

VERON® Hyperbake-ST

Description and Specification

2015.01.14
Rev. Nr. 1

Description

VERON® Hyperbake-ST is a lipolytic enzyme preparation for the treatment of flours. The enzyme is obtained from specific cultures of *Trichoderma reesei*.

- IUB-No.: 3.1.1.3
- CAS-No.: 9001-62-1

Properties

The product has the following characteristics:

- a) solid product
- b) light beige coloured with aromatic smell

Application

VERON® Hyperbake-ST is used for the treatment of flour and for the production of bread improvers. Using VERON® Hyperbake-ST ensures dry and fluffy doughs, which are easily to process due to the improved machine ability. Furthermore you will obtain improved dough and fermentation stability. The baking volume will significantly increase; the crumb will show a whiter appearance.

VERON® Hyperbake-ST can be used to reduce or replace baking emulsifiers, e.g. Datem, which results in similar baking volume, improved shape of the baked goods with similar crumb structure (exchange ratio of 0,8-10ppm for 100g Datem).

In formulations using ingredients containing fats and oils with short fatty acids (e.g. butter), care must be taken that off-flavour does not occur. The influence on taste should be evaluated.

Dosage

0.5 - 3 g / 100 kg flour

The optimum dosage should be determined by means of baking tests.

Specification

The product fulfils the requirements of the FAO/WHO's Joint Expert Committee for Food Additives (JECFA) and Food Chemicals Codex (FCC).

The total viable counts are within the upper limit of $5 \times 10^4 \text{ g}^{-1}$

E.coli: absent in 25g

Composition

Wheat flour, Lipase, Sunflower oil

Storage

Stored in a dry place at room temperature the activity loss will be less than 10 % within one year.

Handling

When handling enzyme products in powder form, direct skin contact and dust formation should be avoided. Enzymes may irritate the skin and eyes; the inhalation of enzyme dust may provoke sensitisation of the respiratory organs.

For further details on the safe handling of our products, please consult our safety data sheet and the technical information sheet "Precautionary measures when handling enzyme products in powder form."

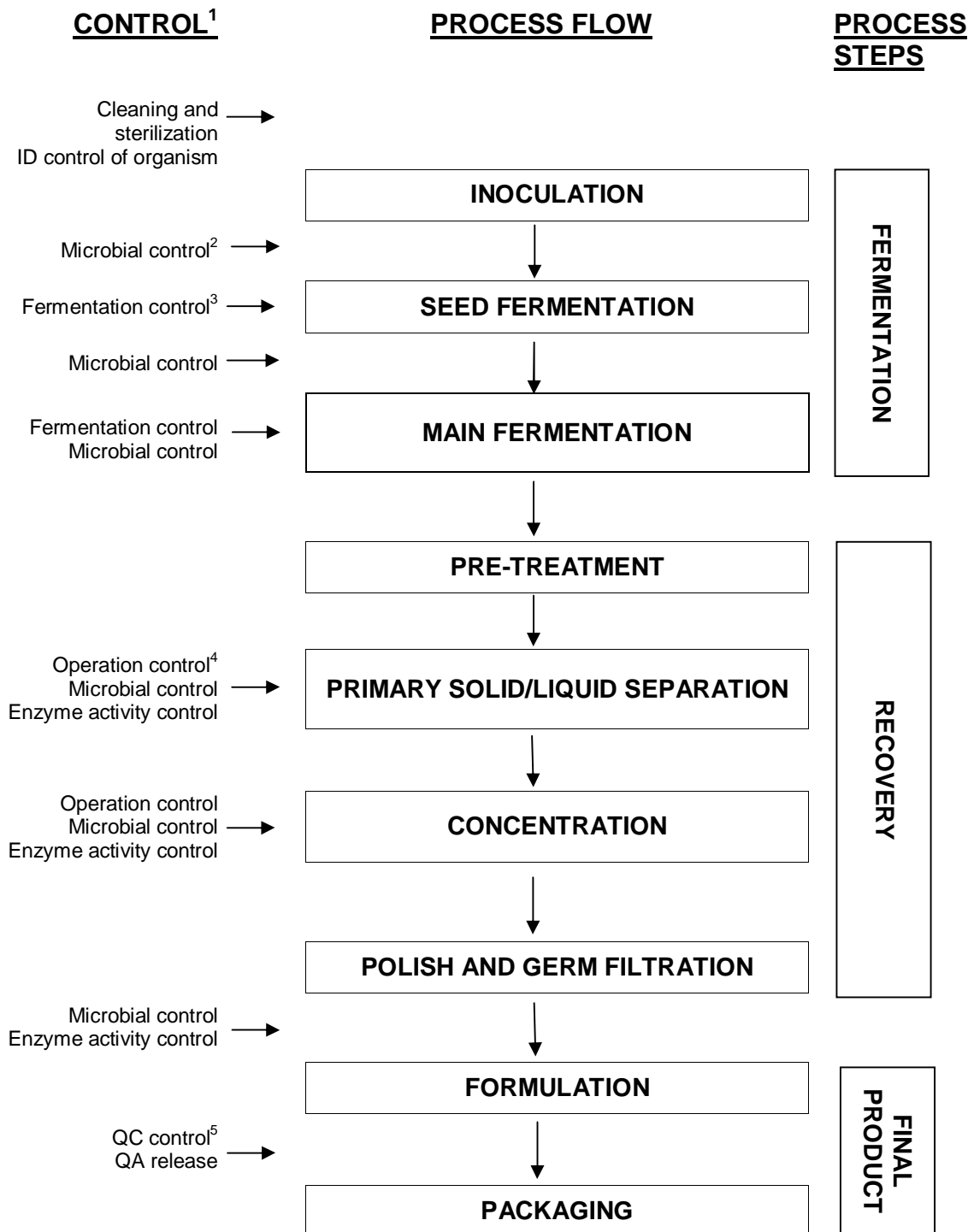
Legal Disclaimer

Due to patent restrictions VERON® Hyperbake-ST may not be used in baking compositions in the following countries or products made therefrom may not be offered or sold, in the following countries: Argentina, Belgium, China, Denmark, France, Germany, Ireland, Italy, Japan, Netherlands, Spain, Switzerland/Liechtenstein, United Kingdom, United States of America before December 9th, 2017.

The product is therefore not commercially available in the above listed countries.

AB Enzymes GmbH is in no way liable for any non-observation of said instructions.

Production Process of Food Enzymes from Fermentation



¹ The controls shown on the flow chart may vary depending on the production set-up. Controls are conducted at various steps throughout the production process as relevant.

² Microbial control: Absence of significant microbial contamination is analyzed by microscope or plate counts

³ During fermentation parameters like e.g. pH, temperature, oxygen, CO₂, sterile air overflow are monitored / controlled.

⁴ Operation control in downstream processes cover monitoring and control of parameters like e.g. pH, temperature

⁵ Final QC control will check that product does live up to specifications like e.g. enzyme activity as well as chemical and microbial specification.

General Directorate for Competition Policy, Consumer Affairs and Fraud Control (DGCCRF)

59 bd Vincent Auriol Tekedoc 233

75703 Paris cedex 13 – FRANCE

File followed by

Department 4B – Quality and Valuation of food

Paris, April 20th, 2017

To:

AB Enzymes

Madam,

Please find attached two opinions from ANSES (Agence Nationale de Sécurité Sanitaire, Alimentation, Environnement, Travail¹) regarding applications for approval of new enzymes as processing aids:

- Endo – 1, 4-beta-glucanase from a genetically modified *Trichoderma reesei* (RF5261) carrying a gene encoding an endo – 1, 4-beta-glucanase from *Trichoderma reesei*, for use in brewing, potable alcohol production, starch processing, and treatment of cereal-grains to be used in manufacturing of baking flour (with the exception of French tradition bread).
- Triacylglycerol lipase from a genetically modified *Trichoderma reesei* (RF10625) carrying a gene encoding for the triacylglycerol lipase from *Fusarium oxysporum*, for use in biscuits, pastries, pastry, breadmaking (with the exception of French tradition bread) and special bread making.

Please note that those 2 opinions will be published online on the Agency website, within the next 2 months. Within this period, you could share your comments on the confidentiality status of any information relating to your industrial know-how. This type of data, if identified as such by the applicant, should be kept confidential. In this case, you should inform us in that regard, in writing and before the publication deadline.

As the Agency did not highlight any safety concerns regarding the use of these enzymes, I hereby inform you that the Annex of the Order of 19 October 2006 on the use of processing aids in the production of foods, will be completed for the intended applications.

In the meantime, pending the publication of the revised Order, the enzyme can already be marketed for the intended purpose.

Please accept, Madam, the expression of my highest consideration.

Signature –

¹ French National Agency for Food, Environmental and Occupational Health and Safety



Ms. Candice Cryne
Regulatory Affairs Specialist (The Americas)
Ontario Canada M6J3L9

Re: GRAS Notice No. GRN 000631

Dear Ms. Candice Cryne:

The Food and Drug Administration (FDA) is responding to the notice, dated February 1, 2016, that you submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on February 12, 2016, filed it on March 10, 2016, and designated it as GRAS Notice No. GRN 000631.

The subject of the notice is triacylglycerol lipase enzyme preparation produced by a genetically modified strain of *Trichoderma reesei* (triacylglycerol lipase enzyme preparation). The notice informs FDA of the view of AB Enzymes that triacylglycerol lipase enzyme preparation is GRAS, through scientific procedures, for use as an enzyme in the manufacture of baked goods, pasta, noodles, and cereal-based snack foods, at a maximum use level of 10 milligrams Total Organic Solids per kilogram (mg TOS/kg) of raw material.

Commercial enzyme preparations that are used in food processing typically contain an enzyme component that catalyzes the chemical reaction as well as substances used as stabilizers, preservatives, or diluents. Enzyme preparations may also contain components derived from the production organism and components derived from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. AB Enzymes' notice provides information about each of these components in the triacylglycerol lipase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, triacylglycerol lipase is identified by the Enzyme Commission Number 3.1.1.3. The accepted name for the enzyme is triacylglycerol lipase; and the systematic name is triacylglycerol hydrolase. This enzyme is also known as lipase; triglyceride lipase; tributyrinase; butyrylase; glycerol ester hydrolase; tributyrinase; Tween hydrolase; steapsin; triacetinase; tributyrin esterase; Tweenase; amno N-AP; Takedo 1969-4-9; Meito MY 30; Tweenesterase; GA 56; capalase L; triglyceride hydrolase; triolein hydrolase; tween-hydrolyzing esterase; amano CE; cacordase; triglyceridase; triacylglycerol ester hydrolase; amano P; amano AP; PPL; glycerol-ester hydrolase; GEH; meito Sangyo OF lipase; hepatic lipase; lipazin; post-heparin plasma protamine-resistant lipase; salt-resistant post-heparin lipase; heparin releasable hepatic lipase; amano CES; amano B; tributyrinase; triglyceride lipase; liver lipase; hepatic monoacylglycerol acyltransferase. The CAS Registry Number for triacylglycerol lipase is 9001-62-1. Triacylglycerol lipase catalyzes the hydrolysis of triacylglycerol to form diacylglycerol and free carboxylate.

AB Enzymes states that the triacylglycerol lipase gene from *Fusarium oxysporum* (Genbank CAB9359) was synthesized (Eurofins, Germany) using the *T. reesei* compatible *Hypocrea jecorina* optimized codon set. The recipient strain used in the construction of the production strain, *T. reesei* RF10625¹, is a genetically modified derivative of *T. reesei* RF4847² with a high capacity for triacylglycerol lipase production. *Trichoderma reesei* RF4847 has been taxonomically identified by the Dutch Culture Collection, CBS; an independent and internationally recognized laboratory. *Trichoderma reesei* RF4847 is constructed by transforming *T. reesei* RF7720 with a purified DNA fragment from a plasmid carrying the synthesized triacylglycerol lipase gene. AB Enzymes states that *T. reesei* is a nonpathogenic, nontoxigenic microbe, and that it has a long history of safe use for the production of enzymes used in food. AB Enzymes also states that the transformed DNA is stably integrated, and does not contain any antibiotic resistance genes. AB confirms that *T. reesei* RF10625 is genetically stable after ten generations.

AB Enzymes states that the triacylglycerol lipase enzyme preparation is produced by a controlled fed-batch submerged fermentation of a selected pure culture of the production strain. The manufacture of triacylglycerol lipase enzyme preparation includes fermentation, processing, and formulation of the final product. Appropriate measures are set in place to control identity, purity, and enzyme-generating ability of the production strain during and after fermentation. During fermentation the enzyme is secreted into the medium, and is separated by centrifugation or filtration, concentrated, and filtered at defined pH and temperature ranges. The enzyme concentrate is formulated as a liquid preparation with sunflower oil and wheat flour. According to AB Enzymes, the raw materials used in the fermentation, recovery, and formulation processes are food grade. The entire process is performed in accordance with Good Manufacturing Practice. AB Enzymes also states that the final triacylglycerol lipase enzyme preparation contains no major food allergens from the fermentation medium.

AB Enzymes states that the triacylglycerol lipase enzyme preparation is tested to ensure compliance with established specifications prior to release, including tests for the absence of the production organism in the final enzyme product. AB Enzymes notes that the triacylglycerol lipase enzyme preparation conforms to the specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 9th edition, 2014; or FCC, 10th edition, 2016), and to the current General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006). AB Enzymes provides data from two non-consecutive triacylglycerol lipase enzyme concentrate batches, to demonstrate that the manufacturing process conforms to set specifications.

AB Enzymes proposes to use triacylglycerol lipase enzyme preparation for baking applications including breads, biscuits, tortillas, cakes, steamed bread and croissants, and cereal-based processing, including pastas, noodles and snacks, at 10 mg TOS/kg of raw material. AB Enzymes states that no enzyme activity is present in the final food. However, in order to estimate dietary exposure to triacylglycerol lipase enzyme preparation, AB Enzymes assumes that the enzyme TOS will remain in the final food. Based on this assumption, AB Enzymes estimates the maximum daily intake of triacylglycerol lipase enzyme TOS from all intended food applications to be 0.09 mg TOS/kg body weight per day (mg TOS/kg bw/d) for a 60 kg adult. AB Enzymes states that triacylglycerol lipase enzyme activity will not produce reaction products that are not already part of the human diet.

AB Enzymes summarizes corroborative toxicological studies, using the triacylglycerol lipase enzyme concentrate, to support the safety of the triacylglycerol lipase enzyme preparation. Tests conducted using bacterial cells showed that triacylglycerol lipase enzyme is not mutagenic. AB Enzymes also demonstrates that the enzyme is not clastogenic to cultured human lymphocytes under the conditions

¹ RF10625 has been deposited at Centraalbureau voor Schimmelcultures (CBS) as CBS 134213.

² RF4847 is a classical mutant derived from the well-characterized strain, *T. reesei* QM6a and has been deposited as CBS 114041.

employed in the study. The results of a 90-day oral toxicity study conducted using rats showed that consumption of triacylglycerol lipase enzyme concentrate did not cause any treatment-related adverse effects at 1000 mg/kg bw/d, the highest dose tested, which corresponds to 1000 mg TOS/kg bw/d of the triacylglycerol lipase enzyme. Based on the highest dose tested in the 90-day study, and the estimated maximum daily intake from the proposed use levels of triacylglycerol lipase enzyme preparation, i. e., 1000 mg TOS/kg bw/d and 0.09 mg TOS/kg bw/d, respectively, AB Enzymes calculates the margin of safety to be approximately 11,000.

AB Enzymes discusses potential food allergenicity of triacylglycerol lipase enzyme. AB Enzymes conducted an amino acid sequence homology search for triacylglycerol lipase enzyme against known allergens using the publicly available Food Allergy Research and Resource Program (FARRP) database, and the Allergen Database for Food Safety (ADFS). Amino acid identity matches greater than 35% over 80 amino acids were not found; no matches of contiguous stretches of eight amino acids shared between the triacylglycerol lipase enzyme amino acid sequence and known allergens were found. Based on the results obtained from the alignments and homology search results, AB Enzymes concludes that the triacylglycerol lipase enzyme does not show significant homology to any known allergen, and that the risk of allergic responses from oral consumption of triacylglycerol lipase enzyme is low.

Based on the data and information summarized above, AB Enzymes concludes that triacylglycerol lipase enzyme preparation is GRAS for its intended use.

Allergen Labeling

The Federal Food, Drug, and Cosmetic Act (FD&C Act) requires that the label of a food that is or contains an ingredient that bears or contains a “major food allergen” declare the presence of the allergen (section 403(w)). The FD&C Act defines a “major food allergen” as one of eight foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) or a food ingredient that contains protein derived from one of those foods. Triacylglycerol lipase enzyme preparation produced by a genetically modified strain of *T. reesei* may require labeling under the FD&C Act, because it may contain protein derived from wheat. Questions about the submission of petitions or notifications for exemptions from food allergen labeling requirements should be directed to the Division of Biotechnology and GRAS Notice Review in the Office of Food Additive Safety. However, questions about food labeling in general should be directed to the Office of Nutrition, Labeling, and Dietary Supplements.

Standards of Identity

In the notice, AB Enzymes states its intention to use triacylglycerol lipase enzyme preparation in food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Section 301(l) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

Section 301(l) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(l)(1)-(4) applies. In its review of AB Enzymes’s notice that triacylglycerol lipase enzyme preparation produced by a genetically modified strain of *T. reesei* is GRAS

for the intended uses, FDA did not consider whether section 301(l) or any of its exemptions apply to foods containing triacylglycerol lipase enzyme preparation. Accordingly, this response should not be construed to be a statement that foods that contain triacylglycerol lipase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(l).

Conclusions

Based on the information provided by AB Enzymes, as well as other information available to FDA, the agency has no questions at this time regarding AB Enzymes' conclusion that triacylglycerol lipase enzyme preparation produced a genetically modified strain of *T. reesei* is GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of triacylglycerol lipase enzyme preparation produced a genetically modified strain of *T. reesei*. As always, it is the continuing responsibility of AB Enzymes to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter responding to GRN 00 0631, as well as a copy of the information in this notice that conforms to the information in the GRAS exemption claim (proposed 21 CFR 170.36(c)(1)), is available for public review and copying at www.fda.gov/grasnoticeinventory.

Sincerely,

Dennis M. Keefe -S

Dennis M. Keefe, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

Digitally signed by Dennis M. Keefe -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA,
ou=People, 0.9.2342.19200300.100.1.1=1300072773,
cn=Dennis M. Keefe -S
Date: 2016.07.18 11 30:02 -04'00'

ANALYSIS OF SAFETY BASED ON PARIZA/JOHNSON DECISION TREE

Pariza and Johnson have published updated guidelines for the safety assessment of microbial enzyme preparations (2001)¹ from the 1991 IFBC Decision Tree². The safety assessment of a given enzyme preparation is based upon an evaluation of the toxigenic potential of the production organism. The responses below follow the pathway indicated in the decision tree as outlined in Pariza and Johnson, 2001. The outcome of this inquiry is that triacylglycerol lipase enzyme preparation from *Trichoderma reesei* (*T.reesei*) strain RF10625 expressing the gene encoding triacylglycerol from *Fusarium oxysporum* is "ACCEPTED" as safe for its intended use.

