Toxicology Review of Standardized Allergenic Extract, Timothy Grass (Phleum Pratense)

BLA: 125473

Sponsor: Merck Sharp & Dohme Corp.

Product: GRASTEK

Reviewer name: Nabil Al-Humadi and Steven Kunder

Division name: OVRR/DVRPA

Proposed use: Disease modifying treatment of diagnosed Timothy and related grass pollen induced allergic rhinitis, with or without conjunctivitis, in adults and children 5 years of age and older.

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1 INTRODUCTION:

MK-7243 is standardized allergenic extract, Timothy grass (*Phleum pratense*) sublingual tablets 2800 BAU (bioequivalent allergen unit). MK-7243 is indicated for the disease modifying treatment of diagnosed Timothy and related grass pollen induced allergic rhinitis, with or without conjunctivitis, in adults and children 5 years of age and older.

The drug substance is a standardized allergen extract from Timothy grass pollen (*Phleum pratense*) sourced from the United States (US). Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc, in collaboration with ALK-Abelló A/S, based the development program of nonclinical and clinical studies for MK-7243 in the treatment of seasonal grass pollen allergies on experience with the marketed subcutaneous immunotherapy (Alutard SQ® *Phleum pratense,* ALK-Abelló A/S).

The clinical trial experience with MK-7243 includes 19 prospective clinical studies. Thirteen of the prospective clinical studies are considered pivotal development studies. Seven of these are phase 1 or phase 2 studies, and 6 are phase 3 efficacy and safety trials including adult and pediatric studies.

Specific immunotherapy with allergen products is the repeated administration of small doses of allergens to allergic individuals in order to activate immunomodulatory mechanisms and provide sustained relief of symptoms, reduced need for symptomatic medications, and improvement in quality of life during subsequent natural allergen exposure. For the treatment of seasonal allergies, the goal is to achieve clinical benefit during the first and subsequent seasons following initiation of therapy. The additional important therapeutic rationale for long-term treatment is to develop disease-modification, with symptom relief and reduced need for anti-allergy medication that persists after the treatment periods ends.

The systemic toxicity of *Phleum pratense* allergens has been assessed up to a period of 6 months of repeated administration in mice and up to 12 months repeated administration in dogs along with genetic toxicology and reproductive toxicology assessments. The dose-dependent increases in allergen-specific IgG measured in mice demonstrated a specific immune response as a result of allergen exposure.

2 STABILITY SUMMARY AND CONCLUSION:

The stability studies included three primary stability batches of SCH 697243 drug substance (DS), which are stabilized as (b)(4) The batches were manufactured by the representative and proposed (b)(4) process at the ALK-Abelló A/S facility in Hørsholm, Denmark.

The proposed shelf life for the SCH 697243 drug substance (DS), when stored at (b)(4) , is (b)(4) from the date of manufacture.

3 SEROLOGY STUDIES:

Four (2 in mice and 2 in dogs) serology studies were submitted to support the pre-clinical studies in this BLA. The results of these studies are summarized below:

3.1 STUDY NO. 1: FOUR WEEKS + RECOVERY STUDY (MPI, 944-002) IN (b)(4) DOGS

In this study, six dogs/sex/group (12 in total) served as a control group (group 1) and received placebo tablet. Groups 2 and 3 consisted of four dogs/sex/group (8 in total), were treated sublingually at a dose levels of 25000 and 75000 SQ units, respectively. Group 4 had six dogs/sex/group (12 in total) treated with 500000 SQ units. Two animals/sex selected from groups 1 and 4 were treated daily for four weeks and then allowed a four weeks recovery period.

Plasma IgG levels were determined by an (b)(4) in which (b)(4)

Compared to the mice studies, the background level is less consistent as seen in the placebo group and 500000 SQ group at the pretest time point. Specific IgG antibodies were significantly higher in group 4 when compared to the placebo group as shown in the table below.

Table 1: Serum IgG in four-week dog study

Comparisons	P-value
Placebo versus 25.000	0.06
Placebo versus 75.000	0.30
Placebo versus 500.000	0.0036

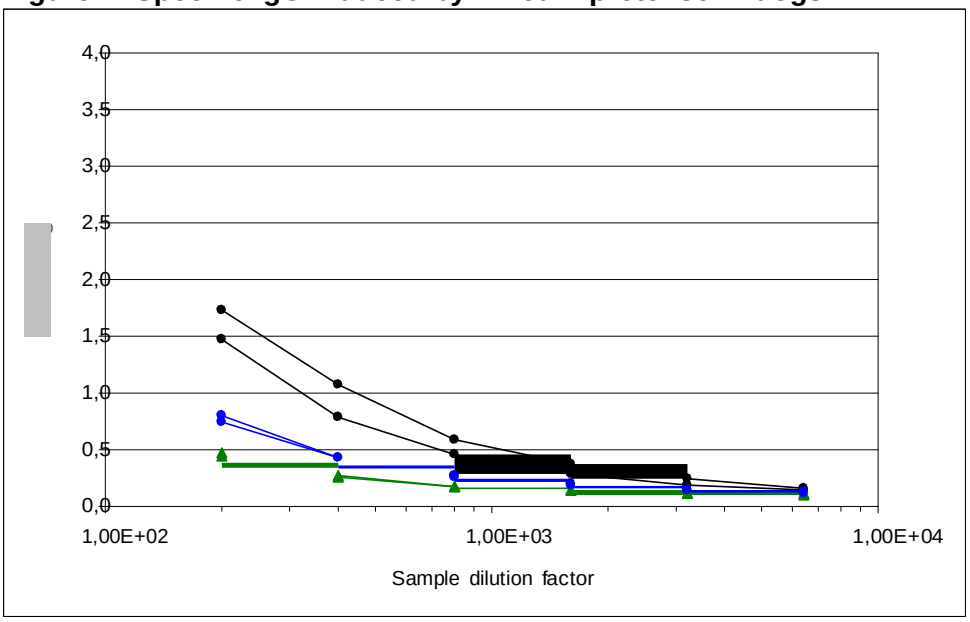
3.2 STUDY NO. 2: FIFTY TWO WEEK CHALLENGE + RE-CHALLENGE STUDY ((b)(4), LEA 001) IN (b)(4) DOGS

Animals (eight dogs/sex/group) in groups 2, 3, and 4 were treated with the test article (tablets) at dosages of 25000, 75000 or 500,000 SQ/day, respectively, for 52 weeks. Control group received a placebo tablet at the same frequency. An additional four animals/sex were included in the control group and group 4 (recovery groups). These animals were maintained for a further 8 weeks at the end of the 52 weeks treatment period without further dosing. The potential for hypersensitivity in response to a re-challenge was assessed at the end of the week 8 recovery phase with one further week of daily dose administration in these remaining animals.

Plasma IgG levels were determined by an (b)(4) in which (b)(4)

In this study an increase in specific IgG is reported in both group 1 (placebo) and group 4 (500000 SQ).

Figure 1: Specific IgG induced by Phleum pretense in dogs



Titration curves of Phleum pratense specific IgG in plasma samples from dogs depicting measured (b) (4) values as a function of dilution factor of samples. Three dogs from 25.000 SQ group, week 52 are depicted. All samples measured in duplicate. Black dots = Positive control. Blue dots = group 4. Green triangle = group 1.

In the four weeks study (study no. 1 above), daily sublingual doses of *Phleum pratense* in a tablet formulation at doses up to 500000 produced significant higher levels of specific IgG compared to placebo treated animals.

There is a discrepancy between the two dog studies as an increase in the specific IgG level is reported in the first study, while the second study does not produce this difference.

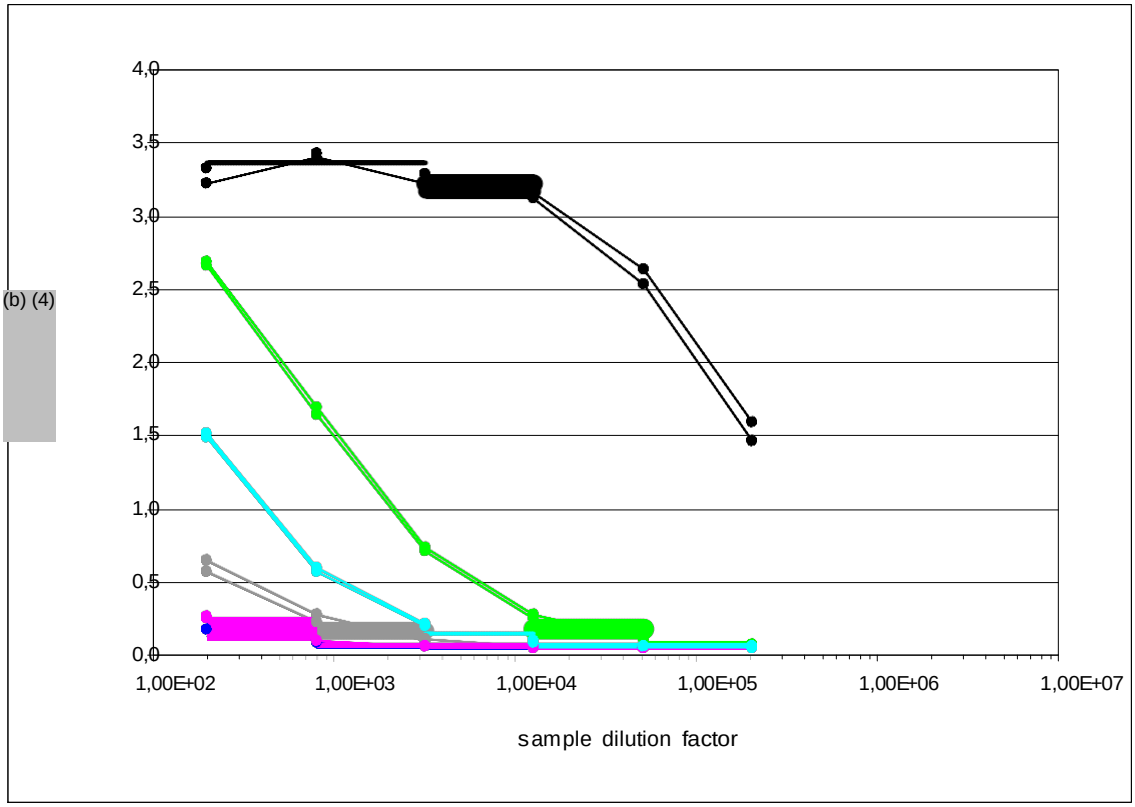
3.3 STUDY NO. 3: FOUR WEEKS STUDY (MPI, 944-001) IN MICE

Three groups of male and female (b)(4) mice (6 mice/sex/group, 12 in total) received the test article daily (in the sublingual area of the buccal cavity) for four weeks at dose levels of 25,000, 75,000 and 500,000 SQ, respectively. The control group (6 mice/sex, 12 in total) was treated with distilled water daily for four weeks. Three additional groups (10 mice/sex/group, 20 in total) received the test article daily for four weeks, followed by a four week without treatment, followed by a seven to nine day challenge period, at daily dose levels of 25,000, 75,000 and 500,000 SQ-U, respectively. A control group of 10 mice/sex (20 in total) followed the same challenge dose regimen. Groups 1, 2, and 3 were treated sublingually in the mouth once daily at a fixed volume of 5 µl. Group 4 (500,000 SQ-U) was treated with two 5 µl (between one and 10 minutes apart) doses.

Plasma IgG levels were determined by an (b)(4) in which (b)(4)

Daily sublingual doses of *Phleum pratense* (aqueous extract of grass pollen) at doses of up to 500,000 SQ-U per day for 28 days, produced serum levels of *Phleum pratense* specific IgG antibodies that were proportional to the dose level and the time length of the treatment.

Figure 2: Specific IgG induced by Phleum pretense in four week study mice



Titration curves of Phleum pratense specific IgG in plasma samples from mice depicting measured [redacted] values as a function of dilution factor of the samples. Positive Phleum pratense control (●) from [redacted] mice immunised i.p., unimmunised [redacted] mice (●), mouse from 500.000 SQ group (●), mouse from 75.000 SQ group (●), mouse from 25.000 SQ group (●) and mouse from placebo SQ group (●). All samples are measured in duplicate.

The levels of specific IgG were increased in all groups of mice. As indicated in the table below, the serum levels of specific IgG antibodies were proportional to the dose level as well as to the length of treatment.

Table 2: Number of mice with increased serum levels of Phleum pratense specific IgG antibodies.

Treatment Dose – Number of weeks	Number of animals with Phleum pratense specific IgG* (%)	Number of animals tested
Placebo – 4 w	0	12
25.000 SQ – 4 w	1 (8%)	12
75.000 SQ – 4 w	1 (9%)	11
500.000 SQ – 4 w	8 (67%)	12
Placebo – 9 w	0	19

25.000 SQ – 9 w	2 (11%)	18
75.000 SQ – 9 w	7 (39%)	18
500.000 SQ – 9 w	8 (72%)	11

* Animals with IgG levels higher than 0.4 (b) (4) (this is the general background level of the pretest and placebo values).

3.4 STUDY NO. 4: FIFTEEN WEEK CHALLENGE STUDY ((b)(4) , LAE 003) IN MICE

The immunogenic potential of *Phleum pratense* (aqueous extract of grass pollen**)** was assessed in (b)(4) mice following administration into the sublingual area of the buccal cavity. Animals were treated over a period of 15 weeks followed by 4 weeks off dose, one further week of treatment (challenge), and a four week recovery period.

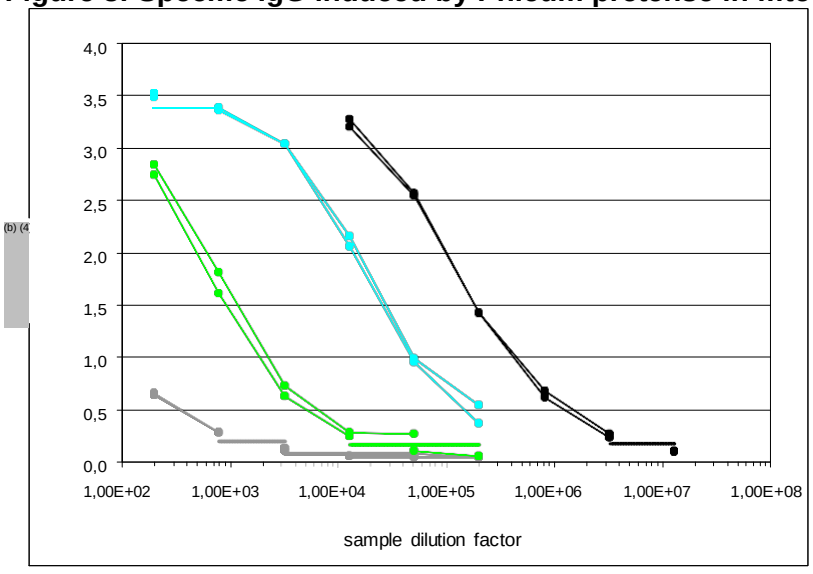
Groups 2, 3, and 4 (10/sex/group) were treated sublingually at fixed dosages of 25,000, 75,000, or 500,000 SQ-U/day, respectively. Group1 (control), were treated with distilled water. Groups 1, 2, and 3 were treated with 5µl/day and group 4 was treated with 10µl/day. A recovery group consisting of 6 male and 6 female mice were attached to groups 1 and 4.

Serum IgG levels were determined by an (b)(4) in which (b)(4)

Phleum pratense (aqueous extract of grass pollen) at doses of up to 500,000 SQ-U per day for 21 weeks produced serum levels of *Phleum pratense* specific IgG antibodies that were proportional to the dose and time length of sublingual treatment.

In the placebo groups of 15 and 21 weeks, one and four animals had serum antibody levels higher than background, respectively. This might be related to the fact that the mice in this study were fed a standard mouse chow. Standard mouse chow might contain components of plant origin that cross-react with antibodies generated against *Phleum pratense*.

Figure 3: Specific IgG induced by Phleum pretense in fifteen week study mice



Titration curves of Phleum pratense specific IgG in plasma samples from mice depicting measured (b)(4) values as a function of dilution factor of the samples. Positive Phleum pratense control (●) from (b)(4) mice immunised i.p., unimmunised (b)(4) mice (●), mouse from 500.000 SQ group (●,●). All samples are measured in duplicate.

Increased serum levels of specific IgG were observed in all groups of mice receiving sublingual doses of *Phleum pratense*. As indicated in the table below, the serum levels of specific IgG antibodies were proportional to the dose level as well as to the length of treatment.

Table 3: Number of mice with increased serum levels of Phleum pratense specific IgG antibodies.

Treatment dose	Week -1	Week 15	Week 21
Placebo	0/32	1/32	4/19
25.000 SQ	0/20	15/19	13/19
75.000 SQ	0/20	17/20	17/19
500.000 SQ	0/32	30/31	19/21

Results are indicated as number of animals with Phleum pratense specific antibodies (IgG levels higher than 0.4(b)(4) /number of animals tested.

4 TOXICITY STUDIES SUBMITTED TO SUPPORT THIS BLA:

4.1 GENERAL TOXICOLOGY STUDIES

1. Study number LEA 003/024445: *Phleum pratense* toxicity study by buccal cavity administration to (b)(4) mice for 15 weeks followed by 4 weeks off dose and one further week of treatment and a 4 week recovery period.
2. Study number 001-033458: *Phleum pratense* toxicity study by sublingual tablet administration to (b)(4) dogs for 52 weeks followed by 8 weeks off dose and one further week of treatment.
3. Study # 3 [002-032364]: *Phleum pratense* toxicity study by buccal cavity administration to (b)(4) mice for 26 weeks followed by a 5 week recovery period.
4. Study #4 [944-001]: A subchronic oral toxicity study of *Phleum pratense* grass extract in mice
5. Study #5 [944-002] A subchronic oral toxicity study of timothy grass extract in dogs

4.2 REPRODUCTIVE STUDIES:

1. Study # 4 [004-023818]: *Phleum pratense* preliminary study of effects on embryo-fetal toxicity in the (b)(4) mouse by buccal cavity administration.
2. Study #7 [LEA 008/033623]: *Phleum pretense* combined study on fertility and embryo-fetal development in the (b)(4) mouse by buccal cavity administration
3. Study #8 [LEA 010/042144] Pretense: pre- and post-natal development study in the (b)(4) mouse by buccal cavity administration

4.3 GENE TOXICOLOGY STUDIES:

1. Study # 2325/2: *Phleum pratense* grass extract: reverse mutation in four histidine-requiring strains *of Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli*

2. Study # 2325/4: *Phleum pratense* grass extract: Mutation at the
3. Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the (b)(4)
4. Study # 2325/6: *Phleum pratense* grass extract: reverse mutation in four histidine-requiring strains *of Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli*

5 SUMMARY OF TOXICOLOGY STUDIES:

5.1 STUDY # 1 [LEA 003/024445]: (PHLEUM PRATENSE TOXICITY STUDY BY BUCCAL CAVITY ADMINISTRATION TO (b)(4) MICE FOR 15 WEEKS FOLLOWED BY 4 WEEKS OFF DOSE AND ONE FURTHER WEEK OF TREATMENT AND A 4 WEEK RECOVERY PERIOD.

5.1.1 Précis:

In this multiple dose toxicology study, mice were treated with Phleum Pratense, or distilled water. Animals (10/group/sex]) in groups 1, 2, and 3 were treated by sublingual dose of 5 µL per mouse per day. For group 4, the dose volume was 10 µL (2 of 5 µL doses) per mouse per day. Mice were treated for 15 weeks followed by 4 weeks off dose and one further week of treatment and a 4 week recovery period. Study duration was 24 weeks. The proposed clinical dose was used in this study.

Title and study number: Phleum pratense toxicity study by buccal cavity administration to (b)(4) mice for 15 weeks followed by 4 weeks off dose and one further week of treatment and a 4 week recovery period. Study number: LEA 003/024445
Performing laboratory: (b)(4)
(b)(4)
Study initiation date: May 08, 2002
Final report date: March 11, 2004
Test article batch/lot:

Test article	Batch No.
Phleum Pratense	23 April 2002/L (Batch (b)(4)) LBA 2009/11 June 2002 (Batch (b)(4))

Animal species and strain: (b)(4) mice
Breeder/supplier: (b)(4).
Number of animal per group and sex: 10 per sex per group
Age: 39-43 days
Body weight range: 29.9-39.6 g for males and 21.9-29.1 g for females
Route and site of administration: Sublingual administration.
Volume of injection: The dose volume was 5 µL per mouse per day for groups 1, 2, and 3 and 10 µL (2 of 5 µL doses) per mouse per day for group 4
Frequency of administration and study duration: Test article were administered into the sublingual area of the buccal cavity over a period of 15 weeks followed by 4 weeks off dose, and one further week of treatment and a four week recovery period. Study duration was 24 weeks.
Dose: Animals were treated with fixed dosages of 25,000, 75,000 and 500,000 SQ-U/day.
Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the BLA. Stability analysis was not provided in this study.
Means of administration: Sublingual administration
Report status: Final report

5.1.2 Experimental design:

Animals were randomized and assigned to 4 different groups. Each group consisted of 10 males and 10 females. Animals in groups 1, 2, and 3 were treated with a 5 µL per day and group 4 were treated with 10 µL (2 of 5 µL doses) per day by sublingual route. The control group received 10 µL of distilled water. A recovery group consisting of 6 male and 6 female mice was attached to group 1 (control) and group 4 (500,000 SQ-U/day). Study duration was 24 weeks. The details of the study design are listed in the following table:

Table 4: Experimental design

Group	Test material	Dose Volume µl/mouse/day	Dose Level SQ-U/day	Number of Animals (#/sex/group) Main	Number of Animals (#/sex/group) Recovery
1 (control)	Distilled Water	5	0	10	6
2	Phleum Pratense	5	25,000	10	0
3	Phleum Pratense	5	75,000	10	0
4	Phleum Pratense	10	500,000	10	6

5.1.3 Methods:

Randomization procedure: Yes.
Statistical analysis plan: Yes.
The following parameters were evaluated: clinical observations (twice daily), detailed observations (daily during the first week of treatment, twice weekly during weeks 2 to 4 [middle and end of each week], weekly during week 5 to 15 and again daily during week 20), physical examination (weekly), body weights (weeks -1 and 0 and weekly throughout the treatment and recovery period, and before necropsy), food consumption (week -1 and weekly throughout the treatment and recovery period), body temperature (not recorded), ophthalmoscopy (not recorded), clinical chemistry(not recorded), hematology (bone marrow samples were obtained from the femur during necropsy of all animals at the scheduled terminal kill). Immunoassay includes lymphocyte immunophenotyping, natural killer cell assay, and lymphocyte proliferation assays (weeks 20 and 24), anti-Phleum pratense antibody (prior to the commencement of treatment and during Weeks 5, 10, 15, 19 and 20 of treatment and then in week 24 (week 4 of recovery)). Organ weight, macroscopic examination, and tissue collection (terminal necropsy at weeks 20 and 24).

Table 5: Study parameters and schedule

Parameters	Frequency of Testing
Clinical observation[1]	Twice daily

[1] Cageside observations include mortality, morbidity, general health and signs of toxicity.

Parameters	Frequency of Testing
Clinical signs[2]	Daily during the first week of treatment, twice weekly during weeks 2 to 4 [middle and end of each week], weekly during week 5 to 15 and again daily during week 20
Physical examination	Weekly
Body weight	Weeks -1 and 0 and weekly throughout the treatment and recovery period, and before necropsy
Food consumption	Week -1 and weekly throughout the treatment and recovery period
Body temperature	Not recorded
Ophthalmologic exam	Not recorded
Clinical chemistry	Not recorded
Hematology	Bone marrow samples were obtained from the femur during necropsy of all animals at the scheduled terminal kill
Anti-Phleum pratense antibody*	Prior to the commencement of treatment and during weeks 5, 10, 15, 19, and 20 of treatment and then in week 24 (week 4 of recovery)
Immunoassay includes lymphocyte immunophenotyping, natural killer cell assay, and lymphocyte proliferation assays	Weeks 20 and 24
Necropsy	Weeks 20 and 24
Organ weight, macroscopic examination, and tissues for histopathology	Weeks 20 and 24

* Blood was collected from retro-orbital sinus.

5.1.4 Results:

Morbidity and mortality: There were four unscheduled deaths, all of which were associated with routine blood sampling procedures.

5.1.4.1 SYSTEMIC TOXICITY:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, or body weight.

Mean body weight gain for group 4 females (500,000 SQ-U/day) were significantly lower than the concurrent controls throughout the treatment period (weeks 0-20). This decrease in body weight gain was not reported in the recovery groups.

Food consumption for group 4 females was significantly lower than the concurrent controls throughout the treatment period (weeks 0-20). Food consumption for groups 3 and 4 males were slightly lower than the concurrent controls throughout the treatment period (weeks 0-20).This decrease in food consumption was not reported in the recovery

[2] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

groups. Reduced food conversion efficiency reported for group 4 females suggests that the reduced bodyweight gain was not entirely attributable to reduced food intake.

Bone marrow smears: No changes in the cellularity, distribution, and morphology and myeloid:erythroid ratio was reported when assessed visually.

5.1.4.2 IMMUNOPHENOTYPING RESULTS:

Table 6: Immunophenotyping: T, B, and NKR cells numbers.

GROUP NUMBER (n)	TOTAL T CELLS CD3+	TOTAL B CELLS CD45RA+	NKR CELLS CD3-NKR+	CD4+ T CELLS CD3+CD4+CD8-	CD8+ T CELLS CD3+CD4+CD8+
	Cells/spleen ($x10^8$) ± SD[+]	Cells/spleen ($x10^8$) ± SD	Cells/spleen ($x10^8$) ± SD	Cells/spleen ($x10^8$) ± SD	Cells/spleen ($x10^8$) ± SD
1M (10)	0.32 ± 0.17	0.66 ± 0.39	0.03 ± 0.01	0.23 ± 0.10	0.07 ± 0.05
2M (9)	0.22 ± 0.09	0.47 ± 0.20	0.02 ± 0.01	0.16 ± 0.06	0.06 ± 0.02
3M (9)	0.24 ± 0.06	0.50 ± 0.20	0.03 ± 0.01	0.17 ± 0.03	0.06± 0.02
4M (9)	0.20 ± 0.15	0.46 ± 0.41	0.02 ± 0.01	0.14** ± 0.09	0.05 ± 0.04
1F (10)	0.29 ± 0.13	0.75 ± 0.48	0.03± 0.01	0.20 ± 0.10	0.06 ± 0.03
2F (10)	0.22 ± 0.09	0.49 ± 0.20	0.02 ± 0.01	0.15 ± 0.06	0.06 ± 0.03
3F (10)	0.23 ± 0.08	0.45* ± 0.21	0.03 ± 0.02	0.16 ± 0.06	0.06 ± 0.02
4F (10)	0.22* ± 0.08	0.39** ± 0.15	0.02 ± 0.01	0.17 ± 0.07	0.05 ± 0.02

Table of immunophenotyping: T, B, and NKR cells numbers. [+] SD = Standard deviation. * Significantly different at $p \leq 0.05$. ** Significantly different at $p \leq 0.01$.

