144	L05	M	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused, Skeletal, Sternebrae, 4th & 5th sternebrae, Fused, Skeletal, Ribs, Between 4th & 5th rib, Right, Intercostal
2	LD	146	R03	M	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused
2	LD	147	R01	F	Skeletal, Ribs, 5th & 6th rib, Right, Fused, Distal part
2	LD	147	R03	F	Skeletal, Ribs, 3rd rib, Right, Branched, Distal part
2	LD	149	R01	M	Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
2	LD	150	R02	F	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused
2	LD	151	L02	F	Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
3	MD	161	L01	M	Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
3	MD	161	R01	F	Skeletal, Skull, Parietal, Right, Hole, large
3	MD	161	R05	M	Skeletal, Caudal vertebrae, 14th & 15th caudal centrum, Fused
3	MD	162	L01	F	Fresh Visceral, Arteries, Subclavian artery, Malpositioned, Rt subclavian from pulmo.trunk
3	MD	165	L01	F	Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused
3	MD	167	L02	F	Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
3	MD	167	L03	F	Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused
3	MD	167	L05	F	Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
3	MD	171	L04	M	Skeletal, Caudal vertebrae, 12th & 13th caudal centrum, Fused
3	MD	173	L02	M	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused
3	MD	173	R02	M	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused
3	MD	173	R03	M	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused
3	MD	176	R05	F	Skeletal, Lumbar vertebrae, All lumbar arches, Misshapen, Forced apart
3	MD	177	L02	F	Skeletal, Caudal vertebrae, 10th & 11th caudal centrum, Fused Skeletal, Caudal vertebrae, 12th & 13th caudal centrum, Fused

GROUP	DOSE	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
3	MD	178	L02	M	Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
3	MD	178	L04	F	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
3	MD	178	R01	F	Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
3	MD	179	R03	F	Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
4	HD	181	R01	M	External, Limbs, Hindlimb, Right, Amelia, External, Trunk, Thorax/Abdomen, Gastroschisis External, Trunk, Trunk, Short External, Forepaws/digits, Paw, Left, Malrotated, Inward Skeletal, Lumbar vertebrae, 1st-3rd lumbar centrums, Absent Skeletal, Sternebrae, 5th-6th sternebrae, Absent Skeletal, Thoracic vertebrae, 6th-13th thoracic centrums, Absent Skeletal, Ribs, 4th and 8th, short Skeletal, Ribs, 5th-8th rib, Right, Absent Skeletal, Ribs, 9th-12th rib, Bilateral, Absent Skeletal, Thoracic vertebrae, 5th-7th thoracic arches, Right, Absent Skeletal, Thoracic vertebrae, 4th-7th thoracic arch, misshapen Skeletal, Thoracic vertebrae, 8th-13th thoracic arches, bilateral, absent Skeletal, Pelvic girdle, Pubis, Right, Absent Skeletal, Lumbar vertebrae, 1st- 3rd, & 5th lumbar arches, Absent Skeletal, Lumbar vertebrae, 4th lumbar centrum, misshapen Skeletal, Thoracic vertebrae, 3rd-5th thoracic centrum, misshapen
4	HD	181	R05	F	Skeletal, Sternebrae, 4th & 5th sternebrae,Fused
4	HD	183	L01	M	Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
4	HD	183	L02	F	Skeletal, Caudal vertebrae, 8th caudal centrum, Misaligned
4	HD	183	L06	F	Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
4	HD	183	L07	M	External, Mouth/jaw, Palate, Cleft External, General, General, Markedly stunted External, Mouth/jaw, Lip, Cleft, Upper Lip Skeletal, Skull, Maxilla, Misshapen
4	HD	183	R01	M	External, Mouth/jaw, Palate, Cleft External, Mouth/jaw, Lip, Cleft, Upper Lip Skeletal, Skull, Maxilla, Misshapen Skeletal, Skull, Orbit, Right, Small
4	HD	184	L03	F	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
4	HD	184	R01	M	Skeletal, Sternebrae, 4th & 5th sternebrae, Fused

GROUP	DOSE	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
4	HD	184	R02	M	Fresh Visceral, Abdomen, Adrenal gland, Left, Absent Fresh Visceral, Kidneys, Kidney, Left, Absent Fresh Visceral, Urogenital system, Ureter, Left, Absent
4	HD	186	L02	M	Skeletal, Forelimbs, Radius, Right, Absent Skeletal, Caudal vertebrae, 12th & 13th caudal centrum, Fused Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused Skeletal, Forelimbs, Ulna, Right, Short
4	HD	186	R04	F	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
4	HD	190	R02	F	Skeletal, Skull, Parietal, Left, Hole, large
4	HD	190	R04	F	External, Forepaws/digits, All digits, Bilateral, Brachydactyly External, Forepaws/digits, Paw, Bilateral, Small Skeletal, Forepaws, 2^{nd}-5^{th} digits, right or bilateral, absent – middle phalanx, Right, Absent
4	HD	190	R05	M	External, Tail, Tail, Kinked Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused Skeletal, Ribs, 4th & 5th rib, Right, Fused, Proximal part
4	HD	191	R02	M	Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused
4	HD	191	R03	F	Fresh Visceral, Heart, Heart, Microcardia Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
4	HD	192	L01	F	Fresh Visceral, Abdomen, Adrenal gland, Right, Absent Fresh Visceral, Kidneys, Kidney, Right, Absent Fresh Visceral, Urogenital system, Ureter, Right, Absent
4	HD	192	L03	F	External, Forepaws/digits, 2nd digit, Left, Brachydactyly Skeletal, Sternebrae, 1^{st} & 2^{nd} sternebrae, Fused Skeletal, Sternebrae, 3^{rd} & 4^{th} sternebrae, Fused Skeletal, Sternebrae, 4^{th} & 5^{th} sternebrae, Fused Skeletal, Forepaws, 2^{nd}– 4^{th} digit - distal phalanx, Left, Absent
4	HD	192	L04	F	Skeletal, Sternebrae, 2^{nd} & 3^{rd} sternebrae, Fused Skeletal, Sternebrae, 3^{rd} & 4^{th} sternebrae, Fused Skeletal, Sternebrae, 4^{th} & 5^{th} sternebrae, Fused
4	HD	195	L01	F	Skeletal, Sternebrae, 3^{rd} & 4^{th} sternebrae, Fused Skeletal, Sternebrae, 4^{th} & 5^{th} sternebrae, Fused
4	HD	195	R01	M	Skeletal, Sternebrae, 3^{rd} & 4^{th} sternebrae, Fused
4	HD	195	R03	F	Skeletal, Sternebrae, 2^{nd} & 3^{rd} sternebrae, Fused Skeletal, Sternebrae, 3^{rd} & 4^{th} sternebrae, Fused Skeletal, Sternebrae, 4^{th} & 5^{th} sternebrae, Fused
4	HD	195	R04	M	Skeletal, Sternebrae, 3^{rd} & 4^{th} sternebrae, Fused Skeletal, Sternebrae, 4^{th} & 5^{th} sternebrae, Fused

GROUP	DOSE	DAM # (LITTER)	FETUS #	SEX	MALFORMATION(S)
4	HD	195	R06	M	Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
4	HD	198	R03	M	External, Trunk, Trunk, Umbilical Hernia, Omphalocele Skeletal, Sternebrae, 1st & 2nd sternebrae, Fused Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused
4	HD	199	L03	F	Skeletal, Forelimbs, Radius, Left, Absent Skeletal, Sternebrae, 1st & 2nd sternebrae, Fused Skeletal, Sternebrae, 2nd & 3rd sternebrae, Fused Skeletal, Sternebrae, 3rd & 4th sternebrae, Fused Skeletal, Sternebrae, 4th & 5th sternebrae, Fused Skeletal, Forelimbs, Ulna, Left, Misshapen Skeletal, Shoulder girdle, Spina Scapula, Bilateral, Misshapen Skeletal, Forelimbs, Ulna, Left, Short

9.3 Prenatal and Postnatal Development

Study title: HMR1726 – Exploratory oral pre-and postnatal developmental toxicity study in rats

Study no.:	DPN0029
Study report location:	EDR 4.2.3.5.3.1
Conducting laboratory and location:	Sanofi-Aventis US Inc. Malvern, PA 19335 USA
Date of study initiation:	November 18, 2008
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Teriflunomide, W001, 100%

Methods

Doses:	GD6-LD13: 0 (C), 0.3 (LD), 0.6 (MD), 1.0 (HD) mg/kg/day GD6-GD20: 1.0 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	2% potato starch mucilage
Species/Strain:	Rat/Sprague Dawley, Timed Pregnant Crl:CD(SD)
Number/Sex/Group:	6 F/group
Study design:	F0 dosing: GD6 through LD14
Deviation from study protocol:	None

Dosing solution analysis determined that the formulations of teriflunomide utilized on study were within the 85-115% of nominal.

Mortality was checked twice daily.

- The MD and HD groups were sacrificed on LD8 or LD9 due to increased mortality and toxicity in the F1 pups.

Clinical signs were evaluated in the F0 generation female rats at least twice daily, except for the day of termination in which signs were monitored once. During non-dosing phases, the clinical signs were recorded once daily; however, during the dosing phase, clinical signs were noted pre- and post-dose.

Summary of clinical signs – F0

Observation	Group 1 0 mg/kg/day	Group 2 0.3 mg/kg/day	Group 3 0.6 mg/kg/day	Group 4 1.0 mg/kg/day	Group 5 1.0 mg/kg/day (GD6-20)
Total Animals Examined	6	6	6	6	6
Pale; Eye(s)	1	0	0	0	0
All Pups Dead/Missing	0	0	0	2	0
Euthanized as per Study Director[a]	0	0	6	4	0

[a] Groups 3 and 4 were terminated early on LD8 or LD9

- In the F1 pups prior to weaning, there was a dose-related increase in adverse clinical signs particularly at the MD and the HD. In some cases, these toxicities were also present at the LD. Based upon these adverse clinical signs, the sponsor terminated the MD and the HD group on LD8 or LD9. These data are summarized in the reviewer-generated table, below.

Adverse clinical signs in F1 pups

			Teriflunomide					
	C		**LD**		**MD**		**HD**	
Total litters:	5		6		6		6	
Total pups:	61		86		76		83	
Clinical sign	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Activity, decreased	0	0	0	0	2	2	1	2
Cool to touch	0	0	4	4	8	7	13	17
Abdomen discoloration	0	0	0	0	3	2	1	1
Forepaw discoloration	0	0	0	0	15	15	16	21

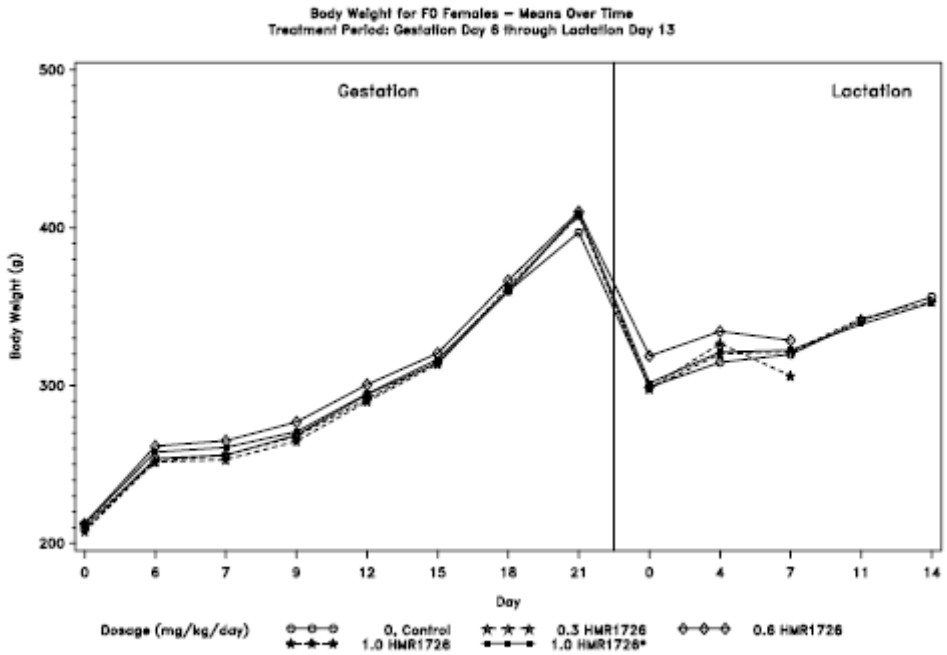
			Teriflunomide					
	C		LD		MD		HD	
Total litters:	5		6		6		6	
Total pups:	61		86		76		83	
Hindpaw discoloration	0	0	0	1	10	9	15	15
Found dead	0	0	0	1	1	0	16	16
Forepaw—Malrotated digits	0	0	0	0	21	18	21	23
Hindpaw—Malrotated digits	0	0	7	5	21	24	13	15
Forepaw—Malrotated	0	0	4	4	22	18	20	23
Hindpaw—Malrotated	0	0	0	1	17	13	17	17
Missing	0	0	0	0	1	1	4	9
No milk, stomach	0	0	0	0	14	13	19	20
Pale body	0	0	0	0	3	4	12	15
Umbilical hernia	0	0	1	0	3	3	5	3
Tail, wavy	0	0	0	0	17	18	16	15

Based on the adverse signs shown in the table above, the sponsor's selection of the LD in this study as the HD for the pivotal pre- and post-natal study is adequate, due to the presence of malformations at the MD and HD.

Body weight was recorded for F0 females on GD0, 6, 7, 9, 12, 15, 18, and 21, as well as on LD0, 4, 7, 11, and 14.

- The results of the body weight for F0 females is shown in the sponsor's figure 1 (below).

Figure 1 - Body Weight for F0 Females- Means Over Time



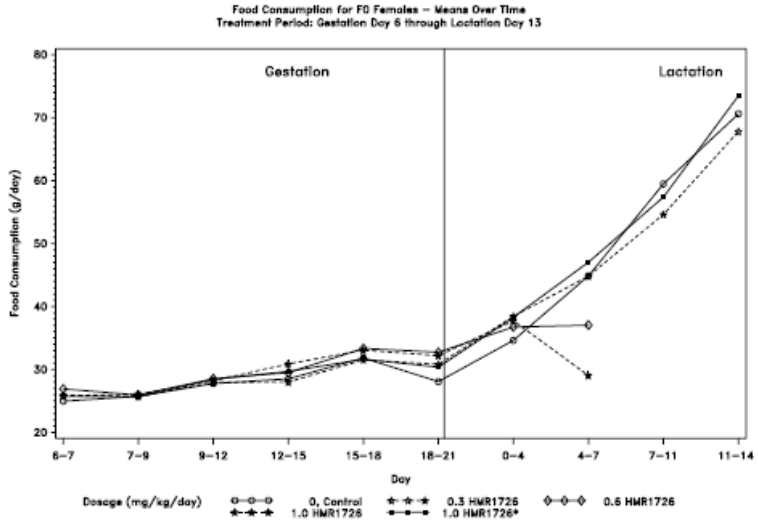
Gestation=All pregnant females; Lactation=All females delivering live pups. Sample sizes may not be constant over time.
01APR09–13:43
*: Group dosed during Gestation Days 6–20

Best Available Copy

Food consumption was recorded on the same schedule as that for body weight.

