

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**APPLICATION NUMBER: NDA 20905**

**PHARMACOLOGY REVIEW(S)**

**Pharmacology Review**

NDA: 20-905

Sponsor: Hoechst Marion Roussel Inc. MO

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Drug: Arava, Leflunomide

Category: An antiproliferative and immunosuppressive agent

Indication: Rheumatoid Arthritis

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**Introduction:**

The NDA is submitted for the marketing approval of leflunomide for the treatment of rheumatoid arthritis. Leflunomide shares several pharmacological properties that contribute to its antiinflammatory effect. The published literature on Leflunomide suggests that the basic structure of the compound was originated from the agricultural herbicide research project. Further modification of a chemical series and identification of its pharmacodynamic properties led to the discovery of Leflunomide. One of the major pharmacological effects of the drug is related to the inhibition of pyrimidine synthesis that prevents proliferative actions of lymphocytes. However, tyrosine kinase and  $PLA_2$  inhibition may also contribute to its efficacy. The efficacy and safety of the drug are considerably related to the antiproliferative effect of the drug. Several preclinical *in vitro* and *in vivo* studies have been conducted for the safety and efficacy of the drug in the review.

The application is submitted for marketing the drug to treat signs and symptoms of rheumatoid arthritis. The sponsor has also claimed that the product has beneficial effect on the improvement of x-ray scores and prevents deterioration of the disease condition. The product was reviewed under an \_\_\_\_\_ dated March 16, 1993, Jan 3, 1994 and March 28, 1995. These reviews are attached with this NDA so as to avoid repetition of some of the data analysis. The mutagenicity and reproductive sections of the data have been reviewed by Dr. Will Coulter, HFD-550. Dr. Coulter's review will be attached with this review also.

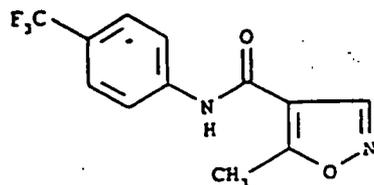
**Chemistry:**

Leflunomide, HWA 486 is chemically N-(4'-trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide. CAS 75706-12-6, mol. wt. 270.2. It is a white powder, pKa 10.8 at 23C and practically insoluble in water. It is soluble in methanol, ethanol, isopropanol, ethyl acetate etc. The drug exists in two polymorphic forms. The sponsor needs to state that polymorphic forms in safety studies did not differ from batch to batch so that the drug substance would confound the results of the safety studies. The drug will be marketed as 10, 20 and 100 mg tablets. The inactive ingredients do not have safety issues. The structure of Leflunomide, its primary and active metabolite A771726 and A813226, an intermediate in the metabolism of A771726 are shown below.

## 2. PRESENTATION OF THE TEST SUBSTANCES

### 2.1 HWA 486

Structural formula:



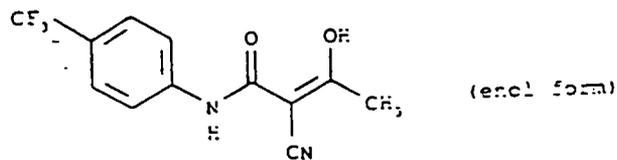
Chemical name: N-(4-trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide

Empirical formula:  $C_{12}H_9F_3N_2O_2$

Molar mass: 270.21 g mol<sup>-1</sup> (non-labelled)

### 2.2 A77 1726

Structural formula:



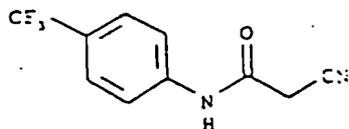
Chemical name: 2-cyano-3-hydroxy-N-(4-trifluoromethylphenyl)-crotonamide

Empirical formula:  $C_{12}H_9F_3N_2O_2$

Molar mass: 270.21 g mol<sup>-1</sup> (non-labelled)

### 2.3 A81 3226

Structural formula



Chemical name: N-(4-trifluoromethylphenyl)-2-cyanoacetamide

**Human experience:**

Clinical efficacy of Leflunomide has been investigated for rheumatoid arthritis and for chronic inflammation of polyarticular joints. The sponsor stated that coadministration of Leflunomide with 10-25 mg dose of methotrexate, NSAIDs and low doses of corticosteroid improved efficacy to the coadministered drugs in patients who were otherwise refractory to the treatment. Increase safety concerns due to coadministration of above agents were not indicated in the summary report. The efficacy was studied at 10 and 20 mg daily oral dose for one year. The NDA is submitted for the marketing approval of Leflunomide at 20 mg maximum daily doses.

**Pharmacology:**

Most of the pharmacology data were reviewed under \_\_\_\_\_ dated Aug. 3, 1989 and \_\_\_\_\_ dated March 16, 1993. The review of the data suggests that Leflunomide shares several pharmacological effects of which inhibition of pyrimidine synthesis by inhibition of dihydroorotate dehydrogenase (DHODH) is one of the important pharmacodynamic effects. The  $IC_{50}$  of A 771726 for inhibition of DHODH was 81 nM *in vitro* in mouse spleen membranes. The *in vitro*  $IC_{50}$  in human and rat recombinant enzymes was 1  $\mu$ M and 19  $\mu$ M, respectively. Uridine counteracted the inhibition. Leflunomide has weak PG cyclooxygenase, 5-lipoxygenase and  $PLA_2$  inhibitory effect. It is not known whether these effects contribute to the efficacy of Leflunomide in RA. The *in vivo* activity was shown in several experimental models of inflammation, autoimmunity and transplantation.

**Special pharmacologic studies:**

Anti allergic effect in rat skin PCA tests:

page 5-18100, vol. 55:

Male S-D rats were injected intradermally with IgE directed for dinitrophenol. Twenty-four hours later, animals were injected i.v with DNP and Evan's blue dye. Blue color at the site of injection was noted as the sign of anaphylactic reactions. Pretreatment of animals with A 771726A at 1-10 mg/kg i.v doses immediately before Evan's blue dye, inhibited the allergic reaction. Therefore, Leflunomide is considered to have antiallergic activity.

Immunogenicity to Leflunomide and A771726 in guinea-pigs:

Page 5-18115, vol. 55:

Male Hartley guinea-pigs \_\_\_\_\_ were used in the study. Animals were given Leflunomide at 0.2 mg and 2.0 mg orally and 2.0 mg s.c. The doses for A 771726 were 0.02 and

2.0 mg s.c. Both these compounds were given for sensitization. Concentrations that would not inhibit immunoglobulin formation were chosen. Animals were challenged with leflunomide and A 1771726 at 0.1 and 0.25 mg i.v doses, respectively. Leflunomide and A 771726 with guinea pig serum albumin were also used as a challenging dose at 0.25 and 0.05 mg, respectively.

Guinea-pigs were orally treated with Leflunomide at 0.1 or 1.0 % suspensions five times per week for three weeks for sensitization. Blood was collected at the end of 11 days after the last dose for PCA reactions. The sensitization was also conducted by s.c route at 2 mg for Leflunomide, 0.02 and 2.0 mg A 771726 on a weekly basis for 4 weeks. At 11 days post sensitization, blood was collected and serum separated for the PCA test. Ovalbumin was also used as a positive control in the experiment.

Animals sensitized according to the above method were challenged 13 days after the last dose of sensitization for examining the potential for an anaphylactic response. Leflunomide-guinea pig serum albumin mixtures, A771726 guinea-pig serum albumin mixtures, Leflunomide and A 771726 in methylcellulose suspensions and propylene glycol solutions were used as the challenging dose. Each antigen preparation was administered i.v into the ear vein. Systemic anaphylactic reactions were monitored.

For the PCA test, sensitized serum was diluted and injected intracutaneously in the back after shaving. Four hours after the serum injections, animals were treated with Leflunomide, A 771726 and their mixtures with guinea-pig serum albumin by i.v route. Animals were also given Evan's blue dye for localization of the cutaneous anaphylactic response. Ovalbumin challenge was given as a positive response for the PCA test.

Results of the test showed that Leflunomide or A 771726 failed to show cutaneous anaphylaxis either by a direct sensitization technique or by a passive sensitization technique. These data suggest that in the guinea-pig model, Leflunomide or its active metabolite is devoid of inducing cutaneous allergic reactions.

Mouse model of local anaphylaxis:

Page 5-18136, vol 55:

CD male mice were given i.p injections of ovalbumin in Al(OH)<sub>3</sub> for sensitization. An acute reaction in the hind paw to the subplantar injections of ovalbumin was induced about 7 days after the sensitization. The response elicited as an acute inflammatory swelling. One hour after the challenge, animals were sacrificed and the weight of the hind paw was determined. Animals were treated per oral once or twice per day either on days 4, 5, 6 and 7 or on day 7 before the challenge.

Results showed that leflunomide at 30 mg/kg doses given on days 4, 5, 6 and 7 inhibited the acute anaphylactic response by 100%. CSA at 100 mg/kg dose showed about 50% inhibition. Methotrexate at 1 mg/kg dose showed about 94% under conditions similar to Leflunomide treatment. Several nonsteroidal antiinflammatory agents showed 0-44% inhibition.

None of the above agents showed inhibition of acute anaphylactic response when given one hour before the challenge. In similar experiments, cyclophosphamide showed 74% inhibition of the anaphylactic response.

Above data suggest that leflunomide and other immunosuppressants are capable inhibiting the Immunogenicity when the animals were treated during the sensitization phase.

Acute anaphylaxis in rats:

Page 5-18160, vol 55:

Wistar rats were sensitized by the i.p injections of ovalbumin in  $Al(OH)_3$ . On day 10, animals were challenged by subplantar injections of ovalbumin in the hind paw. Paw inflammation was assayed by determining the weight of the ankle. Animals were pretreated with Leflunomide, CSA, Methotrexate, indomethacin and tenidap during 0-4 and 7-10 days.

In another set of experiment, animals were treated with intradermal injections of serum containing antibodies. Rats were treated i.v with the antigen (ovalbumin) one day after the passive sensitization along with Evan's blue dye. The cutaneous anaphylaxis was examined from the intensity of the blue color. Leflunomide, A 771726, CSA, methotrexate, indomethacin, tenidap, cyproheptadine and cromolyn were used as the reference drugs. The treatment was given on days -3, -2, -1 and day 0 by oral route. The single iv treatment was also given on day 0 prior to the challenge.

Leflunomide, CSA and methotrexate inhibited paw edema completely, NSAIDs had minor effect when the treatment was given during the sensitization period. The antigen specific serum Ig E and Ig G were also inhibited completely.

Results of the passive cutaneous anaphylaxis test showed that Leflunomide had only 16% inhibition at 10 mg/kg oral doses given on days 3, 2, 1 days before the challenge and on the day of the test. C.S.A. and methotrexate was also marginally active. Cyproheptadine and Cromolyn showed 71 and 90% inhibition at 10 mg/kg dose.

Data suggest that Leflunomide was marginally effective in the model of active anaphylaxis.

**Guinea-pig maximization test:**

Page 5-18200 vol 55:

The experiment was conducted in Hartley female guinea-pigs. Two intradermal injections of 50% Freund's adjuvant, Leflunomide in liquid paraffin and Leflunomide in adjuvant were made in guinea-pigs on study day 1. On study day 8, 0.5 g of substance in a cellulose patch was given to injection site. These two treatments were for induction and recording any primary irritancy for the drug. On day ten, the sites were examined for irritancy. Dermal challenge was given on day 22 by applying 0.5 g of the test substance in the cellulose patch. The application area was kept occluded for 24 hours. Following the challenge, animals were examined for the skin lesions.

A dose of 0.2% intradermal injections were given for Leflunomide based on the preliminary study that a higher dose would give greater irritancy. However, during the induction injections in Freund's adjuvant, there was erythema and edema. The sponsor stated that use of sodium dedesylsulfate for the induction was withdrawn on day 7. Dermal challenge on day 24 did not show any erythema.

Therefore, Leflunomide is devoid of contact sensitizing properties in the guinea-pig model.