Absolute number and percentage of the splenic lymphocyte subsets were measured using (b) (4) . A trend of reduced lymphocyte numbers in all treated groups was reported. Statistical significant reduction in CD4+ T lymphocytes was reported in group 4 males. A trend of reduced B lymphocyte numbers and percentages was reported in all female groups. This reduction was statistically significant in groups 3 and 4. The percentage B lymphocytes reduction was accompanied by a corresponding decrease in the percent T lymphocytes which reached statistical significance in group 4 females.

Table 7: Immunophenotyping: Natural killer (NKR) cells:

GROUP NUMBER (N)	LYTIC UNITS GEOMETERIC MEAN (95% CI)	RELATIVE TO THE CONTROL	LYTIC UNITS/ SPLEEN (X103) GEOMETERIC MEAN (95% CI)	RELATIVE TO THE CONTROL
1M (10)	136.1 (67.92 - 272.79)	----	0.10 (0.05 - 0.20)	----
2M (9)	69.42 (34.12 - 141.26)	0.51 (0.27 - 0.98)	0.19 (0.09 - 0.37)	1.81 (1.01 - 3.25)
3M (9)	59.97 (29.63 - 121.35)	0.44 (0.23 - 0.84)	0.20 (0.10 - 0.40)	1.93 (1.08 - 3.44)
4M (9)	80.29 (39.68 - 162.47)	0.59 (0.31 - 1.14)	0.12 (0.06 - 0.23)	1.12 (0.62 - 2.03)
1F (10)	95.83 (55.20 - 166.37)	----	0.17 (0.10 - 0.29)	----
2F (10)	55.94 (32.22 - 97.11)	0.58 (0.28 - 1.23)	0.26 (0.15 - 0.43)	1.48 (0.74 - 2.93)

3F (10)	68.15 (39.25 - 118.32)	0.71 (0.34 - 1.50)	0.18 (0.11 - 0.31)	1.05 (0.52 - 2.09)
4F (10)	78.16 (45.02 - 135.69)	0.82 (0.39 - 1.70)	0.14 (0.08 - 0.24)	0.83 (0.42 - 1.63)

Table of immunophenotyping: Natural killer cells activity. n = Number of animals
Lytic units are the predicted ratio giving 20% lysis.
Lytic units/spleen is lytic units expressed relative to spleen cell counts.
Main phase test groups (log data) compared to control using Williams test. No statistically significant differences from controls, $p>0.05$.
95% CI = 95% confidence intervals for the geometric mean or ratio relative to control. The population geometric mean or ratio should be within this interval 95% of the time.

Natural killer (NKR) cell function (cells per lytic unit) in both sexes were decreased in groups 2, 3, and 4 when compared to control group. In the meantime, lytic units per spleen (total level of NKR activity per spleen) in these groups were not different from the control group.

Table 8: Splenocyte proliferation

Group Number (N)	Mean CPM (N=3) ± SD Control	Mean CPM (N=3) ± SD Con A 0.2 µg/ml	Mean CPM (N=3) ± SD Con A 1.0 µg/ml
1M (10)	4197 ± 3011	16184 ± 1662	60980 ± 22353
2M (9)	2947 ± 1939	15267 ± 9741	60827 ± 15492
3M (9)	3578 ± 1244	19180 ± 6608	51241 ± 12957
4M (9)	2720 ± 1628	18021 ± 9655	60824 ± 24285
1F (10)	181 ± 155	4801 ± 5644	16778 ± 16825
2F (10)	134 ± 95	4437 ± 5217	14512 ± 13822
3F (10)	213 ± 127	5036 ± 4166	14057 ± 11920
4F (10)	307 ± 233	11640 ± 4805	21819 ± 8423

Splenocyte proliferation was increased by Con A stimulation in treated male groups. This increase was comparable to the vehicle control group. Because proliferation did not occur, the data obtained from the female animals (processed on day 1) could not be interpreted. Data processed on day 2 indicated that the intra-group responses were highly variable across all groups, with 2 animals failing to respond per group. Day 2 female data indicated that the splenocytes from group 4 animals were responding better to 0.2ug/ml ConA compared to the vehicle control.

Table 9: Mean body and organ weights:

SEX GROUPS	MALES (WEEK 20/ WEEK 24) 1 (CONTROL)	MALES (WEEK 20/ WEEK 24) 2	MALES (WEEK 20/ WEEK 24) 3	MALES (WEEK 20/ WEEK 24) 4	FEMALES (WEEK 20/ WEEK 24) 1 (CONTROL)	FEMALES (WEEK 20/ WEEK 24) 2	FEMALES (WEEK 20/ WEEK 24) 3	FEMALES (WEEK 20/ WEEK 24) 4
NUMBER OF ANIMALS	10/10	9/5	9/5	9/5	10/10	10/10	10/10	10/10
BODY WEIGHT (terminal)	44.6/46.8	43.7/NC	42.2/ NC	42.8/45.9	36.1/36.4	35.2/ NC	34.3/ NC	32.2*/32.8
BRAIN	0.50/0.53	0.50/ NC	0.49/ NC	0.48/0.49 ++	0.51/0.53	0.53/ NC	0.52/ NC	0.52/0.52
ADRENALS	NC	NC	NC	NC	NC	NC	NC	NC
EPIDIDYMIDES	0.12/0.13	0.12/ NC	0.14/ NC	0.11/0.12	NA	NA	NA	NA
HEART	0.28/0.28	0.29/ NC	0.30/ NC	0.28/0.24	0.21/0.21	0.22/ NC	0.20/ NC	0.18**/0.19
KIDNEYS	0.71/0.88	0.74/ NC	0.67/ NC	0.69/0.76	0.47/0.45	0.49/ NC	0.47/ NC	0.45/0.44
LIVER	2.15/2.24	2.10/ NC	2.10/ NC	2.02/2.14	1.86/1.77	1.89/ NC	1.74/ NC	1.61/1.65
LUNGS AND BRONCHI	0.38/0.39	0.36/ NC	0.38/ NC	0.36/0.37	0.34/0.39	0.31/ NC	0.31/ NC	0.30/0.35
ILLIAC LYMPH NODES Right	NC	NC	NC	NC	NC	NC	NC	NC
ILLIAC LYMPH NODES Left	NC	NC	NC	NC	NC	NC	NC	NC
SALIVARY GLAND	0.26/0.25	0.25/ NC	0.23/ NC	0.26/0.25	0.15/0.13	0.17/ NC	0.14/ NC	0.13/0.13
SEMINAL VESICLES	0.40/0.44	0.38/ NC	0.35/ NC	0.38/0.38	NA	NA	NA	NA
SPLEEN	0.12/0.10	0.11/ NC	0.10/ NC	0.10/0.10	0.14/0.15	0.12/ NC	0.12/ NC	0.11*/0.11
TESTES	0.29/0.29	0.26/ NC	0.27/ NC	0.28/0.29	NA	NA	NA	NA
PITUITARY	NC	NC	NC	NC	NC	NC	NC	NC
PROSTATE	0.07/0.06	0.06/ NC	0.07/ NC	0.07/0.05	NA	NA	NA	NA
THYROID and PARATHYROID	NC	NC	NC	NC	NC	NC	NC	NC
THYMUS	0.03/0.03	0.03/ NC	0.03/ NC	0.03/0.03	0.04/0.03	0.03/ NC	0.03/ NC	0.03/0.03
OVARIES					NC	NC	NC	NC
UTERUS AND CERVIX					0.29/0.23	0.25/ NC	0.27/ NC	0.31/0.24

Table of organ weight: Absolute weights are expressed as mean (grams). NC = Not collected. NA = Not applicable. Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase and recovery phase of the study (main phase organ weight/recovery phase organ weight). $^{++}$ Significantly different at $p \leq 0.01$ Students' *t* test. * Significantly different at $p \leq 0.05$ Williams' test. ** Significantly different at $p \leq 0.01$ Williams' test.

Female body weight was decreased in group 4 at weeks 20 and 24. Brain weight was decreased in group 4 males at week 24. Heart weight was decreased in group 4 males at week 24. The decrease in heart weight was significant in group 4 females at week 20. Male kidney weight was decreased in group 3 at week 20 and in group 4 at week 24. Female's liver weight was decreased in group 4 males at weeks 20 and 24. The reduction in male's kidney and female's liver weights was not significant. Female's spleen weight was significantly decreased in group 4 at weeks 20 and 24.

5.1.4.3 CLINICAL CHEMISTRY RESULTS:

Not collected.

5.1.4.4 MACROPATHOLOGY:

No test article-related effect on macroscopic evaluation was reported.

Terminal sacrifice

Table 10: Macroscopic findings at terminal sacrifice.

Group	Findings
1M	Opaque in eyes (1/10)*; congested lungs and bronchi (3/10); enlarged spleen (1/10); sternum misshapen bone (1/10)
2M	Opaque in eyes (1/9); unilaterally small eyes (2/9); swelling (1/9) and small (1/9) Harderian glands; congested lungs and bronchi (2/9); enlarged spleen (1/9); hairloss (1/9) and scab (1/9) in skin
3M	Unilaterally small eyes (1/9); congested lungs and bronchi (5/9); forestomach depression in stomach (1/9); swelling in tail (1/9)
4M	Congested lungs and bronchi (4/9); forestomach depression in stomach (1/9); cystic preputial glands (1/9); hairloss in skin (1/9)
1F	Opaque in eyes (2/10); unilaterally small eyes (1/10); congested lungs and bronchi (4/10); thin optic nerves (1/10); periovarian sac distension (6/10) and cyst(s) (1/10) in ovaries; enlarged spleen (2/10); fluid distention in uterus (4/10); thickened uterus (1/10); hairloss in skin (2/10)
2F	Opaque in eyes (1/10); congested mandibular lymph nodes (1/10); congested lungs and bronchi (4/10); periovarian sac distension (3/10) and cyst(s) (5/10) in ovaries; fluid distention in uterus (1/10); thickened uterus (1/10)
3F	Opaque in eyes (1/10); congested lungs and bronchi (4/10); periovarian sac distension (3/10) and cyst(s) (4/10) in ovaries; enlarged spleen (1/10); forestomach raised area in stomach (1/10); fluid distention in uterus (3/10); thickened (3/10) and fluid swelling (1/10) uterus; enlarged lumbar lymph node (1/10); enlarged renal lymph node (1/10); hairloss in skin (1/10)
4F	Opaque in eyes (2/10); swelling Harderian glands (1/10); congested (1/10) and mass (1/10) in lungs and bronchi; periovarian sac distension (3/10) and cyst(s) (4/10) in ovaries; fluid distention in uterus (3/10); thickened uterus (3/10); regional to mass bronchial lymph node (1/10)

* (number of animals with the observation/total number of animals in the group).

5.1.4.5 MICROSCOPIC FINDINGS:

Test article-related reduced incidences of extramedullary haemopoiesis in the spleens of group 4 females were reported.

Table 11: Test article-related reduced incidences of extramedullary haemopoiesis

Group	1F	2F	3F	4F
Dosage (SQ-U/day)	0	25,000	75,000	500,000
No.examined	10	0	1	10
Extramedullary haemopoiesis: Slight Moderate Total	 5 2 7	 - - -	 1 0 1	 1 0 1a

a = p<0.05 for a two-tailed Fisher's exact test

Bronchioalveolar adenoma (single neoplasm), was reported in the lung of one group 4 females. Other changes reported were considered to be within normal limits for (b)(4) mice of this age.

Terminal sacrifice

Table 12: Microscopic findings at terminal sacrifice.

Group	Findings
1M	Moderate keratitis in eyes (1/10); moderate lenticular degeneration in eyes (1/10); slight (2/10) and moderate (4/10) acinar necrosis/atrophy/basophilia with inflammation in Harderian glands; minimal (1/10) and slight (2/10) perivascular lymphoid aggregations in kidneys; slight cortical tubular basophilia (1/10); minimal (1/10), slight (4/10), and moderate (1/10) lymphoid aggregates in lachrymal glands; slight focal hepatocyte necrosis in liver (2/10); slight sinus histiocytosis in mesenteric lymph node (1/10); slight sinus histiocytosis in retrophar lymph node (1/10); slight alveolar hemorrhage in lungs and bronchi (1/10); slight gliosis in optic nerves (1/10); slight inflammation in pancreas (1/10); slight extramedullary hemopoiesis in spleen (1/10); minimal dilated glands in stomach (1/10); refluxed seminal colloid plug in urinary bladder (2/10); moderate scab in skin (1/10); slight epidermal ulceration in skin (1/10); moderate epidermal hyperplasia in skin (1/10)
2M	Traumatic disruption in eyes (2/9); moderate acinar necrosis/atrophy/basophilia with inflammation in Harderian glands (1/2); slight alveolar hemorrhage in lungs and bronchi (1/10); moderate extramedullary hemopoiesis in spleen (1/1)
3M	Traumatic disruption in eyes (1/9); slight alveolar hemorrhage in lungs and bronchi (1/10); abscess in tail (1/9)
4M	Minimal focal fusiform cell hyperplasia in adrenals (1/9); slight x-zone vacuolation in adrenals (1/9); spermatocele (2/9) and reduced numbers of spermatozoa (1/9) in epididymides; minimal (1/9), slight (3/9), and moderate (4/9) acinar necrosis/atrophy/basophilia with inflammation in Harderian glands; minimal cortical tubular basophilia (2/9); slight (3/9) and moderate (1/9) lymphoid aggregates in lachrymal glands; slight inflammation in lachrymal glands (1/9); slight focal hepatocyte necrosis in liver (1/10); slight alveolar hemorrhage in lungs and bronchi (1/10); slight extramedullary hemopoiesis in spleen (2/9); minimal epithelial hyperplasia in stomach (1/9); minimal (1/9) and slight (1/9) seminiferous tubular atrophy in testes; moderate seminiferous tubular dilatation in testes (1/9); minimal epithelial hyperplasia in stomach (1/9); minimal and slight seminiferous tubular atrophy in testes (1/9); moderate seminiferous tubular dilatation in testes (1/9); follicular cyst in thyroids (1/9); cystic atrophy in preputial glands (1/9)

Group	Findings
1F	Slight (8/10) and moderate (1/10) focal fusiform cell hyperplasia in adrenals; congestion in adrenals (1/10); minimal (4/10) and moderate (1/10) x-zone vacuolation in adrenals; traumatic disruption in eyes (2/10); minimal (1/10), slight (5/10), and moderate (4/10) acinar necrosis/atrophy/basophilia with inflammation in Harderian glands; slight (3/10) and moderate (1/10) perivascular lymphoid aggregations in kidneys; minimal (1/10) and slight (3/10) cortical tubular basophilia; pelvis haemorrhage in kidneys (3/10); minimal medullary cyst in kidneys (2/10); minimal (2/10) and moderate (1/10) lymphoid aggregates in lachrymal glands; slight sinus histiocytosis in mesenteric lymph node (1/10); slight gliosis in optic nerves (1/10); slight degenerate fibers in optic nerves (1/10); cystic ovarian bursa in ovaries (4/10); paraovarian cyst in ovaries (5/10); slight focal acinar cell hypertrophy in pancreas (1/10); moderate acinar atrophy in salivary glands (1/10); slight lymphoid aggregates in salivary glands (1/10); slight (5/10)and moderate (2/10) extramedullary hemopoiesis in spleen; minimal dilated glands in stomach (2/10); slight lymphoid hyperplasia in thymus (1/10); minimal dilated glands in stomach (2/10); slight lymphoid hyperplasia in thymus (1/10); slight luminal dilatation in uterus (3/10); slight edema in uterus (1/10)
2F	Traumatic disruption in eyes (1/10); follicular cyst in ovaries (1/7); cystic ovarian bursa in ovaries (4/7); paraovarian cyst in ovaries (3/7); slight luminal dilatation in uterus (2/2)
3F	Traumatic disruption in eyes (1/10); slight alveolar hemorrhage in lungs and bronchi (1/10); follicular cyst in ovaries (2/7); cystic ovarian bursa in ovaries (4/7); paraovarian cyst in ovaries (4/7); hemorrhage in ovaries (1/7); slight extramedullary hemopoiesis in spleen (1/1); slight parakeratosis and hyperkeratosis in stomach (1/10); slight parakeratosis and hyperkeratosis in stomach (1/10); slight luminal dilatation in uterus (5/6); slight luminal thrombus in uterus (1/6); slight generalized increased cellularity in lumbar lymph node (1/10)
4F	Minimal (2/10), slight (3/10), and moderate (2/10) focal fusiform cell hyperplasia in adrenals; minimal (2/10), slight (4/10), and moderate (1/10) x-zone vacuolation in adrenals; slight ceroid accumulation in adrenals (1/10); slight keratitis in eyes (1/10); loss of outer nuclear layer in the retina (2/10); slight (4/10) and moderate (2/10) acinar necrosis/atrophy/basophilia with inflammation in Harderian glands; minimal (2/10) and slight (3/10) perivascular lymphoid aggregations in kidneys; minimal cortical tubular basophilia (3/10); pelvis haemorrhage in kidneys (1/10); slight focal hepatocyte necrosis in liver (1/10); minimal focal inflammation in liver (2/10); slight sinus histiocytosis in mesenteric lymph node (2/10); bronchioloalveolar adenoma in lungs and bronchi (1/10); cystic ovarian bursa in ovaries (4/4); paraovarian cyst in ovaries (7/10); slight extramedullary hemopoiesis in spleen (1/10); slight lymphoid hyperplasia in thymus (1/10); slight lymphoid hyperplasia in thymus (1/10); follicular cyst in thyroids (1/10); slight luminal dilatation in uterus (5/10); slight endometrial hyperplasia in uterus (1/10)

Table of microscopic findings at terminal sacrifice.
* (number of animals with the observation/total number of animals in the group).

An extensive number of tissues were examined for histology. No increased incidences of histological findings indicative of potential adverse events were observed in the treated groups relative to the controls.

Table 13: Test article related effects

Test article related effects
↓ T, B, and natural killer cells ↓ Body weight (group 4) ↓ Food consumption (group 4) ↓ Extramedullary haemopoiesis (group 4 females) ↓ Spleen weight (group 4 females)

5.1.5 Assessment:

No test article-related, mortality, nor any toxicologically relevant changes in clinical signs, or food consumption were reported.

Body weights were decreased in group 4 males and females. This decrease might be related to the reduction in food consumption in these groups. At recovery period, the reductions in body weight and food consumption were not reported.

Hematopoiesis is the formation and development of blood cells. In the embryo and fetus, it takes place in a variety of sites including the liver, spleen, thymus, lymph nodes, and bone marrow. From birth throughout the rest of life it takes place in the bone marrow with a small amount occurring in lymph nodes. Extramedullary hematopoiesis is the formation and development of blood cells outside the bone marrow, as in the spleen, liver, and lymph nodes. Reduction in extramedullary haemopoiesis in the spleen of group 4 females were reported. The reduction in the extramedullary haemopoiesis in the spleen might be related directly or indirectly to the reduction in group 4 female's spleen weight.

Immune function (splenic natural killer cell activity or T lymphocyte mitogen responsiveness) was unaffected by test article treatment. The absence of histopathological changes to the white pulp of the spleen and lack of biologically significant changes in splenic lymphocyte numbers supported this conclusion.

Based on the overall findings in this study, it can be concluded that in (b)(4) mice administration of Phleum Pratense had limited adverse effects in terms of systemic toxicity at the dose level of 500,000 SQ-U/day. No Adverse effects were reported at dose levels 25,000 SQ-U/day and 75,000 SQ-U/day.

GLP study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

5.1.6 Conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues reported in this study.

5.2 STUDY # 2 [001-033458]: PHLEUM PRATENSE TOXICITY STUDY BY SUBLINGUAL TABLET ADMINISTRATION TO (b)(4) DOGS FOR 52 WEEKS FOLLOWED BY 8 WEEKS OFF DOSE AND ONE FURTHER WEEK OF TREATMENT.

5.2.1 Précis:

In this multiple dose toxicology study, (b)(4) dogs were treated with *Phleum pratense* (at one of the following dose concentrations: 25,000, 75,000, or 500,000 SQ-U/day) or placebo. Animals (4/sex/group) were treated by administering tablets into the sublingual area of the buccal cavity. Animals were treated over a period of 52 weeks followed by 8 weeks off dose and one further week of treatment. Two animals per sex per group were assigned as recovery groups (groups 1 and 4 only). Animals in the main and recovery groups were sacrificed at weeks 52 and 61, respectively. The proposed clinical dose was used in this study, 25000 SQ-U.

Title and study number: *Phleum pratense* toxicity study by sublingual tablet administration to (b)(4) dogs for 52 weeks followed by 8 weeks off dose and one further week of treatment. Study number: LEA 001/033458
Performing laboratory: (b)(4)

Study initiation date: May 15, 2002
Final report date: March 30, 2004
Test article batch/lot:

Test article	Batch No.
Phleum Pratense	Shipment 1. 28062D456/26122D457/26132D458 Shipment 2. 28062H534/26122H527/26132H528/26132H529
Placebo Tablets	28062D456, 28062H534,

Animal species and strain: (b)(4) dogs
Breeder/supplier: (b)(4) .
Number of animal per group and sex: 4/sex/group for the main study and 2/sex/group for the recovery groups.
Age: 20-24 weeks
Body weight range: 7.3-10.8 kg for males and 6.4-10.1 kg for females
Route and site of administration: Sublingual administration.
Dose concentrations: Control group animals each received 1 placebo tablet per day, group 2 animals each received one 25,000 SQ-U tablet per day, group 3 animals each received three 25,000 SQ-U tablets per day and group 4 animals each received four 125,000 SQ-U tablets per day. The doses remained constant for the duration of the treatment period irrespective of the individual bodyweights.
Frequency of administration and study duration: Test article were administered into the sub lingual area of the buccal cavity over a period of 52 weeks followed by 8 weeks off dose and one further week of treatment.
Dose: Animals were treated with fixed dosages of 25,000, 75,000 and 500,000 SQ-U/day.
Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the BLA. Stability analysis were not provided in this study.
Means of administration: Sublingual administration
Report status: Final report

5.2.2 Experimental design:

Animals were randomized and assigned to 4 different groups. Control group animals each received 1 placebo tablet per day, group 2 animals each received one 25,000 SQ-U tablet per day, group 3 animals each received three 25,000 SQ-U tablets per day and group 4 animals each received four 125,000 SQ-U tablets per day. The doses remained constant for the duration of the treatment period irrespective of the

individual bodyweights. A recovery group consisting of 2 male and 2 female dogs was attached to group 1 (control) and group 4 (500,000 SQ-U/day). Study duration was 52 weeks followed by 8 weeks off dose and one further week of treatment. The details of the study design are listed in the following table:

Table 14: Experimental design

Group	Test material	Dose Level SQ-U/day	Number of tablets per day	Concentration of tablets/day	Number of Animals (#/sex/group) Main	Number of Animals (#/sex/group) Recovery
1 (control)	Placebo	0	1	0	4	2
2	Phleum Pratense	25,000	1	25,000	4	0
3	Phleum Pratense	75,000	3	25,000	4	0
4	Phleum Pratense	500,000	4	125,000	4	2

5.2.3 Methods:

Randomization procedure: Yes.
Statistical analysis plan: Yes.
The following parameters were evaluated: clinical observations (twice daily), detailed observations (daily during the first week of treatment and week 61 [re-challenge period], twice weekly during weeks 2 to 26 [middle and end of each week] and weekly until end of dosing period), physical examination (weekly), body weights (week 0, weekly thereafter throughout the treatment and recovery periods and before necropsy), food consumption (daily during the acclimatization period and throughout the study), body temperature (not recorded), ophthalmoscopy (before treatment commenced and during weeks 9, 25, 52, 60 [recovery week 8] and 61 [re-challenge] of the study), electrocardiography and blood pressure (during the pre-treatment period for the three standard limb leads [I, II and III] and the three augmented limb leads [aVR, aVL and aVF] and on weeks 10, 26, 52, 60 [recovery week 8] and 61 [re-challenge] of the study [2 and 24 hours after dose administration]), hematology, clinical chemistry, and anti-*Phleum pratense* antibodies (before treatment commenced and during weeks 10, 26, 39, 52, 60 [recovery week 8] and 61 [re-challenge]). Urinalysis (before treatment commenced and during weeks 9, 25, 38, 51, 60 [recovery week 8] and 61 [re-challenge]). Organ weight, macroscopic examination, and tissue collection (terminal necropsy at weeks 52 and 61).

Table 15: Study parameters and schedule

Parameters	**Frequency of Testing**
Clinical observation[3]	Twice daily

[3] Cageside observations include mortality, morbidity, general health and signs of toxicity.

Parameters	Frequency of Testing
Clinical signs[4]	Daily during the first week of treatment and week 61 (re-challenge period), twice weekly during weeks 2 to 26 (middle and end of each week) and weekly until end of dosing period
Physical examination	Weekly
Body weight	Week 0, weekly thereafter throughout the treatment and recovery periods and before necropsy
Food consumption	Daily during the acclimatization period and throughout the study
Body temperature	Not recorded
Ophthalmologic exam	Before treatment commenced and during weeks 9, 25, 52, 60 (recovery week 8) and 61 (re-challenge) of the study
Electrocardiography and blood pressure	During the pre-treatment period for the three standard limb leads [I, II and III] and the three augmented limb leads [aVR, aVL and aVF] and on weeks 10, 26, 52, 60 [recovery week 8] and 61 [re-challenge] of the study [2 and 24 hours after dose administration]
Clinical chemistry*	Before treatment commenced and during weeks 10, 26, 39, 52, 60 [recovery week 8] and 61 [re-challenge]
Hematology*	Before treatment commenced and during weeks 10, 26, 39, 52, 60 [recovery week 8] and 61 [re-challenge]
Anti-Phleum pratense antibody*	Before treatment commenced and during weeks 10, 26, 39, 52, 60 [recovery week 8] and 61 [re-challenge]
Urinalysis	Before treatment commenced and during weeks 9, 25, 38, 51, 60 [recovery week 8] and 61 [re-challenge]
Necropsy	Terminal necropsy at weeks 52 and 61
Organ weight, macroscopic examination, and tissues for histopathology	Terminal necropsy at weeks 52 and 61

* Blood was collected from the jugular vein.

5.2.4 Results:

Morbidity and mortality: No test article-related morbidity or mortality was reported.

[4] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

5.2.4.1 SYSTEMIC TOXICITY:

No treatment-related changes in clinical signs, body weight, food consumption, ophthalmic examination, urinalysis, organ weights, and macroscopic examination were reported.

Vomiting was reported in all groups, including the control group, during the study.

Group 3 (75,000 SQ-U/day) females' body weight were lower than groups 1, 2, and 4 during the study. Since group 4 (500,000 SQ-U/day) body weights were not different from the control, the decrease in group 3 is questionable.

When compared to pre-treatment, lower heart rates for all groups of males and females (including control group) were reported. A tendency for lower heart rates in all males' treated groups, with the degree of difference greater at the 24 hour post-dose time point than at 2 hours post dose, were reported. In males, at week 26, 24 hours post dosing, the most significant decrease were reported. Longer PR and QT intervals were accompanied this decrease. Significant decrease in heart rate were reported 24 hours post dosing at week 26 in groups 2, 3, and 4 males. Heart rates at 2 hours post dosing in week 52 were higher than those at 24 hour post dosing in week 26.

At 2 hours post dosing in week 10 and at 24 hours post dosing in week 52, blood pressure increased in group 4 males. This increase was not reported in females or at week 26.

5.2.4.2 HEMATOLOGY:

All treated groups were reported with a trend of higher group mean and individual monocyte values when compared with controls. These differences from controls were noted in weeks 26, 39, and 52.