- The food consumption in F0 females is shown in the sponsor's figure 2 (below). The only significant effect on food consumption occurred at the MD and HD groups during the period of LD4-7.

Figure 2 - Average Daily Food Consumption for F0 Females – Means Over Time



Gestation=All pregnant females; Lactation=All females delivering live pups. Sample sizes may not be constant over time.
01APR09–13:43
*: Group dosed during Gestation Days 6–20

Study title: HMR1726 - Oral pre-and postnatal developmental toxicity study in rats

Study no.:	DPN0331
Study report location:	EDR 4.2.3.5.3.1
Conducting laboratory and location:	Sanofi-Aventis Deutschland GmbH Frankfurt, Germany
Date of study initiation:	August 09, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, W001, 100.5%

Methods

Doses:	0 (C), 0.05 (LD), 0.10 (MD), 0.30 (HD) mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	2% potato starch mucilage
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	24 F/group
Satellite groups:	n/a
Study design:	F0 dosing: GD6 through LD20
Deviation from study protocol:	None

The dose selection for this study was based upon preliminary findings from a range-finding pre-postnatal toxicity study (DPP0029, reviewed above).

Observations and Results

Dosing Solution Analysis determined that the formulations of teriflunomide utilized on study were within the 85-115% of nominal.

F_0 Dams

Survival was monitored twice daily (pre- and post-dose during dosing period); once daily on weekends and holidays and on day of termination.

- All dams survived to terminal sacrifice.

Clinical signs were monitored twice daily (pre- and post-dose during dosing period); once daily on weekends and holidays and on day of termination.

- There was no drug-related clinical sign in this study.

Body weight was determined on the following days: GD0, 3, 6, 7, 9, 12, 15, 18, 21, and 25 (GD25 only if animal did not deliver), and on LD0, 4, 7, 11, 14, 18, and 21.

- There was no effect of teriflunomide on mean body weight.

Mean body weight gain was decreased in the HD dams during the intervals of GD6-7 (-50%), GD7-9 (-19.3%), and GD12-15 (-14.2%).

Food consumption (g/animal/day or g/100g body weight/day) was measured on GD0, 3, 6, 7, 9, 12, 15, 18, 21, and on LD0, 4, 7, 11, 14, 18, and 21.

- There was no effect of teriflunomide on food consumption.

Necropsy was performed and the abdominal and thoracic cavities were evaluated for macroscopic observations.

- There were no treatment-related findings.

Toxicokinetics were not performed in this study.

Calculated reproductive indices for the F0 generation.

- Mean Gestation Length = Total No. of gestation days / No. of females delivering
- Parturition Index (%) = (No. of females that deliver / No. of females with implantation sites) x 100
- Post-implantation Loss (%) = [(No. of implantations– No. of viable pups) / No. of implantation sites] x 100
- Number of male and female pups on PND0, 4 (pre-culling), 7, 11, 14, and 21 (preweaning)
- Sex Ratios on PND0 [% male = (No. of male pups / No. of pups) X 100]
- Live Birth Index (%) = (No. of pups born alive / No. of pups born) X 100
- Postnatal Day 4 Pup Viability Index (%) = (No. of pups surviving to PND4 (preculling)/ No. of pups born alive) x 100
- Postnatal Day 7 Pup Viability Index (%) = (No. of pups surviving to PND7 / No. of pups alive on PND4 (after culling)) x 100
- Postnatal Day 11 Pup Viability Index (%) = (No. of pups surviving to PND11 / No. of pups alive on PND7) x 100
- Postnatal Day 14 Pup Viability Index (%) = (No. of pups surviving to PND14 / No. of pups alive on PND11) x 100
- Postnatal Day 21 Pup Viability Index (%) = (No. of pups surviving to PND21 / No. of pups alive on PND14) x 100
- Weaning Index (%) = (No. of pups surviving to PND21 / No. of pups alive on PND4 (after culling)) x 100

In the following sponsor's Tables, summary data are provided for mortality, pregnancy, and parturition data (Tables 1 and 10) and for mating and pregnancy (Table 11) of F0 dams.

- Mean post-implantation loss was notable at LD (+17.1%) but not at MD (-2.4%) or the HD (-29.3%); the lack of a dose response suggests this effect was not teriflunomide-related.

Table 1 - Summary of F0 female mortality, pregnancy and parturition data

Group Dose (mg/kg/d)	Group 1 0	Group 2 0.05	Group 3 0.10	Group 4 0.30
Mated females	24	24	24	24
pregnant females (with implant)	23 (95.8%)	23 (95.8%)	20 (83.3%)	24 (100%)
Pregnant females surviving at GD21	23	23	20	24
Delivered females	22	23	20	24
Pregnant females not delivered	1	0	0	0
Delivered females surviving to weaning pups	22	22	20	24
Non pregnant females	1	1	4	0
Non pregnant females surviving at GD21	1	1	4	0
Non pregnant females surviving at GD25	1	1	4	0

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Table 10 - Parturition index for F0 females

Group	HMR1726 (mg/kg/d)	Parturition index (%)	
1	0	95.65	
2	0.05	100.00	NS
3	0.10	100.00	NS
4	0.30	100.00	NS

Parturition index (%): 100*(number of females that delivered)/(number of females with implants)
*: Significant trend ($p \leq 0.05$) through indicated dose level, NS: No significant trend ($p > 0.05$) through indicated dose level, NT: Not tested
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Table 11 - Mating and pregnancy data for F0 females

Group	HMR1726 (mg/kg/d)		Gestation length	Implantations	Postimplantation loss	Litter size	Percentage of males	Live birth index
1	0	Mean	21.7	14.5	8.2	13.4	47.6	100.0
		Std	0.40	1.74	10.14	2.46	17.55	0.00
		N	22	22	22	22	22	22
2	0.05	Mean	21.7 NS	14.8	9.6 NS	13.8	49.5 NS	95.3 NS
		Std	0.42	1.65	20.71	2.17	13.56	20.84
		N	23	23	23	23	23	23
3	0.10	Mean	21.6 NS	15.3	7.8 NS	14.1	51.5 NS	100.0 NS
		Std	0.31	1.84	10.61	2.36	17.07	0.00
		N	20	20	20	20	20	20
4	0.30	Mean	21.6 NS	14.8	5.8 NS	14.0	48.5 NS	99.7 NS
		Std	0.29	2.48	6.52	2.49	14.98	1.46
		N	24	24	24	24	24	24

N: Delivered females
Implantations: sum by delivered females of the right and left implantation sites
Postimplantation loss by female: 100 * ((total number of implants) - (total number of born-alive pups)) / (total number of implants)
Litter size: Number of pups per litter
Percentage of males by female: 100 * (total number of males) / (total number of pups)
Live birth index: 100 * (number of pups born alive) / (total number of pups)
*: Significant trend ($p \leq 0.05$) through indicated dose level, NS: No significant trend ($p > 0.05$) through indicated dose level, NT: Not tested
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F1 Generation

Survival was checked daily for pups, prior to weaning and was checked twice daily for pups, post-weaning.

Pre-weaning (PND0-21):

- As shown in sponsor's Table 12, a majority of the F1 pups survived; no losses were noted in C or MD groups.
- In the LD group, 1 dam had all dead pups (8 total) and one dam had 1 pup die after birth.
- In the HD group, one pup died after birth.
- Prior to PND4 culling, losses occurred in each of the LD (3 pups, 2M and 1F) and MD (3 pups, 2M and 1F) groups; however, none occurred in the HD group.
- 1 HD pup was found dead on PND16 (umbilical hernia).

Post-weaning (PND21-63):

- PND21, LDF (#234), accidental death.
- PND28, 2 deaths in C (M, #106; F, #413); no clinical signs were noted prior to death.
- PND47, HDM (#195); no clinical signs were noted prior to death.

Clinical signs were checked daily commencing on PND0.

- Clinical signs were limited to the HD group and included malrotated limbs—forepaw, both in 3M [#190, 389, and 390] and 2F [#277, 489] and impaired coat growth (whole body) in 5M [#177, 178, 179, 378, 391] and 9F [#277, 278, 289, 291, 478, 477, 479, 489, 491].
- Impaired coat growth persisted until PND23.

Body weights were recorded at each stage of development, including:

Pre-weaning: PND0, 4, 7, 11, 14, and 21 (individual weights and sex confirmation performed)

- At PND21, HD F1 pups had decreased mean fetal weights compared to C (M, -4%; F, -6%).
- An increased incidence in the number of litters with a mean weight of <50 g at PND21 was notable in the HD group (6/24) compared to C (0/22), LD (1/22), and MD (0/20).

Post-weaning: individual weights were collected once weekly beginning PND21 and on day of completion of vaginal opening or balanopreputial separation.

- No treatment-related change in either mean body weight or mean body weight gain was observed.

F1 Gestation: GD0, 4, 8, 11, and 14

- No treatment-related change in either mean body weight or mean body weight gain was observed.

Food consumption was recorded for pups during post-weaning period (once weekly PND28 to necropsy) and in F1 genstation (GD0, 4, 8, 11, and 14), but it was not measured during the pre-weaning period.

- At the HD, there was an increase in food consumption in females during GD4-8 that was +10.8% and at this same period, the relative food consumption was increased +8.8%. A relative increase also occurred during the interval of GD0-4 of +7.5%. In males, there was an increase in food consumption from PND42-49 of +8.3%.

Physical development was evaluated for specific developmental landmarks at the following times:

Eye Opening: PND11

- There was no treatment-related effect.

Pupillary Light Reflex: PND19 (only)

- HD group, 3F pups from 2 different litters had no papillary reflex; of these 3F and 2F (one per litter) also had corneal opacity present.

Righting Reflex: PND21

- There was no treatment-related effect.

Vaginal opening: PND25 until criterion met and body weight was measured

- No treatment-related effect was observed (see sponsor's Table 25).

Balanopreputial separation: PND30 until criterion met and body weight was measured

- No treatment-related effect was observed (see sponsor's Table 25).

Neurological assessment was evaluated in the F1 generation by three primary tests.

Passive avoidance: An Avoidance Monitor System was used to evaluate learning and memory on PND28 (± 5 days) and exactly 7 days later on PND35 (± 5 days), passive avoidance and auditory startle were evaluated. The following datasets were recorded and reported (see sponsor's Table 35):

a) Number of crossings light to dark
b) Latency period, and
c) Number of trials to reach criteria.

During the learning phase of this task, on PND28 during the 2nd trial, MD and HD females had increased latency compared to C [ss].

- Startle reflex and habituation: was utilized to evaluate hearing and auditory reflex development on PND60. The same animals that underwent evaluation for passive avoidance were used for this study. The following datasets were collected for animal responses (starting input, maximum input, time to maximum input, and mean input); the data were reported as mean amplitude of response for each block of 10 trials.
- Unremarkable, no treatment-related effects were observed.

Motor activity: was evaluated on PND65 in the animals selected for reproductive assessment. The datasets collected for each animal included: number of central and peripheral zone entries, rearing movements, distance traveled, total time and rest time (central and peripheral zones) for each of three ten minute intervals (see sponsor's Table 31).

At the HD (both sexes), the same parameters were recorded:

- increased overall peripheral zone distance traveled (M, +8.7%; F, +6.3%),
- increased number of entries in the peripheral zone for each interval 1-3 and overall,
- increased distance traveled in the central zone at each interval and overall, and increased number of entries in central zone at each interval 1-3 and overall.

Reproductive assessment was performed on all animals that were at least 85 days old, each F1 female was placed into cohabitation with an F1 male of the same dosage group (avoided sibling mating). Cesarean sections were performed on F1 females on GD14; F1 males were sacrificed following completion of Cesarean sections in the females. The following indices were determined:

1. Mean time to insemination = Collective sum of days until insemination / No. of inseminated females
2. Female Mating Index (%) = (No. of females inseminated / No. of females paired with males) x 100
3. Female Fertility Index (%) = (No. of females pregnant / No. of females paired with males) x 100
4. Pregnancy Index (%) = (No. of females pregnant / No. of females inseminated) x 100
    - As shown in sponsor's Text tables 3 and 4, there was no effect of teriflunomide on any of the male/female mating performance evaluations and fertility was unaffected.
    - The pregnancy rates of mated females were C (20/23), LD (21/23), MD (24/24), and HD (22/23); all females were sperm positive, even though 3 C, 2 LD, and 1 HD mated females were not pregnant.

5. Pre-implantation Loss (%) = [(No. of corpora lutea – No. of implantation sites) / No. of corpora lutea] x 100
6. Post-implantation Loss (%) = [(No. of implantations – No. of viable embryos) / No. of implantation sites] x 100
7. If 100% total post-implantation loss is >n=2 in a single group, then the Gestation Index (%) = (No. of females with live litters / No. of females pregnant) x 100 was calculated
    - The Cesarean section data for the F1 generation is shown in sponsor's Table 38; there were no teriflunomide-related changes.

Necropsy was performed to determine pregnancy status, the number of corpora lutea, the total number and location of implantations, number of viable or dead embryos, number of resorptions, and a gross examination of placentas.

Sponsor data summaries for F1 pups (pre-weaning)

Table 12 - Summary of surviving male and female pups until weaning

Group	HMR1726 (mg/kg/d)	Sex	Number of litters at birth	Number of pups[1] at birth	Number of surviving pups on postnatal day 0	4[bc]	4[ac]	7	11	14	21[bc]
1	0	males	22	141	141	141	84	84	84	84	84
		females	22	154	154	154	92	92	92	92	92
		all	22	295	295	295	176	176	176	176	176
2	0.05	males	23	156	153	151	89	89	89	89	89
		females	23	161	155	154	86	86	86	86	86
		all	23	317	308	305	175	175	175	175	175
3	0.10	males	20	145	145	143	81	81	81	81	81
		females	20	137	137	136	79	79	79	79	79
		all	20	282	282	279	160	160	160	160	160
4	0.30	males	24	164	164	164	94	94	94	94	94
		females	24	171	170	170	96	96	96	96	95
		all	24	335	334	334	190	190	190	190	189

[1]: Live and dead pups at birth, [bc]: before culling, [ac]: after culling
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Table 18 - Physiological development data for F1 pups

Group	HMR1726 (mg/kg/d)		Eye Opening: Median day criterion achieved males		Eye Opening: Median day criterion achieved females		Eye Opening: Median day criterion achieved all	Pupillary Light Reflex: Percentage of pups responding males		Pupillary Light Reflex: Percentage of pups responding females		Pupillary Light Reflex: Percentage of pups responding all	Righting Reflex: Percentage of pups responding males		Righting Reflex: Percentage of pups responding females		Righting Reflex: Percentage of pups responding all
1	0	Mean	14.3		14.2		14.3	100		100		100	100		100		100
		Std	0.53		0.53		0.55	0		0		0	0		0		0
		N	22		22		22	22		22		22	22		22		22
2	0.05	Mean	14.3	NS	14.1	NS	14.2	100	NT	100	NS	100	100	NT	100	NT	100
		Std	0.61		0.51		0.57	0		0		0	0		0		0
		N	22		22		22	22		22		22	22		22		22
3	0.10	Mean	14.4	NS	14.3	NS	14.4	100	NT	100	NS	100	100	NT	100	NT	100
		Std	0.6		0.54		0.59	0		0		0	0		0		0
		N	20		20		20	20		20		20	20		20		20
4	0.30	Mean	14.3	NS	14.2	NS	14.3	100	NT	95.8	NS	97.8	100	NT	100	NT	100
		Std	0.78		0.64		0.67	0		14.12		8.45	0		0		0
		N	24		24		24	24		24		24	24		24		24

N: Number of litters
*: Significant trend ($p \leq 0.05$) through indicated dose level, NS: No significant trend ($p > 0.05$) through indicated dose level, NT: Not tested

The following sponsor's table summarizes the necropsy findings in F1 pups up to weaning (PND21).

