

Summary for the mouse carcinogenicity data:

The male mice showed high incidences of malignant lymphoma that was statistically significant in the trend test. The maximum incidences of lymphoma in the historical control was 12%. The incidences in the carcinogenicity experiment showed almost 17%. There was an increase in the leydig cell adenoma in the testes.

Female mice showed high incidences of adenoma and carcinoma of the lung. The combined incidences were 15/70 or 21%. The historical control for bronchoalveolar adenocarcinoma was 15.9%. The sponsor stated that there was no statistical significance for the finding when a trend test was applied. The statistical reviewer needs to analyze these data after appropriate adjustment for mortality. The malignant lymphoma in the female mice may not be of significance due to a high incidences in the control.

Out of non neoplastic changes in the male mice, hypospermatogenesis was observed in 6% of mice at 15 mg/kg and the control group showed 1%. Other observed changes were related to the immunosuppression. Male mice at 15 mg/kg dose showed a high incidence of necrosis in the liver. Female mice showed an increase in the fibrosis in the uterus. The animals also showed skin toxicity, i.e dermatitis and ulcer of the skin. There was a tendency for developing alopecia in female mice. The control animals showed lenticular opacity. The treated animals at the high dose also showed higher incidences of opacity in the lens in eyes. It could be an age-related change or drug-related change. However, comparing the chronic toxicity data in rats, it appears that the change in the lens could be treatment-related.

The exposure data suggest that A 771726, the active metabolite was accumulated in the serum. The data at 5 and 15 mg/kg doses suggest that an increase in the dose over 15 mg/kg would have not increased the levels of A 771726 further. These data also support the dose selection for the carcinogenicity study.

It can be concluded from the carcinogenicity data that oral treatment of male and female mice up to 15 mg/kg dose reached a maximum tolerated dose for the carcinogenicity bioassay based on the increased mortality in male mice. The male mice showed higher immunosuppression than the female mice. An increased incidences of dermatitis and ulcer of the skin, hypospermatogenesis and fibrosis in the cervical uterus were observed as the long term toxicity to Leflunomide.

Male mice showed malignant lymphoma and Leydig cell adenoma in the testes. Female mice showed an increase incidence of adenoma and carcinoma in the lung. However, statistical significance of the findings needs to be confirmed. The sponsor stated that historical control data for the specific strain of mice for malignant lymphoma in male and lung carcinoma for female are not available.

- Malignant lymphoma in male mice
- Bronchoalveolar adenocarcinoma in lungs in the female mice
- Necrosis of the liver in female mice
- Hypospermatogenesis
- Fibrosis of the cervical uterus
- Alopecia in female mice
- Increased dermatitis and ulcer of the skin
- Increased incidences of lenticular opacity in male and female mice

Toxicokinetics and ADME:

TFMA levels in mice following three months of oral treatment with TFMA:

Page 5-18306, vol 56:

NMRI mice were treated with TFMA by oral gavage at 10, 32 and 100 mg/kg doses. TFMA levels in the serum were determined at the end of 1 and 3 months

Maximum absorption time (T_{max}) was about 1-2 hours at all doses tested. At the end of 28 doses, C_{max} in males were 137, 433 and 5513 ng/ml at 10, 32 and 100 mg/kg doses, respectively. The corresponding C_{max} was 454, 1108, and 3742 ng/ml at 10, 32 and 100 mg/kg doses, respectively. The data suggest that there was an accumulation of the drug at 100 mg/kg dose in male mice. The levels were dose proportionate in female mice.

The concentration data at 2 hour post dose after 28 doses in male mice were 137, 396 and 1960 ng/ml at 10, 32 and 100 mg/kg. The C_{2hr} at the end of 92 dose in male mice were 168, 908 and 2592 ng/ml. The concentration at 2 hour post dose in female mice after 28 doses was 227, 860, 1842 ng/ml. At the end of 92 doses, the C_{2hr} in female mice were 502, 983, and 2750 ng/ml.

The AUC at the end of 28 doses were 1957, 6086, 29430 ng.h/ml in male mice at 10, 32, 100 mg/kg. The AUC in female mice were 3211, 12382 and 26153 at 10, 32 and 100 mg/kg doses, respectively. The data at the end of 92 doses are not given.

It is concluded that female mice had a greater exposure than male mice. The exposure in male

mice at 100 mg/kg dose was higher than that expected.

Toxicokinetics of A771726 levels in serum following 1 month iv doses in rats:

Page 5-18365, vol 56:

The serum levels of A771726 were detected at 24 hours (trough) at the end of 29 days of the treatment. The study was conducted for determining absolute bioavailability of the metabolite in rats. The concentrations are shown in the following table.

Dose, mg/kg	Number of animals	C _{mean} (μg/ml)	Conc/Dose
3.2	M, 10	2.18	0.68
	F, 14	1.31	0.40
8.0	M, 6	1.18	0.148
	F, 11	1.29	0.161
20.0	F, 3	0.86	0.043

The data suggest that at higher doses the levels of A771726 were reduced than that compared to the lower doses. It is possible that A 771726 may have an induction effect.

Toxicokinetics of A771726 in Wistar rats

Page 5-18403, vol 56:

A 771726 was administered iv in male and female rats at 0.25 and 1.0 mg/kg doses for one month. The C_{max} (μg/ml), AUC (μg.h/ml) and C_{24 hrs} (μg/ml) after 28 days are shown in the following table.

Dose mg/kg	Sex	C _{max}	C _{max} /Dose	AUC	AUC/Dose	C _{24 hrs}	C ₂₄ /Dose
0.25	M	1.07	4.28	3.45	13.8	0.25	1.00
	F	1.40	5.60	4.06	16.2	0.21	0.84
1.0	M	5.97	5.97	16.5	16.5	0.75	0.75
	F	6.76	6.76	17.1	17.1	1.16	1.16

The data suggest that the increase in the exposure was dose proportionate. Female rats showed higher exposure and higher trough levels after one month of iv dosing. However, statistical significance of the difference is not known.

Toxicokinetics of A 771726 for one month study in dogs:

Page 5-18590, vol 56:

One month iv toxicity was conducted at 0.8, 2.5 and 8 mg/kg doses in beagle dogs. Blood samples were collected at 1, 2, 3, 5, 7 and 22 hours after the 1st and 28th doses. Serum levels of A 771726 were determined at 0.1-0.2 $\mu\text{g}/\text{ml}$ limits of detection. AUC ($\mu\text{g}\cdot\text{h}/\text{ml}$) normalized to dose after first and 28 doses are shown in the following table.

Dose (mg/kg)	1 Dose, M	1 Dose, F	28 Dose, M	28 Dose, F
0.8	26.0	25.1	107	132
2.5	41.9	47.6	169	223
8.0	75	83	164	154

The data suggest that AUC was dose proportionate at 0.8 and 2.5 mg/kg as it reached the steady state. However, female dogs showed a higher exposure than the male dogs.

Toxicokinetics of TFMA following oral doses of Leflunomide during organogenicity in rabbits:

Page 5-18668, vol 57:

Pregnant rabbits were dosed at 1 and 10 mg/kg/oral doses of Leflunomide from gestation day 6 to day 16. Plasma levels of TFMA were determined on days 6 and 16. The study was conducted in Himalayan Rabbits. The limit of detection was 2 ng/ml. There were no detectable levels of TFMA at 1 mg/kg dose. The data at 10 mg/kg are shown below.

Dose mg/kg	C _{max} ng/ml, Day 6	T _{max} hr, Day 6	AUC ng·h/ml, Day 6	C _{max} , Day 16	T _{max} , Day 16	AUC, Day 16
10	15.2	2	262	64.4	1	1135

Above data suggest that TFMA was accumulated on the repeated oral dosing in rabbits.

Toxicokinetics of A 771726 following oral dosing in pregnant rabbits during organogenicity:

Page 5-18693, vol 57:—

Leflunomide was administered to Himalayan Rabbits at 1 and 10 mg/kg doses per oral during days 6 to 16 of pregnancy. Plasma levels of A 771726 were determined on days 6 and 16 at 0.1 $\mu\text{g/ml}$ limits of detection. The C_{max} ($\mu\text{g/ml}$), T_{max} (hr) and AUC ($\mu\text{g.h/ml}$) are shown in the following table.

Dose mg/kg	C_{max} , Day 6	T_{max} , Day 6	AUC Day 6	C_{max} Day 16	T_{max} Day 16	AUC Day 16
1	4.10	1.0	52.5	4.25	5.0	32.7
10	61.6	2.0	1052	101	1.0	1717
Dose Normalized	6.10		105	10.1		172

The data clearly suggest that at 10 mg/kg dose of Leflunomide, there was an increase in the accumulation of A 771726 in the plasma of the pregnant rabbits that could have contributed to the toxicity in the fetuses.

The audited report has been resubmitted in page 5-18719 vol 57.

Disposition of ^{14}C -Leflunomide in mice at 3 mg/kg oral or iv dose:

Page 5-18958, vol 58:

The radio tracer Leflunomide was given in male Swiss Webster mice. Blood samples were pooled for plasma level determinations. Plasma, urine and whole blood samples were assayed for total radioactivity.

Leflunomide was stable in mouse plasma at 0C. However at 37C, it was completely metabolized within 10 hours. $T_{1/2}$, T_{max} , C_{max} and AUC_{0-∞} for A 771726 were 10.6 hr, 0.5 hr, 13.5 $\mu\text{g eq/ml}$ 250 $\mu\text{geq.h/ml}$, respectively after the oral dose. No parent compound was detected in the plasma.

The $T_{1/2}$, T_{max} , C_{max} and AUC_{0-∞} after the 3 mg/kg iv dose were 11.1 hr, 2 hr, 14.9 $\mu\text{g eq/ml}$, and 262 $\mu\text{g eq.h/ml}$, respectively. The recovery of radioactivity was 30% of dose in the urine and 72%

of the dose in the feces after oral dosing. The excretion of radioactivity following iv dose was 24% in the urine and 77% in the feces. Most of the radioactivity was excreted within 24 hours and almost complete excretion was noted within 168 hours.

Data suggest that most of the radioactivity was excreted in the feces in mice irrespective of the route of dosing.

Dose proportionality of Leflunomide in male rats:

Page 5-18983, vol 58:

Male S-D rats were given single oral doses of Leflunomide at 5, 10 and 20 mg/kg. Plasma levels of A771726 were determined. The data are shown in the following table.

Parameter	5 mg/kg	10 mg/kg	20 mg/kg
C_{max} $\mu\text{g/ml}$	21.2	31.7	77.9
C_{max}/Dose	4.24	3.17	3.90
T_{max} (hr)	0.75	3.0	4.0
$T_{1/2}$ (hr)	3.38	3.58	3.48
AUC _{0-24 hrs} $\mu\text{g}\cdot\text{hr/ml}$	165	331	1098
AUC/dose	33	33.1	54.9

The data suggest that up to 10 mg/kg dose, the kinetics were linear after a single oral dose in male rats. Above the 10 mg/kg dose, the kinetics were not linear.

Effect of food on oral kinetics in rats:

Page 5-19008, vol 58:

Following a single oral dose at 10 mg/kg in male SD rats, kinetics of A 771726 were determined in the fasted and fed states. Following table suggest the differences between fasted and fed state.

Parameters	Fasted	Fed
C_{max} $\mu\text{g/ml}$	34.5	25
T_{max} (hr)	3.0	4.0

$T_{1/2}$ (hr)	3.34	5.49
AUC _{0-∞} $\mu\text{g}\cdot\text{h}/\text{ml}$	376	339
% relative Bioavailability	111	100

Above data suggest that bioavailability of the drug was better in the fasted state and food decreased the bioavailability by about 11%.

Bioavailability of A 771726 in rats:

Page 5-19029 vol 58:

Leflunomide was given orally and iv in male SD rats at 1 mg/kg. A771726 was also given by iv route at 3 mg/ml concentration. The dose has not been indicated. Animals were fasted overnight and 4 hours post dose. Data from a table suggest that the dose level achieved was about 3.8 mg/kg. The bioavailability of Leflunomide measured by A 771726 was 86% in rats when compared with the iv dose of A 771726. However, iv dose of Leflunomide showed a lower bioavailability when compared to iv dose of A 771726. The data suggest that first pass metabolism of Leflunomide generates A771726, the active metabolite.

For dogs, absolute bioavailability of Leflunomide assayed as A 771726 was about 76% (see page 5-19077, vol 58). The dose of Leflunomide was 3 mg/kg. A similar study was conducted at 5 mg/kg oral or iv doses of Leflunomide and 5 mg/kg dose of A 771726 in male beagle dogs. Summary of the data for plasma A 771726 kinetics are shown in the following table.

Parameter	5 mg/kg/oral Leflun.	5 mg/kg iv Leflun.	5 mg/kg iv A 771726
C _{max} $\mu\text{g}/\text{ml}$	26.22	21.5	61.8
T _{max} hr	3.0	4	0.02
$T_{1/2}$ hr	23.5	15.9	17.16
AUC _{0-∞} ($\mu\text{g}\cdot\text{h}/\text{ml}$)	702	542	883
V _d (ml)	3538	2452	2607
Cl _r (ml/min)	105	107	108
%F	80	61	

Data suggest that first pass metabolism of the drug increases the biological half-life after oral dosing and the bioavailability of A 771726 was more after the oral dose of Leflunomide.

drug substance showed a higher bioavailability.