Decision Tree:

1. **Is the production strain genetically modified?** *Trichoderma reesei* strain RF10625 was genetically modified to express triacylglycerol from *Fusarium oxysporum*.
Yes go to #2;
2. **Is the production strain modified using rDNA techniques?** Yes go to #3a;
3.
 - 3a. **Does the expressed enzyme product which is encoded by the introduced DNA have a history of safe use in food?** Yes, Go to 3c;
 - 3c. **Is the test article free of transferable antibiotic resistance gene DNA?** Yes, transferable DNA was not detected in the lipase enzyme preparation manufactured using *T. reesei* and production process described herein. Additionally, no antibiotic resistance gene has been integrated. Go to 3e;
 - 3e. **Is all other introduced DNA well characterized and free of attributes that would render it unsafe for constructing microorganisms to be used to produce food-grade products?** Yes, inserted DNA is well characterized. Go to 4;
4. **Is the introduced DNA randomly integrated into the chromosome?** Yes, go to #5;
5. **Is the production strain sufficiently well characterized so that one may reasonably conclude that unintended pleiotropic effects which may result in the synthesis of**

¹ Pariza M.W. and Johnson E.A. Reg. Toxicol. Pharmacol. Vol. **33** (2001) 173-186

² IFBC (International Food Biotechnology Committee), Chapter 4: Safety Evaluation of Foods and Food Ingredients Derived from Microorganisms in Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification, Regulatory Toxicology and Pharmacology. Vol. **12**:S1-S196 (1990).

toxins or other unsafe metabolites will not arise due to the genetic modification method that was employed? Yes, there is no concern for pleiotropic effects. Go to #6;

- 6. Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via this evaluation procedure?** Yes, *T. reesei* has been demonstrated as a safe production host and methods of modification have been well documented. Safety of this organism has been evaluated and confirmed through toxicological testing as described herein. **ACCEPTED**

REPORT



Lipase produced with *Trichoderma reesei*: *Salmonella typhimurium* reverse mutation assay

Study Director:

Test Facility:

Harlan
Cytotest Cell Research GmbH (Harlan CCR)
In den Leppsteinswiesen 19
64380 Rossdorf/Germany

Sponsor:

AB Enzymes GmbH
Feldbergstr. 78
64293 Darmstadt
Germany

Study Monitor:

Harlan Study Number:

1544902

Study Completion Date:

15 August 2013
Final

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STUDY DIRECTOR STATEMENT OF GLP COMPLIANCE

Harlan Cytotest Cell Research GmbH (Harlan CCR)
In den Leppsteinswiesen 19
64380 Rossdorf
Germany

Harlan Study Number: **1544902**
Study Title: Lipase produced with *Trichoderma reesei*:
Salmonella typhimurium reverse mutation assay

This study was performed in compliance with “Chemikaliengesetz” (Chemicals Act) of the Federal Republic of Germany, “Anhang 1” (Annex 1); in its currently valid version. These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI).

This report fully and accurately reflects the procedures used and data generated. There were no circumstances considered to have affected the integrity of the study or the validity of the data.

Date: 15 August 2013

QUALITY ASSURANCE STATEMENT

Harlan Study Number: **1544902**

Study Title: Lipase produced with *Trichoderma reesei*:
Salmonella typhimurium reverse mutation assay

The general facilities and activities are inspected at least once a year and the results are reported to the relevant responsible person and management.

Study-related procedures conducted at the test facility were audited and inspected. The details of these audits and inspections are given below.

Dates and Types of QA Inspections			Reported to the relevant Study Director and Test Facility Management
Date of Inspection	Type of Inspection	Phase Inspected	Report Date
12 April 2013	Study Plan Verification	N/A	12 April 2013
21 May 2013	Process – based	Test system preparation and application	21 May 2013
07 August 2013	Report Audit	N/A	07 August 2013

This statement confirms that this report reflects the raw data and the procedures followed.

Quality Assurance:

Date: 15 August 2013

SUMMARY

This study was performed to investigate the potential of Lipase produced with *Trichoderma reesei* to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and TA 102.

The assay was performed in two independent experiments both with and without liver microsomal activation. To verify a minor increase in strain TA 98 without S9 mix, confirmatory experiments IIa and IIb were performed with strain TA 98 without S9 mix as pre-incubation assay. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment IIa and IIb
without filtration: 2500; 5000; 7500; and 10000 µg/plate

Experiment IIb with filtration: 2500 and 5000 µg/plate

No precipitation of the test item occurred up to the highest investigated dose.

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation. Only strain TA 102 showed a minor reduction in the number of revertants in experiment II with S9 mix at 5000 µg/plate.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Lipase produced with *Trichoderma reesei* at any dose level, neither in the presence nor absence of metabolic activation (S9 mix).

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

Conclusion

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, Lipase produced with *Trichoderma reesei* is considered to be non-mutagenic in this *Salmonella typhimurium* reverse mutation assay.

GENERAL INFORMATION

Schedule

Experimental Starting Date: 30 April 2013

Experimental Completion Date: 04 July 2013

Additional Responsibilities

Harlan Cytotest Cell Research GmbH (Harlan CCR)

Deviations from Study Plan

Deviation

The maximum dose level was 10000 µg/plate in strain TA 98 in the pre-incubation assay without S9 mix (Experiment IIa and IIb) to verify the results.

This deviation to the study plan, however, does not affect the validity of the study.

Archiving

Unless instructed otherwise by the Sponsor, the study plan, all raw data, specimens (if any) and the final report will be retained in the Harlan Cytotest Cell Research GmbH archive for at least 3 years. Thereafter, the material will be transferred to the GLP archive of Harlan Laboratories Ltd. in Füllinsdorf, Switzerland, for further archiving up to a total archiving period of 15 years.

No data will be discarded without contacting the Sponsor to obtain their written consent.

A sample of the test item will be archived two years after the expiration date provided by the sponsor. If no expiration date is given, the archiving period will be the required 15 years. Thereafter the samples will be discarded without further notice.

1 INTRODUCTION AND PURPOSE

The experiments were performed to assess the potential of the test item to induce gene mutations by means of two independent *Salmonella typhimurium* reverse mutation assays. Experiment I was performed as a plate incorporation assay. Since a negative result was obtained in this experiment, experiment II was performed as a pre-incubation assay.

The most widely used assays for detecting gene mutations are those using bacteria. They are relatively simple and rapid to perform, and give reliable data on the ability of an agent to interact with DNA and produce mutations.

Reverse mutation assays determine the frequency at which an agent abolishes or suppresses the effect of the forward mutation. The genetic target presented to an agent is therefore small, specific and selective. Several bacterial strains, or a single strain with multiple markers are necessary to overcome the effects of mutagen specificity. The reversion of bacteria from growth-dependence on a particular amino acid to grow in the absence of that amino acid (reversion from auxotrophy to prototrophy) is the most widely used marker.

The *Salmonella typhimurium* histidine (his) reversion system measures his⁻ → his⁺ reversions. The *S. typhimurium* strains are constructed to differentiate between base pair (TA 1535, TA 100, TA 102) and frameshift (TA 1537, TA 98) mutations.

According to the direct plate incorporation or the pre-incubation method the bacteria are exposed to the test item with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a dose response effect several dose levels with adequately spaced concentrations were tested. The maximum dose level was 5000 µg/plate with the exception for strain TA 98 without mix in experiment IIa and IIb. The maximum dose level was 10000 µg/plate to verify the results.

To validate the test, reference mutagens were tested in parallel to the test item.

1.1 Guidelines / Regulations

This study was designed to be compatible with the procedures indicated by the following internationally accepted guidelines and recommendations:

"Ninth Addendum to OECD Guidelines for Testing of Chemicals", Section 4, No. 471:
"Bacterial Reverse Mutation Test", adopted July 21, 1997

"Commission Regulation (EC) No. 440/2008 B13/14", dated May 30, 2008

2 TEST AND REFERENCE ITEM

2.1 Test Item

Information as provided by the Sponsor.

Identification:	Lipase produced with <i>Trichoderma reesei</i>
Batch:	LP 12136B3; RF 10625
Purity:	TOS value: 94,38 (=Total Organic Substance)
Expiry Date:	November 2014
Storage Conditions: (provided by the Sponsor)	At room temperature, moisture protected
Stability in Solvent:	1 day in water at room temperature

The test item concentrations was administered on TOS level = Proteine, peptides, carbohydrates, and fat

3 MATERIALS AND METHODS

3.1 Test System

3.1.1 Characterisation of the *Salmonella typhimurium* Strains

The histidine dependent strains are derived from *S. typhimurium* strain LT2 through mutations in the histidine locus. Additionally due to the "deep rough" (*rfa*⁻) mutation they possess a faulty lipopolysaccharide envelope, which enables substances to penetrate the cell wall more easily. A further mutation (deletion of the *uvrB* gene) causes an inactivation of the excision repair system. The latter alteration also includes a deletion in the nitrate reductase and biotin genes. In the strains TA 98, TA 100, and TA 102 the R-factor plasmid pKM 101 carries *umu* DC analogous genes that are involved in error-prone repair and the ampicillin resistance marker. The strain TA 102 does not contain the *uvrB*⁻-mutation. Additionally TA 102 contains the multicopy plasmid pAQ1, which carries the *hisG428* mutation and a tetracycline resistance gene. TA 102 contains the ochre mutation in the *hisG* gene.

When summarized, the mutations of the bacterial strains used in this study can be described as follows:

<i>Salmonella typhimurium</i>		
Strains	Genotype	Type of mutations indicated
TA 1537	<i>his C 3076; rfa</i> ⁻ ; <i>uvrB</i> ⁻	frame shift mutations
TA 98	<i>his D 3052; rfa</i> ⁻ ; <i>uvrB</i> ⁻ ; R-factor	" "
TA 1535	<i>his G 46; rfa</i> ⁻ ; <i>uvrB</i> ⁻	base-pair substitutions
TA 100	<i>his G 46; rfa</i> ⁻ ; <i>uvrB</i> ⁻ ; R-factor	" "
TA 102	<i>his G 428; rfa</i> ⁻ ; <i>uvrB</i> ⁺ ; R-factor	" "

Regular checking of the properties of the *Salmonella typhimurium* strains regarding the membrane permeability, ampicillin resistance; UV sensitivity, and amino acid requirement as well as normal spontaneous mutation rates is performed in Harlan CCR according to B. Ames et al. and D. Maron and B. Ames. In this way it is ensured that the experimental conditions set down by Ames are fulfilled.

The bacterial strains TA 1535, TA 1537, TA 98, TA 100, and TA 102 were obtained from Trinova Biochem GmbH (35394 Gießen, Germany).

3.1.2 Storage

The strain cultures are stored as stock cultures in ampoules with nutrient broth + 5 % DMSO in liquid nitrogen.

3.1.3 Precultures

From the thawed ampoules of the strains 0.5 mL bacterial suspension was transferred into 250 mL Erlenmeyer flasks containing 20 mL nutrient medium. A solution of 20 µL ampicillin

(25 µg/mL) was added to the strains TA 98, TA 100, and TA 102. This nutrient medium contains per litre:

8 g Nutrient Broth
5 g NaCl

The bacterial cultures were incubated in a shaking water bath for 4 hours at 37° C. The optical density of the bacteria was determined by absorption measurement and the obtained values indicated that the bacteria were harvested at the late exponential or early stationary phase (10^8 - 10^9 cells/mL).

3.1.4 Selective Agar

The plates with the selective agar were obtained from E. Merck.

3.1.5 Overlay Agar

The overlay agar contains per litre:

7.0 g Agar Agar
6.0 g NaCl
10.5 mg L-Histidine x HCl x H₂O
12.2 mg Biotin

Sterilisations were performed at 121 °C in an autoclave.

3.2 Test Item Preparation

On the day of the experiment, the test item Lipase produced with *Trichoderma reesei* was suspended in deionised water. The solvent was chosen because of its solubility properties and its relative nontoxicity to the bacteria.

3.3 Controls

3.3.1 Negative Controls

Concurrent untreated and solvent controls were performed.

3.3.2 Positive Control Substances

Without metabolic activation

Strains: TA 1535, TA 100
Name: sodium azide, NaN_3
Purity: at least 99 %
Dissolved in: deionised water
Concentration: 10 µg/plate

Strains: TA 1537, TA 98
Name: 4-nitro-o-phenylene-diamine, 4-NOPD
Purity: > 99.9 %
Dissolved in: DMSO (purity >99 %)
Concentration: 10 µg/plate in strain TA 98, 50 µg/plate in strain TA 1537

Strain: TA 102
Name: methyl methane sulfonate, MMS
Purity: > 99.0 %
Dissolved in: deionised water
Concentration: 2.0 µL/plate

With metabolic activation

Strains: TA 1535, TA 1537, TA 98, TA 100, TA 102
Name: 2-aminoanthracene, 2-AA
Purity: 97.5 %
Dissolved in: DMSO (purity >99 %)
Concentration: 2.5 µg/plate (10.0 µg/plate in TA 102)

The stability of the positive control substances in solution is unknown but a mutagenic response in the expected range are sufficient evidence of biological stability.

3.4 Mammalian Microsomal Fraction S9 Mix

Due to the limited capacity for metabolic activation of potential mutagens in *in vitro* methods an exogenous metabolic activation system is necessary.

Phenobarbital/ β -naphthoflavone induced rat liver S9 were used as the metabolic activation system. The S9 was prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 was routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test.

The protein concentration of the S9 preparation was 44.9 mg/mL (Lot. No.: 150213) in both experiments.

3.4.1 S9 Mix

An appropriate quantity of S9 supernatant is thawed and mixed with S9 cofactor solution, to result in a final concentration of approx. 10 % v/v in the S9 mix. Cofactors are added to the S9 mix to reach the following concentrations in the S9 mix:

8 mM	MgCl ₂
33 mM	KCl
5 mM	glucose-6-phosphate
4 mM	NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment, the S9 mix is stored in an ice bath. The S9 mix preparation is performed according to Ames et al.

3.5 Pre-Experiment for Toxicity

To evaluate the toxicity of the test item a pre-experiment was performed with all strains used. Eight concentrations were tested for toxicity and mutation induction with each 3 plates. The experimental conditions in this pre-experiment were the same as described for the experiment I below (plate incorporation test).

Toxicity of the test item can be evident as a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.

The pre-experiment is reported as main experiment I, since the following criteria are met:

Evaluable plates (>0 colonies) at five concentrations or more in all strains used.

3.6 Dose Selection

In the pre-experiment the concentration range of the test item was 3 – 5000 µg/plate. The pre-experiment is reported as experiment I. Since no relevant toxic effects were observed 5000 µg/plate were chosen as maximal concentration.

The concentration range included two logarithmic decades. The following concentrations were tested in experiment II:

33; 100; 333; 1000; 2500; and 5000 µg/plate

To verify a the small increase in strain TA 98 without S9 mix confirmatory experiments IIa and IIb were performed with the following concentrations.

Experiment IIa and IIb

without filtration: 2500; 5000; 7500; and 10000 µg/plate

Experiment IIb with filtration: 2500 and 5000µg/plate

3.7 Experimental Performance

For each strain and dose level, including the controls, three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates:

Experiment I (Plate Incorporation)

100 µL Test solution at each dose level (solvent or reference mutagen solution (positive control)),

500 µL S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),

100 µL Bacteria suspension (cf. test system, pre-culture of the strains),

2000 µL Overlay agar

Experiment II (Pre-Incubation)

In the pre-incubation assay 100 µL test solution (solvent or reference mutagen solution (positive control)), 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and incubated at 37 °C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on minimal agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37 °C in the dark.

3.8 Data Recording

The colonies were counted using the Petri Viewer Mk2 (Perceptive Instruments Ltd, Suffolk CB9 7BN, UK) with the software program Ames Study Manager (v.1.21). The counter was connected to a PC with printer to print out the individual values and mean values of the plates for each concentration together with standard deviations and enhancement factors as compared to the spontaneous reversion rates (see tables of results). Due to contamination and wide spread bacteria colony growth the colonies were partly counted manually.

3.9 Acceptability of the Assay

The *Salmonella typhimurium* reverse mutation assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of our historical data
- the positive control substances should produce a significant increase in mutant colony frequencies
- a minimum of five analysable dose levels should be present with at least three dose levels showing no signs of toxic effects, evident as a reduction in the number of revertants below the indication factor of 0.5.

3.10 Evaluation of Results

A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and TA 102) or thrice (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

3.11 Biometry

According to the OECD guideline 471, a statistical analysis of the data is not mandatory.

4 RESULTS AND DISCUSSION

The test item Lipase produced with *Trichoderma reesei* was assessed for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and TA 102.

The assay was performed in two independent experiments both with and without liver microsomal activation. To verify a minor increase in strain TA 98 without S9 mix, confirmatory experiments IIa and IIb were performed with strain TA 98 without S9 mix as pre-incubation assay. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment IIa and II b
without filtration: 2500; 5000; 7500; and 10000 µg/plate

Experiment IIb with filtration: 2500 and 5000µg/plate

No precipitation of the test item occurred up to the highest investigated dose.

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation. Only strain TA 102 showed a minor reduction in the number of revertants in experiment II with S9 mix at 5000 µg/plate.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Lipase produced with *Trichoderma reesei* at any concentration level, neither in the presence nor absence of metabolic activation (S9 mix). A minor increase in the number of revertant colonies was observed in experiment I in strain TA 98 in the absence of metabolic activation. The threshold of two was not reached and the mean value of the revertant colonies was in the range of the historical control data of the solvent control. In experiment II, a minor increase was also observed in strain TA 98 in the absence of metabolic activation. The threshold of two was just not reached and the mean value of the revertant colonies was just in the upper limit of the historical solvent control range. To verify these repeated minor increases in strain TA 98 a confirmatory experiment (reported as experiment IIa) was performed as a pre-incubation assay up to a concentration of 10000 µg/plate. In experiment IIa a test item related intense microbial contamination was observed in nearly all concentrations. Since toxins of the microbial contamination can affect the results of this assay, this experiment was repeated with and without sterile filtrated test item solution (reported as experiment IIb). Based on the limited solubility at these high concentrations the concentrations of 7500 and 10000 µg/plate could not

be filtrated. No increase in the number of revertant colonies was observed in the assay with sterile test item solution, therefore the test item is judged as non mutagenic in this assay.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase in induced revertant colonies.