The test was repeated with A771726 (page 5-18220, vol 55) in female Hartley guinea-pigs. 1% A 771726 was used for the induction in Freund's Adjuvant. The challenge dose was given by a dermal patch of 5% preparation in petrolatum. During the dermal induction, erythema was observed. However, there was no sign of contact sensitization property of A 771726 after a challenging dose.

**Delayed hypersensitivity test in rats:**

Page 5-18238, vol 55:

The delayed hypersensitivity reactions were induced by sensitization of male Wistar rats with s.c injections of 1 mg methylated bovine serum albumin. Challenge doses (0.5 mg subplantar injections) were given on 7, 10 or 14 days after the initial injection. Paw edema was determined as the sign of delayed hypersensitivity reaction. Leflunomide, cyclosporine (CSA), methotrexate, indomethacin and tenidap were orally given days 0-4 and 7-10 or days 7-10 or on the day of the challenge. At 10 mg/kg dose, Leflunomide showed 65%, CSA showed 74% and methotrexate showed 85% (at 0.3 mg/kg dose) inhibition when treated on days 0-4 and 7-10. NSAIDs showed about half inhibition. Acute treatment on the day of challenge at 10 mg/kg doses of Leflunomide or CSA, 0.5 mg/kg methotrexate showed 60, 17 and 75% inhibition, respectively. The data suggest that Leflunomide inhibited late allergic response.

Similar experiments were conducted in male CD-1 mice (page 5-18271, vol 55). Leflunomide treatment during days 4 to 7 after initial sensitization showed a dose related inhibition of delayed inflammation with  $ED_{50}$  at 22 mg/kg per oral. No inhibitory effect was observed at 30 mg/kg dose of Leflunomide when the treatment was given on the day of the challenge.

The data suggest that Leflunomide inhibited the delayed immunological activation process and it does not have any effect on the effector phase of the response.

#### Acute safety studies:

##### Oral:

Mice (Hoe:NMRKf-SPF71 strain) treated at 500 mg/kg died within 24 hours. Lacrimation, and trembling were noted. A dose of 200 mg/kg did not show mortality in mice. The report is submitted in page 5-3629, vol 23.  $LD_{50}$  values in different animal species are shown in page 5-3644, vol 23.  $LD_{50}$ s for mouse, rat and rabbit were 445, 235, 132 mg/kg per oral, respectively. Symptoms for mouse were tremors, for the rat convulsions. Rabbits showed ulcers in the pylorus region of the stomach. Dogs showed emesis at 25 mg/kg dose without mortality.

##### i.p.

Median lethal dose given by i.p route was between 200-400 mg/kg in rats. At 400 mg/kg dose, reduced motility, bristling fur, crawling and squatting were noted (page 5-3690, vol 23).  $LD_{50}$  in mice after i.p dose was 185 mg/kg (page 5-3701, vol 23). Blepharophimosis, lacrimation and depressed respiration were noted at death. Death occurred within 2-24 hours.

TFMA acute oral toxicity in mice was 400 mg/kg/oral. Death occurred between 1 hour to 12 hours after dosing. Cyanosis and deep narcosis were observed at death.

These data suggest that reduced movement, lacrimation, emesis, tremor, convulsions and ulcer in the pylorus of stomach were signs of acute toxicity of Leflunomide.

#### Chronic toxicity study of Leflunomide in rats: Immunological investigations:

##### Page 5-18293 vol 55:

A group of male and female rats were treated for 12 weeks with 50 ppm of Leflunomide in the diet. After 12 weeks, the dose was reduced to 20 ppm for a total of 24 months. The sponsor has not stated reasons for the reduction of the dose. However, a similar reduction in the dose was made due to the poor tolerability of the drug. The adjusted dose was 0.9 mg/kg per day for male and 1.27 mg/kg per day for female. The spleen and thymus were removed and weighed after the

treatment. Also Sheep RBC were injected i.p for the determination of antibody formation. Spleen cells were assayed for plaque formation in the presence of SRBC.

Results of the assay suggest splenocyte from male and female rats treated with Leflunomide had an increase proliferative response after two years of drug administration in the *ex vivo* assay. However, plaque forming cells and antibodies to SRBC were reduced significantly in the leflunomide treated animals. The tables below support the conclusion.

| Time       | % change from Control, Female | % change from Control, Male |
|------------|-------------------------------|-----------------------------|
| 2 nd week  | -79                           | -79                         |
| 4 th week  | -78                           | -88                         |
| 12 th week | -72                           | -98                         |
| 12 month   | -98                           | nd                          |
| 24 month   | -93                           | -33                         |

The antibody titre to SRBC in male and female rats are shown below.

| Time       | Untreated, M | HWA 486, F | Untreated, M | HWA 486, F |
|------------|--------------|------------|--------------|------------|
| 2nd week   | 32           | 8          | 4            | 8          |
| 4th week   | 32           | 8          | 16           | 32         |
| 12th week  | 8            | 0          | 32           | 1          |
| 12th month | 128          | 16         | 64           | 16         |
| 24th month | nd           | nd         | nd           | nd         |

nd=not done as per foot note, see page 5-18303

Data suggest that the drug has an inhibitory effect on the antibody production to SRBC without affecting the proliferative signals. T and B cell mitogen induced proliferation was also not inhibited by the Leflunomide treatment. The weight of thymus and spleen did not change significantly.

The data suggest that leflunomide at the dose used was more effective in the inhibition of antibody formation than lymphocyte proliferation.

**One year oral toxicity study in dogs:**

Page 5-8907, vol : 37: \_

The study was conducted at \_\_\_\_\_ according to the GLP.  
The drug was administered in gelatin capsules. The study design is shown below:

| Group | Number of animals | Dose, mg/kg      |
|-------|-------------------|------------------|
| 1     | 4 M, 4 F          | Control capsules |
| 2     | 4 M, 4 F          | 0.25 mg/kg       |
| 3     | 4 M, 4 F          | 0.8 mg/kg        |
| 4     | 4 M, 4 F          | 2.50 mg/kg       |

Male and female beagle dogs of Hoe:BEAK strain were used in the study. Body weight of the male dogs averaged 12.3 kg and 11.6 kg for the female. These animals were about 9 months old. Before the dosing schedule, blood chemistry, hematology, ophthalmological examinations, ECG and urine analysis were conducted. These parameters were also investigated during the experimental period. Intermediate values 01 were taken on the day 42, intermediate values 02 were collected on the day 91, intermediate values 03 were taken on the day 175, premature final values 01 were taken on the day 190, intermediate values 04 were taken on days 273-275 and the final values were taken on the day 364-366 of the experiment. Toxicokinetics from the serum samples were conducted at 1, 2, 3 and 20 hours post dose on days 9, 48, 91, 170, 268 and 359.

Mortality, clinical signs, body weight, food consumption were also monitored during the study. At necropsy, organ weight, macroscopic and microscopic examinations were conducted.

**Results:**

One female dog (#6645) at 0.8 mg/kg dose was killed on day 191 due to the poor health condition. The rest of the animals survived during the study.

**Clinical condition:**

Reddish and dry skin were observed in two females from day 274 at the high dose group. Alopecia was also observed in the high dose. The sponsor has not indicated how long alopecia was observed in the animals. Alopecia is a common side effect of anti metabolites.

Body weights (g) of the animals are shown in the following table.

| Day     | Gr 1, M | Gr 1, F | Gr 2, M | Gr 2, F | Gr 3, M | Gr 3, F | Gr 4, M | Gr 4, F |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 1       | 11625   | 11675   | 12075   | 11425   | 12750   | 11075   | 12850   | 12250   |
| 365     | 15425   |         | 15500   |         | 16075   |         | 15525   |         |
| 370-372 |         | 14225   |         | 14525   |         | 15533   |         | 15325   |

The weight gain was 3800, 3425, 3325 and 2675 g for male dogs at 0, 0.25, 0.8 and 2.5 mg/kg doses, respectively. The data suggest that male dogs at 2.5 mg/kg dose showed a reduction of the body weight gain.

The weight gain for the female animals was 2550, 3100, 4458 and 3075 g at 0, 0.25, 0.8 and 2.5 mg/kg doses, respectively. Apparently the female animals did not show a reduction of the weight gain.

Neither individual nor summary data for the ophthalmological examinations could be located in the submission. However, the narrative in the result section stated that there were no ophthalmological changes observed in these animals.

#### ECG:

One male dog at 2.5 mg/kg dose (#6635) showed a decrease in the HR to 40 beats per minute at the final evaluation. The animal showed lower HR on previous occasion also. However, it showed lowest HR at the final observation period. However, QRS and QT intervals did not change substantially. Female dogs did not show any drug related changes.

#### Hematology:

There were no treatment related changes in the WBC counts at the end of the experiment (days 364-366) in the treated dogs. RBC counts showed statistically significant decrease in the female dogs at 2.5 mg/kg at the end of the experiment. The RBC counts for the female dogs in the control group were  $6.94 \times 10^{12}/L$  and that at 2.5 mg/kg were  $6.03 \times 10^{12}/L$ . The hemoglobin levels were decreased from 152 g/L in the control to 134 g/L at 2.5 mg/kg dose of Leflunomide at the end of the experiment in female dogs. The changes were statistically significant. Heinz bodies were detected at 2.5 mg/kg dose in male dogs on days 364. Thrombocyte counts were significantly increased in male and female dogs on days 42, 91, 175 (males only) and 365 (females only). Lymphocyte counts and other hematological parameters were not affected by the treatment. The thrombocyte counts ( $10^9/L$ ) are shown following table.

| Days | Control      | 0.25 mg/kg    | 0.80 mg/kg    | 2.5 mg/kg    |
|------|--------------|---------------|---------------|--------------|
| 42   | 387 M, 385 F | 372 M, 431 F  | 400 M, 432* F | 449*M, 453*F |
| 91   | 380 M, 364 F | 359 M, 386 F  | 375 M, 473*F  | 441*M, 468*F |
| 175  | 380 M, 429 F | 413 M, 507* F | 387 M, 482*F  | 469 M, 519*F |
| 364  | 399 M, 421 F | 353 M, 473* F | 400 M, 407 F  | 464 M, 533*F |

\* statistically significant

The mechanism for the increase in the platelet counts that occurred mostly in female is not clearly understood.

#### Clinical Chemistry:

There was a tendency for an increase in the inorganic phosphate levels in the serum in male and female animals. During the treatment period, the increase reached statistical significance in male or female animals. At the last observation point, the control female had inor. Phos of 1.80 mmol/L and at 2.5 mg/kg dosed female had 1.85 mmol/L that was statistically significant. The control male had 1.47 mmol/L and at 2.5 mg/kg, the inorg. Phos had 1.50 mmol/L in the serum, the change was not statistically significant. At some observation points e.g about six months of the treatment, showed significant elevation of the serum calcium from 2.62 to 2.70 mmol/L at 2.5 mg/kg dose in male dogs. These data are shown in the following table.

