

Toxicology Review of House Dust Mites Allergen Extract

BLA: 125592

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Sponsor: Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc.

Product: House Dust (*Dermatophagoides farina*) & Mites (*Dermatophagoides pteronyssinus*) Allergen Extract Sublingual Tablet (Sublingual Immunotherapy [SLIT])

Proposed indication: Treatment of house dust mite (HDM) induced allergic rhinitis, with or without conjunctivitis, in adults 18 through 65 years of age

Recommended dose: 12 DU/tablet

Reviewers name: Ching-Long Joseph Sun (general and developmental toxicology studies) and Nabil Al-Humadi (genotoxicity studies)

Division name: OVRR/DVRPA

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Introduction:

Allergic rhinitis (hay fever or pollinosis) is an inflammation in the nose which occurs when the immune system overreacts to allergens in the air (1). Runny or stuffy nose (clear fluid), sneezing, red, itchy, and watery eyes, and swelling around the eyes are some of the signs and symptoms (2). Seasonal, which symptoms develops during specific times of the year, are pollen related (4).

In 1859, pollen was identified as the cause of allergic rhinitis by Charles Blackley (6). In 1906, Clemens von Pirquet determined the mechanism (5). The link with hay came about due to an early (and incorrect) theory that the symptoms were brought about by the smell of new hay (7, 8).

Pollen, pet hair, dust, or mold is typically the environmental allergens which trigger allergic rhinitis (4). Inherited genetics and environmental exposures contribute to the development of allergies (4). The underlying mechanism involves IgE antibodies attaching to the allergen and causing the release of inflammatory chemicals such as histamine from mast cells (3). Allergic rhinitis symptoms resemble those of the common cold; however, they often last for more than two weeks and typically do not include a fever (4).

A number of medications (nasal steroids, antihistamines such as diphenhydramine, cromolyn sodium, and leukotriene receptor antagonists such as montelukast) may improve symptoms (9). Allergen immunotherapy (exposing patients to larger and larger amounts of allergen) is often effective therapy.

Between 10–30% of people, in western countries, are affected in a given year (3, 6). It is most common between the ages of twenty and forty (3).

MK-8237 (allergen extract) is indicated (to be used in adults 18 through 65 years of age) as immunotherapy for the treatment of house dust mite-induced allergic rhinitis, with or without conjunctivitis.

MK-8237 contains two drug substances, each of which consists of a (b) (4) from two cultivated house dust mite species, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. Development Units (DU) is used to measure the biological potency of the (b) (4). This is based on the total allergenic activity and the content of the two major allergens.

Because the mouse demonstrated primary pharmacodynamics effects to allergen immunotherapy, it was considered the choice of species for the nonclinical toxicological assessment for MK-8237.

Limited animal models that specifically address the efficacy and/or mechanisms of SLIT with HDM extract are available. Mice treated with SLIT with *D. farinae* extract displayed reduced airway hyper-reactivity, reduced eosinophil infiltration to airways, and reduced lung inflammation upon intranasal challenge with HDM allergen extract compared to a buffer SLIT-treated group. Concomitantly, an increase in regulatory T cells in the spleen was reported (10). In mice displaying airway inflammation and asthma symptoms prior to treatment, sublingual administration of HDM

extract improved lung function and reduced eosinophil attraction to airways (11). A combined sublingual/swallow route of delivery in HDM-sensitized and an airway-challenged rat has also been shown to be efficacious (12). In an acute mouse model of HDM-allergic airway inflammation, prophylactic treatment with *D. farinae* extract prevented the subsequent HDM-induced airway resistance compared to buffer SLIT-treated mice upon methacholine challenge (Flexivent) (13). The finding that prophylactic SLIT with HDM extract prevented the airway hyper-reactivity induced by subsequent exposure to HDM allergens in mice supported these data (14).

The safety of the mixture of *D. farinae* and *D. pteronyssinus*, the allergen extracts in MK-8237, was evaluated in the following studies:

- 1- 26 week repeat-dose study in mice,
- 2- in vitro and in vivo genotoxicity studies,
- 3- embryo-fetal development studies in mice, and
- 4- oral mucosal irritation study in rabbits with the MK-8237 tablet.

Clinical studies:

Eight trials that enrolled over 4,300 subjects, 2,734 of whom received at least 1 dose of MK-8237 were included in the clinical development program. An overview of the trials included in the clinical development program is in the table below:

Trial	Phase	Region	Age / years	Main Objective	Inclusion Criterion	MK-8237 Doses (DU) once daily	Subjects receiving MK-8237	Subjects receiving Placebo
MT-01 [Ref. 5.3.5.1: P011]	I	EU	18-63	Tolerability and safety	AA ± AR	1, 2, 4, 8, 16, 32	54	17
MT-03 [Ref. 5.3.5.1: P013]	I	EU	5-14	Tolerability and safety	AA ± AR	0.5, 1, 3, 6, 9, 12	54	18
P008 [Ref. 5.3.5.1: P008]	I	US	12-17	Tolerability and safety	AR ± asthma	6, 12	130	65
MT-02 [Ref. 5.3.5.1: P012]	II	EU	14-74	Efficacy in AA	AA + AR	1, 3, 6	461	143
P003 [Ref. 5.3.5.1: P003]	II	EU	18-65	Efficacy in AR (EEC)	AR ± asthma	6, 12	83	41
MT-04 [Ref. 5.3.5.1: P014]	III	EU	17-83	Efficacy in AA	AA + AR	6, 12	557	277
MT-06 [Ref. 5.3.5.1: P015]	III	EU	18-66	Efficacy in AR	AR ± AA	6, 12	654	338
P001 [Ref. 5.3.5.1: P001]	III	NA	12-85	Efficacy in AR	AR ± asthma	12	741	741
Total	-	-	-	-	-	-	2734	1640

AR: allergic rhinitis; AA: allergic asthma; DU: development unit; EEC: environmental exposure chamber; EU: European Union;

Data Source: [Ref. 5.3.5.1: P011, P013, P008, P012, P003, P014, P015, P001]

A development program in Japan is being conducted by Torii Pharmaceutical Co., Ltd (herein referred to as Torii), in partnership with ALK, in addition to the 8 trials conducted by the sponsor (P001, P003, P008) and ALK (MT-01, MT-02, MT-03, MT-04, MT-06).

Subjects reporting a history of symptoms to house dust exposure, reporting medication use to treat those symptoms, and demonstrating IgE reactivity by skin prick test (SPT) and serum specific IgE to *D. farinae* and/or *D. pteronyssinus* were enrolled.

Adolescents and adults with symptomatic HDM-induced allergic rhinitis (AR) with/without conjunctivitis and with/without asthma as a primary diagnosis were recruited for safety and efficacy trials. Subjects with a history of asthma were eligible for the trials. In order to be eligible, subjects were required to demonstrate a forced expiratory volume in 1 second (FEV1) ≥ 70% of predicted in MT-06 and P003 and ≥ 80% in P001. About 37.0% of subjects in the AR/C phase III trials (MT-

06, P001) had concomitant asthma. The majority of subjects had sensitivities to other allergens in addition to HDM.

Continuous exposure to high doses of the offending allergen is required for allergen-specific tolerance and disease-modifying treatment effects of immunotherapy (15). Within the first 4 weeks of treatment, the immunologic changes in specific antibodies and T-cell regulation are detectable. Clinical studies with sublingual tablet immunotherapy for seasonal allergens have demonstrated that clinical benefit can be observed as early as 4 to 12 weeks after treatment initiation (16, 17, 18). In both phase III AR/C trials, subjects received treatment for up to 12 months with efficacy measured during the last 8 weeks of treatment.

