Toxicology Review of Ragwitek

(Short Ragweed Pollen Allergen Extract)
STN: 125478.0
Type and date of submission: Original; March 11, 2013
Applicant: Merck
Product: RagwitekR [Allergen Extract, Short Ragweed (*Ambrosia artemisiifolia*)] sublingual tablets
Cross references: IND 12970
Proposed indication: As immunotherapy for diagnosed ragweed pollen induced allergic rhinitis, with or without conjunctivitis, in adults 18 years of age and older
Recommended clinical dose: 1 sublingual tablet (12 Amb a 1-U) for adults 18 years of age and older
Reviewer: Ching-Long Joseph Sun, Ph. D., Division of Vaccines and Related Products Applications
The following toxicological study reports, conducted using *Phleum pretense (Timothy Grass)*, were submitted. These reports are irrelevant and not reviewed under this application as they were not conducted using the product, *Ambrosia artemisiifolia (Ragweed)*.
07231- Report on serology of mice included in repeated dose toxicology (study #07231)
10-042144 - Pre- and post-natal development study in the (b)(4) mouse by buccal cavity administration
10106 - Report on serology of dogs Included in repeated dose toxicology
1-033458 - 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
2-032364 - Toxicity study by buccal cavity administration to (b)(4) mice for 26 weeks followed by a 5 week recovery period
2325-2 - Reverse Mutation in four histidine-requiring strains of *Salmonella Typhimurium* and two tryptophan-requiring strains of *Excherichia Coli*
2325-4 - Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the -------(b)(4)----------
2325-6 - Reverse Mutation in four histidine-requiring strains of *Salmonella Typhimurium* and two tryptophan-requiring strains of *Excherichia Coli*
3-024445 - Toxicity Study by buccal cavity dministration 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
4-023818 - Preliminary study of effects on embryo-fetal toxicity in the (b)(4) mouse by buccal cavity administration
5-023911 - Single dose toxicity study by buccal cavity administration to (b)(4) mice with a 14 day observation period
6-023930 - Single dose toxicity study by intravenuous administration to (b)(4) mice with a 14 or 15-day observation period
8-033623 - Combined study of effects on fertility and embryo-fetal development in the (b)(4) mouse by buccal cavity administration

944-001 - Subchronic oral toxicity study in mice
944-002 - Subchronic oral toxicity study in dogs
The following two submitted toxicological study reports, conducted using *Ambrosia artemisiifolia (ragweed),* are reviewed:

- 012/053003-Toxicity study by buccal cavity administration to (b)(4) mice for 4 weeks followed by 4 weeks off-dose and one further week of treatment
- 1001345-Combined (b)(4) assay in the stomach and a ------(b)(4)---------- test in rats

Review
Study title and number: Toxicity study by buccal cavity administration of *Ambrosia artermisiifolia* to (b)(4) mice for 4 weeks followed by 4 weeks off-dose and one further week of treatment (Study # LEA012/053003)
Performing laboratory: ----------(b)(4)----------
Initiation date: June 28, 2005
Final report date: February 10, 2006
Test article batch/lot #: -----(b)(4)-------
Animal species and strain: Crl:----(b)(4)-------- mouse
Breeder/supplier: -------(b)(4)----------
Number of animals per sex per group: 10 (main study); 4 (off-dose period or re-challenge period; 9 (satellite group for antibody assay)
Age: 38-42 days
Body weight range: 26.9-37.0g (males) and 23.5-29.4g (females)
Route and site of administration: Sublingual area of the buccal cavity
Volume of administration: 5 ul one time for the two low- dose groups s or two times (1 to 10 minutes apart) for the high-dose group
Frequency of administration and study duration: Daily for 4 week of treatment period and 1 week of re-challenge period; 9 week
Dose: 12, 35 and 70 Amb a 1-U/day
Stability: The study report stated that the sponsor was responsible for the characterization of the test substance and the documentation of the methods of synthesis, fabrication or derivation and stability. The certificate of Analysis is presented in Annex 1.
Means of administration: a calibrated pipette
Report status: Final
Experimental design