Groups 3 and 4 males' mean MCHC (mean cell haemoglobin concentration) was decreased in week 52 with statistical significance ($p<0.05$) being attained. Group 4 values recorded during the recovery and re-challenge phase of the study were essentially similar to those of concurrent controls.

Reticulocytes levels were decreased in group 3 males at week 39. Large unstained cells levels were increased in groups 2, 3, and 4 males at week 39. Basophils levels were increased in group 4 females at week 52.

Table 16: Hematology Results

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)	NOT OF NOTE
RED BLOOD CELLS	Reticulocytes: Pre-dose F ↓ ≤0.6 G3 Week 39 M ↓ ≤0.6 G3	Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC)
WHITE BLOOD CELLS	Monocyte count Pre-dose M ↑ ≥1.8 G4 Eosinophils count Pre-dose M ↑ ≥2.0 G2, Pre-dose M ↑ ≥1.8 G4 Large Unstained Cells (LUC) Week39 M ↑ ≥1.8 G2, Week39 M ↑ ≥2.0 G3, Week39 M ↑ ≥1.6 G4 Basophils: Week 52 F ↑ ≥1.8 G4,	Macrophage Total leukocytes (WBC) Neutrophil count Lymphocyte count
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Prothrombin time Mean platelet volume Fibrinogen Platelet count
OTHERS		Bone marrow cytology

5.2.4.3 CLINICAL CHEMISTRY:

ALT levels were increased in group 3 males (76%) and females (62%) at week 26. One animal (# 949) in group 3 males was reported with high alanine amino-transferase (ALT) values throughout the treatment period. The greatest difference from control values being recorded at week 39. All other biochemistry parameters recorded for this animal were within the expected ranges. There were no gross macropathology or organ weight

changes associated with the ALT increase in this animal. Phosphorus levels were increased significantly in groups 2, 3, and 4 males.

Globulin a1 levels were increased in group 3 males at week 52. Globulin a2 levels were increased in group 3 females at week 52. Gamma globulin and triglyceride levels were decreased in group 3 females at week 52.

No differences between groups 1 and 4 in biochemistry values, during the recovery and re-challenge phase of the study, were recorded.

Table 17: Clinical Chemistry Results

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Alanine aminotransferase (ALT): Week 26 M ↑ ≥1.8 G3, Week 26 F ↑ ≥1.6 G3, Week 39 M ↑ ≥2.4 G3, Week 52 M ↑ ≥1.8 G3	Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids Lactate dehydrogenase (LDH) Aspartate aminotransferase (AST)
B) HEPATOBILIARY		Gamma-glutamyl transferase (GGT) Total bile acids Alkaline phosphatase (ALP) Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein, ND, fibrinogen, ND (also under coagulation),
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	a1 globulin: Week 52 M ↑ ≥1.8 G3 a2 globulin: Week 52 F ↑ ≥1.8 G3 Gamma globulin: Week 52 F ↓ ≤0.3 G3 Triglycerides: Week 52 F ↓ ≤0.6 G3	Albumin (A) A/G ratio Cholinesterase Total protein Creatine kinase Total cholesterol

5.2.4.4 MEAN BODY AND ORGAN WEIGHTS

Table 18: Mean body and organ weights

SEX **GROUPS**	**MALES (WEEK 52/ WEEK 61)** **1 (CONTROL)**	**MALES (WEEK 52/ WEEK 61)** **2**	**MALES (WEEK 52/ WEEK 61)** **3**	**MALES (WEEK 52/ WEEK 61)** **4**	**FEMALES (WEEK 52/ WEEK 61)** **1 (CONTROL)**	**FEMALES (WEEK 52/ WEEK 61)** **2**	**FEMALES (WEEK 52/ WEEK 61)** **3**	**FEMALES (WEEK 52/ WEEK 61)** **4**
NUMBER OF ANIMALS	4	4	4	4	4	4	4	4
BODY WEIGHT (terminal)	14425	14250	14725	14950	11925	12400	11450	12575
BRAIN	86.8	86.1	81.9	83.4	75.4	79.7	78.5	75.9
ADRENALS	1.37	1.50	1.40	1.32	1.54	1.50	1.51	1.57
EPIDIDYMIDES	5.03	5.38	4.94	5.50				
HEART	119.6	117.2	122.6	119.7	91.1	102.5	90.8	106.6
KIDNEYS	60.1	61.8	65.1	65.9	51.0	51.9	51.9	52.2
LIVER	387	375	405	399	379	400	342	390
LUNGS AND BRONCHI	105	106	106	107	88.2	88.7	86.4	91.6
ILLIAC LYMPH NODES Right	NC	NC	NC	NC	NC	NC	NC	NC
ILLIAC LYMPH NODES Left	NC	NC	NC	NC	NC	NC	NC	NC
SALIVARY GLAND	12.6	12.7	14.5	13.1	10.2	10.8	10.1	10.6
SEMINAL VESICLES	NC	NC	NC	NC				
SPLEEN	171	128	155	120	106	120	112	100
TESTES	24.3	22.8	19.9	20.8				
PITUITARY	0.08	0.08	0.07	0.08	0.08	0.08	0.06	0.07
PROSTATE	11.3	10.7	14.2	12.7				

SEX GROUPS	MALES (WEEK 52/ WEEK 61) 1 (CONTROL)	MALES (WEEK 52/ WEEK 61) 2	MALES (WEEK 52/ WEEK 61) 3	MALES (WEEK 52/ WEEK 61) 4	FEMALES (WEEK 52/ WEEK 61) 1 (CONTROL)	FEMALES (WEEK 52/ WEEK 61) 2	FEMALES (WEEK 52/ WEEK 61) 3	FEMALES (WEEK 52/ WEEK 61) 4
NUMBER OF ANIMALS	4	4	4	4	4	4	4	4
THYROID and PARATHYROID	0.86	0.74	0.77	0.77	0.68	0.79	0.83	0.96
THYMUS	9.32	7.91	11.7	8.26	8.36	9.02	9.69	12.6
OVARIES					1.72	1.67	1.54	1.49
UTERUS AND CERVIX					15.3	14.3	12.1	7.6

Table of organ weight: Absolute weights are expressed as mean (grams). NC = Not collected. NA = Not applicable.

Male's salivary gland weight was increased 15% in group 3 at week 52. Spleen weight was decreased in groups 2 and 4 males at week 52 by 25% and 30%, respectively. Spleen weight was increased by 13% in group 2 females at week 52. Testes weight was decreased at week 52 by 18% and 14% in groups 3 and 4, respectively. At week 52, prostate weight was increased by 26% and 12% in groups 3 and 4, respectively. At week 52, thyroid weight was decreased by 14%, 11%, and 11% in groups 2, 3, and 4 males, respectively. At week 52, thyroid weight was increased by 16%, 22%, and 41% in groups 2, 3, and 4 females, respectively. At week 52, thymus weight was decreased by 15% and 11% in groups 2 and 4 males, respectively, and increased by 26% in group 3 males. At week 52, thymus weight was increased by 16% and 51% in groups 3 and 4 females, respectively. At week 52, ovaries weight was decreased by 11% and 13% in groups 3 and 4, respectively. At week 52, uterus weight was decreased by 21% and 50% in groups 3 and 4, respectively.

5.2.4.5 MACROPATHOLOGY:

No test article-related effect on macroscopic evaluation was reported.

Terminal sacrifice

Table 19: Macroscopic findings at terminal sacrifice.

Group	Findings
1M	Abnormal contents in gall bladder (1/4)*; haemocyst in heart (1/4); unilaterally absent (1/4) and enlarged (1/4) kidneys; pale area in lungs and bronchi (1/4); enlarged spleen (2/4); raised area in buccal cavity (2/4)
2M	Abnormal contents in gall bladder (1/4); congested area in stomach (1/4)
3M	Pale area in lungs and bronchi (2/4); raised area in buccal cavity (2/4); congested retropharyngeal lymph node (1/4)
4M	Abnormal contents in gall bladder (2/4); pale area in lungs and bronchi (1/4); adhesions in spleen (1/4); raised area in buccal cavity (1/4)
1F	Abnormal contents in gall bladder (2/4); pale area in lungs and bronchi (2/4); cyst in thyroids (1/4); congested retropharyngeal lymph node (2/4)
2F	Abnormal contents in gall bladder (1/4); cyst in pituitary (1/4); congested retropharyngeal lymph node (1/4)
3F	Abnormal contents in gall bladder (2/4); pale area in lungs and bronchi (2/4); congested urinary bladder (1/4)

4F	Pale area in lungs and bronchi (1/4); asymmetrical ovaries (1/4); congested retropharyngeal lymph node (1/4)

* (number of animals with the observation/total number of animals in the group).
Table of macroscopic findings at terminal sacrifice.

5.2.4.6 MICROSCOPIC FINDINGS:

Terminal sacrifice

Table 20: Microscopic findings at terminal sacrifice.

Group	Findings
1M	Minimal cortical vacuolation (zona glomerulosa) in adrenals (2/4); minimal cortical vacuolation (zona fasciculata/reticularis) in adrenals (1/4); minimal dilated glands containing necrotic debris in duodenum (1/4); minimal (2/4) and slight (1/4) interstitial inflammatory cell foci in epididymides; minimal lymphoid cell aggregates in epididymides (1/4); minimal arteritis/periarteritis in epididymides (1/4); moderate spermatocele granuloma in epididymides (1/4); minimal degenerate spermatogenic cells in ducts in epididymides (1/4); minimal erythrophagocytosis in epididymides (1/4); minimal inflammatory cells synovium in femur (1/4);slight synovial proliferation in femur (1/4); minimal mucosal congestion in ileum (1/4); minimal mucosal congestion in jejunum (1/4) Kidneys: Minimal (2/4) and slight (1/4) papilla mineralization; minimal hyperplasia of urothelium-papilla (1/4); minimal interstitial inflammatory cells (1/4) Liver: Minimal parenchymal inflammatory cell foci (3/4) Lungs: Minimal perivascular inflammatory/lymphoid cells (2/4); minimal peribronchiolar inflammatory/lymphoid cells (1/4); minimal aggregations of alveolar macrophages (1/4); slight focal foamy alveolar macrophages (1/4); minimal focal cholesterol cleft granulomata (1/4); Slight increased germinal centre development in mandibular lymph node (2/4); slight pigmented macrophages in mandibular lymph node (1/4); slight (1/4) and moderate (1/4) increased germinal centre development in mesenteric lymph node; minimal (1/4), slight (1/4), and moderate (1/4) sinus erythrocytosis/erythrophagocytosis in mesenteric lymph node; slight periductal inflammatory cells in nictitans glands (1/4); slight (3/4) and moderate (1/4) dilated ducts in nictitans glands; minimal periductal lymphoid cells in esophagus (2/4); minimal periglandular lymphoid cells in esophagus (1/4); minimal periglandular inflammatory cells in esophagus (1/4); minimal degenerate fibers in optic nerves (1/4); cyst in parathyroids (1/4); cyst in pars distalis in pituitary (1/4); minimal lymphocytic infiltration in prostate (1/4); minimal (1/4) and slight (3/4) acinar atrophy in prostate; slight acinar distension in prostate (2/4); minimal periductal lymphoid cells in salivary glands (1/4); minimal siderophages in red pulp in spleen (1/4); slight fibrosis/granulation tissue adjacent to epiphyseal plate in sternum and marrow (1/4); minimal mineralization in stomach (1/4); slight seminiferous tubular degeneration in testes (1/4); cyst in thyroids (1/4); ectopic thymic tissue in thyroids (1/4); prominent C-cells in thyroids (1/4); minimal ventral subepithelial perivascular lymphoid cells in tongue (2/4); minimal dorsal subepithelial perivascular lymphoid cells in tongue (1/4); minimal ventral subepithelial perivascular lymphoid cells in [tip] tongue (1/4); minimal dorsal subepithelial perivascular lymphoid cells in [tip] tongue (1/4); slight arteritis/periarteritis and minimal myofibre regeneration in [tip] tongue (1/4); B-squamous cell papilloma in buccal cavity (2/4); slight (2/4) and moderate (1/4)

Group	Findings
	increased germinal center development in retropharyngeal lymph nodes; minimal increased cellularity of paracortex in retropharyngeal lymph nodes (1/4); minimal (3/4) and slight (1/4) gingival sulcus subepithelial inflammation in LR jaw administration site
2M	Minimal cortical vacuolation (zona glomerulosa) in adrenals (1/4); minimal interstitial inflammatory cell foci in epididymides (2/4); minimal lymphoid cell aggregates in epididymides (1/4); slight arteritis/periarteritis in epididymides (1/4) <u>Kidney:</u> Minimal papilla mineralization (4/4); minimal hyperplasia of urothelium-papilla (1/4) <u>Liver:</u> Minimal parenchymal inflammatory cell foci (4/4) <u>Lungs:</u> Minimal perivascular inflammatory/lymphoid cells (2/4) Slight increased germinal centre development in mandibular lymph node (2/4); minimal pigmented macrophages in mandibular lymph node (1/4); slight (3/4) and moderate (1/4) increased germinal centre development in mesenteric lymph node; minimal increased cellularity of paracortex in mesenteric lymph node (2/4); minimal (1/4) and slight (3/4) sinus erythrocytosis/erythrophagocytosis in mesenteric lymph node; minimal (1/4), slight (2/4), and moderate (1/4) dilated ducts in nictitans glands; minimal periductal lymphoid cells in esophagus (1/4); minimal periglandular lymphoid cells in esophagus (1/4); minimal periductal lymphoid cells in pancreas (1/4); cyst in parathyroids (1/4); cyst in pars distalis in pituitary (1/4); minimal lymphocytic infiltration in prostate (1/4); minimal (1/4) and slight (2/4) acinar atrophy in prostate; minimal acinar distension in prostate (2/4); minimal periductal lymphoid cells in salivary glands (1/4); slight focal ductal dilatation and acinar atrophy in salivary glands (1/4); minimal focal fibrosis and inflammation in salivary glands (1/4); minimal siderophages in red pulp in spleen (1/4); minimal fibrosis/granulation tissue adjacent to epiphyseal plate in sternum and marrow (1/4); minimal mucosal lymphoid cells in stomach (1/4); slight mucosal lymphoid follicles in stomach (1/4); minimal mineralization in stomach (1/4); slight mucosal congestion in stomach (1/4); minimal multinucleate spermatids in testes (1/4); cyst in thymus (1/4); cyst in thyroids (2/4); minimal ventral subepithelial perivascular lymphoid cells in tongue (1/4); minimal (2/4) and slight (1/4) dorsal subepithelial perivascular lymphoid cells in tongue; minimal dorsal subepithelial perivascular inflammatory cells in tongue (1/4); slight dorsal epithelial and subepithelial inflammatory cells in [tip] tongue (1/4); minimal ventral subepithelial perivascular lymphoid cells in [tip] tongue (1/4); minimal dorsal subepithelial perivascular lymphoid cells in [tip] tongue (3/4); minimal ventral subepithelial perivascular inflammatory cells in [tip] tongue (1/4); minimal dorsal lymphoid cells in muscle in [tip] tongue (2/4); slight (2/4) and moderate (2/4) increased germinal center development in retropharyngeal lymph nodes; minimal increased cellularity of paracortex in retropharyngeal lymph nodes (3/4); minimal gingival sulcus subepithelial inflammation in LR jaw administration site (4/4)
3M	Minimal cortical vacuolation (zona glomerulosa) in adrenals (1/4); minimal cortical inflammatory cells in adrenals (1/4); minimal (2/4) and slight (1/4) interstitial inflammatory cell foci in epididymides; slight lymphoid cell aggregates in epididymides (3/4); slight arteritis/periarteritis in epididymides (2/4); minimal mucosal congestion in jejunum (1/4) <u>Kidney:</u> Minimal (2/4) and slight (2/4) papilla mineralization; slight hyperplasia of urothelium-papilla (1/4)

Group	Findings
	Liver: Minimal (3/4) and slight (1/4) parenchymal inflammatory cell foci Lungs: Minimal (3/4) and slight (1/4) perivascular inflammatory/lymphoid cells; minimal peribronchiolar inflammatory/lymphoid cells (2/4); minimal prominent alveolar macrophages (2/4); moderate alveolar septal fibrosis and epithelial hyperplasia (1/4); minimal alveolar congestion (1/4); moderate epithelial hyperplasia in terminal bronchiole (1/4); slight (1/4) and moderate (1/4) pleural fibrosis Slight increased germinal centre development in mandibular lymph node (1/4); minimal increased cellularity of paracortex in mandibular lymph node (1/4); minimal (1/4) and slight (1/4) pigmented macrophages in mandibular lymph node; slight increased germinal centre development in mesenteric lymph node (3/4); minimal increased cellularity of paracortex in mesenteric lymph node (1/4); minimal (1/4), slight (2/4), and moderate (1/4) sinus erythrocytosis/erythrophagocytosis in mesenteric lymph node; minimal periductal inflammatory cells in nictitans glands (2/4); minimal interstitial inflammatory cells in nictitans glands (1/4); slight dilated ducts in nictitans glands (4/4); minimal (1/4) and slight (1/4) periductal lymphoid cells in esophagus; minimal periductal inflammatory cells in esophagus (1/4); minimal periglandular lymphoid cells in esophagus (2/4); minimal periarterial inflammatory cells-adventitia in esophagus (1/4); minimal (1/4) and slight (1/4) acinar cell degranulation in pancreas; cyst in parathyroids (1/4); minimal lymphocytic infiltration in prostate (2/4); minimal (1/4) and slight (1/4) acinar atrophy in prostate; minimum acinar distension in prostate (2/4); minimal periductal lymphoid cells in salivary glands (2/4); minimal interstitial lymphoid cells sublingual in salivary glands (1/4); minimal siderophages in red pulp in spleen (2/4); minimal mucosal lymphoid cells in stomach (1/4); minimal mineralization in stomach (1/4); minimal (2/4) and slight (1/4) mucosal congestion in stomach; minimal (2/4) and moderate (1/4) seminiferous tubular degeneration in testes; minimal (1/4) and slight (1/4) inflammatory cell infiltration in testes; minimal involution/atrophy in thymus (1/4); cyst in thyroids (1/4); prominent C-cells in thyroid (2/4); minimal interstitial inflammatory cells in thyroids (3/4); minimal ventral subepithelial perivascular lymphoid cells in tongue (2/4); minimal dorsal subepithelial perivascular lymphoid cells in tongue (1/4); minimal dorsal subepithelial perivascular inflammatory cells in tongue (1/4); minimal dorsal subepithelial mast cells in [tip] tongue (1/4); minimal ventral subepithelial perivascular lymphoid cells in [tip] tongue (1/4); minimal dorsal subepithelial perivascular lymphoid cells in [tip] tongue (3/4); B-squamous cell papilloma in buccal cavity (2/4); slight (2/4) and moderate (1/4) increased germinal center development in retropharyngeal lymph nodes; minimal (1/4) and slight (1/4) increased cellularity of paracortex in retropharyngeal lymph nodes; moderate sinus erythrocytosis/erythrophagocytosis in retropharyngeal lymph node (1/4); minimal (2/4) and slight (2/4) gingival sulcus subepithelial inflammation in LR jaw administration site; minimal plant material and inflammatory exudate in gingival sulcus in LR jaw administration site (1/4)
4M	Minimal cortical vacuolation (zona glomerulosa) in adrenals (3/4); minimal cortical vacuolation (zona fasciculata/reticularis) in adrenals (2/4); minimal perivascular inflammatory cells in meninges in brain (1/4); minimal mucosal congestion in caecum (1/4); minimal (3/4) and moderate (1/4) interstitial inflammatory cell foci in epididymides; minimal lymphoid cell aggregates in epididymides (1/4); slight (2/4) and moderate (1/4) arteritis/periarteritis in epididymides; moderate spermatocele granuloma in epididymides (1/4); slight degenerate spermatogenic cells in ducts in epididymides (1/4); minimal cystoid degeneration at ora serrata in eyes (1/4); minimal inflammatory cells synovium in femur (1/4); minimal synovial proliferation in

Group	Findings
	femur (1/4); slight arteritis/periarteritis in heart (1/4); minimal mucosal congestion in ileum (2/4) Kidney: Minimal (2/4) and slight (2/4) papilla mineralization; minimal cortical tubular basophilia (1/4); minimal interstitial inflammatory cells (1/4) Liver: Minimal parenchymal inflammatory cell foci in liver (4/4) Lungs: Minimal perivascular inflammatory/lymphoid cells (2/4); minimal prominent alveolar macrophages (2/4); slight arteritis/periarteritis of bronchial arteries (1/4); moderate alveolar septal fibrosis and epithelial hyperplasia (1/4); slight alveolar congestion (1/4); slight pleural fibrosis (1/4) Minimal increased cellularity of paracortex in mandibular lymph node (1/4); moderate increased germinal centre development in mesenteric lymph node (3/4); slight increased cellularity of paracortex in mesenteric lymph node (1/4); minimal (1/4) and slight (2/4) sinus erythrocytosis/erythrophagocytosis in mesenteric lymph node; minimal periductal inflammatory cells in nictitans glands (3/4); minimal (1/4) and slight (1/4) dilated ducts in nictitans glands; minimal periductal lymphoid cells in esophagus (2/4); minimal periductal inflammatory cells in esophagus (1/4); minimal periglandular lymphoid cells in esophagus (2/4); minimal acinar cell necrosis in pancreas (1/4); cyst in parathyroids (1/4); cyst in pars distalis in pituitary (1/4); moderate lymphocytic infiltration in prostate (1/4); minimal (1/4) and slight (1/4) acinar atrophy in prostate; minimal acinar distension in prostate (3/4); minimal periductal lymphoid cells in salivary glands (3/4); slight increased germinal center development in spleen (1/4); moderate subcapsular organizing hemorrhage in spleen (1/4); minimal fibrosis/granulation tissue adjacent to epiphyseal plate in sternum and marrow (1/4); slight mucosal lymphoid cells in stomach (1/4); minimal mucosal congestion in stomach (2/4); slight prominent germinal centers [antrum] in stomach (1/4); minimal mucosal inflammatory cells [antrum] in stomach (1/4); minimal seminiferous tubular degeneration and vacuolation in testes (1/4); slight (1/4) and moderate (1/4) arteritis/ periarteritis in thymus; cyst in thymus (1/4); minimal (1/4) and slight (1/4) involution/atrophy in thymus; cyst in thyroids (1/4); prominent C-cells in thyroids (1/4); minimal interstitial inflammatory cells in thyroids (1/4); minimal dorsal subepithelial perivascular lymphoid cells in [tip] tongue (1/4); minimal mucosal lymphoid cells in trachea (1/4); B-squamous cell papilloma in buccal cavity (1/4); minimal (1/4), slight (1/4), and moderate (1/4) increased germinal center development in retropharyngeal lymph nodes; minimal increased cellularity of paracortex in retropharyngeal lymph nodes (2/4); slight generalized decreased cellularity in retropharyngeal lymph node (1/4); slight sinus erythrocytosis/erythrophagocytosis in retropharyngeal lymph node (1/4); minimal (3/4) and slight (1/4) gingival sulcus subepithelial inflammation in LR jaw administration site; slight plant material and inflammatory exudate in gingival sulcus in LR jaw administration site (1/4)
1F	Minimal cortical vacuolation (zona glomerulosa) in adrenals (1/4); minimal cortical vacuolation (zona fasciculata/reticularis) in adrenals (1/4); minimal meningeal fibrosis (cerebrum) in brain (2/4); minimal mucosal congestion in duodenum (1/4) Kidney: Minimal (1/4) and slight (3/4) papilla mineralization; minimal hyperplasia of urothelium-papilla (1/4); minimal (2/4) and slight (2/4) cortical tubular vacuolation; moderate cortical tubular dilatation (1/4); minimal interstitial inflammatory cells (1/4)

Group	Findings
	Liver: Minimal parenchymal inflammatory cell foci in liver (2/4) Lungs: Minimal (2/4) and slight (1/4) perivascular inflammatory/lymphoid cells (2/4); minimal prominent alveolar macrophages (2/4); minimal (1/4) and slight (1/4) focal foamy alveolar macrophages; slight alveolar septal fibrosis and epithelial hyperplasia (2/4); minimal focal cholesterol cleft granulomata (1/4); minimal alveolar congestion (1/4) Slight increased germinal centre development in mandibular lymph node (1/4); minimal increased cellularity of paracortex in mandibular lymph node (1/4); minimal sinus erythrocytosis/erythrophagocytosis in mandibular lymph node (1/4); slight (2/4) and moderate (1/4) increased germinal centre development in mesenteric lymph node; slight increased cellularity of paracortex in mesenteric lymph node (1/4); minimal (1/4) and slight (3/4) sinus erythrocytosis/erythrophagocytosis in mesenteric lymph node; slight periductal inflammatory cells in nictitans glands (1/4); slight (1/4) and moderate (2/4) dilated ducts in nictitans glands; minimal (2/4) and slight (1/4) periductal lymphoid cells in esophagus; minimal acinar cell degranulation in pancreas (1/4); cyst in parathyroids (2/4); cyst in pars distalis in pituitary (1/4); minimal periductal lymphoid cells in salivary glands (1/4); minimal interstitial lymphoid cells sublingual in salivary glands (3/4); minimal siderophages in red pulp in spleen (2/4); slight trabecular organizing hemorrhage in spleen (1/4); minimal (2/4) and slight (1/4) mucosal congestion in stomach; cyst in thymus (1/4); slight involution/atrophy in thymus (1/4); cyst in thyroids (1/4); ectopic thymic tissue in thyroids (1/4); prominent C-cells in thyroids (2/4); minimal interstitial inflammatory cells in thyroids (1/4); slight ventral subepithelial perivascular lymphoid cells in tongue (1/4); minimal dorsal subepithelial perivascular lymphoid cells in tongue (4/4); minimal ventral subepithelial perivascular lymphoid cells in [tip] tongue (1/4); minimal dorsal subepithelial perivascular lymphoid cells in [tip] tongue (3/4); slight lateral epithelial hyperplasia and subepithelial fibrosis in [tip] tongue (1/4); slight (1/4) and moderate (2/4) increased germinal center development in retropharyngeal lymph nodes; minimal (1/4) and slight (1/4) increased cellularity of paracortex in retropharyngeal lymph nodes; slight (1/4) and moderate (1/4) sinus erythrocytosis/erythrophagocytosis in retropharyngeal lymph node; minimal (3/4) and slight (1/4) gingival sulcus subepithelial inflammation in LR jaw administration site
2F	Minimal cortical vacuolation (zona glomerulosa) in adrenals (3/4); minimal cortical vacuolation (zona fasciculata/reticularis) in adrenals (1/4); minimal mucosal congestion in duodenum (2/4); minimal mucosal congestion in ileum (1/4); minimal mucosal congestion in jejunum (1/4) Kidney: Slight papilla mineralization (4/4); slight cortical tubular vacuolation (3/4); minimal subepithelial inflammatory cells in pelvis (1/4) Liver: Minimal (2/4) and slight (1/4) parenchymal inflammatory cell foci Lungs: Minimal perivascular inflammatory/lymphoid cells (1/4); minimal prominent alveolar macrophages (2/4); minimal alveolitis (1/4); minimal alveolar hemorrhage (1/4) Slight (3/4) and moderate (1/4) increased germinal centre development in mesenteric lymph node; minimal increased cellularity of paracortex in mesenteric