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| Parameter                      | Days | Control     | 0.25        | 0.80          | 2.50 mg/kg    |
|--------------------------------|------|-------------|-------------|---------------|---------------|
| Calcium(mmol/L)                | 42   | 2.75M,2.68F | 2.79M,2.79F | 2.82M,2.85*F  | 2.85*M,2.78F  |
| Inorg PO <sub>4</sub> (mmol/L) | 42   | 1.85M,1.82F | 1.82M,1.80F | 1.90M,1.80F   | 2.09*M,1.81F  |
| Bilirubin(umol/L)              | 42   | 2.0M,1.9F   | 2.1*M,1.8F  | 2.3*M,1.8F    | 2.2*M,2.9F    |
| ASAT(u/L)                      | 42   | 11M,10F     | 12M,11F     | 10M,12F       | 16*M,13F      |
| ALAT(u/L)                      | 42   | 18M,19F     | 22*M,15F    | 22*M,19F      | 22*M,18F      |
| CK((u/L)                       | 42   | 33M,19F     | 32M,37F     | 28M,33F       | 69*M,27F      |
| Calcium                        | 91   | 2.83M,2.76F | 2.78M,2.77F | 2.66M,2.73F   | 2.84M,2.88F   |
| InorgPO4                       | 91   | 1.66M,1.67F | 1.67M,1.66F | 1.74M,1.75F   | 1.89*M,1.78F  |
| Bilirubin                      | 91   | 4.4M,4.4F   | 4.6M,4.4F   | 4.4M,3.8F     | 4.6*M,5.3     |
| Urea(mmol/L)                   | 91   | 5.17M,5.17F | 5.54M,5.06F | 5.83M,6.26F   | 5.37M,5.74*F  |
| Cholesterol(mmol/L)            | 91   | 4.32M,4.06F | 4.53M,3.62F | 3.78M,3.43F   | 4.11*M,4.30*F |
| Total Lipids(g/L)              | 91   | 6.45M,6.31F | 6.58M,5.86F | 6.64*M,6.18*F | 6.98*M,7.12*F |
| ASAT                           | 91   | 13M,13F     | 15M,16F     | 16*M,17F      | 20*M,17F      |
| CK                             | 91   | 28M,18F     | 21M,31F     | 22M,27F       | 59*M,23F      |
| Calcium                        | 175  | 2.62M,2.62F | 2.60M,2.58F | 2.69*M,2.63F  | 2.70*M,2.65F  |
| Bilirubin                      | 175  | 3.3M,3.2F   | 3.6M,3.8F   | 3.8M,3.4F     | 5.5*M,4.3F    |
| Bilirubin                      | 273  | 2.9M,2.7F   | 3.0M,2.8F   | 2.9M,3.1F     | 3.6*M,3.4F    |
| Uric acid                      | 273  | 22M,19F     | 23M,22F     | 22M,23F       | 22M,24*F      |
| Calcium                        | 364  | 2.72M,2.75F | 2.67M,2.76F | 2.77M,2.82F   | 2.69M,2.84F   |
| InorgPO4                       | 364  | 1.47M,1.80F | 1.39M,1.81F | 1.60M,1.83F   | 1.50M,1.85*F  |
| Bilirubin                      | 364  | 4.0M,4.0F   | 4.0M,4.4F   | 4.5*M,4.6F    | 5.1*M,5.9*F   |
| Cholesterol                    | 364  | 4.33M,4.72F | 4.6M,4.81F  | 4.57M,5.48F   | 4.34M,6.56*F  |

|              |     |             |              |              |               |
|--------------|-----|-------------|--------------|--------------|---------------|
| TG           | 364 | 0.7M,0.69F  | 0.65M,0.65F  | 0.56M,0.72F  | 0.59M,0.94*F  |
| Total Lipids | 364 | 7.25M,7.61F | 7.37M,7.34*F | 7.47*M,8.31F | 7.12*M,9.74*F |

\*statistical significance

A significant elevation of serum bilirubin from 4.0 (M) and 4.0 (F) to 5.1 and 5.9  $\mu\text{mol/L}$  was observed in male and female dogs at 2.5 mg/kg. These data were statistically significant.

There was a significant increase in the cholesterol and triglyceride (TG) levels in the female dogs that are shown in the following table.

| Parameter           | Control | 0.25 mg/kg | 0.80 mg/kg | 2.5 mg/kg |
|---------------------|---------|------------|------------|-----------|
| cholesterol, mmol/L | 4.72    | 4.81       | 5.48       | 6.56      |
| TG, mmol/L          | 0.69    | 0.65       | 0.72       | 0.94      |

Transaminase activity did not change significantly at the end of the treatment period. The gamma globulin proteins were not affected by the treatment. Alpha<sub>1</sub> globulin protein levels were increased from 0.034(M), 0.043(F) in the control to 0.039(M) and 0.049(F), respectively. These changes were statistically significant.

Creatinine levels were not determined in the urine. Also other excretory markers of kidney function were not determined.

At necropsy, the female # 6645, that was killed in moribund condition showed cachexia.

The organ weight data suggest that there was an increase in the weight of heart from 130 mg/kg in the control to 154 mg/kg at 2.5 mg/kg dose in male dogs. The weight of the lungs was 125, 155, 144 and 141 mg/kg at control, low mid and high doses respectively in male dogs.

The histopathology data are shown below. Figure in the parentheses represents number examined.

| Lesions   | Gr 1 M | Gr 1 F | Gr 2 M | Gr 2 F | Gr 3 M | Gr 3 F | Gr 4 M               | Gr 4 F |
|---|--------|--------|--------|--------|--------|--------|----------------------|--------|
| Kidney tubular atrophy<br>Round cell infiltration<br>Cortical scars | 1(4)   |        |        |        |        |        | 1(4)<br>1(4)<br>1(4) |        |
| Spleen Lymphocyte<br>depletion                                      |        |        |        |        |        | 1(4)   |                      |        |
| Thymus involution   |        |        |        |        |        | 1(4)   |                      |        |
| Bone marrow, reduced<br>hemopoiesis                                 |        |        |        |        |        | 1(4)   |                      |        |
| Testes, focal tubular atrophy                                       | 0(4)   |        | 2(4)   |        | 1(4)   |        | 1(4)                 |        |

The testicular lesions at the low, mid and high doses were between minimal to slight. Thymus involution and reduced hemopoiesis in the mid dose female dog was marked. Kidney tubular atrophy in the high dose male was slight to moderate.

Histology data suggest that the female dog in the mid dose suffered from excessive immunosuppression. The data suggest that the treatment induced immunosuppression to the animal. However, 2.5 mg/kg dose was tolerated for one year. There was kidney lesions in one high dose male. Testicular atrophy was seen at all doses of Leflunomide that needs to be addressed in the reproductive safety of the label. The changes in the bone structure were not evaluated in the study.

Toxicokinetics:

Page 5-9509, vol 38:

The serum levels of A771726 in dogs were assayed  
The data in  $\mu\text{g/ml}$  over the period of one year for  $C_{\text{max}}$  are shown in the following table.

| Days | 0.25, M | 0.25, F | 0.8, M | 0.8, F | 2.5, M | 2.5 F |
|------|---------|---------|--------|--------|--------|-------|
| 9    | 0.74    | 1.41    | 3.43   | 3.38   | 8.83   | 16.4  |
| 48   | 1.01    | 0.61    | 2.93   | 2.34   | 9.75   | 13.3  |
| 91   | 0.49    | 0.49    | 3.99   | 2.86   | 12.1   | 16    |
| 170  | 0.57    | 0.77    | 3.50   | 2.57   | 12.4   | 10.6  |
| 268  | 0.70    | 1.04    | 4.65   | 3.66   | 12.2   | 13.4  |
| 359  | 1.33    | 0.75    | 4.32   | 4.12   | 15.7   | 16.4  |

The data revealed that there was a dose dependent increase in the  $C_{max}$  in male and female dogs. The time to reach peak levels was about 2 to 2.5 hours for most of the cases.

The area under the curve ( $\mu\text{g}\cdot\text{h}/\text{ml}$ ) is shown in the following table.

| Day | 0.25, M | 0.25, F | 0.8, M | 0.8, F | 2.5, M | 2.5, F |
|-----|---------|---------|--------|--------|--------|--------|
| 9   | 9.12    | 16.3    | 43.1   | 37.3   | 128    | 202    |
| 48  | 12.0    | 8.45    | 40.6   | 34.1   | 143    | 165    |
| 91  | 7.26    | 5.56    | 52.0   | 35.1   | 179    | 190    |
| 170 | 8.35    | 9.79    | 54.4   | 41.2   | 186    | 159    |
| 268 | 9.75    | 11.9    | 63.9   | 50.1   | 200    | 183    |
| 359 | 16.4    | 9.21    | 56.0   | 52.3   | 235    | 207    |

Above data suggest that there was an increase in the exposure at 2.5 mg/kg dose in male and female dogs.

Conclusions of the one year dog study:

Dogs treated with leflunomide at 0.25, 0.8 and 2.5 mg/kg doses in gelatin capsules showed signs of immunosuppression at the mid dose. Long term safety study of immunosuppressants is often confounded with secondary infections. Therefore, the doses for the safety studies are selected at a range that would prevent overt toxicities and lethality due to secondary infections. On the basis of the limitation, the dose selection for the study is considered to be appropriate. Data from several published reports showed that Leflunomide had significant immunosuppressant activity in the canine model of transplantation. Therefore, selection of the dog model for chronic toxicity testing is reasonable.

Chronic treatment of Leflunomide in dogs showed alopecia and reddish skin lesions at the high dose. Heinz bodies were detected specially at 2.5 mg/kg dose. Thrombocyte counts were elevated.

Although histological changes in the liver were not observed, elevation of the serum bilirubin and lipid levels at several time points during the treatment suggests that the drug may have a deleterious effect on the liver if it is continued beyond one year. Serum calcium levels were also elevated mostly in male dogs at 2.50 mg/kg dose during the treatment days 42 and 175. Inorganic phosphate levels in the serum were elevated at 2.5 mg/kg in male dogs on days 42 and 91. The inorganic phosphate levels in female dogs were increased on the day 364.

Calcium and A 771726 bind to serum albumin. If one displaces other, an increase in the free form of the drug or calcium is anticipated. In the presence of the kidney toxicity, the excretion of calcium may be affected and the levels of serum calcium may be increased. The changes in the calcium and phosphate levels may reflect kidney toxicity. It is also possible that these blood chemistry data may be associated with degenerative changes in the bone. However, there is no data available in the study report to indicate that the treatment was associated with changes in the bone density (no histological data on the bone have been provided in the report). One male dog at 2.5 mg/kg dose showed kidney tubular atrophy. It appears there may be an effect of the drug on the kidney. These data may be compared to toxicity studies in other species and that for the human safety.

The major histological changes other than immunosuppression were tubular atrophy in testes at all doses tested. The finding may be a reflection of sexual maturity of the dogs deployed in the study or resulted from the side effect of the drug. Incidences of testicular atrophy in rats (AT6179) and impaired spermatogenesis in the six month dog study (AT6236, also see page 5-8692, vol 35 of the NDA) reviewed for dated March 16, 1993 (pages 15 and 19) suggest that tubular atrophy of testes may be related to the treatment.

Therefore, Leflunomide was tolerated up to 2.5 mg/kg (50 mg/m<sup>2</sup>) for one year. Some of the toxicities observed in the study were tubular atrophy of testes, immunosuppression, kidney tubular atrophy, intermittent alterations of the calcium and phosphate metabolism. Clinical significance of the change is unknown at this time.

The kinetic data suggest that there was a dose proportionate exposure and the exposure at the high dose was more than expected. The no effect dose for the study is 0.25 mg/kg (5.0 mg/m<sup>2</sup>).

#### Oral Carcinogenicity study in rats:

Page 5-14376, vol.1.49, Report # 97.0558:

The study was conducted in accordance to the GLP between March 1, 1994 to March 22, 1996. Leflunomide is also referred as HWA 486. Male and female Wistar rats with average weight 124 g (M) and 108 g (F) at the beginning of the dosing were used in the experiment. These rats were about 6 weeks of age at the beginning of the study. Animals were treated with the vehicle or the drug once a day by oral gavage. Duration of the treatment was 24 months except the high dose males which were treated for 84 weeks. Body weight, food consumption, mortality, hematology, blood chemistry, urine analysis, serum levels of the drug, organ weights, macroscopic and microscopic pathology were conducted during the study. Laboratory tests were conducted on week 54-56, week 84 for high dose males and week 104-105 for rest of animals.

The drug substance was suspended in 2% starch mucilage for oral administration. The stability of

the suspensions was monitored. The suspensions were administered by stomach tube.

The dosage groups are shown in the following table.

| Group | Male | Female | Dose (mg/kg) | Conc ( $\mu$ g/ml) | Vol (ml/kg) | Animals (M) | Animals (F) |
|-------|------|--------|--------------|--------------------|-------------|-------------|-------------|
| 1     | 50   | 50     | 0            | 0                  | 2.5         | 1-50        | 51-100      |
| 2     | 50   | 50     | 0            | 0                  | 2.5         | 101-150     | 151-200     |
| 3     | 60   | 60     | 0.50         | 0.2                | 2.5         | 201-260     | 261-320     |
| 4     | 60   | 60     | 1.25         | 0.5                | 2.5         | 321-380     | 381-440     |
| 5     | 80   | 80     | 3.00         | 1.2                | 2.5         | 441-520     | 521-600     |
| 6     | 80   | 80     | 6.00         | 2.4                | 2.5         | 601-680     | 681-760     |

The animals that died or were killed for humane reasons were dissected immediately for the pathological examinations of the tissues. Surviving animals were killed one day after the last dose.