5 CONCLUSION

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

6 REFERENCES

- Ames, B.N., J. McCann, and E. Yamasaki (1977)
Methods for detecting carcinogens and mutagens with the Salmonella/mammalian
microsome mutagenicity test
In: B.J. Kilbey et al. (Eds.) "Handbook of Mutagenicity Test Procedures" Elsevier,
Amsterdam, 1-17
- de Serres F.J. and M.D. Shelby (1979)
Recommendations on data production and analysis using the Salmonella/microsome
mutagenicity assay
Mutation Res. 64, 159-165
- Hollstein, M., J. McCann, F.A. Angelosanto and W.W. Nichols (1979)
Short-term tests for carcinogens and mutagens
Mutation Res. 65, 133-226
- Maron D.M., J. Katzenellenbogen and B.N. Ames (1981)
Compatibility of organic solvents with the Salmonella/Microsome Test
Mutation Res. 88, 343-350
- Maron D.M., Ames, B.N. (1983)
Revised methods for the Salmonella mutagenicity test
Mutation Res. 113, 173-215

Study Name: 1544902			Study Code: Harlan CCR 1544902				
Experiment: 1544902 VV Plate			Date Plated: 30/04/2013				
Assay Conditions:			Date Counted: 07/05/2013				
Metabolic <u>Activation</u>	Test <u>Group</u>	Dose Level <u>(per plate)</u>	Revertant Colony Counts (Mean ±SD)				
			<u>TA 1535</u>	<u>TA 1537</u>	<u>TA 98</u>	<u>TA 100</u>	<u>TA 102</u>
Without Activation	Deionised water		16 ± 1	9 ± 3	23 ± 3	98 ± 9	336 ± 34
	Untreated		12 ± 1	7 ± 1	29 ± 7	91 ± 1	315 ± 5
	Lipase produced with Trichoderma reesei	3 µg	13 ± 4	9 ± 1	22 ± 1	96 ± 13	295 ± 3
		10 µg	20 ± 1	11 ± 3	24 ± 0	91 ± 6	313 ± 13
		33 µg	18 ± 3	8 ± 2	27 ± 4	95 ± 7	355 ± 12
		100 µg	16 ± 1	10 ± 2	25 ± 7	108 ± 4	359 ± 29
		333 µg	18 ± 4	13 ± 1	25 ± 3	93 ± 5	303 ± 5
		1000 µg	19 ± 2	9 ± 1	24 ± 4	100 ± 8	374 ± 19
		2500 µg	19 ± 3	12 ± 5	30 ± 1	120 ± 11	322 ± 6
	5000 µg	16 ± 1	13 ± 1	42 ± 4	148 ± 15	201 ± 4	
	NaN3	10 µg	1985 ± 100			2115 ± 76	
	4-NOPD	10 µg			266 ± 27		
	4-NOPD	50 µg		75 ± 13			
MMS	2.0 µL					3631 ± 419	
With Activation	Deionised water		15 ± 2	18 ± 2	42 ± 2	109 ± 2	372 ± 41
	Untreated		14 ± 4	16 ± 2	49 ± 5	118 ± 18	395 ± 79
	Lipase produced with Trichoderma reesei	3 µg	14 ± 6	14 ± 2	31 ± 6	82 ± 2	350 ± 15
		10 µg	11 ± 3	15 ± 4	33 ± 6	79 ± 7	382 ± 33
		33 µg	14 ± 3	15 ± 3	36 ± 2	84 ± 1	355 ± 28
		100 µg	15 ± 5	19 ± 1	32 ± 4	77 ± 3	370 ± 53
		333 µg	13 ± 2	22 ± 3	37 ± 3	81 ± 10	292 ± 15
		1000 µg	19 ± 3	20 ± 3	36 ± 6	85 ± 5	276 ± 41
		2500 µg	14 ± 4	19 ± 6	40 ± 4	97 ± 10	222 ± 25
	5000 µg	17 ± 3	17 ± 7	36 ± 9	125 ± 24	195 ± 23	
	2-AA	2.5 µg	437 ± 17	309 ± 50	2031 ± 324	2325 ± 282	
	2-AA	10.0 µg					2093 ± 212
	Key to Positive Controls						
NaN3	sodium azide						
2-AA	2-aminoanthracene						
MMS	methyl methane sulfonate						
4-NOPD	4-nitro-o-phenylene-diamine						

Table 2 Summary of Experiment II

Study Name: 1544902

Experiment: 1544902 HV2 Pre

Assay Conditions:

Study Code: Harlan CCR
1544902

Date Plated: 23/05/2013

Date Counted: 28/05/2013

<u>Metabolic Activation</u>	<u>Test Group</u>	<u>Dose Level (per plate)</u>	<u>Revertant Colony Counts (Mean \pmSD)</u>				
			<u>TA 1535</u>	<u>TA 1537</u>	<u>TA 98</u>	<u>TA 100</u>	<u>TA 102</u>
Without Activation	Deionised water		13 \pm 1	10 \pm 2	28 \pm 6	97 \pm 10	327 \pm 1
	Untreated		14 \pm 2	10 \pm 4	23 \pm 4	95 \pm 9	323 \pm 12
	Lipase produced	33 μ g	12 \pm 4	9 \pm 3	27 \pm 8	105 \pm 14	353 \pm 19
	with	100 μ g	14 \pm 5	10 \pm 2	31 \pm 5	112 \pm 5	361 \pm 14
	Trichoderma	333 μ g	17 \pm 6	11 \pm 1	29 \pm 6	114 \pm 4	367 \pm 13
	reesei	1000 μ g	17 \pm 3	12 \pm 2	31 \pm 10	115 \pm 7	359 \pm 3
		2500 μ g	22 \pm 5	12 \pm 0	40 \pm 3	149 \pm 11	311 \pm 22
		5000 μ g	27 \pm 1	15 \pm 2	54 \pm 4	167 \pm 8	205 \pm 2
	NaN3	10 μ g	1706 \pm 66			1870 \pm 57	
	4-NOPD	10 μ g			336 \pm 10		
	4-NOPD	50 μ g		69 \pm 13			
	MMS	2.0 μ L					3213 \pm 137
With Activation	Deionised water		20 \pm 7	20 \pm 1	44 \pm 7	157 \pm 4	496 \pm 18
	Untreated		15 \pm 2	24 \pm 5	53 \pm 10	145 \pm 7	488 \pm 35
	Lipase produced	33 μ g	20 \pm 9	18 \pm 3	39 \pm 8	103 \pm 20	448 \pm 72
	with	100 μ g	18 \pm 8	20 \pm 8	35 \pm 11	106 \pm 11	445 \pm 33
	Trichoderma	333 μ g	19 \pm 7	31 \pm 4	47 \pm 12	97 \pm 12	386 \pm 21
	reesei	1000 μ g	17 \pm 5	32 \pm 3	61 \pm 6	125 \pm 12	361 \pm 16
		2500 μ g	26 \pm 2	34 \pm 8	60 \pm 11	131 \pm 17	302 \pm 18
		5000 μ g	31 \pm 7	36 \pm 8	66 \pm 5	149 \pm 13	186 \pm 30
	2-AA	2.5 μ g	344 \pm 18	271 \pm 13	2071 \pm 148	2808 \pm 152	
	2-AA	10.0 μ g					2651 \pm 228

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
MMS	methyl methane sulfonate
4-NOPD	4-nitro-o-phenylene-diamine

Table 3 Summary of Experiment IIa

Study Name: 1544902

Experiment: 1544901 HV2a Pre

Assay Conditions:

Study Code: Harlan CCR
1544902

Date Plated: 19/06/2013

Date Counted: 25/06/2013

<u>Metabolic Activation</u>	<u>Test Group</u>	<u>Dose Level (per plate)</u>	<u>Revertant Colony Counts (Mean ±SD)</u>
<u>TA 98</u>			
Without Activation	Deionised water		22 ± 2
	Untreated		24 ± 3
	Lipase produced with	2500 µg	33 ± 1
	Trichoderma reesei	5000 µg	40 ± 8 ^{C M}
		7500 µg	31 ± 3 ^{C M}
		10000 µg	22 ± 7 ^{C M}
	4-NOPD	10 µg	380 ± 24
<u>Key to Positive Controls</u>		<u>Key to Plate Postfix Codes</u>	
4-NOPD	4-nitro-o-phenylene-diamine	C	Contaminated
		M	Manual count

Table 4 Summary of Experiment IIb

Study Name: 1544902

Experiment: 1544902 HV2b

Assay Conditions:

Study Code: Harlan CCR

1544902

Date Plated: 01/07/2013

Date Counted: 04/07/2013

Without Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)	
			<u>TA 98</u>	
Without Filtration	Deionised water		39 ± 2	^{B M}
	Untreated		44 ± 8	^{B M}
	Lipase produced with	2500 µg	56 ± 6	^{B M C}
	Trichoderma reesei	5000 µg	42 ± 1	^{B M C}
		7500 µg	74 ± 20	^{B M C}
		10000 µg	50 ± 2	^{B M C}
	4-NOPD	10 µg	364 ± 22	^{B M}
With Filtration	Deionised water		38 ± 3	^{B M}
	Untreated		38 ± 4	^{B M}
	Lipase produced with	2500 µg	54 ± 4	^{B M}
	Trichoderma reesei	5000 µg	58 ± 9	^{B M}
		7500 µg		
		10000 µg		
	4-NOPD	10 µg	342 ± 41	^{B M}
Key to Positive Controls			Key to Plate Postfix Codes	
4-NOPD	4-nitro-o-phenylene-diamine		B	Extensive bacterial growth
			M	Manual count
			C	Contaminated

Table 5 Individual Results of Experiment I

Study Name: 1544902
 Experiment: 1544902 VV Plate
 Assay Conditions:

Study Code: Harlan CCR 1544902
 Date Plated: 30/04/2013
 Date Counted: 07/05/2013

Without metabolic activation						
Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Lipase produced with <i>Trichoderma reesei</i>	3 µg	13.0	3.6	0.8	9, 14, 16
		10 µg	19.7	0.6	1.2	19, 20, 20
		33 µg	17.7	3.2	1.1	19, 20, 14
		100 µg	16.3	1.2	1.0	17, 15, 17
		333 µg	18.0	4.4	1.1	21, 13, 20
		1000 µg	19.3	2.1	1.2	21, 17, 20
		2500 µg	19.0	3.0	1.2	22, 19, 16
		5000 µg	15.7	0.6	1.0	16, 16, 15
	Deionised water		16.0	1.0		17, 15, 16
	Untreated Control		12.3	0.6		12, 13, 12
TA 1537	Lipase produced with <i>Trichoderma reesei</i>	3 µg	9.0	1.0	1.0	10, 9, 8
		10 µg	11.0	2.6	1.2	9, 10, 14
		33 µg	8.0	2.0	0.9	10, 6, 8
		100 µg	10.0	1.7	1.1	9, 12, 9
		333 µg	12.7	0.6	1.4	13, 13, 12
		1000 µg	9.0	1.0	1.0	10, 9, 8
		2500 µg	12.3	4.5	1.4	8, 17, 12
		5000 µg	12.7	1.2	1.4	14, 12, 12
	Deionised water		9.0	2.6		8, 12, 7
	Untreated Control		7.0	1.0		7, 6, 8
TA 98	Lipase produced with <i>Trichoderma reesei</i>	3 µg	21.7	1.2	0.9	21, 23, 21
		10 µg	24.0	0.0	1.0	24, 24, 24
		33 µg	27.0	4.0	1.2	23, 31, 27
		100 µg	25.3	6.8	1.1	23, 33, 20
		333 µg	25.0	2.6	1.1	28, 24, 23
		1000 µg	24.3	3.8	1.0	20, 26, 27
		2500 µg	30.0	1.0	1.3	29, 31, 30
		5000 µg	41.7	4.2	1.8	37, 45, 43
	Deionised water		23.3	3.1		26, 24, 20
	Untreated Control		29.0	7.2		27, 23, 37
TA 100	Lipase produced with <i>Trichoderma reesei</i>	3 µg	95.7	12.7	1.0	104, 81, 102
		10 µg	91.3	5.5	0.9	85, 94, 95
		33 µg	95.3	7.4	1.0	98, 87, 101
		100 µg	108.0	4.0	1.1	104, 112, 108
		333 µg	93.3	5.0	1.0	88, 98, 94
		1000 µg	99.7	8.1	1.0	95, 109, 95
		2500 µg	119.7	10.7	1.2	108, 122, 129
		5000 µg	148.3	15.2	1.5	132, 162, 151
	Deionised water		97.7	9.3		87, 104, 102
	Untreated Control		91.3	1.2		92, 92, 90

Study Name: 1544902
 Experiment: 1544902 VV Plate
 Assay Conditions:

Study Code: Harlan CCR 1544902
 Date Plated: 30/04/2013
 Date Counted: 07/05/2013

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 102	Lipase produced with Trichoderma reesei	3 µg	294.7	3.1	0.9	298, 292, 294
		10 µg	312.7	12.6	0.9	311, 301, 326
		33 µg	354.7	12.4	1.1	347, 369, 348
		100 µg	359.3	29.1	1.1	332, 356, 390
		333 µg	302.7	4.5	0.9	298, 303, 307
		1000 µg	374.3	18.5	1.1	374, 393, 356
		2500 µg	322.0	6.2	1.0	329, 320, 317
		5000 µg	201.0	3.6	0.6	205, 200, 198
	Deionised water		336.0	33.6		333, 371, 304
	Untreated Control		314.7	4.7		320, 311, 313
TA 1535	NaN3	10 µg	1985.3	99.7	124.1	1964, 1898, 2094
TA 1537	4-NOPD	50 µg	74.7	13.3	8.3	90, 67, 67
TA 98	4-NOPD	10 µg	266.0	26.5	11.4	237, 272, 289
TA 100	NaN3	10 µg	2115.3	76.1	21.7	2112, 2041, 2193
TA 102	MMS	2.0 µL	3631.0	418.9	10.8	4113, 3355, 3425

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Study Name: 1544902
 Experiment: 1544902 VV Plate
 Assay Conditions:

Study Code: Harlan CCR 1544902
 Date Plated: 30/04/2013
 Date Counted: 07/05/2013

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Lipase produced with Trichoderma reesei	3 µg	14.3	5.7	1.0	16, 19, 8
		10 µg	11.0	2.6	0.7	14, 10, 9
		33 µg	13.7	3.2	0.9	15, 10, 16
		100 µg	14.7	4.5	1.0	19, 10, 15
		333 µg	13.3	1.5	0.9	13, 15, 12
		1000 µg	19.3	2.5	1.3	22, 17, 19
		2500 µg	13.7	4.0	0.9	16, 16, 9
		5000 µg	17.3	2.5	1.2	15, 20, 17
	Deionised water		15.0	2.0		17, 15, 13
	Untreated Control		14.0	3.6		15, 17, 10
TA 1537	Lipase produced with Trichoderma reesei	3 µg	14.3	1.5	0.8	16, 14, 13
		10 µg	15.3	3.5	0.9	15, 19, 12
		33 µg	14.7	2.5	0.8	17, 15, 12
		100 µg	19.3	0.6	1.1	20, 19, 19
		333 µg	22.3	3.2	1.2	20, 26, 21
		1000 µg	19.7	2.5	1.1	22, 17, 20
		2500 µg	19.3	5.9	1.1	26, 15, 17
		5000 µg	16.7	7.0	0.9	16, 10, 24
	Deionised water		18.0	1.7		17, 20, 17
	Untreated Control		15.7	1.5		14, 16, 17
TA 98	Lipase produced with Trichoderma reesei	3 µg	31.0	6.1	0.7	28, 38, 27
		10 µg	32.7	6.4	0.8	29, 40, 29
		33 µg	35.7	1.5	0.8	36, 34, 37
		100 µg	32.0	3.6	0.8	28, 35, 33
		333 µg	37.3	3.1	0.9	38, 34, 40
		1000 µg	36.0	5.6	0.9	31, 42, 35
		2500 µg	40.3	4.0	1.0	36, 44, 41
		5000 µg	36.3	8.5	0.9	45, 36, 28
	Deionised water		42.0	1.7		43, 40, 43
	Untreated Control		49.0	5.0		49, 54, 44
TA 100	Lipase produced with Trichoderma reesei	3 µg	82.0	1.7	0.8	80, 83, 83
		10 µg	79.0	6.9	0.7	71, 83, 83
		33 µg	84.0	1.0	0.8	84, 83, 85
		100 µg	76.7	3.1	0.7	74, 76, 80
		333 µg	81.0	10.1	0.7	70, 83, 90
		1000 µg	85.3	4.5	0.8	81, 85, 90
		2500 µg	97.0	10.4	0.9	90, 92, 109
		5000 µg	125.0	24.4	1.2	142, 97, 136
	Deionised water		108.7	2.1		107, 111, 108
	Untreated Control		118.0	18.2		127, 130, 97

Study Name: 1544902
 Experiment: 1544902 VV Plate
 Assay Conditions:

Study Code: Harlan CCR 1544902
 Date Plated: 30/04/2013
 Date Counted: 07/05/2013

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 102	Lipase produced with Trichoderma reesei	3 µg	349.7	14.7	0.9	361, 333, 355
		10 µg	382.3	33.3	1.0	419, 354, 374
		33 µg	354.7	27.5	1.0	383, 328, 353
		100 µg	369.7	52.8	1.0	325, 356, 428
		333 µg	291.7	14.6	0.8	287, 280, 308
		1000 µg	276.0	41.0	0.7	313, 232, 283
		2500 µg	222.0	25.1	0.6	248, 198, 220
		5000 µg	194.7	23.1	0.5	221, 178, 185
	Deionised water		371.7	40.6		355, 418, 342
	Untreated Control		395.3	79.1		332, 370, 484
TA 1535	2-AA	2.5 µg	437.0	17.4	29.1	417, 449, 445
TA 1537	2-AA	2.5 µg	309.3	50.1	17.2	258, 312, 358
TA 98	2-AA	2.5 µg	2030.7	323.8	48.3	1779, 1917, 2396
TA 100	2-AA	2.5 µg	2324.7	282.5	21.4	2559, 2011, 2404
TA 102	2-AA	10.0 µg	2093.0	212.1	5.6	2233, 1849, 2197

Key to Positive Controls

2-AA 2-aminoanthracene

Table 6 Individual Results of Experiment II

Study Name: 1544902
 Experiment: 1544902 HV2 Pre
 Assay Conditions:

Study Code: Harlan CCR 1544902
 Date Plated: 23/05/2013
 Date Counted: 28/05/2013

Without metabolic activation						
Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Lipase produced with <i>Trichoderma reesei</i>	33 µg	12.0	4.0	0.9	12, 16, 8
		100 µg	14.3	5.1	1.1	10, 20, 13
		333 µg	17.3	5.8	1.4	14, 24, 14
		1000 µg	17.0	3.5	1.3	19, 19, 13
		2500 µg	21.7	5.0	1.7	21, 17, 27
		5000 µg	27.3	0.6	2.2	28, 27, 27
	Deionised water		12.7	1.2		12, 14, 12
	Untreated Control		14.3	2.3		13, 13, 17
TA 1537	Lipase produced with <i>Trichoderma reesei</i>	33 µg	9.0	2.6	0.9	7, 12, 8
		100 µg	10.3	1.5	1.1	12, 10, 9
		333 µg	11.3	1.2	1.2	12, 12, 10
		1000 µg	12.3	2.1	1.3	10, 13, 14
		2500 µg	12.0	0.0	1.2	12, 12, 12
		5000 µg	15.0	1.7	1.6	14, 17, 14
	Deionised water		9.7	2.1		12, 8, 9
	Untreated Control		9.7	3.5		6, 10, 13
TA 98	Lipase produced with <i>Trichoderma reesei</i>	33 µg	27.3	8.1	1.0	26, 20, 36
		100 µg	31.3	4.6	1.1	26, 34, 34
		333 µg	29.0	6.0	1.0	35, 29, 23
		1000 µg	31.3	9.9	1.1	20, 38, 36
		2500 µg	40.3	3.1	1.4	43, 41, 37
		5000 µg	54.3	4.0	1.9	59, 52, 52
	Deionised water		28.0	6.2		30, 21, 33
	Untreated Control		23.3	4.2		20, 22, 28
TA 100	Lipase produced with <i>Trichoderma reesei</i>	33 µg	105.3	14.2	1.1	118, 90, 108
		100 µg	111.7	4.9	1.2	106, 115, 114
		333 µg	113.7	4.0	1.2	109, 116, 116
		1000 µg	114.7	7.1	1.2	107, 116, 121
		2500 µg	149.0	11.4	1.5	144, 162, 141
		5000 µg	167.3	7.6	1.7	162, 164, 176
	Deionised water		96.7	9.6		107, 95, 88
	Untreated Control		95.3	9.2		106, 90, 90

Study Name: 1544902
 Experiment: 1544902 HV2 Pre
 Assay Conditions:

Study Code: Harlan CCR 1544902
 Date Plated: 23/05/2013
 Date Counted: 28/05/2013

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 102	Lipase produced with Trichoderma reesei	33 µg	352.7	18.6	1.1	344, 340, 374
		100 µg	361.0	14.2	1.1	350, 377, 356
		333 µg	366.7	12.7	1.1	381, 357, 362
		1000 µg	358.7	3.1	1.1	358, 356, 362
		2500 µg	311.3	21.5	1.0	333, 311, 290
		5000 µg	205.3	1.5	0.6	204, 207, 205
	Deionised water		327.0	1.0		327, 326, 328
	Untreated Control		323.3	11.6		334, 311, 325
TA 1535	NaN3	10 µg	1706.3	65.6	134.7	1770, 1710, 1639
TA 1537	4-NOPD	50 µg	69.0	13.0	7.1	61, 62, 84
TA 98	4-NOPD	10 µg	336.0	10.0	12.0	336, 346, 326
TA 100	NaN3	10 µg	1869.7	57.1	19.3	1829, 1935, 1845
TA 102	MMS	2.0 µL	3213.0	137.0	9.8	3314, 3268, 3057

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Study Name: 1544902
 Experiment: 1544902 HV2 Pre
 Assay Conditions:

Study Code: Harlan CCR 1544902
 Date Plated: 23/05/2013
 Date Counted: 28/05/2013