Group	Findings
	lymph node (2/4); minimal (1/4) and slight (3/4) sinus erythrocytosis/erythrophagocytosis in mesenteric lymph node; minimal interstitial inflammatory cells in mammary protocol (1/4); minimal (1/4) and slight (2/4) periductal inflammatory cells in nictitans glands; slight (3/4) and moderate (1/4) dilated ducts in nictitans glands; slight squamous metaplasia (developmental anomaly) in nictitans glands (1/4); minimal periductal lymphoid cells in esophagus (1/4); minimal periductal inflammatory cells in esophagus (1/4); minimal periglandular inflammatory cells in esophagus (2/4); minimal interstitial lymphoid cells in pancreas (1/4); minimal periductal lymphoid cells in pancreas (1/4); cyst in parathyroids (2/4); ectopic thymic tissue (1/4); cyst in pars distalis in pituitary (1/4); minimal mucosal congestion in rectum (1/4); minimal periductal lymphoid cells in salivary glands (3/4); minimal siderophages in red pulp in spleen (2/4); minimal mucosal lymphoid cells in stomach (1/4); minimal (1/4) and slight (1/4) mucosal congestion in stomach; ectopic thymic tissue in thyroids (1/4); minimal ventral subepithelial perivascular lymphoid cells in tongue (1/4); minimal (3/4) and slight (1/4) dorsal subepithelial perivascular lymphoid cells in tongue; minimal ventral subepithelial perivascular lymphoid cells in [tip] tongue (1/4); minimal dorsal subepithelial perivascular lymphoid cells in [tip] tongue (2/4); slight ventral subepithelial perivascular inflammatory cells in [tip] tongue (1/4); minimal mucosal lymphoid cells in trachea (1/4); slight increased germinal center development in retropharyngeal lymph nodes (2/4); slight increased cellularity of paracortex in retropharyngeal lymph nodes (1/4); minimal (1/4) and slight (1/4) sinus erythrocytosis/erythrophagocytosis in retropharyngeal lymph node; minimal gingival sulcus subepithelial inflammation in LR jaw administration site (4/4); minimal intraepithelial hair shafts in gingival sulcus in LR jaw administration site (1/4)
3F	Minimal cortical vacuolation (zona glomerulosa) in adrenals (4/4); minimal cortical vacuolation (zona fasciculata/reticularis) in adrenals (2/4); minimal meningeal mineralization (cerebrum) in brain (1/4); minimal mucosal congestion in duodenum (2/4) <u>Kidney:</u> Minimal (1/4) and slight (3/4) papilla mineralization; minimal hyperplasia of urothelium-papilla (3/4); minimal (3/4) and moderate (1/4) cortical tubular vacuolation; slight subepithelial inflammatory cells in pelvis (1/4) <u>Liver:</u> Minimal parenchymal inflammatory cell foci (2/4) <u>Lungs:</u> Minimal perivascular inflammatory/lymphoid cells (1/4); minimal prominent alveolar macrophages (4/4); minimal aggregations of alveolar macrophages (1/4); slight focal foamy alveolar macrophages (1/4); minimal alveolitis (2/4); slight pneumonitis (1/4); slight (1/4) and moderate (1/4) alveolar septal fibrosis and epithelial hyperplasia Minimal increased cellularity of paracortex in mandibular lymph node (2/4); slight (2/4) and moderate (1/4) increased germinal centre development in mesenteric lymph node; minimal increased cellularity of paracortex in mesenteric lymph node (2/4); slight sinus erythrocytosis/erythrophagocytosis in mesenteric lymph node (3/4); minimal (2/4) and slight (1/4) periductal inflammatory cells in nictitans glands; minimal (1/4) and slight (1/4) interstitial inflammatory cells in nictitans glands; minimal (1/4) and slight (3/4) dilated ducts in nictitans glands; minimal periductal lymphoid cells in esophagus (1/4); minimal periductal inflammatory cells in esophagus (2/4); minimal periglandular lymphoid cells in esophagus (2/4); minimal periglandular inflammatory cells in esophagus (2/4); minimal interstitial inflammatory

Group	Findings
	cells in pancreas (1/4); minimal interstitial fibrosis in pancreas (1/4); cyst in parathyroids (1/4); cyst in pars distalis in pituitary (1/4); minimal (2/4) and slight (1/4) periductal lymphoid cells in salivary glands; minimal interstitial lymphoid cells sublingual in salivary glands (2/4); minimal siderophages in red pulp in spleen (1/4); minimal mucosal lymphoid cells in stomach (1/4); minimal mineralization in stomach (1/4); minimal mucosal congestion in stomach (2/4); cyst in thyroids (2/4); minimal interstitial inflammatory cells in thyroids (2/4); minimal (2/4) and slight (1/4) dorsal subepithelial perivascular lymphoid cells in tongue; slight dorsal subepithelial perivascular inflammatory cells in tongue (1/4); minimal dorsal inflammatory cells in muscle in tongue (1/4); minimal ventral subepithelial perivascular lymphoid cells in [tip] tongue (4/4); minimal dorsal and ventral lymphoid cells in muscle in [tip] tongue (1/4); slight goblet cell hyperplasia in trachea (1/4); minimal submucosal congestion in urinary bladder (1/4); slight increased germinal center development in retropharyngeal lymph nodes (2/4); minimal increased cellularity of paracortex in retropharyngeal lymph nodes (2/4); minimal sinus erythrocytosis/erythrophagocytosis in retropharyngeal lymph node (2/4); minimal gingival sulcus subepithelial inflammation in LR jaw administration site (4/4); minimal intraepithelial hair shafts in gingival sulcus in LR jaw administration site (1/4)
4F	Minimal cortical vacuolation (zona glomerulosa) in adrenals (2/4); slight cortical vacuolation (zona fasciculata/reticularis) in adrenals (3/4); minimal mucosal congestion in colon (1/4); minimal mucosal congestion in ileum (1/4) <u>Kidney:</u> Minimal (2/4) and slight (2/4) papilla mineralization; minimal (3/4) and slight (1/4) cortical tubular vacuolation; minimal cortical tubular basophilia (1/4); minimal cortical tubular dilatation (1/4); minimal interstitial inflammatory cells (1/4) <u>Liver:</u> Minimal parenchymal inflammatory cell foci (4/4) <u>Lungs:</u> Minimal perivascular inflammatory/lymphoid cells (2/4); minimal (2/4) and slight (1/4) prominent alveolar macrophages; minimal (1/4) and slight (1/4) focal foamy alveolar macrophages; slight alveolar septal fibrosis and epithelial hyperplasia (1/4); minimal focal cholesterol cleft granulomata (1/4); minimal alveolar osseous metaplasia (2/4) Slight increased germinal centre development in mandibular lymph node (1/4); minimal increased cellularity of paracortex in mandibular lymph node (2/4); minimal pigmented macrophages in mandibular lymph node (1/4); slight (1/4) and moderate (1/4) increased germinal centre development in mesenteric lymph node; minimal increased cellularity of paracortex in mesenteric lymph node (3/4); minimal (2/4) and slight (2/4) sinus erythrocytosis/erythrophagocytosis in mesenteric lymph node; minimal periductal inflammatory cells in nictitans glands (4/4); slight interstitial inflammatory cells in nictitans glands (1/4); minimal (2/4) and moderate (1/4) dilated ducts in nictitans glands; minimal (1/4) and slight (1/4) periductal lymphoid cells in esophagus; minimal periductal inflammatory cells in esophagus (1/4); minimal periglandular inflammatory cells in esophagus (1/4); prominent adipocytes in parathyroids (1/4); cyst in pars distalis in pituitary (1/4); minimal dilated glands containing necrotic debris in rectum (1/4); minimal (1/4) and slight (1/4) periductal lymphoid cells in salivary glands; minimal interstitial lymphoid cells sublingual in salivary glands (1/4); scab in skin (1/4); minimal siderophages in red pulp in spleen (2/4); minimal mineralization in stomach (1/4); minimal (2/4) and slight (1/4) mucosal congestion in stomach; cyst in thymus (1/4); cyst in thyroids (3/4); minimal lymphoid cells in thyroids (1/4); minimal ventral subepithelial perivascular lymphoid cells in

Group	Findings
	tongue (1/4); minimal (2/4) and slight (1/4) dorsal subepithelial perivascular lymphoid cells in tongue; minimal dorsal epithelial and subepithelial inflammatory cells in [tip] tongue (1/4); minimal ventral subepithelial perivascular lymphoid cells in [tip] tongue (2/4); minimal dorsal subepithelial perivascular lymphoid cells in [tip] tongue (2/4); slight myofibre regeneration in [tip] tongue (1/4); minimal dorsal lymphoid cells in muscle in [tip] tongue (1/4); slight goblet cell hyperplasia in trachea (1/4); slight (3/4) and moderate (1/4) increased germinal center development in retropharyngeal lymph nodes; minimal increased cellularity of paracortex in retropharyngeal lymph nodes (1/4); moderate sinus erythrocytosis/erythrophagocytosis in retropharyngeal lymph node (1/4); minimal gingival sulcus subepithelial inflammation in LR jaw administration site (3/4); minimal intraepithelial hair shafts in gingival sulcus in LR jaw administration site (1/4)

* (number of animals with the observation/total number of animals in the group).
Table of microscopic findings at terminal sacrifice.

An extensive number of tissues were examined for histology. No increased incidences of histological findings indicative of potential adverse events were observed in the treated groups relative to the controls.

Anti-Phleum Pratense antibodies
The results of the anti-Phleum pratense antibodies were not presented in this report.

Table 21: Test article related effects

Test article related effects
↓ Heart rate (group 4)
↑ Blood pressure (group 4)
↑ Monocyte levels (all treated groups)
↑ Basophils levels (group 4)
↓ MCHC (groups 3 and 4 at week 52)
↑ Large unstained cells levels (groups 2, 3, and 4 males)
↑ Phosphorus (groups 2, 3, and 4 males)

5.2.5 Assessment:
No treatment-related mortality or changes in clinical signs, body weight, food consumption, ophthalmic examination, urinalysis, organ weights, and macroscopic examination were reported.

Heart rate decrease 24 hours post treatment at week 26 in group 4 males might be test article-related. This decrease was not significant in the recovery group (week 52) and there were signs of improvements. No signs of heart rate decrease in females' groups were reported at week 26.

At 2 hours post dosing in week 10 and at 24 hours post dosing in week 52, blood pressure increased in group 4 males. This increase was not reported in females or at week 26.

The increase in monocyte and basophil levels might be an indication of an immune response related to test article treatment.

Mean corpuscular hemoglobin concentration (MCHC) is the average concentration of hemoglobin in red blood cells. MCHC is used to help diagnose the type (cause) and severity of anemia. When MCHC is low, this might be an indication of iron-deficiency anemia. It is reported as part of a standard complete blood count. It is calculated by dividing the hemoglobin by the hematocrit. The slight decrease in MCHC levels was reported in both males and females at week 52. The decrease was 1% and 2% in males and females, respectively. This slight decrease has no toxicological importance.

Large unstained cells is the measurement of the large, peroxidase-negative, cells which cannot be further characterized (i.e. as large lymphocytes, virocytes, or stem cells) present in a biological specimen. There were slight increase in large unstained cells in groups 2, 3, and 4 males only. This increase was not reported in females' groups.

Phosphorus is an essential dietary nutrient. Phosphorus is concentrated in the bones and every cell of the body contains phosphorus. Kidneys are the primary regulators of phosphorus balance in the body. Abnormally high or low levels may cause serious metabolic problems. Phosphorus levels were increased 8%, 15%, and 9% at week 26 in groups 2, 3, and 4 males, respectively. This increase was not reported at week 39. Phosphorus levels were increased 10%, 17%, and 10% at week 52 in groups 2, 3, and 4 males, respectively. Since the increase was not time responsive (not reported at week 39) and was not reported in females, no toxicological significance is associated with this increase.

GLP study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

5.2.6 Conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues reported in this study.

5.3 STUDY # 3 [002-032364]: PHLEUM PRATENSE TOXICITY STUDY BY BUCCAL CAVITY ADMINISTRATION TO (b)(4) MICE FOR 26 WEEKS FOLLOWED BY A 5 WEEK RECOVERY PREIOD.

5.3.1 Précis:

In this multiple dose toxicology study, mice were treated with *Phleum pratense* (at one of the following dose concentrations: 25,000, 75,000, or 500,000 SQ-U/day) or distilled water. Animals (10/sex/group) were treated by administering 5 µl into the sublingual area of the buccal cavity. Animals were treated over a period of 26 weeks followed by 5 weeks of recovery period. Six animals per sex per group were assigned as recovery groups (groups 1 and 4 only). Animals in the main and recovery groups were sacrificed at weeks 26 and 31, respectively. The proposed clinical dose was used in this study.

Title and study number: *Phleum pratense* toxicity study by buccal cavity administration to (b)(4) mice for 26 weeks followed by a 5 week recovery period. Study number: 002-032364

Performing laboratory: (b)(4)

Study initiation date: May 03, 2002

Final report date: March 02, 2004
Test article batch/lot:

Test article	Batch No.
Phleum Pratense	46655

Animal species and strain: (b)(4) mice
Breeder/supplier: (b)(4).
Number of animal per group and sex: 10/sex/group for the main study and 6/sex/group for the recovery groups.
Age: 40-44 days
Body weight range: 26.2-35.9 g for males and 20.0 -27.7 g for females
Route and site of administration: Oral administration (into the sublingual area of the buccal cavity)
Dose concentrations: Control group animals each received 5 µl/day of distilled water, group 2 animals each received 5 µl/day of 5,000 SQ-U (final concentration 25,000), group 3 animals each received 5 µl/day of 15,000 SQ-U (final concentration 75,000), and group 4 animals each received two 5 µl/day of 50,000 SQ-U (final concentration 500,000). The doses remained constant for the duration of the treatment period irrespective of the individual bodyweights.
Frequency of administration and study duration: The volume administered to each animal was constant (independent of bodyweight) at 5µl/mouse/day (groups 1, 2 and 3) and 10µl/mouse/day (group 4). The dose was administered to group 4 animals as two separate 5µl doses, given 1-10 minutes apart. Study duration was 31 weeks.
Dose: Animals were treated with fixed dosages of 25,000, 75,000 and 500,000 SQ-U/day. Animals were treated daily.
Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the BLA. Stability analysis was not provided in this study.

Means of administration: Oral administration (into the sublingual area of the buccal cavity).
Report status: Final report

5.3.2 Experimental design:

Animals were randomized and assigned to 4 different groups. Animals in groups 1, 2, 3, and 4 were treated with 0, 25,000, 75,000 and 500,000 SQ-U/day of test article, respectively. A recovery group consisting of 6 males and 6 females mice was attached to group 1 (control) and group 4 (500,000 SQ-U/day). Study duration was 26 weeks followed by 5 weeks recovery period. The details of the study design are listed in the following table:

Table 22: Experimental design

Group	Test material	Dosage µl/mouse/day	Dosage SQ-U	Number of Animals (#/sex/group) Main	Number of Animals (#/sex/group) Recovery
1 (control)	Distilled water	5	0	10	6
2	Phleum Pratense	5	25,000	10	0

3	Phleum Pratense	5	75,000	10	0
4	Phleum Pratense	10	500,000	10	6

5.3.3 Methods:

Randomization procedure: Yes.
Statistical analysis plan: Yes.
The following parameters were evaluated: clinical observations (twice daily), detailed observations (daily during the first week of treatment and twice weekly during weeks 2 to 4 and weekly during weeks 5 to 26), body weights (weeks -1, 0, and weekly thereafter throughout the treatment and recovery periods and before necropsy), food consumption (week -1 and weekly throughout the treatment and recovery period), body temperature (not recorded), hematology (weeks 8, 25, and week 4 of recovery), clinical chemistry (weeks 13, 26, and week 5 of recovery). Organ weight, macroscopic examination, and tissue collection (terminal necropsy at weeks 26 and 31).

Table 23: Study parameters and schedule

Parameters	Frequency of Testing
Clinical observation[5]	Twice daily
Clinical signs[6]	Daily during the first week of treatment and twice weekly during weeks 2 to 4 and weekly during weeks 5 to 26
Body weight	Weeks -1, 0, and weekly thereafter throughout the treatment and recovery periods and before necropsy
Food consumption	Week -1 and weekly throughout the treatment and recovery period
Body temperature	Not recorded
Ophthalmologic exam	Not recorded
Clinical chemistry*	Weeks 13, 26, and week 5 of recovery
Hematology*	Weeks 8, 25, and week 4 of recovery
Anti-Phleum pratense antibody	Not measured
Necropsy	Terminal necropsy at weeks 26 and 31
Organ weight, macroscopic examination, and tissues for histopathology	Terminal necropsy at weeks 26 and 31

* Blood was collected from the retro-orbital sinus.

5.3.4 Results:

Morbidity and mortality: There were three unscheduled deaths two of which were associated with blood sampling procedures.

5.3.4.1 SYSTEMIC TOXICITY:

No treatment-related changes in clinical signs, organ weights, and macroscopic examination were reported.

[5] Cageside observations include mortality, morbidity, general health and signs of toxicity.
[6] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Decrease in body weight gains for groups 2 and 4 males were reported during the weeks 0-26 and this decrease attained significance in group 4. This decrease was not reported in group 3 males and all females' groups. This decrease was not reported in group 4 males and females during the recovery period (weeks 26-31).

Significant decrease in food intake for group 4 males was reported during the weeks 1-26. This decrease was not reported in groups 2 and 3 males and all treated females groups during this period (week 1-26). This decrease was not reported during the recovery period (weeks 27-31).

5.3.4.2 HEMATOLOGY:

Minor inter-group differences from controls in the hematological parameters (weeks 8 and 25) were reported. Some of these differences were attained a level of statistical significance.

Monocyte levels were significantly decreased in groups 3 and 4 males at week 8. Hematocrat levels were significantly increased in group 4 females at week 8. Hemoglobin levels were significantly increased in group 4 females at week 8. Eosinophil levels were decreased in groups 2, 3, and 4 males at week 25. Eosinophil levels were decreased in group 2 females at week 25. Large unstained cell levels were decreased in group 2 females and group 4 males at weeks 25 and 31, respectively.

In recovery groups, hematology parameters did not reveal any changes that were considered attributable to previous treatment.

Table 24: Hematology Results

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes
WHITE BLOOD CELLS	Monocyte count Week 8 M ↓ ≥0.7 G3 Eosinophils count Week 25 M ↓ ≥0.6 G2, Week 25 M ↓ ≥0.7 G3 Week 25 M ↓ ≥0.7 G4 Week 25 F ↓ ≥0.7 G2 Large Unstained Cells (LUC) Week 25 F ↓ ≥0.6 G2 Week 31 M ↓ ≥0.5 G4	Macrophage Total leukocytes (WBC) Neutrophil count Lymphocyte count Basophils
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Prothrombin time Mean platelet volume Fibrinogen Platelet count
OTHERS		Bone marrow cytology

5.3.4.3 CLINICAL CHEMISTRY:

Inter-group differences from controls in the blood chemistry parameters (weeks 13 and 26) were reported. Some of these differences were attained a level of statistical significance. In recovery groups, blood chemistry parameters did not reveal any changes that were considered attributable to previous treatment.

Significant decrease in ALP levels in group 4 females at week 13 were reported. Significant decrease in total protein and albumin levels in groups 2, 3, and 4 females at week 13 were reported. Significant increase in gamma globulin levels in group 4 females at week 13 were reported. Significant decrease in urea levels in group 4 males at week 13 were reported. Significant increase in phosphorus levels in group 4 males at week 13 were reported. Significant decrease in sodium levels in groups 2, 3, and 4 females at week 13 were reported. Significant increase in potassium levels in group 4 males at week 26 were reported. Significant increase in triglyceride levels in groups 3 and 4 females at week 26 was reported. Significant increase in triglyceride levels in group 4 males at week 31 was reported. A decrease in triglyceride levels in group 4 females at week 31 was reported. Significant decrease in total protein levels in group 4 females at week 26 was reported. Significant decrease in albumin and A/G levels in group 4 males at week 31 was reported.

A decrease in ALT (45%) and AST (44%) levels in group 4 females at week 31 was reported.

Table 25: Clinical Chemistry Results

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Alanine aminotransferase (ALT): Week 31 F ↓ ≤ 0.6 G4 Aspartate aminotransferase (AST) Week 31 F ↓ ≤ 0.6 G4	Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids Lactate dehydrogenase (LDH)
B) HEPATOBILIARY		Gamma-glutamyl transferase (GGT) Total bile acids Alkaline phosphatase (ALP) Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein, ND, fibrinogen, ND
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Triglycerides: Week 26 F ↑ ≥1.6 G3 Week 26 F ↑ ≥1.7 G4 Week 31 F ↓ ≤0.7 G3	Albumin (A) A/G ratio Cholinesterase Total protein Creatine kinase Total Cholesterol

ND= not determined

5.3.4.4 MEAN BODY AND ORGAN WEIGHTS:

Significant increase in spleen weight (after adjustment for bodyweight) in group 3 females at week 26 were reported. Because spleen weights for group 4 (500,000 SQ-U/day) females and spleen weights for all males treated groups were comparable with those of controls, this increase was not toxicologically significant. An increase in lungs weights were reported in groups 2, 3, and 4 males were reported at week 26. Slight decrease in heart weight was reported in group 4 females at week 31. A decrease in uterus weight (50%) was reported in group 4 at week 31. This decrease is questionable as the standard deviation value was high (0.313) in control group when compared to group 4 (0.044).

No treatment related-effects on organ weights were reported in recovery groups.

Table 26: Organ weights

SEX GROUPS	males (week 26/ week 31)* 1 (CONTROL)	males (week 26/ week 31)* 2	males (week 26/ week 31)* 3	males (week 26/ week 31)* 4	females (week 26/ week 31) 1 (CONTROL)	females (week 26/ week 31) 2	females (week 26/ week 31) 3	females (week 26/ week 31) 4
NUMBER OF ANIMALS	10/5	10	10	10/6	10/6	10	10	10/5
BODY WEIGHT (terminal)	41.0/47.2	39.3	41.4	38.8/44.1	32.1/33.4	31.7	31.2	31.3/31.4
BRAIN	0.49/0.48	0.48	0.50	0.49/0.49	0.49/0.49	0.50	0.51	0.50/0.48
ADRENALS	NC	NC	NC	NC	NC	NC	NC	NC
EPIDIDYMIDES	0.11/0.11	0.11	0.11	0.11/0.11				
HEART	0.23/0.28	0.26	0.25	0.22/0.29	0.18/0.20	0.19	0.21	0.18/0.17
KIDNEYS	0.68/0.69	0.62	0.69	0.60/0.63	0.40/0.42	0.44	0.44	0.43/0.44
LIVER	2.01/2.33	1.93	2.10	1.89/2.24	1.61/1.57	1.59	1.67	1.58/1.50
LUNGS AND BRONCHI	0.26/0.38	0.35	0.32	0.30/0.34	0.28/0.33	0.29	0.32	0.28/0.30
ILLIAC LYMPH NODES Right	NC	NC	NC	NC	NC	NC	NC	NC
ILLIAC LYMPH NODES Left	NC	NC	NC	NC	NC	NC	NC	NC
SALIVARY GLAND	0.24/0.23	0.25	0.26	0.24/0.27	0.15/0.14	0.16	0.15	0.14/0.13
SEMINAL VESICLES	0.41/0.49	0.40	0.45	0.37/0.41				
SPLEEN	0.10/0.11	0.09	0.09	0.09/0.11	0.10/0.10	0.11	0.13	0.10/0.10
TESTES	0.26/0.26	0.26	0.25	0.25/0.26				
PITUITARY	NC	NC	NC	NC	NC	NC	NC	NC
PROSTATE	0.06/0.05	0.06	0.06	0.06/0.05				
THYROID and PARATHYROID	NC	NC	NC	NC	NC	NC	NC	NC
THYMUS	0.03/0.03	0.03	0.02	0.03/0.03	0.03/0.03	0.03	0.03	0.03/0.03
OVARIES					NC	NC	NC	NC
UTERUS AND CERVIX					0.25/0.40	0.31	0.33	0.30/0.20

Table of organ weight: Absolute weights are expressed as mean (grams). NC = Not collected. NA = Not applicable. * Results in groups 1 and 4 presented as values collected from terminal and recovery groups (week 26/week 31)

5.3.4.5 MACROPATHOLOGY:

No test article-related effect on macroscopic evaluation was reported.

Terminal sacrifice// recovery sacrifice

Table 27: Macroscopic findings at terminal and recovery (groups 1 and 4) sacrifice.

Group	Findings (Main// Recovery)
1M	Hairloss and scab in skin (1/4)// Congested lungs and bronchi (2/5); enlarged mandibular lymph node (1/5); hairloss in skin (1/5)
2M	Congested lungs and bronchi (7/10); forestomach depression (1/10); misshapen sternum and marrow (1/10); animal thin (1/10); hairloss in skin (1/4)// NA

3M	Congested lungs and bronchi (3/10); animal thin (2/10); swelling in tail (1/10); hairloss in skin (1/4)// NA
4M	Congested lungs and bronchi (3/10); enlarged mandibular lymph node (1/10); forestomach depression (1/10); small eyes (1/10); misshapen sternum and marrow (1/10); animal thin (3/10); hairloss and scab in skin (1/4)// Congested lungs and bronchi (1/6); granular kidneys (1/6); enlarged spleen (1/6); opaque in eyes (1/6)
1F	Congested lungs and bronchi (3/10); unilaterally enlarged kidneys (1/10); forestomach depression (1/10); small eyes (1/10); perivarian sac distension in ovaries (5/10); cyst in ovaries (1/10); fluid distention in uterus (1/10); hairloss in skin (1/4)// pale liver (1/6); small and opaque eyes (1/6); cyst and perivarian sac distension in ovaries (2/6); fluid distention (1/6), thickened (1/6), and dark (1/6) uterus; thin animal (1/6); swelling in tail (1/6)
2F	Congested lungs and bronchi (3/10); cyst in pancreas (1/10); forestomach depression (1/10); perivarian sac distension in ovaries (5/10); cyst in ovaries (1/10); dark area in ovaries (1/10); fluid distention (4/10) and thickened (1/10) uterus; animal thin (2/10); hairloss in skin (1/4)// NA
3F	Congested lungs and bronchi (5/10); pale area (1/10) and enlarged (1/10) liver; enlarged spleen (3/10); small eyes (1/10); perivarian sac distension in ovaries (5/10); fluid distention in uterus (4/10); animal thin (3/10); hairloss in skin (2/4)// NA
4F	Congested lungs and bronchi (3/10); enlarged mandibular lymph node (1/10); small eyes (1/10); opaque in eyes (2/10); perivarian sac distension in ovaries (5/10); cyst in ovaries (2/10); fluid distention in uterus (4/10); animal thin (1/10); hairloss in skin (2/4)// perivarian sac distension in ovaries (3/5); thin animal (1/6); swelling in tail (1/6)

* (number of animals with the observation/total number of animals in the group). NA = Not applicable.

Table of macroscopic findings at terminal and recovery (groups 1 and 4) sacrifice.

5.3.4.6 MICROSCOPIC FINDINGS:

Terminal sacrifice// Recovery sacrifice

Table 28: Microscopic findings at terminal sacrifice.