Text table 2 – Summary of necropsy findings in pups

Group	Culled Day 4	Culled Day 21	Died
Control	Short tail, anal atresia (1), necrotic tail tip (1), hematoma at liver lobe (1)	No abnormalities	-
0.05 mg/kg/d	Depression at parietal bone (2)	No abnormalities	Autolysis (8) Not suckled (2)
0.10 mg/kg/d	Eschar at toe (1)	No abnormalities	Autolysis (1) Not suckled (1)
0.30 mg/kg/d	No abnormalities	No abnormalities	Autolysis (1)

Table 25 - Sexual maturation landmarks for F1 animals

			Preputial Separation (males)				Vaginal Opening (females)			
Group	HMR1726 (mg/kg/d)		age		body weight		age		body weight	
1	0	Mean	31.5		121.7		33.5		117.7	
		Std	1.4		13.32		1.86		8.44	
		N	48		48		48		48	
2	0.05	Mean	32	NS	124.8	NS	32.9	NS	114.7	NS
		Std	1.34		13.16		1.44		8.12	
		N	47		47		47		47	
3	0.10	Mean	32.8	NS	131	NS	33.3	NS	116.3	NS
		Std	1.14		13.28		1.94		12.06	
		N	48		48		48		48	
4	0.30	Mean	31.7	NS	123.5	NS	33.2	NS	114.1	NS
		Std	1.46		12.32		1.8		8.87	
		N	48		48		48		48	

N: Pups selected for the F1 study (excluding males death before PND30 and females death before PND25)
*: Significant trend ($p \leq 0.05$) through indicated dose level, NS: No significant trend ($p > 0.05$) through indicated dose level, NT: Not tested
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Table 35 - Summary of F1 female passive avoidance data

			Learning								Memory				
Group	HMR1726 (mg/kg/d)		3rd suc. rk		Lat. 1		Lat. 2		Lat. 3		Nb crossing		3rd suc. rk		Lat. 1		Nb crossing	
1	0	Mean	8		11.7		6.5		33.6		4.3		6		36.4		1.9	
		Std	2.1		7.31		3.25		22.76		1.93		2.5		25.22		1.65	
		N	24		24		24		24		24		19		19		19	
2	0.05	Mean	8	NS	9.2	NS	6.9	NS	24.4	NS	5.2	NS	5	NS	44.7	NS	1.2	NS
		Std	1.9		5.53		7.25		23.35		2.46		1.9		20.95		1.34	
		N	23		23		23		23		23		18		18		18	
3	0.10	Mean	8	NS	12.7	NS	10.8	*	21.0	NS	4.3	NS	5	NS	45.4	NS	1.5	NS
		Std	1.4		11.71		8.27		18.02		1.55		2.3		22.39		2.11	
		N	24		24		24		24		24		22		22		22	
4	0.30	Mean	8	NS	12.7	NS	9.2	*	30.6	NS	4.6	NS	6	NS	45.0	NS	1.9	NS
		Std	1.9		12.27		6.86		23.57		1.93		2.8		21.51		2.16	
		N	24		24		24		24		24		20		20		20	

Animals which do not cross during the first 3 trials of the learning phase are not included in the statistical analysis.
Animals which are not successful during the learning evaluation are not included in the statistical analysis of the memory evaluation.
3rd suc. rk: Rank of the third consecutive success, Lat.: Latency period, Nb crossing: Number of crossings from light to dark
*: Significant trend ($p \leq 0.05$) through indicated dose level, NS: No significant trend ($p > 0.05$) through indicated dose level, NT: Not tested
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In the following sponsor data tables, the results of the F1 generation reproductive analyses are provided.

Text table 3 – Time to insemination

Group	Day 1 to 4	Day 5 to 8	Day 9 to 13	Day 14 to 17	Day 18 to 21	Mean
Control	20	1	1	1	-	3.3
0.05 mg/kg/d	21	-	1	1	-	3.5
0.10 mg/kg/d	23	-	1	-	-	2.9
0.30 mg/kg/d	22	-	1	-	-	2.8

Text table 4 – Mating, pregnancy and fertility indices (%)

Dose (mg/kg/d)	Control	0.05 mg/kg/d	0.10 mg/kg/d	0.30 mg/kg/d
Mated/cohabited	100	100	100	100
Pregnant/mated	87	91	100	96
Pregnant/cohabited	87	91	100	96

10 Special Toxicology Studies

10.2 Immunotoxicity

Study title: HMR-1726—A One Month Immunotoxicity Study in Rats

Study no.:	IMM0121
Study report location:	EDR 4.2.3.7.2.1
Conducting laboratory and location:	Sanofi-Aventis Recherche & Développement Montpellier Cedex, France
Date of study initiation:	25 January 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Teriflunomide, Lot #W001, 99.1% purity

Methods

Doses:	0 (C), 1 (LD), 3 (MD), 6 (HD) mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral (gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	2% potato mucilage
Species/Strain:	Rat/Sprague Dawley Crl:CD®(SD)
Number/Sex/Group:	10/sex/group
Age:	6-7 Weeks
Weight:	M, 166-209 g; F, 126-157 g
Satellite groups:	
Unique study design:	Positive Control: Cyclophosphamide 10 mg/kg (C+) 10 days of dosing on Days: 13, 14,15,16,17, 20, 21, 22, 23, and 24
Deviation from study protocol:	None

Observations and Results

Mortality was checked twice daily.

- No deaths occurred on study.

Clinical Signs were checked at least once daily.

- Neither treatment of teriflunomide nor cyclophosphamide resulted in any clinical signs.

Body Weights were once prior to dosing (Day -1) and weekly commencing on Day 8.

- Unremarkable, there was no notable change in mean absolute body weight in teriflunomide-treated groups.
- A significant decrease was reported in mean body weight for the positive control group (both sexes) on Days 22 (M, -6.1% and F, -8%) and 27 (M, -7.1% and F, -7.3%).
- Mean body weight change from Days 1-22 was significantly reduced in HDM (-9.4%) and
- In C+M, group mean body weight change from Days 1-22 and 1-27 were significantly reduced compared to vehicle control by -13.4% and -14.5%, respectively; however, the group mean body weight change in C+F was unaffected at any interval.

Food Consumption was recorded weekly commencing on Day 1.

- In HD group, males had slightly lower (-7.8%) mean food consumption during Week 1 than controls; however, females were unaffected.
- C+M, decreased mean food consumption compared to CM was noted during Weeks 2, 3, and 4 by -5.1%, -11.3%, and -16.5%, respectively.
- C+F, decreased mean food consumption compared to CF was noted during Weeks 3 and 4 by -10.6%, and -13.5%, respectively.

Hematology and Immunotoxicology was evaluated on Day 28 for hematology and lymphocyte subpopulation analysis, and on Days 20 and 29 for specific immunoglobulin analyses to KLH. WBC, RBC, Hgb, Hct, RDW, MCV, MCH, MCHC, Plt, Neutr, Eos, Baso, Lymphocytes, Monocytes, LUCs, Retic., Retic absolute, and Retic Hgb content were measured.

Hematology

- Cyclophosphamide (C+) significantly altered several hematological parameters in both sexes, including:
    - Increased compared to C (vehicle): RDW (M, +1.3%; F,) [ss], Reticulocyte absolute (M, +79%; F,) [ss], Reticulocyte % (M, +3.35%; F,) [ss],
    - Decreased compared to C (vehicle): Platelet count (M, -23.3%; F,) [ss], WBC count (M, -72%; F,) [ss], Neutrophils (M, -33%; F,) [ss]
- Teriflunomide-treatment altered some erythrocyte parameters.
    - Males, dose-dependent significant decrease in MCH compared to control (-2.7%, -2.9%, and -4.8% for LD, MD, and HD, respectively); dose-related increase in mean absolute number of reticulocytes in MD and HD groups (+8.2% and +12.9%) compared to controls. In HD group,
    - Females,

Lymphocyte subpopulations were analyzed by flow cytometric analysis of peripheral blood for the following lymphocyte subpopulations: T cells (CD3+/CD4+, CD3+/CD8+), B and NK cells. Both absolute cell numbers and percent were determined.

- Cyclophosphamide (C+) significantly reduced total peripheral blood lymphocytes present as well as each of the subpopulations of T, B, and NK cells examined (see reviewer-generated table below).
- Decreased T cell subpopulations and B cells were observed in a dose-dependent manner in females treated with teriflunomide; however, there was no effect in males.

Effect of Cyclophosphamide (C+) or Teriflunomide on Mean Peripheral Blood Lymphocyte Subpopulations in Rats following Repeated Dosing

Treatment Group	Total Lymphocytes		T cells				B cells		NK cells	
			CD3+/CD4+		CD3+/CD8+					
	M	F	M	F	M	F	M	F	M	F
Control	10.324	7.150	3.314	2.971	1.285	1.143	5.345	2.750	0.171	0.085
C+	-80	-78	-64	-68	-33	-49	-100	-100	-85	-84
LD	+15	+17	+10	0	+18	+2	+20	+35	-30	+110
MD	+10	-12	-2	-19	+17	-26	+8	-3	+10	+63
HD	-2	-26	-9	-28	-12	-30	+4	-24	-4	+41

Control values reported as absolute numbers with units of (10E9/L)
Treatment Groups, C+, LD, MD, and HD reported as percent of control values

TDAR

- **T cell Dependent Antibody Response (TDAR)** was initiated by an intravenous administration of KLH antigen on Day 15, analysis of the primary anti-KLH IgM and IgG response on Day 20, a booster administered intravenously on Day 24, and an analysis of the secondary anti-KLH IgM and IgG responses on Day 29.
- As shown in the reviewer-generated table (below), there was a significant reduction in the primary and secondary antibody titer response to KLH challenge following treatment with the positive control, cyclophosphamide. Likewise, treatment with teriflunomide resulted in a dose-dependent suppression of both primary and secondary antibody titers in response to KLH challenge. The suppression of antibody titers at both the MD and HD were greater than that achieved with the positive control. Taken together, these data suggest that teriflunomide is an immunosuppressant that may interfere with antigen response or immunity development in the human.

Antibody Titers in response to KLH antigen administered on Days 15 and 24

Treatment Group	Primary Response (Day 20)				Secondary Response (Day 29)			
	IgM		IgG		IgM		IgG	
	M	F	M	F	M	F	M	F
Control	4.89 ± 0.254	5.221 ± 0.203	4.92 ± 0.379	5.101 ± 0.203	4.836 ± 0.222	4.957 ± 0.256	5.83 ± 0.475	5.830 ± 0.291
C+	-43.9%	-33.2%	-49.2%	-35.7%	-43.2%	-28.2%	-45.8%	-28.1%
LD	-5.7%	-25.4%	-10.8%	-28.9%	+3.7%	-3.0%	-6.2%	-16.5%

Treatment Group	Primary Response (Day 20)				Secondary Response (Day 29)			
	IgM		IgG		IgM		IgG	
	M	F	M	F	M	F	M	F
MD	-34.6%	-53.1%	-41.1%	-59.0%	-20.2%	-40.6%	-35.4%	-55.6%
HD	-49.3%	-59.4%	-57%	-61.9%	-37.4%	-52.2%	-54.1%	-67.9%

Control values reported as absolute numbers with units of log (X+1)
Treatment Groups, C+, LD, MD, and HD reported as percent of control values

10.3 Mechanistic studies (not included elsewhere)

One of the primary toxicities noted in the label of ARAVA® (leflunomide), the prodrug of teriflunomide, was liver toxicity that was identified in both toxicology species as well as during the clinical trials.

Study Title: Cytotoxicity assessment of HWA486 and HMR1726 in rat primary hepatocyte cultures.

Study No. MCT0070

The purpose of this *in vitro* study was to measure hepatocyte toxicity. In this study, both the active metabolite HMR1726 (teriflunomide, Batch No. W001) and its parent HWA486 (leflunomide, Batch No. L01000-05-01) were assessed, as well as, the positive control, ketoconazole.

Hepatocytes were isolated from male SD rats and placed into primary culture (18 hr) prior to 24 hr incubation with increasing concentrations of test articles. Cytotoxicity was evaluated using two measures—cell viability and membrane integrity. Hepatocyte viability was measured as total cellular ATP using a luciferase-based assay. According to sponsor, the luminescent signal is proportional to ATP present and the ATP concentration is directly proportional to the number of metabolically active cells. Hepatocyte membrane integrity was measured indirectly by quantification of lactate dehydrogenase concentration in the supernatant.

Concentrations evaluated

HMR1726 (μM): 2, 4, 8, 16, 32, 64, 128, and 256*
HWA486 (μM): 2, 4, 8, 16, 32, 64, 128, 256, and 1024**
Ketoconazole (μM): 2, 4, 8, 16, 32, 48, 64, and 96

*Highest concentration tested justified based upon an approximation of the limit of solubility in DMSO.
**Sponsor did not provide a justification for the highest concentration of HWA486 tested.