Group	**Test article**	**Amb a 1-U concentration/ml**	**Amb a 1-U dose/mouse/day**	**No. of animals/sex/group/sacrifice time point**
1	Control (water)	--	--	10(treatment), 4 (off-dose), 4 (re-challenge) and 9 (satellite)*
2	*Ambrosia artemissiifolia*	2400	12	Same as above
3	*Ambrosia artemissiifolia*	7000	35	Same as above
4	*Ambrosia artemissiifolia*	7000	70	Same as above

*Satellite study: 9/sex/group was assigned for antibody assay only
Methods:

Endpoint	**Methodology**
Hematology	----------(b)(4)----------
Clinical chemistry	----------(b)(4)----------
Electrophoretic protein fractions	-----(b)(4)----------

Randomization procedure: The animals were removed from transit boxes and allocated to study cages. No specific randomizing method was employed.
Statistical analysis plan: For categorical data, the proportion of animals was analyzed using Fisher's exact test for each treatment group versus the control. For continuous data, (b)(4)- test was first applied to test the homogeneity of variance between the groups. For bodyweight, food consumption, organ weight and clinical pathology data, the following sequence of tests was used: If 75 % of the data were the same values, a frequency analysis was applied. Treatment groups were compared using a (b)(4) test for a trend in proportions and pairwise Fisher's Exact test for each group against the control. If ---(b)(4)-- test for variance homogeneity was not significant, then parametric analysis was applied. If the F1 test for monotonicity of dose-response was not significant, ---(b)(4)--- test for a monotonic trend was applied. It the F1 test was significant, --(b)(4)-- test was performed. If ---(b)(4)-- test was significant using logarithmic and square-root transformations, then non-parametric test s were applied. If the H1 test for monotonicity of dose-response was not significant, (b)(4)-- test for monotonic trend was applied. If the H1 test was significant, -(b)(4) test was performed. The following parameters were evaluated

Parameters	**Frequency of Testing**
Cage observations	Twice daily
Clinical observations	Prior to, immediately and 1-2 hours after each dose and as late as possible in the working days daily during the first week of treatment, twice weekly during weeks 2-4, daily during the first week of the off-dose period, once weekly thereafter and daily during re-challenge period
Body weight	One week prior to, day 0 and twice weekly thereafter
Body temperature	Not monitored
Food consumption	One week prior to and weekly thereafter
Ophthalmologic exam	Prior to and week 4 in groups 1 and 4
Clinical chemistry Electrophoretic protein analysis	At terminations in weeks 4, 8 and 9 under isoflurane thru the retro-orbital
Hematology	Same as above
Coagulation	Not performed
Antibody assay	Prior to dosing in first 3 animals/sex, at terminations of main study in the next 3

Parameters	Frequency of Testing
	animals/sex and at terminations of off-dose and re-challenge periods in the last 3 animals/sex
Necropsy	Weeks 4, 8 and 9
Tissues for histopathology	Same as above

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

SYSTEM	ORGAN COLLECTED	ORGAN NOT COLLECTED
digestive	Large intestine (cecum, colon, rectum); small intestine (duodenum, jejunum, ileum), esophagus, gall bladder, liver*, salivary gland* (submandibular, sublingual), pancreas, stomach	
	Lung with bronchi*, trachea, inclusion of nasal cavity, paranasal sinuses and nasopharynx with tongue	
	Aorta, heart*	
immunologic/	Sternum, femur, lymph node (mandibular, mesenteric, retropharygeal), spleen*, thymus*	
urogenital	Epididymis*, kidneys*, ovaries, prostate*, seminal vesicle*, testes*, urinary bladder, uterus (with cervix)*, vagina	Fallopian tubes
neurologic	Brain*, optic nerve, sciatic nerve, spinal cord (cervical, lumbar, mid-thoracic)	
hormonal	Adrenals, mammary glands, thyroid (with parathyroid glands), pituitary glands	

SYSTEM	ORGAN COLLECTED	ORGAN NOT COLLECTED
other	Eyes, optic nerve, skeletal muscle, skin, tongue, lachrymal gland, Harderian gland	
Gross Lesions		