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Lipase produced with Trichoderma reesei	33 µg	19.7	9.5	1.0	9, 27, 23
		100 µg	18.0	7.8	0.9	23, 9, 22
		333 µg	19.3	6.7	1.0	27, 16, 15
		1000 µg	17.0	5.3	0.9	15, 23, 13
		2500 µg	26.0	2.0	1.3	24, 26, 28
		5000 µg	31.3	6.7	1.6	33, 24, 37
	Deionised water		20.0	6.9		28, 16, 16
	Untreated Control		15.3	2.1		17, 13, 16
TA 1537	Lipase produced with Trichoderma reesei	33 µg	18.0	2.6	0.9	19, 15, 20
		100 µg	20.3	7.6	1.0	17, 15, 29
		333 µg	30.7	4.0	1.6	35, 27, 30
		1000 µg	32.0	3.5	1.6	30, 30, 36
		2500 µg	34.0	7.8	1.7	29, 43, 30
		5000 µg	36.0	7.9	1.8	33, 30, 45
	Deionised water		19.7	1.2		19, 19, 21
	Untreated Control		23.7	4.5		24, 28, 19
TA 98	Lipase produced with Trichoderma reesei	33 µg	38.7	7.5	0.9	30, 43, 43
		100 µg	35.3	11.0	0.8	48, 29, 29
		333 µg	47.0	12.3	1.1	56, 33, 52
		1000 µg	60.7	6.1	1.4	66, 54, 62
		2500 µg	60.0	10.5	1.4	70, 49, 61
		5000 µg	65.7	4.6	1.5	63, 71, 63
	Deionised water		43.7	7.4		52, 41, 38
	Untreated Control		53.0	9.5		47, 64, 48
TA 100	Lipase produced with Trichoderma reesei	33 µg	102.7	19.7	0.7	113, 115, 80
		100 µg	106.3	11.2	0.7	94, 116, 109
		333 µg	97.3	11.5	0.6	86, 109, 97
		1000 µg	124.7	11.7	0.8	116, 120, 138
		2500 µg	131.3	17.0	0.8	148, 114, 132
		5000 µg	149.3	12.7	0.9	163, 138, 147
	Deionised water		157.3	4.2		156, 162, 154
	Untreated Control		144.7	6.5		138, 145, 151

Study Name: 1544902
 Experiment: 1544902 HV2 Pre
 Assay Conditions:

Study Code: Harlan CCR 1544902
 Date Plated: 23/05/2013
 Date Counted: 28/05/2013

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 102	Lipase produced with Trichoderma reesei	33 µg	448.0	72.2	0.9	523, 442, 379
		100 µg	445.0	33.2	0.9	435, 418, 482
		333 µg	386.3	21.4	0.8	363, 405, 391
		1000 µg	361.0	16.1	0.7	356, 348, 379
		2500 µg	301.7	18.0	0.6	303, 283, 319
		5000 µg	186.0	30.0	0.4	175, 163, 220
	Deionised water		495.7	17.6		516, 485, 486
	Untreated Control		488.3	34.8		450, 497, 518
TA 1535	2-AA	2.5 µg	344.0	18.2	17.2	365, 335, 332
TA 1537	2-AA	2.5 µg	271.3	13.3	13.8	280, 278, 256
TA 98	2-AA	2.5 µg	2070.7	147.6	47.4	1980, 1991, 2241
TA 100	2-AA	2.5 µg	2808.3	152.3	17.8	2819, 2955, 2651
TA 102	2-AA	10.0 µg	2650.7	228.4	5.3	2609, 2446, 2897

Key to Positive Controls

2-AA 2-aminoanthracene

Table 7 Individual Results of Experiment IIa

Study Name: 1544902
 Experiment: 1544901 HV2a Pre
 Assay Conditions:

Study Code: Harlan CCR 1544902
 Date Plated: 19/06/2013
 Date Counted: 25/06/2013

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 98	Lipase produced with Trichoderma reesei	2500 µg	33.3	0.6	1.5	33, 33, 34
		5000 µg	39.7	8.1	1.8	49 C M, 36 C M, 34 C M
		7500 µg	30.7	3.2	1.4	32 C M, 33 C M, 27 C M
		10000 µg	22.3	6.5	1.0	16 C M, 22 C M, 29 C M
	Deionised water		22.0	2.0		24, 22, 20
	Untreated Control		24.0	3.5		22, 28, 22
TA 98	4-NOPD	10 µg	380.0	23.6	17.3	370, 363, 407

Key to Positive Controls

4-NOPD 4-nitro-o-phenylene-diamine

Key to Plate Postfix Codes

C Contaminated
 M Manual count

Table 8 Individual Results of Experiment IIb

Study Name: 1544902
 Experiment: 1544902 HV2b
 Assay Conditions:

Study Code: Harlan CCR 1544902
 Date Plated: 01/07/2013
 Date Counted: 04/07/2013

Without metabolic activation (without filtration)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 98	Lipase produced with Trichoderma reesei	2500 µg	56.3	5.5	1.4	62 B M C, 56 B M C, 51 B M C
		5000 µg	42.3	0.6	1.1	42 B M C, 43 B M C, 42 B M C
		7500 µg	74.0	20.4	1.9	97 B M C, 67 B M C, 58 B M C
		10000 µg	49.7	1.5	1.3	48 B M C, 51 B M C, 50 B M C
	Deionised water		39.3	1.5		39 B M, 38 B M, 41 B M
	Untreated Control		44.0	7.5		43 B M, 52 B M, 37 B M
TA 98	4-NOPD	10 µg	364.0	22.1	9.3	356 B M, 389 B M, 347 B M

Key to Positive Controls

4-NOPD 4-nitro-o-phenylene-diamine

Key to Plate Postfix Codes

B Extensive bacterial growth
 M Manual count
 C Contaminated

Without metabolic activation (with filtration)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 98	Lipase produced with Trichoderma reesei	2500 µg	53.7	4.0	1.4	50 B M, 58 B M, 53 B M
		5000 µg	57.7	9.5	1.5	47 B M, 65 B M, 61 B M
		7500 µg				
		10000 µg				
	Deionised water		38.3	3.2		42 B M, 37 B M, 36 B M
	Untreated Control		38.3	4.0		34 B M, 42 B M, 39 B M
TA 98	4-NOPD	10 µg	342.3	41.0	8.9	357 B M, 296 B M, 374 B M

Key to Positive Controls

4-NOPD 4-nitro-o-phenylene-diamine

Key to Plate Postfix Codes

B Extensive bacterial growth
 M Manual count

APPENDICES

Appendix 1 Historical Data

These data represent the laboratory's historical control data from January 2011 until December 2011 representing approx.550 experiments (TA 102 the historical data are based on approx. 200 experiments).

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA 1535	Solvent control	14	2.37	9	23	20	3.75	11	35
	Untreated control	14	2.87	7	25	20	3.82	10	32
	Positive control	1751	226.44	710	2385	367	93.12	126	703
TA1537	Solvent control	12	3.31	5	26	16	4.34	7	30
	Untreated control	12	4.03	5	29	18	4.92	6	33
	Positive control	88	38.92	61	448	342	144.31	77	809
TA 98	Solvent control	30	5.60	17	47	40	6.08	21	58
	Untreated control	31	6.36	17	55	42	6.83	24	68
	Positive control	372	78.05	158	595	2167	717.60	249	4089
TA 100	Solvent control	142	29.42	86	243	156	29.4	99	249
	Untreated control	150	28.19	86	248	163	31.26	94	281
	Positive control	1741	488.75	569	3082	2642	796.59	825	4503
TA 102	Solvent control	380	43.64	305	510	502	89.88	321	677
	Untreated control	372	41.71	300	479	511	87.27	315	659
	Positive control	2499	898.06	1080	4678	2329	598.00	1091	3972

Mean = mean value of revertants/plate

SD = standard deviation

Min = minimal value/Max = maximal value

Appendix 2 Monitoring Authority Statement of GLP Compliance**Gute Laborpraxis/Good Laboratory Practice****GLP-Bescheinigung/Statement of GLP Compliance**

(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)



Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Harlan Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according chemVwV-GLP Nr. 5.3/OECD guidance)

2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften
3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)
8 Analytische Prüfungen an biologischen Materialien

2 Toxicity studies
3 Mutagenicity studies
8 Analytical studies on biological materials

25. April, 23./25. und 26. Juli 2012

Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.



Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Im Auftrag

Hess. Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz,
Mainzer Straße 80 D65189 Wiesbaden
(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority)

Appendix 3 Certificate of Analysis

		NOVALAB OY	I(2)
ANALYSIS REPORT		Order: 1300613	 Finnish Accreditation Service T071 (EN ISO/IEC 17025)
Roal Oy Tykkimäentie 15, PL 57		Date: 22.3.2013	
05201 Rajamäki			
Order name:	ROAL Oy, Enzyme sample		
Sample:	13MU0113 LP 12136B3		
Sampling time:			
Sample arrived:	8.3.2013		
Analysis started:	14.3.2013		

Analysis		Result	Method
Ash	%	0,91	Novalab 009*
Moisture	%	4,7	Novalab 010*
Protein	%	70,1	Novalab 001.A, kjeldahl, calculated*
Carbonhydrates	%	24,0	Calculated
Energy value	kJ/100 g	1610	Calculated
Fat	%	0,28	Novalab 076*

*Accredited method

Sample code: Dry enzyme sample LP 12136B3

Methods:

Moisture: AOAC 2000 950.46 (39.1.02) modified

Protein: AOAC 2000 2001.11 (4.2.11) modified

Fat: NMKL 131:1989 modified

Ash: NMKL 173:2005 modified

Carbohydrate: By difference 100 % - (moisture+protein+fat+ash)%

Energy value: Calculated on the basis of contents of protein, fat and carbohydrate. Factors protein and carbohydrate 17 kJ/g, fat 37 kJ/g

Measurement uncertainty:

Moisture: ± 2 relative-%

Ash: ± 10 relative-%

Fat: ± 100 relative-%

Protein: ± 5 relative-%

Carbonhydrates, calculated: ± 5 relative-%

Energy, calculated: ± 5 relative-%

Results apply only to samples analysed. Partial copying of the certificate is prohibited without the laboratory's permission.Office and laboratory
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FI-03600 Karkkila
Finlandpuh (09) 2252 860
fax (09) 2252 8660
www.novalab.fiBank
Länsi-Uudenmaan Op
Karkkila
FI43 5297 2820 0007 16Business ID 0733227-8
Location Karkkila
VAT reg.



2(2)

Novalab Oy**Delivery**

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Office and laboratory
Lepolantie 9
FI-03600 Karkkila
Finland

puh (09) 2252 860
fax (09) 2252 8660
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Bank
Länsi-Uudenmaan Op
Karkkila
FI43 5297 2820 0007 16

Business ID 0733227-8
Location Karkkila
VAT reg.

REPORT (PART I OF II)

Lipase produced with *Trichoderma reesei*: 90-Day Oral Toxicity (Gavage) Study in the Wistar Rat

Test Facility: Harlan Laboratories Ltd.
Zelgliweg 1
4452 Itingen / Switzerland

Sponsor: AB Enzymes
Feldbergstrasse 78
64293 Darmstadt / Germany

Harlan Study Number: D80691

Study Completion Date: 30-Apr-2014

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STUDY DIRECTOR STATEMENT OF GLP COMPLIANCE

Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen / Switzerland

Harlan Study Number: D80691

Study Title: Lipase produced with *Trichoderma reesei*: 90-Day Oral Toxicity (Gavage) Study in the Wistar Rat

This study was performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice adopted May 18th, 2005 [SR 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 (ENV/MC/CHEM(98)17) and adopted November 26th, 1997 by decision of the OECD Council [C (97)186/Final].

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI).

This report fully and accurately reflects the procedures used and data generated. There were no circumstances considered to have affected the integrity of the study or the validity of the data.

The signature of any principal investigator is included in their respective statements of compliance and to this report.

QUALITY ASSURANCE STATEMENT

Harlan Study Number: D80691

Study Title: Lipase produced with *Trichoderma reesei*: 90-Day Oral Toxicity (Gavage) Study in the Wistar Rat

The general facilities and activities are inspected at least once a year and the results are reported to the relevant responsible person and management.

Study-related procedures conducted at the test facility were audited and inspected. The details of these audits and inspections are given below.

Dates and Types of QA Inspections			Reported to the Relevant Study Director and Test Facility Management
Date of Inspection	Type of Inspection	Phase Inspected	Report Date
06/09-Sep-2013	Study plan verification	N/A	09-Sep-2013
16-Sep-2013	Study-based	Test item preparation, test system preparation and application	16-Sep-2013
18-Sep-2013	Study-based	Clinical observation	18-Sep-2013
18-Sep-2013	Study-based	Formulation analysis	19-Sep-2013
12-Dec-2013	Study-based	Clinical observation	12-Dec-2013
17-Dec-2013	Study-based	Test system preparation and application, necropsy	17-Dec-2013
21-Feb-2014	Report audit	Appendix formulation analysis	21-Feb-2014
28/31-Mar 2014 to 01 to 04-Apr-2014	Report audit	N/A	07-Apr-2014

Verification of study plan amendments for GLP compliance was performed during the course of the study.

This statement confirms that this report reflects the raw data and the procedures followed.

The signature of any principal investigator is included in their respective statements of compliance and to this report.

SUMMARY

General

In this subchronic toxicity study, Lipase produced with *Trichoderma reesei* was administered daily by oral gavage to SPF-bred Wistar rats of both sexes at dose levels of 50, 200 and 1000 mg/kg body weight/day for a period of 92/93 days. A control group was treated similarly with the vehicle, bidistilled water, only.

The groups comprised 10 animals per sex which were sacrificed after 92/93 days of treatment.

Clinical signs, outside cage observation, food consumption and body weights were recorded periodically during the acclimatization, treatment and recovery periods. Functional observational battery, locomotor activity and grip strength were performed during week 13.

At the end of the dosing and the treatment-free recovery period, blood samples were withdrawn for hematology and plasma chemistry analyses. Urine samples were collected for urinalyses. All animals were killed, necropsied and examined post mortem. Histological examinations were performed on organs and tissues from all control and high dose animals, and all gross lesions from all animals.

Mortality / Viability

There was not test item-related mortality.

Clinical Signs (Daily and Weekly)

There were no test item-related findings in the daily or weekly observations

Functional Observational Battery

There were no test item-related findings in the functional observation battery at week 13.

Grip Strength and Locomotor Activity

Grip strength and locomotor activity of test item-treated rats were unaffected.

Food Consumption

There were no test item-related effects.

Body Weights

There were no test item-related effects.

Ophthalmoscopic Examinations

There were no test item-related effects.

Hematology / Clinical Biochemistry / Urinalysis

There were no changes of toxicological relevance.

Organ Weights

There were no test item-related effects.

Macroscopic / Microscopic Findings

There were no unscheduled deaths.

There were no treatment related macroscopic or microscopic findings.

Conclusion

Based on the results of this study, 1000 mg/kg body weight/day of Lipase produced with *Trichoderma reesei* was established as the no-observed-effect-level (NOEL) and 1000 mg/kg body weight/day as the no-observed-adverse-effect-level (NOAEL).

GENERAL INFORMATION

Schedule

Experimental Starting Date:	10-Sep-2013
Delivery of Animals:	10-Sep-2013
Randomization:	11-Sep-2013
Acclimatization:	10 to 15-Sep-2013
Administration / Treatment:	16-Sep to 16/17-Dec-2013
Termination (Necropsy):	17/18-Dec-2013
Experimental Completion Date:	29-Apr-2014

Animal Welfare

This study was performed in an AAALAC-accredited laboratory in accordance with the Swiss Animal Protection Law under license no. 27.

The conduct of the study may be reviewed, as part of the Harlan Laboratories Ltd. Ethical Review Process.

Accreditation

Harlan Laboratories Ltd. / Switzerland is accredited as a test laboratory for analysis in the fields of clinical chemistry, hematology, blood-coagulation and urine diagnostics in accordance with the Standard ISO/IEC 17025 under accreditation number STS 085 by the Swiss Accreditation Service.

Multi – Site Study Details

Phase Number:	14001
Test Site:	Propath GmbH Muttenserstrasse 30 4133 Pratteln / Switzerland

Test Site QA:

Certus Quality Assurance Services

Erlensträsschen 73
4125 Riehen / Switzerland

Additional Responsibilities

Deputy Study Director:

Planning Coordinator:

Technical Coordinator:

Necropsy / Histotechnology:

Clinical Laboratory Investigations:

Formulation Analysis:

Responsible Scientist for Peer
Review:

Archiving

Unless instructed otherwise by the Sponsor, the study plan, all raw data (paper and electronic), a sample of the test item(s), specimens (as long as the quality permits evaluation) and the final report will be retained in the Harlan Laboratories Ltd., 4452 Itingen / Switzerland archives for at least ten years after which instructions will be sought as to further retention or disposal. Further retention or return of the data will be chargeable to the Sponsor.

Frozen samples will be discarded after the results are deemed acceptable. No material or data will be discarded without the Sponsor's written consent.

The original final pathology phase report, all slides, dispatch list(s) and raw data provided were sent to the test facility.

1 INTRODUCTION

1.1 Purpose

The purpose of this oral toxicity study was to assess the cumulative toxicity of Lipase produced with *Trichoderma reesei* when administered daily to rats by gavage for a period of 92/93 days.

This study should provide a rational basis for toxicological risk assessment in man.

1.2 Data Requirements / Test Guidelines

The study procedures indicated in this report meet or exceed the requirements of the following guidelines:

- "Repeated Dose 90-Day Oral Toxicity Study in Rodents", OECD Guidelines for the testing of Chemicals, Section 4, Health Effects, Number 408, 21 September 1998.
- Directive 96/54/EC, B. 26. "Subchronic Oral Toxicity", 30 September 1996, including Additional Testing for Neurotoxicity.

2 TEST / CONTROL ITEM

2.1 Test Item

The test item and all information described below was supplied by the Sponsor.

Identification:	Lipase produced with <i>Trichoderma reesei</i>
Description:	Light brownish powder
Batch Number:	LP 12136B3; RF 10625
Purity/Concentration as Supplied:	UVCB substance; not formulated (microbial food enzyme)
Purity/Concentration for Formulation:	TOS value: 94.38%
Correction for Purity:	Yes
Expiry Date (Retest Date):	30-Nov-2014 (defined by Harlan Laboratories)
Storage Conditions:	Room temperature in the original container (dry)
Safety Precautions:	Routine hygienic procedures (gloves, goggles, face mask).
Analytical Standard:	The test item served as analytical standard and reference item.

2.2 Vehicle and Control Item

Identification:	Bidistilled water
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3 MATERIALS AND METHODS

3.1 Test System

Animals:	Rat, RccHanTM: WIST(SPF)
Rationale:	Recognized by international guidelines as a recommended test system.
Breeder:	Harlan Laboratories, B.V. Kreuzelweg 53 5961 NM Horst / Netherlands
Number of Animals:	Group 1: 10 males and 10 females Group 2: 10 males and 10 females Group 3: 10 males and 10 females Group 4: 10 males and 10 females Group 10: 2 males and 2 females
Total Number of Animals:	42 males and 42 females
Age (at Delivery):	Ca. 7 weeks
Body Weight Range (at Acclimatization):	Males: 193 - 242 g (mean 211 g) Females: 130 - 160 g (mean 141 g)
Identification:	Acclimatization: Cage card and tail mark (later ear tattoo) Treatment: Cage card and individual ear tattoo
Randomization:	Randomly allocated to groups by body weight.
Acclimatization:	Under test conditions after health examination. Only animals without any visible signs of illness were used for the study.

3.2 Allocation

The group identification and animal numbers assigned to treatment are stated in the following table:

Allocation and Dose Levels mg/kg/day	Group 1 Control* 0	Group 2 50 mg/kg/day	Group 3 200 mg/kg/day	Group 4 1000 mg/kg/day	Group 10 Reserve Animals**
Males	01 - 10	11 - 20	21 - 30	31 - 40	100-101
Females	41 - 50	51 - 60	61 - 70	71 - 80	200-201

* Control animals were treated with the vehicle only

** Reserve animals were removed from the study after randomization. Any raw data collected during on reserve animals were not reported but retained in the raw data.

3.3 Husbandry

Room Numbers, Itingen:

Room no. 129B

Conditions:

Standard laboratory conditions. Air-conditioned with 10 - 15 air changes per hour, continuously monitored environmental conditions (temp. range: 22 ± 3 °C; relative humidity range: 30 - 70%). Values outside of these ranges occasionally occurred and are considered not to have any influence on the study. Therefore, these data are not reported but are retained at Harlan Laboratories Ltd. The light cycle was set to 12-hour fluorescent light / 12-hour dark cycle with at least eight hours music during the light period.

Accommodation:

In groups of two (reserve animals) or five (test animals) in Makrolon type-4 cages with wire mesh tops and standard softwood bedding (J. Rettenmaier & Söhne GmbH & Co. KG, 73494 Rosenberg / Germany, imported by Provimi Kliba AG, 4303 Kaiseraugst / Switzerland) including paper enrichment (Enviro-dri from Lillico, Biotechnology, Surrey / UK).