Plasma and RBC levels of A 771726 and TFMA in rhesus monkey after oral doses of Leflunomide:

Page 5-19176, vol 58:

Three female monkeys were given oral dose of Leflunomide at 6 mg/kg. Levels of A 771726 in the plasma and erythrocytes were measured. Data showed that the plasma half life of A 771726 is about 18 hours in monkeys and the AUC ∞ was 1391 $\mu\text{g}\cdot\text{h}/\text{ml}$. For erythrocytes, the half life and AUC ∞ of A 771726 were 17.9 hr and 358 $\mu\text{g}\cdot\text{hr}/\text{ml}$, respectively. The data suggest that about 25% of the drug is partitioned in the erythrocytes.

Metabolism of Leflunomide in rats, dogs and mice:

Page 5-19052, vol 58:

^{14}C -labeled Leflunomide was given to male CD mice, SD rats and a beagle dog orally as suspensions as a single oral dose. Urine samples were collected up to 48 hours. Excretion of radioactivity was 33.7, 10.1 and 15% for rats, mice and dog, respectively. Hydroxy isomers of Leflunomide or hydroxy A771726 were detected in mice and rats. Dog urine did not show these metabolites possibly due to lack of a treatment of the urine with hydrolyzing enzymes.

A 14-day oral toxicity in rats at 2.5 and 10 mg/kg/day:

Page 5-19184 vol 58:

Male SD rats were given oral doses of Leflunomide as suspensions at 2, 5 and 10 mg/kg per day for 14 days. Blood samples were collected for determining plasma A 771726 levels. The method does not indicate when the samples were collected. However, Appendix II suggest that the samples were collected at 0, 1, 5, 10, 15, 30 45 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 hours following 14 days of the treatment. Data are shown in the following table.

Parameter	2 mg/kg	5 mg/kg	10 mg/kg
Cmax ($\mu\text{g}/\text{ml}$)	5.34	7.33	14.6
Normalized Cmax	5.34	2.93	2.92
Tmax (hr)	1.0	1.0	2.0
T $\frac{1}{2}$ (hr)	4.09	2.78	2.82

AUC 0-24hr ($\mu\text{g}\cdot\text{h}/\text{ml}$)	50.5	86.1	101.8
AUC normalized	50.5	34.4	20.4
AUC 0- ∞ ($\mu\text{g}\cdot\text{h}/\text{ml}$)	46.3	75.5	111.6
AUC normalized, 0- ∞	46.3	30.2	22.3

The data suggest that the exposure was not dose proportionate.

Toxicokinetics of A 771726 in rats and dogs in plasma were determined at the end of six months of dosing in rats and dogs (page 5-19213, vol 58). Some of the data were discussed in review pages 10-13 dated March 16, 1993. Only additional information will be added here.

The AUC of A 771726 in male rats at 0.5, 1.0, 2.0 and 4.0 mg/kg doses were 9.79, 18.05, 28.69 and 47.86 $\mu\text{g}\cdot\text{h}/\text{ml}$, respectively. AUC for female rats were 8.53, 19.98, 33.90 and 51.14 $\mu\text{g}\cdot\text{h}/\text{ml}$ at 0.5, 1.0, 2.0 and 4.0, respectively. The data suggest that the exposure at the end of six month was dose proportionate except that the exposure was reduced slightly at 4 mg/kg dose.

The AUCs of A 771726 in dogs at the end of six months at 0.8, 2.5 and 8.0 mg/kg doses were 62.03, 293.8 and 561.9 $\mu\text{g}\cdot\text{h}/\text{ml}$. The data showed that the exposure was not dose proportionate.

Protein binding in monkey plasma:

Page 5-19233 vol 59:

At the concentration range of 0.5 to 100 $\mu\text{g}/\text{ml}$ binding of ^{14}C A771726 to the plasma protein was 98.89 to 99.36%. Human plasma protein binding was 99.38% and that for the rat and dog was 96.01 and 97.24%, respectively. These data suggest that active metabolite of Leflunomide has high plasma protein binding. Therefore, it may show drug interactions with concomitant use of NSAIDs and methotrexate.

Absorption, metabolism and excretion of ^{14}C -Leflunomide in mice at 16 mg/kg:

Page 5-19244 vol 59:

Following a single oral dose in male and female CD mice, urine and feces were collected for radiochemical analysis. 45-70% of the radioactivity was excreted within 48 hours in the feces. About 16-26% of the radioactivity was excreted in the urine. There was no unchanged drug excreted from the system. The metabolic pattern was similar to rat, dog and human. TFMA oxanilic acid and a glucuronide conjugated metabolite were the major metabolites identified by TLC. Excretion of radioactivity in female mice was higher.

Metabolism of Leflunomide in rats and dogs:

Page 5-19267 vol 59:

S-D rats from Charles River (gender not specified) and male beagle dogs were used in the study. ^{14}C -Leflunomide was orally given at 16 mg/kg as a solution. ^{14}C TFMA and acetyl TFMA were given orally, however, the dose is not mentioned in the protocol. *In vitro* incubations of Leflunomide were also carried out in the presence of intestinal micro flora of rat and dog feces for 16 hrs at 37C. Metabolites from urine and feces were characterized.

Dogs:

An oral dose of 16 mg/kg of Leflunomide showed 63-66% of the radioactivity in the urine and 15-26% of radioactivity in the feces in male and female dogs. These percentages are expressed as the % of total recovery of radioactivity during 7 days. The iv dose showed 24-33% of total recovery in the urine and 57% of the radioactivity in the feces. It appears from the data that there may be a route dependent disposition of Leflunomide so that after metabolic changes in the liver from the oral dose, most of the metabolites excreted as the polar metabolites in the urine.

^{14}C TFMA at 5 mg/kg oral dose showed 75% of the dose excreted in the urine and 2.96 % of the dose was excreted in the feces. Most of the urinary radioactivity was due to TFMA. The other metabolite was 2-amino-5-trifluoromethyl phenol (hydroxylated metabolite). The data suggest that TFMA oxalate was not excreted in the dog urine as a metabolite of TFMA.

The excretion of radioactivity from ^{14}C carbonyl labeled Leflunomide was 44% of the dose in the urine and 29% of the dose in the feces after 168 hours. The excretion of radioactivity from ^{14}C benzene ring labeled Leflunomide accounted for 64% of the total radioactivity in the urine and 22% in the feces.

Incubation of 267 $\mu\text{g/ml}$ of A771726 with the dog micro flora showed a level of TFMA of 14.8 ng/ml within 24 hours. A similar experiment in the presence of 267 $\mu\text{g/ml}$ concentration of A771726 in the presence of gut micro flora from rats showed 3.3 ng/ml of TFMA at the end of 16 hours of the incubation. Therefore, there was a difference between rats and dogs with respect to TFMA generation. The generation of TFMA *in vitro* in the presence of micro flora was greater in dogs than that of rats.

The kinetics measured by the total radioactivity in plasma, blood and RBC in dogs after a single dose of 16 mg/kg/oral of Leflunomide are shown in the following table. The data show the differences in the position of the label at carbonyl vrs benzene ring of Leflunomide.

Parameters	Plasma	Plasma	Blood	Blood	RBC	RBC
	¹⁴ C-ring	¹⁴ C- carb.	¹⁴ C-ring	¹⁴ C-carb	¹⁴ C-ring	¹⁴ C-carb
C _{max} (μgeq/ml)	46.1	82.5	31.0	53.3	9.5	20.6
T _{max} (hr)	12.0	6.0	12.0	8.0	12.0	8.0
T _{1/2} (hr)	11.8	23.4	23.2	30.7	22.7	43.4
AUC (μgeq/ml.hr)	2012	3499	1409	2579	462.9	1459
C _{max} Carb/Ring	56%		58%		46%	

Most of the radioactivity excreted in the urine and feces had the ring structure. Some of the Leflunomide or A 771726 molecules were hydrolyzed in dogs to form TFMA in the gut. When TFMA was given orally, it was hydroxylated and excreted as a conjugated metabolite rather than TFMA oxalate. The differences in the metabolites in the urine following administration of ¹⁴C-Leflunomide, ¹⁴C-TFMA and ¹⁴-carbonyl Leflunomide suggest that TFMA oxalate was excreted as a metabolite of Leflunomide without formation of TFMA in the dog plasma. When TFMA alone was given, it was metabolized to a hydroxylated product. These data indicate that TFMA is not a precursor for the formation of TFMA oxalate in the dog. Therefore, metabolism of A 771726 to TFMA oxalate took place without the formation of TFMA in the plasma in dogs.

It was concluded that TFMA is metabolized in dogs to form hydroxylated metabolite that is excreted in the urine as a glucuronide conjugated product.

Rats:

Following an oral dose of 16 mg/kg of Leflunomide, the excretion of radioactivity was 65.11% of the dose in urine and 31.48% of the dose in the feces up to 96 hours in male and female rats. There were no gender differences in the excretion pattern. When ¹⁴C labeled TFMA was administered at 16 mg/kg dose, the excretion of radioactivity was 91% of the dose within 48 hours. Data from the ¹⁴C labeled at the ring or carbonyl group were almost similar.

The whole blood to the plasma ratio of radioactivity at 16 mg/kg dose of ¹⁴C -TFMA was 64.90 in rats indicating that large amount of TFMA is taken up in the erythrocyte pool. TFMA levels in the whole blood and plasma following 16 mg/kg dose of Leflunomide was less than 0.0065 μg/ml. The corresponding total radioactivity was 21.47 μg eq/ml in the whole blood. Therefore, very minute amount of TFMA is formed in vivo following Leflunomide dosing. Concentrations of TFMA in the micro flora from the rat gut contents was 3.3 to 4 ng/ml up to 16 hours of incubation when 267 μg/ml concentration of A 771726 was added. Similarly the concentration of TFMA in the rat micro flora at 267 μg/ml concentration of Leflunomide was 1.3 to 5.6 ng/ml

within 16 hours. These studies were conducted in the *in vitro* system. The data suggest that Leflunomide or A 771726 does not hydrolyze under the influence of intestinal flora enzymes *in vitro* to form TFMA as a major metabolite in rats.

These data suggest that the major urinary metabolite in rats following Leflunomide dosing is oxalate of TFMA. It is not formed from the generation of TFMA as the degradation product of leflunomide *in vivo*. However, traces of TFMA were detected in the blood following an oral dose of Leflunomide.

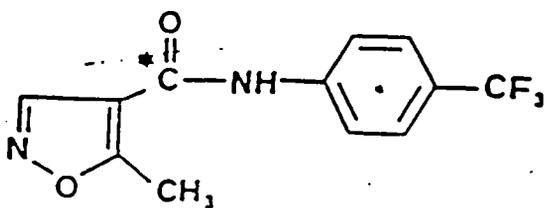
Human:

Maximum Levels of TFMA in the plasma following a 100 mg daily dose of Leflunomide for 14 days in human subjects were less than 10.8 ng/ml. The data suggest that formation of TFMA may not be a major step for the metabolic disposition of Leflunomide or A 771726 in humans.

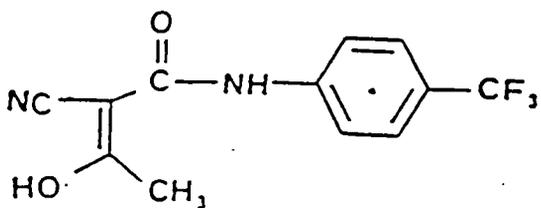
Conclusion of the experiment:

Above data suggest that Leflunomide is excreted as TFMA oxalate in rats and dogs without formation of TFMA as the precursor. However, some amount of TFMA (ng quantity) is formed in dogs, rats and human from Leflunomide. Among these species, dog showed most of the TFMA that have been formed by the hydrolysis at the amide bond as suggested from the experiment with carbonyl and ring labeled Leflunomide. In dogs, TFMA is hydroxylated and excreted as a conjugate. In rats, TFMA is acetylated, oxidized and excreted as TFMA oxalate. The species difference in the metabolism of TFMA is due to inability of dogs to acetylate TFMA. Hydroxylated TFMA may be responsible for Heinz body formation in the RBC in dogs. It should be reiterated that A 771726 is the major active metabolite of Leflunomide formed by the hydrolysis of the isoxazole ring in the liver. Structures of the compounds discussed in the text are shown in the following page.

**APPEARS THIS WAY
ON ORIGINAL**



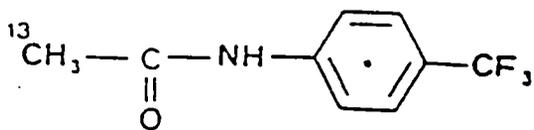
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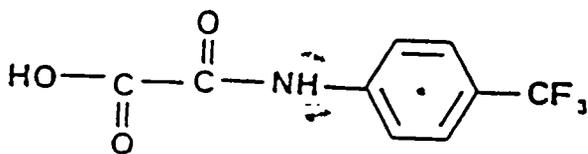
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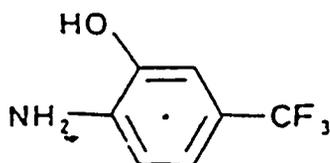
Trifluoromethyl aniline
(TFMA)



N-acetyl-TFMA



TFMA-N-oxalate



2-amino-5-trifluoromethyl-
phenol

Autoradiography and tissue distribution of Leflunomide and A 771726 in rats:

Page 5-19314 vol 59: —

¹⁴C labeled Leflunomide in the aniline ring and ¹⁴C A771726 were used in the study. S-D (male) and male Lister hooded rats were used in the study. ¹⁴C-Leflunomide were conducted in SD rats and hooded rats. for ¹⁴C-A771726 were conducted in SD rats. Tissue distribution of radioactivity following ¹⁴C-Leflunomide was studied in SD rats. Oral doses of Leflunomide were given as suspensions at 16 mg/kg. PEG based i.v. formulation was injected at 3 mg/kg dose. Oral or iv doses of A 771726 were 3 mg/kg.