Table of Histology – Complete tissues were examined in groups 1 and 4. All gross lesions tissues from all groups were examined.
Results:
Mortality: All animals survived until the scheduled termination.
Clinical observation: There were no clinical signs considered to be associated with treatment.
Food consumption: There was slight lower mean food consumption in the females of group 3 following the off-dose period which continued into the re-challenge period. Overall, food consumption was not affected.
Body weights: The data revealed higher weight gain in the treated males groups and the females in group 2 and 3. The females in groups 4 showed similar weight gain to that of concurrent control. All treated male groups and females in groups 2 and 3 had lower weight gain compared with control at the end of off-dose period. The magnitude of body weight changes were within 6-7 %.
Ophthalmoscopy: There were no treatment related effects.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	**END POINTS DIFFERENT THAN THE CONCURRENT CONTROL**	**NOT OF NOTE**
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR B) HEPATOBILIARY		Aspartate aminotransferase Alanine aminotransferase Glutamate dehydrogenase (not determined) Sorbitol dehydrogenase (not determined) Total bile acids (not

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	**END POINTS DIFFERENT THAN THE CONCURRENT CONTROL**	**NOT OF NOTE**
		determined)
Total bilirubin Week 4 Group 3; ↑1.5 (F)* Week 9 Group 2; ↑4 (F)* Group 3; ↑2 (F)* Group 4; ↑1.5 (F)*	Alkaline phosphatase Gamma-glutamyl transferase	
ACUTE PHASE REACTANTS		Fibrinogen Alpha-1 globulin Alpha-2 globulin Beta globulin Gamma globulin
KIDNEY FUNCTION		Creatinine Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Albumin Globulin (calculated) A/G Ratio Total Cholesterol Creatinine kinase (not determined) Cholinesterase (not determined) Total protein Triglycerides

Table of Clinical Chemistry Results
*: In the absence of pathological changes or dose related trend, these elevated bilirubin levels in females are not considered treatment- related.

HEMATOLOGY		
MEASUREMENT RELATED TO	**END POINTS DIFFERENT THAN THE CONCURRENT**	**Not of NOTE**
red blood cells		Hematocrit Hemoglobin Conc. Mean Corp. Hb. Mean Corp. Volume Mean Corp. Hb. Conc. Total Erythrocyte Count Reticulocytes
white blood cells	Total leukocytes Week 4	Basophils count

HEMATOLOGY		
MEASUREMENT RELATED TO	**END POINTS DIFFERENT THAN THE CONCURRENT**	**Not of NOTE**
	Group 3; ↑1.6 (M) Group 4;↓ 0.6 (F) Lymphocyte count Week 4 Group 3; ↑1.7 (M) Group 4:↓ 0.6 (F) Week 8 Group 3; ↑1.8 (M) Neutrophil count Week 4 Group 4; ↓0.6 (F) Eosinophil count Week 4 Group 4; ↓0.5 (F) Monocyte Week 4 Group 4;↓ 0.6 (F) Large unstained cells Week 4 Group 4;↓ 0.4 (F)	
clotting potential		Activated partial-thromboplastin time (not determined) Prothrombin time (not determined) Platelet count Fibrinogen (not determined)

Table of Hematology Results

High WBC counts and elevated lymphocytes in group 3 males at end of treatment period and continued at end of off-dose period for the latter. In contrast, treated group females showed low WBC counts with low values for majority of the subtypes after the treatment period. Given the inconsistency in white cell findings between males and females, it is unlikely that these effects are treatment related.

Immunogenicity evaluation: The conduct of the analyses was the responsibility of the applicant/sponsor and the results are not included in this report according to the testing facility.

Organ weights: There were no treatment-related organ weight changes.

Gross pathology: There were no treatment-related gross findings.

Microscopic findings: In the mandibular lymph node, minimal increased germinal center development was seen in four males receiving 70 Amb a 1-U/day and two male controls. In the submandibular salivary gland, slight or moderated degranulation of striated ducts was seen in two males in group 4. The low incidence of the former is considered unlikely to be treatment related. The latter was probably due to normal physiological secretion of saliva and therefore not considered to be toxicologically significant.