Diet:

Pelleted standard Harlan Teklad 2914C (batch nos. 29/13 and 35/13) rat / mouse maintenance diet (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland) was available *ad libitum*. The feed batches were analyzed for contaminants.

Results of respective analyses for contaminants are included in Appendix I on p. 309 .

Water:

Community tap-water from Itingen was available *ad libitum* in water bottles. Results of bacteriological assay, chemical and contaminant analyses of respective samples are included in Appendix II on p. 316 .

3.4 Dose Formulations

The dose formulations were corrected to 100% according to its active ingredient purity of 94.38%.

The dose formulations were prepared weekly. Based upon the results of dose formulation analyses performed during a non-GLP dose range finding study (Harlan Laboratories study

D80680), the stability of the test item formulations was considered to be sufficient to justify weekly preparation.

The test item was weighed into a glass beaker on a tared Mettler balance and the vehicle was added. The mixtures were stirred using a magnetic stirrer and used at room temperature (20 ± 5 °C).

Homogeneity of the test item in the vehicle was maintained during the daily administration period using a magnetic stirrer.

3.4.1 Storage of Dose Formulations

The dose formulations were stored in glass beakers at room temperature (20 ± 5 °C).

3.4.2 Analysis of Dose Formulations

The analysis was performed by Harlan Laboratories Ltd. using a TOS method provided by the Sponsor.

After experimental start and during weeks 6 and 12, duplicate samples of the control group as well as three samples (top, middle and bottom) of about 2 g of each concentration were taken prior to dosing for analysis of homogeneity and concentration. Duplicate samples of about 2 g of each concentration were taken to confirm stability (4 hour and 7 days).

The samples were delivered to the analytical department (Harlan Laboratories Ltd., Analytics, Zelgliweg 1, 4452 Itingen / Switzerland) and stored there at -20 ± 5 °C until analysis.

The test item was used as analytical standard.

Dose formulation samples (primary samples and duplicates) were discarded upon written confirmation by the study director.

The results of analysis were summarized in an appendix and attached to the report (see Appendix III on p. [321](#)).

3.5 Treatment

Method:	Oral, by gavage
Rationale for Method:	Administration by gavage is a common and accepted route of exposure for studies of this type.
Frequency of Administration:	Daily
Dose Levels:	Group 1: 0 mg/kg/day Group 2: 50 mg/kg/day Group 3: 200 mg/kg/day Group 4: 1000 mg/kg/day
Rationale for Dose Level Selection:	The dose levels were selected based on a previous dose range finding toxicity study in Wistar rats, Harlan Laboratories study D80680.
Dose Volume:	10 mL/kg body weight
Dose Concentrations:	Group 1: 0 mg/mL/day Group 2: 5 mg/mL/day Group 3: 20 mg/mL/day Group 4: 100 mg/mL/day
Duration of Pre-Randomization Phase:	1 day
Duration of Acclimatization Phase:	5 days
Duration of Treatment Phase:	92/93 days

3.6 Phase Designation

Phase 1:	Pre-randomization phase
Phase 2:	Acclimatization phase
Phase 3:	Treatment phase

3.7 Activities and Observations

3.7.1 Viability / Mortality

Observations for viability / mortality were recorded twice daily.

3.7.2 Daily Observations

The animals were observed for clinical signs once before commencement of administration as well as daily on days 1 - 92/93 (twice daily during days 1 - 3) during the treatment period.

3.7.3 Weekly Behavioral Observations

The animals were observed in their home cages, outside their home cages in a standard arena and in the hand. These observations were performed once before commencement of administration and once weekly (weeks 1 to 12) thereafter.

	SCORE	PARAMETER	D	W 1 - 12	F 13
APPEARANCE	1 - 3	Piloerection	X	X	X
	1 - 3	Salivation	X	X	X
	1	Hunched posture	X	X	X
MOTOR	1 - 3	Ataxia	X	X	X
	1 - 3	Tremor/twitching	X	X	X
	1	Prostration	X	X	X
	1	Circling		X	X
	1 - 3	Spasm		X	X
BEHAVIOR	1 - 3	Hyperactivity	X	X	X
	1 - 3	Somnolence	X	X	X
	1 - 3	Increased exploration		X	X
	1 - 3	Reduced grooming		X	X
	1 - 3	Vocalisation		X	X
RESPIRATION	1	Dyspnea	X	X	X
	1	Tachypnea	X	X	X
	1	Bradypnea	X	X	X
REFLEXES	1	Blink		X	X
	1	Pinna		X	X
	1	Iridic light reflex		X	X
	1	Push-off (hind leg)		X	X
	1	Pain response		X	X
	1	Startle/hearing		X	X
	1	Righting reflex		X	X
MISCELLANEOUS	1 - 3	Lacrimation		X	X
	1	Limbs cyanotic		X	X
	1	Mydriasis		X	X
	1	Miosis		X	X
	1	Exophthalmos		X	X
	1 - 3	Reduced muscle tone		X	X

D: Daily observations

W1 - 12: Weekly behavioral observations (weeks 1 - 12)

F13: Functional Observational Batter (week 13)

3.7.4 Functional Observational Battery (Screen)

During week 13, relevant parameters (presented in Section 3.7.3) from a modified Irwin screen test were evaluated in all animals. The results are present in the summary and individual tables of the weekly detailed clinical observations under week 13.

Grip Strength

Forelimb and hindlimb grip strength measurements were performed using a push-pull strain gauge (Mecmesin, AFG 25N). The animals were placed with the forepaws inside a triangular grasping ring and with the hind paws outside a triangular grasping ring. Using one hand, the animals were held towards the base of the tail and steadily pulled away or towards the ring until the grip was broken. Each measurement was repeated three times, the means were calculated and recorded.

Locomotor Activity

Locomotor (decreased or increased) activity was measured quantitatively with AMS Föhr Medical Instruments GmbH (FMI) and DeMeTec GmbH Activity Monitor System. Animals were monitored during the fourth treatment week for a 60-minute period and the total activity of this time period was recorded.

Low beams count was reported in 10-minute intervals as well as the total activity of the measuring period.

3.7.5 Food Consumption

The food consumption was recorded once during the acclimatization period and weekly thereafter, using an on-line electronic recording system consisting of a Mettler balance connected to the Harlan Laboratories computer.

3.7.6 Body Weights

Body weights were recorded weekly during acclimatization, treatment and recovery periods and before necropsy, using an on-line electronic recording system consisting of a Mettler balance connected to the Harlan Laboratories computer.

3.8 Clinical Laboratory Investigations

Blood and Urine Sampling:

After 13 Weeks:

17/18-Dec-2013

Blood samples were drawn by sublingual puncture from all animals under light isoflurane anesthesia. The animals were fasted in metabolism cages for approximately 18 hours before blood sampling but allowed access to water *ad libitum*. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms.

Urine was collected during the 18 hours fasting period into a specimen vial, using a metabolism cage.

In the summary and individual tables the names of some parameters have been abbreviated.

Detailed methodology, abbreviations and general remarks are described in Appendix IV on p. [329](#).

Clinical laboratory data are expressed, with a few exceptions, in general accordance with the International System of Units (SI).

3.8.1 Hematology

The following hematology parameters were determined:

Complete Blood Cell Count

Erythrocyte count	Differential leukocyte count:
Hemoglobin	Neutrophils
Hematocrit	Eosinophils
Mean corpuscular volume	Basophils
Red cell volume distribution width	Lymphocytes
Mean corpuscular hemoglobin	Monocytes
Mean corpuscular hemoglobin concentration	Large unstained cells
Hemoglobin concentration distribution width	Platelet count
Reticulocyte count	
Reticulocyte maturity index (low, medium, high fluorescence)	
Leukocyte count, total	

Hemoglobin Derivatives

Methemoglobin	Heinz bodies (slides were prepared but not evaluated)
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Coagulation

Prothrombin time (= Thromboplastin time)	Activated partial Thromboplastin time
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3.8.2 Clinical Biochemistry

The following clinical biochemistry parameters were determined:

Glucose	Gamma-glutamyl-transferase
Urea	Creatine kinase
Creatinine	Sodium
Bilirubin, total	Potassium
Cholesterol, total	Chloride
Triglycerides	Calcium
Phospholipids	Phosphorus
Aspartate aminotransferase	Protein, total
Alanine aminotransferase	Albumin
Lactate dehydrogenase	Globulin
Alkaline phosphatase	Albumin/Globulin ratio
Bile acids	

3.8.3 Urinalysis

The following urine parameters were determined:

Physical Examination

Urine volume (18 hour)	Color
Specific gravity (relative density)	Appearance

Chemical Examination

pH value	Urobilinogen
Nitrite	Bilirubin
Protein	Erythrocytes
Glucose	Leukocytes
Ketones	

3.9 Pathology

3.9.1 Necropsy

Sacrifice:

After 13 Weeks:

17/18-Dec-2013

All animals were weighed and necropsied. Descriptions of all macroscopical abnormalities were recorded. All animals were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination.

Samples of the following tissues and organs were collected from all animals at necropsy and fixed in neutral phosphate buffered 4% formaldehyde solution unless indicated otherwise.

Tissues / Organs	Weight	Collect	Examine
Adrenal glands	X	X	X
Aorta		X	X
Bone (sternum, femur including joint)		X	
Bone marrow (sternum)		X	X
Brain - including section of medulla/pons, cerebral and cerebellar cortex	X	X	X
Cecum		X	X
Colon		X	X
Duodenum		X	X
Epididymides (fixed in modified Davidson's solution)	X	X	X
Esophagus		X	X
Eyes w/optic nerve (fixed in Davidson's solution)		X	X
Harderian gland (fixed in Davidson's solution)		X	
Heart including auricles	X	X	X
Ileum, with Peyer's patches		X	X
Jejunum		X	X
Kidneys	X	X	X
Larynx		X	X
Lacrimal gland, exorbital		X	
Liver	X	X	X
Lungs, filled w/formalin at necropsy		X	X

Tissues / Organs	Weight	Collect	Examine
Lymph nodes - mesenteric and mandibular		X	X
Mammary gland area		X	X
Nasal cavity		X	
Ovaries	X	X	X
Pancreas		X	X
Pharynx		X	
Pituitary gland		X	X
Prostate gland and seminal vesicles incl. coagulating glands		X	X
Rectum		X	X
Salivary glands - mandibular, sublingual		X	X
Sciatic nerve		X	X
Skeletal muscle		X	
Skin		X	X
Spinal cord - cervical, midthoracic, lumbar		X	X
Spleen	X	X	X
Stomach		X	X
Testes (fixed in modified Davidson's solution)	X	X	X
Thymus	X	X	X
Thyroid (incl. parathyroid gland, if possible)		X	X
Tongue		X	
Trachea		X	X
Ureters		X	
Urinary bladder, filled w/formalin at necropsy		X	X
Uterus (incl. oviducts, cervix and vagina)	X	X	X
All gross lesions		X	X

3.9.2 Organ Weights

The organs from animals listed in the table in Section 3.9.1 were weighed before fixation and recorded on the scheduled dates of necropsy. Relative organ weights were calculated on the basis of the body weight and brain weight.

The terminal body weight was recorded immediately prior to necropsy and the organ to terminal body weight ratios as well as organ to brain weight ratios were determined.

3.9.3 Histotechnique

All organ and tissue samples, as defined under Histopathology (see Section 3.9.4), were processed, embedded and cut at an approximate thickness of 2 to 4 micrometers and stained with hematoxylin and eosin.

3.9.4 Histopathology

Slides of all organs and tissues listed in the table in Section 3.9.1 collected at scheduled sacrifices from all animals of the control and high-dose groups and all gross lesions from all animals were examined by the study pathologist. A description of all abnormalities is included (see Appendix V on p. 342). Attempts were made to correlate gross observations with microscopic findings. The stage of estrus was evaluated. The pathology phase report is attached.

A peer review was performed by W. Henderson. The findings of the study pathologist and the peer-reviewing pathologist compared favorably. The peer review report is retained in the raw data.

3.10 Data Compilation

The TOX-CONTROL LIMS computer was used to sort and present data for inclusion in the report. All electronically recorded data are conserved on digital medium. The macroscopical findings and associated data were transferred electronically to Propath for compilation of the pathology report.

Locomotor activity was recorded on-line, and the results were printed and transcribed into the computer system for compilation and analysis. Grip strength values were recorded on data sheets and then transcribed into the computer system for compilation and analysis.

Severity grades for clinical symptoms were generally assigned as follows: 0 = not present, 1 = present / slight, 2 = moderate, 3 = marked.

3.11 Statistical Analysis

The following statistical methods were used to analyze body weight, food consumption, grip strength, locomotor activity, clinical laboratory data, ophthalmoscopy, organ weights and ratios as well as macroscopic findings:

- The Dunnett-test [see References (1)] (many to one t-test) based on a pooled variance estimate was applied if the variables could be assumed to follow a normal distribution for the comparison of the treated groups and the control groups for each sex.

- The Steel-test [see References (2)] (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution.
- Fisher's exact-test [see References (3)].

4 RESULTS AND DISCUSSION

4.1 Analysis of Dose Formulations

(See Appendix III on p. [321](#))

The linearity of the analytical system used for sample analyses was demonstrated with a good relationship between peak areas measured and calibration solution concentrations. All calibration points used met the acceptance limit of $\pm 20\%$ variation from the calibration curve derived by linear regression analysis. The coefficients of determination (R^2) calculated were found to exceed 0.99.

The application formulations investigated during the study were found to comprise Lipase produced with *Trichoderma reesei* in the range of 86.5% to 114.2%, meeting the required content limit of $\pm 20\%$ with reference to the nominal content.

As single results did not deviate more than 5.5% (acceptance criterion: $<15\%$) from the corresponding mean, Lipase produced with *Trichoderma reesei* was considered to be homogeneously distributed in the dose preparations.

In addition, the test item was found to be stable with reference to the TOC method in application formulations when kept up to eight days at room temperature due to recoveries which met the variation limit of 10% from the time-zero (homogeneity) mean.

In conclusion, the results indicate the accurate preparation and storage of the test item Lipase produced with *Trichoderma reesei* in vehicle during this study.

4.2 Observations

4.2.1 Viability / Mortality

(See Individual Tables on p. [123](#))

All animals survived the scheduled treatment or recovery periods.

4.2.2 Daily Observations

(See Summary Tables on p. [45](#) , Individual Tables on p. [132](#))

There were no test item-related findings of toxicological relevance at any dose level.

A small number of typical background findings were noted, including hairless areas or sores, ear fissures, and in one male treated with 50 mg/kg/day, a small nodule in the left axillary region.

4.2.3 Weekly Behavioral Observations

(See Summary Tables on p. 54 , Individual Tables on p. 142)

There were no test item-related clinical observations evident during the weekly behavioral observations (weeks 1 to 12) at any dose level.

4.2.4 Functional Observational Battery (Screen)

There were no clinical observations evident during the functional observational battery (week 13) at any dose level.

Grip Strength

(See Summary Tables on p. 59 , Individual Tables on p. 147)

The mean fore- and hind limb grip strength values of the test item-treated rats compared favorably with those of the respective control rats. The single statistically significant difference (increased, $p < 0.05$) of the males treated with 1000 mg/kg/day was considered to be of no toxicological relevance.

Locomotor Activity

(See Summary Tables on p. 62 , Individual Tables on p. 156)

There were no test item-related differences in the mean locomotor activity at any dose level.

4.2.5 Food Consumption

(See Figures on p. 32 , Summary Tables on p. 67 , Individual Tables on p. 165)

There were no differences in the mean daily food consumption. The statistically significant differences in the relative food consumption values ($p < 0.05$ and $p < 0.1$) noted in males during days 85 - 92 were considered to result from an outlying control value.

4.2.6 Body Weights

(See Figures on p. 38 , Summary Tables on p. 77 , Individual Tables on p. 183)

There were no test item-related differences in the mean body weights or mean body weight gain.

4.2.7 Ophthalmoscopic Examinations

(See Summary Tables on p. 87 , Individual Tables on p. 209)

There were no test item-related ophthalmoscopic changes at any dose level.

Typical background findings were noted (uni- or bilateral corneal opacity, persistent hyaloid vessel or persistent pupillary membrane) in males and females of all groups. The severity and incidence of these findings at the end of the treatment period were similar.

4.3 Clinical Laboratory Investigations

4.3.1 Hematology

(See Summary Tables on p. 92 , Individual Tables on p. 223)

There were no test item-related differences of toxicological relevance in the hematology parameters. All differences either remained within the ranges of the historical control values or were unrelated to dose and therefore considered to be of no toxicological relevance.

In males, statistically significant differences included reduced hemoglobin distribution width ($p < 0.01$, 1000 mg/kg/day). The mean relative neutrophil count was increased ($p < 0.01$, 200 mg/kg/day) and mean relative lymphocyte counts were decreased ($p < 0.05$, 50 and 200 mg/kg/day) when compared with the controls. The mean absolute eosinophil count was elevated ($p < 0.05$, 50 mg/kg/day). The mean relative prothrombin time was higher ($p < 0.01$, 1000 mg/kg/day).

In females, the mean leukocyte count was reduced ($p < 0.05$, 200 mg/kg/day); the relative differential leukocyte count in this dose group showed elevated mean relative eosinophils ($p < 0.01$), reduced lymphocytes ($p < 0.05$). The absolute differential leukocyte count showed significantly higher eosinophils ($p < 0.05$, 1000 mg/kg/day), reduced lymphocytes ($p < 0.05$, 200 mg/kg/day) and increased mean prothrombin times ($p < 0.05$, 1000 mg/kg/day).

4.3.2 Clinical Biochemistry

(See Summary Tables on p. 98 , Individual Tables on p. 240)

There were no test item-related differences of toxicological relevance in the clinical biochemistry parameters. All differences either remained within the ranges of the historical control values or were unrelated to dose and therefore considered to be of no toxicological relevance.

In males, a small number of statistically significant differences without toxicological relevance were noted. Elevated glucose and reduced calcium levels in males at 1000 mg/kg/day (both

p<0.05) were evident, as were significant differences in chloride levels in males at 300 and 1000 mg/kg/day (both p<0.05). The mean potassium level in males at 1000 mg/kg/day was significantly elevated (p<0.05) and exceeded the upper limit of the historical control values. The mean total bilirubin levels of all treated males were reduced and the males treated with 1000 mg/kg/day attained statistical significance (p<0.01) when compared with the control males. All exceeded the lower range of the historical control data. The difference in potassium (although statistically significant, p<0.05) noted in males treated with 1000 mg/kg/day only marginally exceeded the upper limit of the historical control data. A reduction in total bilirubin, as seen in the test item-treated males, is not generally associated with toxicological relevant changes.

In females treated with 200 mg/kg/day and 1000 mg/kg/day, only the mean sodium levels were significantly elevated (both p<0.01) but remained within the ranges of the historical control data.

4.3.3 Urinalysis

(See Summary Tables on p. [103](#) , Individual Tables on p. [257](#))

The urinalysis parameters were unaffected in males and females at all dose levels.

4.4 Pathology

4.4.1 Organ Weights

(See Summary Tables on p. [106](#) , Individual Tables on p. [266](#))

There were no test item-related changes in the mean absolute or relative organ weights at any dose level.

4.4.2 Macroscopic Findings

(See Summary Tables on p. [119](#) , Individual Tables on p. [291](#))

There were no test item-related changes.

4.4.3 Microscopic Findings

(See Appendix V on p. [342](#))

There were no treatment related morphological alterations. All recorded microscopic findings were within the range of background pathology encountered in Wistar rats of this age and occurred at similar incidences and severity in both control and treated rats.

5 DISCUSSION AND CONCLUSION

Oral administration of Lipase produced with *Trichoderma reesei* to Wistar rats at doses of 100, 300 and 1000 mg/kg/day, for 92/93 days resulted in no test item-related deaths, no relevant findings during daily observations or during weekly observations (weeks 1 - 12), during the functional observational battery (week 13), no differences of toxicological relevance in the fore- and hind limb grip strength values or locomotor activity, no differences of toxicological relevance in the mean food consumption or mean body weight development, no test item-unrelated differences in the ophthalmoscopy, no test item-related effects upon hematology, clinical biochemistry and urinalysis, and no toxicologically relevant differences in mean absolute and relative organ weights, macroscopic or microscopical findings.