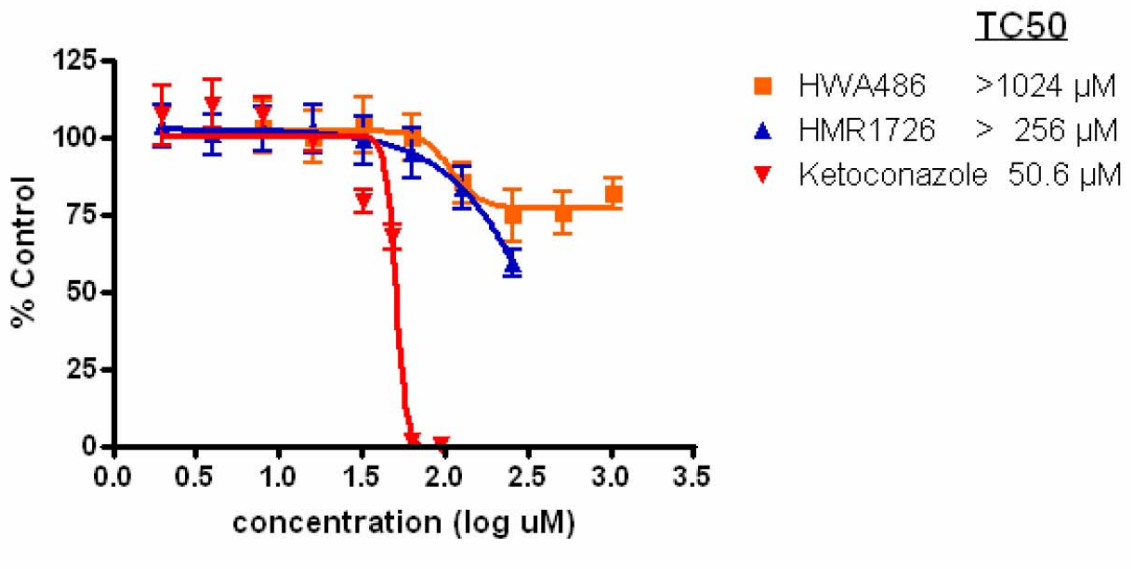
Results

As anticipated, ketoconazole decreased rat primary hepatocyte viability and membrane integrity in a concentration-dependent manner (steep DR curve); the calculated 50% cytotoxicity (TC50) measured for each parameter was 50.6 µM and 56.8 µM, respectively.

Treatment of hepatocyte cultures with either HMR1726 or HWA486 decreased total cellular ATP in a concentration-dependent manner; however, neither test article resulted in at least 50% hepatocyte toxicity. Thus, a TC50 was not calculated. Hepatocyte membrane integrity was unaffected by treatment with either HMR1726 or HWA486 at any concentration tested.

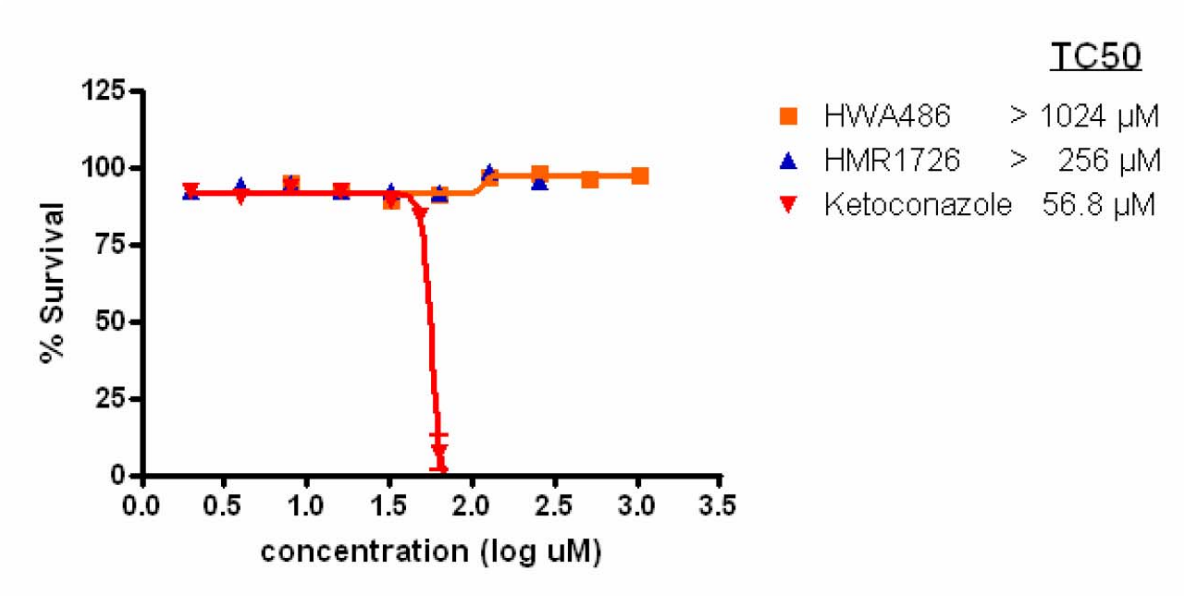
The results of these two studies are presented in sponsor's Figures 1 and 2, below).

Figure 1 - SUMMARY OF TOTAL CELLULAR ATP CONTENT



Values were compared to the vehicle control (0.25 % DMSO). Values represent the mean ± standard deviation (n=6).

Figure 2 - SUMMARY OF CELL MEMBRANE INTEGRITY



Values represent compound treated compared with Vehicle control treated with Triton-X 100 treated cells (total LDH release). Values represent the mean ± standard deviation (n=3).

Study Title: Cytotoxicity assessment of H HWA486 and HMR1726 in human primary hepatocyte cultures.

Study No. MCT0071

The purpose of this *in vitro* study was to measure hepatocyte toxicity from primary cultures of human liver cells (Caucasian male, 28 yo, COD was anoxia). In this study, both the active metabolite HMR1726 (teriflunomide, Batch No. W001) and its parent drug HWA486 (leflunomide, Batch No. L01000-05-01) were assessed, as well as ketoconazole as a positive control. Cytotoxicity was evaluated using two measures—cell viability and membrane integrity. Primary human hepatocyte cultures established 72 hr earlier were purchased and pre-incubated for 5 hr prior to a 24-hr incubation with increasing concentrations of either test articles or the positive control, ketoconazole.

Concentrations evaluated

HMR1726 (µM): 4, 8, 16, 32, 64, 128, 256, and 512*
HWA486 (µM): 4, 8, 16, 32, 64, 128, 256, and 512*
Ketoconazole (µM): 2, 4, 8, 16, 32, 48, 64, and 96

*Highest concentration tested justified based upon an approximation of the limit of solubility in DMSO.

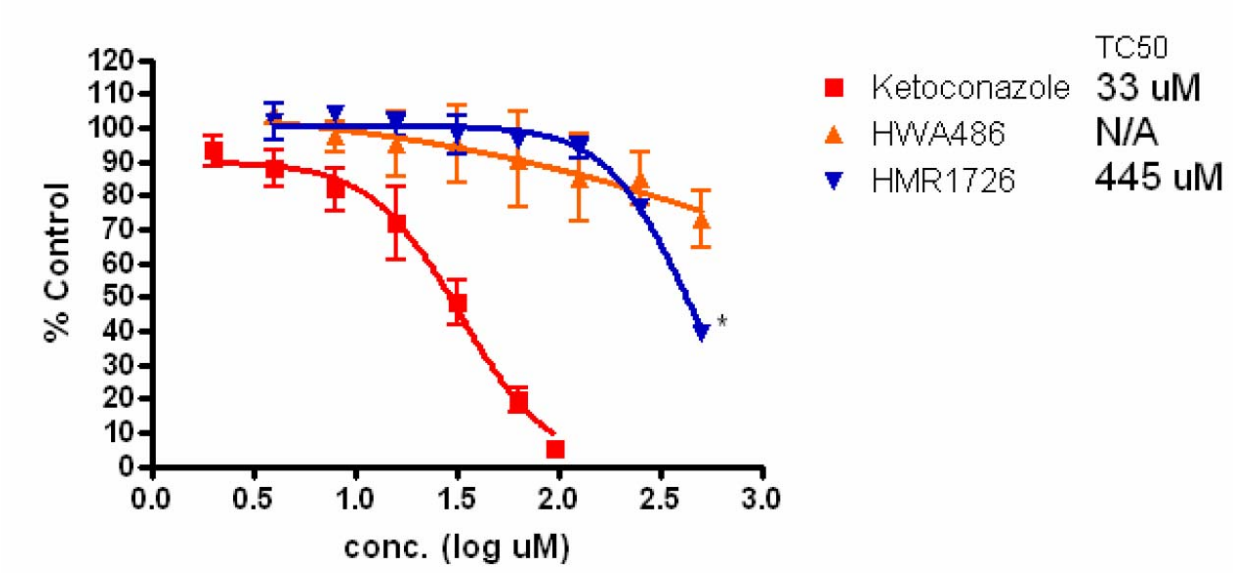
Results

As anticipated, ketoconazole decreased human primary hepatocyte viability in a concentration-dependent manner; the calculated 50% cytotoxicity (TC50) was 33 μM. However, due to a low amount of LDH in the supernatant, the membrane integrity study was not conducted.

Treatment of hepatocyte cultures with HMR1726 or HWA486 decreased total cellular ATP in a concentration-dependent manner. At the highest concentration of HMR1726 tested (512 μM), a precipitate was noted; the calculated TC50 was 445 μM. In contrast, HWA486 at concentrations up to 512 μM did not reach at least 50% cytotoxicity.

The results are shown below in sponsor's Figure 1.

Figure 1 – Summary of Total Cellular ATP Content



* Denotes precipitation of test article at concentration noted.

Values were compared to the vehicle control (0.25 % DMSO). Values represent the mean +/- standard deviation (n=6).

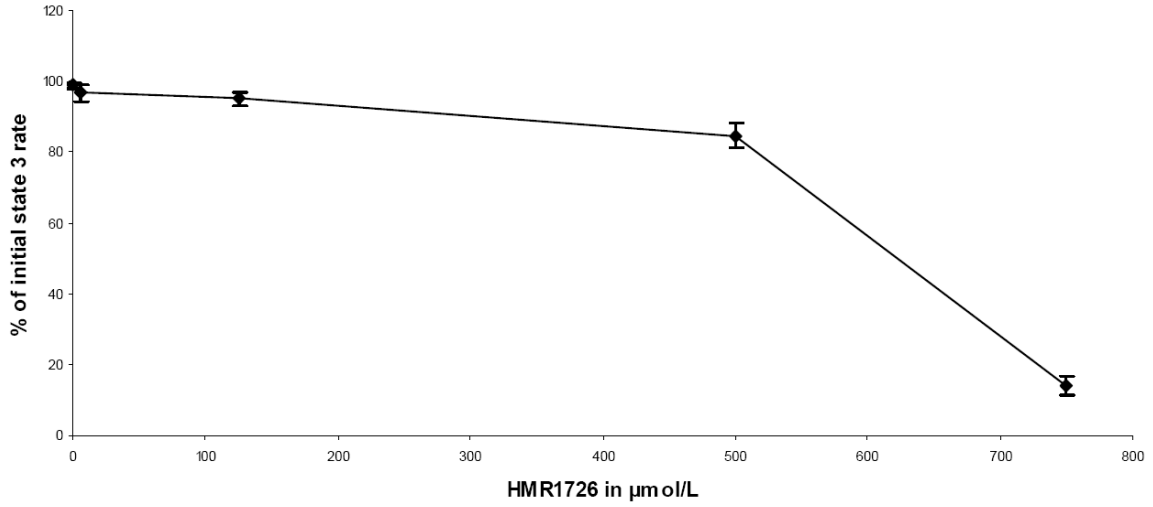
Study Title: Mitochondrial toxicity investigation with HMR1726 on rat liver isolated mitochondria.

Study No.: MCT0078

The purpose of this *in vitro* study was to determine if HMR1726 treatment affected oxygen consumption (respiration) of mitochondria isolated from rat liver. This study was designed to determine whether the test article acted as a respiratory inhibitor and/or an uncoupling agent for either a complex 1 substrate (glutamate-malate) or complex 2

substrate (succinate). Three experimental series were conducted to determine the effect of HMR1726 (6.25, 125, 500, and 750 μM) on mitochondrial oxygen consumption that was either ADP-dependent (state 3 respiration) or ADP-independent (state 4 respiration). In the first experiment, HMR1726 was evaluated in state 3 respiration in the presence of complex 1 substrates, glutamate and malate. As shown in sponsor's Figure 1, liver mitochondrial respiration was unaffected by DMSO vehicle or low concentrations of HMR1726 (6.25 and 125 μM); however, weak and marked inhibition was seen at HMR1726 concentrations of 500 μM (15%) and 750 μM (85%), respectively.

Figure 1 - Effect of HMR1726 on state 3 rat liver mitochondrial respiration with glutamate-malate as substrates.

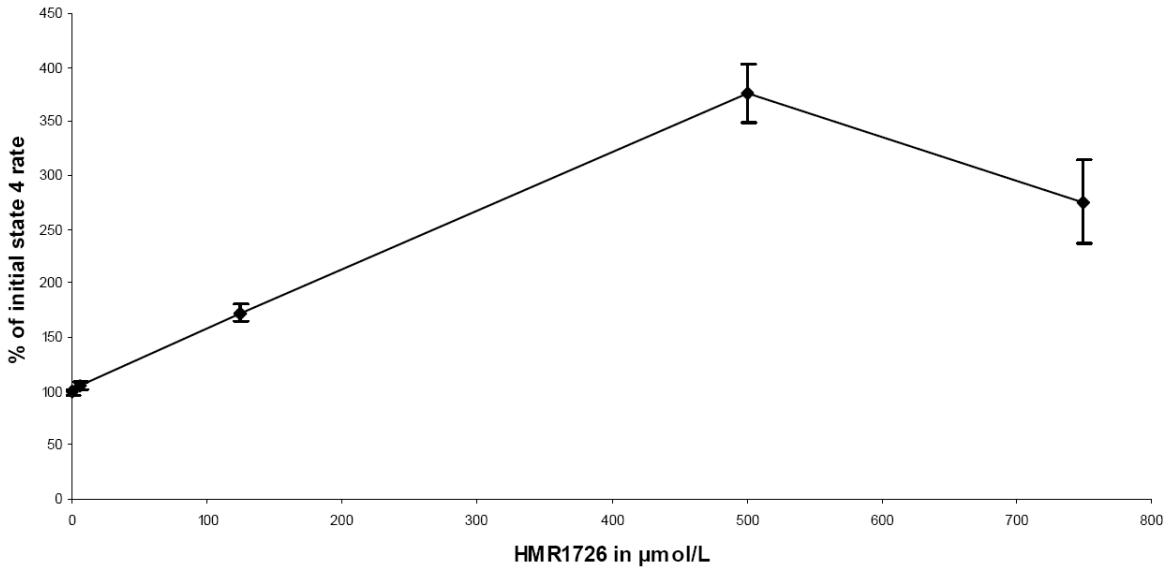


Results are represented as mean values from 3 experiments ± SD and are expressed as a percentage of initial state 3 oxygen consumption rate.

In the second experiment, increased oxygen consumption during state 4 mitochondrial respiration was seen at all concentrations of HMR1726 tested. In contrast, the DMSO vehicle had no effect on oxygen consumption. A peak increase of 375% was achieved following HMR1726 at a concentration of 500 μM. Thus, at all tested concentrations, HMR1726 resulted in an uncoupling of rat liver mitochondrial respiration; this uncoupling effect of HMR1726 was not modified by oligomycin. These data suggest that HMR1726

acts as a true uncoupler of rat liver mitochondrial respiration.