Serial blood samples were collected for determining the radioactivity in the whole blood and plasma. after iv doses was conducted immediately, 2 and 48 hours post dose. The autoradiography of the SD rats was done at 6, 18 and 48 hours post dose. For the hooded rats, was done at 48 hours. Above studies referred to Leflunomide. Autoradiography after oral doses of A 771726 in SD rats was done at 0.33, 3 and 24 hours after dosing. In the case of iv dosing, was done at 0.5 and 24 hours postdose.

S-D rats were also placed in the metabolism cage after an oral dose of 16 mg/kg. Animals were sacrificed at 6, 18 and 48 hours post dose. Tissue distribution of radioactivity was determined.

Results:

Following an oral dose of 16 mg/kg of Leflunomide in SD rats, the peak radioactivity ($\mu\text{g eq/g}$) in the blood and plasma were 62.55 and 29.05 at 360 and 240 minutes post dose, respectively. Thereafter, the level of radioactivity was reduced.

The radioactivity at 120 min after 3 mg/kg iv dose of Leflunomide, was 7.26 ($\mu\text{g eq/g}$) in the whole blood and 5.75 ($\mu\text{g eq/g}$) in the plasma. Dose normalized maximum radioactivity after the oral dose in the whole blood was greater than the iv dose. The result suggests that elimination of Leflunomide from the body after the iv dose may be faster than its active metabolite A 771726 in the rats. This contributed to the lower level of radioactivity after the iv dose.

Oral dose of A771726 at 3 mg/kg showed the level of radioactivity of 8.36 and 11.78 $\mu\text{g eq/g}$ at 10 min post dose. Differences in the radioactivity in the whole blood and plasma between oral and iv doses were minimal. However, these data do not support the above conclusion that Leflunomide clearance is faster than A 771726.

Maximum radioactivity in the tissue was observed at 6 hour in SD rats compared to 18 and 48 hour dosing. Data are shown in the following table.

Tissues	Radioactivity % of Total dose	Tissue/Plasma
Blood	7.04	0.79
Heart	0.43	0.34
Kidney	1.55	0.68
Liver	7.60	0.80
Skin	12.89	0.29
GI tract	22.01	0.65
Lung	0.57	0.39
Brown fat	0.1	0.68
Marrow	0.02	0.35
Bone	0.02	0.11
Brain	0.09	0.03
Spleen	0.25	0.15
Testis	0.39	0.15
Thymus	0.26	0.18

Several photographs are presented to show distribution of radioactivity.

The data suggest that skin and liver showed higher radioactivity than other organs. Toxicity of Leflunomide in these organs needs to be closely evaluated.

Above experiment was repeated in SD rats at 16 mg/kg oral doses of ¹⁴C-Leflunomide for 7 days (page 5-19350, vol 59). was performed at several time points after the final dose. Radioactivity in the blood, liver and nonextractable radioactivity in the liver were determined.

suggest that elimination of radioactivity was prolonged following repeated oral of doses Leflunomide. About 60-77% of the total radioactivity in the liver was irreversibly bound to the protein. T_{1/2} for elimination of radioactivity in the liver was 75 hours and that from the blood was 170 hours.

Whole body autoradiography in pregnant SD rats:

Page 5-19359, vol: 59.

¹⁴C Leflunomide was administered at 16 mg/kg dose on day 14 and 18 for examining placental transfer of the drug or its metabolites. Food was withheld from the animals. The doses were given as oral suspensions. The animals were sacrificed at day 4, 8, 24 and 48 hours after dosing. Animals were killed by ether anesthesia, frozen sections were prepared.

Within four hours after dosing, the radioactivity was distributed mainly in the liver, kidney, blood, marrow, GI tract, amniotic fluid, skin and fat both on days 14 and 18. Radioactivity was also seen in the placenta, adrenal and lung. The distribution of radioactivity in the brain was less than peripheral organs. This signified that the drug has major effect in the peripheral organs than CNS although hemorrhage was observed in the long term safety studies in rats.

The major differences in the distribution of the radioactivity between day 14 and 18 studies were related to fetuses. The radioactivity was observed in the _____ in the fetal tissues on gestation day 18 that continued up to 24 hours after dosing. Fetal distribution of the radioactivity on the gestation day 14 was minimal.

The data suggest that Leflunomide or its metabolite is distributed in the fetuses through the placental barrier in pregnant rats mostly in the post organogenicity stage of development.

Whole body _____ in pregnant rabbits:

Page 5-19389, vol 59:

The whole body _____ was also examined in pregnant NZ rabbits after dosing animals on gestation days 10 and 13 at 15 mg/kg dose. ¹⁴C-Leflunomide was given orally as suspensions and animals were sacrificed for autoradiography at 4, 24 and 48 hours post dose.

The sponsor stated that animals showed a different distribution of radioactivity at 4 hours post dose on gestation day 10 than what was expected. Misdosing may contributed to the discrepancy. The distribution of radioactivity at 4 hour post dose on gestation day 13 was observed in the liver, kidney, lung, blood, urine, mammary glands and GI tract. The copy of the images showed a background radioactivity in the brain. The data suggest that radioactivity did not pass through the blood brain barrier. There was transfer of radioactivity across the placenta to the fetus. However, unlike the rat experiment, the rabbit study was conducted early in the development of the fetus. Still there was evidence of transplacental distribution of the radioactivity from ¹⁴C -Leflunomide.

Both pregnant rat and the rabbit studies suggest that Leflunomide or its metabolites crossed the placental barrier. The distribution was greater during the fetal development stage.

Metabolic fate of Leflunomide in rats and dog:

Page 5-19409 vol 59:

The metabolic fate of Leflunomide was determined in rats and dogs *in vivo* using ^{14}C Leflunomide at the aniline ring. The experiments were conducted in male and female SD rats and male beagle dogs. Animals were fasted 16 hours pre dose and 4 hour post dose. The oral dose was given at 16 mg/kg as a single dose. Intravenous doses were given at 3 mg/kg. Suspensions and solutions were prepared for oral dosing in dogs.

Oral absorption of Leflunomide was determined by the collection of blood from the portal vein. During the process heparinized blood from a donor animal was infused through the renal vein. *In vitro* metabolism of Leflunomide in blood, plasma, liver, kidney and lung homogenate from rats and blood, plasma from dogs were carried out.

The report suggests that metabolism of Leflunomide in the gut micro flora was investigated in rats and dogs. However, results of a similar experiment have been discussed above.

Glucuronidase or sulfatase conjugation products were identified by corresponding enzyme hydrolysis. There is a similarity of some data between this report and that of Page 5-19267, vol 59 that would not be discussed once again.

Results:

Results of the mass balance study in dogs using several formulations and routes are presented in the following table. Results are expressed as the percent of recovery of radioactivity over 7 days. Each data point represents one dog.

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Route, Formulation	Urine	Feces
p.o, solution	63.20	25.87
p.o, solution	65.24	14.93
p.o, solution	37.83	33.16 (dog vomited)
p.o suspensions	33.20	60.62
p.o suspension	52.79	41.62
iv	24.26	56.74
iv	32.87	57.40

The data suggest that most of the radioactivity was excreted in the urine in dogs after oral doses as a solution. However, the radioactivity excreted from the suspensions in dogs did not confirm above findings.

Excretion of the radioactivity in the urine in rats was faster than that of dogs. Most of the radioactivity in the rat urine was excreted within 72 hours, whereas it took 144 hours for excretion of the radioactivity from the dog urine. Similar data were observed for the excretion of the radioactivity in the feces.

Biliary excretion of the radioactivity within 28 hours post oral dose was less than 10% of the total oral dose in rats. Reabsorption of ^{14}C labeled compound in the intestine when excreted with bile accounted for 2% radioactivity. The data suggest that Leflunomide or its metabolites did not undergo enterohepatic circulation in rats.

An intravenous dose (3 mg/kg) in dogs showed 38% of the dose recovered in the bile. The data indicate that biliary excretion of the drug or its metabolites is greater in dogs than that of rats.

Metabolite pattern in urine:

About 45-50% of the total dose was excreted as a single metabolite in the rat urine, almost no parent compound was excreted in the urine in rats. Other minor metabolites accounted for 5% of the radioactivity. About 15 to 25% of the total dose in dogs was excreted in the urine as the same metabolite as identified in the rat urine with a R_f of 0.17 when chloroform, ethyl acetate, ethanol, water and ammonia was used as the solvent at 63:5:30:1:1 ratios (solvent system A). The sponsor stated that they have used solvent H, however, a representative chromatogram using the solvent system H have not been provided.

TLC analyses of the urine samples from rats and dogs suggested that all urine samples contained one major metabolite that was very polar. The sponsor has not clearly stated the identity of the metabolite. However, the reviewer perceived that the major urinary metabolite would be a TFMA derivative since TFMA alone partitioned at a different Rf close to 0.7. It was also suggested from the enzyme studies that the metabolite was not sulfate or glucuronide conjugate.

Metabolite pattern in bile:

Radioactive metabolites from the bile samples from rats dosed orally and dogs dosed iv were separated

In the presence of solvent system A, bile samples from both rats and dogs did not show unchanged Leflunomide and TFMA within 4-6 hours after the dosing. Active metabolite A 771726 was identified in rats and dogs (band 1). There was another metabolite that was identified in the bile from rats and dogs. This metabolite was detected near the origin (band 2). These two bands accounted for 6% of the oral dose in rats and 55% of the iv dose in dogs. The band 2 is different from the major urinary metabolite.

The TLC bands in the solvent system H showed unchanged Leflunomide, A 771726 (active metabolite) and TFMA. Other than these bands, a band at Rf 0.43 was detected in the bile in dogs and rats.

Metabolites in the Feces:

Fecal metabolites in the solvent system A in rats and dogs were partitioned to A 771726, Leflunomide and TFMA. Dogs showed higher levels of TFMA than rats. In addition to these bands, a band near the origin (polar metabolite) was detected in the feces from rats and dogs which was similar to that in the bile. However, its proportion in the feces was less than that found in the bile. It was reasoned that the biliary metabolite in the solvent system A near the origin was further degraded in the gut. The sponsor stated that fecal samples showed similar pattern of metabolites when solvent systems H and B (Toluene/ methanol) were used.

Metabolites in the plasma:

Plasma samples: Oral doses in rats did not show unchanged Leflunomide. Active metabolite A 771726 were detected in the rat plasma after the oral dose of Leflunomide. At the end of 24 hours, about 20% of the total radioactivity was detected as the slow moving polar band.

Oral doses of Leflunomide solution in dogs showed unchanged Leflunomide that peaked within 20 minutes after the dosing. Intravenous doses of Leflunomide showed A 771726 as the major

metabolite (active) in the plasma.

Metabolism in the gut micro flora:

The results of the metabolism of Leflunomide in gut micro flora *in vitro* from rats and dogs suggest that breakdown of Leflunomide to A 771726 and TFMA would take place in the gut. Breakdown of Leflunomide to A 771726 and TFMA did occur in control samples without gut micro flora also. Therefore, metabolism of Leflunomide may also take place in the absence of the micro flora from the intestinal tract.

Glucuronidation:

Urinary and biliary metabolites from the rat did not contain any glucuronide and sulphate conjugated products. However, about 3% of the total dose was excreted as the glucuronide conjugated product of A 771726 in the dog bile. Polar metabolites from the urine samples from the dog did not have any glucuronide conjugated metabolites.

In vitro metabolism in the presence of blood, plasma, liver, kidney and lung homogenate with or without oxygen suggest that metabolism of Leflunomide to form A 771726 as the active metabolite mostly took place in the liver under the anaerobic conditions. However, the experimental protocol was not adequate to infer that drugs that interact with the P 450 system would not interfere with the metabolism of Leflunomide. More experimental data using microsomal proteins and inhibitors of P-450 systems would provide insight to the mechanism.

Portal vein metabolites:

Intra duodenal administration of ¹⁴C-Leflunomide suspensions in rats and subsequent collection of total blood from the portal vein showed that only 20% of the total radioactivity was appeared in the portal vein within one hour. However, 80% of the radioactivity was due to A 771726. The data suggest that opening of the ring of Leflunomide could take place both in the GI tract as well as through anaerobic metabolism in the liver.

Summary of the metabolism experiments in rats and dogs:

¹⁴C-Leflunomide was used for isolation and characterization of metabolites in the urine, feces and bile from rats and dogs. The separation of metabolites were done by using several solvent systems.

Leflunomide breaks down to A 771726 (active metabolite) and to a some extent to TFMA in the guts and liver in rats and dogs. The opening of the ring mostly possible under the anaerobic condition. Kidney is the major route of excretion of Leflunomide metabolites after oral doses in

the rats and dogs. Major urinary metabolite is a polar metabolite that migrated near the origin in the ~~which~~ which remains unidentified from rat and dog urine samples. The rate of excretion of Leflunomide or its metabolites in rats was faster than that in dogs. Fecal samples in rats and dogs showed TFMA, A 771726, Leflunomide and a polar band near the origin in system. Glucuronide or sulfate conjugated products were not excreted in rats. However, 3% of the dose excreted as A771726 glucuronide product in the bile in dogs.

The sponsor stated that mixed function oxidase systems were not involved in the metabolism of Leflunomide to A 771726. However, there was no direct evidence to suggest that drugs involved in the metabolism by P-450 systems would not interfere with the metabolism of Leflunomide from this experiment.