Animals were killed by exsanguination under anesthesia (Hostaket) at 50-100 mg/kg the end of the experiment. Exsanguination was conducted by severing the vena cava canalis. Animals that died or killed in moribund conditions were dissected immediately.

#### Results:

The cumulative mortality and animals killed due to a poor health condition before the terminal sacrifice are listed in the following table.

| Wk  | Gr 1 | Gr 1 | Gr 2 | Gr 2 | Gr 3 | Gr 3 | Gr 4 | Gr 4 | Gr 5 | Gr 5 | Gr 6 | Gr 6 |
|-----|------|------|------|------|------|------|------|------|------|------|------|------|
|     | M    | F    | M    | F    | M    | F    | M    | F    | M    | F    | M    | F    |
| 84  |      |      |      |      |      |      |      |      |      |      | 54   |      |
| 106 | 16   | 11   | 18   | 12   | 22   | 20   | 19   | 18   | 23   | 15   |      | 27   |
| %   | 32   | 22   | 36   | 24   | 36.7 | 34   | 31.6 | 30   | 28.8 | 18.7 | 67.5 | 33.8 |

Both male and female rats showed higher mortality than the average control at the highest dose studied. Twenty six-male rats in group 6 survived and were killed on week 84. The data suggest that the high dose (6 mg/kg) showed higher mortality than the control. The pathologist's report

suggests that most of the deaths at 3 and 6 mg/kg were related to the bone marrow and hematopoietic suppression may be due to the immunosuppressive effect of the drug.

**Clinical signs:**

The sponsor has not provided any summary table for the clinical signs. However, the reviewer created a comprehensive list of clinical signs shown below. The figure in the parentheses represents the week when the sign was first observed.

| Signs   | 0 mg/kg          |        | 0.5 mg/kg        |   | 1.25 mg/kg       |   | 3.0 mg/kg        |   | 6 mg/kg        |   |                  |
|---|------------------|--------|------------------|---|------------------|---|------------------|---|----------------|---|------------------|
|   | M;               | F      | M;               | F | M;               | F | M;               | F | M;             | F |                  |
| Alopecia  | 6(8);<br>12(2)   |        | 5(7);<br>15(11)  |   | 10(3);<br>15(7)  |   | 7(5);<br>22(5-9) |   | 1(9);<br>25(2) |   | 9(6);<br>17(4)   |
| Corneal opacity, vascularization, dullness and injury | 1(90);           |        | 3(90);<br>1(91)  |   | 1(43);<br>1(58)  |   | 2(33,81);        |   | ;1(56)         |   | 2(18);<br>5(71)  |
| Cloudy lens   | 2(90);<br>4(91)  |        | 5(86);<br>4(91)  |   | 5(90);<br>4(72)  |   | 4(90);<br>11(85) |   | ;8(91)         |   | 3(72);<br>15(89) |
| Opaque lens   |                  |        | 1(102);<br>1(91) |   | 3(102);<br>3(91) |   | 1(102);<br>4(91) |   | ;7(91)         |   | ;7(91)           |
| Exophthalmos  | 1(90);<br>1(102) |        | 1(87);<br>1(102) |   |                  |   | 1(96);<br>1(98)  |   | ;3(59)         |   | 2(59);<br>3(66)  |
| Mandibular anomaly                                    |                  | ;1(22) |                  |   |                  |   |                  |   | ;1(7)          |   | 1(6);1(13)       |
| Lid margin blood encrust                              | 3(43);1(72)      |        | 1(91);<br>1(21)  |   | 4(12-24);        |   | 3(72);           |   | 3(14);<br>3(7) |   | 4(7);4(85)       |

|   |        |                 |            |                 |        |                 |
|---|--------|-----------------|------------|-----------------|--------|-----------------|
| Upper incisor oblique/ fallen or broken | 2(81)  |                 | 2(15)2(63) | 2(9-46)         |        | 1(51)2(11)      |
| Lower incisor trimmed, fallen out       | ;2(76) | 1(86);          |            | 1(82);<br>1(75) | ;1(9)  | 1(61);<br>2(21) |
| Ulceration in hind limb                 | 3(86); | 1(69);<br>2(94) | 6(76);     | 1(5);<br>2(85)  |        | ;3(85)          |
| Reddish Urine                           | ;1(83) | ;1(44)          |            |                 | 1(44); |                 |
| Swollen eyelids                         |        |                 | 1(11);     |                 |        |                 |
| Enlg Testes                             |        | 1(85);          | 1(88);     |                 |        |                 |
| Uterine Prolapse                        |        | ;1(44)          |            |                 |        |                 |
| Eyeball gross destruction               | ;1(71) |                 |            |                 |        |                 |
| Open wound in throat/hind limb          | ;1(40) |                 |            |                 |        |                 |
| Diarrhea                                | 2(85); |                 |            |                 |        |                 |

**Conclusion for the clinical findings:**

The clinical signs observed during the study suggest that animals in the control and treated groups showed alopecia, corneal opacity and opacity of the lens in rats. However, a comparison of the dose effects among various treated groups showed that female rats at 1.25 mg/kg (gr 4), 3.00

mg/kg (gr 5) and 6.00 (gr 6) showed increased incidences of alopecia and opacity of lens. The lenticular changes were affected from weeks 85-91 of the treatment. Alopecia was noticed as early as two weeks of the treatment in the control and treated groups. Female rats at 3.00 and 6.00 mg/kg doses also showed an increase in the incidence of exophthalmos from the treatment week 59. Increased cloudiness, vascularization and dullness were observed at 6.0 mg/kg doses in female rats from weeks 71 onwards. The sponsor stated that total numbers of opacity and cloudiness in the lens in Wistar WU rats were 8 in male and 8 in female out of 100 rats per sex in the control groups of the experiment. It should be mentioned in this regard that male rats also showed an increase in the opacity and cloudiness in lens. However, incidences in female rats were higher.

Body weights (g) at the beginning and end of the treatment period in male rats are shown in the following table.

| Day              | Gr I    | Gr II   | Gr III    | Gr IV      | Gr V      | Gr VI     |
|------------------|---------|---------|-----------|------------|-----------|-----------|
|                  | 0 mg/kg | 0 mg/kg | 0.5 mg/kg | 1.25 mg/kg | 3.0 mg/kg | 6.0 mg/kg |
| Day 1, Mean      | 125.2 g | 124.2 g | 125.8 g   | 124.4 g    | 122.5 g   | 122.3 g   |
| SD               | 8.3     | 8.3     | 8.0       | 7.1        | 7.4       | 8.0       |
| n                | 50      | 50      | 60        | 60         | 80        | 80        |
| Day 582,583,wk83 | 551.4   | 539.2   | 534.3     | 537.5      | 521.7     | 514.7     |
| SD               | 49.1    | 53.0    | 48.1      | 41.4       | 47.8      | 44.6      |
| n                | 45      | 48      | 48        | 53         | 71        | 26        |
| Day 722,wk103    | 543.2   | 517.8   | 526.8     | 507.9      | 514.9     |           |
| SD               | 59.2    | 59.0    | 60.4      | 58.8       | 53.7      |           |
| n                | 32      | 36      | 40        | 45         | 59        |           |

The body weight gain during the treatment period in male rats was 418, 393, 401, 383.5, 392.4 and 392.4 G for groups 1, 2, 3, 4, 5 and 6, respectively. The data suggest that there was no effect of the treatment on the body weight of rats for the treated groups 3, 4 and 5. For group 6, at day 583, about 7% decrease in the body weight was noticed in the male rats.

The body weight ( g) for female rats during the treatment is shown in the following table.

| Day           | Gr I  | Gr II | Gr III | Gr IV | Gr V  | Gr VI |
|---------------|-------|-------|--------|-------|-------|-------|
| Day 1, Mean   | 109.3 | 108.8 | 109.5  | 108.7 | 107.0 | 107.3 |
| SD            | 6.7   | 8.7   | 7.1    | 7.4   | 7.4   | 6.6   |
| N             | 50    | 50    | 60     | 60    | 80    | 80    |
| Day 722, Mean | 330   | 324.5 | 331.4  | 317.5 | 324.0 | 315.4 |
| SD            | 44.6  | 41.0  | 43.3   | 28.9  | 42.9  | 32.5  |
| N             | 38    | 41    | 41     | 43    | 66    | 55    |

The weight gain during the treatment was 220, 215.7, 221.9, 208.8, 217 and 208.1 G for groups 1, 2, 3, 4, 5 and 6, respectively. Group 6 female rats lost about 4% body weight or body weight gain compared to the average of two controls.

Therefore, body weight gain was comparable between the control and treatment groups in male and female animals. The food consumption was also unaffected by the treatment.

#### Hematology:

The interim values were obtained at week 56 from the first surviving 10 M/F animals from groups 2-6. The second sets of data were collected from all surviving male rats at week 84 from group 6 referred as final value 1. On week 104-105 a final hematology data were collected from first 10 M/F rats in the groups 2-5 and first 10 F from group 6, these data were referred as the final value 2.

Data on week 56 did not show any changes in the treated groups for the erythrocyte parameters including Heinz bodies compared to the control in male rats. **There were no significant changes in the leukocyte counts.** However, at 6 mg/kg doses, the count was increased from  $4.0 \times 10^9/L$  in the control group to  $4.5 \times 10^9/L$  in male rats (statistically not significant). A similar increase in the leukocyte counts was also observed at 3 and 6 mg/kg doses in female rats that was not statistically significant. The changes in the leucocyte counts might be due to the biological variation.

Male rats at 6 mg/kg dose did not show statistically significant changes on week 84 compared to the control on week 56. There were no data from the control animal on week 84.

The leukocyte counts in male rats on week 104 were reduced significantly from 3.9 in the control

to 2.6 and  $3.0 \times 10^9/L$  at 1.25 and 3.00 mg/kg doses, respectively. On the other hand, the leukocyte counts in female rats were increased from  $2.3 \times 10^9/L$  to  $4.2 \times 10^9/L$ . However, it was not statistically significant.

These data suggest that the decrease in the leukocyte counts in male rats may be due to its immunosuppressive action. However, its onset was after 84 weeks. A similar effect was not observed in the female rats.

#### Clinical Chemistry:

These data were collected at the same time point as that of the hematology data. The method does not state clearly whether whole blood or plasma levels were reported. The following table represents the statistically significant changes in male and female animals during the interim analysis on week 56.

| Male                            | Control | 0.5 mg/kg | 1.25 mg/kg | 3.00 mg/kg | 6.00 mg/kg |
|---------------------------------|---------|-----------|------------|------------|------------|
| Bilirubin( $\mu\text{mol/L}$ )  | 4.9     | 5.0       | 5.0        | 4.2*       | 4.3*       |
| Creatinine( $\mu\text{mol/L}$ ) | 37      | 39        | 34         | 36         | 40*        |
| ALAT(GPT, $\text{u/L}$ )        | 33      | 36        | 47         | 36         | 56*        |
| TG ( $\text{mmol/L}$ )          | 2.51    | 2.40      | 1.81       | 2.03       | 1.64*      |
| <b>Female</b>                   |         |           |            |            |            |
| Uric acid( $\mu\text{mol/L}$ )  | 54      | 46        | 45         | 48         | 45*        |
| Calcium ( $\text{mmol/L}$ )     | 2.53    | 2.55      | 2.59       | 2.55       | 2.63*      |
| Glucose( $\text{mmol/L}$ )      | 6.06    | 5.89      | 5.78       | 5.34*      | 5.32*      |
| Total Lipids( $\text{g/L}$ )    | 5.33    | 5.88      | 5.74       | 5.46       | 6.34*      |
| ASAT(GOT, $\text{u/L}$ )        | 50      | 52        | 46         | 49         | 41*        |

The bilirubin levels were significantly reduced from  $4.9 \mu\text{mol/L}$  to  $4.2-4.3 \mu\text{mol/L}$  at 3 and 6 mg/kg doses in male rats on week 56. The transaminase activity (ALAT) and creatinine levels were also significantly increased at 6 mg/kg in male rats on week 56. The transaminase activity (GOT) was decreased at 6 mg/kg in female rats on week 56.