The potency unit that was used during clinical development is the Development Unit (DU). The DU is determined at the (b) (4)

comparison to the DU of the in-house reference (IHR). The sum of 0.5 DU of *Dermatophagoides pteronyssinus* and 0.5 DU of *Dermatophagoides farinae* equal to one DU in the HDM tablet. The proposed commercial term is defined as SQ-HDM, where SQ designates the method for standardization based on biological potency, major allergen content, and complexity of the allergen extract, and HDM is the abbreviation for house dust mite. The terms SQ-HDM and DU are equivalent.

Stability Summary:

For 26-week toxicity study: Stability as (b) (4)

Stability as (b) (4)

Stability for (b) (4)

For 7-day toxicity study: The stability of dosing formulations had been guaranteed for (b) (4). The expiration date was stated in sections 12.1 and 12.2 (supplement 1 and supplement 2, GMP).

For reproductive study: The dosing formulations (45, 90 and 180 DU/mL) had been confirmed to be stable, as described in supplement 1, for (b) (4) and in supplement 2 for (b) (4) when stored at (b) (4).

For genotoxicology studies the following expiration dates for each batch number were reported:

Test article	Batch number	Purity*	Potency**	Expiration date
<i>Dermatophagoides farinae</i> ⁺	FARAPI2023	43.9%	0.91	10/26/2011
<i>Dermatophagoides pteronyssinus</i> ⁺	PTEAPI2023	47.6%	1.08	10/26/2011
<i>Dermatophagoides pteronyssinus</i> ⁺	PTE2012	45.5	1.20	22 Mar 2012
<i>Dermatophagoides farinae</i> ⁺	FAR2012	56.7	0.85	15 Mar 2012
<i>Dermatophagoides farinae</i> ⁺	FAR2015	45.9%	0.77	10/20/2012
<i>Dermatophagoides pteronyssinus</i> ⁺	PTE2015	53.2%	0.91	10/13/2012

⁺House dust mite extracts

(b) (4)

Toxicity studies submitted to support this BLA:

General toxicology studies:

- 1- Toxicity study by sublingual administration to (b) (4) mice for 26 weeks followed by a 4-week recovery (Study # VPQ0001).
- 2- TO-203 Tablet: Oral mucosal irritation study by 7 days repeated sublingual administration to rabbit (Study # P130040).

Reproductive Study:

Subcutaneous dose study for effects of mixture of *Dermatophagoides farina* and *Dermatophagoides pteronyssinus* allergen extract on embryo-fetal development in mice (study #P120737)

Preliminary study on embryo-fetal development in mice (The study is not reviewed given it's being preliminary in nature)

Genotoxicology studies:

- 1- *Dermatophagoides farina* and *Dermatophagoides pteronyssinus* (house dust mite extracts): Reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli*. Study number: 2325/11.
- 2- Reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli* using a (b) (4) methodology. Study number: 8244469.
- 3- Induction of chromosome aberrations in cultured human peripheral blood lymphocytes. Study number: 8258885.
- 4- Chromosomal aberration test of allergen extract (b) (4) obtained from two types of house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) in cultured human peripheral blood lymphocytes. Study number: F960 (247-021).
- 5- Combined Comet assay in the liver and stomach and a bone marrow micronucleus test in treated rats. Study number: 8247727.

Package insert: The applicant submitted the updated package insert which contains the PLLR compliant sections.

General toxicology studies review:

Study # 1: Title and study number: Toxicity study by sublingual administration to (b) (4) mice for 26 weeks followed by a 4-week recovery (Study # VPQ0001)

Performing laboratory: (b) (4)

Initiation date: July 12, 2012

Final report date: November 13, 2013

Batch/lot number of test article: *Der far* (142212) and *Der pte* (142213)

Animal species and strain: (b) (4) mouse

Breeder/supplier: (b) (4)

Number of animal per sex per group: 12 or 18

Age: 36-42 days

Body weight range: 25.8-37.1 g (males) 22.2-29.6 g (females)

Route and site of administration: Buccal, sublingual

Volume of administration: 5 ul (groups 1, 2 and 3); 5 ul 2 times 5-20 minutes apart (group 4)

Frequency of administration and study duration: Daily for 26 weeks; 40 weeks

Dose/animal: 0.9, 3.5 or 14 DU

Stability: The certificate of analysis provided by the applicant is presented in annex 1. Doses prepared for weeks 1, 2, 13 and 26 were dispatched to the responsible analyst for analysis of achieved concentration. The results of these analyses are presented in annex 2.

Means of administration: Not specified

Report status: Final

Group	Test Material	Dose (DU/day)	Volume (uL)	No. /sex/necropsy*	No. /sex/necropsy**
1	Saline	0	5	12	6
2	<i>Der far</i>	0.9	5	12	0

Group	Test Material	Dose (DU/day)	Volume (uL)	No. /sex/necropsy*	No. /sex/necropsy**
	and <i>Der pte</i> mixture				
3	<i>Der far</i> and <i>Der pte</i> mixture	3.5	5	12	0
4	<i>Der far</i> and <i>Der pte</i> mixture	14	10 (2 x 5)	12	6

*: Week 26

**: Week 30

Table 1: Experimental design (study # 1).

Methods:

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

Randomization procedure: Animals were randomly selected and assigned to study groups without specifying the methodology.

Statistical analysis plan: A parametric analysis was performed if Bartlett's test for variance homogeneity was not significant. The F1 approximate test was applied. If the F1 approximate test for monotonicity of dose response was not significant, Williams' test for a monotonic trend was applied. If the F1 approximate test was significant, Dunnett's test was performed instead. Where there were only two groups, comparisons were made using t-tests.

A non-parametric analysis was performed if Bartlett's test was still significant following both logarithmic and square-root transformations. The H1 approximate test, the non-parametric equivalent of the F1 test described above, was applied. If the H1 approximate test for monotonicity of dose-response was not significant, Shirley's test for a monotonic trend was applied. If the H1 approximate test was significant, Steel's test was performed instead. Where there were only two groups, comparisons were made using Wilcoxon rank sum tests.

Parameters	Frequency of Testing
Cage-side observations	Twice daily for mortality and ill-health
Physical examination	Weekly
Signs with dosing	Daily during first week, twice weekly during 2-26 at pre-dose, end of dosing, 1-2 hours after dosing and end of day
Body weight	Days -7, -3, -2, -1, 1 and 2, then weekly and before necropsy
Food consumption	Daily for week -1 and week 1, then weekly
Clinical chemistry	Weeks 26 and 30 thru the orbital sinus under isoflurane
Hematology	Weeks 13, 26 and 30 thru the orbital sinus under isoflurane

Parameters	Frequency of Testing
Immunogenicity	Not performed
Ophthalmic examination	Pre-dose and week 26 (groups 1 and 4)
Necropsy	Weeks 26 and 30 (groups 1 and 4)
Tissues for histopathology	Same as above

Table 2: Parameters evaluated (study # 1).

Postmortem procedures: The following tissues were collected at necropsy and were microscopically examined in main study groups 1 and 4 and premature deaths. Site of administration (tongue) in groups 2 and 3 and abnormalities were microscopically examined. Those tissues marked with an asterisk were weighed.