It can be concluded from the study that A 771726 and TFMA are the known metabolites in rats and dogs, two unknown polar metabolites, one in the urine the other from the feces have not been identified. Metabolites of Leflunomide are excreted in the urine and feces.

Site of Metabolism of Leflunomide following intraduodenal doses at 16 mg/kg in dogs:

Page 5-19463, vol 59:

Bile, blood samples from portal vein, vena cava and jugular vein were collected for assaying Leflunomide and its metabolites. Data suggest that gut tissues contribute to 10% of the metabolism of Leflunomide and first pass metabolism in the liver was about 79%. Thus GI mucosa and liver balance the formation of the active metabolites in the event if one organ fails to metabolize Leflunomide.

In vivo and in vitro metabolism of Leflunomide, A771726 and A 813226:

Page 5-19486, vol 59:

The structure of A 813226 is shown in page 4.

Liver microsomes, S-9 supernatant from rats and human liver samples and P-450 inhibitors were used for the understanding of the metabolic pathway of Leflunomide and A 771726. In vivo studies in rats were conducted in SD male rats using cyano labeled with ^{14}C -A771726 at 3 mg/kg iv dose.

Microsomal preparations obtained from rats without a pretreatment with inducers did not show metabolic turn over of ^{14}C -A771726. Induction of P-450 in rats by phenobarbitone, dexamethasone and use of these microsomal preparations suggest that CYP3A was mainly involved as the major P450 system in the metabolism of Leflunomide and A 771726 in vitro.

Total metabolic turnover in dexamethasone treated microsome was 50% of which 21% was oxanilic acid (Comp I) and 10% glycol anilide (comp III). These two metabolites were considered to be major urinary metabolites in vivo in rats. Use of an inhibitor of CYP 3A, troleandomycin also supports that CYP 3A was involved in the metabolism. Therefore, aerobic processes also contribute to the metabolism to Leflunomide in the rats in addition to anaerobic processes as suggested in a separate study mentioned earlier.

Relatively modest metabolic turn over in the presence of phenobarbital induced liver microsomal preparations suggest that CYP2B is also involved to a minor extent for the metabolism of A 771726 in vitro in rats.

In another experiment, both microsomal and cytosolic fractions of liver from dexamethasone treated rats were used to investigate in vitro metabolism of A 771726 in comparison to commercially available liver fractions. Formation of Trifluorooxanilic acid (Compound I) and glycol anilide (compound III) was identified and confirmed from the dexamethasone induced liver fractions from rats. The soluble fraction (cytosol) and microsomes treated with carbon monoxide also showed similar metabolic profiles. The data suggest that both the soluble and microsomal fractions from dexamethasone induced rat liver contain enzymes that would metabolize A 771726. It should be reiterated that in a previous study, involvement of mixed function oxidase systems was ruled out for the ring opening of Leflunomide. Results of this study also suggest that metabolism of leflunomide is not completely dependant on mixed function oxidase systems.

In vivo studies in rats were conducted with ¹⁴C-A 771726 at the CN site. Urine and fecal samples were collected following iv administration at 3 mg/kg. Total radioactivity excreted in the urine and feces at 48 hours post dose was 12.4% and 48.3% of the dose, respectively.

The cyano group in A 771726 formed thiocyanate ions in rats when the carbon in the cyano group was labeled. Thiocyanate was detected in the urine and plasma but not in the feces. Thiocyanate formation was catalyzed by rhodanase. ¹⁴C labeled A813226 (structural analog of A 771726, chemically N-(4-trifluoromethyl)-2-cyano acetamide) given intravenously to rats, showed thiocyanate in the urine and plasma. The rest of the molecule gave oxanilic acid. Since oxanilic acid was also excreted from A 771726 dosing in the rat urine, A 813226 formation from the oral dose of Leflunomide was considered to be an intermediate in the metabolic pathway of Leflunomide.

Microsomal systems and cytosol from human liver catalyzed metabolism of Leflunomide to A 771726 by 95 and 72%, respectively. The data again support that opening of isoxazole ring is not completely dependent on mixed function oxidase systems. The opening of the ring was much faster at a lower concentrations. Metabolism of ¹⁴C-Leflunomide other than ring opening was minimal but observed at higher amount than the control samples with no cofactors or with heat

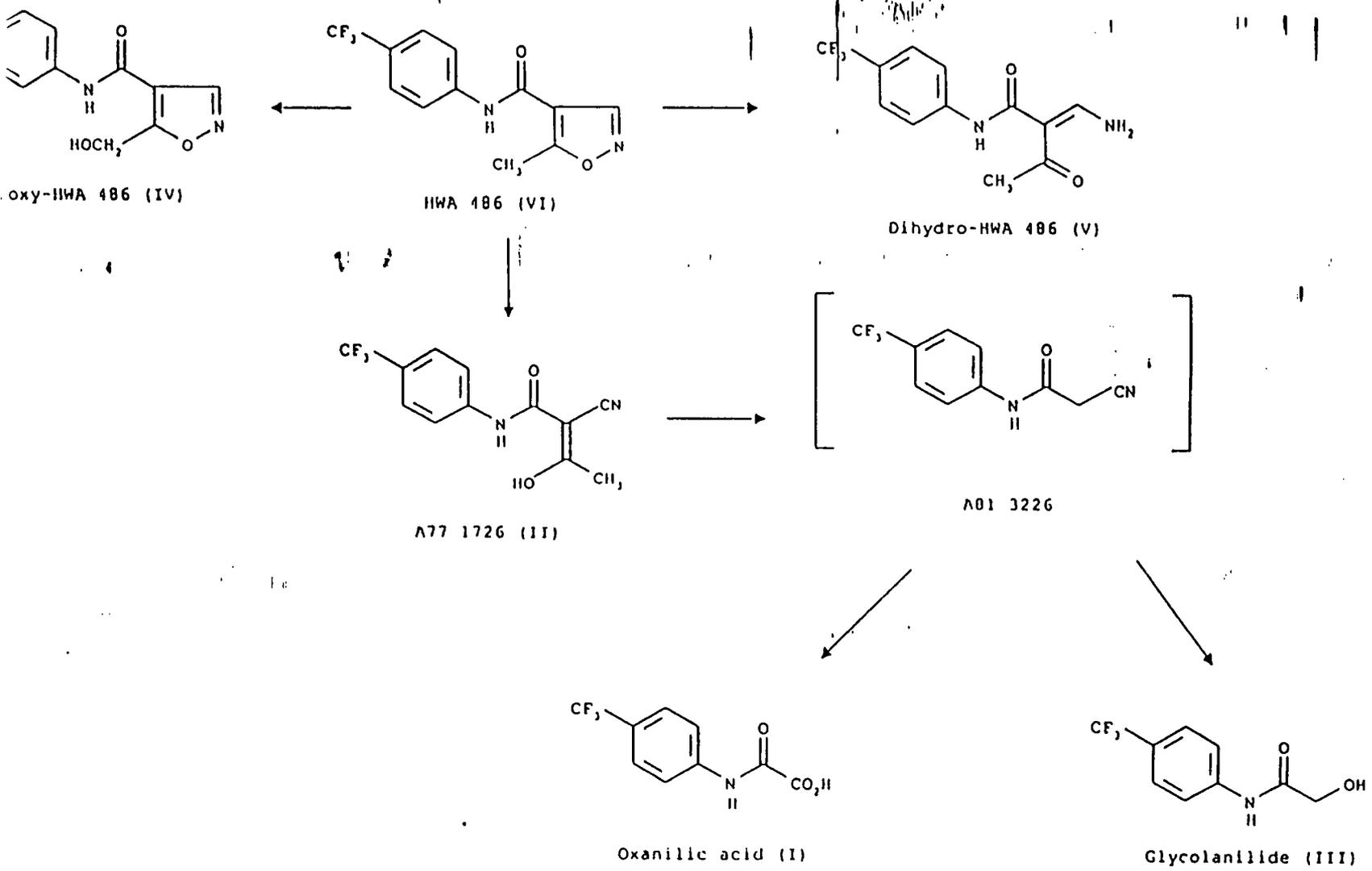
inactivated microsome. The sponsor attempted to use inhibitors of P450 systems. However, the data were inconclusive. Formation of A 771726 from Leflunomide was identified in human GI tissues. Both cytosol and microsomes were involved in the process. The data suggest that the isoxazole ring opening could take place in the GI and liver in humans. Therefore, first pass metabolism of the drug took place both in the GI tract as well as in the liver. Cloned P450 system in which human P450 was individually expressed in insects showed minimal metabolic activity other than isoxazole ring opening. Although CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 isozymes showed the formation of oxanilic acid, glycol anilide, hydroxy Leflunomide, dihydro Leflunomide to a different proportion, the amount of these metabolites in the cloned system was not high.

Conclusion of metabolism studies using human and rat tissues:

Data from the human liver microsomal system and cytosol purchased from IAM, Pennsylvania suggest that microsomal proteins and cytosolic fractions were able to metabolize Leflunomide to A 771726, an active metabolite. Human microsomes from subjects with high P4503A4 activity showed four metabolites of Leflunomide in addition to A 771726 i.e. oxanilic acid, glycoanilide, hydroxymethyl Leflunomide and dihydro Leflunomide. Qualitatively similar data were obtained from cDNA for several human P450 isozymes expressed in insects. Involvement of CYP 4503A4 (major) and other CYP isozymes was suggested. The metabolism of Leflunomide was not dependant on P450 mixed function oxidase systems. Both GI tissues and liver were involved in the first pass metabolism of Leflunomide in the human. It should be noted that qualitatively similar metabolites were also detected in the rodents and dogs. Formation of A 813226 was suggested as an intermediate for the formation of oxalinic acid in rats. Some of the intermediate was also excreted as thiocyanate in the rat urine. The proposed pathway of the metabolism in the human system and the structures are shown below.

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Fig. 15: Proposed *in vitro* metabolic pathways of HWA 486 and A77 1



The *in vitro* metabolism of A771726 or Leflunomide in human microsomal system was slow and P 450 was not absolutely necessary for isoxazole ring opening. The data suggest that the possibility of drug-drug interactions for Leflunomide is low.

Summary of the Drug metabolism and kinetics:

Oral administration of Leflunomide generates an open ring compound (A 771726) as an active metabolite. Both GI tissues and liver are involved in the first pass metabolism. Opening of the ring in the liver involves both P450 systems and cytosolic fractions of the liver as suggested from the experiments in human and rat liver homogenate. P450 3A (induced by Dexamethasone) also plays an important role for the opening of Leflunomide isoxazole ring. Human liver microsomes metabolized Leflunomide further to form TFMA oxanilic acid, glycoanilide, hydroxymethyl Leflunomide and dihydro Leflunomide. However, formation of these metabolites by human P 450 system was slightly over control (control liver homogenate were obtained without induction of P 450). The sponsor concluded from the data that metabolism of Leflunomide by P450 systems other than opening the isoxazole ring in human liver samples *in vitro* was minimal. Therefore, metabolism of Leflunomide involves both P450 and cytosolic constituents of the liver. Mouse, rats and dogs showed qualitatively similar metabolic pattern.

A definite possibility for the drug-drug interactions of Leflunomide or A 771726 at the P450 site may not take place as evident from the metabolism data in the presence of cytosolic and microsomal fractions of the liver homogenate. However, drug interactions at the plasma protein binding site e.g. with methotrexate or NSAIDs may not be ruled out based on the data on plasma protein binding. *In vitro* plasma protein binding of A 771726 is between 96 to 99.38% in monkey, human, rat and dog plasma. Although preclinical drug interaction studies have not been reported in the NDA, clinical studies with Leflunomide and methotrexate have been planned to investigate the pharmacodynamics of the combination therapy.

Leflunomide is metabolized and excreted as TFMA oxalate in rats and dogs without formation of TFMA as an intermediate. The TFMA oxanilic acid is formed from A 771726 via formation of a cyanide containing intermediate A 813226 after the oral doses in rats. The intermediate A 813226 also forms some amount of thiocyanate and it can be detected in the plasma and urine. However, nanogram quantity of TFMA is also formed from Leflunomide in the gut and liver in rats and dogs. Humans also showed TFMA in the plasma in nanogram quantities. The formation of TFMA was greater in dogs compared to rats. TFMA is acetylated and oxidized to TFMA oxalinic acid in rats. However, TFMA is hydroxylated in dogs and excreted as a conjugated product. The difference in the metabolism of TFMA in rats and dogs is due to the lack of acetylating enzymes in dogs. Hydroxylated TFMA may be responsible for increased incidences of anemic changes in dogs through the formation of Heinz bodies.

Metabolic profiles of Leflunomide in mice are similar to rats, dogs and humans. Oxanilic acid of TFMA and a glucuronide conjugated metabolite (aglycon not identified) were the major urinary metabolites identified by . Fecal excretion of radioactivity was 49-74% and urinary excretion of radioactivity was 16-26% in mice. However, structure of the fecal metabolite was not identified although it appeared to be a single metabolite in the . (thin layer chromatography) system. Plasma half life ($T_{1/2}$) of A 771726 in male mice was 10.6 hours after an oral dose of 3 mg/kg.

Plasma half life of A 771726 in several species were determined at several doses and durations of the treatment. The ^{14}C Leflunomide at the ring or carbonyl group showed half-life for excretion of radioactivity as 11.8 and 23.4 hours in the dog plasma for ring or carbonyl labeled Leflunomide, respectively. The plasma $T_{1/2}$ in dogs for A 771726 was 23.5 hours after oral dose of 5 mg/kg of Leflunomide. In dogs, exposure of A 771726 at the end of six months was reduced at 8 mg/kg dose compared to 2.5 mg/kg dose. The oral bioavailability in dogs was 76%.