Based on the results of this study, 1000 mg/kg body weight/day of Lipase produced with *Trichoderma reesei* was established as the no-observed-effect-level (NOEL) and as the no-observed-adverse-effect-level (NOAEL).

6 REFERENCES

1. C.W. Dunnett:
A Multiple Comparison Procedure for Comparing Several Treatments with a Control,
J. Amer. Stat. Assoc. 50, 1096-1121 (1955).
2. R.G. Miller:
Simultaneous Statistical Inference, Springer Verlag, New York (1981).
3. R.A. Fisher:
Statistical Methods for Research Workers, Oliver and Boyd, Edinburgh (1950).

7 FIGURES

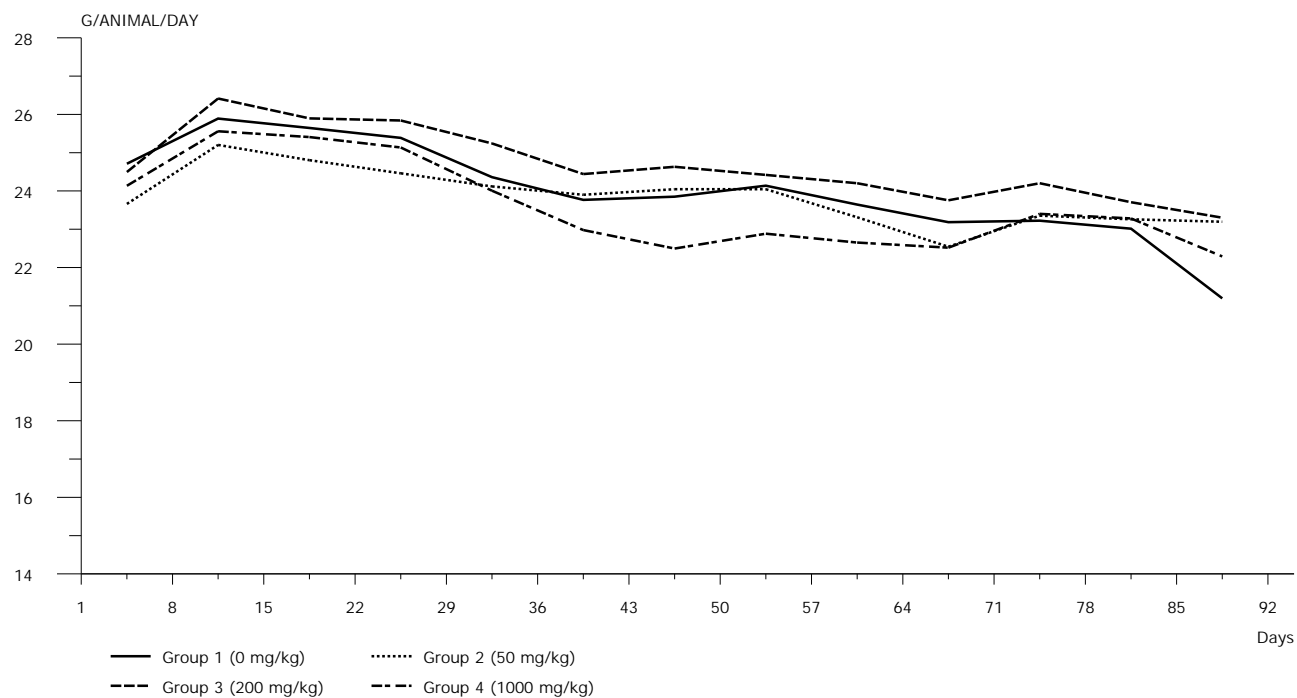
FOOD CONSUMPTION (G/ANIMAL/DAY) - GRAPHICS

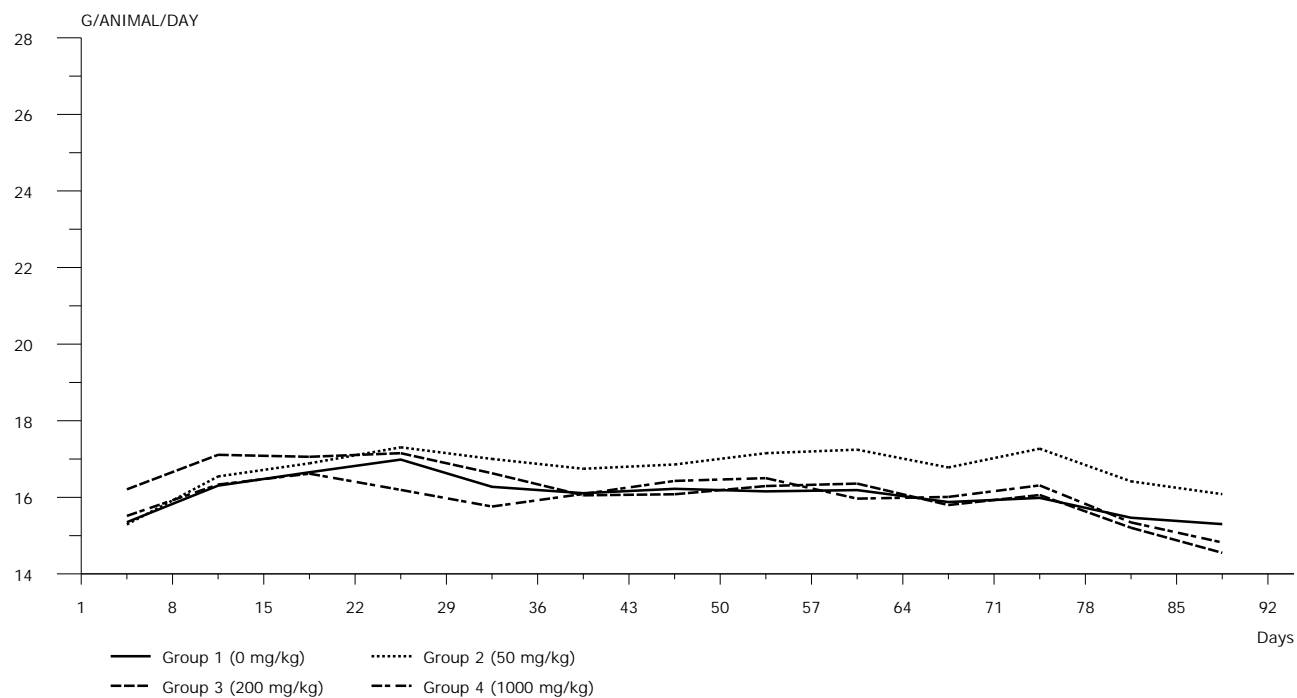
Data excluded from Summary Report

Not Reported

All Study Phases

Cage	13	Male	Group 10	Reserve Removed
Cage	26	Female	Group 10	Reserve Removed

**FOOD CONSUMPTION (G/ANIMAL/DAY) - GRAPHICS
MALES****TREATMENT**

**FOOD CONSUMPTION (G/ANIMAL/DAY) - GRAPHICS
FEMALES****TREATMENT**

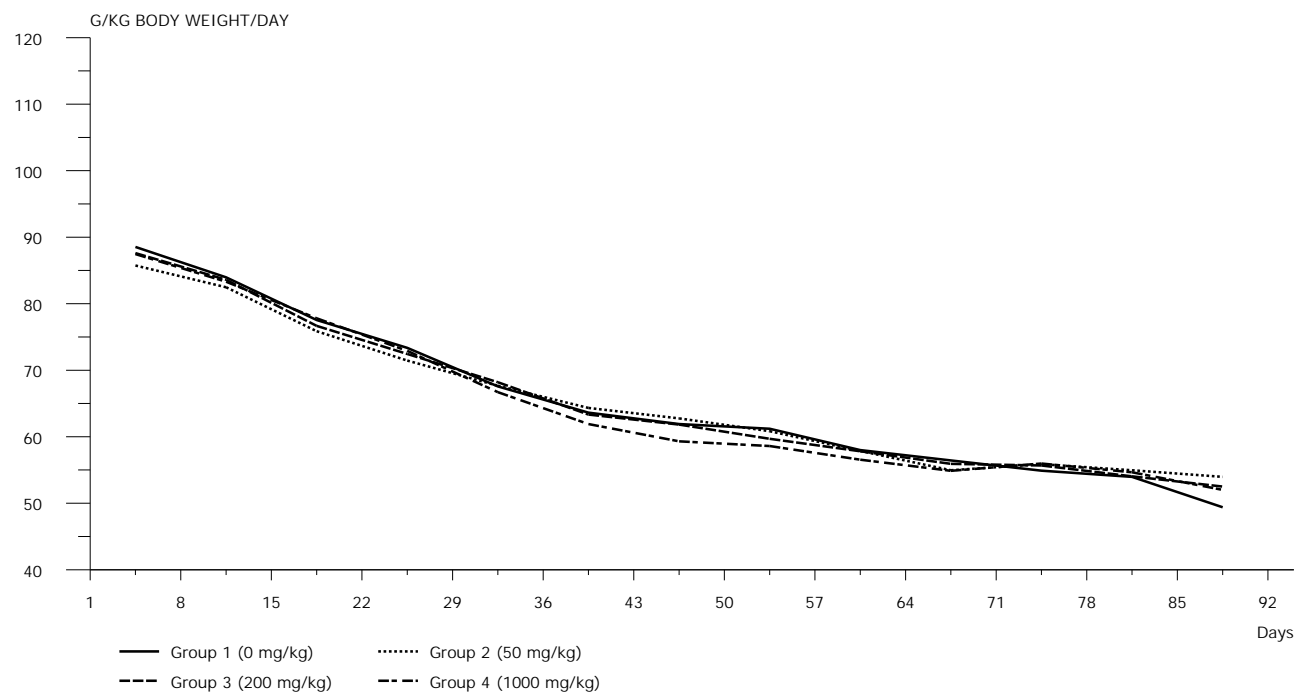
RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) - GRAPHICS

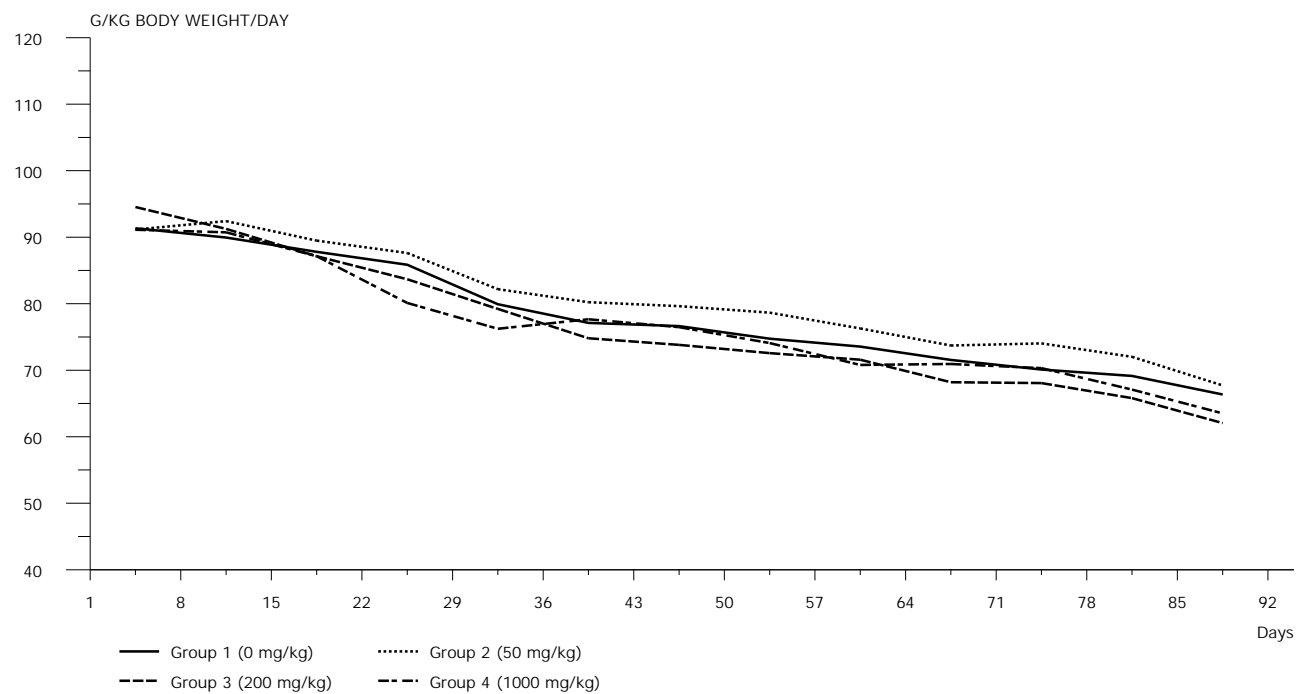
Data excluded from Summary Report

Not Reported

All Study Phases

Cage	13	Male	Group 10	Reserve Removed
Cage	26	Female	Group 10	Reserve Removed

**RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) - GRAPHICS
MALES****TREATMENT**

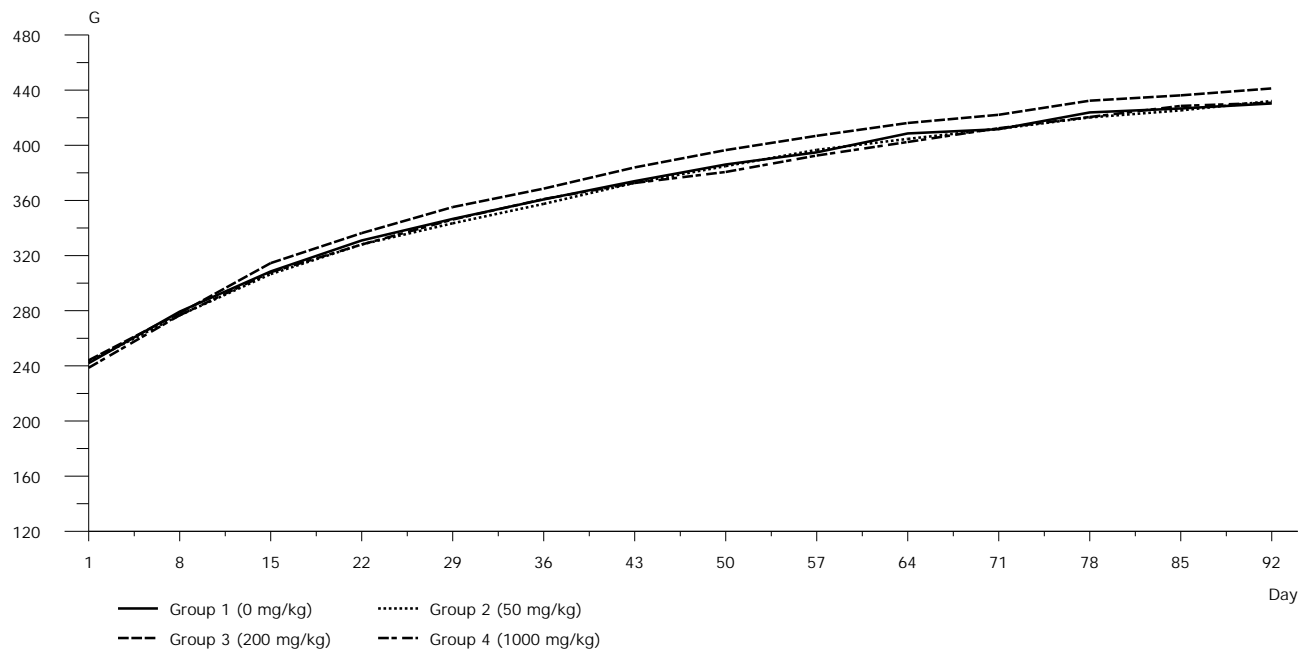
**RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) - GRAPHICS
FEMALES****TREATMENT**

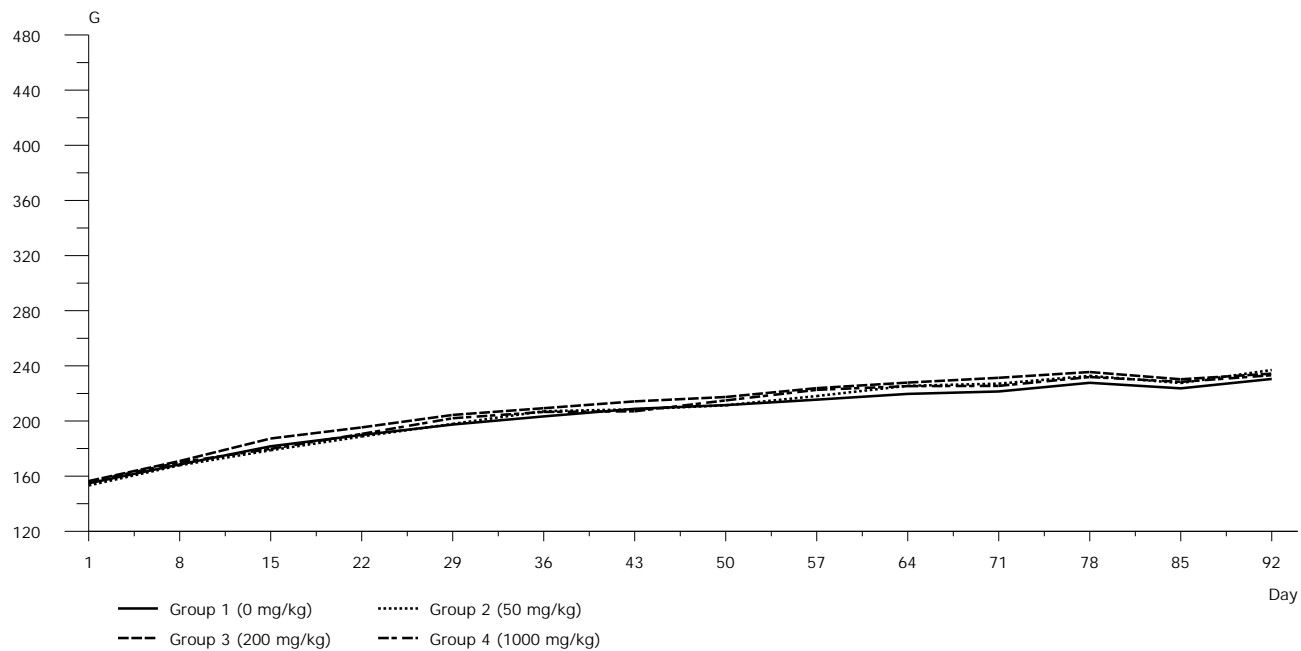
BODY WEIGHTS (G) - GRAPHICS**Data excluded from Summary Report**

Not Reported

All Study Phases

Animal	100	Male	Group 10	Reserve Removed
Animal	101	Male	Group 10	Reserve Removed
Animal	200	Female	Group 10	Reserve Removed
Animal	201	Female	Group 10	Reserve Removed