SYSTEM	ORGAN COLLECTED	ORGAN NOT COLLECTED
Digestive	Large intestine (cecum, colon, rectum); small intestine (duodenum, jejunum, ileum), liver*, stomach , pancreas	
Respiratory	Lungs with trachea, head sectioning (nasal cavity, paranasal sinuses and nasopharynx)	
Cardiovascular	Heart*, aorta	
Immunologic/ Hematopoietic	Bone marrow smear, bone (femur and joint, sternum), lymph nodes (mandibular, mesenteric, left axillary and retropharyngeal), spleen* , thymus*	
Urogenital	Epididymes, kidneys*, urinary bladder, uterus, prostate, testis*, seminal vesicle, ovary, vagina	Fallopian tube, cervix
Neurologic	Brain*, spinal cord	
Hormonal	Adrenals*, thyroid with parathyroid gland, pituitary gland*	
Other	Skeletal muscle, sciatic nerve, eyes, optic nerve , Peyer's patches, tongue, salivary glands (submandibular, parotid and sublingual), Harderian glands, skin with mammary glands,	

Table 3: Tissues collected at necropsy (study # 1).

Results:

Mortality and clinical observations: One female in group 1, two females in group 2, two males and one female in group 3 and one male in group 4 were sacrificed during the study for welfare reasons. There were no test article related mortalities or clinical signs. No local reactions were observed at the site of administration (buccal).

Body weights and food intake: Compared to the saline group no significant changes were observed of the treatment groups.

Ophthalmic examinations: No abnormalities were observed.

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		Glutamic oxalacetic transaminase (not determined) Gamma glutamyl transferase (not determined) Alanine aminotransferase Aspartate aminotransferase Sorbitol dehydrogenase (not determined) Total bile acid (not determined)
		Total bilirubin Alkaline phosphatase
KIDNEY FUNCTION		Creatinine Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Albumin Globulin A/G ratio Cholesterol Cholinesterase (not determined) Total protein Triglyceride Fibrinogen Creatine kinase (not determined) Lactate dehydrogenase (not determined)

Table 4: Clinical chemistry results (study # 1).

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. Reticulocyte Red blood cell Thrombocyte
WHITE BLOOD CELLS		WBC Differential leukocyte count Large unstained cells
CLOTTING POTENTIAL		Activated partial-thromboplastin time (not determined) Prothrombin time (not determined) Platelet

Table 5: Hematology results (study # 1).

Organ weights: There were no treatment related effects.

Gross pathology: The macroscopic examination revealed no intergroup differences.

Microscopic pathology: Minimal focal stomach ulceration was observed in two high-dose females and one low dose male. Given that low incidence of these findings, they were not considered test article related. There were following incidental findings: Malignant astrocytoma was observed in the brain of a male mid-dose group at early sacrifice due to its poor clinical condition. A similar isolated occurrence was reported in a carcinogenicity study at the testing facility. Thus, this was considered incidental due to the single incidence in a mid dose group. Bronchiolo-alveolar adenoma was seen in one male each in high and mid-dose groups. This is a commonly occurring neoplasm in mice and the incidence (8.3%) in this study was within the historical incidence range observed at the testing facility (0-14%). Bronchiolo-alveolar carcinoma was observed in one female in low dose group. This tumor type is a commonly occurring neoplasm in mice and being a single incidence in low dose group and was considered incidental. Endometrial polys observed in the uterus of one female each in low and mid dose groups were considered incidental. There were no test article-related microscopic findings after 4-week of recovery except one single incidence of lymphoma in the thymus and mesenteric lymph node in a high dose female. This is a commonly neoplasm in (b) (4) -mice observed from carcinogenicity studies at the testing facility as early as week 9 of these studies.

Assessment

There were no test articles-related effects on clinical signs, mortality, body weights, food consumption, ophthalmic examinations, hematology and clinical chemistry. Based on the overall evaluation of the study report, it can be concluded that twenty six weeks daily sublingual administrations of the allergen extracts in (b) (4) (a mixture of *der far* and *der pte*) were well-tolerated in mice at doses up to 14 DU.

GLP study deviations or amendments: No deviation from protocol is indicated.

BLA 125592

Study # 2: Study title: TO-203 Tablet: Oral mucosal irritation study by 7 days repeated sublingual administration to rabbit (Study # P130040)

Performing laboratory: (b) (4)

Initiation date: July 29, 2013

Final report date: February 19, 2014

Batch/lot number of test article: 1290424 (low dose, 6 DU), 1290427 (high dose, 12 DU)

Animal species and strain: (b) (4) rabbits

Breeder/supplier: (b) (4)

Number of animal per group: 3 males

Age: 14 weeks

Body weight range: 2.23-2.27 kg

Route and site of administration: Sublingual

Number of tablets of administration: 2

Frequency of administration: Daily for 7 days

Dose/animal: 12 or 24 DU

Stability: The stability of dosing formulations had been guaranteed for 3 years at room temperature. The expiration date was stated in sections 12.1 and 12.2 (supplement 1 and supplement 2, GMP).

Means of administration: Under isoflurane anesthesia, two tablets were placed tandem using tweezers on the anterior part and the other on the posterior part of the sublingual site and retained for 10 minutes and removed.

Report status: Final

Experimental design

Group	Test Material	Dose (DU/day)	Number of tablets)	No. /necropsy
1	Placebo	0	2	3
2	Low dose	12	2	3
3	High dose	24	2	3

Table 6: Experimental design (study # 2).

Randomization procedure: Animals were randomly selected and assigned to study groups by the stratified randomization (b) (4) on the basis of body weight.

Statistical analysis: No statistical analysis was performed.

The following parameters were evaluated

Parameters	Frequency of Testing
Clinical observations	Twice daily (before and 1-7 hours after dosing)
Dosing site examination	Twice daily (before dosing and after removal of tablet) and once on day 8
Body weight	Daily before dosing
Necropsy	Day 8
Tissues for histopathology	Tongue and the floor of the buccal cavity

Table 7: Parameters evaluated (study # 2).

Results:

Examination of the dosing site: No change in buccal mucosa was observed.

Clinical observations: No death occurred or abnormal sign in behaviors was observed.

Body weights: No differences in body weight gains were observed.

Histopathology: No test article related finding was detected.

Assessment:

There were no irritable effects on buccal mucosa caused by TO-230 tablet up to 24 DU following sublingual dosing for 7 days. There were no test article related changes in clinical observation, body weight and no gross or histopathological observation in the dosing site.

Study # 3: Study title and number: Subcutaneous dose study for effects of mixture of *Dermatophagoides farina* and *Dermatophagoides pteronyssinus* allergen extract on embryo-fetal development in mice (study #P120737)

Performing laboratory: (b) (4)

Initiation date: February 25, 2013

Final Report date: November, 8, 2013

Test article batch/lot: 121-256

Animal species and strain: (b) (4); (b) (4) mouse

Breeder/supplier: (b) (4)

Number of female animal per group: 22

Age: 10-11 weeks

Average body weight: 23-32 g

Route and site of administration: Subcutaneous; back

Volume of injection: 10 ml/kg (0.23-0.32 ml/animal)

Frequency of administration and study duration: Daily on gestation days (GDs) 6-17; 1 month

Dose: 450, 900, or 1800 DU/kg or 12.5, 25, or 50 DU/animal

Stability: The dosing formulations (45, 90 and 180 DU/mL) had been confirmed to be stable as described in supplement 1 for (b) (4) when stored at (b) (4) and in supplement 2 for (b) (4) when stored at (b) (4)

Means of administration: A syringe with a 26G needle

Report status: Final

Experimental design

Group	Test article	Dose DU/kg	Dose DU/ animal	Dosing volume ml*	No. of animals	Number of pregnant animals	No. of Cesarean
1	Control	0	0	0.28	22	19	19
2	Low	450	12.5	0.28	22	17	16**
3	Mid	900	25	0.28	22	20	20
	High	1800	50	0.28	22	20	18**

*: Average based on body weights of 0.23-0.32 g

**: 1 and 2 dams for groups 2 and 4, respectively, had early delivery before GD 18 and were excluded

Table 8: Experimental design (study # 3).