Group	Findings (Main// Recovery)
1M	Focal fusiform cell hyperplasia in adrenals (2/10); lymphoid aggregates in lachrymal glands (8/10); gliosis in optic nerves (1/9); aggregates of lymphocytes in pancreas (2/10); inflammation in prostate (1/10); lymphoid aggregates in salivary glands (1/10); extramedullary hemopoiesis in spleen (1/10); dysplasia in stomach (1/10); lymphoid aggregations in urinary bladder (2/10); scab (1/1), epidermal ulceration (1/10), and epidermal hyperplasia (1/10) in skin; dermal inflammation in skin (1/1)// sinus histiocytosis in mandibular lymph node (1/1); alveolar hemorrhage (1/2) and alveolar epithelial hyperplasia (1/2) in lungs Kidneys: Cortical cyst (1/10); cortical tubular basophilia (5/10); perivascular lymphoid aggregations (5/10); dilated medullary tubules (2/10) thrombus (1/10) Liver: Focal inflammation (1/10); perivascular lymphocytic infiltration (1/10)
2M	Epithelial hyperplasia (1/1), submucosal inflammation (1/1), and ulceration in the nonglandular region of the stomach
3M	Keratin cyst in tail (1/1)

4M	Focal fusiform cell hyperplasia in adrenals (3/10); lymphoid aggregates in lachrymal glands (4/10); gliosis in optic nerves (2/10); lymphoid aggregates in salivary glands (2/10); epithelial hyperplasia (1/10), submucosal inflammation (1/10), and dilated glands (1/10) in stomach; prominent residual bodies in testes (1/10); lymphoid hyperplasia in thymus (2/10); lymphoid aggregations in urinary bladder (2/10); scab (1/1), epidermal ulceration (1/10), and epidermal hyperplasia (1/10) in skin; dermal inflammation in skin (1/1)// extramedullary hemopoiesis in spleen (1/1)
	
	Kidney:
	Cortical tubular basophilia (4/10); perivascular lymphoid aggregations (1/10); dilated medullary tubules (1/10); simple tubular hyperplasia (1/10)// perivascular lymphoid aggregations (1/1); glomerulonephritis (1/1)

Group	Findings (Main// Recovery)
1F	Focal fusiform cell hyperplasia (8/10) and x zone vacuolation (9/10) in adrenals; keratitis (1/10), loss of outer nuclear layer of retina (3/10), and scleritis (2/10) in eyes; lymphoid aggregates in lachrymal glands (2/10); sinus histiocytosis in mandibular lymph node (1/10); cystic ovarian bursa in ovaries (7/10); focal acinar cell hypertrophy in pancreas (1/10); focal lymphocytic infiltration in pituitary (1/10); lymphoid aggregates in salivary glands (1/10); extramedullary hemopoiesis in spleen (3/10); dilated glands (1/10) and squamous cyst in stomach; lymphoid hyperplasia in thymus (1/10); lymphoid aggregations in urinary bladder (4/10); luminal dilatation (2/10) and edema (1/10) in uterus// loss of outer nuclear layer of retina (1/1) and traumatic disruption (1/1) in eyes; cystic ovarian bursa (3/3); luminal dilatation (3/3), edema (1/3), and endometrial hyperplasia (1/3) in uterus; keratin cyst in tail (1/1) <u>Kidney:</u> Cortical tubular basophilia (1/10); perivascular lymphoid aggregations (1/10); dilated medullary tubules (1/10); cortical scarring (1/10) <u>Liver:</u> Focal inflammation (1/10); focal hepatocyte necrosis (2/10)// increased glycogen vacuolation (1/1)
2F	Cystic ovarian bursa in ovaries (5/6); pancreatic duct cyst in pancreas (1/1); epithelial hyperplasia (1/1), submucosal inflammation (1/1), and ulceration in the nonglandular region of the stomach; luminal dilatation in uterus (4/4)
3F	Cystic ovarian bursa in ovaries (5/5); extramedullary hemopoiesis in spleen (3/3); luminal dilatation in uterus (4/4) <u>Liver:</u> Focal hepatocyte necrosis (1/1)

Group	Findings (Main// Recovery)
4F	Focal fusiform cell hyperplasia (6/9) and x zone vacuolation (4/9) in adrenals; keratitis (1/9), loss of outer nuclear layer of retina (2/9), scleritis (1/9), lenticular degeneration (2/9), and anterior chamber inflammatory cells (1/9) in eyes; lymphoid aggregates in lachrymal glands (3/9); generalized increased cellularity in mandibular lymph node (1/9); alveolar hemorrhage in lungs and bronchi (1/9); cystic ovarian bursa in ovaries (8/9); haemosiderin within cleft in pituitary (1/9); lymphoid aggregates in salivary glands (1/9); extramedullary hemopoiesis in spleen (2/9); dilated glands in stomach (3/9); lymphoid hyperplasia (2/8) and medullary atrophy (1/8) in thymus; luminal dilatation in uterus (5/9); cystic ovarian bursa (3/3); keratin cyst in tail (1/1) <u>Kidney:</u> Perivascular and peripelvic lymphoid aggregations (3/9); dilated medullary tubules (1/9); cortical scarring (1/10) <u>Liver:</u> Focal inflammation (2/9); prominent increase in mitotic activity (1/9)

* number of animals with the observation/total number of animals in the group.

An extensive number of tissues were examined for histology. No increased incidences of histological findings indicative of potential adverse events were observed in the treated groups relative to the controls.

Table 29: Test article related effects

Test article related effects
↓ Body weight gain (group 2 and 4 males)
↓ Food intake (group 4 males)
↓ Monocyte levels (groups 3 and 4 males) W*8
↓ Eosinophil levels (groups 2, 3, and 4 males) W25
↓ Eosinophil levels (group 2 females) W25
↓ Total protein and albumin (groups 2, 3, and 4 females) W13
↓ Na (groups 2, 3, and 4 females) W13
↑ Triglyceride (groups 3 and 4 females) W26
↑ Triglyceride (group 4 males) W31
↓ ALT and AST (group 4 females) W31
↑ Lung weight (groups 2, 3, and 4 males) W26
↓ Uterus weight (group 4 females) W31

*W = Week

5.3.5 Assessment:

No treatment-related mortality or changes in clinical signs, organ weights, and macroscopic examination were reported.

A decrease in body weight gain and food intake in group 4 males were reported during the main study period (weeks 1-26). This decrease was not reported in group 4 females during weeks 1-26 and group 4 males and females during the recovery period (weeks 26-31).

Monocytes are part of the body's immune system and play multiple roles in immune function. Eosinophils are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. They are granulocytes that develop during haematopoiesis in the bone marrow before migrating into blood. The decrease in monocytes and eosinophils levels were not consistent as it was not seen in female groups and was not reported in recovery groups.

The total protein test measures the total amount of two classes of proteins (albumin and globulin) found in the fluid portion of the blood. Albumin helps prevent fluid from leaking out of blood vessels. Globulins are an important part of the immune system. When the total protein levels are low, a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly, could be suggested. Low levels of protein may be reported in severe malnutrition and with conditions that cause malabsorption, such as celiac disease or inflammatory bowel disease (IBD). Total protein levels in this study were low in groups 2, 3, and 4 females and at week 13 only. This decrease was not reported in males and there were no time response (weeks 26 and 31 results did not show any differences from control group).

Sodium maintains the body's fluid and electrolyte balance, acid-base balance, muscle contractions, and nerve transmission. Too much sodium in the diet may have harmful health effects, including high blood pressure, increased risk for stroke, heart failure, osteoporosis, stomach cancer and kidney disease. The decrease in sodium level in this study were reported in females and at week 13 only. No toxicological significance was concluded from this finding as it was reported at week 13 only and was not reported in males.

Triglycerides are absorbed into the body through the small intestine after they are made in the liver or consumed in the diet. Triglycerides never travel to their destination in the body alone. Triglycerides are attached to a protein and become a lipoprotein referred to as a chylomicron or a very low-density lipoprotein (VLDL). These lipoproteins are not very dense or heavy. Therefore, along with low-density lipoproteins (LDL), they run the risk of potentially contributing to heart disease. No clear tendency of persistent increases in triglyceride levels in this study were reported.

The increases in ALT and AST were not associated with any histopathological changes and was reported in recovery groups of females only. The toxicological importance of this increase is questionable.

Lung weight increase was associated with lungs and bronchi congestion reported in groups 2, 3, and 4 males. No clear association between the decrease in uterus weight and the luminal dilatation reported in group 4 females.

GLP study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity, or interpretation of the results.

5.3.6 Conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues reported in this study.

5.4 STUDY NO. 4: TITLE AND STUDY NUMBER: A SUBCHRONIC ORAL TOXICITY STUDY OF TIMOTHY GRASS EXTRACT IN DOGS, STUDY NO. 944-002

5.4.1 Précis:

A 28-day study was conducted to evaluate the potential subchronic toxicity of *Phleum pratense* allergen tablets in (b)(4) dogs.
There were no unscheduled deaths in the study. There were no test article-related clinical signs or effects on body weight or food consumption during the treatment and recovery periods. There were no test article-related effects on ophthalmoscopy, physical examination, electrocardiography or clinical pathology. There were no test article-related organ weight changes or macroscopic findings. Similarly, there were no microscopic changes observed in any tissue including the oral cavity. Under the conditions of this study, four weeks of daily sublingual doses of *Phleum pratense* allergen at 25,000, 75,000 and 500,000 SQ-T produced no treatment- related effects.

Performing laboratory: (b)(4)
Study initiation date: March 14, 2002
Final Report date: April 14, 2003
Test article batch/lot: 28062B425, 26122B427, 26132B441
Animal species and strain: (b)(4) dog
Breeder/supplier: (b)(4)
Number of animal per group and sex: 6/sex control, 4/sex 25,000 SQ dose group, 4/sex 75,000 SQ dose group, 6/sex 500,000SQ dose group
Age: 5 months
Body weight range: males, 5.5-12.0 kg; females, 5.0-10.0 kg
Route and site of administration: oral, sublingual
Volume of injection: not applicable
Frequency of administration and study duration: daily, 28 days of administration
Dose: vehicle control, 25,000 SQ, 75, 000 SQ and 500, 000 SQ
Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use vials (one vial per dose). Stability studies were performed by the sponsor of the IND on the same batches of vaccine and adjuvant control as used in this study (see appendix).
Means of administration: oral (tablet)
Report status: final

Table 30: Experimental design

Group	Treatment SQ units	Number of Animals (#/sex/group) Treatment phase	Number of Animals (#/sex/group) Recovery phase
1	0=control	10	2
2	25,000	8	2
3	75,000	8	2
4	250,000	10	2

Table 31: Methods

Endpoint	Methodology

Hematology	(b)(4)
Clinical chemistry	(b)(4)
Coagulation	(b)(4)

Randomization procedure: All animals available for the random selection process had body weights that falling within ±20% of the mean body weight for each sex.

Animals considered suitable for study will be weighed prior to treatment and randomized, by sex, into treatment groups using a standard, by weight, block randomization procedure.

Statistical analysis
The comparisons to be used in the statistical analyzes are between the control and treatment groups. If more than one set of comparisons is required, all analyzes will be conducted separately on each set unless stated otherwise. Data for each sex within a set will also be analyzed separately.

The raw data will be tabulated within each time interval, and the mean and standard deviation or median (categorical variables) will be calculated for each endpoint by sex and group. For each endpoint, treatment groups will be compared to the control group using the analysis outlined below. Data for some endpoints, as indicated, will be transformed by either a log or rank transformation prior to conducting the specified analysis.

Group pair-wise comparisons were used for body weight, food consumption, hematology, clinical chemistry, and organ weights. For each specified endpoint and for all collection intervals, Levene's test was used to assess homogeneity of group variances. If Levene's test is not significant ($p<0.01$), Dunnett's test will be used to compare each treatment group with the control group. If Levene's test is significant {$p<0.01$), comparisons with the control group will be made using Welch's t-test with a Bonferroni conection. Results of all pair-wise comparisons will be reported at the 0.05 and 0.01 significance levels. All endpoints will be analyzed using two-tailed tests unless indicated otherwise. Log transformation was used for evaluation of leukocyte counts.

Table 32: Study parameters and schedule

Parameters	**Frequency of Testing**
Cageside observation[7]	2x daily
Clinical observations[8]	weekly
Body weight	Prior to first administration, weekly
Food consumption	weekly
Body temperature	Not measured
Ophthalmologic exam	Prior to study, at sacrifice
Clinical chemistry*	Pretest, at sacrifice, and on two/sex for Groups 1 and 4 only at recovery.
Hematology**	Pretest, at sacrifice, and on two/sex for Groups 1 and 4 only at recovery.

[7] Cageside observations include mortality, morbidity, general health and signs of toxicity.
[8] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Parameters	Frequency of Testing
Coagulation***	Pretest, at sacrifice, and on two/sex for Groups 1 and 4 only at recovery.
Immunological response	Pretest, at sacrifice
Necropsy	Day 28, day 56
Tissues for histopathology	At necropsy

*(indicate blood collection site) **(indicate blood collection site) ***(indicate blood collection site). (NC = not collected)

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. All tissues were examined for histology.

Table 33: Listing of tissues examined

Organ/Tissue	Collected	Not collected
Adrenal glands		
Aorta		
Bone (sternum & femur)		
Bone marrow (sternum & femur)	X!*	
Brain (cerebrum, cerebellum, medulla/ pons, and olfactory bulb)	X!	
Cervix	X!	
Colon	X	
Duodenum	X	
Epididymides	X*	
Esophagus	X	
Eyes (optic nerve)	X	
Fallopian tubes (oviduct)		x
Gall bladder	X	
Gross lesions (if any)	X	
Harderian gland (if rat, mouse or hamster)		
Heart	X*	
Ileum	X	
Injection site(s)		x
Jejunum	X	
Kidneys	X*	
Lacrimal glands		
Larynx		
Liver	X*	
Lung (main-stem; bronchi)	X	
Lymph nodes (cervical)	X	
Lymph nodes (mandibular)	X	
Lymph nodes (mesenteric)	X	
Mammary glands	X	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		x
Ovaries	X*	
Pancreas		
Parathyroid	X*	
Peyer's patch (if applicable)		
Pituitary gland	X*	

Organ/Tissue	Collected	Not collected
Prostate	X*	
Rectum		x
Salivary glands (mandibular)	X*	
Sciatic nerve		
Skeletal muscle	X	
Skin		x
Spinal cord (cervical, lumbar, thoracic)	X	
Spleen	X*	
Stomach (squamous and glandular)	X	
Testes	X*	
Thymus	X*	
Thyroid (w/ parathyroid glands)	X*	
Tongue	X	
Trachea	X	
Ureters		x
Uterus (w/ cervix)	X*	
Urinary bladder	X	
Vagina	X	

Table of Histology – Tissues examined. All dose groups collected and examined.

5.4.2 Results:

Organ weights for recovery animals were conducted only on controls and high dose groups.
Morbidity and mortality: All animals survived to their scheduled termination.
Clinical observations: No treatment-related observations were observed.
Food consumption: No treatment-related differences in food consumption were observed.

Table 34: Clinical Chemistry Results

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤0.7)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤0.7)**)**	NOT OF NOTE
LIVER FUNCTION: B) HEPATOBILIARY		Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein, ND, fibrinogen, ND
KIDNEY FUNCTION		Creatinine Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase Total protein Fasting triglycerides
MUSCLE INJURY		Creatine phosphokinase (CPK)[9] ND

Table of Clinical Chemistry Results
ND = not determined

Table 35: Hematology Results.

[9] Serum CPK activities in the range of 2000 too 3000 IU/liter following intramuscular dosing should be considered to have significant potential for human toxicity (Gray, Fundamental and Applied Tox 1:290, 1981). Minor increases in CPK serum levels (2 to 3 fold elevations) may be indicative of a febrile response (Mukhutdinova, Bulletin of Experimental Bio Med: 128: 674, 1999)

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great 1.5[10], ie, ≥1.6 or ≤ 0.7	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes
WHITE BLOOD CELLS	Total leukocytes (WBC), day 56 recovery group 4 males, inc 1.5x	Basophils, eosinophils count lymphocyte count Macrophage/monocyte count Neutrophil count Large unstained cells (LUC)
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen, ND
OTHERS		Bone marrow cytology

Table of Hematology Results.
ND = not determined

Table 36: Organ weights

SEX **GROUPS**	**MALES** **1 (CONTROL)**	**MALES** **2**	**MALES** **3**	**MALES** **4**	**FEMALES** **1 (CONTROL)**	**FEMALES** **2**	**FEMALES** **3**	**FEMALES** **4**
NUMBER OF ANIMALS Study/recovery	4/2	4	4	4/2	4/2	4	4	4/2
BODY WEIGHT kg (terminal)	8.0±0.40/ 9.0±0.56	8.5±0.56/	8.3±0.49	8.8±0.89/ 8.1±0.65	6.7±0.43/ 7.1±0.12	6.3±0.54	6.3±0.45	6.4±0.51/ 6.5±0.60
BRAIN	74.92±2.49/ 74.19±1.09	74.36±8.69	76.60±7.46	74.79±5.61/ 68.05±4.22	67.60±3.23/ 66.62±1.07	66.32±10.78	71.68±5.05	70.21±3.53/ 69.04±1.43
ADRENALS	0.84±0.23/ 0.98±0.05	0.92±0.16	0.87±0.20	1.12±0.22/ 0.70±0.05	0.81±0.11/ 0.90±0.07	0.78±0.04	0.82±0.09	0.81±0.12/0.83 ±0.19
EPIDIDYMIDES	1.49±0.50/ 2.12±0.29	1.53±0.24	1.59±0.30	1.65±0.39/ 1.44±0.07				
HEART	65.38±8.78/ 83.06±5.62	62.97±10.15	72.79±9.00	69.52±5.77/ 62.99±6.84	56.27±4.11/ 66.07±1.82	53.75±6.78	48.02±6.14	54.03±6.76/ 52.66±0.32
KIDNEYS	43.62±3.83/ 48.54±0.21	40.14±7.37	45. 54±4.36	48.23±3.99/ 37.03±6.30	35.09±2.28/ 36.50±4.30	35.26±3.64	34.58±4.27	33.63±4.17/ 36.02±1.50
LIVER	200.71±7.28/ 291.23±21.03	233.41±17.07	249.32±14.82	241.47±23.66/ 210.41±5.76	180.00±7.51/ 198.06±6.77	176.53±19.7 8	168.50±13. 12	184.80±30.65/ 164.79±6.37

[10] With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

SEX GROUPS	MALES 1 (CONTROL)	MALES 2	MALES 3	MALES 4	FEMALES 1 (CONTROL)	FEMALES 2	FEMALES 3	FEMALES 4
SPLEEN	37.05±6.15/ 51.41±6.52	33.83±10.77	49.71±9.32	27.91±5.32/ 34.12±12.09	35.08±13.47/ 44.05±18.05	41.08±8.78	39.59±5.88	38.67±13.32/ 38.39±2.69
TESTES	5.89±2.41/ 8.79±0.76	5.23±1.98	7.78±1.86	7.96±2.83/ 7.65±0.92				
THYROID and PARA-THYROID	0.70±0.22/ 0.88±0.05	0.76±0.13	0.73±0.17	0.76±0.11/ 0.76±0.15	0.53±0.10/ 0.61±0.30	0.54±0.08	0.55±0.18	0.68±0.07/0.59 ±0.08
THYMUS	8.04±4.47/ 5.08±0.93	11.30±3.66	7. 07±0.80	9.09±5.25/ 8.92±0.82	10.18±1.12/ 5.62±0.70	8.94±3.96	6.37±1.56	8.34±3.61/ 8.59±2.65
OVARIES				0.50±0.08/ 0.59±0.18	0.55±0.06	0.49±0.07	0.45±0.05/ 0.62±0.09	0.50±0.08/ 0.59±0.18
UTERUS				1.65±0.50/ 2.28±1.35	1.68±0.63	1.31±0.17	1.73±0.94/ 4.62±2.88	1.65±0.50/ 2.28±1.35

Table of organ weight. Absolute weights are expressed as mean (grams, unless otherwise noted).
*different from controls at P≤0.05; **different from controls at P≤0.01.

Table 37: Gross Pathology

Group	Findings
1M	NF
2M	Spleen discoloration, mild, 1/4
3M	NF
4M	NF
1F	NF
2F	NF
3F	NF
4F	NF

NF = no findings

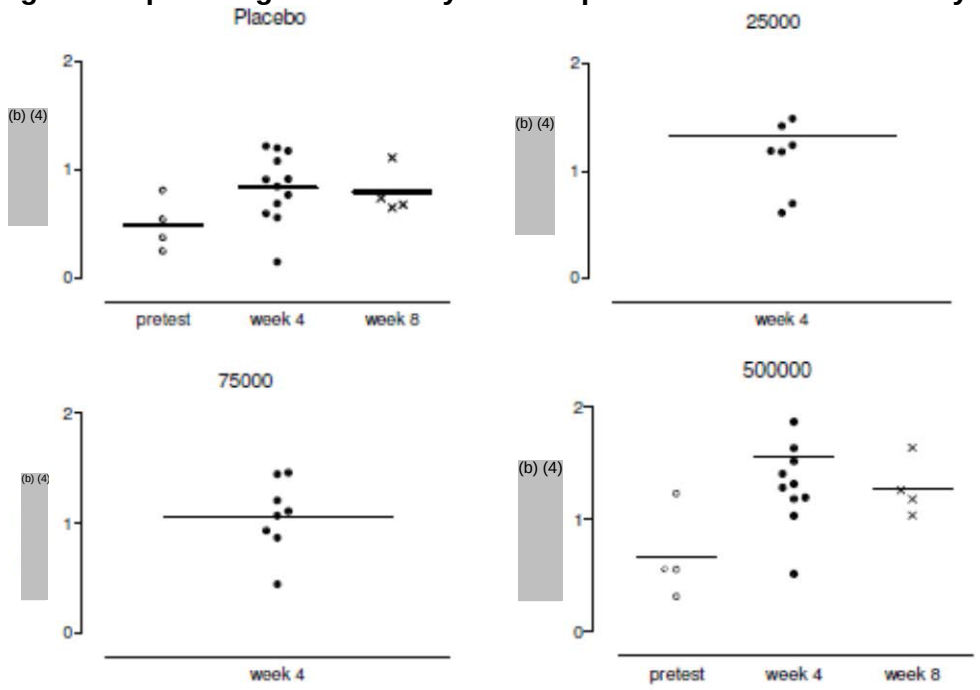
Table 38: Microscopic findings

Groups	Findings
1 M	NF
2 F	NF
2 M	Pituitary cyst,1/4 Prostate, cystic dilation, 1/4 Trachea, mild inflammation 1/4
2 F	Trachea, lymphocytic infiltrate, 1/4
3 M	NF
3 F	NF
4 M	Brain, fibrosis mild 1/4 Kidney Lymphocytic infiltrate:1/4 Pituitary cyst, 1/4
4 F	Liver, mononuclear cell infiltrate, 1/4

NF = no findings

Serology: Daily sublingual doses of the test article at doses up to 500000 for four weeks, produced significant higher levels of specific IgG compared to placebo treated animals. A dose response to the test article was not observed.

Figure 4: Specific IgG induced by Phleum pretense in four week study in dogs



Each point in each group represents the level of specific IgG in a dog plasma sample (mean values of duplicates in the dilution 1:200). The line indicates the mean value of the group at a given time point. Figure from sponsor submission.

Test article related effects:
Organ weights either not increasing or slightly decreasing in the group 4 recovery dogs are difficult to interpret in the absence of accompanying histopathology as well as the small recovery group size (n=2).

5.4.3 Assessment:

Doses of 25,000, 75,000 and 500,000 SQ units, 2500 SQ/kg, 7500 SQ/kg, and 50,000 SQ/kg, respectively, respectively were evaluated in this study. The absolute clinical dose, 1500 SQ/kg, is exceeded by these doses. The dog doses are transformed to 1555 SQ/kg, 4166 SQ/kg and 27,777 SQ/kg, respectively, which are equivalent to, or exceed the clinical dose.
No overt treatment-related toxicity was observed following oral tablet administration of the test article daily for 28 days and following recovery.

5.5 STUDY NO. 5: TITLE AND STUDY NUMBER: A SUBCHRONIC ORAL TOXICITY STUDY OF PHLEUM PRETENSE GRASS EXTRACT IN MICE, STUDY NO.944-001

Précis: The objective of this study was to evaluate the potential subchronic toxicity of the test article, *Phleum pratense* (aqueous extract of grass pollen) when administered

daily in the sublingual area of the buccal cavity. In addition, a separate challenge phase of the study was conducted in which animals were administered the test article for four weeks, followed by a four-week drug holiday, followed by a seven to nine-day challenge period with the test article (to assess hypersensitivity potential).

Performing laboratory: (b)(4)
Study initiation date: March 1, 2002
Final Report date: April 11, 2003
Test article batch/lot: 0000044495
Animal species and strain: mouse, (b)(4)
Breeder/(b)(4)
Number of animal per group and sex: 12, main study; 20 challenge phase
Age: 8 weeks
Body weight range: males, 28.9-34.6 g; females, 21.7-24.8 g
Route and site of administration: oral sublingual
Volume of administration: 5 µL, groups 1-3, 5 µL twice daily, group 4
Frequency of administration and study duration: Four main study groups received the control or test article daily for four weeks at dose levels of 0, 25,000, 75,000, and 500,000 SQ units. Four additional groups received control or test article for four weeks, followed by a four week drug holiday, followed by a challenge dose of seven to nine days, at dose levels of 0, 25,000, 75,000, and 500,000 SQ units.
Dose: 0, 25,000, 75,000, and 500,000 SQ units.
Stability: Analysis of homogeneity of the test article under test conditions was not performed as part of the study. Potency was tested following the end of dosing and was maintained.
Means of administration: pipette
Report status: final

Table 39: Experimental design

Group	Treatment, SQ units, grass extract	Number of Animals (#/sex/group) Treatment phase	Number of Animals (#/sex/group) Challenge phase
1, 5	Control (0)	12	20
2, 6	25, 000	12	20
3, 7	75, 000	12	20
4, 8	500, 000	12	20

Table 40: Methods

Endpoint	Methodology
Hematology	(b)(4)
Clinical chemistry	(b)(4)
Coagulation	(b)(4)

Randomization procedure: Animals found suitable for study were weighed prior to treatment and randomized into control and treatment groups, by sex, utilizing a standard, by weight, block randomization procedure. All mice selected for study had body weights that were within ±20% of the mean weight for each sex.

Statistical analysis plan: The comparisons used in the statistical analyzes are between the control and treatment groups. If more than one set of comparisons is required, all analyzes will be conducted separately on each set unless stated otherwise. Data for each sex within a set will also be analyzed separately.

The raw data was tabulated within each time interval, and the mean and standard deviation or median (categorical variables) will be calculated for each endpoint by sex and group. For each endpoint, treatment groups will be compared to the control group using the analysis outlined below. Data for some endpoints, as indicated, was transformed by either a log or rank transformation prior to conducting the specified analysis.
Group Pair-Wise Comparisons were used for body weight, food consumption, hematology, clinical chemistry, and organ weights. For each specified endpoint and for all collection intervals, Levene's test was used to assess homogeneity of group variances. If Levene's test is not significant ($p<0.01$), Dunnett's test will be used to compare each treatment group with the control group. If Levene's test is significant ($p<0.01$), comparisons with the control group will be made using Welch's t-test with a Bonferroni conection. Results of all pair-wise comparisons will be reported at the 0.05 and 0.01 significance levels. All endpoints will be analyzed using two-tailed tests unless indicated otherwise. Log transformation was used for evaluation of leukocyte counts.