**BODY WEIGHTS (G) - GRAPHICS
MALES****TREATMENT**

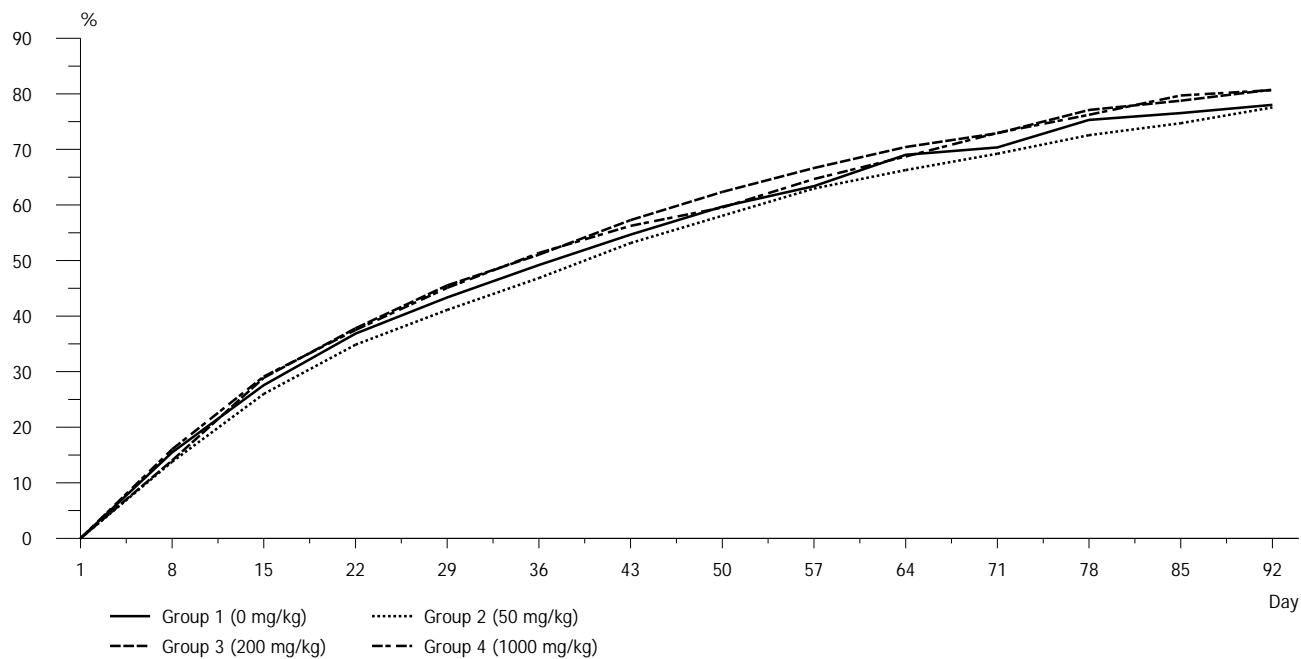
**BODY WEIGHTS (G) - GRAPHICS
FEMALES****TREATMENT**

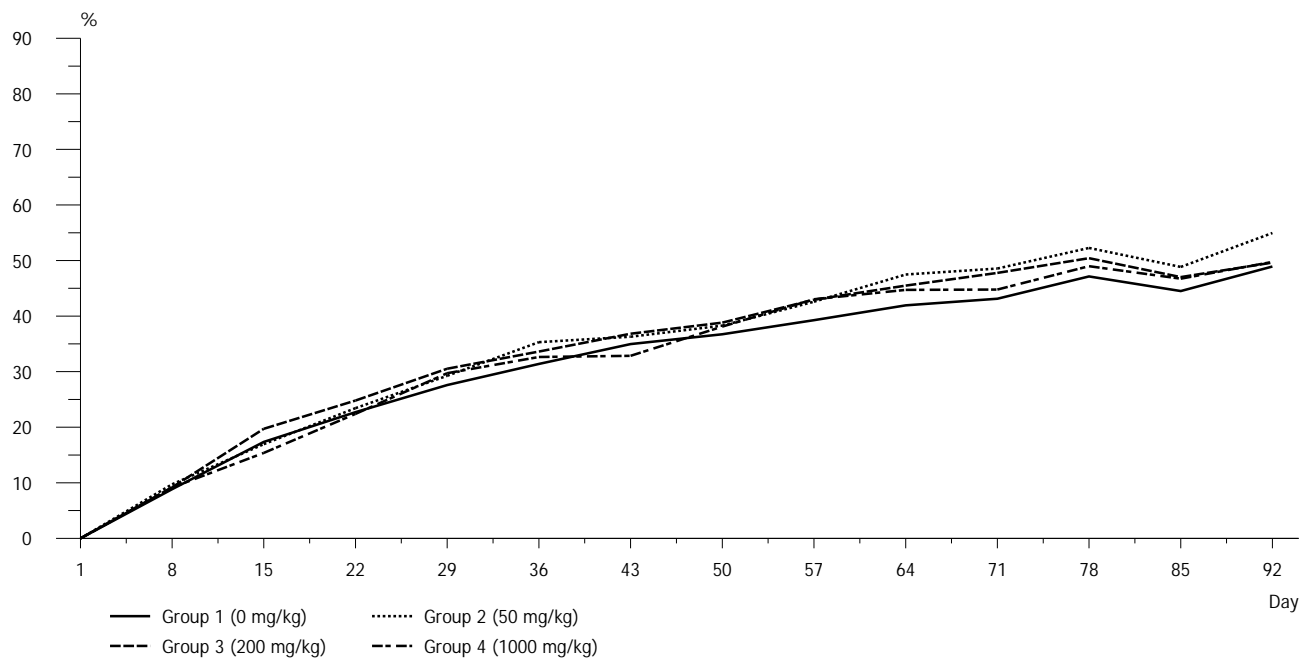
BODY WEIGHT GAIN (%) - GRAPHICS**Data excluded from Summary Report**

Not Reported

All Study Phases

Animal	100	Male	Group 10	Reserve Removed
Animal	101	Male	Group 10	Reserve Removed
Animal	200	Female	Group 10	Reserve Removed
Animal	201	Female	Group 10	Reserve Removed

**BODY WEIGHT GAIN (%) - GRAPHICS
MALES****TREATMENT**

**BODY WEIGHT GAIN (%) - GRAPHICS
FEMALES****TREATMENT**

8 SUMMARY TABLES

DAILY OBSERVATIONS - SUMMARY**Affected animals as percentage to observed animals**

0 0%
< between 1% and 9%
1 between 10% and 19%
2 between 20% and 29%
... ...
9 between 90% and 99%
A 100%

Data excluded from Summary Report

Not Reported

All Study Phases

Animal	100	Male	Group 10	Reserve Removed
Animal	101	Male	Group 10	Reserve Removed
Animal	200	Female	Group 10	Reserve Removed
Animal	201	Female	Group 10	Reserve Removed

Incomplete Recordings

Selection of Findings

All findings reported

DAILY OBSERVATIONS - SUMMARY
MALES**ACCLIMATIZATION**

Weeks / Days

1

1 2 3 4 5

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

APPEARANCE

- FISSURES (3)

RIGHT EAR	G	.	.	1	1	1
	%	.	.	1	1	1

No further abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

G : Rounded group means of grades of affected animals

% : Affected animals as percentage to observed animals (See explanation on cover page)

TREATMENT

1 2 3 4 5 6
1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7

No abnormality recorded.

No further abnormality recorded.

No abnormality recorded.

No further abnormality recorded.

% : Affected animals as percentage to observed animals (See explanation on cover page)

DAILY OBSERVATIONS - SUMMARY
MALES

TREATMENT

Weeks / Days
 7 8 9 10 11 12
 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7

Group 1 (0 mg/kg)

APPEARANCE

- SCABS (3)

RIGHT EYE

[illegible]

No further abnormality recorded.

Group 2 (50 mg/kg)

APPEARANCE

- HAIR LOSS (3)

POSTERIOR DORSUM

[illegible]

- FISSURES (3)

RIGHT EAR

[illegible]

No further abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

APPEARANCE

- HAIR LOSS (3)

NECK (CERVICAL)

G	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
%	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

- SCABS (3)

NECK (CERVICAL)

[illegible]

No further abnormality recorded.

G : Rounded group means of grades of affected animals

% : Affected animals as percentage to observed animals (See explanation on cover page)

DAILY OBSERVATIONS - SUMMARY
MALES**TREATMENT**

Weeks / Days												
1 3							1 4					
1	2	3	4	5	6	7	1	2	3	4	5	6

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

APPEARANCE

- HAIR LOSS (3)	
POSTERIOR DORSUM	G 1 1 1 1 1 1 1 1 1
	% 1 1 1 1 1 1 1 1 1

- FISSURES (3)	
RIGHT EAR	G 1 1 1 1 1 1 1 1 1
	% 1 1 1 1 1 1 1 1 1

- NODULE (1)	
LEFT AXILLARY REGION	G . . . 1 1 1 1 1 1
	% . . . 1 1 1 1 1 1

No further abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

G : Rounded group means of grades of affected animals

% : Affected animals as percentage to observed animals (See explanation on cover page)

**DAILY OBSERVATIONS - SUMMARY
FEMALES****ACCLIMATIZATION**

Weeks / Days

1

1 2 3 4 5

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

G : Rounded group means of grades of affected animals

% : Affected animals as percentage to observed animals (See explanation on cover page)

**DAILY OBSERVATIONS - SUMMARY
FEMALES****TREATMENT**

Weeks / Days																																									
1							2							3							4							5							6						
1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

G : Rounded group means of grades of affected animals

% : Affected animals as percentage to observed animals (See explanation on cover page)

**DAILY OBSERVATIONS - SUMMARY
FEMALES****TREATMENT**

Weeks / Days

7		8		9		10		11		12										
1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

G : Rounded group means of grades of affected animals

% : Affected animals as percentage to observed animals (See explanation on cover page)

**DAILY OBSERVATIONS - SUMMARY
FEMALES****TREATMENT**

Weeks / Days													
1 3							1 4						
1	2	3	4	5	6	7	1	2	3	4	5	6	7
<hr/>													

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

G : Rounded group means of grades of affected animals

% : Affected animals as percentage to observed animals (See explanation on cover page)

WEEKLY BEHAVIORAL OBSERVATIONS - SUMMARY**Affected animals as percentage to observed animals**

0 0%
< between 1% and 9%
1 between 10% and 19%
2 between 20% and 29%
... ...
9 between 90% and 99%
A 100%

Data excluded from Summary Report

Not Reported

All Study Phases

Animal	100	Male	Group 10	Reserve Removed
Animal	101	Male	Group 10	Reserve Removed
Animal	200	Female	Group 10	Reserve Removed
Animal	201	Female	Group 10	Reserve Removed

Incomplete Recordings

Selection of Findings

All findings reported

**WEEKLY BEHAVIORAL OBSERVATIONS - SUMMARY
MALES****ACCLIMATIZATION**

Weeks

0

1

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

G : Rounded group means of grades of affected animals

% : Affected animals as percentage to observed animals (See explanation on cover page)

**WEEKLY BEHAVIORAL OBSERVATIONS - SUMMARY
MALES****TREATMENT**

Weeks

0										1			
1	2	3	4	5	6	7	8	9	0	1	2	3	-

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

G : Rounded group means of grades of affected animals

% : Affected animals as percentage to observed animals (See explanation on cover page)

**WEEKLY BEHAVIORAL OBSERVATIONS - SUMMARY
FEMALES****ACCLIMATIZATION**

Weeks

0

1

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

G : Rounded group means of grades of affected animals

% : Affected animals as percentage to observed animals (See explanation on cover page)

**WEEKLY BEHAVIORAL OBSERVATIONS - SUMMARY
FEMALES****TREATMENT**

Weeks

0										1			
1	2	3	4	5	6	7	8	9	0	1	2	3	-

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

G : Rounded group means of grades of affected animals

% : Affected animals as percentage to observed animals (See explanation on cover page)

GRIP STRENGTH - SUMMARY**Data excluded from Summary Report**

Not Reported

All Measurements

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Reported Parameter

Parameter	Statistical Testing
-----------	---------------------

DURING WEEK 13

Grip Fore	GRIP FORELIMB	DUNNETT, MEAN
Grip Hind	GRIP HINDLIMB	DUNNETT, MEAN

DURING WEEK 13

Grip Fore	GRIP FORELIMB	DUNNETT, MEAN
Grip Hind	GRIP HINDLIMB	DUNNETT, MEAN

Statistical Methods

DUNNETT	DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not significant (-)
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GRIP STRENGTH - SUMMARY
DURING WEEK 13
FEMALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
GRIP STRENGTH					

Grip Fore	MEAN	1.32	1.45 -	1.41 -	1.52 *
KILOGRAM	ST.DEV.	0.20	0.10	0.16	0.18
	MINIMUM	0.91	1.30	1.16	1.27
	MAXIMUM	1.62	1.59	1.62	1.83
	N	10	10	10	10
Grip Hind	MEAN	1.11	1.09 -	1.01 -	1.07 -
KILOGRAM	ST.DEV.	0.15	0.08	0.10	0.09
	MINIMUM	0.84	0.97	0.87	0.86
	MAXIMUM	1.39	1.21	1.23	1.17
	N	10	10	10	10

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

GRIP STRENGTH - SUMMARY
DURING WEEK 13
MALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
GRIP STRENGTH					

Grip Fore	MEAN	1.86	1.96 -	1.99 -	2.01 -
KILOGRAM	ST.DEV.	0.25	0.07	0.17	0.13
	MINIMUM	1.31	1.88	1.72	1.82
	MAXIMUM	2.16	2.12	2.21	2.23
	N	10	10	10	10
Grip Hind	MEAN	1.33	1.34 -	1.35 -	1.36 -
KILOGRAM	ST.DEV.	0.08	0.10	0.11	0.08
	MINIMUM	1.21	1.17	1.17	1.22
	MAXIMUM	1.43	1.44	1.49	1.43
	N	10	10	10	10

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

LOCOMOTOR ACTIVITY - SUMMARY**Data excluded from Summary Report**

Not Reported

All Measurements

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Reported Parameter

Parameter	Statistical Testing
-----------	---------------------

DURING WEEK 13

0-10 MIN	LOCOMOTOR ACTIVITY	DUNNETT, MEAN
10-20 MIN	LOCOMOTOR ACTIVITY	DUNNETT, MEAN
20-30 MIN	LOCOMOTOR ACTIVITY	DUNNETT, MEAN
30-40 MIN	LOCOMOTOR ACTIVITY	DUNNETT, MEAN
40-50 MIN	LOCOMOTOR ACTIVITY	DUNNETT, MEAN
50-60 MIN	LOCOMOTOR ACTIVITY	DUNNETT, MEAN
Total	LOCOMOTOR ACTIVITY	DUNNETT, MEAN

DURING WEEK 13

0-10 MIN	LOCOMOTOR ACTIVITY	DUNNETT, MEAN
10-20 MIN	LOCOMOTOR ACTIVITY	DUNNETT, MEAN
20-30 MIN	LOCOMOTOR ACTIVITY	DUNNETT, MEAN
30-40 MIN	LOCOMOTOR ACTIVITY	DUNNETT, MEAN
40-50 MIN	LOCOMOTOR ACTIVITY	DUNNETT, MEAN
50-60 MIN	LOCOMOTOR ACTIVITY	DUNNETT, MEAN
Total	LOCOMOTOR ACTIVITY	DUNNETT, MEAN

Statistical Methods

DUNNETT	DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not significant (-)
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LOCOMOTOR ACTIVITY - SUMMARY
DURING WEEK 13
FEMALES

		Group 1	Group 2	Group 3	Group 4
		0 mg/kg	50 mg/kg	200 mg/kg	1000 mg/kg
LOCOMOTOR ACTIVITY					

0-10 MIN	MEAN	411	459 -	395 -	479 -
	ST.DEV.	79	115	103	96
	MINIMUM	342	220	260	375
	MAXIMUM	616	604	564	638
	N	10	10	10	10
10-20 MIN	MEAN	199	199 -	193 -	199 -
	ST.DEV.	82	113	71	68
	MINIMUM	1	11	111	24
	MAXIMUM	301	344	349	258
	N	10	10	10	10
20-30 MIN	MEAN	162	151 -	136 -	164 -
	ST.DEV.	157	97	97	71
	MINIMUM	0	0	15	19
	MAXIMUM	532	282	281	274
	N	10	10	10	10
30-40 MIN	MEAN	181	183 -	115 -	135 -
	ST.DEV.	174	133	82	82
	MINIMUM	5	0	4	6
	MAXIMUM	574	381	271	258
	N	10	10	10	10
40-50 MIN	MEAN	146	121 -	107 -	87 -
	ST.DEV.	107	90	82	83
	MINIMUM	8	0	0	2
	MAXIMUM	337	262	221	214
	N	10	10	10	10
50-60 MIN	MEAN	98	93 -	68 -	98 -
	ST.DEV.	93	95	107	87
	MINIMUM	2	0	0	0
	MAXIMUM	257	221	323	236
	N	10	10	10	10

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

LOCOMOTOR ACTIVITY - SUMMARY
DURING WEEK 13
FEMALES

		Group 1	Group 2	Group 3	Group 4
		0 mg/kg	50 mg/kg	200 mg/kg	1000 mg/kg

LOCOMOTOR ACTIVITY

Total	MEAN	1196	1205 -	1013 -	1163 -
	ST.DEV.	555	473	283	261
	MINIMUM	560	346	604	743
	MAXIMUM	2583	1848	1511	1524
	N	10	10	10	10

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

LOCOMOTOR ACTIVITY - SUMMARY
DURING WEEK 13
MALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
LOCOMOTOR ACTIVITY					

0-10 MIN	MEAN	468	412 -	509 -	463 -
	ST.DEV.	148	97	102	81
	MINIMUM	300	244	376	375
	MAXIMUM	769	578	700	602
	N	10	10	10	10
10-20 MIN	MEAN	313	218 -	258 -	271 -
	ST.DEV.	121	46	108	131
	MINIMUM	125	142	32	116
	MAXIMUM	538	308	423	559
	N	10	10	10	10
20-30 MIN	MEAN	199	123 -	219 -	168 -
	ST.DEV.	124	73	116	103
	MINIMUM	25	35	32	42
	MAXIMUM	491	266	376	338
	N	10	10	10	10
30-40 MIN	MEAN	190	139 -	145 -	117 -
	ST.DEV.	92	77	99	85
	MINIMUM	48	14	19	0
	MAXIMUM	331	286	355	248
	N	10	10	10	10
40-50 MIN	MEAN	98	102 -	107 -	110 -
	ST.DEV.	73	80	128	87
	MINIMUM	14	1	0	7
	MAXIMUM	202	254	364	266
	N	10	10	10	10
50-60 MIN	MEAN	166	83 -	118 -	160 -
	ST.DEV.	145	67	117	153
	MINIMUM	0	4	0	14
	MAXIMUM	431	189	384	410
	N	10	10	10	10

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

LOCOMOTOR ACTIVITY - SUMMARY
DURING WEEK 13
MALES

		Group 1	Group 2	Group 3	Group 4
		0 mg/kg	50 mg/kg	200 mg/kg	1000 mg/kg

LOCOMOTOR ACTIVITY

Total	MEAN	1434	1076 -	1355 -	1288 -
	ST.DEV.	477	204	403	327
	MINIMUM	903	851	897	934
	MAXIMUM	2283	1493	1992	1946
	N	10	10	10	10

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

FOOD CONSUMPTION (G/ANIMAL/DAY) - SUMMARY**Data excluded from Summary Report**

Not Reported

All Study Phases

Cage	13	Male	Group 10	Reserve Removed
Cage	26	Female	Group 10	Reserve Removed

**FOOD CONSUMPTION (G/ANIMAL/DAY) - SUMMARY
MALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
ACCLIMATIZATION					
Days 1-6	MEAN	22.6	22.3 -	23.0 -	22.9 -
	ST.DEV.	1.1	0.6	1.6	1.5
	N	3	3	3	3
TREATMENT					
Days 1-8	MEAN	24.7	23.7 -	24.5 -	24.1 -
	ST.DEV.	1.7	0.7	1.1	1.9
	N	3	3	3	3
Days 8-15	MEAN	25.9	25.2 -	26.4 -	25.6 -
	ST.DEV.	1.7	1.0	0.7	1.6
	N	3	3	3	3
Days 15-22	MEAN	25.6	24.8 -	25.9 -	25.4 -
	ST.DEV.	1.9	0.8	1.0	0.6
	N	3	3	3	3
Days 22-29	MEAN	25.4	24.5 -	25.8 -	25.1 -
	ST.DEV.	1.7	0.9	0.7	1.4
	N	3	3	3	3
Days 29-36	MEAN	24.4	24.1 -	25.2 -	24.0 -
	ST.DEV.	1.7	0.5	0.7	2.1
	N	3	3	3	3
Days 36-43	MEAN	23.8	23.9 -	24.4 -	23.0 -
	ST.DEV.	1.5	0.3	0.9	1.9
	N	3	3	3	3
Days 43-50	MEAN	23.9	24.0 -	24.6 -	22.5 -
	ST.DEV.	1.3	0.5	0.7	2.2
	N	3	3	3	3
Days 50-57	MEAN	24.1	24.0 -	24.4 -	22.9 -
	ST.DEV.	1.6	0.6	0.9	1.5
	N	3	3	3	3
Days 57-64	MEAN	23.6	23.3 -	24.2 -	22.7 -
	ST.DEV.	1.0	0.5	0.9	1.8
	N	3	3	3	3
Days 64-71	MEAN	23.2	22.5 -	23.8 -	22.5 -
	ST.DEV.	0.9	1.8	1.3	1.6
	N	3	3	3	3