Randomization procedure: Not specified.

Statistical analysis plan: Bartlett's test was performed to compare the variances among the control and test article groups. If variance of the data was homogeneous, Dunnett's multiple comparison was performed against the control group. If the variance of the data was heterogeneous, Steel's multiple test was performed against the control group. F-test was performed to compare the

variances among the control and high dose groups. If variance of the data was homogeneous, Student's t-test was performed relative to the control group. If the variance of the data was heterogeneous, Aspin- Welch t-test was performed relative to the control group. For the sex ratio of live fetuses the Chi-square test was used for comparisons between the control group and the test article groups. For the indexes and incidences the Wilcoxon rank-sum test was performed between the control group and the test article groups.

The following parameters were evaluated

Parameters	Frequency of Testing
Mortality and clinical observations	Twice daily during dosing period and daily during other times
Body weight	GDs 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18
Food consumption	GDs 1, 2, 4, 6, 8, 10, 12, 14, 16 and 18
Scheduled maternal euthanasia for numbers of corpora lutea, implantations, early resorptions, late resorptions, dead/live fetuses, placenta and external anomalies	GD 18
Scheduled fetal euthanasia skeletal (1/2 litters) and visceral (1/2 litters) examinations in control and high-dose groups only	GD 18

Table 9: Parameters evaluated (study # 3).

Results:

Dams

Mortality: One animal in low dose group died on GD 15. This animal was necropsied and no abnormalities were macroscopically observed at the necropsy. The death was judged not to be treatment-related, since it was not dose-dependent. Additionally, this animal was non-pregnant. Therefore, the data obtained from this animal was excluded from the evaluation. All other mice survived to scheduled euthanasia.

Pregnancy: There were 3, 5, 2, and 2 non-pregnant animals in groups 1, 2, 3 and 4 respectively.

Delivery: One and two dams in groups 2 and 4, respectively, had early delivery before the scheduled necropsy on GD18. The body weight, food consumption on GD 18 and cesarean sections from these animals were excluded.

Clinical signs: No findings were observed at any dose level.

Body weights: No differences in body weights were observed.

Food consumption: Food consumption was unaffected except a slight transient increase on GD8 in high-dose group.

Necropsy: No organs or tissues were preserved for microscopic examination since no gross findings were observed.

Fetuses

Number of corpora lutea, implantations, pre-implantation losses, post-implantation losses, live fetuses, sex ratio, and body weights of the live fetuses: There were no treatment-related changes except the percent of late resorption was increased at low dose group (2.48%) and mid-dose group (4.77%) vs 0.44% in control group. However the incidences were within the historical control data range of 0-6.02% at the testing facility as provided in amendment 2 and the findings were considered to be incidental.

Visceral examinations: No abnormalities or variations were observed.

Skeletal examinations: The incidence of fused sternebrae was observed in 2 of 105 fetuses (1.9%) or 2/18 litters in control group and 4 of 104 fetuses (3.9%) or 4/18 litters in high-dose group. The incidence in fetuses was above the upper limit of the historical data at the test facility (2/140 fetuses, 1.4%, 4 studies, 2003-2013). Therefore this skeletal malformation finding was considered to be test article-related. The progress of ossification of sacrocaudal body was lower (11.857) in high-dose group. However, the incidence was around the upper limit of historical data at the testing facility (11.609, 4 studies, 2003-2013). This finding was not considered to be test article-related.

GLP study deviations or amendments: There were neither deviations from the study protocol nor unpredicted events.

Assessment

There were no test article-related effects on clinical signs, body weight, and food consumption in dams. There were no visceral anomalies and variations. However, an increased incidence of fused sternebrae was observed in high-dose group above the historical control incidence range. This finding should be described in section 8.1 of the PI.

Genotoxicology studies:

Study # 1: Title and study number: *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (house dust mite extracts): Reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli*. Study number: 2325/11.




Introduction

The Ames test is to evaluate the mutagenic potential of a test article by measuring genetic activity in one or more histidine-requiring strains of *Salmonella typhimurium* in the absence and presence of a liver metabolizing system (19). This assay has the ability to detect genetically active compounds of most chemical classes, with around 80-90% sensitivity and specificity (20). Several *Escherichia coli* (*E. coli*) strains have also been used for mutagen screening (21). The derivatives of WP2, a tryptophan-requiring strain in which agents causing base substitution mutations can increase the frequency of Trp⁺ revertants is prominent among these (22). It has been proposed that a combination of repair-proficient and repair-deficient plasmid-containing strains of *E. coli* NM be used to detect a range of oxidative mutagens and cross-linking agents, and to complement the specificity of the *S. typhimurium* strains (20). The following bacterial strains were used in this study:

Organism	Strain	Type of mutation	Mutant gene
<i>S. typhimurium</i>	(b) (4)	(b) (4)	histidine
<i>S. typhimurium</i>	(b) (4)	(b) (4)	histidine
<i>S. typhimurium</i>	(b) (4)	(b) (4)	histidine
<i>S. typhimurium</i>	(b) (4)	(b) (4)	histidine
<i>E. coli</i>	(b) (4)	(b) (4)	tryptophan
<i>E. coli</i>	(b) (4)	(b) (4)	tryptophan

Table 10: Bacterial strains (genotox study # 1).

(b) (4)



Evaluating the mutagenic activity of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (house dust mite extracts), by examining its ability to revert four strains of *Salmonella typhimurium* and two strains of *E. coli* in the absence and presence of a ^{(b) (4)} liver metabolizing system (S-9), was the objective of this study.

Performing laboratory: (b) (4)



Study initiation date: June 28, 2006

Final report date: May 9, 2009

Test article, batch/lot:

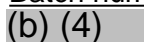
Test article

Dermatophagoides farinae (house dust mite extracts)

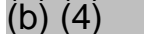
Dermatophagoides pteronyssinus (house dust mite extracts)

Batch number

(b) (4)