Area under the concentration curve (AUC) of A 771726 in male and female rats at 0.5, 1.0, 2.0 and 4.0 mg/kg oral doses were dose proportionate at the end of six months. There was no gender differences in the exposure in the experiment. The kinetic data in rats suggest that oral bioavailability of A 771726 was 80% compared to iv A 771726 and 61% compared to an iv dose of Leflunomide. Due to the first pass metabolism of Leflunomide after an oral dose, bioavailability of A 771726 after an oral dose of Leflunomide was greater than iv Leflunomide. Food reduced the bioavailability by about 10% in rats. The plasma $T_{1/2}$ of A 771726 in rats was between 2.78 to 4.09 hours after oral doses from 2-10 mg/kg. The plasma $T_{1/2}$ of A 771726 in monkeys was about 18 hours.

The pharmacokinetics of A 771726 in pregnant rabbits was determined during organogenesis. The AUC on days 6 and 16 of pregnancy was 52.5 and 37.7 ($\mu\text{g}\cdot\text{hr}/\text{ml}$) at 10 mg/kg/oral dose, respectively. In a separate studies in pregnant rats and rabbits, ^{14}C -Leflunomide and its metabolites were distributed in the placenta and fetuses during the gestation period. The doses were 15 mg/kg/oral for rabbits and 16 mg/kg/oral in rats.

Distribution of radioactivity after an oral dose of ^{14}C Leflunomide was assessed in rats and rabbits using techniques. The distribution of radioactivity in the rat suggests that skin, liver, GI tract were major organs of radioactivity. Rats showed 60-77% irreversibly bound radioactivity in the liver following 7 days treatment in the binding study. in the pregnant rabbits showed that Leflunomide or its metabolites distributed to the placenta and fetuses during organogenesis. However, distribution of the radioactivity to the brain was minimal.

It is concluded that in large animals clearance of Leflunomide or its metabolites was slower than small rodents. The drug or its metabolites are distributed in the fetuses. Leflunomide is metabolized in the gut and liver. The metabolic pattern in humans and animals are qualitatively similar.

NDA Summary:

Leflunomide is an immunosuppressant having isoxazole structure. It is metabolized *in vivo* in the liver and gut to form an open ring structure (A771726) that possesses greater immunosuppressant effects than Leflunomide. *In vitro* studies in rats showed inhibition of Con A induced lymphoproliferation by Leflunomide and it is about 3-fold less effective than A 771726. The *in vitro* inhibitory potency (IC_{50}) of A 771726 for Con A-induced lymphoproliferation was about $0.09 \mu\text{M}$ when rat splenic lymphocytes were used. A maximum about 74% inhibition of Con A induced lymphoproliferation was noted in the human lymphocyte system at 2.5 to $10 \mu\text{M}$. Several pharmacological studies also showed an increase in the cell proliferation at a lower concentration. Mixed lymphocyte reaction assay in mouse *in vitro* showed lymphocyte proliferation at $100 \mu\text{M}$ of Leflunomide.

In vivo potency of Leflunomide was shown at 2 mg/kg/oral dose given prophylactically in adjuvant induced arthritic rats. However, the antiinflammatory effect of Leflunomide was not evident in Type II collagen induced arthritis model in mice at 10 mg/kg dose. Type II collagen induced arthritis in rats was inhibited by Leflunomide at 10 mg/kg per day oral dose when treated during the sensitization phase. Leflunomide showed inhibition of DNA autoantibody formation in MRL mouse at 20 and 35 mg/kg doses, the model represents experimental model of lupus.

The mode of action of Leflunomide or A 771726 in RA is not clear. However, the antiinflammatory activity in RA may be due to its inhibition of pyrimidine synthesis by inhibition of dihydroorotate dehydrogenase. The enzyme inhibition was reversed by pyrimidine base i.e uridine.

Leflunomide or A 771726 showed antiallergic reactions in the skin when IgE induced cutaneous inflammation was monitored in rats. It is devoid of inducing allergic reaction in guinea pigs. The antiallergic response was also demonstrated in the active anaphylaxis in rats. Leflunomide or A 771726 did not show contact sensitization in the guinea pig skin test. Several *in vivo* studies also suggest that Leflunomide and its active metabolite inhibits antibody formation in rats.

Acute oral overdose toxicity is expected at 500 mg/kg (1500 mg/m^2) in mice, 235 mg/kg (1386 mg/m^2) in rats and 132 mg/kg (1478 mg/m^2) in rabbits. Dogs tolerated a dose of 25 mg/kg per oral. Signs of overdose toxicity at these doses were reduced movement, lacrimation, tremor, convulsion, ulcer in the pyloric stomach and emesis. This information should be indicated in the overdose section of the label.

A six month oral toxicity in Wistar rats was performed at 0.5, 1.0, 2.0 and 4.0 mg/kg/day/oral as suspensions. The report was reviewed under _____ dated March 16, 1993. Mortality was reported at 2 mg/kg (n=2) and 10 mg/kg (n=10). There was one mortality reported at 0.5 mg/kg dose also. It is not clear from the toxicity data whether mortality at 0.5 mg/kg was treatment-

related. Female rats at 4 mg/kg dose showed anemic changes. GPT and GOT activities were elevated at all doses in male rats. Organ weight data showed an elevation of the weight of lungs and liver in female rats at 4 mg/kg/oral dose. Sign of immunosuppression was evident through the depletion of hematopoietic cells in the bone marrow. Plasma levels of A 771726 at one hour after the 177 doses of Leflunomide were 1.8, 3.7, 7.7 and 14 $\mu\text{g/ml}$ at 0.5, 1.0, 2.0 and 4.0 mg/kg doses, respectively. On the basis of the data, 1.0 mg/kg/oral doses was considered to be the dose tolerated for six months.

The sponsor proposed that carcinogenicity study should be conducted at 0.5, 1.25 and 3.0 mg/kg oral doses in rats. The CAC executive committee suggested that the doses for the rat carcinogenicity study should be 0.1, 0.3 and 1.0 mg/kg/oral. It was also suggested that the high dose groups should have higher number of animals due to expected mortality from the treatment with the immunosuppressant. The sponsor conducted the carcinogenicity test in rats at 0.5, 1.25, 3.0 and 6.0 mg/kg oral doses. An increase in the mortality was observed at 6 mg/kg dose. Statistical review of the data suggest that intercurrent mortality trend was significant among male rats. Based on the data, it is concluded that the dosing schedule in the rat carcinogenicity study reached the maximum tolerated dose.

Chronic toxicity point of view, oral treatment of rats for two years with Leflunomide showed an increase in the incidences of alopecia, corneal opacity and opacity of lens. Changes in the eye were observed around week 85. Male and female rats at 6 mg/kg dose showed an increase in the liver toxicity. It is important to note that male and female rats at 3 mg/kg and higher doses showed an increase in the inorganic phosphate. This may be an important safety issue for the long term use of Leflunomide in the bone. However, no abnormalities in the bone reported so far in the preclinical study. It is possible that the increase in the inorganic phosphate levels in the serum could have been due to the kidney toxicity. However, kidney toxicities in rats were not evident from the data.

Histological examinations showed an increase hemorrhage in the brain and spinal cord, necrosis of the liver and oligospermia.

Although several possibilities of neoplastic lesions were described in the review, hepatocellular adenocarcinoma, endometrial stromal sarcoma and uterine polyps in female rats were considered to be of significance. However, statistical review of the data suggest that there was a statistical significance only for the uterine polyps in rats with a P value of 0.0121. The sponsor stated that the historical control in the Wistar rats for uterine polyps was between 0-8.2%. Although the sponsor provided the historical control data, the high incidences of uterine polyps in the high dose group is alarming. The issue needs to be discussed in the CAC-EC meeting.

The high incidences of uterine glandular polyps may be associated with post menstrual bleeding. Therefore, human data should be compared for any increase in the post menstrual bleeding

disorders.

Serum levels of A 771726 were determined during the carcinogenicity study. The CAC during the review of the protocol suggested that blood samples should be collected from satellite groups for toxicokinetics. It appears that the blood samples for the toxicokinetic study was conducted like the population PK study. Blood samples were taken from orbital plexus from non fasted rats. The exposure data ($\mu\text{g}\cdot\text{h}/\text{ml}$) at 6 mg/kg oral dose in male and female rats are shown below.

Gender	Day 380	Day 555	Day 724
Male	40.9	27.2	-
Female	45.6	50.7	22.4

Above data should be used for the determination of exposure ratios between animal to human at a maximum recommended dose. Since exposure to rats was reduced after one year of dosing (possibly due to the liver toxicity or due to the aging process), the reviewer recommends that maximum exposure achieved during the study in the rats should be compared to the human exposure. Gender differences in the kinetics were not observed in the chronic rat study. However, a decrease in the exposure to A 771726 at the end of 78 to 104 weeks in rats may reflect toxicity involving organs that metabolized Leflunomide. In this context, additional safety to the liver and GI tract beyond one year of the treatment needs to be carefully monitored in the clinical trial. Rats also showed TFMA levels in the serum. Ratio of maximum serum C_{max} of A 771726 to TFMA in male rats at 6 mg/kg dose is 723. Similarly, maximum serum C_{max} of A 771726 to TFMA in female rats at 6 mg/kg dose is 486. Therefore, levels of TFMA compared to A 771726 (active metabolite) is minimal.

Dose finding study for the mouse carcinogenicity experiment was conducted. Leflunomide was given by oral gavage at 3, 10 and 30 mg/kg. The study was reviewed on Jan 3, 1994. Weight gain/loss at 10 mg/kg dose was 22.9% (loss) in male and 14% (gain) in female. Weight gain/loss at 30 mg/kg dose was 66.7% (loss) in male and 48.6% (loss) in female mice. Centrilobular hypertrophy of the liver was noticed at 30 mg/kg in male and female rats. There was sign of immunosuppression at 10 and 30 mg/kg as evident from the thymus atrophy. There was no treatment related mortality. The reviewer recommended that 3-10 mg/kg may be considered as the maximum dose for the mouse carcinogenicity study. The sponsor proposed that the carcinogenicity study would be conducted at 3, 10 and 30 mg/kg/oral doses for 18 months in the protocol. The CAC-EC suggested that 15 mg/kg may be chosen as the MTD and the experiments should be conducted for 24 months. Accordingly, the sponsor selected 1.5, 5 and 15 mg/kg/oral doses for a 24 month study for carcinogenicity evaluation.

The mortality trend in male mice was significantly higher than the control. The reviewer concluded that the experiment was conducted at the maximum tolerated dose.

Chronic treatment in mice for two years showed hypospermatogenesis, immunosuppression (pharmacodynamic response), fibrosis of the uterus, dermatitis and ulcer of the skin, alopecia, lenticular opacity and necrosis of the liver. It should be noted that the distribution of radioactive Leflunomide or its metabolites in the rat skin was high. A similar drug distribution data for mice are not available. Although several *in vivo* tests in rats and guinea-pigs suggested that the drug does not have immediate or delayed allergic response, an increase incidence of alopecia in rats, dermatitis and ulcer in the mouse skin suggest that Leflunomide treatment would render patients susceptible to skin toxicity. The toxicity may also be mechanism based because antimetabolite type of drugs would slow the growth and differentiation of epithelial cells.

Changes in the lens for cataract formation were studied in female brown Norway rats (page 5-3468, vol 22). Cataract was induced by naphthalene and streptozotocin. Leflunomide up to 3 mg/kg dose for 41 days did not show cocataractogenic effect in the experimental model.

Several neoplastic changes are listed the review. The biostatistical review showed a statistically significant increase in the increase in the incidences of malignant lymphoma in male mice ($P=0.0017$). This finding is not surprising for any immunosuppressant. Another finding of significance is Bronchoalveolar adenoma and carcinoma in female mice ($P=0.0025$). It should be mentioned that both neoplastic lesions in mice were possibly related to the immunosuppression.

Toxicokinetic data were collected from the satellite groups at the end of one year and two years of dosing in mice. However, AUC was not calculated. The sponsor provided data at 2 and 24 hours post dose on day 724. Therefore, differences in the kinetics between first and second year of the treatment can not be examined for the mice study. The exposure data provided by the sponsor suggest that the exposure at 15 mg/kg dose reached a maximal level when compared to that obtained at 5 mg/kg dose. The exposure data support that the experiment was conducted at the MTD.

The exposure ratios at the maximum doses in the rat and mouse carcinogenicity studies to that of human exposure at the maximum recommended dose needs to be determined for the label. The exposure data for the mouse is not available in the submission. The reviewer asked the kinetics reviewer to provide the human exposure data. The PK reviewer suggested that 1512 $\mu\text{g}\cdot\text{h}/\text{ml}$ would be the human exposure at 25 mg oral dose of Leflunomide after 100 mg loading dose. However, it is the best estimation because the reviewer derived the data from the trough level at 25 mg dose. The maximum recommended human dose is 20 mg daily. As per request of the review division, the sponsor provided human exposure data and animal to human exposure ratios dated June 24, 1998. In this amendment the human exposure reported by the sponsor was 1512 $\mu\text{g}\cdot\text{h}/\text{ml}$ that is similar to the data provided by the PK reviewer.

The maximum exposure data considered by the sponsor for the rat carcinogenicity study for the purpose of determining the rat to human ratio was $39.0 \mu\text{g}\cdot\text{h}/\text{ml}$. This is an average exposure in male and female rats at $6 \text{ mg}/\text{kg}$ at the end of 79 weeks. Since male rats at $6 \text{ mg}/\text{kg}$ dose were sacrificed at the time point, the exposure at this time point was chosen for male and female rats for a comparison to the human exposure. The ratio of maximum rat to human exposure for the carcinogenicity was 0.025.