Figure 2 - Effect of HMR1726 on state 4 rat liver mitochondrial respiration with glutamate-malate as substrates.



Results are represented as mean values from 3 experiments ± SD and are expressed as a percentage of initial state 4 oxygen consumption rate.

Study Title: Effect of HMR1726 on rat liver mitochondrial permeabilization.

Study No.: MCT0079

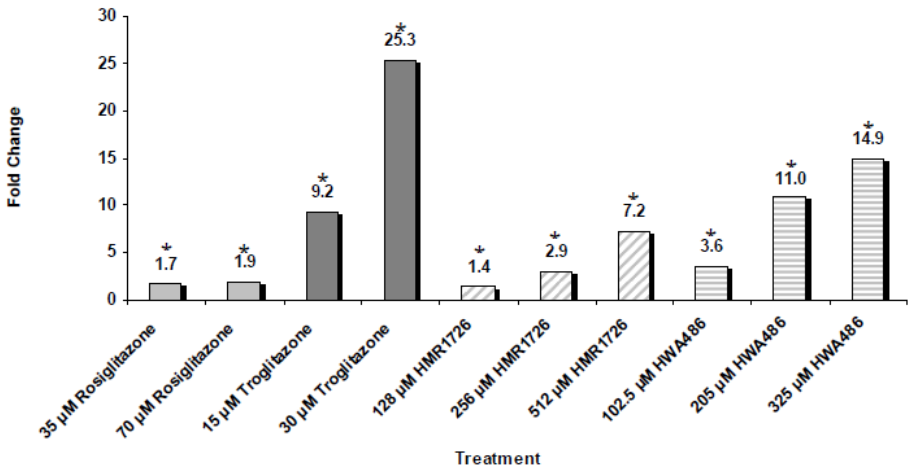
In this non-GLP study, the effect of teriflunomide to modify the mitochondrial membrane permeabilization (MMP) was measured as a loss of the inner transmembrane potential or as a modification of mitochondrial permeability transition as determined by mitochondrial swelling. At concentrations of teriflunomide ranging from 6.25 to 750 µM, there was no increase in mitochondrial swelling. In contrast, calcium (50 µM) treatment of mitochondria increased swelling. Teriflunomide did, however, depolarize the inner mitochondrial membrane when treated at 125, 500, and 750 µM concentrations; no effect was observed at a concentration of 6.25 µM. As teriflunomide has been associated with mitochondrial toxicity, this may be the mechanism of such toxicity.

Study Title: HMR1726 and HWA486—Measurement of Superoxide Anion Generation in H4IIE Rat Hepatoma Cell Line by Flow Cytometry.

Study No.: MCT0082

In this non-GLP in vitro study, teriflunomide and leflunomide (HWA486) were examined to determine if these compounds generated superoxide anion in a rat hepatoma cell line. This study also utilized two control drugs, including rosiglitazone (negative) and troglitazone (positive) as well as, ketoconazole as a standard control to determine cell viability. Teriflunomide was tested at concentrations of 4, 8, 16, 32, 64, 128, 256, and 512 μM (up to its solubility limit that produced no cell death at 1 hr of treatment), and leflunomide was tested at concentrations of 8-1024 μM. As shown in sponsor's Figure 4, there was a concentration-dependent increase in superoxide anion generation in rat hepatoma cells following treatment of teriflunomide or leflunomide.

Figure 4 - Intracellular Superoxide Anion Generation (Fold Change)



An * Indicates the treatment was significantly different ($p<0.05$) from DMSO vehicle control after multiplicity adjustment according to

Study Title: Exploration of the Apoptotic Potential and Cytotoxicity of HMR1726 and HWA486 in Rat Primary Hepatocytes.

Study No.: MCT0083

In this non-GLP in vitro study, the potential of teriflunomide and leflunomide to generate apoptosis in rat primary hepatocytes was determined and compared to that achieved with staurosporine (positive control). Staurosporine treatment resulted in a concentration-dependent and time-dependent decrease in cellular ATP content (a measure of cell cytotoxicity), increased LDH activity in cell culture media at all time points (a measure of hepatocyte membrane integrity), and increased caspase-3/7 activity. Based on the findings with staurosporine, this study was a valid study. Rat hepatocytes incubated with teriflunomide resulted in both a concentration- and time-dependent decrease in cellular ATP content (see sponsor's Table 1). In contrast, hepatocytes treated with leflunomide resulted in variable findings of cytotoxicity with the greatest effect observed after 2 hr incubation.

Table 1 - SUMMARY OF TC_{50} FOR TOTAL CELLULAR ATP CONTENT

	2 Hours	4 Hours	6 Hours	Comments
HWA486	201µM	>1024µM	323µM	Precipitation observed at ≥ 256 µM
HMR1726	>512µM	495µM	406µM	No precipitation observed
STS	6.6µM	2.8µM	2.4µM	No precipitation observed

Both leflunomide and teriflunomide increased LDH activity in a concentration-dependent manner after a 6-hr incubation; the effect of teriflunomide resulted in an approximate 50% loss in cell membrane activity (Figure 8), whereas, leflunomide (Figure 7) had a more limited loss of cell membrane integrity of 25%.

Figure 8 - HMR1726 Related Cytotoxicity – 6 Hours Incubation

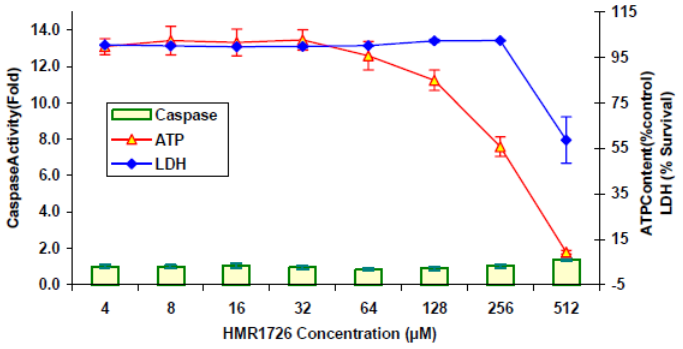


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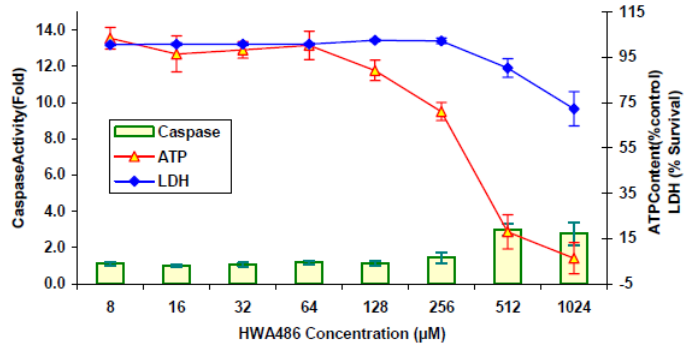
Figure 7 - HWA486 Related Cytotoxicity – 6 Hours Incubation



10.5 Metabolites

The sponsor submitted 9 studies to evaluate the toxicity and genotoxicity of 4-trifluoromethylaniline (4-TFMA), a metabolite of teriflunomide. The toxicity studies included a single oral dose and a 3-month repeat dose toxicity study. The genotoxicity studies included both *in vitro* and *in vivo* assessments of mutagenicity and chromosomal aberrations.

In the first GLP/QA study (No. 013570; initiated June 15, 1994), mice (n =2/sex/400 mg/kg dose; 1 fasted female/1000 mg/kg dose) were administered a single oral (gavage) dose of 4-TFMA in a vehicle of 2% potato starch mucilage. The mice used in this study were immature ICR mice of strain Hsd/01a:ICR. Mice surviving the initial dose of 4-TFMA were observed for 3-4 weeks and body weights were measured weekly. The organs (heart, lungs, liver, spleen, kidneys, brain, and adrenals) of animals that died on study or at terminal sacrifice were examined macroscopically. Soon after dose administration (within minutes), clinical signs including crawling, cyanosis, and abdominal position were observed up until the following night (at 400 mg/kg, group). Cyanosis was observed up to study Day 2 in this same group. Animals dosed with 1000 mg/kg 4-TFMA had decreased depth of breathing, irregular respiration, and coma prior to death. One male (400 mg/kg) died within 1 hr 15 min of administration, the second death occurred in the 1 female dosed at 1000 mg/kg and occurred after 1.5 days. In the two animals that died on study, the lungs were found to be light brown or grey in color; however, the survivors had no such lung findings. It was also noted that in survivors, the body weight was normal and unremarkable.

In the second GLP/QA study (No. 014803; initiated June 6, 1995), a three-month repeat oral dose study was conducted in mice (n=20/sex/group (main study). Mice (10/sex/group; TK at Day 28 and 2/sex/group at end of study) were administered doses of 4-TFMA of 0 (C), 10 (LD), 32 (MD), and 100 (HD) mg/kg/day by oral gavage. The vehicle was a solution of potato starch. Animals were dosed and monitored daily for mortality and clinical signs; 7 deaths occurred in the HD group (4 M—Days 19, 49, 71, 77 and 3 F—Days 7, 27, 39). The majority of clinical signs were observed at the HD (both sexes) and included: cyanosis, panting, gasping, general condition (poor-very poor), drawn in flanks, urine-reddish in color, squatting or prone position, bristling coat, trembling, decreased spontaneous activity, narrowed palpebral fissures and uncoordinated, stilted and ataxic gait. In MD (both sexes), decreased spontaneous activity occurred commencing on Day 79; and in LD (both sexes), ruffled coat was observed and considered treatment-related. Body weight and food consumption was measured once weekly. In HDM, body weight was similar to control although slightly reduced. In contrast, in females, there was an increase in body weight at the HD. No treatment-related findings were found upon macroscopic eye examinations. Clinical pathology was examined at the end of the study. The hematology parameters assessed were RBC ct, Hgb, Hct, MCV, MCH, MCHC, Leukocyte ct, platelet ct, differential, Retic., and Heinz bodies. The clinical chemistry parameters examined were AST, ALT, AP, uric acid, Bilirubin (total), creatinine, and urea. The results of the hematology and clinical chemistry analyses are consistent with a 4-TFMA-induced hemolytic anemia in both sexes. In males, a dose-dependent effect was observed and was present in females at the MD and HD; there were decreased RBC counts,

increased Reticulocyte counts, decreased Hct, and increased MCH. Additionally, in males, there was also a decrease in Hgb at the HD, and a moderate increase in MCV at MD and HD levels; in females, the increase in MCH occurred at all doses. Consistent with the observation of hemolytic anemia, at the HD, polychromasia was observed and up to 30% of the RBC had nuclear fragments present. These results suggest that the hematopoietic system was responding to the hemolytic anemia. Consistent with this interpretation, the only notable clinical chemistry change was a marked increase in bilirubin (total) in males (+160% of control) and females (+85% of control). At necropsy, the following organs were weighed and absolute and relative weights (body weight-corrected) were recorded for adrenals, brain, heart, kidney, liver, lung, spleen uterus, ovary, and testes. In males, at the HD, increased absolute heart (16%) and spleen (666%) weights were found that were also significantly increased when normalized to body weight; liver weight was significantly increased relative to body weight. Consistent with the hemolytic anemia observed in male dose groups treated with TFMA, there was an increased absolute spleen weight at the LD (60%) and MD (187%); both of these values were statistically significant when analyzed relative to body weight. In females, at all doses, spleen weight (absolute) was increased by 29%, 179%, and 551% for LD, MD, and HD, respectively; each of these values was statistically significant when analyzed relative to body weight. At necropsy, the pathologist noted test article and dose-related findings in the spleen and liver of 4-TFMA treated groups [Note: D1 = LD; D2 = MD; D3 = HD]; these data are summarized in sponsor's table, below.

Group		D1	D2	D3
Animals /Group (examened)		20	20	19
Spleen	Discoloration	16	19	17
	Size-Change	1	14	19
Liver	Discoloration	1	4	15

A full genotoxicity battery was conducted on 4-TFMA to determine the mutagenic and clastogenic potential of this metabolite of teriflunomide. Two Ames tests were submitted (Study Nos. 008508, initiated 05/88; 012797, initiated: 07/93); however, the first study was inadequate as neither *Salmonella* strain TA102 nor the *E. coli* strain WP2uvrA was evaluated. The second study was adequate and complete. In each test, 4-TFMA was mutagenic in a concentration-dependent manner in two salmonella strains TA100 and TA1535, and this effect occurred in the absence and in the presence of metabolic activation (S9). The results of the second study for the positive strains are shown in sponsor's Tables 1, 2, and 5 (below). The positive control results are shown in sponsor's Tables 11 and 11a.