Assessment

There were no treatment-related effects on clinical signs, mortality, body weights, food consumption, hematology, blood chemistry, ophthalmology, macroscopic pathology and organ weights.
Finding of increased germinal center development in the mandibular lymph node may be indicative of an immune response to the exposure of the animals to the antigen. However, similar findings were found in two control animals. In addition, such findings in the retropharygeal lymph node, which drains both the buccal cavity and mandibular lymph node, were absent.
Antibody assay was not conducted by the testing laboratory and provided in the study report.
Based on overall findings in the mice study, it can be concluded that the daily oral buccal administration of *Ambrosia artermissifolia* at doses up to 70 Amb a 1-U/day for 4-5 weeks was well tolerated other than the expected immune response, increased germinal center development in the mandibular lymph node.
GLP study deviations or amendments: No protocol amendments were recorded in the draft report that influenced the quality, integrity or interpretation of the results.
Study title: Combined –b(4)-- assay in the stomach and a bone marrow micronucleus test in rats (Study # 1001345)
Testing laboratory: ----------(b)(4)----------
Testing date: January 19, 2012 to June 7, 2010
Animal species and strain: ---(b)(4)---- rat
Breeder/supplier: --------(b)(4)----------
Number of males/group: 5
Age: 7-8 weeks
Body weight range: 217-251 g
Route of administration: Oral gavage
Volume of injection: 20 ml/kg for *Ambrosia artemisiifolia* and vehicle or 10 ml/kg for EMS (ethyl methane sulfonate, positive control)
Frequency of administration and study duration: 3 daily doses; 3 days
Dose: 375, 750 and 1500 mg/kg
Test article batch no: -(b)(4)--
Stability: Data provided by the sponsor indicated that the test article formulations were stable for 24 hours when protected from light and store refrigerated or 3 hours at 15-25oC. According to the study report, all formulation were used by the testing facility within one hour and were protected from light and stored at room temperature. Determination of stability and characteristics of the test article were the responsibility of the sponsor.
Means of administration: Gavage
Report status: Final
Study design:

Dose group	**Dose level**	**No. of male animals/group**

Dose group	Dose level	No. of male animals/group
1	Water (negative control, vehicle)	5
2	375 mg/kg	5
3	750mg/kg	5
4	1500 mg/kg	5
5	EMS (positive control) 200 mg/kg	5

Randomization procedure: Animals were randomized to groups using a system of random numbers.
Statistical plan: Statistical analysis was performed for micronucleus number using a 2 x 2 contingency table to determine chi-square. Probability values of smaller or equal to 0.05 were accepted as significant. A test for liner trend was used to evaluate possible dose-response relationships.
Three hours after the last injection, the animals were euthanized by sodium pentobarbital. The stomach and one femur were removed.
Stomach slides from negative control and treatment groups were prepared for –b(4)- analysis.
Measurements of tail moment and tail intensity were obtained from 100 cells per animal. The number of clouds (cytotoxicity, necrosis or apotosis) out of 100 cells was scored for each slide.
Bone marrow from the isolated femur of all groups was extracted with fetal calf serum for micronucleus test. The cell suspensions were centrifuged. The centrifuge was spread on slides. The smears were stained. The polychromatic/normochromatic erythrocyte ratio was determined by analyzing at least 2000 erythrocytes.
The data were considered valid if the following criteria were met:

- The vehicle control treatments demonstrated low levels of-b(4)- cytotoxicity.
- The vehicle control data were consistent with the laboratory's historical control data, and
- The positive control induced a marked increase in –b(4)-- parameters and the frequency of MN PCE.

The test article was considered as positive in this study if all criteria listed below for either DNA damage and/or micronucleus induction were met.

DNA damage: A dose related change in tail moment or tail intensity was observed in any tissue between the vehicle and test article groups, or at least a single dose group.
Induction of micronuclei:

- A statistically significant increase in the frequency of MN PCE occurred at one of more dose levels
- The incidence and distribution of MN PCE in individual animals at such a point exceeded the laboratory's historical vehicle control data
- A dose-response trend in the proportion of MN PCE was observed.