For the mouse carcinogenicity study, the maximum exposure ratio between mouse to human would be 1.6 based on AUC of $2380 \mu\text{g}\cdot\text{h}/\text{ml}$ at $15 \text{ mg}/\text{kg}$ provided by the sponsor. The exposure ratio data between the rat and mouse indicate that mouse tolerated higher exposure to the Leflunomide or its metabolites.

Chronic toxicity of Leflunomide in dogs was examined in a one year study. The maximum dose was $2.5 \text{ mg}/\text{kg}/\text{oral}$. Anemic changes due to Heinz body formation were noted. The elevation of bilirubin and abnormal lipid metabolism suggest that Leflunomide treatment affected the liver. Inorganic phosphate levels in the serum were elevated similar to that observed for the rats. Tubular atrophy in testes was noted. The maximum exposure ratio between dogs to humans was calculated to be $235/1512$ or 0.15.

Reproductive toxicity (segment I, I and III) were conducted in Wistar rats. Segment II study was also conducted in Himalayan rabbits. These studies were reviewed by Dr. Coulter, HFD-550 dated June 8, 1998.

Results of segment I study in rats suggest that there was no impairment of fertility in male and female rats up to $4 \text{ mg}/\text{kg}$ ($23.6 \text{ mg}/\text{m}^2$).

Segment II study in Wistar rats showed toxicity to fetuses at $15 \text{ mg}/\text{kg}$ ($88.5 \text{ mg}/\text{m}^2$). At this dose, a reduction in the body weight of dams was observed. The plasma exposure data in rats at $15 \text{ mg}/\text{kg}$ dose is not available for comparison to the human exposure. However, the sponsor submitted an extrapolated data at $15 \text{ mg}/\text{kg}$ dose in normal rats i.e. $C_{\text{max}} 50 \mu\text{g}/\text{ml}$ and AUC $200 \mu\text{g}\cdot\text{h}/\text{ml}$ in non pregnant rats. Using the data provided by the sponsor, the exposure rat to human would be $200/1512=0.13$.

In vitro study in rat embryo limb cells also showed inhibition of cell proliferation in the presence of Leflunomide or A 771726.

Segment II teratogenicity study in Himalayan rabbits suggests that $10 \text{ mg}/\text{kg}/\text{oral}$ dose is tolerated by dams but fetal defects were noted at $10 \text{ mg}/\text{kg}$ ($111.7 \text{ mg}/\text{m}^2$). The maximum exposure ratio between pregnant rabbits to humans would be about 1.13.

Segment III reproductive safety study in Wistar rats showed that treatment of pregnant rats with Leflunomide during pregnancy (postimplantation period), delivery and nursing at 1.25 mg/kg (7.4 mg/m²) showed toxicity to pups. This may be due to impaired nursing or Leflunomide-induced toxicity in pups. It is possible that the metabolites are excreted in the milk and responsible for the toxicity to pups. There are no data on excretion of the metabolites in the milk. Pups that survived did not show impairment of reproductive functions. It can be concluded that Leflunomide does not cause genetic defects that impaired the reproductive functions of the second generation of rats. Pups of F₂ generation did not show any abnormality. The maximum exposure in female rats at 1.25 mg/kg dose was 11.9 µg.h/ml. The rat to human exposure ratio would be 0.008.

Reproductive safety data suggest that the drug affected pre and post natal development of pups in rats at maternally tolerated doses. Unless specific reproductive safety in humans are available, a pregnancy category C may be given to the product. The definite human adverse experience to the reproductive organs or pregnancy is unknown at the present time.

Ames test data suggest that Leflunomide was not mutagenic in the presence or absence of metabolic activation. Unscheduled DNA synthesis was not affected in the presence or absence of metabolic activation *in vitro* in a human cell line.

In vitro forward mutation assay at HGPRT loci in Chinese Hamster Lung fibroblast cells did not show mutagenicity.

In vivo studies in mice did not show mutagenicity in the micronucleus test at 150 mg/kg/oral (885 mg/m²) to 200 mg/kg/oral (1180 mg/m²) doses. In vivo cytogenetic test in the Chinese Hamster bone marrow cells showed an increase in the aberrations at 300 and 600 mg/kg when gaps were included for the data analysis. Results of *in vivo* cytogenetic test in Chinese Hamster suggest that Leflunomide may be mutagenic. The results were discussed in page 15 of dated March 16, 1993.

Mutagenicity of a minor metabolite TFMA was examined in several tests. It was mutagenic in Ames test *in vitro*, gene mutation assay at HGPRT locus and chromosomal aberration assay in Chinese Hamster lung fibroblast cells *in vitro*.

In vivo cytogenetic assays in the bone marrow cells in Chinese Hamster and mouse micronucleus tests did not show mutagenicity to TFMA.

A published report (Environ. Mutagenesis 5:803-811, 1983) in page 5-20883 vol 62 has been cited for the mutagenicity tests of TFMA. The report stated in page 807 that TFMA is not mutagenic in Ames test and UDS (unscheduled DNA synthesis and repair) test. However, results of the assay conducted by the sponsor suggest that TFMA is mutagenic in Ames test. The proposed label indicates that TFMA is non mutagenic in the UDS assay. The full report for the

UDS assay is not available. Unless a full GLP audited report is provided by the sponsor, it is not appropriate to include results of the UDS study in the package insert. The maximum levels of TFMA was 10.7 ng/ml-obtained from the data submitted for the PK review. A 771726 C_{max} levels in the steady state at 25 mg daily dose after 100 mg loading dose was 57.2 $\mu\text{g/ml}$. The ratio of A 771726 to TFMA would be about 5345.

Leflunomide undergoes first pass metabolism in the gut and liver. It is converted to an open ring structure. Experiments with the cytosolic fraction of the liver homogenate suggest that the metabolism of Leflunomide involves both P450 and soluble fractions of the liver. P 4503A (dexamethasone sensitive) contributes to the metabolism of Leflunomide. Rodents, dogs and humans metabolized Leflunomide similarly. TFMA oxanilic acid, glycoanilide, hydroxy methyl Leflunomide and dihydro Leflunomide are metabolites of A 771726. Traces of TFMA are formed in most of the species. Metabolism of TFMA is different in dogs than other species due to lack of an acetylating enzyme in dogs.

Leflunomide and A 771726 have high plasma protein binding. Therefore, interactions with NSAIDs and other drugs at the plasma protein binding sites may be examined in the future. Presently, the sponsor is conducting a combination study with methotrexate. Results of the study would provide information on the drug-drug interactions.

Plasma half life ($T_{1/2}$) of Leflunomide in dogs was 23.5 hours at 5 mg/kg dose and oral bioavailability was about 76%. $T_{1/2}$ of Leflunomide in rats was between 2.78 to 4.09 hours and oral bioavailability was 80%. The plasma $T_{1/2}$ in monkey was about 18 hours. $T_{1/2}$ of A 771726 in mice was 10.6 hours at 3 mg/kg/oral dose. Large animals showed a longer plasma half life of elimination of Leflunomide or A 771726. Human $T_{1/2}$ for leflunomide (measured as A771726) was about 15 days in RA patients and 8-9 days in healthy volunteers. That led to higher exposure of A 771726 in humans compared to animals. When the exposure ratios between maximum tolerated doses in the animal toxicity studies were compared to that of maximum recommended human dose, except mouse to human ratio, most of the ratios were less than one. It can be suggested from the exposure ratios that Leflunomide is more toxic to humans than animals.

Leflunomide or its metabolites were distributed to the fetuses. However, minimal drug was distributed in the brain. It should be noted that higher incidences of hemorrhage in the brain and spinal cord were noted in the carcinogenicity studies. Other than GI and liver, a high amount of radioactivity was present in the skin. These data substantiate cutaneous toxicities e.g. ulcer, dermatitis and alopecia. In the radio tracer studies, about 60-77% of the radioactivity was bound irreversibly in the liver in rats. The irreversibly bound drug or its metabolites may contribute to the hepatic toxicity after chronic administration.

Conclusion and Recommendation of the NDA:

Leflunomide is an immunosuppressant effective in several *in vitro* and *in vivo* experimental models of inflammation. The mode of action of Leflunomide may be due to the inhibition of dihydroorotate dehydrogenase. This leads to the inhibition of pyrimidine synthesis.

Leflunomide may exist in two polymorphic forms. Therefore, relative proportion of each form in the batches used for the safety studies need to be provided before the approval of the drug.

Acute, chronic, reproductive safety and mutagenicity studies were conducted for the approval of leflunomide. The NDA is approvable for the chronic treatment of rheumatoid arthritis at a maximum recommended dose of 20 mg/kg/day/oral with a recommended loading dose. The recommendation is made on the basis of the results of the preclinical safety studies, understanding of the preclinical safety concerns of the similar products for the treatment of arthritis. Clinical safety and efficacy of the drug has to be evaluated for further consideration and for the approval. However, following recommendations and concerns need to be addressed in the label of the drug.

- Acute Safety:

Over dose toxicity studies indicated that Leflunomide is lethal at 500 mg/kg (1500 mg/m²) in mice, 235 mg/kg (1386 mg/m²) in rats and 132 mg/kg (1478 mg/m²) in rabbits. Doses up to 25 mg/kg (500 mg/m²) was tolerated in dogs. Signs of over dose toxicity were reduced movements, lacrimation, tremor, convulsions, ulcer in the pyloric stomach and emesis.

- Carcinogenicity:

Leflunomide treatment up to 6 mg/kg (35.4 mg/m²) showed statistically significant increased incidences of uterine polyps in a two year carcinogenicity study in rats. The ratio of rat to human exposure to A771726 at the dose was 0.025.

In a two year carcinogenicity study in mice up to 15 mg/kg (45 mg/m²) dose showed statistically significant increase in the incidences of malignant lymphoma in male mice and Bronchoalveolar adenocarcinoma in lungs for female mice. The mouse to human exposure ratio at the maximum dose was 1.6.

- **Reproductive toxicity:**

Leflunomide treatment in male and female rats did not show any adverse effects on the fertility. However, toxicities to fetuses were observed in rats at 15 mg/kg (88.5 mg/m²) and rabbits at 10 mg/kg (111.7 mg/m²) when treated during organogenicity. The exposure ratios between rat to human was 0.13 and that for the rabbit would be 1.13. When rats were treated with Leflunomide at 1.25 mg/kg (7.4 mg/m²) during pregnancy and nursing, there was an increase in the post natal mortality of pups. The exposure ratio between rat to human at that dose was 0.008.

- **Pregnancy Category:**

Based on the animal reproductive safety data, the pregnancy category C can be given to the product. However, long term toxicity studies in rats, mice and dogs suggest that chronic treatment with Leflunomide would induce oligospermia, uterine polyps, and fibrosis of uterus. If these toxicities and non-reproductive organ toxicities render the drug of little benefit to pregnant women, based on the risk to benefit of the class of antimetabolite, the pregnancy category X may be allotted. However, the risk and benefit of the drug needs to be examined by the medical reviewer.

- **Mutagenicity:**

Leflunomide is not mutagenic in *in vitro* tests e.g. Ames Assay, unscheduled DNA synthesis and repair assay, HGPRT gene mutation in Chinese Hamster lung fibroblast cells and *in vivo* micronucleus test in mice. However, it showed an increase in aberrations in bone marrow cells *in vivo* in female Chinese Hamsters.

A minor metabolite Trifluoromethyl aniline showed mutagenicity in *in vitro* tests e.g. Ames test, HGPRT gene and chromosomal aberration tests in Chinese Hamster lung fibroblast cells. *In vivo* studies in mouse micronucleus test and Chinese Hamster bone marrow cells did not show mutagenicity.

- **Chronic toxicity:**

Several chronic toxicities were observed in animals. The clinical reviewer needs to compare the findings with the clinical safety data base.

Alopecia, oligospermia, corneal and lens opacity, necrosis of the liver, increase calcium (females after week 56) and phosphate levels in the serum were noted in rats. The kinetics of A 771726 was reduced at the end of one year perhaps due to increase liver and GI toxicities. It is also possible that the change in the kinetics after the first year of the treatment may be related to the

aging process. There may be a possibility that the efficacy of the drug would be lowered at the end of second year of treatment compared to that in the first year. If so, a long term monitoring (beyond one year) of safety and efficacy of the drug is recommended.

Chronic treatment for two years in mice showed hypospermatogenesis, uterine fibrosis, dermatitis, ulceration in the skin, alopecia, necrosis in the liver and lenticular opacity.

One year safety study in dogs showed alopecia, liver toxicity, increase inorganic phosphate, tubular atrophy of testes and kidney tubular atrophy.

When these side effects are combined, effect on skin, liver, possible bone degeneration due to an increase in the calcium and or phosphate release, opacity of the eye, changes in the testes and spermatogenesis are considered to be side effects that need to be addressed in the label. It is also possible that the increased calcium and inorganic phosphate levels in the serum may reflect abnormal tubular function in the kidney.

The reviews need to be presented in the CAC and reproductive safety committee for a discussion.