Table 1: Mutagenicity experiment with 4-Trifluoromethyl-anilin
with and without metabolic activation

TA 100

Number of revertant colonies per plate and mean values
using Salmonella typhimurium strain TA 100

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	155.7	166	159	142
u.c.	-	201.3	205	191	208
4	-	189.3	187	176	205
20	-	192.0	196	183	197
100	-	189.3	216	174	178
500	-	348.3	346	361	338
2500	-	57.3	30	67	75 (ibl)
5000	-	0.0	0	0	0 (nbl)
0	+	182.7	197	172	179
u.c.	+	216.3	224	217	208
4	+	219.3	216	213	229
20	+	217.7	224	216	213
100	+	303.7	307	286	318
500	+	541.0	579	548	496
2500	+	792.0	824	764	788
5000	+	84.3	138	62	53 (ibl)

Compound dissolved in 100 microliter DMSO
- : absence
+ : presence
u.c. : untreated control
(ibl) : incomplete bacterial lawn
(nbl) : no bacterial lawn

Table 2: Mutagenicity experiment with 4-Trifluoromethyl-anilin with and without metabolic activation

TA 1535

Number of revertant colonies per plate and mean values using Salmonella typhimurium strain TA 1535

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	9.0	8	10	9
u.c.	-	12.7	11	12	15
4	-	13.7	16	15	10
20	-	14.7	10	15	19
100	-	30.0	28	35	27
500	-	86.3	74	78	107
2500	-	13.7	8	12	21 (ibl)
5000	-	0.0	0	0	0 (nbl)
0	+	16.0	17	19	12
u.c.	+	11.7	10	10	15
4	+	15.7	18	12	17
20	+	17.0	16	19	16
100	+	51.3	47	51	56
500	+	139.3	149	131	138
2500	+	145.0	190	278	267
5000	+	39.0	14	21	82 (ibl)

Compound dissolved in 100 microliter DMSO

- : absence
+ : presence
u.c. : untreated control
(ibl) : incomplete bacterial lawn
(nbl) : no bacterial lawn

Table 5: Mutagenicity experiment with 4-Trifluoromethyl-anilin with and without metabolic activation

WP2uvrA

Number of revertant colonies per plate and mean values using Escherichia coli strain WP2uvrA

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	38.7	42	37	37
u.c.	-	43.3	46	38	46
4	-	39.3	41	41	36
20	-	46.7	45	51	44
100	-	55.3	47	59	60
500	-	118.7	96	137	123
2500	-	181.7	160	187	198
5000	-	22.7	24	28	16 (ibl)
0	+	41.3	48	35	41
u.c.	+	43.7	52	41	38
4	+	46.0	43	47	48
20	+	46.7	43	43	54
100	+	58.0	70	55	49
500	+	105.0	128	84	103
2500	+	317.7	331	302	320
5000	+	326.0	329	313	336

Compound dissolved in 100 microliter DMSO

- : absence
+ : presence
u.c. : untreated control
(ibl) : incomplete bacterial lawn

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Table 11 : mutability (positive controls) and sterility test of the experiment with 4-Trifluoromethyl-anilin

Number of revertant colonies per plate and mean values using Salmonella typhimurium strains and Escherichia coli

Strain	Compound	Dose ug/plate	metab. Activ.	Mean value	Colonies per plate		
TA100	Sodium-azide	1	-	456.7	474	423	473
TA1535	Sodium-azide	1	-	336.7	334	330	346
TA1537	9-Aminoacridine	50	-	91.3	81	103	90
TA98	2-Nitrofluorene	2.5	-	769.0	792	833	682
WP2uvrA	MNNG	2.5	-	154.7	157	149	158
	4-Trifluoromethyl-anilin	5000	-	0.0	0	0	0

- : absence

Table 11a : mutability (positive controls) and sterility test of the experiment with 4-Trifluoromethyl-anilin

Number of revertant colonies per plate and mean values using Salmonella typhimurium strains and Escherichia coli

Strain	Compound	Dose ug/plate	metab. Activ.	Mean value	Colonies per plate		
TA100	2-Aminoanthracen	0.5	+	524.3	510	563	500
TA1535	2-Aminoanthracen	1	+	142.7	152	130	146
TA1537	2-Aminoanthracen	1	+	161.0	155	157	171
TA98	2-Aminoanthracen	0.5	+	1031.7	980	1097	1018
WP2uvrA	2-Aminoanthracen	10	+	347.7	404	312	327
	S-9 mix	500 ul	+	0.0	0	0	0
	4-Trifluoromethyl-anilin	5000 ug	+	0.0	0	0	0

\+ : presence

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Two chromosomal aberration tests in V79 Chinese hamster cells were performed (Study Nos. 013344, initiated: 11/93; 013555 initiated: 1/94, Batch Wa73). Both studies were adequate and valid. In the first study, 4-TFMA increased the mutation rate greater than that observed in the negative control either in the absence or the presence of metabolic activation; this effect occurred in a concentration-dependent manner (-S9, 50-250 μg/mL; +S9, 100-500 μg/mL). In the second study, 4-TFMA was evaluated at concentrations ranging from 10-100 μg/mL (-S9) and 0-500 μg/mL (+S9) after 18 and 28 hr. In the main study, there was no significant increase in chromosomal aberrations excluding gaps in the 18-hr incubation of 4-TFMA in the absence of metabolic activation; in contrast, the number of chromosomal aberrations was significantly increased compared to the negative control after a 28-hr incubation. 4-TFMA cultured in the presence of metabolic activation had significantly increased numbers of chromosomal aberrations excluding gaps after both 18-hr (250 and 500 μg/mL) and 28-hr (500 μg/mL) incubations. In a repeat experiment, similar results were observed, except that the concentration at which a significant effect occurred in the presence of metabolic activation at 18 hr was 500 μg/mL.

In Study No. 017876, the mutagenic potential of oral (gavage) administered 4-TFMA was tested in an unscheduled DNA synthesis (UDS) in rat liver by an *in vivo/in vitro* method. In this study, Han Wistar rats (range-finding, n=6/sex; main study, n=38/sex) were utilized. Animals were administered either vehicle control (1% carboxymethyl cellulose) or 4-TFMA (range-finding, 100 or 200 mg/kg; main study, 40 or 100 mg/kg) in a dose volume of 10 mL/kg. In the range-finding study, groups of 3 rats/sex were utilized; in the main study, groups of 5 rats/sex were dosed. The main study included two positive control groups, 2-acetamidofluorene (75 mg/kg) and dimethylnitrosamine (10 mg/kg). In the range-finding study, death occurred in 1M and 1F at the 200 mg/kg dose; therefore, the main study was conducted at 100 mg/kg and 40 mg/kg. In the main study, the 100 mg/kg dose after 12 hr resulted in 3 deaths (1M, 2F) and the clinical signs included lethargy, piloerection, protruding eyes, pale colored eyes, abnormal breathing, and cold to the touch. At the 40 mg/kg dose, animals had

piloerection and blue/gray extremities. As conducted, this study was a valid and adequate study. The negative control was within the historical range for the testing laboratory, and the two positive controls were significantly different from the negative control. In contrast, there was no significant increase in UDS from hepatocytes isolated within 2-4 hr or 12-14 hr following a single dose 4-TFMA (40 or 100 mg/kg). These data suggest that 4-TFMA tested at a dose up to 100 mg/kg had no effect on UDS in hepatocytes isolated from treated Wistar rats; thus, under the conditions of this assay, 4-TFMA was not shown to be mutagenic.

In Study No. 017736, 4-TFMA was tested in a mammalian erythrocyte micronucleus test in NMRI mice. Mice were treated with a single intramuscular dose of 4-TFMA of 8, 25, 80 or 100 mg/kg (male) and 20, 60, 200 or 300 mg/kg (female). Deaths occurred at doses of 80, 100, and 300 mg/kg of 4-TFMA (these animals were replaced and survived after treatment). The following clinical signs were noted postdose: decreased spontaneous activity, coat bristling, squatting posture, stilted gait, cyanosis, palpebral fissure narrow, forward crawling, unsteady gait, stupor, no placing reflex, general poor condition, tremor, and reduced placing reflex. These toxicities were observed for up to 48 hr postdose. Analysis of bone marrow erythrocytes isolated from negative control animals in each sex at 12, 24, or 48 hr postdose showed minimal presence of micronuclei (male, 1.8 to 2.6; female 2.0-2.4); whereas, the positive control in each sex was significantly elevated 24 hr postdose (male, 49.6; female, 37.8). In contrast, none of the 4-TFMA doses examined, at any time point, increased the number of micronucleated polychromatic erythrocytes. These data suggest that under the conditions of this *in vivo* assessment, 4-TFMA is not a clastogen.

In Study No. 014161, an *in vitro* chromosome aberration test was conducted in bone marrow cells of Chinese hamster for 4-TFMA. As conducted, the assay was a valid and adequate assessment. In the negative control group, the mean number of chromosomal aberrations without gaps was 0.0% at 12, 24, or 48 hr. The positive control substance, cyclophosphamide (50 mg/kg) increased the number of chromosomal aberrations excluding gaps to 18% at 24 hr. At 12, 24, or 48 hr postdose, the number of chromosomal aberrations observed in animals treated with 4-TFMA (75 mg/kg) was 0.2%, 0.2%, and 0.4%, respectively. The lack of a significant treatment effect suggests that 4-TFMA was not clastogenic in this *in vitro* assessment.

10.6 Impurities

The sponsor has conducted 15 studies to evaluate the toxicity of (b) (4) impurities.

In a non-GLP non-QA study (Study No. 2000-0732), the acute oral toxicity of the (b) (4) impurity (200, 315, 400, and 500 mg/kg) was determined in male (5/group) and female (5/group) SD rats; the lethal dose was estimated for males at >500 mg/kg and for females between 400 and 500 mg/kg. In HDF, decreased mean body weight gain was observed between study Days 1 to 8 that recovered on Days 8-15; 3 HDF died on study. Clinical signs following administration of (b) (4) included uncoordinated

gait, hypoactivity, prone position, cyanosis, decreased or no righting reflex, decreased or no paw reflex to pinching, squatting posture, stupor, ataxia, irregular respiration, panting, decreased respiratory rate, decreased body temperature, bristling coat, serous eye discharge, and drawn in flanks. Upon macroscopic examination of the premature decedents, target organs identified were lung, liver, small intestine, and body cavity. The findings noted were several dark red patches in the lung, several beige patches were found on the liver, beige mucous in the small intestine, and the body cavity was filled with a light brown fluid. In contrast, none of the survivors had any findings upon macroscopic exam at necropsy.

In a 1-month GLP/QA study (No. F2000tox0804) repeat dose toxicity study with recovery (23-24 days) of (b) (4) (0, 1, 5, 25 mg/kg) was conducted in SD rats (n=15/sex/group). No mortality, change in behavior, body weight, body weight gain or feed consumption was attributed to (b) (4) treatment. In contrast, treatment-related findings in clinical pathology (hematology and clinical chemistry) were found consistent with the development of oxidative hemolysis. Reduction in rbc count, Hgb, and Hct were found in all dosed males and in MD and HD females; each of these findings was biologically and statistically significant. Increased MCV was found in MD and HD groups of both sexes; this effect was also biologically relevant and statistically significant compared to C. Consistent with oxidative hemolysis, reticulocytes were increased in a dose-dependent manner that was statistically significant in LD, MD, and HD groups (+13 and +27, and +196% (males) and +242, 535 and 881% (females) of Control, respectively). Heinz bodies were absent in Control and LD groups (both sexes), but present in both MD and HD groups (both sexes). Measurement of methemoglobin demonstrated increased values at all doses in a dose-dependent manner. The increase in methemoglobin was still present in recovery animals. Several alterations of clinical chemistry were treatment-related, including: a) increased total bilirubin in LD, MD, HD (males) and HDF, b) increased AST and ALT in MDM and HD (both sexes), c) increased calcium in LD, MD, and HD (females), d) increased sodium, creatinine, and albumin in HDF, e) decreased globulin in HD (both sexes), and f) decreased triglycerides MDM and HDM. Macroscopic examination at necropsy identified enlarged spleen and discolored kidneys; these findings were supported by microscopic findings. A dose-dependent increase in spleen weight relative to body weight was found in males compared to control of +12.4%, +60%, and +214% for LD, MD, and HD, respectively. In females, a similar increase in relative spleen weight was found in MD and HD compared to control of +49% and +248%, respectively; in HDF, there was an increase in relative heart weight (+12%) compared to control. Upon microscopic examination of the spleen, there was a correlation between enlargement and occurrence of extramedullary hematopoiesis and hemosiderin deposition in terminal animals at the MD and HD. In recovery animals, there was no sign of extramedullary hematopoiesis; however, there was still evidence of hemosiderin deposition at the MD and HD. Based on the presence of oxidative hemolysis in all male dose groups and changes in clinical pathology—hematology and clinical chemistries, a NOAEL was not established in this study.

In a GLP/QA study (No. F2001tox0191), the oral (gavage) toxicity of [redacted] (0, 0.1, and 0.3 mg/kg) was re-tested in a 28-day repeat dose study with a 28-day recovery in SD rats (n=15/sex/group) to ascertain a NOEL for the oxidative hemolysis observed in the previous study. In contrast to the previous study (F2000tox0804), there were no [redacted] effects observed at either dose of 0.1 or 0.3 mg/kg; thus, the NOEL in this study was considered 0.3 mg/kg.

In a GLP/QA Study No. F2000tox0714, the [redacted] impurity (Batch No. C009, 100.2%) was evaluated in an Ames test in Salmonella strains TA100, TA98, TA1535, TA1537, and TA102. In this study, precipitation of the impurity occurred at a concentration of 5000 μg/plate. Based on the results of the cytotoxicity study, the concentration range selected for the definitive mutagenicity study in the absence and presence of metabolic activation was the following:

Without metabolic activation:
Strains: TA100, TA1535, TA98, and TA102 (5, 16, 50, 160, 500, and 1600 μg/plate).
Strain: TA1537 (1.6, 5, 16, 50, 160, and 500 μg/plate)

With metabolic activation:
Strains: TA100 and TA102 (5, 16, 50, 160, 500, and 1600 μg/plate)
Strains: TA1535, TA1537, and TA98 (16, 50, 160, 500, 1600, and 5000 μg/plate)

Administration of the positive control to each of the tester strains in the absence and presence of metabolic activation resulted in clear and statistically significant increases in the number of revertant colonies. In contrast, incubation of the [redacted] impurity with any of the tester strains in the absence or presence of metabolic activation did not increase the number of revertants. Taken together, these data suggest that under the conditions of this study, [redacted] was not a mutagen.

The sponsor conducted two *in vitro* mutation assays of [redacted] (Study Nos. 2004-0837 and LYM0229) in mouse lymphoma L5178Y cells. In the first study, the concentrations of [redacted] (Batch Hau5284-2, 99.8% purity) (as determined in a cytotoxicity study) were 12.5, 25, 50, 75, 100, 125, 150, and 250 μg/mL (no metabolic activation) and 25, 50, 100, 125, 150, 175, 200, 250, and 300 μg/mL (with metabolic activation). In this study, the positive controls utilized were 4-nitroquinoline-1-oxide (without metabolic activation) and benzo(a)pyrene (with metabolic activation); each of these positive controls significantly increased the mutant frequency compared to the negative solvent control. In the absence of metabolic activation, [redacted] did not increase the mutant frequency at any concentration tested in a 3-hr assay. The concentration range was adequate as moderate cytotoxicity was observed at the highest concentration tested. In a separate assay (24 hr), [redacted] increased the mutant frequency at the lowest concentration of 25 μg/mL and at concentrations ranging from 75 to 137.5 μg/mL; however, the increased mutant frequency did not reach all of the mutagenicity criteria—the increased mutant frequency did not exceed the range of historical negative control results. In a second 24-hr assay, several but not all the criteria specified for a mutagen were met. There was a significant increase in mutant frequency at all concentrations analyzed (25-137.5 μg/mL), and the increase at the highest concentration was greater

than the historical negative control; however, the mean plating efficiency of the negative control was below the sponsor's acceptable range of 70-130%, and thus the sponsor considered that this repeat assay was not valid. In the presence of metabolic activation, two studies were conducted, and in each case, cytotoxicity was observed; however, there was no change in the mutant frequency. These data suggest that in the presence of metabolic activation, (b) (4) is not a mutagen; however, in the absence of metabolic activation, the mutagenicity of (b) (4) was inconclusive.
In the second study conducted (LYM0229) the concentration range of (b) (4) that produced positive or inconclusive results in the first study were examined. In addition, the effect of uridine to inhibit any effect of (b) (4) on DHDOH was examined. Similar to the previous study, (b) (4) at high concentrations resulted in cytotoxicity; however, there was no increase in the mutant frequency at any of the concentrations of impurity tested. Likewise, the addition of uridine had no effect on the mutant frequency at any concentration of (b) (4). These data suggest that under the conditions of this study, (b) (4) was not a mutagen.