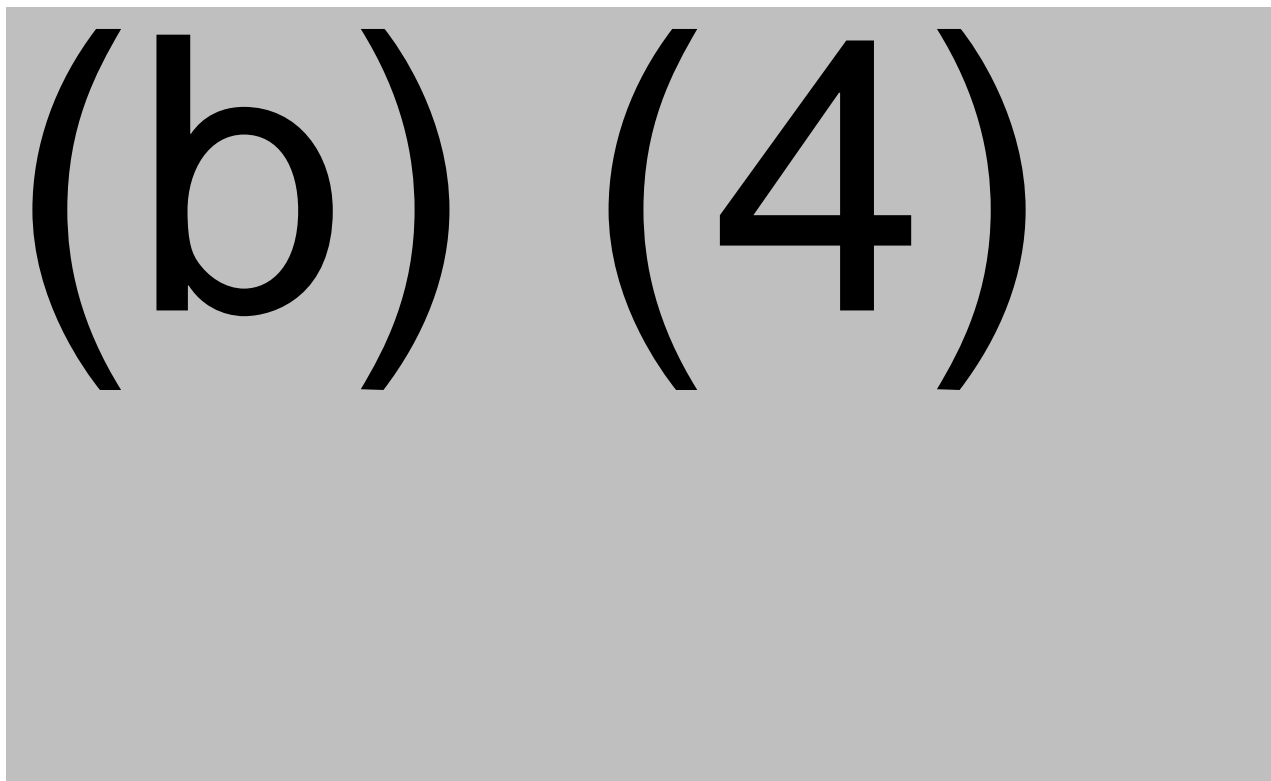


(b) (4)



Materials and methods:

(b) (4)



(b) (4)

The mutation data were evaluated as follows:

No significant increases in revertant numbers due to the *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (house dust mite extracts) treatments of the tester strains (in the absence and in the presence of S-9) were reported. Thus, no evidence of any *Dennatophagoides farinae* and *Dermatophagoides pteronyssinus* (house dust mite extracts) mutagenic activity were reported.

Conclusion

It was concluded that *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (house dust mite extracts) did not induce mutation in four histidine-requiring strains of *Salmonella typhimurium* ((b) (4)) and two tryptophan-requiring strains of *E. coli* ((b) (4)).

Study # 2: Title and study number: Reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli* using a (b) (4) methodology. Study number: 8244469.

Objective

Evaluating the mutagenic activity of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (house dust mite extracts), by examining its ability to revert four strains of *Salmonella typhimurium* and two strains of *E. coli* in the absence and presence of a (b) (4) liver metabolizing system (S-9), was the objective of this study. A (b) (4) procedure was used for all treatments in this study.

Performing laboratory: (b) (4)

Study initiation date: June 02, 2011

Final report date: September 06, 2011

Test article, batch/lot:

Test article	Batch number	Purity*	Potency**	Expiration date
<i>Dermatophagoides farinae</i> (house dust mite extracts)	(b) (4)	43.9%	0.91	10/26/2011
<i>Dermatophagoides pteronyssinus</i> (house dust mite extracts)	(b) (4)	47.6%	1.08	10/26/2011

Materials and methods:

(b) (4)

(b) (4)

Conclusion

It was concluded that *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (house dust mite extracts) did not induce mutation in four histidine-requiring strains of *Salmonella typhimurium* (b) (4) and two tryptophan-requiring strains of *E. coli* (b) (4).

Study # 3: Title and study number: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes. Study number: 8258885.

Introduction

Number of human genetic diseases is recognized by chromosome defects (26).

To identify agents that cause structural chromosome aberrations in cultured mammalian cells, the in vitro chromosome aberration test is used (27). Human peripheral blood lymphocytes are used in this assay because the cells are only used in short-term culture and maintain a stable karyotype (28). Experiments with these cells can be performed in conjunction with a (b) (4) liver metabolizing system (S-9). This is because, for a short incubation periods, no toxicity is induced by the liver homogenate itself. The assay is not specifically designed to evaluate potential to induce aneuploidy or polyploidy but the increases in numerical chromosome aberrations can be detected.

Objective

Evaluating the clastogenic potential of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (house dust mite extracts) by examining its effects on the chromosomes of cultured human peripheral blood lymphocytes treated in the absence and presence of S-9, was the objective of this study.

Performing laboratory: (b) (4)

Study initiation date: April 02, 2012

Final report date: September 21, 2012

Test article, batch/lot:

Test article	Batch number	Purity*	Potency**	Expiration date
<i>Dermatophagoides farinae</i> (house dust mite extracts)	(b) (4)	45.9%	0.77	10/20/2012
<i>Dermatophagoides pteronyssinus</i> (house dust mite extracts)	(b) (4)	53.2%	0.91	10/13/2012
(b) (4)				

Materials and methods:

Dermatophagoides pteronyssinus, (b) (4) and *Dermatophagoides farinae*, (b) (4) concentration ranges tested:

In cultures treated with the test article in the absence and presence of S-9, no increases in the frequency of cells with numerical aberrations, which exceeded the concurrent vehicle controls and the normal ranges, were reported.

Conclusion

The test article induced structural chromosome aberrations starting at concentration of 2250 µg/mL (extract) after 20 hour exposure without the presence of the liver microsomal enzymes (S9). No results for 20 hour exposure with the presence of liver microsomal enzymes (S9) were reported.

The human dose at 12 DU [6 DU is from Der p and 6 DU from Der f] tablet will have 240-840 µg of extract by dry weight. Exposure to this low level for extended period of time is of concern.

Study # 4: Title and study number: Chromosomal aberration test of allergen extract (b) (4) obtained from two types of house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) in cultured human peripheral blood lymphocytes. Study number: F960 (247-021).

Purpose

To assess the *in vitro* induction of chromosomal aberration potential of the test substance in cultured human peripheral blood cells. In addition, 3 lots of the test substances were used to evaluate the reproducibility of the results.

Performing laboratory: (b) (4)

Sponsor: (b) (4)

Study initiation date: January 21st, 2015

Final report date: March 31st, 2015

Test article, batch/lot:

Test article	Batch number	Expiration date	Stability
<i>Dermatophagoides farinae</i>	(b) (4)	June 12, 2015	(b) (4)
	(b) (4)	May 19, 2016	
	(b) (4)	May 20, 2016	
<i>Dermatophagoides pteronyssinus</i>	(b) (4)	July 9, 2015	
	(b) (4)	May 17, 2016	
	(b) (4)	May 18, 2016	

Materials and methods:

Dermatophagoides pteronyssinus, (b) (4) and *Dermatophagoides farinae*, (b) (4) concentration ranges tested:

(b) (4)

(b) (4)

Conclusion:

Allergen extract (b) (4) obtained from two types of house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) did not induce chromosomal aberrations (negative) in cultured human peripheral blood lymphocytes under the conditions in this study. In addition, because all lots of each test substance showed negative results, the reproducibility of results of the chromosomal aberration potential was confirmed.