Results:
Selection of doses: The maximum achievable dose of 1500 mg/kg was administered in a range-finding study where no clinical sign of toxicity or loss of body weight were observed. Thus it confirmed the maximum achievable/feasible dose for the main study.
Clinical signs and body weights: No clinical signs of toxicity and no effect on body weights were observed.
Analysis of –b(4)--- data:

Treatment (mg/kg)	**Cells Scored**	**Tail Intensity**	**Tail Moment**
Vehicle	500	2.37	0.25
Ambrosia artemisiifolia (375)	500	3.23	0.30
Ambrosia artemisiifolia (750)	500	3.99	0.39
Ambrosia artemisiifolia (1500)	500	2.88	0.28
EMS (200)	600	26.28	3.67

Group of rats treated with the test article exhibited tail moments and tail intensities that were higher than the vehicle control group. However the increases were less than twice the vehicle control and were not clearly dose-related.
Analysis of micronucleus data:

Treatment (mg/kg)	**Cell Total**	**%PCE**	**MN PCE**	**MN PCE/ 2000 PCE**	**% MN PCE**

Treatment (mg/kg)	Cell Total	%PCE	MN PCE	MN PCE/ 2000 PCE	% MN PCE
Vehicle	10000	44.80	19	3.80	0.19
Ambrosia artemisiifolia (375)	10000	44.50	11	2.20	0.11
Ambrosia artemisiifolia (750)	10000	45.30	12	2.40	0.12
Ambrosia artemisiifolia (1500)	10000	41.12	12	2.40	0.12
EMS (200)	12000	42.72	270	45.00	2.25

Groups of rats treated with the test article exhibited %PCE values that were similar to vehicle controls. Their frequencies of MN PCE were also similar to and not statistically significant different from those seen in vehicle controls for all dose groups.
Conclusion: The positive control yielded significant increases in these parameters. The test article did not induce DNA damage in the stomach of male rats following oral gave administration of doses up to 1500 mg/kg. At these same dose levels, it did not induce any increase in MN PCE in the bone marrow.
GLP Study Deviations or amendments: Any protocol deviations were noted in the final report. All were minor and considered unlikely to impact on the study outcome.
Assessment
The *Ambrosia artemissifolia* powder extract induced no DNA damages in the stomach and no clastogenic activity in rats treated with oral doses up to 1500 mg/kg.
Conclusions
A toxicity study with *Ambrosia artemesiifolia* extract in mice dosed up to 4-5 weeks revealed no safety issues at doses up to 70 Amb a 1-U/day which is approximately 6-fold based on absolute value or 11666-fold based on 1-U/kg the human dose of 12 Amb a 1-U/day.
No animal reproductive and development studies were performed with the product to assess its teratogenic potential. Negative study results from a different product are not relevant and cannot be substituted for it in terms of its pregnancy category designation. Thus, a pregnancy category C instead of B would be appropriate in section 8.1.
A core of genetic studies has not been performed with the product. These studies, which are designed to identify genotoxic hazard, include: (1) A test for gene mutation in

bacteria. (2) An in vitro assessment of chromosome damage. And (3) an in vivo test for chromosome damage. A combined in vivo –b(4)-assay and micronucleus test in rats was performed with the product. Negative results from only one assay could not constitute that the product does not pose an overall genotoxic risk for humans. Thus only the negative results of this study could be described in section 13.1.
Impairment of fertility of the product has not been investigated in animals.
Description of the study results from a general toxicity study in mice is not warranted in section 13.2 of the labeling and should be deleted.
The above-mentioned revisions/recommendations in sections 8.1, 13.1 and 13.2 of the labeling are shown below:

8.1 Pregnancy

Pregnancy Category C.

Animal reproduction studies have not been performed with –b(4)----------. . There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, ---b(4)---------- should only be continued during pregnancy if clearly needed.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No long term studies have been performed in animals to evaluate the carcinogenic potential of *Ambrosia artemisiifolia.*

Ambrosia artemisiifolia did not cause clastogenic effect in an in vivo combined –b(4)--assay and micronucleus assay in rats.

Impairment of fertility has not been investigated with *Ambrosia artemisiifolia*.

13.2 Animal Toxicology and/or Pharmacology

(Delete)

Concurrence: Martin D. Green, Ph. D. Division of Vaccines and Related Products Applications