Asoke Mukherjee
Pharmacologist

cc:
Orig. NDA #20-905
HFD-550/Div. File
HFD-550/Reviewer
HFD-550/CSO
HFD-345
R/D Init. by:
F/T by:
A:NDA20905.apr

Andrea B. Weir
26 Aug 98 (see Aug 25, 1998
memorandum)

MEMORANDUM

Date: August 25, 1998
From: Andrea B. Weir, Ph.D., Pharmacology Team Leader, HFD-550
Subject: Pharmacology/Toxicology Review for NDA 20-905, Leflunomide

The 1-year toxicology study conducted in the dog and the 2-year carcinogenicity studies conducted in the rat and the mouse are pivotal studies for NDA 20-905 for leflunomide. These three studies were evaluated in two reviews, one dated July 1, 1998, and the other dated August 18, 1998. In general, the clinical and pathology interpretations presented in the July 1, 1998 review do not reflect those of the undersigned. The interpretations in the review dated August 18, 1998 reflect the opinion of the undersigned. A copy of the August 18, 1998 review is attached to this memorandum.

Andrea B. Weir, Ph.D.
Pharmacology Team Leader, HFD-550

cc: NDA File/NDA 20-905

Review and Evaluation of Pharmacology and Toxicology Data
Division of Anti-Infective Drug Products, HFD-520

NDA #: 20,905

DRUG NAMES: HWA 486; A 77 1486 A

CATEGORY: Antirheumatic compound

NUMBER OF VOLUMES: 1,335

DATE CDER RECEIVED: 3/9/98

DATE ASSIGNED: 8/2/98

DATE REVIEW STARTED: 8/8/98

DATE 1ST DRAFT COMPLETED: 8/18/98

DATE REVIEW ACCEPTED BY TEAM LEADER: *August 18, 1998*

REVIEW OBJECTIVES: To review the 1 year dog oral study and the rat and mouse carcinogenicity studies.

TOXICOLOGY:

1. Testing for Toxicity by Repeated Oral Administration to Dogs Over 1 Year (capsules); Report #96.0239. This study was conducted by Hoechst Marion Roussel Preclinical Development, Frankfurt am Main, Germany, and was initiated on June 29, 1994. A GLP compliance statement is included in the report.

Animals: 4 beagle dogs/sex/dose, aged 9 months at study initiation.

Doses administered: 0 (empty capsule), 0.25, 0.80, or 2.5 mg/kg/d orally for 1 year. Doses were based upon a 3 month oral toxicity study and a 6 month oral toxicity study with doses of 4, 8, and 16 mg/kg/d and 0.8, 2.5, and 8.0 mg/kg/d. In both of these studies, the test compound induced a transient anemia in the mid and high dose animals.

Parameters evaluated: Toxicokinetics (at 1, 2, 3 and 20 hours after the 9th, 48th, 91st, 170th, 268th, and 359th treatments), mortality, clinical signs, feed consumption, body weight, neurologic status, ophthalmoscopy, EKG, hematology, clinical chemistries, urinalyses, gross necropsy, organ weights (adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pancreas, pituitary, prostate, spleen, testes, thymus, thyroid with parathyroid, uterus) and histopathology (adrenals, aorta, sternal bone marrow, brain stem, cecum, femoral head, cerebral cortex, cerebellum, cervical lymph node, colon, diaphragm, duodenum, epididymides, eyes with optic nerve, femur, stomach fundus, gall bladder, heart, ileum, iliac lymph node, jejunum, kidneys, liver, lungs, medulla oblongata, esophagus, ovaries, pancreas, pituitary, pylorus, prostate, rectum, salivary glands, skeletal muscle, sciatic nerve, mammary gland and skin, spleen, testes, thymus, thyroid with parathyroid, tongue, tonsils, trachea, urinary bladder, uterus, vagina, gross lesions).

Results: Mortality/morbidity: One mid dose female (#6645) had to be terminated on Day 191 due to poor condition.

Clinical signs: Two high dose females showed reddish-dry skin from Day 274 until the end of the study. Their hypogastric areas were dotted with pustules and the haircoats were rough, or alopecic.

Significant differences from controls were noted in body weight, feed consumption, neurologic examination, gross necropsy, or organ weights.

Hematology: Reticulocytes were significantly increased in the mid and high dose males and all dosed females at the interim testings, but no significant differences from controls were noted at study termination. Heinz bodies within red cells were increased in the high dose males, and low and mid dose females. No significant differences from controls were found in hematocrit, hemoglobin concentration or erythrocyte counts.

The premature decedent showed a marked reticulocytosis, Heinz bodies, and Howell-Jolly bodies in the red cells before termination.

Mid and high dose females showed significantly increased leukocyte counts at mid study and increased thrombocyte counts in all females at study termination. Although the values reached statistical significance, no toxicological significance is attributed to this finding.

Clinical chemistries: Total (all males mid-study, mid and high dose males (5.1 umol/L) at study termination) and direct (all males mid study, high dose females at study termination) bilirubin were statistically and toxicologically significantly increased when compared to controls (4.0 umol/L for both males and females). All other changes (increased cholesterol in high dose males mid-study, high dose females at study termination; AST in mid and high dose males mid-study, increased A&T in all dosed males mid-study; globulin levels of various sub-types at all test points in all dosed animals) were considered spontaneous and not treatment-related.

Organ weights: Absolute heart weights in males were increased (17%) in high dose males, but no histologic correlate was reported.

Histopathologic evaluation: The premature decedent mid dose female showed markedly decreased hematopoiesis (red cell and white cell lines), marked thymic involution and splenic lymphocytic depletion. A compound-induced toxicity of the hematopoietic system could not be definitively ruled out in this animal. Hypertrophy of skeletal muscle and diaphragm was reported as an incipient sign of degeneration.

Although testicular tubular atrophy was reported in all dose groups (0/4 controls, 2/4 low dose, 1/4 mid dose, 1/4 high dose), the focal nature of the finding and minimal severity coupled with lack of findings in other male reproductive organs makes this finding possibly compound-related but toxicologically insignificant.

No other consistent histologic lesions were found in any dose group. It is an unusual finding for there to be so few histologic findings, whether toxicologically significant or not, in a dog study of this duration.

Toxicokinetic portion: Samples were collected on Days 9, 48, 91, 170, 268, and 359, at 1, 2, 3, and 20 hours post-dosing. No Day 1 samples were taken. Time to reach peak concentration was 1-3 hours post-dosing. Tmax did not appear to increase with dose or duration. There was a slight increase in exposure with increasing dose level in females at mid-study, but not in males. By the study termination, there were no significant differences between dose levels for normalized Cmax or AUC.

The sponsor did not conclusively rule out the possibility of hematopoietic toxicity, as there were significant increases in reticulocytes in all dosed groups at some point in the study, as well as Heinz body formation. Both findings were reported for the premature decedent female. However, no concomitant changes were found in erythrocyte counts, hemoglobin concentrations or hematocrit. They concluded that oral administration of HWA 486 induced transient hematologic effects in doses as low as 0.25 mg/kg given for up to 1 year.

2. HWA 486 (A 77 1486) Oral Carcinogenicity Study in Mice (Administration by Stomach Tube); Report #013384.

This study was conducted by Hoechst Marion Roussel, Frankfurt Am Main, Germany, and was initiated on June 6, 1994. A compliance statement is included in the report.

7. 50 CD1 mice/sex/dose for main study and 16/sex/dose for toxicokinetics, except the high dose group that had 7. dose.

Doses administered: 0 (2 potato starch mucilage [2 control groups]), 1.5, 5.0, or 15 mg/kg/d by gavage once/day for 2 years. Toxicokinetic animals were dosed daily for 1 year.

Doses were chosen based on a 3 month study where 30 mg/kg/d induced anemia, extramedullary hematopoiesis, centrilobular hepatocellular hypertrophy, and thymic atrophy.

Parameters evaluated: Clinical signs, body weight, feed consumption, ophthalmoscopy, neurologic evaluation, palpation for nodules, hematology, toxicokinetics (after 12 months [8/sex/dose] at 2, and 24 hours after the last dose, and at 24 months [5/sex/dose group] for both the main metabolite and a minor metabolite (4-trifluoromethylanilin), gross dissection, organ weights (heart, lungs, liver, kidneys, spleen, adrenals, testes, ovaries, brain), and histopathology (adrenals, aorta, bone marrow, eyes with optic nerves, brain, diaphragm, epididymides, esophagus, femur, gall bladder, heart, intestines (large and small), kidneys, liver, lungs, lymph nodes, medulla oblongata, nasal septum, pancreas, parathyroid, pituitary, prostate, salivary glands, ovaries, sciatic nerve, seminal vesicle, skeletal muscle, spleen, skin with mammary gland, spinal cord (three sections), sternum, stomach, testes, thymus, thyroid, tongue, trachea, tumors (if present), urinary bladder, uterus, vagina).

Results:

Mortality:

mg/kg	Males					Females				
	0	0	1.5	5.0	15.0	0	0	1.5	5.0	15.0
Number	50	50	50	50	70	50	50	50	50	70
Week										
	-	-	1 (gavage)	1	-	1	-	1	1 (gavage)	2
27-52	3	1	-	2	4	1	1	1	-	1
53-78	2	2	2	-	11	1	1	1	-	1
79-106	11	6	14	12	20	14	10	6	7	10
% deaths	32	18	34	30	50	44	28	24	18	22.9

Thus, it appears that the mortality rate in the high dose males was significantly higher than other dose groups.

Body weight:

Weight Gain in Grams and % Difference from Control #1

Sex	Control #1	Control #2	1.5 mg/kg	5.0 mg/kg	15 mg/kg
M	13.7	14.5 (+5.8%)	12.0 (-12.4%)	12.4 (-9.5%)	11.2 (-18.2%)
F	10.1	8.9 (-11.9%)	9.8 (-3%)	9.2 (-8.9%)	8.0 (-20.8%)

Statistical significance was reached in the mid and high dose animals, and the weight gain difference in the low dose females was considered treatment-related. However, overall means for the two control groups shows a significant difference between them, which exaggerates the compound effect in males, but lessens the effect in females.

Feed consumption: There was a slight decrease in absolute consumption in the high dose males, but the toxicologic significance is not clear since the females actually lost more weight than the males.

Clinical signs: Disseminated alopecia was noted in high dose females.

Ophthalmoscopy: Lens opacity was noted in 12%, 24%, 24%, 14% and 24.3% in controls #1 and 2, low, mid and high dose group males, respectively, and 18%, 26%, 18%, 20% and 31.4% in the same groups in females, respectively. As the

ity in the control groups was so high, the slight increase noted in the high dose animals is of questionable toxicologic significance.

Hematology: Statistically significant increases in rbc counts (8.27 vs. 9.12 in control and high dose females, respectively), hemoglobin (128 g/L in control females vs. 135 g/L in high dose females) and hematocrit were found in all treated females. However, these differences were not considered toxicologically significant as they are consistently less than 10% different, and there were no increases in reticulocytes, normoblasts or Howell-Jolly bodies in any of the dose groups. In the high dose animals of both sexes, there was a marked increase in Heinz body formation (8/25 high dose males, 13/44 in high dose females; 2/16 in each of the control groups).

Slight dose-dependent decreases were noted in thrombocytes (statistically significant in the high dose males). White blood cell counts were 11% decreased in the high dose males, but no dose-relationship was evident, and the absolute numbers were within limits reported for other dose groups at interim test points and no differences from controls were noted for the females. Therefore, neither of these findings is considered toxicologically significant.

Gross necropsy: No treatment-related findings were reported.

Organ weights: Relative spleen and brain weights were increased in mid and high dose females, as well as all treated males. No differences were found in absolute weights. Thus, this finding is attributed to the decreased total body weights.

Histopathology: Autolysis was higher than expected in the low dose males (8/50), and high dose males (9/70).

Extramedullary hematopoiesis was slightly increased in the treated females when compared to controls.

High dose males showed an increase in malignant lymphomas (17.4% vs. 8% for the combined male controls, 4% for the low dose males, and 8% for the mid dose males). Published historical control ranges are from 0-22% for (strain) mice. Statistical analysis by the CDER statistician (B. Taneja, Ph.D., dated 6/22/98) indicated a significant linear dose tumor-trend for malignant lymphoma in male mice at $p=0.0017$.

An increased incidence of nematode infestation was found in this group. The sponsor attributed this finding to "a slightly depressed immunity" making them more susceptible to this infestation. However, it is not clear whether the stated immunosuppression (unclear) is due to the increased malignancies (lymphoma) found in this group, or to a compound-related effect.

Incidence of Pulmonary Masses in Mice Treated with HWA 486 for 2 Years

Males

Females

	<u>Control 1</u>	<u>Control 2</u>	<u>Low</u>	<u>Mid</u>	<u>High</u>	<u>Control 1</u>	<u>Control 2</u>	<u>Low</u>	<u>Mid</u>	<u>High</u>
N with adenoma	4	4	1	8	10	2	0	3	4	6
N with carcin.	13	5	11	4	8	1	1	4	5	9
N total	17	9	12	12	18	3	1	7	9	15

Pulmonary bronchiolo-alveolar adenomas and carcinomas were increased in the compound-treated females, and adenomas were found in the mid and high dose males. Variability in the control males was high. Trend analysis did show significance ($p=0.0025$). As both adenoma and carcinoma levels are within published current (within 5 years) U.S. historical control ranges for these masses, a treatment-related association cannot be made.

Although there was a slight apparent increase in uterine/cervical fibrosis (2/99 in controls, 4/70 in high dose), and cystic endometrial hyperplasia in the high dose females when compared to controls, these findings are common in aged animals, and would be presumptuous to assign a treatment effect to them.

Tnsor concluded that lifetime administration of HWA 486 to mice caused decreased body weight development in mid and high dose animals, disseminated alopecia in females, increased red cell parameters in females, and markedly increased Heinz body formation in most high dose animals.