The data on week 84 for male rats at 6 mg/kg dose are shown in the following table. Since there was no corresponding control data, the statistical significance could not be determined.

| Parameters | 6 mg/kg, week 56 | 6 mg/kg, week 84 |
|------------|------------------|------------------|
| Uric acid  | 55               | 118              |
| Creatinine | 40               | 74               |
| ALAT       | 56               | 45               |

Above data showed an increase in the uric acid, creatinine and a decrease in the ALAT activity when compared to that of the data on week 56 from same animals.

It appears that the male rats at the high dose had kidney and liver toxicity.

The clinical chemistry data on week 104 for male rats groups 1-5 and female rats groups 1-6 are presented in the following table.

| Male          | Control | 0.5 mg/kg | 1.25 mg/kg | 3.0 mg/kg | 6 mg/kg |
|---------------|---------|-----------|------------|-----------|---------|
| Inorg Phos.   | 2.29    | 2.37      | 2.23       | 2.42*     |         |
| ASAT(GOT)     | 47      | 34*       | 32*        | 33*       |         |
| <b>Female</b> |         |           |            |           |         |
| Calcium       | 2.36    | 2.41      | 2.41       | 2.54*     | 2.48*   |
| Inorg Phos    | 1.56    | 1.73*     | 1.84*      | 2.45*     | 2.11*   |
| Serum Glucose | 4.86    | 5.24      | 5.33       | 5.48*     | 5.44*   |

Inorganic phosphate levels were significantly increased and GOT activity was significantly decreased at 3 mg/kg dose in male rats.

Female rats showed a dose related increase in the calcium, inorganic phosphate and glucose levels.

Conclusion for the clinical chemistry:

Above data on the serum chemistry parameters suggest that there may be drug related toxicities in the kidney and liver in male and female rats. It is also important to note that increase in the inorganic phosphate at the end of 2 years of treatment in male and female rats at 3-6 mg/kg doses may be related to the kidney toxicity or an effect related to the bone metabolism that was not present at the end of one year of dosing. The finding has significant importance to the long

term treatment of the drug for joint diseases.

**Electrophoresis Data:**

Changes in the globulin proteins measured by electrophoretic separation techniques are shown in the following table.

| Parameter                | Sex | Week | Control | 0.5    | 1.25   | 3.0    | 6.0 mg/kg |
|--------------------------|-----|------|---------|--------|--------|--------|-----------|
| γ-globulin               | M   | 56   | 0.048   | 0.043  | 0.040  | 0.033* | 0.033*    |
| Albumin                  | F   | 56   | 0.571   | 0.622* | 0.629  | 0.634* | 0.644*    |
| γ-globulin               | F   | 56   | 0.135   | 0.115* | 0.122* | 0.177* | 0.112*    |
| γ <sub>2</sub> -globulin | F   | 56   | 0.051   | 0.046  | 0.045  | 0.049  | 0.043*    |
| β-globulin               | F   | 56   | 0.139   | 0.124* | 0.124* | 0.117* | 0.123*    |
| γ-globulin               | F   | 56   | 0.059   | 0.047* | 0.037* | 0.034* | 0.034*    |
|                          |     |      |         |        |        |        |           |
| γ-globulin               | M   | 104  | 0.060   | 0.056  | 0.047* | 0.042* |           |
| γ-globulin               | F   | 104  | 0.061   | 0.054  | 0.059  | 0.054  | 0.050*    |

The gamma globulin levels at 3 and 6 mg/kg doses in male rats and at 0.5-6.0 mg/kg doses in female rats were reduced significantly on week 56.

At the end of week 104 similar decrease in the gamma globulin levels were observed at 1.25 mg and 3.0 mg/kg doses in male rats and 6.0 mg/kg doses in the female rats.

The decrease in the gamma globulin levels suggest that the drug may inhibit lymphocyte derived antibody synthesis due to immunosuppression beginning week 54.

Urine chemistry data did not show abnormalities except changes in the specific gravity.

**Organ Weight:**

Organ weights relative to the body weight in male rats showed a statistically significant increase in the weight of lungs at 1.25 (1%) and 3.0 mg/kg (8%). Similarly, statistically significant increase in the weight of the testes was noted at 3 mg/kg dose. The increase was 1.8%. The percent increase in the normalized data was determined from the respective control.

The normalized weight of the liver was increased significantly at 3.0 (4%) and 6.0 (6%) mg/kg doses in female rats.

The data suggest that liver and testes may be the target organ of toxicity following chronic treatment with Leflunomide. The changes in the lung in male rats may be due to the secondary changes due to the immunosuppressant properties of the drug.

Increased palpable masses were observed in female rats. The data for control 1, control 2, 0.5, 1.25, 3.0 and 6.0 mg/kg doses were 8, 16, 21.7, 23.3, 15.0 and 6.3%, respectively.

#### Macroscopic Pathology:

Most of the changes in the male rats were observed at 3 and 6 mg/kg doses. The changes were discolored pancreas, testes, consistency changes in the bone marrow and reddish lymph nodes. Changes in the bone marrow and lymph nodes were also observed in female rats at 6 mg/kg dose.

#### Histological Findings:

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#### Neoplastic Findings:

Neoplastic lesions in male rats are shown in the following table. Number in the parenthesis represents number of animals examined. Number of animals were 50, 50, 60, 60, 80 and 80 for grs 1, 2, 3, 4, 5 and 6, respectively. Only those tumors that have a possibility of a significance and greater than the control are listed here. It appears that there may be typographical errors for the total number of tissues examined for the malignant lymphoma. However, the problem has been corrected in the statistical database.

| Organ                                    | Gr 1  | Gr2   | Gr 3   | Gr 4   | Gr 5   | Gr 6  |
|--|-------|-------|--------|--------|--------|-------|
| Hemolymphoret sys,<br>Lymphoma Malignant | 0(1)  | 0(3)  | 1(3)   | 0(3)   | 1(1)   |       |
| Skin, Squamous cell carcinoma            | 0(50) | 0(50) | 0 (60) | 1 (59) | 1 (80) | 0(79) |
| Cerebellum, Medulloblastoma              | 0(50) | 0(50) | 0(60)  | 0(59)  | 1(80)  | 0(80) |
| Hepatocellular Adenoma                   | 0(49) | 0(50) | 0(59)  | 1(59)  | 1(80)  | 1(77) |

Neoplastic lesions in the female rats are similarly shown in the following table. Number of rats were 50, 50, 60, 60, 80 and 80 for gr 1, gr 2, gr 3, gr 4, gr 5 and gr 6, respectively.

| Organ                       | Gr 1  | Gr 2  | Gr 3  | Gr 4  | Gr 5  | Gr 6  |
|-----------------------------|-------|-------|-------|-------|-------|-------|
| Hepato cell. Adenoma        | 0(48) | 1(50) | 0(60) | 2(59) | 1(80) | 4(80) |
| Hepato cell Carcinoma       | 0(48) | 0(50) | 0(60) | 1(59) | 0(80) | 0(80) |
| Hepato. Carc + Aden.        | 0(48) | 1(50) | 0(60) | 3(59) | 1(80) | 4(80) |
| Uterus, glandular polyps    | 0(50) | 0(50) | 0(60) | 1(60) | 0(80) | 4(80) |
| Endomet. Strom. Sarcoma     | 0(49) | 0(50) | 0(60) | 0(60) | 2(80) | 0(79) |
| Malignant Thymoma           | 0(36) | 0(33) | 0(38) | 0(38) | 0(53) | 1(57) |
| Benign Thymoma              | 1(36) | 2(33) | 1(38) | 1(38) | 7(53) | 5(57) |
| Total Thymoma               | 1(36) | 2(33) | 1(38) | 1(38) | 7(53) | 6(57) |
| Malig. schwannoma of vagina | 0(48) | 0(48) | 0(56) | 0(59) | 0(80) | 1(78) |
| Clitoral gland adenoma      | 0(2)  |       |       |       | 0(1)  | 1(1)  |

## Non Neoplastic Findings in male rats:

Figure in the parenthesis represents the number of animals examined for each lesion

| Organ                          | Gr 1  | Gr 2  | Gr 3  | Gr 4  | Gr 5  | Gr 6   |
|--------------------------------|-------|-------|-------|-------|-------|--------|
| Medulla Oblangata, Hemorrhage  | 1(47) | 1(47) | 2(55) | 0(54) | 2(75) | 28(76) |
| Cer Spinal Cord Hemor.         | 0(38) | 0(43) | 0(51) | 0(53) | 1(64) | 37(76) |
| Thorc. Spinal Cord Hemor.      | 0(43) | 0(45) | 0(57) | 0(56) | 3(77) | 29(74) |
| Lung Infl. Lymph. Cell         | 1(50) | 1(50) | 2(60) | 0(60) | 9(80) | 6(80)  |
| Alveolar edema                 | 2(50) | 1(50) | 1(60) | 0(60) | 1(80) | 14(80) |
| Atrophy, Duod, Jej, Ilium      | 4(43) | 1(38) | 0(48) | 0(50) | 2(68) | 13(43) |
| Centrilob Necrosis, Liver      | 0(50) | 1(51) | 2(60) | 0(60) | 1(80) | 8(80)  |
| Inflammation, Pancreas         | 1(50) | 0(50) | 3(60) | 1(60) | 2(80) | 16(80) |
| Extra Med Myelopoiesis, Spleen | 2(50) | 1(50) | 2(60) | 1(60) | 0(80) | 11(80) |

|                              |       |       |       |       |       |        |
|------------------------------|-------|-------|-------|-------|-------|--------|
| Hemorrhage, Urinary bladder  | 0(47) | 0(48) | 0(60) | 0(58) | 0(80) | 10(77) |
| Testes, oligospermia         | 0(50) | 0(50) | 1(60) | 2(59) | 4(80) | 5(80)  |
| Panmyelopathy, congestion    | 2(50) | 0(50) | 4(60) | 4(59) | 9(80) | 50(80) |
| Thymus, Atrophy              | 8(30) | 6(23) | 8(35) | 2(30) | 6(55) | 32(50) |
| Cer. Lymph node Hemor.       | 3(44) | 2(34) | 8(60) | 5(60) | 3(75) | 40(80) |
| Mes. Lymph. node Hemor.      | 7(45) | 5(46) | 6(56) | 3(55) | 7(70) | 41(74) |
| Iliac. Lymph. node Hemor.    | 4(41) | 3(39) | 5(51) | 4(47) | 5(66) | 40(70) |
| Corneal Vascularization, eye | 2(49) | 1(50) | 4(60) | 0(60) | 1(80) | 9(80)  |
| Femur Endostosis             | 0(49) | 0(46) | 1(59) | 0(58) | 0(79) | 3(79)  |
| Sternum, deformation         | 0(50) | 0(50) | 0(59) | 0(57) | 0(80) | 1(80)  |

## Non neoplastic lesions, female rats:

| Organ                                     | Gr 1  | Gr 2   | Gr 3  | Gr 4  | Gr 5   | Gr 6   |
|---|-------|--------|-------|-------|--------|--------|
| Medulla Oblangata, Hemor                  | 0(41) | 2(45)  | 3(59) | 1(54) | 2(77)  | 8(78)  |
| Spinal Cord, Hemor                        | 0(43) | 2(46)  | 1(58) | 1(55) | 4(73)  | 22(76) |
| Alveolar edema, lung                      | 0(49) | 1(50)  | 1(60) | 0(60) | 0(80)  | 6(80)  |
| Inflam. Lymphoid Cell, lung               | 0(49) | 0(50)  | 1(60) | 2(60) | 10(80) | 5(80)  |
| Ulceration, forestomach                   | 0(49) | 0(50)  | 0(60) | 2(60) | 0(80)  | 1(80)  |
| Focal necrosis, Liver                     | 2(48) | 1(50)  | 1(60) | 2(59) | 5(80)  | 7(80)  |
| Bone marrow, panmyelopathy and congestion | 0(49) | 0(50)  | 0(60) | 0(60) | 5(80)  | 25(80) |
| Thymus atrophy                            | 3(36) | 3(33)  | 3(38) | 1(38) | 2(53)  | 15(57) |
| Hemorrhage, lymph nodes combined          | 4(41) | 11(46) | 7(56) | 6(54) | 41(78) | 38(77) |
| Eyes, Lenticular Degeneration             | 4(48) | 6(50)  | 1(59) | 6(59) | 29(79) | 36(80) |
| Corneal Vascularization                   | 0(48) | 1(50)  | 0(59) | 0(59) | 0(79)  | 3(80)  |

**Reviewer's Comment on neoplastic lesions:**

The carcinogenicity findings are reviewed by the biostatistician. The pharmacology and statistical reviews will be further discussed in the CAC executive committee of CDER. The reviewer's recommendations will include the findings of the statistical reviewer. Same process will be applied for the mice study.