Table 41: Study parameters and schedule

Parameters	**Frequency of Testing**
Cageside observation[11]	2x daily
Clinical observations[12]	weekly
Body weight	At receipt, prior to randomization, weekly
Food consumption	weekly
Body temperature	NC
Ophthalmologic exam	NC
Clinical chemistry*	Pretest (orbital sinus), sacrifice (cardiac puncture)
Hematology**	Pretest (orbital sinus), sacrifice (cardiac puncture)
Coagulation***	nc
Immunological response	Pretest (orbital sinus), sacrifice (cardiac puncture)
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	nc
Necropsy	Day 28, day 63
Tissues for histopathology	Day 28, day 63

*(indicate blood collection site) **(indicate blood collection site) ***(indicate blood collection site). (NC = not collected)

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Table 42: Listing of tissues examined

[11] Cageside observations include mortality, morbidity, general health and signs of toxicity.
[12] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Organ/Tissue	Collected	Not collected
Adrenal glands	X*!	
Aorta	X!	
Bone (sternum & femur)	X!	
Bone marrow (sternum & femur)	X	
Brain (cerebrum, cerebellum, medulla/ pons, and olfactory bulb)	X*!	
Cervix	X with uterus!	
Colon	X!	
Duodenum	X!	
Epididymides	X*!	
Esophagus	X!	
Eyes (optic nerve)	X!	
Fallopian tubes (oviduct)		X
Gall bladder	X!	
Gross lesions (if any)	X!	
Harderian gland (if rat, mouse or hamster)	X!	
Heart	X*!	
Ileum	X!	
Injection site(s)		X
Jejunum	X!	
Kidneys	X*!	
Lacrimal glands	X!	
Larynx		X
Liver	X*!	
Lung (main-stem; bronchi)	X*!	
Lymph nodes (cervical)		X
Lymph nodes (mandibular)	X!	
Lymph nodes (mesenteric)	X!	
Mammary glands	X!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)		X
Ovaries	X*!	
Pancreas	X!	
Peyer's patch (if applicable)		X
Pituitary gland	X*!	
Prostate	X!	
Rectum	X!	
Salivary glands (mandibular)	X!	
Sciatic nerve	X!	
Skeletal muscle	X!	
Skin	X!	
Spinal cord (cervical, lumbar, thoracic)	X!	
Spleen	X*!	
Stomach (squamous and glandular)	X!	
Testes	X*!	
Thymus	X*!	
Thyroid (w/ parathyroid glands)	X*!	

Organ/Tissue	Collected	Not collected
Tongue	X!	
Trachea	X!	
Ureters		X
Uterus (w/ cervix)	X*!	
Urinary bladder	X!	
Vagina	X!	
Zymbal's gland (if applicable)		X

Table of Histology – Tissues examined

5.5.1 Results:

Morbidity and mortality: One male in the 25,000 SQ unit low dose group was sacrificed in extremis on Day 14. One male in the 25,000 SQ unit challenge group was found dead on Day 13. Neither of these deaths appeared to be test article-related. Two females in the 500,000 SQ unit (Challenge)group were found dead, one on Day 26, and the second on Day 63 (during the challenge phase). The female found dead on Day 63 had decreased activity, impaired righting reflex, and weight loss prior to death. There was no histopathologic finding associated with the death. All other mice survived until terminal necropsy.

Clinical examination: There were no test article-related observations seen during the main or challenge studies.
Body weight: There was no test article-related effect seen on body weights. Body weights in the 500,000 SQ unit challenge group were statistically significantly different from the control weights on several weeks over the course of the study. This difference was not progressive, sometimes greater or less than control values, and not seen in the comparison groups at the same dose level. This decrease did not appear to be test article-related. Body weights in the main study females at 500,000 SQ units also showed a decrease at Week 1 but returned to similar weights as the other groups.

Food consumption: There were no test article-related effects seen on food consumption. While statistically significant differences were seen, they occurred sporadically and were not consistent over time or dose, did not appear to be test article-related.

Table 43: Clinical Chemistry results

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤0.7)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤0.7)**)**	NOT OF NOTE
LIVER FUNCTION: A) HEPATOCELLULAR		Alanine aminotransferase (ALT or SGPT) Aspartate aminotransferase (AST or SGOT) Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids
LIVER FUNCTION: B) HEPATOBILIARY		Alkaline phosphatase (ALP) Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein, ND, fibrinogen, ND
KIDNEY FUNCTION		Creatinine Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase ND Total protein Fasting triglycerides
MUSCLE INJURY		Creatine phosphokinase (CPK)[13] ND

Table of Clinical Chemistry Results
ND = not determined

Table 44: Hematology Results

[13] Serum CPK activities in the range of 2000 too 3000 IU/liter following intramuscular dosing should be considered to have significant potential for human toxicity (Gray, Fundamental and Applied Tox 1:290, 1981). Minor increases in CPK serum levels (2 to 3 fold elevations) may be indicative of a febrile response (Mukhutdinova, Bulletin of Experimental Bio Med: 128: 674, 1999)

MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE IF GREAT 1.514, IE, ≥1.6 OR ≤ 0.7	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes ND
WHITE BLOOD CELLS		Basophils, eosinophils count ND lymphocyte count Macrophage/monocyte count Neutrophil count ND Total leukocytes (WBC) Large unstained cells (LUC) ND
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time ND Platelet count Prothrombin time ND Mean platelet volume Fibrinogen ND
OTHERS		Bone marrow cytology ND

Table of Hematology Results. (Instructions to reviewers - list the differences in endpoints relative to concurrent controls used in the study. Statistical differences of (P ≤ 0.05) and biological relevance should be used in an assessment of biological meaning. In other words some statistical differences are not biologically meaningful and some lack of statistical difference obscures meaningful biological differences. Differences are relative to concurrent placebo unless otherwise indicated.

Systemic toxicity:

Table 45: Organ Weights

[14] With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

SEX GROUPS DOSE IN SQ UNITS	MALES 1 (CONTROL)	MALES 25000	MALES 75000	MALES 500000	FEMALES 1 (CONTROL)	FEMALES 25000	FEMALES 75000	FEMALES 500000
NUMBER OF ANIMALS	12/20	11/19	12/18	12/19	12/20	12/20	12/19	12/18
BODY WEIGHT (TERMINAL) G	26/30	26/29	26/29	26/28	20/21	20/22	19/22	20/22
BRAIN G	0.47/0.51	0.49/0.49	0.48/0.51	0.47/0.50	0.46/0.48	0.46/0.48	0.48/0.50	0.46/0.50
ADRENALS MG	9/7	8/6	9/8	8/7	11/10	10/11	9/12	13/10
EPIDIDYMIDES G	0.20/0.11	0.11/0.11	0.11/0.11	0.09/0.11				
HEART G	0.18/0.21	0.17/0.19	0.18/0.20	0.17/0.19	0.14/0.15	0.14/0.15	0.14/0.16	0.13/0.15
KIDNEYS G	0.57/0.66	0.58/0.64	0.53/0.63	0.55/0.62	0.32/0.39	0.34/0.38	0.34/0.41	0.34/0.39
LIVER G	1.21/1.52	1.21/1.44	1.25/1.43	1.15/1.28**	0.87/0.97	0.86/0.99	0.82/1.01	0.84/1.03
LUNG G	0.22/0.26	0.30/0.23	0.24/0.25	0.21/0.24	0.18/0.20	0.19/0.24	0.19/0.22	0.19/0.22
PITUITARY MG	3/3	3/3	2/3	3/3	3/3	3/3	3/3	3/3
SPLEEN G	0.06/0.08	0.06/0.07	0.07/0.07	0.05/0.07	0.05/0.07	0.05/0.07	0.06/0.07	0.06/0.07
TESTES G	0.23/0.24	0.25/0.23	0.25/0.24	0.24/0.23				
THYROID AND PARATHYROID MG	5/5	5/5	6/4	5/4	5/5	5/5	5/5	5/5
THYMUS G	0.03/0.02	0.03/0.02	0.03/0.02	0.03/0.02	0.03/0.03	0.03/0.02	0.03/0.03	0.03/0.03
OVARIES					25/26	28/32	27/31	25/29
UTERUS					0.14/0.22	0.14/0.25	0.15/0.21	0.17/0.22

Table of organ weight. Absolute weights are expressed as mean (grams).
*different from controls at P≤0.05; **different from controls at P≤0.01.

Table 46: Gross Pathology

Group	Findings
1M	NF
2M	NF
3M	NF
4M	NF
1F	Ovary cyst, 1/20
2F	Ovary cyst, 3/20
3F	NF
4F	ovary cyst, 2/20

NF = no findings

Table 47: Microscopic findings

Groups	Findings interim	Terminal (post challenge)
1M		Kidney, lymphocyte infiltrate, 1/12; mineralization, 3/12 Thymus, lymphoid necrosis, slight, 1/12
2M	Kidney, lymphocyte infiltrate, 1/12; mineralization, 3/12 Liver, inflammation, slight, 2/12 Thymus, lymphoid necrosis, slight, 4/12	Pituitary cyst, slight, 1/12
3M	Thymus, lymphoid necrosis, slight, 1/12	

Groups	Findings interim	Terminal (post challenge)
4M	Adrenal, cortex, pigment mild 1/12; kidney, nephropathy, slight, 1/12 Liver, inflammation, slight, 1/12 Thymus, lymphoid necrosis, severe, 1/12	Kidney,; mineralization, 1/12 Liver, inflammation, slight, 4/12; single cell necrosis 1/12 Lymph node, mesenteric, hyperplasia, 6/12 Thymus, lymphoid necrosis, mild, 3/12
1F	Thymus, lymphoid necrosis, slight, 6/12	
2F	Adrenal cortex, hyperplasia, trace, 4/12 Adrenal cortex, hyperplasia, trace, 6/12	
3F		
4F	Adrenal cortex, hyperplasia, trace, 6/12 kidney, nephropathy, slight, 1/12 Thymus, lymphoid necrosis, mild, 4/12, moderate, 2/12	Adrenal cortex, hyperplasia, trace, 1/12 kidney, nephropathy, slight, 1/12 Liver, inflammation, slight, 2/12 Spleen, hyperplasia, mild, 3/12; macrophages, 5/12

(NF = no findings) (finding may be lumped in a manner similar to that for gross pathology)

Table 48: Test article related effects

Summary	Daily Dose (SQ-unit) 0(Control)	Daily Dose (SQ-unit) 0(Control)	Daily Dose (SQ-unit) 25,000	Daily Dose (SQ-unit) 25,000	Daily Dose (SQ-unit) 75,000	Daily Dose (SQ-unit) 75,000	Daily Dose (SQ-unit) 500,000	Daily Dose (SQ-unit) 500,000
Number of Toxicity Animals **Number of Challenge Animals[a]**	M:12 M:20	F:12 F:20	M:12 M:20	F:12 F:20	M:12 M:20	F:12 F:20	M:12 M:20	F:12 F:20
NoteworthyFindings								
Mortality: Toxicity Animals	0/12	0/12	1/12	0/12	0/12	0/12	0/12	0/12
Mortality: Challenge Animals	0/20	0/20	1/20	0/20	0/20	0/20	0/20	2/20[b]
Body Weight (%) Week 4[c]	33.42g	26.42g	3.1	0.04	2.5	1.1	-1.0	0.53
Body Weight Challenge group (%)[a,d] Week 9	37.29g	27.72g	-1.8	3.2	-1.9	2.5	-4.3	1.4
Food Consumption (%)[c] **Week 4**	6.01g/animal	6.48g/animal	4.7	-11.6	5.0	-12.7	-5.3	-10.6
Food Consumption Challenge group (%)[a,d]	6.01g/animal /day	6.13g/animal /day	4.7	2.8	5.8	-7.5	-1.5	-12.4
Clinical Observations	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum Chemistry[e]	-	-	-	-	-	-	-	-
Urinalysis/Urine Chemistry	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-

- = No noteworthy findings

* = Considered test article-related # / # = number affected / number examined NA = Not applicable M = Male F = Female

a: Challenge group animals received test or control article for four weeks, followed by a four week drug holiday, followed by a challenge dose of seven to nine days.

b: There was no microscopic cause of death found. Therefore, the cause of death is unknown, but because the deaths occurred in the high dose group, they may be, but probably are not, test article-related.
c: For controls, group means are shown. For dose groups, percent differences from controls are shown.
d: At end of challenge dosing period. For controls, group means are shown. For dose groups, percent differences from controls are shown.
e: Serum samples were also used for assessment of *Phleum pratense* specific antibodies ((b)(4)). Results indicated that the serum levels of *Phleum pratense* specific IgG antibodies were proportional to the dose and time length of the sublingual treatment.

Table 49: Summary

Daily Dose (SQ-unit)	0(Control)	0(Control)	25,000	25,000	75,000	75,000	500,000	500,000
Number of Toxicity Animals **Number of Challenge Animals**[a]	M:12 M:20	F:12 F:20	M:12 M:20	F:12 F:20	M:12 M:20	F:12 F:20	M:12 M:20	F:12 F:20
NoteworthyFindings								
Mortality: Toxicity Animals	0/12	0/12	1/12	0/12	0/12	0/12	0/12	0/12
Mortality: Challenge Animals	0/20	0/20	1/20	0/20	0/20	0/20	0/20	2/20[b]
Body Weight (%) Week 4[c]	33.42g	26.42g	3.1	0.04	2.5	1.1	-1.0	0.53
Body Weight Challenge group (%)[a,d] **Week 9**	37.29g	27.72g	-1.8	3.2	-1.9	2.5	-4.3	1.4
Food Consumption (%)[c] **Week 4**	6.01g/animal /day	6.48g/animal/ day	4.7	-11.6	5.0	-12.7	-5.3	-10.6
Food Consumption Challenge group (%)[a,d] **Week 9**	6.01g/animal /day	6.13g/animal/ day	4.7	2.8	5.8	-7.5	-1.5	-12.4
Clinical Observations	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum Chemistry[e]	-	-	-	-	-	-	-	-
Urinalysis/Urine Chemistry	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-

- = No noteworthy findings * = Considered test article-related # / # = number affected / number examined NA = Not applicable M = Male F = Female

a: Challenge group animals received test or control article for four weeks, followed by a four week drug holiday, followed by a challenge dose of seven to nine days.
b: There was no microscopic cause of death found. Therefore, the cause of death is unknown, but because the deaths occurred in the high dose group, they may be, but probably are not, test article-related.
c: For controls, group means are shown. For dose groups, percent differences from controls are shown.
d: At end of challenge dosing period. For controls, group means are shown. For dose groups, percent differences from controls are shown.
e: Serum samples were also used for assessment of *Phleum pratense* specific antibodies. Results indicated that the serum levels of *Phleum pratense* specific IgG antibodies were proportional to the dose and time length of the sublingual treatment.

5.5.2 Assessment

There were no test article-related observations, or effects on body weight, body weight gain or food consumption. There were no test article-related effects on clinical pathology parameters, organ weights, or on macro- or microscopic observations. Any findings do not appear to be treatment-related, but typical findings for mice.
Under the conditions of this study, daily sublingual doses of *Phleum pretense* (aqueous extract of grass pollen) at doses of up to 500,000 SQ-U per day for 28 days, or after a four-week drug holiday followed by an additional challenge, produced no adverse effects. The doses of 500,000 SQ-U (25,000,000 SQ-U/kg), 75,000 SQ-U (3,750,000SQ/kg) and 25000 SQ-U (1,250,000 SQ/kg) in mice compare to the clinical dose of 75,000 SQ-U (1500 SQ-U/kg). The dose multiple afforded by this study in mice provides an acceptable safety margin.

6 REPRODUCTION TOXICITY STUDY:

6.1 STUDY # 1 [004-023818]: PHLEUM PRATENSE PRELIMINARY STUDY OF EFFECTS ON EMBRYO-FETAL TOXICITY IN THE (b)(4) MOUSE BY BUCCAL CAVITY ADMINISTRATION.

Key study findings: No significant findings were reported.
Study no.: 004-023818
Conducting laboratory and location: (b)(4)
Date of study initiation: 07/04/2002
Date of study completion: 10/14/2003
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity:

Test article	Batch/Lot #'s	Purity %
Phleum Pratense	5,000 SQ-U/µl-HRC14.6.2002/1; 15,000 SQ-U/µl- HRC14.6.2002/2; 50,000 SQ-U/µl- HRC14.6.2002/3	NR*

* NR = Not reported

Animal species and strain: Female mice of the (b)(4) strain
Breeder/supplier: (b)(4)
Number of animal per group and sex: Thirty-two females. Six animals were assigned to each of the four groups
Age: 8-9 weeks.
Body weight range: 25-27 grams
Route and site of administration: Sublingual area of the buccal cavity
Volume of injection: 5 (groups 1, 2, and 3) or 10 (group 4) µl/mouse/day
Frequency of administration and study duration: Daily from day 6 to day 15 after mating. Study duration was 17 days.
Dose: 0, 25,000, 75,000, and 500,000 SQ-U/mouse for groups 1, 2, 3, and 4, respectively.
Stability:
No stability results were reported.
6.1.1

6.1.2 Methods

6.1.3 Study design:

Mice were treated daily from day 6 to day 15 after mating. Animals in groups 1-3 were dosed with 5 µl/mouse/day and animals in group 4 were dosed with 10 µl/mouse/day. Animals were assigned to 4 different groups and each group contained 6 animals. The details of the study design are listed in the following table:

Table 50: Study design

Group	Treatment	Dosage Volume (µl/mouse/day)	Concentration (SQ-U/µl)	Dosage (SQ-U/mouse)	Number of Animals
1	Control	5	0	0	6
2	Phleum Pratense	5	5,000	25,000	6
3	Phleum Pratense	5	15,000	75,000	6
4	Phleum Pratense	10	50,000	500,000	6

6.1.3.1 PARAMETERS AND ENDPOINTS EVALUATED:

The following parameters were evaluated: Viability observation (once each day), full physical examination (days 0, 4, 8, 12, and 17 after mating), detailed clinical observations (daily throughout the treatment period), maternal body weight (days 0, 3 and 6-17 after mating), food consumption (days 0-2, 3-5, 6-9, 10-13, and 14-16 after mating). Macroscopic pathology examination, litter parameters, and fetal examinations (day 17 after mating). The following litter parameters were examined: number of copora lutea in each ovary, number of implantation sites, number of resorption sites, and number and distribution of fetuses in each uterine horn. Fetuses were weighed, sexed and examined for any external abnormalities. Placental weights and abnormalities were also recorded. The followings have been examined under low magnification: The neck and the thoracic and abdominal cavities.

Randomization**:** Yes
Statistical methods**:** No

6.1.4 Results

Maternal findings:

Mortality/Clinical signs:

No test article-related effects on mortality or clinical observations were reported.

6.1.5

Food consumption and body weight gain:

No test article-related effects on body weight gain or food consumption were reported. Some inter-animal variability in food consumption was reported.

Table 51: Summary of body weight changes during gestation

Group	0-6 Days	6-10 Days	6-14 Days	6-17 Days
1	3.1±0.9*	3.5±0.8	13.9±1.4	25.6±2.6
2	2.7±0.9	3.0±1.4	12.4±3.7	23.5±6.6

3	3.2±1.1	3.4±1.1	15.5±3.0	27.6±4.1
4	2.8±1.3	3.4±0.6	13.0±0.6	25.6±1.7

* Body weight ± standard deviation.

Table 52: Summary of group mean values (g/mouse/day) of food consumption during gestation

Group	0-2 Days	6-9 Days	10-13 Days	14-16 Days
1	7.6±1.7*	5.9±3.1	7.3±3.8	8.3±1.2
2	5.3±1.0	6.3±1.4	5.5±1.1	8.3±1.4
3	5.4±1.3	4.9±2.5	5.8±3.6	8.7±1.1
4	5.5±0.9	6.7±1.1	8.0±1.8	8.7±1.1

* Food consumption ± standard deviation.

Necropsy findings:
No findings related to test article treatment were reported.

Embryo-fetal findings:
Pregnancy status
Two animals were found to be non-pregnant at necropsy. These were females 8 and 19 from groups 2 and 4, respectively.

Litter data
There were no test article-related effects on the mean numbers of corpora lutea, implantations, the numbers of live young *in-utero* at termination, or the percentage of males in the litters. However, early and late resorption and percent implantation loss were higher in group 4 when compared to control group. Summary of the results are listed in the following table:

Table 53: Summary of litter data at gestation day 17

Group	Corpora Lutea*	Implant-ations*	Resor-ptions Early	Resor-ptions Late	Resor-ptions Total	Live Young Male*	Live Young Female*	Live Young Total*	Sex Ratio (%M)	Implan-tation Loss (%) Pre-	Implan-tation Loss (%) Post-
1	15.2 ± 1.5	15.3 ± 1.5	0.2	0.3	0.5	7.3 ± 1.4	7.5 ± 2.3	14.8 ± 1.7	50.1	0.0	3.3
2	13.8 ± 1.5	14.0 ± 2.9	0.4	0.0	0.4	6.8 ± 1.6	6.8 ± 4.1	13.6 ± 3.0	53.8	4.6	3.2
3	15.7 ± 2.1	16.0 ± 1.3	0.5	0.0	0.5	8.3 ± 2.2	7.2 ± 1.0	15.5 ± 1.8	53.2	2.1	3.1
4	16.0 ± 0.7	15.6 ± 1.1	0.4	0.4	0.8	8.6 ± 2.5	6.2 ± 1.1	14.8 ± 1.8	57.2	4.9	5.3

* Value ± standard deviation.

Placental, litter and fetal weights
No test article-related effects on placental, litter, and fetal weights were reported. Summary of the results are listed in the following table:

Table 54: Summary of placental, litter, and fetal weights at gestation day 17.

Group	Placental	Litter	Fetal	Fetal	Fetal

	Weight*	Weight*	Weights Males*	Weights Females*	Weights Overall*
1	0.12 ± 0.02	16.1 ± 2.04	1.13 ± 0.11	1.05 ± 0.08	1.09 ± 0.09
2	0.13 ± 0.01	15.1 ± 3.83	1.12 ± 0.09	1.07 ± 0.08	1.10 ± 0.07
3	0.13 ± 0.02	17.5 ± 2.30	1.15 ± 0.06	1.10 ± 0.06	1.13 ± 0.06
4	0.12 ± 0.01	15.9 ± 1.71	1.10 ± 0.13	1.06 ± 0.11	1.08 ± 0.12

* Value ± standard deviation.

Fetal findings

One fetus in litter 11 (group 2) and one fetus in litter 21 (group 4) exhibited cleft palate. Since cleft palate was reported in control litter 2, this finding was not considered test article-related. Exencephaly was reported in one fetus in litter 12 (group 2) and one fetus in litter 20 (group 4). This finding was reported in one animal of groups 2 and 4 and none was reported in group 3. Thus, this finding was not clearly related to test article treatment. There were no conclusive effects of treatment with *Phleum pratense* on fetuses. Summary of fetal findings are listed in the table below:

Table 55: Summary of fetal abnormalities at necropsy

Group 1 (Control)	Litter Size	Fetus Number	Weight (g) (Sex M/F)	Necropsy Observations
1	16	--	--	NAD
2	13	L7	0.99(M)	Cleft palate
3	17	L5	0.61(F)	Shiny skin
4	16	--	--	NAD
5	13	--	--	NAD
6	14	R11	0.68(F)	Shiny skin
Group 2 (25,000 SQ/U/Mouse/Day	**Litter Size**	**Fetus Number**	**Weight (g) (Sex M/F)**	**Necropsy Observations**
7	12	--	--	NAD
8	NP			
9	09	--	--	NAD
10	16	--	--	NAD
11	16	L1 R10	1.29(M) 1.21(M)	Hemorrhage on nares Cleft palate
12	15	L2	0.96(F)	Exencephaly
Group 3 (75,000 SQ/U/Mouse/Day)	**Litter Size**	**Fetus Number**	**Weight (g) (Sex M/F)**	**Necropsy Observations**
13	16	--	--	NAD
14	14	--	--	NAD

15	16	--	--	NAD
16	16	--	--	NAD
17	18	--	--	NAD
18	13	--	--	NAD
Group 4 (500,000 SQ/U/Mouse/Day)	**Litter Size**	**Fetus Number**	**Weight (g) (Sex M/F)**	**Necropsy Observations**
19	NP			
20	13	R7	0.85(F)	Exencephaly, eyelids not apparent
21	16	R10	0.99(M)	Cleft palate
22	15	--	--	NAD
23	13	--	--	NAD
24	17	--	--	NAD

Only fetuses with observations are reported
M/F = Male/Female
NAD = No abnormalities detected
NP = Not pregnant

6.1.6 Summary:

The objective of this study was to evaluate the effect of *Phleum pratense* vaccine, administered sublingually, on pregnant mice. Animals (6/group) were assigned to 4 different groups and treated by 5 (groups 1, 2, and 3) or 10 (group 4) µl/mouse/day, of control or test article sublingually. Terminal sacrifice necropsies were conducted on study day 17.

Clinical symptoms, mortality, body weight, food consumption, litter data, placental, litter, and fetal weights, fetal and placental abnormalities and necropsy findings were evaluated. Gross necropsy was performed on litters and fetuses.

Caesarian sectioning:
No test article-related treatment, at 500,000 SQ-U from day 6 to day 15 of gestation, systemic toxicity were reported. There were a tendency of increases in resorptions and % implantation loss in group 4 when compared to control group. However, these increases did not reach significance but might be test article-related.

Natural delivery and F1 generation:
No animals were assigned for natural delivery and F1 generation investigation.

6.1.7

6.1.8 Conclusions

The administrations of Phleum pratense on SD's 6 to 15 at 5 or 10 µl/mouse/day via the buccal cavity did not give an indication of adverse effects on the litters or their fetuses.