6 pages have been determined to be not releasable: (b)(4)

Study # 5: Title and study number: Combined Comet assay in the liver and stomach and a bone marrow micronucleus test in treated rats. Study number: 8247727.

Introduction

The common genotoxic events are chromosome defects, point mutations, base pair deletions, DNA-DNA and DNA-protein cross-links. These events have the potential to trigger a number of human genetic diseases (26). In vitro testing for chemicals abilities to produce such genetic changes in cells are used. In vivo, chemicals may react differently. This is because where metabolic systems other than liver cytochrome P448/P450 operate, and where the dynamic processes of absorption, metabolism, and excretion are involved. Chemicals should therefore also be tested for their genotoxic activity in vivo.

The Comet assay (single cell gel electrophoresis assay) is a technique used for measuring and analyzing DNA strand breaks in individual mammalian cells (29; 30). Briefly, small numbers of cells are suspended in a thin agarose gel on a microscope slide. To be prepared for electrophoreses, the cells are lysed and the DNA subsequently unwound under alkaline conditions (>pH 13). The charged DNA is drawn away from the nucleus during electrophoresis. Relaxed and broken DNA fragments migrate further than undamaged DNA complexes. The extent of DNA damage incurred by the cells could be detected by the degree of DNA migration (31). To increase the sensitivity of the assay, pH levels greater than pH 13 could be used as this enables alkaline labile sites as well as single and double strand DNA breaks to be expressed.

Erythroblasts undergoing their last chromosome replication are the target cells in the bone marrow micronucleus test (32; 33; 34). Chromosome fragments or whole chromosomes, which are unable to attach to the spindle, are left behind as micronuclei when the main nucleus is extruded in the production of the polychromatic erythrocyte (PCE). In these cells, micronuclei can be clearly seen and are more easily counted than structural chromosome aberrations in metaphase cells. Agents which affect spindle formation or function (that is, result in aneuploidy), because they are also formed from intact chromosomes, can be detected.

The Comet and the micronucleus testing (in combination) are anticipated to detect the majority of biologically relevant genotoxins.

Objective

Evaluating the potential of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (house dust mite extracts) to induce DNA damage in the liver and the stomach of treated rats and the potential to induce micronuclei in the polychromatic erythrocytes (PCE) of the bone marrow of the same animals.

Performing laboratory: (b) (4)

Study initiation date: June 02, 2011

Final report date: October 26, 2011

Test article, batch/lot:

<u>Test article</u>	<u>Batch number</u>	<u>Purity*</u>	<u>Potency**</u>	<u>Expiration date</u>
<i>Dermatophagoides pteronyssinus</i> ⁺	PTE2012	45.5	1.20	22 Mar 2012
<i>Dermatophagoides farinae</i>	FAR2012	56.7	0.85	15 Mar 2012

⁺House dust mite extracts

(b) (4)

Stability and homogeneity

Data provided indicated that the test article formulation (Dermatophagoides pteronyssinus, (b) (4) and Dermatophagoides farinae, (b) (4) combined in a 1:1 ratio) in water at 50.1 total mg dry matter/g was stable for (b) (4) when stored at (b) (4).

Materials and methods:

Animal species and strain: (b) (4) rats

Breeder/supplier: For range-finder experiment animals were obtained from (b) (4)

For micronucleus/Comet experiment animals were obtained from (b) (4)

Number of animal per group and sex: 3/sex/group for range-finder groups and 5/sex/group for Micronucleus/Comet experiment.

Age: 8-9 weeks

Body weight range: 226.9-291.7 gram

Route and site of administration: Oral gavage

Volume of injection: 20 mL/kg for all test article and vehicle control treatments and 10 mL/kg for all positive control treatments. Individual dose volumes were based on individual body weight.

Frequency of administration and study duration: The test article, vehicle control and positive control were given as three administrations, at approximately 0, 24 and 45 hours and animals were sampled at approximately 48 hours, i.e. 3 hours after the final administration. It has been reported that this should be sufficient duration for the expression of any genotoxic potential (35; 36; 37; 38; 39; 40; 41).

Dose: 1002 mg/kg/day for range-finder animals. Doses for Micronucleus/Comet experiment are listed in the table below:

Dose levels - Micronucleus/Comet experiment

Group No.	Group Description	Dose level (mg/kg/day)	Animal ID	Sample time (hours after final administration)
1	Vehicle control ^a	0	1-6M	3
2	Dermatophagoides pteronyssinus, (b) (4) and Dermatophagoides farinae, (b) (4)	250	7-12M	3
3	Dermatophagoides pteronyssinus, (b) (4) and Dermatophagoides farinae, (b) (4)	500	13-18M	3
4	Dermatophagoides pteronyssinus, (b) (4) and Dermatophagoides farinae, (b) (4)	1002	19-24M	3
5	Positive control ^b	150	25-30M	3
a	Purified water			
b	(b) (4)			

Table 40: Dose levels-micronucleus/Comet experiment (genotox study # 5).

Means of administration: Oral gavage

Report status: Final

Experimental design

Dermatophagoides pteronyssinus, (b) (4) and Dermatophagoides farinae, (b) (4) concentrations tested

Experiment	Concentration of dosing preparation (mg/mL)	Dose administered (mg/kg/day)
Range-Finder	50.1	1002
Micronucleus/Comet Experiment	12.5	250
	25.0	500
	50.1	1002

Table 41: Dose concentrations tested (genotox study # 5).

Methods:

Parameters	Frequency of Testing
Cageside observation	Twice
Clinical observations	Once daily
Body weight	Each dosing day and each post-dose observation day

Table 42: Parameters evaluated (genotox study # 5).

Tissue samples

The following tissues were removed from all Micronucleus/Comet experiment animals at necropsy:

Group	Micronucleus samples	Comet samples	Histopathology samples
1	BM	L, S	L, S
2	BM	L, S	L, S
3	BM	L, S	L, S
4	BM	L, S	L, S
5	BM	L, S	-
L Liver S Stomach BM Bone Marrow (from 1 femur)			

Table 43: Tissue samples collected (genotox study # 5).

The liver and stomach and one femur were removed from each control (negative and positive) and test article treated animal.

Histopathology samples were preserved in 10% neutral buffered formalin (NBF) and stored at room temperature. No histopathology assessments of these tissues were conducted.

Results:

No test article-related effects were reported on clinical signs and body weight gain in the range-finder experiment at 1002 mg/kg/day dose.

Due to these findings, doses of 250, 500, and 1002 mg/kg/day were selected for testing in the micronucleus/comet experiment. Because no substantial difference in toxicity was reported between males and females in the range-finder experiment, male animals only were used in the micronucleus/comet experiment.

Main Experiment

Raw data

Liver and stomach comet parameters were median tail intensity, median tail moment, % clouds, and % diffused cells. Micronucleus parameters were observed PCE, NCE, and MN PCE numbers together with calculated % PCE and % MN PCE values.

Comet and micronucleus historical vehicle and positive control ranges were listed on page 46.

Validity of study

The data showed that:

1. At least five animals per group were available for analysis.
2. Low levels of cytotoxicity in the Comet assay (i.e. group mean of less than 30% clouds or 30% diffused cells (35)) in the vehicle control group was demonstrated indicating correct preparation of the cell suspensions.
3. The vehicle control data were considered to be consistent with the relevant historical vehicle control data.
4. Marked increase in Comet parameters and a statistically significant increase in the frequency of MN PCE were demonstrated in the positive control chemical (EMS) compared to the concurrent vehicle control groups. The assay data were therefore considered valid.