**Male rats:**

Male rats showed some evidence of malignant lymphoma. Therefore, statistical significance of the finding needs to be compared before reaching a final conclusion. It should be noted that lymphoma is a mechanism-based toxicity for immunosuppressants.

Male rats also showed incidences of adenoma of liver at 1.25-6 mg/kg doses. The incidence did not increase with the dose. The sponsor has not provided historical data for adenoma in the liver. This lesion is also seen in the female rats. The lesion may be related to the treatment.

Squamous cell carcinomas in the skin were noted at 1.5 and 3.0 mg/kg doses. The sponsor has not provided any historical control data for the tumor. Given the fact that male rats in the group VI were killed before the scheduled termination, this lesion may be treatment related. A similar conclusion can be drawn for the incidence of medulloblastoma in the cerebellum.

**Female rats:**

Hepatocellular adenoma in female rats showed an increase in the incidence (5%) at 6 mg/kg dose compared to the control. Carcinoma alone did not show a dose related trend. However, when adenoma and carcinoma were combined, an increase in the liver lesions were present in a dose related manner. The sponsor stated that the historical control for Wistar rats from the supplier for hepatocellular adenoma in female rats was 0-15%. Considering the data, the sponsor stated that the lesions in the liver in female rats were not drug related. The statistical review for the finding needs to be considered.

The female rats showed several lesions in the uterus, e.g., endometrial sarcoma, glandular polyps, endometrial stromal polyps and adenocarcinoma and adenoma of the uterus. Historical controls for glandular polyps of the uterus is 8.2%. Female rats showed benign and malignant thymomas. The effect may be related to immunosuppression. The combined incidences of thymoma in female rats appeared to be dose related and induced by the drug. Although the sponsor suggested that 0-16.7% thymomas were seen in the historical control, it may well be considered as the toxicity of leflunomide. Thymoma may be expected as a consequence of immunosuppression to Leflunomide. Leflunomide treated female rats also showed malignant schwannoma of vagina. The sponsor has not provided the historical incidences and it appears to be a rare tumor. Adenoma of clitoral

glands was also observed at the high doses. This can also be considered as a rare tumor and may be drug related.

Reviewer's comments on non neoplastic lesions:

Male rats:

Hemorrhage in the brain and spinal cord were noticed at 3-6 mg/kg doses. The hemorrhage in the brain or the spinal cord may be associated with myelopathy as suggested by the sponsor.

Otherwise, the method of exsanguination could have contributed to the change. Hemorrhage in the brain was also noted in the female rats.

Inflammation in the lung was observed at 3-6 mg/kg doses characterized by alveolar edema and invasion of inflammatory cells in the lung. Inflammation in the pancreas was observed at 6 mg/kg dose. This might have resulted from the prolonged immunosuppression by leflunomide.

Atrophy of the GI tract and necrosis of the liver were observed at 6 mg/kg dose. It is possible that the effect of the drug on the GI epithelial cell synthesis may be responsible for this side effect as seen with other nucleotide synthesis inhibitors like methotrexate. Leflunomide may have liver toxicity on a chronic administration based on the incidences of necrosis in the liver.

The long term immunosuppression with leflunomide at 3 mg/kg and higher doses was associated with bone marrow congestion. Thymus atrophy and hemorrhage at lymph nodes were seen at 6 mg/kg dose.

As expected from a pyrimidine synthesis inhibitor or a DNA synthesis inhibitor, leflunomide inhibited the development of sperm cells at all doses tested. However, incidences were higher at 3(n=4) and 6 mg/kg(n=5) doses.

An increase in the corneal vascularization was noted at 6 mg/kg doses. It is not clear whether it is a class effect of immunosuppressants.

It is concluded that 6 mg/kg dose was a toxic dose for the long term toxicity to leflunomide. Liver is the target organ of toxicity as characterized by the histological changes and the abnormality in the transaminase activity. An increase in the creatinine levels at 6 mg/kg dose was observed at the end of 56 weeks. However, treatment related changes in the kidney were not evident at 6 mg/kg dose.

A dose of 3 mg/kg was better tolerated for the chronic use in rats. The toxicity at 3 mg/kg dose was hemorrhage in the brain, spinal cord, inflammation of the lung, bone marrow congestion and oligospermia.

An increase in the inorganic phosphate was observed at 3 mg/kg dose at the end of 104 weeks. In the absence of the treatment related pathological changes in the bone or kidney, the significance of the finding is not clear.

**Non neoplastic findings in female rats:**

Hemorrhage in the brain and spinal cord were observed at 3 and 6 mg/kg doses. Inflammation in the lung was present at 3 and 6 mg/kg doses, this effect may be related to the immunosuppressant activity of leflunomide.

The control rats showed incidences of necrosis of the liver (4.16%). However, the incidences in the necrosis of the liver at 3 and 6 mg/kg doses were 6.25 and 7.25%, respectively. Although the magnitude of liver toxicity to Leflunomide in female rats was smaller than that of the male rats, the data indicate that female rats at 6 mg/kg showed an increase in the hepatotoxicity.

Bone marrow congestion was present in animals treated at 3 and 6 mg/kg doses. Atrophy of the thymus was observed at 6 mg/kg dose. These effects were also related to the immunosuppression.

Lenticular degeneration of the eye was observed at 3-6 mg/kg doses. Corneal vascularization was observed at 6 mg/kg dose.

The adverse effects expected from the chronic treatment with leflunomide are brain and spinal cord hemorrhage, necrosis of the liver, oligospermia, lenticular degeneration and corneal vascularization in the eye. These effects were mainly observed at 6 mg/kg dose in both male and female animals. Other toxicities to the long term treatment with leflunomide may be related to the immunosuppression, e.g., bone marrow toxicity, hematopoietic systems. These effects need to be characterized clinically. **Abnormal calcium and phosphate levels in the serum may be due to the altered kidney function or due to an effect on the bone and endocrine functions.** However, treatment related kidney lesions were not evident from the study.

**Toxicokinetics of A 771726 for 2 year toxicity in rats:**

Page 5-17925, vol. 55:

The primary metabolite of leflunomide is A 771726. The kinetics of the metabolite were measured. Since the primary site of the carcinogenicity study is not mentioned in the report, it is necessary to investigate the integrity of the study in relation to the shipment of samples and the laboratories in \_\_\_\_\_ where the assays were conducted.

Blood samples were collected for the determination of serum levels of A 771726. The blood samples were collected on days 380 (54wk), 555 (79wk), 724 (103wk) for male rats except for male rats at 6 mg/kg dose (gr 6). Samples from male rats for gr 6 were collected on day 583 (wk 83).

Blood samples for female rats were collected on days 381 (wk 54), 556 (wk 79) and 724 (wk 103). Blood samples were collected from 6 animals per time point at 0.5, 1, 2 and 4 hours post dose from the retro orbital plexus. A 24-hour post dose sample was also taken on week 79 from these animals for the determination of the trough levels. However, the data point for 24 hour sampling was not considered in the analysis, instead the data point at 0 hour was used for determining the trapezoid areas under the curve. This was done because 24 hour data points were not available for all periods at which blood samples were collected. The sponsor has not provided the differences of A 771726 between 0 and 24 hour samples.

The limit of quantitation was 0.1  $\mu\text{g/ml}$  for samples collected at the end of the dosing period. The area under the curve and  $C_{\text{max}}$  were calculated and normalized to the dose.

Results:  $T_{\text{max}}$  in male and female rats varied between 1-4 hours. Serum  $C_{\text{max}}$  ( $\mu\text{g/ml}$ ) and dose normalized data for male rats are shown in the following table. ND in the table represents not detected.

| Dose mg/kg | Day 380          | Day 380    | Day 555          | Day 555    | Day 723          | Day 723    |
|------------|------------------|------------|------------------|------------|------------------|------------|
|            | $C_{\text{max}}$ | Normalized | $C_{\text{max}}$ | Normalized | $C_{\text{max}}$ | Normalized |
| 0          | ND               | ND         | ND               | ND         | ND               | ND         |
| 0.5        | 1.33             | 2.66       | 1.36             | 2.72       | 0.93             | 1.86       |
| 1.25       | 2.87             | 2.30       | 3.72             | 2.98       | 2.18             | 1.74       |
| 3.0        | 7.26             | 2.42       | 6.77             | 2.26       | 2.41             | 0.803      |
| 6.0        | 13.1             | 2.18       | 11.2             | 1.87       | Killed           | killed     |

The data suggest that the  $C_{\text{max}}$  was dose proportional for A 771726. No accumulation was observed after chronic dosing. However, at the end of two years,  $C_{\text{max}}$  at all dose levels was reduced. This effect may be related to the hepatotoxicity of the drug. The reduction of A 771726 levels after two years may be important because the drug would not induce overt immunosuppression. On the other hand, the liver function needs to be assessed upon chronic administration.

Data from the female rats are similarly presented in the following table.

| Dose mg/kg | Day 381 <sup>~</sup> | Day 381    | Day 556          | Day 556    | Day 724          | Day 724    |
|------------|----------------------|------------|------------------|------------|------------------|------------|
|            | C <sub>max</sub>     | Normalized | C <sub>max</sub> | Normalized | C <sub>max</sub> | Normalized |
| 0          | ND                   |            | ND               |            | ND               |            |
| 0.5        | 3.71                 | 7.42       | 1.80             | 3.60       | 0.79             | 1.58       |
| 1.25       | 3.63                 | 2.90       | 4.25             | 3.40       | 2.10             | 1.68       |
| 3.0        | 8.45                 | 2.82       | 8.14             | 2.71       | 4.56             | 1.52       |
| 6.0        | 13.9                 | 2.32       | 15.0             | 2.50       | 8.99             | 1.50       |

C<sub>max</sub> in female rats was dose proportionate although the variability was more pronounced in females than male rats. There seems to be no differences in the male and female rats with respect to the C<sub>max</sub>. However, female rats also showed a decreased levels at the end of two years that might be due to the toxicity of the liver specially at 6 mg/kg dose. One can also argue that the changes in the kinetics after the first year of the treatment were related to the aging process.

The exposure data for A771726 in male rats are shown in the following table.

| Dose mg/kg | Day 380 AUC(μg h/ml) | Day 380 Norm. | Day 555 AUC | Day 555 Norm. | Day 723 AUC | Day 723 Norm. |
|------------|----------------------|---------------|-------------|---------------|-------------|---------------|
| 0          | ND                   | ND            | ND          | ND            | ND          | ND            |
| 0.5        | 4.43                 | 8.86          | 4.51        | 9.02          | 3.14        | 6.28          |
| 1.25       | 9.36                 | 7.49          | 11.4        | 9.12          | 5.10        | 4.08          |
| 3.0        | 23.4                 | 7.80          | 22.1        | 7.37          | 6.02        | 2.01          |
| 6.0        | 40.9                 | 6.82          | 27.2        | 4.53          |             |               |

The data on exposure to A 771726 in male rats suggests that the exposure was dose proportionate up to week 54. However, data at 6 mg/kg on week 79 and at other doses on weeks 103 suggest that the exposure was reduced. As mentioned for the C<sub>max</sub> data above, the decrease exposure at the end of the dosing period may reflect liver toxicity or age related changes.