6.2 STUDY NO. 2: STUDY TITLE: PHLEUM PRETENSE: PRE- AND POST-NATAL DEVELOPMENT STUDY IN THE (b)(4) MOUSE BY BUCCAL CAVITY ADMINISTRATION

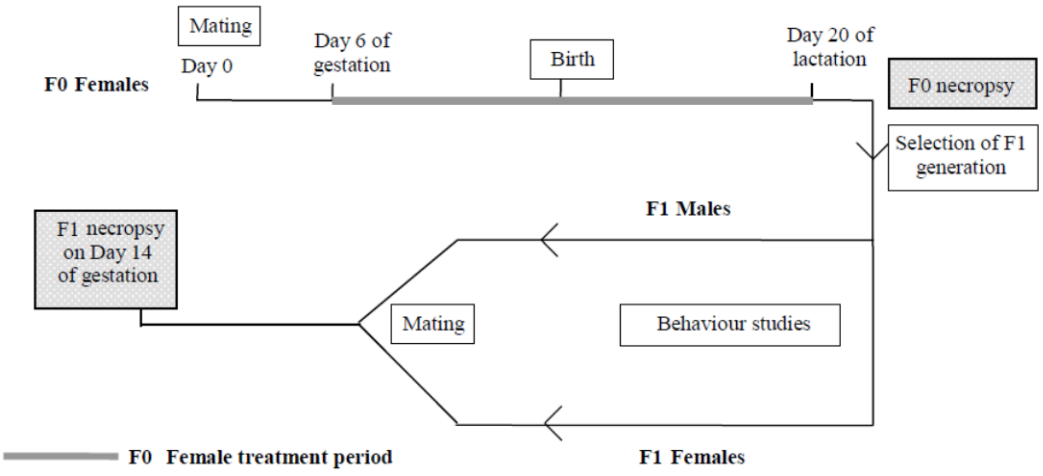
Key study findings: Treatment of F_0 females from Day 6 of gestation to Day 20 of lactation had no effect upon the number of implantation sites, total litter size on Day 1 in F_1 dams, live litter size, sex ratio, body weights, behavioral functions, offspring survival assessed through to weaning or macroscopic pathology. There were no test article-related clinical signs or effects on body weight, sexual maturation or sensory examinations in F_1 animals selected for subsequent mating. There were no test article-related effects on mating performance, fertility, reproductive parameters or macroscopic pathology were observed in F_1 animals.
Study no.: LEA 010/042144
Conducting laboratory and location: (b)(4)

Date of study initiation: February 20, 2004
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: IMP SQ Phleum pretense, PHL 0010 98-103%

6.2.1 Methods

Doses: 0, 25,000, 75,000, 500,000 SQ-U
Species/strain: mouse, (b)(4)
Number/sex/group: F0 females: 24; F1: 22
Route, formulation, volume, and infusion rate: oral, solution in reverse osmosis purifies water

Figure 5: Study design for pre- and post-natal developmental study in mice



Flow chart of study design, from sponsor submission

Parameters and endpoints evaluated:
F0
Mortality and clinical signs
Bodyweight
Food consumption

Gestation length, parturition and gestation length
Gross pathology
F1 litter
Litter size, sex ratio and survival
Bodyweight
Sexual maturation
Sensory examinations
Mating performance and fertility
Gross pathology
Reproductive assessment

Statistical methods:
Statistical analysis were applied where there was indication of possible meaningful intergroup differences. All statistical analyzes were carried out using the individual animal (or litter) as the basic experimental unit.
The following data types were analyzed, in support of interpretation:
F0 female gestation bodyweight change
F0 gestation length and gestation index (analyzed as number of live litters born)
F1 male bodyweight, using weights gains over appropriate study periods.
Motor activity
Accelerating rotarod
Morris water maze, where
For trial time the reciprocal of the mean of three trials was analyzed
For sector count the square root of the mean of the three trials was analyzed
The number of fails or the number of occurrences over the three trials where trial time was maximum (i.e. 120 secs)
For binary (e.g. absence/presence) categorical data, the proportion of animals (or litters/fetuses, as applicable) was analyzed using Fisher.s Exact test (Fisher 1973) for each treated group versus the control.
For continuous data, Bartlett.s test (Bartlett 1937) was first applied to test the homogeneity of variance between the groups. Dependent on the outcome, the following tests were applied:
If 75% of the data (across all groups) were the same value, for example c, then a frequency analysis was applied. Treatment groups were compared using a Mantel test for a trend in proportions (Mantel 1963) and also pairwise Fisher's Exact tests (Fisher 1973) for each dose group against the control both for i) values <c versus values >=c,and for ii) values <=c versus values >c, as applicable.
If Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level, then parametric analysis was applied. If the F1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Williams' test for a monotonic trend (Williams 1971, 1972) was applied. If the F1 test was significant, suggesting that the dose-response was not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead.
If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Shirley's test for a monotonic trend (Shirley 1977) was applied. If the H1 test was significant, suggesting that the dose-response was not monotone, Steel's test (Steel 1959) was performed instead.

Gestation length was analyzed using two-tailed Linear-by-linear tests, with stepdown, using equally spaced scores (Cytel 1995). The test was asymptotic when all four groups included and exact on step-down.
The number of live litters born were analyzed using one-tailed exact Cochran-Armitage tests (Cytel 1995) with step down.

6.2.2 Results F0 Generation

Mortality/Clinical signs:
Two females treated at 25,000 SQ-U/day, one female treated at 75,000 SQ-U/day and one female treated at 500,000 SQ-U/day were killed during early lactation after all the offspring in their litters died; these litters deaths did not appear to be related to treatment. There were no other unscheduled deaths.
Although there were more clinical signs present in the treated groups than in Control, the majority of these were minor in nature (e.g. staining, kinked tail) and not suggestive of an effect of treatment. In addition, three females at 25,000 SQ-U/day and two females at 500,000 SQ-U/day exhibited overactive behavior during late gestation or early lactation. Piloerection was also observed in one female at 25,000 SQ-U/day and four females at 500,000 SQ-U/day during mid-late lactation, either as a post-dosing observation or at routine physical examination on between 1 and 5 days of treatment with no animals showing this sign by the end of the treatment period.

Body weight:
Bodyweight gain at 500,000 SQ-U/day was slightly lower than Control following the start of treatment. This difference attained statistical significance for the periods
Days 6-10 and 6-14 of gestation. Thereafter, bodyweight change was unaffected up to Day 21 of lactation and, overall weight during gestation was only 7% lower than in controls.
There was no effect on bodyweight change of F0 females treated at 25,000 or 75,000 SQ-U/day during gestation or lactation.

Food consumption:
Food consumption values for all treatment groups were similar to that of Control animals during gestation and lactation.

Gestation length, parturition and gestation index
There were no effects on gestation length, parturition and gestation index.
Three Control females and one female from each of the 25,000 and 500,000 SQ-U treated groups were not pregnant. One female in the 500,000 SQ-U treatment group was not observed to litter but was found to have implantation sites at necropsy, and one female at 75,000 SQ-U gave birth to one offspring that was found partially cannibalized. All other animals gave birth to a live litter.

Table 56: Gross pathology

Group		Implantations	Total itter size	Live litter size on Day	Live litter size on Day	Live litter size on Day	Live litter size on Day	Live litter size on Day	Live litter size on Day	Live litter size on Day	Live litter size on Day
			Day	Before cull	Before cull			After cull	After cull		
			1	1	1	4	4	7	11	18	21
1	Mean	13.5	12.6	12.5	12.5	9.8	9.8	9.8	9.8	9.8	9.8

	SD	1.8	2.5	2.4	2.4	0.7	0.7	0.7	0.7	0.7	0.7
	n	21	21	21	21	21	21	21	21	21	21
2	Mean	13.8	13.1	13.0	12.9	10.0	10.0	10.0	10.0	10.0	9.9
	SD	1.9	1.6	1.6	1.6	0.0	0.2	0.2	0.2	0.2	0.3
	n	21	21	21	21	21	21	21	21	21	21
3	Mean	13.7	13.0	12.8	12.7	9.9	9.9	9.9	9.9	9.9	9.9
	SD	2.1	2.2	2.2	2.3	0.3	0.3	0.3	0.3	0.3	0.3
	nn	23	23	23	23	23	23	23	23	23	23
4	Mean	13.0	12.2	12.1	12.1	9.9	9.9	9.9	9.9	9.9	9.9
	SD	1.8	1.5	1.5	1.5	0.4	0.4	0.4	0.4	0.4	0.4
	n	21	21	21	21	21	21	21	21	21	21

F1 litter
Litter size, sex ratio and survival
Litter size – group mean values (F1)

Table 57: Sex ratios

Group	Total on Day 1	Total on Day 1	Total on Day 1		Live (before cull) on Day 1	Live (before cull) on Day 1	Live (before cull) on Day 1	4			Live (after cull) on Day 4	Live (after cull) on Day 4	Live (after cull) on Day 4	21	
	M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
1	Mean 6.1	6.4	48.5	6.1	6.4	48.4	6.1	6.4	48.4		5.0	48.9	4.8	5.0	48.
	SD 1.9	1.5	9.9	1.8	1.5	9.6	1.8	1.5	9.6		0.7	5.9	0.6	0.7	5.9
	n 21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
2	Mean 6.3	6.8	48.2	6.2	6.8	47.9	6.1	6.7	47.7		5.3	47.1	4.6	5.3	46.
	SD 2.2	2.0	14.5	2.0	2.0	14.2	2.1	2.1	14.6		0.9	9.0	1.0	0.9	9.7
	n 21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
3	Mean 6.2	6.7	47.6	6.1	6.7	47.7	6.0	6.7	47.5		5.2	47.7	4.7	5.2	47.
	SD 2.2	2.0	13.1	2.2	2.1	13.4	2.1	2.1	13.3		0.8	8.2	0.9	0.8	8.2
	n 23	23	23	23	23	23	23	23	23	23	23	23	23	23	23
4	Mean 6 4	5 8	53 2	6 4	5 7	53 4	6 4	5 7	53 6		4 6	53 3	5 3	4 6	53
	SD 1 9	2 3	15 7	1 9	2 3	15 5	1 9	2 2	15 4		1 1	10 6	1 1	1 1	10
	n 21	21	21	21	21	21	21	21	21	21	21	21	21	21	21

Sex ratio – group mean values (F1)

Treatment of females from Day 6 of gestation to Day 20 of lactation had no effect upon implantation site counts, total litter size on Day 1 or live litter size and offspring survival assessed through to weaning. Sex ratio was also unaffected.
A total of 4 females experienced total litter loss before weaning. Although there were no total litter losses in the Control group, the low incidence of litter losses, the distribution in the treated groups and the absence of increase/mortality among the litters that survived to weaning, did not implicate maternal treatment with *Phleum pratense* as a cause.

Female 31 treated at 25,000 SQ-U/day lost 6 offspring on Day 5 of lactation (3 males and 2 females found dead, 1 female killed for reasons of animal welfare following partial cannibalization), with the remaining 4 offspring (3 males and 1 female) found dead on Day 6.

On Day 6 this female exhibited overactive behavior. There were no clinical signs present for this litter to indicate a cause of the offspring mortality. All offspring of
Female 33 (treated at 25,000 SQ-U/day) also died prematurely. Three offspring in this litter (2 males and 1 female) were found dead at completion of parturition with no milk in

their stomachs. Two further offspring (1 male and 1 female) were killed for reasons of animal welfare on Day 2 of age. These animals were pale, cold, less active, with dark abdomens.

The abdomen of the male offspring was also distended, and the stomach contained gas and no milk. The female offspring appeared to have been fed. This dam was also observed to be overactive from Day 2 of lactation. The surviving offspring in this litter were pale and underactive, but appeared to be feeding; however, on Day 7 of age/lactation, the remaining offspring were found dead (2 males and 4 females) or killed for reasons of animal welfare (2 males). The 2 males that were killed showed pallor, underactive behavior and one also showed labored respiration. Necropsy revealed some skin abrasions and clotted blood/ subcutaneous hemorrhage in the thoracic cavity of two males and three females.

Female 51 at 75,000 SQ-U/day gave birth to 1 offspring, which was found dead and partially cannibalized to an extent that it was unable to be sexed on Day 1 of age. The small litter size was confirmed by the finding of only 1 uterine implantation site at necropsy.

The litter of Female 83 receiving 500,000 SQ-U/day were cold and underactive with little milk in the stomachs on Day 3 of age, when the dam was observed to be overactive. All the offspring (10 males and 6 females) were therefore killed for reasons of animal welfare. Necropsy examination confirmed that the majority of the offspring had no milk in their stomach.

Bodyweight
Bodyweight and bodyweight change of male and female offspring in treated groups from Day 1 until Day 21 of age was similar to Control values.

Pre-weaning development
The mean age that the surface and air righting reflexes were observed was similar in all groups. A similar percentage of offspring in each group passed the pupil reflex test, and all animals passed the auditory startle response test on Day 20 of age.

Offspring gross pathology
There were no macroscopic observations considered to be related to treatment.
No milk in the stomach was the main finding among offspring dying during early lactation.

Mortality and clinical signs
There was little incidence of clinical signs observed in the selected F1 animals and these did not appear related to maternal treatment.

Bodyweight

Table 58: Bodyweight and bodyweight change - group mean values for females during gestation (F1)

Group		Day 0	Day 3	Day 6	Day 10	Day 14	Days 0-3	Days 0-6	Days 0-10	Days 0-14
1	Mean	30.1	32.3	34.2	38.7	49.8	2.2	4.1	8.6	19.7
	SD	2.8	2.8	3.2	3.4	4.0	0.9	1.4	1.6	2.6

	n	21	21	21	21	21	21	21	21	21
2	Mean	31.4	34.2	36.4	41.5	52.8	2.8	5.0	10.1	21.3
	SD	3.6	4.5	4.8	5.5	6.3	1.4	1.7	2.4	3.5
	n	21	21	21	21	21	21	21	21	21
3	Mean	31.8	34.3	36.5	41.1	52.1	2.5	4.7	9.3	20.3
	SD	3.3	3.5	3.9	4.3	5.1	0.9	1.1	1.4	3.0
	n	21	21	21	21	21	21	21	21	21
4	Mean	29.6	32.5	34.4	39.5	50.1	2.9	4.8	9.9	20.6
	SD	2.6	2.9	3.3	3.6	4.8	0.8	1.3	1.5	2.8
	n	20	20	20	20	20	20	20	20	20

The bodyweight of selected females before pairing or during gestation showed no effects of maternal treatment with the test material. The bodyweight change of males whose mothers were treated at 500,000 SQ-U/day was 12% lower than Control at week 10. The variability did not appear to be significant. No difference was observed in females.

Table 59: Sexual maturation

		Vaginal Opening	Vaginal Opening	Balano-preputial separation	Balano-preputial separation
Group		Age	Bodyweig	Age	Bodyweight
		(days)	(g)	(days)	(g)
1	Mean	27.8	19.4	27.4	22.4
	SD	1.5	1.80	1.1	2.71
	n	22	22	22	22
2	Mean	28.7	19.6	27.2	21.7
	SD	1.9	2.55	1.0	2.23
	n	22	22	22	22
3	Mean	28.5	19.9	27.3	21.3
	SD	2.3	1.76	1.2	2.37
	n	22	22	22	22
4	Mean	28.5	19.8	27.7	21.8
	SD	2.0	2.15	1.1	2.41
	n	22	22	22	22

Sexual maturation – group mean age and bodyweight at attainment (F1)
(Specify methods of statistical analysis): * p<0.05, ** p<0.01

The age and bodyweight of the animals on the day vaginal opening or balano-preputial separation was detected was similar in all groups.

Sensory examinations

Motor activity
Maternal treatment with the test article did not appear to affect the motor activity of their male or female offspring; there were no statistically significant intergroup differences in total levels of low (cage floor) or high (rearing) beam activity.
The high beam activity of males in the 500,000 SQ-U/day was slightly, but consistently lower than in Controls throughout the second half of the one hour monitoring period. However, there was marked inter-animal variability in performance, and none of the

differences for the individual 6-minute recording periods attained statistical significance. Therefore, no effect of material treatment was inferred. Among females in the 500,000 SQ-U/day group, high beam activity was significantly higher at 42 minutes, and low beam activity was significantly higher at 24 minutes, compared with Controls. These differences do not appear to be of toxicological significance.

Neuromuscular co-ordination - accelerating rotarod
All animals in all groups performed well on this test, and there were no effects of treatment detected.

Learning and memory - Morris water maze
The learning and memory in males was considered to have been unaffected by maternal treatment, as judged by similar improvements in trial times and sector entries over the 4 days of testing in the Morris water maze in all groups of males.
There was no conclusive evidence that maternal treatment had affected learning and memory in females. Animals in the 500,000 SQ-U/day groups had noticeably and significantly longer mean trial times on Day 4 of testing compared with Controls. However, this isolated difference was not considered to indicate an adverse effect on learning/memory since; the animals showed progressive improvements in trial times and sector entries throughout the 4 days of testing as in the Controls; the actual improvement in mean trial time over the first 3 days of testing (indicating learning/memory) was 215 compared with 225 in Controls; pool sector entries continued to improve between Days 3 and 4 with the overall percentage improvement in sector entries over the 4 days of testing similar to Controls.

Mating performance and fertility
The percentage of animals in treated groups with a pre-coital interval of 1-4 days exceeded 90% and was unaffected by treatment. Fertility was also unaffected by maternal treatment with Phleum Pratense. One female in each of the 25,000 SQ-U/day and 75,000 SQ-U/day and two females in the 500,000 SQ-U/day groups were not pregnant.

Gross pathology
The macroscopic abnormalities in the selected F1 animals appear to be incidental and not related to treatment.

Table 60: Reproductive Assessment

Group		Corpora lutea	Implantations	Live embryos	Resorptions Early	Resorptions Late	Resorptions Total	Implantation loss (%) Pre-	Implantation loss (%) Post-
1	Mean	15.6	15.1	13.9	1.0	0.2	1.2	3.4	8.2
	SD	2.3	2.0	2.4					
	n	21	21	21	21	21	21	21	21
2	Mean	16.5	15.8	15.0	0.7	0.0	0.7	6.3	4.2
	SD	3.1	2.3	1.9					
	n	21	21	21	21	21	21	21	21
3	Mean	15.9	15.0	14.4	0.6	0.0	0.7	7.1	4.9
	SD	3.3	3.7	3.8					
	n	21	21	21	21	21	21	21	21
4	Mean	15.2	15.0	14.1	0.8	0.2	1.0	2.8	6.4
	SD	2.0	1.8	2.0					
	n	20	20	20	20	20	20	20	20

Litter data – group mean values on Day 14 of gestation (F1)

There was no effect upon mean numbers of corpora lutea, implantations, live embryos or resorptions (early or late) at any dosage.

6.2.3 Summary

Table 61: Summary

Daily Dose (SQ-U/mouse/day)	**0(Control)**	**25,000**	**75,000**	**500,000**
F0 Females:				
No. Evaluated	24	24	24	24
No. Pregnant	21	23	24	23
Mortality	0	2[a]	1[a]	1[a]
No. Aborted or with Total Resorption of Litter	0	0	1[b]	1
Clinical Observations: Gestation Body	-	-	-	-
Weight[c] (%)	55.1g	1.6	-1.1	-3.6
Gestation Body Weight Gain[c] (%)	23.4g	3.4	-3.0	-7.27
Lactation Body Weight[c] (%)	37.8g	0.8	2.4	-3.7

- = No noteworthy findings

a: These females were sacrificed during early lactation after all offspring in their litters died; these litter deaths were not considered to be related to maternal treatment.

b: One female gave birth to one offspring that was found partially cannibalized.

c: At end of gestation or lactation. For controls, group means are shown. For dose groups, percent differences from controls are shown.

Table 62: Doses

F0 Females:				
Gestation Food Consumption[a(%)]	-	-	-	-
Lactation Food Consumption[a(%)]	-	-	-	-
Necropsy Observations	-	-	-	-
Duration of Gestation (Days)	-	-	-	-
Abnormal Parturition **F1**	0	0	0	0
Litters(Preweaning):				
No. Litters Evaluated	21	23	23	22
Mean No. of Implantations	13.5	13.8	13.7	13.0
Mean No. of Live born Pups/Litter	12.5	13.0	12.8	12.1
Postnatal Survival to Day 4: Pups/Litter	12.5	12.9	12.7	12.1
Postnatal Survival to Weaning: Pups/Litter	9.8	9.9	9.9	9.9
No. of Total Litter Losses	0	2	1	1

- = No noteworthy findings

Table 63:

Daily Dose (SQ-U/mouse/day)	**0(Control)**	**25,000**	**75,000**	**500,000**
F1 Litters(Preweaning):				
Pup Body Weights (g)				
Day 1, Male/Female	1.8/1.7	1.8 /1.7	1.7/1.7	1.7/1.7
Day 21, Male/Female	13.3/12.8	13.0/12.6	13.0/12.6	12.7/12.3

Pup Body Weight Change Day 1-21 Male/Female (g)	11.5/11.0	11.2/10.9	11.3/10.9	10.9/10.6
Mean Pup Sex Ratio on Day 1, %M:%F	48.5 : 51.5	48.2 : 51.8	47.6 : 52.4	53.2 : 46.8
Pup Clinical Observations	-	-	-	-
Pup Necropsy Observations	-	-	-	-
Surface Righting Age (Day)	5.9	5.9	5.5	6.0
Air Righting Age (Day)	13.1	13.3	13.3	13.3
Startle Response (% pass)	100.0	100.0	100.0	100.0
Pupil Reflex (% pass)	95.9	99.0	98.3	97.1
F_1 Males (Postweaning):				
No. Evaluated Postweaning	22	22	22	22
Mortality	0	0	0	0
Body Weight at Week 10 (%)	44.6	-2.0	-4.5	-7.8
Body Weight Change Week 1-10 (%)	21.1	-1.9	-3.8	-11.8
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Mean Age of Balano-Preputial Separation (Days)	27.4	27.2	27.3	27.7

- = No noteworthy findings

Table 64:

Daily Dose (SQ-U/mouse/day)	**0(Control)**	**25,000**	**75,000**	**500,000**
F1 Males(Postweaning):				
Neuromuscular Co-ordination- accelerating rotorod	-	-	-	-
Motor Activity	-	-	-	-
Learning and Memory- Morris water maze	-	-	-	-
No. of Males that Mated	22	22	22	22
F1 Females(Postweaning):				
No. Evaluated Postweaning	22	22	22	22
Mortality	0	0	0	0
Premating Body Weight at Week 7 (%)	30.1g	7.6	7.3	1.0
Premating Body Weight Change[a] (%)	10.6g	28.3	22.6	6.6
Gestation Body Weight at Day 14 (%)	49.8g	6.0	4.6	0.6
Gestation Body Weight Change[b] (%)	19.7g	8.1	3.0	4.6

- = No noteworthy findings

a: From weeks 1-7. For controls, group means are shown. For dose groups, percent differences from controls are shown.

b: From days 0-14. For controls, group means are shown. For dose groups, percent differences from controls are shown.

Table 65:

F1Females(Postweaning): Clinical Observations[a]	-	-	-	-
Necropsy Observations	-	-	-	-
Mean Age of Vaginal Patency (Days)	27.8	28.7	28.5	28.5
Neuromuscular co-ordination- accelerating rotarod	-	-	-	-
Motor Activity	-	-	-	-
Learning and Memory- Morris water maze	-	-	-	-
No. of Females that Mated	22	22	22	22
No. of Pregnant Females	22	21	21	20

Reproductive Parameters				
Conception Rate (%)[b]	100	95	95	91
Fertility Index (%)[c]	100	95	95	91
Mean No. of Corpora Lutea	15.6	16.5	15.9	15.2
Mean No. of Implantations	15.1	15.8	15.0	15.0
Mean % Preimplantation Loss	3.4	6.3	7.1	13.9
Mean No. of Live Young (/animal)	13.9	15.0	14.4	14.1

- = No noteworthy findings
a: From weaning to mating.
b: Conception Rate = number of pregnancies / number mated.
c: Fertility Index = number of pregnancies / number of pairings.

6.2.4 Discussion and Conclusions

Treatment of F0 females from Day 6 of gestation to Day 20 of lactation had no effect upon the number of implantation sites, total litter size on Day 1 in F1 dams, live litter size, sex ratio, body weights, behavioral functions, offspring survival assessed through to weaning or macroscopic pathology. There were no test article-related clinical signs or effects on body weight, sexual maturation or sensory examinations in F1 animals selected for subsequent mating. Likewise, no test article-related effects on mating performance, fertility, reproductive parameters or macroscopic pathology were observed in F1 animals.

The absolute doses administered to mice in this study, 25,000, 75,000 and 500,000 SQ-U represent exposures of 1,250,000, 3,750,000 and 25,000,000 SQ-U/kg, respectively. This compares to the clinical dose of 75,000 SQ-U or 1500 SQ-U/kg. When adjusted for body surface area differences, the respective mouse doses are 100, 203, and 1355x the human dose. The large dose multiple relative to the clinical dose also presents a margin for safety.

6.3 STUDY NO. 3: STUDY TITLE: PHLEUM PRETENSE COMBINED STUDY ON FERTILITY AND EMBRYO-FETAL DEVELOPMENT IN THE (b)(4) MOUSE BY BUCCAL CAVITY ADMINISTRATION

6.3.1 Key study findings:

Treatment of male mice with *Phleum pratense* before pairing and female mice before pairing and throughout gestation Day 15 at dosages up to 500,000 SQ-U/day was not associated with any apparent systemic toxicity. Mating performance and fertility was unaffected, and there were no adverse fetal findings. The observed no-observed-adverse-effect level (NOAEL) for fertility and embryo-fetal development within the context of this study was 500,000 SQ-U/day.
Study no.: LEA 008/033623
Volume #, and page #:
Conducting laboratory and location: (b)(4)

Date of study initiation:September 18, 2003
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: IMP SQ Phleum pretense, PHL 0010,

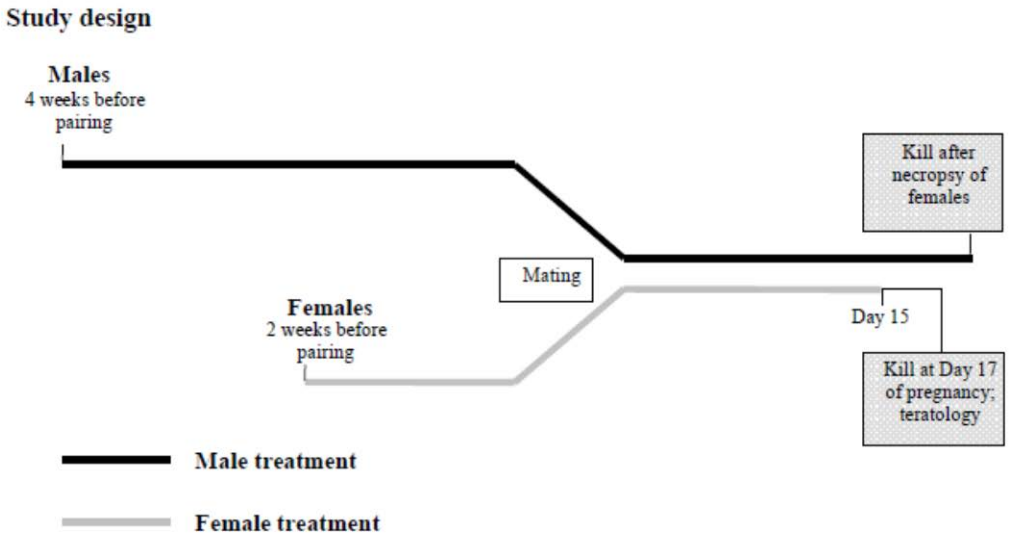
6.3.2 Methods

Table 66: Doses

TEST GROUP	DOSE TO ANIMALS* (SQ-U/DAY) F1 MALES	DOSE TO ANIMALS* (SQ-U/DAY) F1 FEMALES	NUMBER ASSIGNED PER GROUP F1 MALES	NUMBER ASSIGNED PER GROUP F1 FEMALES
1-CONTROL	0	0	48	48
2-LOW	25,000	25,000	24	24
3-MID	75,000	75,000	24	24
4-HIGH	500,000	500,000	24	24

Species/strain: mouse, (b)(4)
Number/sex/group: controls, 48; treated groups, 24
Route, formulation, volume, and infusion rate: oral instillation onto sublingual area of buccal cavity; groups 1-3, 5 µl/mouse/day; group 4, 10 µl/mouse/day
Satellite groups used for toxicokinetics:

Figure 6: Study design for fertility and embryo-fetal development in mice



Study design flowchart from sponsor submission

Duration of Dosing:
Males: Daily for 4 weeks before pairing, throughout pairing until termination after a minimum of 7 weeks treatment
Females: Daily for 2 weeks before pairing, throughout pairing and until Day 15 after mating.