Clinical signs

No test article-related clinical signs of toxicity or body weight gain were reported in the micronucleus/Comet experiment at 250, 500, or 1002 mg/kg/day, or positive control doses. No test article-related macroscopic changes were reported.

Analysis of micronucleus data

No test article-related effect on % PCE values was reported and the values were within acceptable ranges.

No test article-related toxicity to the bone marrow (as would usually be indicated by a notable decrease in % PCE values compared to the vehicle control group or dose dependent decrease) was reported. When compared to control group, group mean frequencies of MN PCE were similar to and not statistically (chi-square) different. Individual frequencies of MN PCE for all treated animals were

consistent with historical vehicle control distribution data and similar to frequencies observed in the concurrent controls.

Dermatophagoides pteronyssinus, (b) (4) and *Dermatophagoides farinae*, (b) (4):
Summary and Statistical Analysis of Micronucleus Data.

Group/Treatment mg/kg/day	Cell Total	% PCE	MN PCE	Mean MN PCE/2000	%MN PCE	SD	Heterogeneity X2	S	Contingency X2C	S
1M Vehicle	12000	50.43	17	2.83	0.14	0.11	8.07	NS		
2M / D. pteronyssinus and D. farinae ¹				3.00						
(250)	12000	53.25	18		0.15	0.05	2.00	NS	0.00	NS
3M / D. pteronyssinus and D. farinae ¹				2.50						
(500)	12000	53.23	15		0.13	0.08	4.61	NS	0.03	NS
4M / D. pteronyssinus and D. farinae ¹				4.17						
(1002)	12000	56.12	25		0.21	0.12	6.45	NS	1.17	NS
5M				27.67						
Positive control, EMS (150)	12000	48.98	166		1.38	0.67			120.61	P≤0.001

Linear trend: z = 1.310 NS

NS = Not significant

MN = Micronucleated

SD = Standard deviation

M = Male

PCE = Polychromatic erythrocyte

¹Dermatophagoides pteronyssinus, frozen droplets and Dermatophagoides farinae, frozen droplets

Table 44: Summary of micronucleus data (genotox study # 5).

Analysis of Comet data

Little or no cytotoxicity, necrosis, or apoptosis in the cell suspensions were reported for both tissues ('cloud' assessment and diffusion slide analysis) of the test article-treated animals.

Tail moments and tail intensities that were similar to the concurrent vehicle control group were reported in both liver and stomach tissues of the test article-treated animals. Comet parameters for test article-treated animals were consistent with the concurrent vehicle control data and considered to be comparable with the laboratory's historical control ranges for each tissue.

Dermatophagoides pteronyssinus, (b) (4) and Dermatophagoides farinae, (b) (4) :
Summary of liver Comet data.

Group/Treatment (mg/kg/day)	Total no. cells scored	Tail Intensity		Tail Moment		Mean % clouds	Mean % Diffused cells
		Mean	SEM	Mean	SEM		
1M							
Vehicle	500	3.24	0.61	0.33	0.06	5.92	3.00
2M / D. pteronyssinus and D. farinae ¹ (250)	600	2.80	0.68	0.29	0.06	4.33	1.33
3M / D. pteronyssinus and D. farinae ¹ (500)	600	3.21	0.71	0.32	0.07	3.92	2.83
4M / D. pteronyssinus and D. farinae ¹ (1002)	600	1.60	0.55	0.17	0.05	3.33	1.67
5M							
Positive control, EMS (150)	550	46.51	2.01	9.08	0.52	2.17	1.50

SEM Standard error of mean

EMS Ethyl methanesulfonate

M Male

¹Dermatophagoides pteronyssinus, frozen droplets and Dermatophagoides farinae, frozen droplets

Table 45: Summary of liver Comet data (genotox study # 5).

Dermatophagoides pteronyssinus, (b) (4) and Dermatophagoides farinae, (b) (4) :
Summary of stomach Comet data.

Group/Treatment (mg/kg/day)	Total no. cells scored	Tail Intensity		Tail Moment		Mean % clouds	Mean % Diffused cells
		Mean	SEM	Mean	SEM		
1M							
Vehicle	600	3.00	0.33	0.28	0.02	14.33	19.33
2M / D. pteronyssinus and D. farinae ¹ (250)	600	2.81	0.31	0.27	0.04	16.00	19.83
3M / D. pteronyssinus and D. farinae ¹ (500)	600	3.26	0.42	0.31	0.03	15.50	18.00
4M / D. pteronyssinus and D. farinae ¹ (1002)	600	2.36	0.29	0.22	0.03	15.00	21.00
5M							
Positive control, EMS (150)	600	30.91	1.87	4.42	0.38	23.58	24.00

SEM Standard error of mean

EMS Ethyl methanesulfonate

M Male

¹Dermatophagoides pteronyssinus, frozen droplets and Dermatophagoides farinae, frozen droplets

Table 46: Summary of stomach Comet data (genotox study # 5).

Conclusion

In conclusion test article-treatment did not induce DNA damage in the liver and the stomach of male rats following oral gavage administration of doses of 250, 500 and 1002 mg/kg/day. These doses did not induce increases in micronucleated polychromatic erythrocytes in the bone marrow.

OVERALL SUMMARY:

GENERAL TOXICOLOGY:

Two studies were submitted to support this BLA. All studies of the test articles were repeated dose studies.

Adequate nonclinical toxicology data were included in these studies. Based on nonclinical toxicity assessments of the above mentioned studies there were no significant safety issues to preclude the BLA from approval. The delivery of an active dose of the product was verified.

REPRODUCTIVE STUDIES:

One reproductive study was submitted to support this BLA. Animals were treated with the test article daily on gestation days (GDs) 6-17. An increased incidence of fused sternebrae was observed in high-dose group above the historical control incidence range. This finding should be described in section 8.1 of the PI.

GENOTOXICOLOGY STUDIES:

Two reverse mutation, two chromosome aberration, and one Comet assay studies were submitted to support this BLA.

It was concluded that *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (house dust mite extracts) did not induce mutation in four histidine-requiring strains of *Salmonella typhimurium* (b) (4)) and two tryptophan-requiring strains of *E. coli* (b) (4)).

Two chromosomal aberration tests were conducted. No chromosomal aberration was reported in the first study. In the second study, test article treatment did not induce structural chromosome aberrations following 3+17 hour treatment in the absence and presence of S-9 when tested to the maximum practicable concentrations (4985 µg/mL). No increases in the frequencies of cells with numerical aberrations were reported.

However, it is concluded that test article treatment induced structural chromosome aberrations in vitro in cultured human peripheral blood lymphocytes following 20+0 hour treatment in the absence of a liver metabolic activation system (S-9) when tested at 2250 µg/mL. (b) (4)

In the Comet study, test article-treatment did not induce DNA damage in the liver and the stomach of male rats following oral gavage administration of doses of 250, 500 and 1002 mg/kg/day. These doses did not induce increases in micronucleated polychromatic erythrocytes in the bone marrow.

There is presently a need to confirm the findings of the cultured human lymphocyte assay using the mouse lymphoma assay to support a weight of evidence approach. The overall data do not suggest the strong possibility of human carcinogenicity; however, the lack of precise knowledge of the exact composition of the product severely limits complementary approach to ruling out any biological significance. The limited bio-distribution of the product in combination with a lack of identification of its components greatly compromises the usefulness of *in vivo* genotoxicity testing. Given the difference

between non mammalian and mammalian genetic structures, a negative finding in the Ames assay is not reassuring of a lack of genotoxicity in mammalian system.