The exposure to A 771726 in female rats is shown in the following table.

| Dose<br>mg/kg | Day 381 | Day 381    | Day 556 | Day 556    | Day 724 | Day 724    |
|---------------|---------|------------|---------|------------|---------|------------|
|               | AUC     | Normalized | AUC     | Normalized | AUC     | Normalized |
| 0             | ND      | ND         | ND      | ND         | ND      | ND         |
| 0.5           | 9.75    | 19.5       | 4.59    | 9.18       | 2.57    | 5.16       |
| 1.25          | 11.9    | 9.52       | 14.1    | 11.3       | 6.78    | 5.42       |
| 3.0           | 26.3    | 8.77       | 22.1    | 7.37       | 12.2    | 4.07       |
| 6.0           | 45.6    | 7.60       | 50.7    | 8.45       | 22.4    | 3.73       |

The data suggest that the exposure was dose proportionate in female rats. However, at the end of the dosing period, the exposure was reduced.

It can be summarized from the data that A 771726 ( an active metabolite of leflunomide) did not accumulate in the serum on chronic administration in rats. There appears to be no differences in the gender for kinetics. There may be a relationship between serum exposure and liver toxicity so that the exposure to the metabolite was reduced at the end of two years or when toxicity to the rats was predominant, e.g., reduced exposures at 6 mg/kg male at the end of week 79. The half life of the drug in rats was not calculated in these studies.

#### Toxicokinetic of TFMA in rats:

Page 5-17986, vol 55:

TFMA is a minor metabolite of leflunomide in humans. It is mostly formed in the dogs and has potential for causing anemic changes. TFMA levels were assayed in rats after chronic dosing for a comparison to the human levels observed in the clinical studies.

The blood samples were collected on days 365, 485, 555 and 723 in male rats and on days 365, 486, 556 and 724 for female rats. It should be noted that last samples from high dose male rats were taken on days 583.

The serum level of TFMA was determined. However, the sponsor stated in the method section that the plasma samples were extracted with hexane. The assay was performed using

The exposure to TFMA was determined using data between 0 and 4-hour sample time.

TFMA was not detected in male and female rats at 0.5 mg/kg dose. The  $T_{max}$  at higher doses was

between 1-4 hours.

TFMA was not detected up to 1.25 mg/kg dose in male rats throughout the observation period. Also, no TFMA was detected on days 723 at 3 mg/kg dose in male rats. The  $C_{max}$  (ng/ml) and dose normalized  $C_{max}$  in male rats are shown in the following table. Male rats at 6 mg/kg were killed after 583 days.

| Dose mg/kg | Days 365  | Days 365   | Days 485  | Days 485   | Days 555  | Days 555   | Days 583       |
|------------|-----------|------------|-----------|------------|-----------|------------|----------------|
|            | $C_{max}$ | Normalized | $C_{max}$ | Normalized | $C_{max}$ | Normalized | $C_{max}$ /Nr. |
| 3.0        | 8.62      | 2.87       | 6.58      | 2.19       | 8.88      | 2.96       | NS             |
| 6.0        | 18.1      | 3.02       | 14.3      | 2.38       | 17.8      | 2.97       | 14.7/ 2.45     |

NS= not sampled

Exposure to TFMA in male rats is shown in the following table. The AUC and dose normalized AUC were calculated in ng.h/ml. No exposure to TFMA was detected at 0.5 to 3.0 mg/kg on day 723. Male rats at 6 mg/kg dose were killed on day 583.

| Dose mg/kg | Day 365 | Day 365 | Day 485 | Day 485 | Day 555 | Day 555 | Day 583   |
|------------|---------|---------|---------|---------|---------|---------|-----------|
|            | AUC     | Norm    | AUC     | Norm    | AUC     | Norm    | AUC/Nr    |
| 3.0        | 29.6    | 9.87    | 22.9    | 7.63    | 24.7    | 8.23    | NS        |
| 6.0        | 58.5    | 9.75    | 48.7    | 8.12    | 57.0    | 9.50    | 46.5/7.75 |

The  $C_{max}$  (ng/ml) and dose normalized  $C_{max}$  for TFMA in female rats is shown in the following table. No TFMA was detected at 0.5 mg/kg.

| Dose mg/kg | Day 365   | Day 365 | Day 486   | Day 486 | Day 556   | Day 556 | Day 724   | Day 724 |
|------------|-----------|---------|-----------|---------|-----------|---------|-----------|---------|
|            | $C_{max}$ | Norm.   | $C_{max}$ | Norm.   | $C_{max}$ | Norm.   | $C_{max}$ | Norm.   |
| 1.25       | 6.0       | 4.8     | 6.43      | 5.14    | 6.2       | 4.96    | ND        |         |
| 3.0        | 12.9      | 4.3     | 12.6      | 4.2     | 13.8      | 4.6     | 5.83      | 1.94    |
| 6.0        | 28.6      | 4.77    | 28.3      | 4.72    | 19.7      | 3.28    | 11.5      | 1.92    |

ND= Not detected

The AUC (ng.h/ml) and normalized AUC (Nr) are shown in the following table.

| Dose mg/kg | Day 365AUC | Day 365 Nr | Day 486AUC | Day 486Nr | Day 556AUC | Day 556Nr | Day 724AUC | Day 724Nr |
|------------|------------|------------|------------|-----------|------------|-----------|------------|-----------|
| 1.25       | 20.8       | 16.6       | 15.3       | 12.2      | 21.2       | 17.0      | ND         |           |
| 3.0        | 47.3       | 15.8       | 40.4       | 13.5      | 42.2       | 14.1      | 16.3       | 5.43      |
| 6.0        | 87.6       | 14.6       | 85.7       | 14.3      | 67.6       | 11.3      | 38.9       | 6.48      |

ND=Not detected

**Conclusion for TFMA kinetics in rats:**

Chronic treatment with Leflunomide for two years accumulated TFMA as a metabolite at 1.25 mg/kg and above doses in rats. The level was decreased after about 80 weeks from that observed after one year of the treatment. A similar observation was made for the active metabolite A 771726. The data suggest that the metabolism of the drug was reduced after one year may be due to the liver toxicity or due to the aging process.

The exposure ratio of A 771726 to TFMA are shown in the table below. The exposures to TFMA at 3 and 6 mg/kg were compared to that of A 771726 at the end of one year in male and female rats.

A 771726 to TFMA exposure ratios in rats after 365 days of treatment.

| Dose mg/kg | Male | Female |
|------------|------|--------|
| 3          | 790  | 556    |
| 6          | 699  | 520    |

The data suggest that TFMA levels in the rats were more than 500 fold lower than the active metabolite of Leflunomide.

**Summary of the carcinogenicity in rats:**

Carcinogenicity to leflunomide was investigated in Wistar rats at 0.5, 1.25, 3 and 6 mg/kg doses for 104 weeks. The treatment did not affect the body weight substantially. However, mortality to male and female rats was increased. The male rats at 6 mg/kg had to be sacrificed about 84 weeks after the initiation of the experiment. Based on the increased mortality and toxicity, the reviewer concluded that the treatment was given to reach the maximum tolerated dose in rats.

metabolite and gender differences in the kinetics in rats. A1771726 exposure was more than 500 fold higher than that for TFMA. The human levels of TFMA is about 10.7 ng/ml at the maximum clinical dose recommended. Therefore, rat to human TFMA ratio at the maximum dose would be 1.68 in male rats and 2.67 in female rats. Data for the rats were taken from day 365 samples.

The exposures to metabolites were reduced one year after dosing. This may indicate an incomplete first pass metabolism of leflunomide.

It is concluded that up to 1.25 mg/kg dose was tolerated for two years in rats. At higher doses, several neoplastic and non neoplastic pathological changes were observed.

#### Major findings of the study:

- Uterine polyps
- Liver toxicity
- Corneal and lenticular opacity
- Alopecia
- Increased serum phosphate
- Oligospermia
- Decrease in the plasma levels of A771726 after one year of the treatment with leflunomide.

#### Oral Carcinogenicity study in mice:

Page 5-12052, vol 44:

The oral carcinogenicity in mice was conducted according to the GLP and final audit of the study was signed off in Aug, 1997. The experiment was conducted in male and female mice, Hsd/Ola:ICR (CD 1) strain. Mice were supplied . The average body weight at the beginning of the dosing was 23.4 g for male and 20.8 g for female and they were approximately 5-7 weeks old. Leflunomide was suspended in starch mucilage for dosing by a stomach tube daily for two years. The batch numbers were L 029-1 and E 040. The suspensions were prepared daily. The stability and homogeneity were determined. The dosage groups are shown in the following table.

| Group | No. Animals, Male    | No. Of Animals, Female | Dose, mg/kg/oral |
|-------|----------------------|------------------------|------------------|
| 1     | 50                   | 50                     | Vehicle          |
| 2     | 50 + 16 for Kinetics | 50 + 16 for kinetics   | Vehicle          |
| 3     | 50 + 16 for Kinetics | 50 + 16 for Kinetics   | 1.5              |
| 4     | 50 + 16 for Kinetics | 50 + 16 for kinetics   | 5.0              |
| 5     | 70 + 16 for Kinetics | 70 + 16 for Kinetics   | 15.0             |

Additional animals for the toxicokinetics were dosed once daily for 373 days. During the study period, body weight, food consumption, survival, clinical conditions, slit lamp examinations of eyes, neurological conditions, teeth and oral mucosa and palpable nodules were examined. Blood samples were taken from orbital plexus from non fasted mice from all mice to be killed for humane reasons and from animals that survived to the scheduled sacrifice. Hematological analyses were conducted from these blood samples. Blood samples were also collected from the satellite animals after one year of dosing at 2 and 24 hours after the dosing. Additional kinetics data were collected at the end of 24 months from first surviving 10 M and 10 F mice for toxicokinetics of serum A771726 and TFMA. There is no indication in the method section about clinical chemistry studies in these mice. At the end of the experiment, organ weights and histology were conducted..

#### Results:

The body weight for male mice at the beginning and end of the experiment is shown in the following table.

| Study day | Cont Gr 1 | Cont Gr2 | Drug 1.5 mg/kg | Drug 5 mg/kg | Drug 15 mg/kg |
|-----------|-----------|----------|----------------|--------------|---------------|
| 1         | 23.3 g    | 24.0 g   | 23.3 g         | 23.6 g       | 22.9 g        |
| 722       | 37.0 g    | 38.5 g   | 35.3 g         | 36.0 g       | 34.1 g        |

The body weight in female mice are shown in the following table.

| Study day | Cont Gr 1 | Cont Gr 2 | Drug, 1.5mg/kg | Drug, 5 mg/kg | Drug, 15 mg/kg |
|-----------|-----------|-----------|----------------|---------------|----------------|
| 1         | 20.9 g    | 21.1 g    | 20.7 g         | 20.8 g        | 20.6 g         |
| 722       | 31.0 g    | 30.0 g    | 30.5 g         | 30.0 g        | 28.6 g         |

The male mice at 15 mg/kg dose showed weight gain. However, the weight gain between day 1

and day 722 was about 10% less than the average of two controls. The final body weight at 15 mg/kg dose about 6% less than the average of two controls.

The female mice at 15 mg/kg dose gained body weight. However, increase in the body weight at the end of day 722 was about 10% less than the combined controls. Also, the final body weight at 15 mg/kg was about 6% less than the control when compared to the average weight for the group at the end of day 722.

The average food consumption during two years was 4.45 g/day in the control male mice. The average food consumption for the male mice treated at 15 mg/kg was 4.3 g/day. The average food consumption for female mice was 4.35 g/day in the control and 4.1 g/day at 15 mg/kg group.

The reduction in the body weight at 15 mg/kg in male and female mice at 15 mg/kg dose partly due to the reduced food intake that may caused by the drug treatment.