The sponsor conducted three *in vitro* chromosome aberration tests (Study Nos. DSE 2002tox0567 (not GLP), 2003-1709 (not GLP), and DSE2005-0280, GLP/QA) in Chinese hamster V79 cells to assess the clastogenicity of the (b) (4) impurity (Batch Nos. C009 and Hau 5284-2). In these studies, (b) (4) was tested at concentrations ranging from 15.63 to 1500 µg/mL in the absence and presence of metabolic activation; the pivotal study was DSE2005-0280. In the pivotal study, two independent mutation tests were performed. As conducted, this study was a valid and adequate assessment of mutagenicity. The positive controls EMS (without metabolic activation) and DMBA (with metabolic activation), each significantly increased the mean number of mutants compared to the negative control. In contrast, none of the (b) (4) concentrations tested increased the mutant frequency. Based on the results of this study, (b) (4) was not a mutagen in V79 Chinese hamster cells.

The sponsor conducted two *in vitro* chromosome aberration tests (Study Nos. MAF0067 and MAF0082) in cultured human peripheral blood lymphocytes. In GLP/QA study MAF0067, (b) (4) was evaluated in the absence and presence of metabolic activation for 3 and/or 20 hr at concentrations of 0, 40, 70, 100, 150, 200, 250, 300, and/or 400 µg/mL. In these studies, the addition of (b) (4) at any of the concentrations tested did not increase the number of micronuclei compared to the solvent control; in contrast, the positive controls MMC (0.2 µg/mL) and cyclophosphamide (7.5 µg/mL), each induced a significant increase in the number of micronuclei. This assay was an adequate and valid study, and the results of this study suggest that (b) (4) is not a clastogen in human peripheral blood lymphocytes. The second study (MAF0082), (b) (4) was tested at lower concentrations and was also negative.

In Study No. DSE 2004-1697, a chromosome aberrations test was conducted in bone marrow of SD rat to assess the clastogenicity of the (b) (4) impurity (Batch No. Hau 5284-2). The rats were administered once daily at oral doses of (b) (4) of 100, 200, and 400 (male) or 50, 100, and 200 mg/kg (female) for two consecutive days. The dose selection was based upon a preliminary dose range-finding study in which severe

clinical signs were observed in animals administered 400 mg/kg (males) and 1/3 F died. The positive control, cyclophosphamide (20 mg/kg) was administered once as a single oral dose. As conducted, the assay was a valid and adequate assessment. Animals were observed for clinical signs and mortality; several clinical signs were noted in males: pale skin, all doses; decreased motor activity and ataxia, MD and HD; drawn in flanks and recumbent posture, HD; in females: pale skin, all doses, decreased motor activity and ataxia, HD. Compared to the negative control results, there was no reduction in the mitotic index at the HD of either sex. The number of micronuclei present was within the historical control range, and there was no dose-related or significant effect observed. Under the conditions of this study, (b) (4) was not clastogenic.

In Study No. HIS1735, the mutagenicity of a putative genotoxic impurity, (b) (4), was tested in an Ames assay. The (b) (4) impurity (concentrations of 5, 16, 50, 160, 500, 1600, and 5000 µg/plate) was evaluated in a full complement of Salmonella strains (TA100, TA1535, TA1537, TA98, and TA102) in the absence and presence of metabolic activation. Based on the results of this study, treatment of the Salmonella strains with (b) (4) was negative in the absence or presence of metabolic activation did not increase the mutation frequency; however, the positive controls significantly increased the number of revertant colonies as expected. As conducted, this study was an adequate assessment of the genotoxic potential of impurity (b) (4), and at the conditions tested, this impurity was found to be negative.

According to the sponsor, some of these studies were conducted due to an initial unfounded genotoxicity concern due to inclusive results from some clastogenicity studies. The following sponsor-provided Tables 16 and 17 summarize the GLP genotoxicity results for impurities (b) (4)

Table 16 - Genotoxicity study results (b) (4)

Test System	Concentration or doses	Result
Ames test, with or without metabolic activation [008508], [012797]	up to 5000 µg/plate	positive
HPRT assay, with or without metabolic activation [013344]	up to 750 µg/mL	positive
In vitro chromosome aberration test, with or without metabolic activation [013555]	up to 650 µg/mL	positive
In vivo unscheduled DNA synthesis in rats [017876]	40 or 100 mg/kg PO	equivocal
In vivo bone marrow micronucleus test in mice [017736], [1998-0642A1]	males: 8, 25, 80 or 100 mg/kg, IP females: 20, 60, 200, 300 mg/kg, IP	negative
In vivo chromosome aberration test in Chinese hamsters [014161]	75 mg/kg, PO	negative

Abbreviations: HPRT = hypoxanthine-guanine-phosphoribosyl transferase, IP = Intraperitoneal, PO = Per os (oral)

Table 17 – GLP Genotoxicity study results - (b) (4)

Test System	Concentration or doses	Result
Ames test, with or without metabolic activation [F2000TOX0714] [2000-067A1]	up to 5000 µg/plate	negative
HPRT assay, with or without metabolic activation [2005-0280]	up to 1500 µg/mL	negative
Screening (non-GLP) chromosomal aberration study in V79 cells [F2002TOX0567]	up to 1000 µg/mL for 3 h	negative
Screening (non-GLP) chromosomal aberration study in V79 cells [2003-1709]	up to 1150 µg/mL for 3 h, up to 80 µg/mL for 24 h	inconclusive
Mouse lymphoma assay, with or without metabolic activation [2004-0837]	up to 300 µg/mL for 3 h, up to 175 µg/mL for 24 h	3 h incubation: negative; 24 h incubation: inconclusive
Repeat mouse lymphoma assay in the absence and presence of uridine [LYM0229]	Up to 150 µg/mL for 24 h	negative
In vitro chromosome aberration test in human lymphocytes [MAF0067]	up to 300 µg/mL for 3 h, up to 400 µg/mL for 20 h	negative
Repeat In vitro chromosome aberration test in human lymphocytes [MAF0082]	up to 250 µg/ml for 3 h, up to 351 µg/ml for 20 h	negative
In vivo chromosome aberration test in rats [2004-1697]	males: 100, 200 or 400 mg/kg, PO females: 50, 100 or 200 mg/kg, PO	negative

Abbreviations: HPRT = hypoxanthine-guanine-phosphoribosyl transferase, PO = Per os (oral)

In a non-GLP study (2004-1939c), impurity (b) (4) was evaluated for its activity on recombinant mouse and human DHODH. This experiment tested the impurity (b) (4) at concentrations up to 1 mM; higher concentrations could not be achieved due to reduced solubility. At up to 1 mM, (b) (4) inhibited mouse DHODH activity by 10% and reduced human DHODH activity by 33-41%. In contrast, the positive control brequinar sodium inhibited both mouse and human DHODH activity, and the IC_{50} values were 284 nM and 9 nM, respectively.

11 Integrated Summary and Safety Evaluation

Teriflunomide (AUBAGIO) is a dihydroorotate dehydrogenase inhibitor that has been submitted as a treatment for relapsing-remitting multiple sclerosis. Teriflunomide is the active metabolite of an approved drug, leflunomide (ARAVA®), which was approved as a treatment for rheumatoid arthritis. In the present application, the sponsor has submitted the nonclinical studies necessary to determine the nonclinical safety profile of teriflunomide.

The pharmacology of both leflunomide and teriflunomide as inhibitors of dihydroorotate dehydrogenase (DHO-DH) is well established from sponsor-conducted studies, as well as from the literature (Knecht and Löffler, 1998). In immune cells, particularly lymphocytes (T and B cells), cellular activation and/or proliferation has been shown to be dependent on *de novo* pyrimidine synthesis by DHO-DH. In human T cells, leflunomide (at concentrations <100 µM) exerts an immunomodulatory effect via

inhibition of DHO-DH (loss of *de novo* pyrimidine synthesis); however, at higher concentrations purine biosynthesis is also restricted, thus reducing ATP-dependent processes, such as T cell signaling (Rűckemann, K., Fairbanks, L.D., Carrey, E.A., Hawrylowicz, C.M., Richards, D.F., Kirschbaum, B., and H.A. Simmonds, 1998, J Biol. Chem., 273 (34): 21682-21691). Likewise, humoral immunity as measured by antibody production by B cells is compromised by inhibition of DHO-DH. In an *in vivo* study by Siemasko, et al. (1996), leflunomide administered to mice decreased both T cell-dependent and T cell-independent antibody responses. The sponsor further confirmed this finding in a immunotoxicity focused 28-day repeat oral dose study of teriflunomide in rat, in which T cell-dependent antibody responses to the antigen KLH were compromised. Additional *in vitro* studies using teriflunomide demonstrated that the loss of antibody response was mediated by inhibition of B cell proliferation that was mediated through inhibition of DHO-DH; this was confirmed by rescue of B cell proliferation by exogenously applied uridine. Taken together, the primary mechanism of teriflunomide to inhibit lymphocyte activity appears to be predominantly mediated through its inhibition of DHO-DH.

The sponsor conducted several *in vivo* pharmacology studies that support its putative effectiveness in patients with multiple sclerosis. Experimental autoimmune encephalomyelitis (EAE) is a well-established model in rodents that is used to determine putative therapies for utility in patients with multiple sclerosis. Similar to the development of multiple sclerosis in humans, development of EAE in rats is a complex process that is not well understood. The sponsor conducted an in vivo and ex vivo study to determine immune cell number and distribution in the spleen, cervical spinal cord, whole blood, and peripheral blood mononuclear cells during the progression of EAE. This study demonstrated the complexity of the immune response in this disease and attempted to understand the role of teriflunomide to attenuate the clinical disease in rats at four stages of the disease course (onset, acute attack, remission, and relapse). Teriflunomide treatment attenuated the clinical disease at each stage of EAE examined yet not all EAE stage dependent cellular changes were attenuated. This further exemplifies that the precise immunological mechanism that confers clinical benefit is not well understood.

In the animal species used for toxicity testing, teriflunomide is rapidly absorbed, highly protein bound, and has a small V_d = 0.073 to 0.163 L/kg (following IV administration), which is less than the total body water of 0.6-0.7 L/kg in the toxicity species. The metabolism of teriflunomide is moderate in all the toxicity species and is found largely unchanged in plasma. In mouse, rat, and dog, the terminal half-life of teriflunomide is long (18-37 h) which, in rats, is attributed to enterohepatic recycling; however, the terminal half-life in rabbit is considerably shorter (4-5 h). Among the toxicology species, the metabolism of teriflunomide is relatively similar, and the human metabolites were represented in at least one animal species. One minor metabolite of teriflunomide is 4-TFMA, (b) (4) was detectable, but at low concentrations, following oral or IV dosing. The excretion of teriflunomide was shown to be mostly in the feces of mouse, rat, and dog and in the urine of rabbits. In female rats,

teriflunomide (approximately 23% of total dose) was excreted in milk. Both rat and dog were good models to evaluate the toxicological effects of teriflunomide.

Toxicology studies of teriflunomide were conducted in mouse, rat, rabbit, and dog. The toxicities associated with repeated teriflunomide exposure were consistent with its mechanism of action. As an inhibitor of de novo pyrimidine synthesis, the toxicities associated were largely associated with organs and systems that actively proliferate, such as, the bone marrow (hematopoietic system), lymph nodes (immune system), pancreas, and the developing fetus. The pivotal toxicology studies were chronic studies conducted in SD rat (6-month) and in Beagle dog (one-year). As conducted, both studies were considered adequate, and each met the regulatory guideline requirements for acceptable toxicology studies.

Repeated administration of teriflunomide in rat and dog resulted in significant toxicities; however, NOAELS were identified in the chronic toxicity studies. Associated with chronic administration, the 6-month Sprague Dawley rat and one-year Beagle dog studies identified several primary target organs, including the bone marrow (rat and dog), pancreas (dog), and the immune system—lymph node, GALT, and spleen in rat and GALT and lymph node in dog.

In the chronic rat study, rats were dosed daily with teriflunomide (0, 0.3, 1.5/9.0, 3.0, and 6.0 mg/kg/day) by oral gavage. Due to a lack of toxicity at the 6 mg/kg/day dose, the dose of the 1.5 mg/kg/day group was increased to 9.0 mg/kg/day on study Day 107. As a result, there were two unscheduled deaths at the HD. These deaths were related to changes in the bone marrow, gastrointestinal tract, and lymphoid tissue. In the premature decedents, several clinical signs were noted, including hypoactivity, unkempt appearance, extremities were pale and/or cool to the touch, colored discharge around the eyes (red, yellow, or clear), and decreased defecation or small feces. These deaths were largely attributable to marked immunosuppression. There was a marked decrease in hematopoietic cells (sternum and the femur), and moderate to marked mucosal atrophy of the gastrointestinal tract that was characterized by a loss of glandular (crypt) epithelium. Consistent with this finding, there was an absence of germinal centers within the lymphoid follicles of the gut-associated lymphoid tissue (GALT) and marked to severe atrophy of the thymus.

In survivors, repeated teriflunomide administration resulted in hemolytic anemia and immunosuppression. At the HD (both sexes), there were several hematological findings, including decreased mean RBC count, Hgb, MCH, and MCHC, as well as increased absolute reticulocyte counts consistent with hemolytic anemia. It is noted, though, that this anemia was regenerative as evidenced by the increase in the reticulocyte count. Several findings could be attributed to the immunosuppressive effects of teriflunomide, such as reduced globulin and total protein observed at MD1, MD2, and HD; these clinical chemistry changes returned to baseline following a 28-day recovery period. Likewise, there was a significant reduction in absolute and relative thymic weight at the MD1, MD2, and HD that correlated with lymphoid atrophy upon microscopic examination. Additional microscopic findings related to

immunosuppression included: an absence of germinal centers and lymphoid atrophy of the GALT in the ileum, absence of germinal centers, lymphoid atrophy in the mesenteric lymph node, and lymphoid atrophy in the spleen; these findings occurred in both sexes at MD1, MD2, and HD. Overall, the hemolytic anemia and immunosuppressive findings were recoverable following a 28-day drug-free period. The NOAEL identified in this study was 0.3 mg/kg/day. On a mg/m^2 basis, the hemolytic anemia and immunosuppressive effects were observed at doses equivalent to 6.3-times and 2.1-times the MRHD of 14 mg/day. At the NOAEL, the dose equivalent is reduced to 0.21-times the MRHD on a mg/m^2 basis. Based upon teriflunomide exposure, the AUC_{0-24} at the NOAEL for the rat is 26.1 μg.h/mL (male) and 29.4 μg.h/mL (female), which is 0.024-times and 0.027-times, respectively, at the exposure expected at the MRHD.