Package Insert Part Review

In compliance with PLLR, section 8.1 is recommended to revise as marked below:

USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk summary

In a developmental study, a vaccine in a liquid formulation containing approximately 1-4 times the human sublingual dose (12 DU) was administered subcutaneously to female mice during gestation. The animal study revealed harmful effects to the fetus due to TRADEMARK [see *Animal Data*]. Epidemiological data suggest that major birth defects occur in 2-4% of the general U.S. population and that miscarriage occurs in 15-20% of clinically recognized pregnancies. However, the incidence of major birth defects and miscarriage in the indicated population is not well established.

Clinical Considerations

Maternal Adverse Reactions

Because systemic and local adverse reactions with immunotherapy may be poorly tolerated during pregnancy, TRADEMARK should be used during pregnancy only if clearly needed.

Animal data

A developmental study was conducted in mice. Doses of 12.5, 25 or 50 DU/animal were administered subcutaneously daily on gestations days 6-17. TRADEMARK exhibited an increased incidence of fused sternebrae at 50 DU dose (4 times the human sublingual dose).

13.1 Genotoxicity data:

Carcinogenesis, Mutagenesis, Impairment of Fertility

Two *in vitro* chromosome aberration assay, an *in vitro* bacterial mutagenesis assay and a combined *in vivo* Comet and micronucleus assay for mutagenicity in rats were performed using HDM (*D. farinae* and *D. pteronyssinus*) allergen extracts. One *in vitro* chromosome aberration assay was positive. However, based on the aggregated results, the weight of evidence approach indicates that the positive results may have little or no human relevance.

Overall conclusion:

Based on the nonclinical toxicity assessments of the *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (house dust mite extracts) vaccine submitted in this BLA, mitotic inhibition were reported and might be test article-related. This finding should be included in section 13.1 of the label.

Internal communication:

The test article induced structural chromosome aberrations starting at concentration of 2250 µg/mL (extract) after 20 hour exposure without the presence of the liver microsomal enzymes (S9). No results for 20 hour exposure with the presence of liver microsomal enzymes (S9) were reported.

The human dose at 12 DU [6DU is from Der p and 6 DU from Der f] tablet will have 240-840 µg of extract by dry weight. Exposure to this low level for extended period of time is of concern.

This finding was not reported in the labeling. Even if the weight of evidence would suggest that it is not a carcinogen, this finding cannot be disregarded from labeling.

Concurrence: Martin D. Green

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Rat liver comet historical control ranges

		Median %Tail Intensity	Median Tail Moment	Mean % clouds	%Diffused cells
Vehicle ranges		59	59	59	53
Number of animals					
Observed range	Minimum	0.13	0.02	0.50	0.00
	Maximum	14.70	1.74	11.00	14.00
95% Reference range	Lower Limit	0.30	0.04	NA	NA
	Upper Limit	8.15	0.81	NA	NA
Positive control (EMS) ranges		53	53	53	46
Number of animals					
Observed range	Minimum	15.39	2.03	0.50	0.00
	Maximum	68.70	16.93	13.50	16.00
95% Reference range	Lower Limit	23.40	3.27	NA	NA
	Upper Limit	64.59	15.85	NA	NA

EMS Ethy methanesulfonate

Table 49: Rat liver Comet historical control ranges.

Range generated using QA audited data from eight studies started between November 2009 and September 2010.

Rat stomach comet historical control ranges

		Median %Tail Intensity	Median Tail Moment	Mean % clouds	%Diffused cells
Vehicle ranges		35	35	35	35
Number of animals					
Observed range	Minimum	0.91	0.13	3.50	6.00
	Maximum	7.65	1.04	20.00	24.00
95% Reference range	Lower				
	Limit	1.51	0.13	NA	NA
	Upper				
	Limit	6.05	0.73	NA	NA
Positive control (EMS) ranges		30	30	30	30
Number of animals					
Observed range	Minimum	14.97	1.77	6.00	8.00
	Maximum	46.03	7.62	28.00	26.00
95% Reference range	Lower				
	Limit	16.34	1.94	NA	NA
	Upper				
	Limit	38.04	5.89	NA	NA

EMS Ethy methanesulfonate

Table 50: Rat stomach Comet historical control ranges.

Range generated using QA audited data from four studies started between November 2009 and September 2010.

Micronucleus historical control ranges – Individual animal data

Control Type		Individual PCE %	Individual frequency of micronucleated PCE per 2000 (%)	Animals (%) with 0,1 (or more) micronuclei (for 2000 PCE scored)							
				0 ≥8	1	2	3	4	5	6	7
Vehicle	Mean	44	2.26 (0.11%)	13.4	27.6	21.9	15.2	12.0	5.0	2.1	1.4
	SD	9.8		1.4							
	Median	45									
	Observed range	16 – 72									
	95% confidence interval	34 – 54									
	for group mean of:	35 – 53									
	4 values	36 – 53									
	5 values	36 – 52									
	6 values	37 – 51									
	7 values	37 – 51									
	8 values	38 – 51									
	9 values										
	10 values										
Positive Control	Mean	38	39 ⁽¹⁾ (1.95%)								
	SD	9.5	-								
	Median	39	23 – 59 ⁽¹⁾								
	Observed range	10 – 61	24 – 57 ⁽¹⁾								
	95% confidence interval	28 – 48	25 – 55 ⁽¹⁾								
	for group mean of:	29 – 47	26 – 54 ⁽¹⁾								
	4 values	30 – 46	27 – 53 ⁽¹⁾								
	5 values	30 – 46	27 – 52 ⁽¹⁾								
	6 values	31 – 45	28 – 52 ⁽¹⁾								
	7 values	31 – 45									
	8 values	31 – 44									
	9 values										
	10 values										

⁽¹⁾ Calculated from square root transformed data.

Table 51: Micronucleus historical control ranges—individual animal data.

Calculated in April 2010 by (b) (4) from studies started between January 2009 and December 2009.

Vehicle statistics based on 283 animals from 38 studies; positive control statistics based on 179 animals from 32 studies.

Micronucleus historical control ranges – Group mean animal data

Control type		#Mean PCE %	#Mean number of micronucleated PCE per 2000
Vehicle	#N	43	43
	Mean SD Median	44.17 ⁽¹⁾	2.21 ⁽²⁾
	Observed range of means	-	-
	95% confidence interval for mean	-	-
		32.41 to 56.26 ⁽¹⁾	0.74 to 4.46 ⁽²⁾
Positive Control	#N	34	34
	Mean SD Median	38.08	40.21
	Observed range of means	6.59	10.84
	95% confidence interval for mean	38.20	39.50
		19.87 to 47.03 24.67 to 51.48	19.67 to 65.67 18.16 to 62.25

Separate mean calculated for each study and experiment.

⁽¹⁾ Calculated from arcsine square root transformed data.

⁽²⁾ Calculated from square root transformed data.

Table 52: Micronucleus historical control ranges–group mean animal data.

Calculated in April 2010 by (b) (4) from studies started between January 2009 and December 2009.

Vehicle statistics based on data from 38 studies; positive control statistics based on data from 32 studies.

Historical vehicle control ranges for human peripheral blood lymphocyte.
Chromosome aberration (b) (4) assay:

(b) (4)

Calculated in April 2010 by (b) (4), from audited report data of studies started between April 2008 and July 2009.