The mortality during the treatment period is shown in the following table.

|       | Gr 1, M | Gr 2, M | Gr 3, M | Gr 4, M | Gr 5, M | Gr 1, F | Gr 2, F | Gr 3, F | Gr 4, F | Gr 5, F |
|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Total | 16      | 9       | 17      | 15      | 35      | 22      | 14      | 12      | 9       | 16      |
| %     | 32      | 18      | 34      | 30      | 50      | 44      | 28      | 24      | 18      | 22.9    |

Animals reserved for the toxicokinetics were sacrificed at the end of one year of the treatment.

The data suggest that the mortality in male mice was higher than the control at the 15 mg/kg dose. However, mortality in the female mice at 15 mg/kg was not increased from the respective control. It is interesting to note that male mice showed more toxicity and immunosuppression than female mice. A similar increase in the toxicity (mortality) in males compared to the females was observed for rats in the carcinogenicity study.

Both male and female mice showed higher incidences of lenticular opacity in both eyes at 15 mg/kg dose. Total number of incidences of lens opacity in both eyes in male mice in the control group was 11 out of 100 animals. The incidences in male mice at 15 mg/kg were 13 out of 70 animals.

The incidences of lens opacity in both eyes for female mice in the control group were 14 out of 100 mice and that for 15 mg/kg group was 12 out of 70 animals.

The trend of increase mortality between 79 to 106 weeks and development of opacity for male mice during this period suggest that the toxicity to the eye was treatment-related and male mice showed greater toxicity in the eye than female mice.

The sponsor stated that there was an increase incidences of alopecia in female mice. However, summary of the incidence table has not been provided.

Palpation of the skin suggest increased incidences of nodules in the male mice at high dose. The incidences are shown in the following table.

| Control 1 | Control 2 | 1.5  | 5    | 15 mg/kg |
|-----------|-----------|------|------|----------|
| 2/50      | 1/50      | 1/50 | 0/50 | 4/70     |

Hematology data in male mice did not show any changes in the RBC, HB and HCT. A slight increase in the Heinz body was seen at 15 mg/kg, however, it is of no significance. The WBC counts were reduced from  $4.2 \times 10^9/L$  in the control to  $3.5 \times 10^9/L$  in male mice at 15 mg/kg. These changes reflect the drug-induced immunosuppression. The differential counts showed a reduction in the lymphocyte bands from 0.56 in the control to 0.49 at 15 mg/kg dose (not statistically significant). An increase in the neutrophil bands was noted at 15 mg/kg dose (0.38 in the control and 0.44 at 15 mg/kg). There was a reduction in the thrombocyte counts from  $1317 \times 10^9/L$  in the control to  $1083 \times 10^9/L$  at 15 mg/kg dose that was statistically significant.

Significant changes in the hematology in female mice are shown in the following table.

| Parameter                  | Group 1, Control | Gr 2, Cont. | 1.5 mg/kg | 5.0 mg/kg | 15 mg/kg |
|----------------------------|------------------|-------------|-----------|-----------|----------|
| Erythrocyte( $10^{12}/L$ ) | 8.03             | 7.83        | 8.80*     | 8.61*     | 8.85*    |
| Hemoglobin(g/L)            | 128              | 127         | 136*      | 136*      | 133*     |
| Hematocrit                 | 0.39             | 0.39        | 0.41*     | 0.41*     | 0.41*    |

Female mice showed significant increases in the RBC counts, Hb and HCT at all treated doses compared to the control. The variation in the data in the treated groups were smaller than that in the control. This change may show statistical significance. However, the biological significance of the finding is unknown.

There was no change in the thrombocyte counts. Female mice did not show significant changes in the WBC counts from the control. However, the lymphocyte band was increased (0.53 in the control and 0.65 at 15 mg/kg) and the neutrophil band was decreased (0.41 in the control and 0.31 at 15 mg/kg) at 15 mg/kg dose compared to the control (statistically not significant).

There were no clinical chemistry data in the result section. It appears from the methods that clinical chemistry tests were not performed.

Organ weight (g) data relative to the body weight showed an increase in the weight of spleen in male mice from 0.231 to 0.379 at 15 mg/kg dose (statistically significant). The change may be related to immunosuppression. There was a significant increase in the weight of the brain from 1.37 to 1.51 at 15 mg/kg dose. The significance of the finding is unknown in the absence of any CNS toxicity.

Female mice showed an increase in the relative weight (g) in the lung from 0.69 in the control to 0.78 at 15 mg/kg dose that may have caused by the inflammatory condition due to immunosuppression as evident from the increase in the alveolar macrophage in the lung for the non-neoplastic finding. The relative weight of the brain in the female mice was increased from 1.68 in the control to 1.78 and 1.80 at 5 and 15 mg/kg doses, respectively. The importance and significance in the increase in the weight of the brain needs to be examined in relation to the pathological changes if any. The radioactive drug distribution study in rats showed a minimal distribution of radioactivity in the brain. If the same distribution pattern exists for mice, it may be inferred that the changes in the weight of the brain may not be caused by the chronic treatment with Leflunomide.

The sponsor has not provided a summary table for macroscopic changes of the organs at necropsy. It was stated that there were no compound-related macroscopic changes observed in the study. Page 5-12719 vol 45 provided an account of intercurrent mortality. The sponsor stated that an increase in dermatitis and ulcer to the skin were observed in 7 out of 44 animals killed for health reason. The table for the non neoplastic changes showed that male mice at 15 mg/kg dose had dermatitis (control 0, gr 5, 2.85%) and ulcer (control 3%, gr 5, 7.1%). This issue needs to be further compared with the clinical adverse drug reactions.

Non neoplastic findings:

Page 5-12758, vol 45:

Male mice:

Following table provides data for the male mice, data in the parentheses indicate number of animals examined. When lesions are pooled for the same organ, it represents total number of incidences regardless of whether it took place in the same animal. Data in excess of the control are shown here.

| Organ/Findings                 | Gr 1, Cont | Gr 2, Cont | Gr 3, 1.5 mg/kg | Gr 4, 5 mg/kg | Gr 5, 15 mg/kg |
|--------------------------------|------------|------------|-----------------|---------------|----------------|
| Meningitis, brain              | 0(50)      | 0(50)      | 0(50)           | 6(50)         | 3(70)          |
| Liver, necrosis                | 1(50)      | 3(50)      | 0(50)           | 2(50)         | 6(69)          |
| Hypospermatogenesis, testes    | 0(50)      | 1(50)      | 1(50)           | 0(50)         | 3(50)          |
| Thyroid Ultimobranchial cyst   | 8(50)      | 8(50)      | 11(50)          | 14(50)        | 16(70)         |
| Spleen, Atrophy                | 1(50)      | 0(50)      | 11(50)          | 4(50)         | 10(70)         |
| Bone marrow, Incr Granulopoies | 2(50)      | 0(50)      | 2(50)           | 7(50)         | 5(70)          |
| Thymus Accidental Atrophy      | 3(50)      | 2(50)      | 8(50)           | 6(50)         | 12(70)         |

Non-neoplastic findings in female mice are similarly presented in the following table.

| Organs/Findings          | Gr 1, Cont | Gr 2, Cont | Gr 3, 1.5 mg/kg | Gr 4, 5 mg/kg | Gr 5, 15 mg/kg |
|--------------------------|------------|------------|-----------------|---------------|----------------|
| Cervical Uterus Fibrosis | 1(50)      | 1(50)      | 3(50)           | 0(50)         | 4(70)          |

Neoplastic Findings in Male mice:

Findings that were in excess of the control are shown in the following table.

| Lesion/Organ                | Gr 1, Cont | Gr 2, Cont | Gr 3, 1.5 mg/kg | Gr 4, 5 mg/kg | Gr 5, 15 mg/kg |
|-----------------------------|------------|------------|-----------------|---------------|----------------|
| Testes, Leydig cell-adenoma | 5(50)      | 2(50)      | 2(50)           | 11(50)        | 6(69)          |
| Blood, Lymphoma malignant   | 3(50)      | 5(50)      | 2(50)           | 4(50)         | 12(69)         |

## Neoplastic Findings in Female mice:

| Lesion/Organ                | Gr 1, Cont | Gr 2, Cont | Gr 3, 1.5 mg/kg | Gr 4, 5 mg/kg | Gr 5, 15 mg/kg |
|-----------------------------|------------|------------|-----------------|---------------|----------------|
| Lung, Bron.alveolar Aden.   | 2(49)      | 0(50)      | 3(50)           | 4(50)         | 6(70)          |
| Lung, Bronchoalveolar Carc. | 1(49)      | 1(50)      | 4(50)           | 5(50)         | 9(70)          |
| Blood, Lymphoma malignant   | 12 (49)    | 10(50)     | 17(50)          | 9(50)         | 14(70)         |

## Toxicokinetics in mice:

Page 5-14311 vol 48:

Serum concentrations of A771726 in the two year carcinogenicity studies were determined in mice. The experiments were performed at Hoechst Marion Roussel Drug Development, Milton Keynes, England. Concentrations at 2 hours and 24 hours post dose were normalized to the dose for comparisons among doses. The toxicokinetic data were collected from the satellite groups at the end of one year and at the end of the study. However, following table depicts the data collected on day 724 for male and female mice.

Although the method section of the carcinogenicity study stated that the kinetics of A 771726 was determined after one year and two years of treatment, the data for days 724 only presented in the results.

Concentrations ( $\mu\text{g/ml}$ ) of A 771726 in serum at 2 and 24 hours post dose.

| Dose mg/kg | Conc. 2 hr | Conc. 2 hr Normalized | Conc.24 hr | Conc.24 hr Normalized. |
|------------|------------|-----------------------|------------|------------------------|
| 1.5, M     | 8.23       | 5.49                  | 5.70       | 3.80                   |
| 1.5, F     | 6.82       | 4.55                  | 5.17       | 3.45                   |
| 5, M       | 44.8       | 8.96                  | 29.8       | 5.96                   |
| 5, F       | 33.5       | 6.70                  | 24.7       | 4.94                   |
| 15, M      | 122        | 8.13                  | 102        | 6.08                   |
| 15, F      | 101        | 6.73                  | 71.7       | 4.78                   |

The data suggest that there was an accumulation of the drug at 5 and 15 mg/kg in male mice may

be due to the lower clearance of the drug at high doses. A similar observation was made for female mice. The exposure to the metabolite at 15 mg/kg almost reached a maximal when data for the mid dose were compared. The gender differences in the kinetics were not apparent in the study.

Although the exposure data were not provided by the sponsor in the NDA, the sponsor submitted the data dated June 24, 1998 upon request. Following table provides the exposure data in  $\mu\text{g}\cdot\text{h}/\text{ml}$  on day 724.

| Dose (mg/kg/day) | Male  | Norm. | Female | Norm. | Mean | Norm. |
|------------------|-------|-------|--------|-------|------|-------|
| 1.5              | 167.2 | 111   | 143.9  | 96    | 156  | 104   |
| 5                | 895.2 | 179   | 698.4  | 139   | 797  | 159   |
| 15               | 2688  | 179   | 2072   | 138   | 2380 | 158   |

TFMA serum levels in mice:

Page 5-14339, vol 48:

The level of TFMA was determined on day 373 of the study. TFMA was not detected at 1.5 mg/kg dose. The levels at 5 and 15 mg/kg (ng/ml) are shown in the following table.

| Dose mg/kg | Conc 2 hr | Normalized | Conc 24 hr | Normalized |
|------------|-----------|------------|------------|------------|
| 5, Male    | 24.4      | 4.88       | ND         |            |
| 5, Female  | 31.3      | 6.27       | 17.9       | 3.58       |
| 15, Male   | 108.6     | 7.24       | 37.4       | 2.49       |
| 15, Female | 151       | 10.1       | 63.9       | 4.26       |

The data suggest that TFMA metabolite accumulated in the serum at 5-15 mg/kg oral doses. Female mice showed higher levels of TFMA at the end of one year. The TFMA levels were 1129 and 668 fold lower than the A 771726 for male and female mice, respectively. Average TFMA levels were 129.5 ng/ml at 15 mg/kg. Considering 10.7 ng/ml as the serum levels of TFMA at the maximum recommended human dose, mice to human TFMA exposure would be 12.10 folds.