In females, the incidences of bronchiolo-alveolar neoplasms were within historical control ranges, while the controls showed a low spontaneous incidence. It is not possible to draw a conclusion concerning the treatment relationship to these neoplasms.

3. Oral Carcinogenicity Study in Rats (Administration by Stomach Tube); Report #97.0558. This study was conducted by Hoechst Marion Roussel, Frankfurt Am Main, Germany, and was initiated on March 1, 1994. A GLP compliance statement is included in the report.

Animals: 50/sex/control and 60/sex/dose Wistar WU rats, aged 6 weeks at study initiation.

Doses administered: 0 (vehicle, 2% potato starch mucilage), 0.5, 1.25, 3.0 or 6.0 mg/kg/d by gavage.

Parameters evaluated: mortality, clinical signs, body weight, feed consumption, neurologic evaluation, hematology, clinical chemistries, urinalyses, toxicokinetics, gross necropsy, organ weights (heart, lungs, liver, kidneys, adrenals, spleen, pituitary, thyroid, testes, ovaries, prostate, brain), and histopathologic evaluation (adrenals, aorta, bone marrow, eyes with optic nerves, brain, cartilage-bone junction, diaphragm, epididymides, esophagus, femur, heart, small and large intestine, kidneys, liver, lungs, lymph nodes, medulla oblongata, nasal septum, pancreas, pituitary, prostate, salivary glands, ovaries, sciatic nerve, seminal vesicle, skeletal muscle, spleen, skin with mammary gland, spinal cord (3 sections), sternum, stomach, testes, thymus, thyroid and parathyroid, tongue, trachea, masses, urinary bladder, uterus, vagina).

Unusual circumstances: Hematology and clinical chemistries were examined in Control #2 only and all treated groups. However, their statistical program could only evaluate in comparison to Control #1, so they exchanged parameters (hematology, clinical chemistries, organ weights, and body weights) between the two control groups.

Results:

Mortality: The 6.0 mg/kg/d males were sacrificed after 84 weeks due to poor health, attributed by the sponsor to hematologic toxicity with bone marrow as the target organ.

Mortality in Rats Treated with HWA 486 for Two Years

Group/ Week	Males						Females					
	1	2	3	4	5	6	1	2	3	4	5	6
1-26	0	1	0	0	0	0	0	0	0	0	0	0
27-52	0	1	5	1	2	4	0	0	1	3	1	1
53-78	2	3	5	4	5	43	1	2	2	5	6	14
79-106	14	13	12	14	16	7	10	10	17	10	8	12
Gavage error	0	0	4	3	13	0	0	0	0	1	0	0
% deaths	32	36	36.7	31.7	28.8	67.5	22	24	33.3	30	18.8	33.8

Body weight: At 6.0 mg/kg, the males had 9.6% lower than the controls by the end of the study. The females were 5.7% less than controls.

No significant differences from controls were noted in feed consumption, lenses (lens opacity and/or cloudy lenses were noted in all dose groups), neurologic evaluation, or urinalyses. Cloudy/opaque lens are a common finding by the end of lifetime studies in rats, and most of these changes were noted from Weeks 85-91 on study.

Alopecia was noted in all HWA 486-treated animals when compared to controls, but the severity was minimal to mild.

ology: Platelets and leukocytes for the mid and high dose males (interim values) were statistically significantly decreased, although within the historical 'normal' range for 2 year Wistar rat studies. The sponsor interprets these findings as "first signs of a compound-related effect". There were no increases in band cells, and the control group had higher maximum total numbers of leukocytes at the final analysis. Additionally, the high dose females had higher leukocyte counts than controls at study termination (~54%). Therefore, it is difficult to assign a compound-related effect to these changes.

Although rbc counts, hemoglobin, and hematocrit were decreased in the high dose males at the 84 week sacrifice, no other groups were tested at those timepoints, and the values were not significantly different from controls at other interim test points.

Hematologic Findings in Red Cells of Prematurely Sacrificed Rats Treated with HWA 486

Dose (mg/kg)	0		0		0.5		1.25		3		6	
N exam/sex	9M	2F	5M	6F	5M	7F	5M	7F	7M	6F	10M	11F
Heinz bodies	0	0	0	1	0	1	1	1	0	1	0	0
Howell-Jolly bodies	0	0	0	0	0	2	0	0	0	0	0	2
Normoblasts	2	0	1	0	1	3	2	4	0	2	4	4

Clinical chemistries: Total bilirubin was decreased in the mid and high dose males (4.2 and 4.3 umol/L at the interim sacrifice compared to 4.9 umol/L), but the values were within 'normal' ranges for ~1 year old Wistar rats. Uric acid and creatinine levels were increased in the high dose males at the 84 week sacrifice, but these values normally rise during the lifespan of male rats, whether treated with test compound or not. No histopathologic correlates were reported. At terminal sacrifice for all other males, no significant differences in creatinine or uric acid were noted between groups.

Gamma globulin levels were statistically significantly decreased in males at >0.5 mg/kg/d, and in females at all dose levels. While the sponsor attributes this finding to immunotoxicity, it is difficult to assign significance to this, as there were no histologic correlates (e.g., lymph node atrophy/degeneration) to substantiate this claim.

Gross necropsy: Red discoloration of the testes, epididymides, lymph nodes, red contents of the urinary bladder, and 'a soft consistency of the bone marrow' were reported in high dose males, but not in other males. Autolysis was pronounced in this group of animals on histologic examination. High dose females showed only the reddened lymph nodes and soft bone marrow, but to a lesser degree.

Hemorrhages of the Medulla Oblongata, Cervical Spinal Cord, and Thoracic Spinal Cord

Organ	Males					Females						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Medulla oblongata	1/47	1/47	2/55	0/54	2/75	28/76	0/41	2/45	3/59	1/54	2/77	8/78
Cerv. spinal cord	3/38	0/43	0/51	0/53	1/64	37/76	0/43	2/46	1/58	1/55	4/73	22/76
Thor. spinal cord	0/43	0/45	0/57	0/56	3/77	29/74	0/49	1/50	1/60	0/60	0/80	6/80

Interpretation of these lesions is difficult as the animals were anesthetized, then exsanguinated by severing the vena cava. No histologic correlates indicating previous hemorrhage (e.g., hemosiderin deposits, and neuronal damage) were reported. It is unclear from the data submitted whether the high dose males with hemorrhagic lesions were autolytic or terminal sacrifice animals.

Organ weights: Lung, testes, and brain weights were increased in the 1.25 and 3 mg/kg (~8%) males, and liver weights were increased from the 3.0 and 6.0 mg/kg (6%) females. No histologic correlates were found, and the differences from controls (Control #1) were usually <10%. Control #2 values were generally higher than the 1.25 and 3.0 mg/kg groups.

Histopathology: Historical data was from reviewed studies in the RITA database, not Hoechst's in-house data. Autolysis was reported in 40/80 high dose male animals examined, with 24 of these being severely autolytic. This makes interpretation of the findings in these animals very difficult to assess. None of the females is reported as autolytic.

No significant lesions were reported in the 0.5 and 1.25 mg/kg/d animals. No compound-related neoplastic effects were reported for any dose group. Glandular stromal polyps were numerically increased (0/100 for controls vs. 4/80 in high dose females), but statistical significance was not reached and the numbers are within published historical control values (up to 11% in the published literature for this strain of rat).

Additionally, thymomas (benign and malignant combined) were increased for the dosed females (3/69 for controls, 1/38 for 0.5 and 1.25 mg/kg groups, respectively, and 7/53 and 6/57 for the 3.0 and 6.0 mg/kg groups), but statistical significance was not reached, and toxicologic significance is uncertain. Historical control values for this lesion are reported at 0-17% in the literature.

Panmyelopathy characterized by depletion and aplasia of the hematopoietic cells of the bone marrow, often with multifocal hemorrhages, was more severe and more frequent in males compared to females. The sponsor stated that this lesion was the cause of death in 44/50 high dose males, and 12/13 high dose females, and in 2/3 premature decedents in the next highest dose group. Hemorrhages in these animals were found in lymph nodes, medulla oblongata and spinal cord, as well as testis, urinary bladder, and epididymides. These hemorrhages were noted at necropsy, but no evidence of previous bleeding abnormalities was found (hemosiderosis, fibrosis, etc.). Additional sites of hemorrhage were the heart and pancreas. Given the number of animals with autolysis at necropsy (see above), it is difficult to determine whether these findings are the result of compound-related toxicity, or the result of autolysis with pooling and congestion in dependent organs after death.

Panmyelopathy in Rats Treated with HMR 486

Organ	Males						Females					
	1	2	3	4	5	6	1	2	3	4	5	6
Bone marrow	n=50	n=50	n=60	n=60	n=80	n=80	n=49	n=50	n=60	n=60	n=80	n=80
Grade 2	1		2	1		6					1	1
Grade 3					2	10					1	4
Grade 4					1	30					1	7
Grade 5						4						1
Av. grade	2.0		2.0	2.0	3.3	3.6					3.0	3.6

The sponsor concluded that lifetime administration of HWA 486 in rats caused bone marrow toxicity in 3.0 and 6.0 mg/kg/d animals, with platelet and leukocyte counts lowered at the lower dose levels. Given the stated level of autolysis in the high dose animals (50%), it appears reasonable to say that compound administration caused significant declines in clinical condition and caused this dose group to be prematurely terminated. However, to attribute the minimal changes in the 3.0 mg/kg group to bone marrow toxicity elicited by compound administration seems to be an exaggerated interpretation of the data.

The degree of autolysis in this study confounds the interpretation of the data and the significance of the impact of the study.

CONCLUSIONS:

One year dog study: Hematology: Reticulocytes were significantly increased in the mid and high dose males and all dosed females at the interim testings, but no significant differences from controls were noted at study termination. Heinz bodies were increased in the high dose males, and low and mid dose females. No significant differences from controls were found in hematocrit, hemoglobin concentration or erythrocyte counts.

The sponsor did not conclusively rule out the possibility of hematopoietic toxicity, as there were significant increases in reticulocytes in all dosed groups at some point in the study, as well as Heinz body formation. Both findings were reported for the premature decedent female. However, no concomitant changes were found in erythrocyte counts, hemoglobin concentrations or hematocrit. They concluded that oral administration of HWA 486 induced transient hematologic effects in doses as low as 0.25 mg/kg given for up to 1 year.

Mouse carcinogenicity study: High dose males showed an increase in malignant lymphomas (17.4% vs. 8% for the combined male controls, 4% for the low dose males, and 8% for the mid dose males). Published historical control ranges are from 0-22% for mice. Statistical analysis by the CDER statistician (B. Taneja, Ph.D., dated 6/22/98) indicated a significant linear dose tumor-trend for malignant lymphoma in male mice at $p=0.0017$.

Rat carcinogenicity study: The sponsor concluded that lifetime administration of HWA 486 in rats caused bone marrow toxicity in 3.0 and 6.0 mg/kg/d animals, with platelet and leukocyte counts also lowered at the lower dose levels. Given the stated level of autolysis in the high dose animals (50%), it appears reasonable to say that compound administration caused significant declines in clinical condition and caused this dose group to be prematurely terminated. However, to attribute the minimal changes in the 3.0 mg/kg group to bone marrow toxicity elicited by compound administration seems to be an exaggerated interpretation of the data. The degree of autolysis in this study confounds the interpretation of the data and the significance of the impact of the study.

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VMO, HFD-520

D.V.M

Orig. NDA
cc:
HFD-520
HFD-520/Pharm Team Ldr/Osterberg
HFD-520/Pharm/Peters
HFD-550/MO/Johnson
HFD-550/Chem/Bhavnagri
HFD-550/CSO/Cook
HFD-550/Pharm Team Ldr/Weir
HFD-550/Pharm/Mukherjee
HFD-550/DepDir/Hyde

Concurrence Only:
HFD-520/REOsterberg
HFD-520/LGavrilovich

1020 8/18/98
AP 8/19/98

HFD 553/200K

NDA 20-905

JUL 10 1998

**THE DIVISION OF ANTI-INFLAMMATORY, ANALGESIC,
AND OPHTHALMIC DRUG PRODUCTS**

**PHARMACOLOGY/TOXICOLOGY REVIEW OF
REPRODUCTIVE AND TERATOGENICITY STUDIES
INITIAL REVIEW**

NDA 20-905

DRUG: Leflunomide Tablets 10 mg, 20 mg, 100 mg
SPONSOR: Hoechst Marion Roussel
SUBMISSION: March 10, 1998
DATE ASSIGNED: May 1, 1998
REVIEW COMPLETED: July 14, 1998
REVIEWER: Almon W. Coulter, Ph.D.

The following reproductive and teratogenicity studies were submitted and reviewed in this report.

Reproduction and Developmental Toxicity Studies	Page
1) Oral Fertility Study of HWA 486 (A77 1486) in Wistar Rats (Effect on Fertility, Pregnancy) and Postnatal Development) Segment I. Document 10463	3
2) Dose-Finding Oral Embryotoxicity Study of HWA 486 (A77 1486A) in Wistar Rats (Segment II). Document 10477	9
3) Oral Embryotoxicity Study of HWA 486 (A77 1486A) in Wistar Rats (Effect on Morphological Development). Document 13220	13
4) A77 1486 (HWA 486) and A77 1726 Study of the Teratogenic Properties in Rat Embryo Limb Bud Cells. Document 11505	16
5) Dose-Finding Oral Embryotoxicity Study of HWA 486 (A77 1486A) in Himalayan Rabbits (Segment II). Document 10476	17
6) Oral Embryotoxicity Study of HWA 486 (A77 1486A) in Himalayan Rabbits (Effect on Morphological Development). Document 13563	19