In the chronic dog study, animals were dosed daily with teriflunomide (0, 0.2, 0.8, 2/4 mg/kg/day) by oral gavage. The initial HD group was 2 mg/kg/day; however, due to the lack of notable toxicity, the dose was increased to 4 mg/kg/day on study Day 192. There was one unscheduled death of a HDF. Prior to death, several clinical signs were notable, including lethargy, increased body temperature, inappetance, ears were cool to the touch, red mucoid feces, and red material on the cage floor. There was a significant body weight loss of 1.9 kg over the 5-week period prior to death. The bone marrow smear had normal erythroid and myeloid precursors; however, the hematology at necropsy showed evidence of regenerative hemolytic anemia. There was significant reduction in RBC (33-39%), Hct, Hgb, platelets, lymphocytes, and marked loss of monocytes (-75%); there was a significant increase in MCV and absolute reticulocytes. A blood test was negative for an infectious agent.

In survivors at the HD (both sexes), there was evidence of regenerative anemia present; the hematology showed reductions in mean RBC, Hgb, Hct and increases in absolute reticulocytes and methemoglobin. In the femur, there was hypocellularity of the bone marrow (2M and 3F; as well as 1 MDF). Upon microscopic examination, there was evidence of degeneration and fibrosis in the pancreas at the HD (4/4, both sexes) and at the MD (1M and 1F). At the HD, this finding in the pancreas was associated with a steady decline in trypsin-immunoreactivity; however, amylase and lipase were unaffected. The significance of this result is unclear. In this study, there was an increase in liver/gallbladder weight in both sexes at the MD and HD; however, there were no microscopic correlates observed. The NOAEL identified in this study was 0.2 mg/kg/day. On a mg/m^2 basis, the hemolytic anemia and the pancreatic fibrosis were observed at doses equivalent to 9.3-times and 1.8-times the MRHD of 14 mg/day. At the NOAEL, the dose equivalent is reduced to 0.46-times the MRHD on a mg/m^2 basis. Based upon teriflunomide exposure, the AUC_{0-24} at the NOAEL for the dog is 26.6 μg.h/mL (male) and 20.2 μg.h/mL (female), which is 0.025-times and 0.019-times, respectively, at the exposure expected at the MRHD.

In the drug label for leflunomide (ARAVA), there is a black box warning for the potential of leflunomide to produce liver toxicity in humans. In addition, the clinical team noted the presence of putative liver toxicities in a few cases in the clinical trials for teriflunomide (the active metabolite of leflunomide). Based on this clinical concern and

the lack of liver-induced toxicities observed in the chronic rat and dog studies, a 1-month repeat intravenous dose toxicity study in rat was conducted to assess teriflunomide-related liver toxicity. In this study, teriflunomide at doses of 0, 3.2, 8, and 20 mg/kg/day was adminstered intravenously; profound toxicity particularly to the liver and hematopoietic system was identified, and considered in many cases to be the cause of premature death. The deaths were related to microscopic findings in the liver that consisted of parenchymal necrosis that were described as having "no reaction" or "poor reaction" and there was a "loss of recognizable lobular configuration". In the periphery of these necrotic foci were variable amounts of rod shaped bacterium (*Clostiridium pilliforme*) that stained poorly with either H&E or PAS alone but well with the combination of these stains. In addition to the liver, the presence of these bacteria in other tissues was noted, in particular, the myocardium of the heart was also found to contain focal lesions. In individual animals, the focal lesions in the heart did not correlate in every case in which the liver was affected. In addition to these findings, notable toxicity to the hematopoietic system was also identified in this study. In particular, bone marrow suppression (moderate to marked reduction or absence of hematopoiesis in the femur and/or sternum) occurred. This was in conjunction with a spectrum of findings present in the spleen, lymph node, and thymus. In several of these animals, bone marrow suppression was further supported by data in 2/3 HDF survivors in which there were decreases in Hgb, Hct, and Rbc count. Based on the severity of findings and presence of liver toxicity at the lowest dose tested in this study, a NOAEL was not established; however, it is notable that many of the deaths in this study were attributed to Tyzzer disease by the study pathologist.

Tyzzer disease is the result of an intracellular infection of rod-shaped bacilli, *Clostiridium pilliforme*, that often is the result of immunosuppression and colonization in rodents (mice, rats, gerbils, hamsters) and rabbits. This disease, though severe in these animals has not, to date, been reported in humans, or transferred to humans from these species. Thus, the lack of a NOAEL to the Tyzzer disease is not of toxicological concern to humans. In contrast, the development of immunosuppression to the point of reduced antibody responses to novel T-dependent antigens is a concern to humans.

Teriflunomide was evaluated in both rats and rabbits for potential reproductive and developmental toxicity. This assessment included studies to evaluate fertility and embryonic development (rats), developmental toxicity (rats and rabbits), and pre-postnatal development (rats). Oral administration of teriflunomide (0, 1, 3, and 10 mg/kg/day) to male rats prior to and throughout mating resulted in a reduction in the mean absolute number of sperm in the cauda epididymis and this reduction was still present when normalized to epididymis weight. One HDM was euthanized prematurely due to marked anemia. The NOAEL identified in this study was 10 mg/kg/day. On a mg/m^2 basis, the hemolytic anemia and the reduction in sperm in the cauda epididymis were observed at doses equivalent to 6.9 times the MRHD of 14 mg/day. At the NOAEL, the dose equivalent is reduced to 2.1 times the MRHD on a mg/m^2 basis. Oral administration of teriflunomide (0, 0.84, 2.6, and 8.6 mg/kg/day) to female rats prior to and throughout mating and up to Day 6 of gestation resulted in several fertility parameters that were affected at the MD and HD, including increased total post-implantation loss, increased early resorptions, and decreased mean number of live

fetuses. In all dose groups, malformations were observed; malformations included short tail, anus atresia, microglossia, cleft palate, absent eye bulge, and micrognathia. In this study, no NOAEL was established.

Teriflunomide was administered once daily at oral doses of 0, 1, 3, and 10 mg/kg/day to mated female rats during Gestation Days 6-17. In this study, maternal and developmental effects of teriflunomide occurred at the MD and HD. At both the MD and HD, there were decreased mean numbers of live fetuses, increased post-implantation loss (via increased early resorptions), and intrauterine deaths (MD 1/20; HD 13/19). At the MD and HD, there were reduced fetal body weights, crown/rump lengths, and placental weights. Multiple external, skeletal, and visceral malformations were identified, including retarded fetus, eye malformation—anopthalmia and aplastic lentis, skull—retarded and reduced orbit, and brain—hydrocephalus. In addition, there were numerous minor defects, variations, and retardations observed in the MD and HD groups. The NOAEL identified in this study was 1 mg/kg/day. On a mg/m^2 basis, the embryolethality and malformations were observed at doses equivalent to 2.1 times the MRHD of 14 mg/day. At the NOAEL, the dose equivalent is reduced to 0.7 times the MRHD on a mg/m^2 basis. Based upon teriflunomide exposure, the plasma $AUC_{0\text{-}24}$ at the NOAEL for the pregnant rat is 110 μg.h/mL (female), which is 0.10-times the exposure expected at the MRHD. It is noteworthy that the reduced number of live fetuses at the HD makes it difficult to evaluate the scope of the malformations.

This concern was addressed, as the sponsor conducted a separate study to assess teriflunomide (10 mg/kg/day) treatment at different developmental stages. In this study, the scope and breadth of the numerous malformations was evident, as was the marked degree of embryolethality. Teriflunomide was administration at different developmental intervals, such as, GD 6-8, GD 9-11, GD 12-14, GD 15-17, and GD 6-17. Most notable in this study, teriflunomide administration during the GD 9-11 interval resulted in complete intrauterine death in all the dams. In contrast, this was not observed with dosing during the GD 6-17 interval; thus, the difference in lethality is unclear. However, this study clearly demonstrated that teriflunomide at a dose that is approximately 6.9 times that of the MRHD (on a mg/m^2 basis) results in extensive embryolethality and teratogenicity. The scope of the malformations includes—external (absent eye bulge, domed cranium, gastroschisis, aglossia, and micrognathia), visceral (anopthalmia, micropthalmia, herniated diaphragm, and severe dilation of the lateral ventricle), and skeletal (thoracic arches—absent, misshapen, and fused; thoracic vertebrae—absent or fused; ribs—fused; sternebrae—BFID or fused; skull—misshapen; mandible—fused; and exocciptal and 1st cervical vertebra—fused) malformations. Importantly, teriflunomide treatment during development results in multiple malformations in the same fetus and in multiple litters. In this study, it is clear that no matter which interval of development teriflunomide is administered, it has significant effects on the developing fetus. At the NOAEL based upon exposure, there is no safety margin at the MRHD.

Teriflunomide was administered once daily at oral doses of 0, 1, 3.5, and 12 mg/kg/day to mated female rabbits during Gestation Days 6-18. In this study, maternal and developmental effects of teriflunomide occurred at the MD and HD. Mean body weight

of HD does was reduced and this correlated with reduced food consumption. At the HD, there was increased total postimplantation loss and there was a decreased number of live fetuses in this group. At the HD, there were a reduced number of live fetuses (-26%), shorter crown/rump length (-7.5%), and reduced live fetal weights in males (-15.3%) and in females (-22%). At the MD, there was a reduction in mean placental weight (-7.3%). Teriflunomide administration resulted in malformations in both the MD and HD groups and included skull abnormalities, sternebrae anomalies, and stunted fetus. The NOAEL identified in this study was 1 mg/kg/day. On a mg/m^2 basis, the embryolethality and malformations were observed at doses equivalent to 4.9 times the MRHD of 14 mg/day. At the NOAEL, the dose equivalent is reduced to 1.4 times the MRHD on a mg/m^2 basis. Based upon teriflunomide exposure, the plasma AUC_{0-24} at the NOAEL for the pregnant rabbit is 59.8 μg.h/mL, which is 0.06-times the exposure expected at the MRHD.

In a pre- and postnatal development study conducted in rats, teriflunomide was administered at oral doses of 0, 0.05, 0.10, and 0.30 mg/kg/day. At the HD, adverse clinical signs were observed in the F1 pups, including: malrotated paws, and impaired coat growth (up to PND23), and a loss of papillary reflex in 3F from 2 separate litters (of these 1 litter had corneal opacity). At the HD, the number of litters in which the mean pre-weaning body weight that was <50 g was notably greater than any other dose group. At the HD, 6/24 litters were affected, whereas the litter incidence in the C was 0/22, LD 1/22 and MD 0/22. No teriflunomide-related effects were observed in a battery of behavioral and developmental assessments in the F1 pups. The NOAEL identified in this study was 0.1 mg/kg/day due to the presence of malformations at the HD. On a mg/m^2 basis, malformations were observed at doses equivalent to 0.21 times the MRHD of 14 mg/day. At the NOAEL, the dose equivalent is reduced to 0.07 times the MRHD on a mg/m^2 basis.

The carcinogenic potential of teriflunomide was evaluated in two rodent bioassays—mouse and rat; each study was considered adequate and valid. In the two-year mouse carcinogenicity study (CAR0092), CD-1/Crl mice were administered teriflunomide at doses of 0, 1, 4, and 12 mg/kg/day by oral gavage for 95 weeks in HDM and 104 weeks for the other groups. Although dosing was stopped at Week 95 in HDM, the group was euthanized at Week 104; this early termination of dosing did not compromise the outcome of this study. In both sexes, at the HD, there was a significant decrease in survival, and the main causes of death were attributed to ulcer/inflammation of the skin or gastrointestinal tract. No drug-related increases of any tumor type were found in this study. On a mg/m^2 basis, teriflunomide was tested at doses that were approximately 0.3, 1.4, and 4.2 times the MRHD of 14 mg/day. Based upon plasma AUC_{0-24}, teriflunomide was tested at exposures approximately 0.2 , 0.9, and 3.4 times the exposure expected at the MRHD of 14 mg/day.

In the two-year rat carcinogenicity study (CAR0093), CD(SD)/Crl rats were administered teriflunomide at doses of 0, 0.5, 1, and 4 mg/kg/day by oral gavage for approximately 104 weeks. In this study, there was a decreased survival of MD and HD males; HDM were euthanized at approximately Week 92 of dosing, and all surviving males in all dose

groups were euthanized at approximately Week 97. There was no effect of teriflunomide on survival in females. In HDF, mean body weight was decreased by more than 10%. Neither the early termination of the males nor the decreased mean body weight in HDF compromised the outcome of this study. No drug-related increases of any tumor type were found in this study. On a mg/m^2 basis, teriflunomide was tested at doses that were approximately 0.3, 0.7, and 1.4 times the MRHD of 14 mg/day. Based upon plasma AUC_{0-24}, teriflunomide was tested at exposures approximately 0.06 times, 0.14 times, and 0.3 times the exposure expected at the MRHD of 14 mg/day.

For the overall conclusions and recommendations, please see the Executive Summary.

Toxicity	Species	NOAEL (mg/kg) M/F	Safety Margin Based on AUC*
Chronic (6 mos) Death, Bone marrow suppression, Decreased body weight, immunosuppression—absent germinal centers	Rat	0.3 mg/kg/day	M: 0.024 F: 0.027
Chronic (1 year) Unscheduled Death—Bone marrow toxicity—hypocellularity [reduced Hgb, RBC, Hct; increased methemoglobin], pancreatic degeneration/fibrosis—decreased trypsin-immunoreactivity,	Dog	0.2 mg/kg/day	M: 0.024 F: 0.019
Carcinogenicity	Mouse	12 mg/kg/day	M: 3.4 F: 2.9
	Rat	4 mg/kg/day	M: 0.25 F: 0.31
Reproductive Toxicity Fertility Male: reduced mean absolute number of sperm in cauda epididymis Female: Total post implantation loss; increased early resorptions, decreased mean number of live fetuses; external malformations—teratogen	Rat	M: 3 mg/kg F: <0.84 mg/kg	M: 2.1** F: <0.6**
EFD Embryolethality—increased complete intrauterine loss; external, visceral, and skeletal malformations both species	Rat	1 mg/kg/day	F: 0.1
	Rabbit	1 mg/kg/day	F:
PPND Reduced survival of F1 pups	Rat	0.1 mg/kg/day	M/F: 0.07**

*$AUC_{0\text{-}24ss}$ in human: 1070 µg.hr/mL at 14 mg/day.
**Based on body surface area at MRHD = 8.63 mg/m^2.

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/s/

RICHARD A HOUGHTLING
07/13/2012

LOIS M FREED
07/13/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 20-2992 **Applicant: Sanofi Aventis US LLC** **Stamp Date: 12 August 2011**

Drug Name: Teriflunomide **NDA Type: Regular**

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X	Human dose multiples are not provided in Sections 8.1, 13.1 Impairment of fertility.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Impurities (b) (4) were evaluated; the data are available for review.
11	Has the applicant addressed any abuse potential issues in the submission?	X		
12	If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

At this time, there are no identified nonclinical review issues.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

RICHARD A HOUGHTLING
09/27/2011

LOIS M FREED
09/28/2011