Parameters and endpoints evaluated:
F0 males
Clinical observations
Body weight
Body weight gain
Food consumption

Conception rate
Fertility index
Gross pathology, males
Organ weight, male reproductive organs

F0 females
Clinical observations
Body weight body weight gain
food consumption
reproductive and fertility parameters
gross pathology
fetal pathology

Statistical methods:
Statistical analyzes were performed automatically by the (b)(4) System on pre-pairing bodyweight and male organ weight data. For the majority of parameters, the similarity of the data was such that analyzes were not considered to be necessary.
All statistical analyzes were carried out separately for males and females, treating the individual animal as the basic experimental unit.
Bartlett's test (Bartlett 1937) was first applied to test the homogeneity of variance between the groups. Using tests dependent on the outcome of Bartlett's test, treated groups were then compared with the Control group, incorporating adjustment for multiple comparisons where necessary. Whenever Bartlett's test was found to be statistically significant, a Behrens-Fisher test was used to perform pairwise comparisons, otherwise a Dunnett's test was used.Significant differences between Control and treated groups were expressed at the 5% ($p<0.05$), or 1% ($p<0.01$) level. Where appropriate, group mean values, each with standard deviation (SD), were calculated from individual data. Mean data after mating was restricted to values for data derived from females with live young killed at Day 17 after mating.

Standard deviations were not calculated for derived data, such as levels of pre- and postimplantation loss, or for the incidence of resorbing fetuses where the distribution of these findings commonly does not conform to the normal statistical distribution.
Data were expressed as group means with standard deviations (SD).

Table 67: Results F0 Generation

	0(Control)	**25,000**	**75,000**	**500,000**
	48	**24**	**24**	**24**
No. Died or Sacrificed Moribund	1	0	0	0
Clinical Observations:	-	-	-	-
Body Weight (%) Day 52	41.2 g	0.2	2.2	0.0
Body Weight Gain[a] (%) Days 0-52	3.1 g	12.9	25.8	-9.7
Food Consumption[a] (%) Days 25-27	6.0 g/animal/day	-3.3	-1.7	-5.0
Necropsy Observations	-	-	-	-
No. of Males that Mated	47	23	24	22
No. of Fertile Males	43	23	20	21
Conception Rate (%)[b]	91	100	83	95

Fertility Index (%)[c]	91	96	83	88
Adult Male Macropathology	-	-	-	-
Organ Weights	-	-	-	-

F0 Males
- = No treatment-related findings

Table 68:

Daily Dose (SQ-U) **F0 Females:** **No. Evaluated**	**0(Control)** **48**	**25,000** **24**	**75,000** **24**	**500,000** **24**
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations	-	-	-	-
Premating Day 14 Body Weight (%)[a]	27.9 g	2.5	3.2	-1.0
Gestation Day 17 body Weight (%)	52.7g	3.2	5.9	-1.3
Gestation Body Weight Gain[a] (%)	25.4 g	3.9	9.1	-0.4
Premating Food Consumption (%)[b]	4.5 g/animal/day	17.8	15.6	-2.2
Gestation Food Consumption (%)	-	-	-	-
Necropsy Observations	-	-	-	-
No. of Pregnant Females	44	23	20	23
No. Aborted or with Total Resorption of Litter	0	0	0	0
Reproductive Parameters Conception Rate (%)[c]	92	96	83	96
Fertility Index (%)[d]	92	96	83	96
Mean No. of Corpora Lutea (/animal)	13.0	13.4	14.1	12.7
Mean No. of Implantations (/animal)	12.4	12.9	13.4	12.3
Mean % Preimplantation Loss (/animal)	8.1	6.4	6.7	4.9
Mean No. of Live Young (/animal)	11.5	12.2	12.6	11.2
Mean No. of Resorptions (/animal)	0.8	0.7	0.8	1.1
Mean % Postimplantation Loss (/animal)	8.0	5.8	5.7	9.3
Adult Female Macropathology	-	-	-	-
Fetal Pathology- No. Evaluated: Fetuses/Litters	496/43	280/23	252/20	258/23
Cleft palate: Fetuses/Litters	0/0	0/1	2/2	2/1
Folded retina: Fetuses/Litters	0/0	2/2	3/2	1/1
Ossification of nasofrontal region: Fetuses/Litters[e]	27/17	9/6	9/7	7/4

- = No treatment-related findings

a: For gestation Days 0 to 17. For controls, group means are shown. For dose groups, percent differences from controls are shown.

b: Day 11-13. For controls, group means (g/animal/day) are shown. For dose groups, percent differences from controls are shown.

c: Conception Rate = number of pregnancies / number mated.

d: Fertility Index = number of pregnancies / number of pairings.

e: Fetuses/litters examined for skeletal findings were 252/43, 136/23, 124/20, and 127/23 respectively for Groups 1 through 4

Table 69: Litter parameters

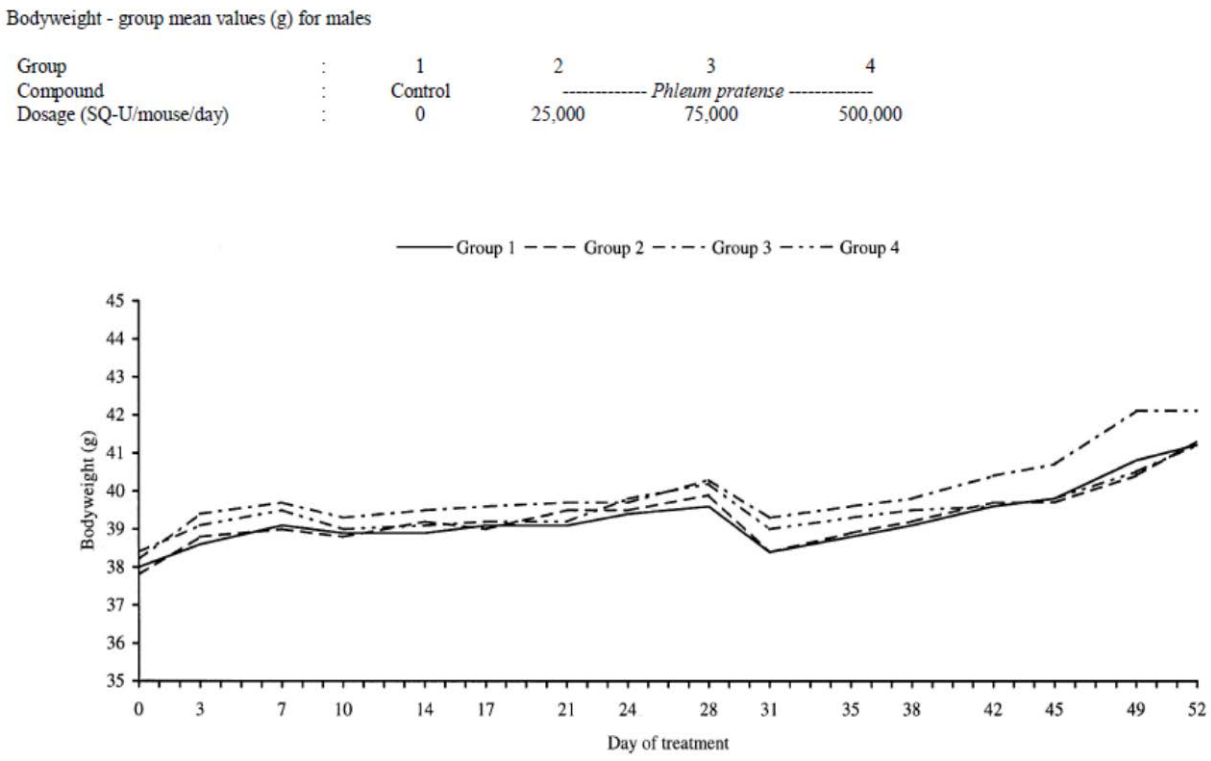
Daily Dose (SQ-U)	0(Control)	25,000	75,000	500,000
Placental Weight (%)	0.12g	-8.3	0.0	0.0
Litter Weight (%)[a]	12.52g	2.4	7.2	-5.8
Fetal Weight (%)	1.09g	-3.7	-2.8	-4.6
Sex Ratio (M:F)	49.6:50.4	48.0:52.0	53.5:46.5	44.5:55.5

- = No noteworthy findings
a: Mean of males and females combined.

Clinical signs and mortality

Control Male 17 was killed for reasons of animal welfare during Week 3 of treatment after showing underactive behavior, piloerection, dark eyes, hunched posture and deep, irregular and noisy respiration (rales). This animal had shown progressive bodyweight loss of 8.9 g during Days 7 to 14 and low food intake from Day 11. Macroscopic necropsy examination did not reveal any abnormalities. There were no unscheduled deaths in the treated groups, and there were no clinical signs apparent at any stage of the study that could be attributed to treatment. No treatment-related clinical signs were observed.

Figure 7: Group body weights, males

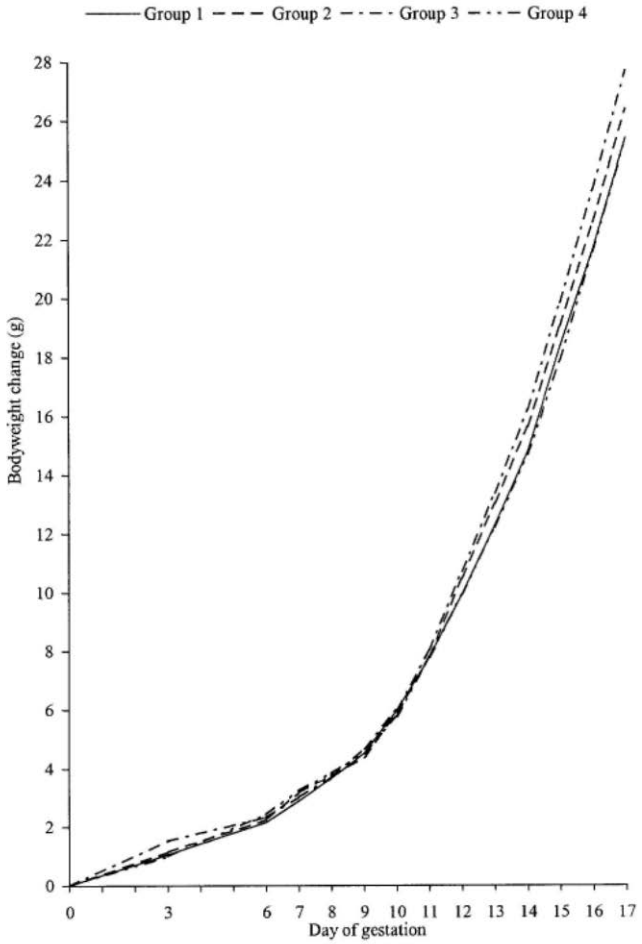


From sponsor submission

Figure 8: Bodyweight change group mean values for females during gestation

Bodyweight change - group mean values (g) for females during gestation

Group	:	1	2	3	4
Compound	:	Control	---------- *Phleum pratense* ----------		
Dosage (SQ-U/mouse/day)	:	0	25,000	75,000	500,000



From sponsor submission

Bodyweight

Bodyweight and bodyweight change was unaffected by treatment at all dosages both for males throughout the study and for females during the pre-pairing and gestation phases. A reduction in bodyweight was noted for males in all groups at the point of pairing which is a typical occurrence during mating.

Food consumption

Although food consumption values showed variability, they did not appear in a treatment-related pattern.

Table 70: Mating performance and fertility - group values

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1 M	47	47	43	100	91	91
2 M	24	23	23	96	100	96
3 M	24	24	20	100	83	83
4 M	24	22	21	92	95	88
1 F	48	48	44	100	92	92
2 F	24	24	23	100	96	96
3 F	24	24	20	100	83	83
4 F	24	24	23	100	96	96

Mating performance and fertility
All animals that were paired mated with the exception of 1 male at 25,000 SQ-U/day and 2 males at 500,000 SQ-U/day. Although all Control animals mated, the small number of treated males that failed to mate and the lack of a dose-response does not support a treatment-related effect.

The majority of animals mated within the first 4 days of pairing, and the distribution of animals with pre-coital intervals greater than 4 days did not suggest a treatment-related effect. The 2 females at 500,000 SQ-U/day with a pre-coital interval of 17 Days mated within 3 days of the replacement of the male partner with a proven male. There were no effects of treatment on the conception rate and fertility index performance or fertility.

Table 71: Organ weights male reproductive organs

group	1	2	3	4
n	47	24	24	24
Terminal body weight	40.7	40.8	41.6	40.9
Testes,	0.27	0.27	0.27	0.26
epididymides	0.10	0.10	0.10	0.10
Seminal vesicles	0.41	0.41	0.43	0.40
prostate	0.017	0.017	0.018	0.018

All weights in grams

Adult macropathology
The type and distribution of macroscopic findings for males and females did not reveal any detrimental effects of treatment. It was noted that no macroscopic abnormalities were detected in the reproductive system of males who failed to mate, whose female partners were not pregnant, or in females who were found to be not pregnant.

Male organ weights
The weights of the testes and accessory sex organs (epididymides, seminal vesicles and prostate) were similar in all groups.

Table 72: Litter data - group mean values on Day 17 of gestation

Group		Corpora Lutea	Implanta -tions	Resor- ptions Early	Resor- ptions Late	Resor- ptions Total	Live young Male	Live young Female	Live young Total	Sex ratio (% M)	Implantation loss (%) Pre-	Implantation loss (%) Post-
1	Mean	13.0	12.4	0.7	0.2	0.8	5.6	5.9	11.5	49.6	8.1	8.0
	SD	2.3	2.5				2.5	2.3	2.7			
	n	43	43	43	43	43	43	43	43	43	43	43

Group		Corpora Lutea	Implanta -tions	Resor- ptions Early	Resor- ptions Late	Resor- ptions Total	Live young Male	Live young Female	Live young Total	Sex ratio (% M)	Implantation loss (%) Pre-	Implantation loss (%) Post-
2	Mean	13.4	12.9	0.5	0.2	0.7	5.8	6.3	12.2	48.0	6.4	5.8
	SD	1.9	2.2				1.9	2.0	2.4			
	n	23	23	23	23	23	23	23	23	23	23	23
3	Mean	14.1	13.4	0.4	0.4	0.8	6.8	5.8	12.6	53.5	6.7	5.7
	SD	2.2	2.0				2.1	1.7	2.2			
	n	20	20	20	20	20	20	20	20	20	20	20
4	Mean	12.7	12.3	0.9	0.3	1.1	5.0	6.2	11.2	44.5	4.9	9.3
	SD	2.1	2.1				2.0	2.3	2.5			
	n	23	23	23	23	23	23	23	23	23	23	23

Litter data

The numbers of corpora lutea, uterine implantations, resorptions, live fetuses and the percentage of males in the litters were similar in all treatment groups. There appeared to be a clustering of a small number of litters containing a dead fetus at 500,000 SQ-U/day (4/23 litters) compared with Control (1/43 litters). However, as there was no effect of treatment at 500,000 SQ-U/day on the mean number of late resorptions per litter, and all the litters involved contained just one dead fetus, this finding does not appear to be a treatment-related effect.

Table 73: Placental, litter and fetal weights - group mean values (g) on Day 17 of gestation

Group		Placental weight	Litter weight	Males	Fetal weight Females	Overall
1	Mean	0.12	12.52	1.12	1.06	1.09
	SD	0.02	3.04	0.08	0.08	0.08
	n	43	43	43	42	43
2	Mean	0.11	12.82	1.06	1.02	1.05
	SD	0.01	2.92	0.07	0.09	0.08
	n	23	23	23	23	23
3	Mean	0.12	13.42	1.08	1.04	1.06
	SD	0.01	2.83	0.10	0.10	0.10
	n	20	20	20	20	20
4	Mean	0.12	11.79	1.06	1.03	1.04
	SD	0.01	3.09	0.12	0.08	0.09
	n	23	23	23	23	23

Placental, litter and fetal weights

Placental, litter and fetal weights were not considered to be affected by treatment. Litter weights and fetal weights were around 6% and 5% lower at 500,000 SQ-U/day respectively, this difference appears to reflect natural variation rather than a treatment-related change due to the inter-group differences observed and the lack of a dose response.

Table 74: Fetal examinations, major abnormalities

Group	Fetuses	Fetuses	Fetuses	Fetuses	Litters	Litters	Litters	Litters
Dose Phleum pratense (SQ-U/mouse/day)	1	2	3	4	1	2	3	4
Number examined	0	25,000	75,000	500,000	0	25,000	75,000	500,000
Number affected	1	5	6	3	1	5	4	2

Cleft palate	0	0	2	2	0	0	2	1
Protruding tongue: misshapen nasal septum and cavities: absent eyelid: exencephaly with absent cerebral hemispheres, flocculus, paraflocculus and pineal gland: misshapen and displaced pituitary	1	0	0	0	1	0	0	0
Misshapen snout and nasal cavities: kinked nasal septum: cleft palate: domed cranium: hydrocephaly	0	1	0	0	0	1	0	0
Exencephaly: open eyelid: protruding	0	1	0	0	0	1	0	0
tongue Folded retina	0	2	3	1	0	2	2	1
Fused basioccipital to ventral arch	0	1	0	0	0	1	0	0
Partially fused ribs	0	0	1	0	0	0	1	0

Fetal pathology

There were a number of differences in fetal pathology findings between the Control and treated groups, but these did not appear to be a treatment-related effect of Phleum Pratense on fetal development or morphology. Two litters at 75,000 and one litter at 500,000 SQ-U/day contained two fetuses with cleft palate. This non dosage-dependant finding fell within the historical control range in terms of the number of litters affected at 500,000 SQ-U/day and not related to treatment. Folded retina was also observed in all treated groups but not Control, in a non dose-dependant manner.
Historical background data from studies assessing this finding occurs between 1991 and 1994, the high incidence of folded retina within the historical data set indicate that it is a common finding in mice, and not treatment related.

6.3.3 Discussion and Conclusions

Treatment of male mice with *Phleum pratense* before pairing and female mice before pairing and throughout gestation at dosages up to 500,000 SQ-U/day was not associated with any apparent systemic toxicity. Mating performance and fertility was unaffected, and there were no adverse fetal findings.

The absolute doses administered to mice in this study, 25,000, 75,000 and 500,000 SQ-U represent exposures of 1,250,000 , 3,750,000 and 25,000,000 SQ-U/kg, respectively. This compares to the clinical dose of 75,000 SQ-U or 1500 SQ-U/kg. When adjusted for body surface area differences, the mouse dose s are 100, 203, and 1355x the human dose. The large dose multiple relative to the clinical dose also presents a margin for safety.

6.3.4 Summary table

Table 75: Summary

Daily Dose (SQ-U)	**0(Control)**	**25,000**	**75,000**	**500,000**
F_0Males: **No. Evaluated**	**48**	**24**	**24**	**24**
No. Died or Sacrificed Moribund	1	0	0	0

Clinical Observations:	-	-	-	-
Body Weight (%) Day 52	41.2 g	0.2	2.2	0.0
Body Weight Gain[a] (%) Days 0-52	3.1 g	12.9	25.8	-9.7
Food Consumption[a] (%) Days 25-27	6.0 g/animal/day	-3.3	-1.7	-5.0
Necropsy Observations	-	-	-	-
No. of Males that Mated	47	23	24	22
No. of Fertile Males	43	23	20	21
Conception Rate (%)[b]	91	100	83	95
Fertility Index (%)[c]	91	96	83	88
Adult Male Macropathology	-	-	-	-
Organ Weights	-	-	-	-

- = No noteworthy findings
a: For controls, group means are shown. For dose groups, percent differences from controls are shown.
b: Conception Rate = number of pregnancies / number mated. c: Fertility Index = number of pregnancies / number of pairings.

Table 76: Summary continued

				500,000 **24**
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations	-	-	-	-
Premating Day 14 Body Weight (%)[a]	27.9 g	2.5	3.2	-1.0
Gestation Day 17 body Weight (%)	52.7g	3.2	5.9	-1.3
Gestation Body Weight Gain[a] (%)	25.4 g	3.9	9.1	-0.4
Premating Food Consumption (%)[b]	4.5 g/animal/day	17.8	15.6	-2.2
Gestation Food Consumption (%)	-	-	-	-
Necropsy Observations	-	-	-	-
No. of Pregnant Females	44	23	20	23
No. Aborted or with Total Resorption of Litter	0	0	0	0
Reproductive Parameters				
Conception Rate (%)[c]	92	96	83	96
Fertility Index (%)[d]	92	96	83	96
Mean No. of Corpora Lutea (/animal)	13.0	13.4	14.1	12.7
Mean No. of Implantations (/animal)	12.4	12.9	13.4	12.3
Mean % Preimplantation Loss (/animal)	8.1	6.4	6.7	4.9
Mean No. of Live Young (/animal)	11.5	12.2	12.6	11.2
Mean No. of Resorptions (/animal)	0.8	0.7	0.8	1.1
Mean % Postimplantation Loss (/animal)	8.0	5.8	5.7	9.3
Adult Female Macropathology	-	-	-	-
Fetal Pathology- No. Evaluated: Fetuses/Litters	496/43	280/23	252/20	258/23
Cleft palate: Fetuses/Litters	0/0	0/1	2/2	2/1
Folded retina: Fetuses/Litters	0/0	2/2	3/2	1/1
Ossification of nasofrontal region: Fetuses/Litters[e]	27/17	9/6	9/7	7/4

- = No noteworthy findings
a: For gestation Days 0 to 17. For controls, group means are shown. For dose groups, percent differences from controls are shown.
b: Day 11-13. For controls, group means (g/animal/day) are shown. For dose groups, percent differences from controls are shown.
c: Conception Rate = number of pregnancies / number mated.
d: Fertility Index = number of pregnancies / number of pairings.

e: Fetuses/litters examined for skeletal findings were 252/43, 136/23, 124/20, and 127/23 respectively for Groups 1 through 4

Table 77: Summary Litters

Daily Dose (SQ-U)	0 (Control)	25,000	75,000	500,000
Placental Weight (%)	0.12g	-8.3	0.0	0.0
Litter Weight (%)[a]	12.52g	2.4	7.2	-5.8
Fetal Weight (%)	1.09g	-3.7	-2.8	-4.6
Sex Ratio (M:F)	49.6:50.4	48.0:52.0	53.5:46.5	44.5:55.5

- = No noteworthy findings
a: Mean of males and females combined.

7 GENETIC TOXICOLOGY STUDIES

7.1 STUDY NO. 1: PHLEUM PRATENSE GRASS EXTRACT: REVERSE MUTATION IN FOUR HISTIDINE-REQUIRING STRAINS OF SALMONELLA TYPHIMURIUM AND TWO TRYPTOPHAN-REQUIRING STRAINS OF ESCHERICHIA COLI. (b)(4) . STUDY NO. 2325-6. MAY 2006

Phleum pratense grass extract was assayed for mutation in four histidine-requiring strains ((b)(4)) of *Salmonella typhimurium*, and two tryptophan-requiring strains (b)(4) of *Escherichia coli*, (b) (4)

(b) (4)

Phleum pratense grass extract was not positive for inducing mutation in four histidine-requiring strains of *Salmonella typhimurium* ((b)(4)) and two tryptophan-requiring strains of *Escherichia coli* ((b)(4)) when tested under the conditions of this study. (b) (4)

7.2 STUDY NO. 2: PHLEUM PRATENSE GRASS EXTRACT: REVERSE MUTATION IN FOUR HISTIDINE-REQUIRING STRAINS OF SALMONELLA TYPHIMURIUM AND TWO TRYPTOPHAN-REQUIRING STRAINS OF ESCHERICHIA COLI. (b)(4) STUDY NO.2325/2. JANUARY, 2006

Phleum pratense grass extract was assayed for mutation in four histidine-requiring strains ((b)(4)) of *Salmonella typhimurium,* and two tryptophan-requiring strains ((b)(4)) of *Escherichia coli,* (b) (4)

(b) (4)

(b) (4)

Phleum pratense grass extract did not induce mutation in four histidine-requiring Strains of *Salmonella typhimurium* ((b)(4)) and two tryptophan-requiring Strains of *Escherichia coli* ((b)(4)) under the conditions of this study. (b) (4)

7.3 STUDY NO. 3: PHLEUM PRATENSE GRASS EXTRACT: MUTATION AT THE THYMIDINE KINASE (TK) LOCUS OF MOUSE LYMPHOMA L5178Y CELLS (MLA) USING THE (b)(4) **STUDY NO. 2325/4. SEPTEMBER 2006**

Phleum pratense grass extract was assayed for its ability to induce mutation at the *tk* locus (5-trifluorothymidine resistance) in mouse lymphoma cells using a (b)(4) protocol. (b) (4)

1 page determined to be not releasable (b)(4)

(b) (4)

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Phleum pratense grass extract did not induce mutation at the *tk*locus of L5178Y mouse lymphoma cells under the conditions of this study. (b) (4)

8 OVERALL SUMMARY:

8.1 GENERAL TOXICOLOGY:

The sponsor submitted toxicology studies conducted in the mouse and dog with dosing periods ranging from 4 to 52 weeks. Doses used in both species included 25,000, 75,000 and 500,000 SQ-U. The doses of 500,000 SQ-U (25,000,000 SQ-U/kg), 75,000 SQ-U (3,750,000SQ/kg) and 25000 SQ-U (1,250,000 SQ/kg) in mice compare to the clinical dose of 75,000 SQ-U (1500 SQ-U/kg). In dogs, the same doses correspond to 50,000, 7,500 and 2,500 SQ-U/kg, respectively. The human dose, 75,000 SQ-U or 1500 SQ-U/kg

Following conversion to human equivalent doses which account for differences in body surface area between animals and humans, the resulting doses exceed the clinical dose by a margin of up to 4100x compared to the highest mouse dose and 50x compared to the highest dog dose.

The lack of toxicologic findings in the repeat dose studies in both the mouse and dog, with the dose margin described above, does not present any safety issues for this product.

8.2 REPRODUCTIVE TOXICOLOGY:

Fertility, developmental and pre- and –post natal reproductive toxicology studies were conducted by the sponsor in the mouse. The doses used, 500,000 SQ-U (25,000,000 SQ-U/kg), 75,000 SQ-U (3,750,000SQ/kg) and 25000 SQ-U (1,250,000 SQ/kg) compare to the clinical dose of 75,000 SQ-U (1500 SQ-U/kg). The lack of toxicologic findings, combined with the dose margin described above, do not indicate reproductive risk.

8.3 GENETIC TOXICOLOGY:

The *in vitro* bacterial reversion assays and *in vitro* mouse lymphoma assay were not positive for genetic toxicity.

Pregnancy Category B.

Reproduction studies have been performed in mice at doses up to 6.7 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to GRASTEK There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, GRASTEK should only be continued during pregnancy if clearly needed.

Treatment with GRASTEK should not be initiated in pregnant women.

Justification: The reproductive toxicology studies conducted by the sponsor support the pregnancy labeling claim.

9 OVERALL CONCLUSION:

Based on nonclinical toxicity assessments, there are no significant safety issues to preclude the BLA from being approved.

Concurrence: Martin D. Green