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**FOOD CONSUMPTION (G/ANIMAL/DAY) - SUMMARY
MALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Days 71-78	MEAN	23.2	23.4 -	24.2 -	23.4 -
	ST.DEV.	1.1	1.0	0.7	1.6
	N	3	3	3	3
Days 78-85	MEAN	23.0	23.3 -	23.7 -	23.3 -
	ST.DEV.	3.2	1.6	1.1	1.5
	N	3	3	3	3
Days 85-92	MEAN	21.2	23.2 -	23.3 -	22.3 -
	ST.DEV.	1.3	1.0	0.9	1.4
	N	3	3	3	3
MEAN OF MEANS Over TREATMENT		24.0	23.8	24.7	23.6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**FOOD CONSUMPTION (G/ANIMAL/DAY) - SUMMARY
FEMALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
ACCLIMATIZATION					
Days 1-6	MEAN	15.6	15.5 -	16.3 -	16.1 -
	ST.DEV.	0.4	0.5	1.0	0.2
	N	3	3	3	3
TREATMENT					
Days 1-8	MEAN	15.4	15.3 -	16.2 -	15.5 -
	ST.DEV.	0.2	0.4	1.0	0.5
	N	3	3	3	3
Days 8-15	MEAN	16.3	16.5 -	17.1 -	16.3 -
	ST.DEV.	0.8	0.7	0.9	0.5
	N	3	3	3	3
Days 15-22	MEAN	16.7	16.9 -	17.1 -	16.6 -
	ST.DEV.	0.4	0.7	1.4	0.5
	N	3	3	3	3
Days 22-29	MEAN	17.0	17.3 -	17.2 -	16.2 -
	ST.DEV.	0.6	0.4	1.1	0.5
	N	3	3	3	3
Days 29-36	MEAN	16.3	17.0 -	16.6 -	15.8 -
	ST.DEV.	0.6	0.6	0.8	0.3
	N	3	3	3	3
Days 36-43	MEAN	16.1	16.7 -	16.1 -	16.1 -
	ST.DEV.	1.0	0.6	0.7	0.3
	N	3	3	3	3
Days 43-50	MEAN	16.2	16.9 -	16.1 -	16.4 -
	ST.DEV.	1.0	0.9	1.3	0.3
	N	3	3	3	3
Days 50-57	MEAN	16.2	17.2 -	16.3 -	16.5 -
	ST.DEV.	0.7	0.5	0.9	0.6
	N	3	3	3	3
Days 57-64	MEAN	16.2	17.2 -	16.4 -	16.0 -
	ST.DEV.	0.8	0.9	0.7	0.7
	N	3	3	3	3
Days 64-71	MEAN	15.9	16.8 -	15.8 -	16.0 -
	ST.DEV.	1.1	0.7	0.6	0.7
	N	3	3	3	3

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**FOOD CONSUMPTION (G/ANIMAL/DAY) - SUMMARY
FEMALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Days 71-78	MEAN	16.0	17.3 -	16.1 -	16.3 -
	ST.DEV.	1.0	0.4	1.1	0.8
	N	3	3	3	3
Days 78-85	MEAN	15.5	16.4 -	15.2 -	15.3 -
	ST.DEV.	0.9	0.4	1.3	0.5
	N	3	3	3	3
Days 85-92	MEAN	15.3	16.1 -	14.6 -	14.8 -
	ST.DEV.	0.8	0.4	1.0	1.0
	N	3	3	3	3
MEAN OF MEANS Over TREATMENT		16.1	16.7	16.2	16.0

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) - SUMMARY

Data excluded from Summary Report

Not Reported

All Study Phases

Cage	13	Male	Group 10	Reserve Removed
Cage	26	Female	Group 10	Reserve Removed

**RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) - SUMMARY
MALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
ACCLIMATIZATION					
Days 1-6	MEAN	108.1	106.0 -	107.0 -	110.1 -
	ST.DEV.	4.5	0.6	2.8	7.6
	N	3	3	3	3
TREATMENT					
Days 1-8	MEAN	88.5	85.7 -	87.6 -	87.5 -
	ST.DEV.	3.9	0.7	1.2	4.6
	N	3	3	3	3
Days 8-15	MEAN	84.0	82.5 -	83.6 -	83.3 -
	ST.DEV.	3.8	1.1	1.7	3.2
	N	3	3	3	3
Days 15-22	MEAN	77.6	75.9 -	76.7 -	77.8 -
	ST.DEV.	3.6	0.8	1.0	2.8
	N	3	3	3	3
Days 22-29	MEAN	73.4	71.5 -	72.4 -	72.9 -
	ST.DEV.	3.1	0.7	1.3	2.3
	N	3	3	3	3
Days 29-36	MEAN	67.6	67.7 -	68.2 -	66.7 -
	ST.DEV.	2.8	0.8	1.7	2.2
	N	3	3	3	3
Days 36-43	MEAN	63.6	64.3 -	63.3 -	61.9 -
	ST.DEV.	2.0	1.3	1.3	1.1
	N	3	3	3	3
Days 43-50	MEAN	61.9	62.7 -	61.8 -	59.3 -
	ST.DEV.	2.0	1.7	1.7	1.4
	N	3	3	3	3
Days 50-57	MEAN	61.2	60.8 -	59.7 -	58.6 -
	ST.DEV.	2.6	1.2	1.5	1.2
	N	3	3	3	3
Days 57-64	MEAN	58.0	57.9 -	57.8 -	56.5 -
	ST.DEV.	0.6	0.8	1.4	0.2
	N	3	3	3	3
Days 64-71	MEAN	56.4	55.0 -	55.9 -	54.9 -
	ST.DEV.	0.2	3.4	0.6	0.9
	N	3	3	3	3

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) - SUMMARY
MALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Days 71-78	MEAN	54.9	55.9 -	55.7 -	56.0 -
	ST.DEV.	0.7	1.6	1.7	0.9
	N	3	3	3	3
Days 78-85	MEAN	54.0	55.0 -	54.0 -	54.7 -
	ST.DEV.	5.8	1.4	1.2	1.1
	N	3	3	3	3
Days 85-92	MEAN	49.4	54.0 **	52.5 *	52.0 *
	ST.DEV.	1.0	0.9	1.2	0.5
	N	3	3	3	3
MEAN OF MEANS Over TREATMENT		65.4	65.3	65.3	64.8

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) - SUMMARY
FEMALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
ACCLIMATIZATION					
Days 1-6	MEAN	111.2	109.9 -	114.9 -	115.0 -
	ST.DEV.	5.0	0.8	6.1	4.3
	N	3	3	3	3
TREATMENT					
Days 1-8	MEAN	91.4	91.2 -	94.5 -	91.1 -
	ST.DEV.	3.2	4.0	4.2	0.9
	N	3	3	3	3
Days 8-15	MEAN	90.0	92.4 -	91.3 -	90.7 -
	ST.DEV.	5.1	2.9	5.5	0.8
	N	3	3	3	3
Days 15-22	MEAN	87.8	89.5 -	87.2 -	87.1 -
	ST.DEV.	3.9	4.2	6.0	1.6
	N	3	3	3	3
Days 22-29	MEAN	85.9	87.6 -	83.7 -	80.1 -
	ST.DEV.	1.7	3.4	3.8	1.0
	N	3	3	3	3
Days 29-36	MEAN	79.9	82.2 -	79.2 -	76.2 -
	ST.DEV.	1.8	4.4	2.5	1.5
	N	3	3	3	3
Days 36-43	MEAN	77.1	80.2 -	74.8 -	77.7 -
	ST.DEV.	5.0	3.3	3.3	0.7
	N	3	3	3	3
Days 43-50	MEAN	76.6	79.6 -	73.8 -	76.5 -
	ST.DEV.	5.3	4.0	5.5	1.4
	N	3	3	3	3
Days 50-57	MEAN	74.7	78.7 -	72.6 -	74.1 -
	ST.DEV.	1.0	3.9	3.0	0.7
	N	3	3	3	3
Days 57-64	MEAN	73.6	76.3 -	71.6 -	70.8 -
	ST.DEV.	2.8	3.9	2.2	0.7
	N	3	3	3	3
Days 64-71	MEAN	71.5	73.7 -	68.2 -	70.9 -
	ST.DEV.	3.4	3.4	3.5	1.2
	N	3	3	3	3

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) - SUMMARY
FEMALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Days 71-78	MEAN	70.1	74.0 -	68.1 -	70.3 -
	ST.DEV.	5.1	0.8	4.9	1.6
	N	3	3	3	3
Days 78-85	MEAN	69.1	72.0 -	65.8 -	67.1 -
	ST.DEV.	5.3	3.5	4.0	0.7
	N	3	3	3	3
Days 85-92	MEAN	66.3	67.7 -	62.0 -	63.5 -
	ST.DEV.	5.0	1.3	5.5	1.8
	N	3	3	3	3
MEAN OF MEANS Over TREATMENT		78.0	80.4	76.4	76.6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

BODY WEIGHTS (G) - SUMMARY**Data excluded from Summary Report**

Not Reported

All Study Phases

Animal	100	Male	Group 10	Reserve Removed
Animal	101	Male	Group 10	Reserve Removed
Animal	200	Female	Group 10	Reserve Removed
Animal	201	Female	Group 10	Reserve Removed

**BODY WEIGHTS (G) - SUMMARY
MALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
PRE-RANDOMIZATION					
Day 1	MEAN	194	193 -	198 -	196 -
	ST.DEV.	13	13	17	14
	N	10	10	10	10
ACCLIMATIZATION					
Day 1	MEAN	210	211 -	213 -	210 -
	ST.DEV.	14	11	16	15
	N	10	10	10	10
TREATMENT					
Day 1	MEAN	242	243 -	244 -	239 -
	ST.DEV.	14	16	20	19
	N	10	10	10	10
Day 8	MEAN	279	277 -	278 -	277 -
	ST.DEV.	15	21	25	20
	N	10	10	10	10
Day 15	MEAN	309	307 -	315 -	308 -
	ST.DEV.	17	25	29	22
	N	10	10	10	10
Day 22	MEAN	331	328 -	336 -	328 -
	ST.DEV.	19	29	34	25
	N	10	10	10	10
Day 29	MEAN	347	343 -	355 -	346 -
	ST.DEV.	20	35	39	28
	N	10	10	10	10
Day 36	MEAN	361	358 -	369 -	361 -
	ST.DEV.	20	41	42	29
	N	10	10	10	10
Day 43	MEAN	374	373 -	384 -	373 -
	ST.DEV.	21	40	45	30
	N	10	10	10	10
Day 50	MEAN	386	385 -	397 -	381 -
	ST.DEV.	20	44	49	32
	N	10	10	10	10
Day 57	MEAN	395	397 -	407 -	393 -
	ST.DEV.	22	43	49	31
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**BODY WEIGHTS (G) - SUMMARY
MALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 64	MEAN	409	405 -	416 -	402 -
	ST.DEV.	22	43	53	35
	N	10	10	10	10
Day 71	MEAN	412	412 -	422 -	412 -
	ST.DEV.	25	45	53	35
	N	10	10	10	10
Day 78	MEAN	424	420 -	432 -	421 -
	ST.DEV.	27	50	56	37
	N	10	10	10	10
Day 85	MEAN	427	425 -	436 -	429 -
	ST.DEV.	29	51	56	36
	N	10	10	10	10
Day 92	MEAN	430	432 -	441 -	431 -
	ST.DEV.	28	48	57	35
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**BODY WEIGHTS (G) - SUMMARY
FEMALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
PRE-RANDOMIZATION					
Day 1	MEAN	130	129 -	128 -	128 -
	ST.DEV.	8	7	7	6
	N	10	10	10	10
ACCLIMATIZATION					
Day 1	MEAN	141	141 -	141 -	140 -
	ST.DEV.	6	8	6	7
	N	10	10	10	10
TREATMENT					
Day 1	MEAN	155	153 -	156 -	156 -
	ST.DEV.	7	10	8	8
	N	10	10	10	10
Day 8	MEAN	169	168 -	171 -	170 -
	ST.DEV.	9	8	12	8
	N	10	10	10	10
Day 15	MEAN	182	179 -	187 -	180 -
	ST.DEV.	9	11	11	10
	N	10	10	10	10
Day 22	MEAN	190	189 -	195 -	191 -
	ST.DEV.	9	10	13	10
	N	10	10	10	10
Day 29	MEAN	197	198 -	204 -	202 -
	ST.DEV.	10	13	14	9
	N	10	10	10	10
Day 36	MEAN	203	207 -	209 -	207 -
	ST.DEV.	8	14	16	10
	N	10	10	10	10
Day 43	MEAN	209	208 -	214 -	207 -
	ST.DEV.	11	16	16	11
	N	10	10	10	10
Day 50	MEAN	212	211 -	217 -	215 -
	ST.DEV.	11	13	18	10
	N	10	10	10	10
Day 57	MEAN	215	218 -	224 -	223 -
	ST.DEV.	13	16	17	9
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**BODY WEIGHTS (G) - SUMMARY
FEMALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 64	MEAN	220	226 -	228 -	225 -
	ST.DEV.	12	18	18	9
	N	10	10	10	10
Day 71	MEAN	221	227 -	231 -	225 -
	ST.DEV.	14	17	17	10
	N	10	10	10	10
Day 78	MEAN	228	233 -	236 -	232 -
	ST.DEV.	16	14	19	10
	N	10	10	10	10
Day 85	MEAN	224	228 -	230 -	228 -
	ST.DEV.	14	15	21	11
	N	10	10	10	10
Day 92	MEAN	231	237 -	234 -	233 -
	ST.DEV.	15	16	22	14
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

BODY WEIGHT GAIN (%) - SUMMARY**Data excluded from Summary Report**

Not Reported

All Study Phases

Animal	100	Male	Group 10	Reserve Removed
Animal	101	Male	Group 10	Reserve Removed
Animal	200	Female	Group 10	Reserve Removed
Animal	201	Female	Group 10	Reserve Removed

**BODY WEIGHT GAIN (%) - SUMMARY
MALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 1	MEAN	0.0	0.0	0.0	0.0
	ST.DEV.	0.0	0.0	0.0	0.0
	N	10	10	10	10
Day 8	MEAN	15.5	13.8 -	14.0 -	16.0 -
	ST.DEV.	2.7	2.5	4.0	2.7
	N	10	10	10	10
Day 15	MEAN	27.6	26.0 -	28.9 -	29.1 -
	ST.DEV.	3.8	3.8	5.4	2.8
	N	10	10	10	10
Day 22	MEAN	36.8	34.9 -	37.8 -	37.5 -
	ST.DEV.	5.2	5.8	8.2	3.2
	N	10	10	10	10
Day 29	MEAN	43.4	41.1 -	45.5 -	45.1 -
	ST.DEV.	6.2	8.1	10.1	5.1
	N	10	10	10	10
Day 36	MEAN	49.2	46.9 -	51.1 -	51.4 -
	ST.DEV.	6.0	10.7	12.1	6.7
	N	10	10	10	10
Day 43	MEAN	54.7	53.1 -	57.3 -	56.2 -
	ST.DEV.	6.0	10.2	12.3	7.1
	N	10	10	10	10
Day 50	MEAN	59.7	58.1 -	62.4 -	59.5 -
	ST.DEV.	6.7	11.5	13.7	7.5
	N	10	10	10	10
Day 57	MEAN	63.4	63.0 -	66.6 -	64.7 -
	ST.DEV.	7.7	11.5	13.5	8.3
	N	10	10	10	10
Day 64	MEAN	69.0	66.3 -	70.4 -	68.7 -
	ST.DEV.	7.3	11.8	15.4	8.9
	N	10	10	10	10
Day 71	MEAN	70.4	69.2 -	72.9 -	73.0 -
	ST.DEV.	9.4	12.4	16.1	9.6
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**BODY WEIGHT GAIN (%) - SUMMARY
MALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 78	MEAN	75.3	72.6 -	77.1 -	76.2 -
	ST.DEV.	9.5	14.1	17.5	8.6
	N	10	10	10	10
Day 85	MEAN	76.5	74.7 -	78.8 -	79.7 -
	ST.DEV.	10.7	14.6	17.8	9.6
	N	10	10	10	10
Day 92	MEAN	78.0	77.6 -	80.8 -	80.7 -
	ST.DEV.	10.0	13.6	17.5	8.3
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**BODY WEIGHT GAIN (%) - SUMMARY
FEMALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 1	MEAN	0.0	0.0	0.0	0.0
	ST.DEV.	0.0	0.0	0.0	0.0
	N	10	10	10	10
Day 8	MEAN	8.9	9.7 -	9.2 -	9.2 -
	ST.DEV.	3.3	4.7	3.3	2.3
	N	10	10	10	10
Day 15	MEAN	17.4	17.0 -	19.7 -	15.4 -
	ST.DEV.	4.7	8.2	2.8	2.6
	N	10	10	10	10
Day 22	MEAN	22.7	23.4 -	24.8 -	22.4 -
	ST.DEV.	5.5	7.2	4.0	4.9
	N	10	10	10	10
Day 29	MEAN	27.6	29.3 -	30.5 -	29.7 -
	ST.DEV.	5.8	5.5	3.8	4.9
	N	10	10	10	10
Day 36	MEAN	31.4	35.3 -	33.6 -	32.6 -
	ST.DEV.	4.6	8.3	4.5	5.2
	N	10	10	10	10
Day 43	MEAN	35.0	36.3 -	36.8 -	32.9 -
	ST.DEV.	6.8	10.8	5.7	3.5
	N	10	10	10	10
Day 50	MEAN	36.7	38.3 -	38.8 -	38.1 -
	ST.DEV.	7.7	10.1	6.9	7.1
	N	10	10	10	10
Day 57	MEAN	39.3	42.6 -	43.0 -	43.0 -
	ST.DEV.	8.3	10.1	7.0	6.8
	N	10	10	10	10
Day 64	MEAN	41.9	47.5 -	45.5 -	44.7 -
	ST.DEV.	7.0	12.4	6.6	5.8
	N	10	10	10	10
Day 71	MEAN	43.1	48.6 -	47.8 -	44.8 -
	ST.DEV.	8.6	12.3	6.8	5.5
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**BODY WEIGHT GAIN (%) - SUMMARY
FEMALES**

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 78	MEAN	47.1	52.3 -	50.4 -	49.0 -
	ST.DEV.	9.4	11.0	8.1	7.6
	N	10	10	10	10
Day 85	MEAN	44.5	48.8 -	47.0 -	46.7 -
	ST.DEV.	7.8	10.8	9.0	6.9
	N	10	10	10	10
Day 92	MEAN	48.9	54.9 -	49.6 -	49.7 -
	ST.DEV.	7.8	10.9	9.9	7.5
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

OPHTHALMOSCOPY**Data excluded from Summary Report**

Not Reported

All Study Phases

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Reported Grades

No grade conversion defined

OPHTHALMOSCOPY
ACCLIMATIZATION, Day 2
MALES

	Group 1		Group 2		Group 3		Group 4	
	0 mg/kg		50 mg/kg		200 mg/kg		1000 mg/kg	
Animals observed	10		10		10		10	
	Mean	%	Mean	%	Mean	%	Mean	%
Unscheduled Findings								
CORNEA								
- CORNEAL OPACITY (3)								
LEFT EYE	1.3	40%	1.3	30% -	1.0	10% -	1.4	50% -
RIGHT EYE	1.0	30%	1.0	10% -	1.0	20% -	1.0	20% -
Total:	2.3		2.3		2.0		2.4	
VITREOUS BODY								
- PERSISTENT HYALOID VESSEL (1)								
LEFT EYE	1.0	10%	1.0	20% -	-	0% -	1.0	10% -
RIGHT EYE	1.0	10%	1.0	20% -	1.0	10% -	1.0	10% -
Total:	2.0		2.0		1.0		2.0	
IRIS								
- PERSISTENT PUPILLARY MEMB (1)								
RIGHT EYE	1.0	10%	-	0% -	-	0% -	-	0% -
Total:	1.0							

% : Percentage of affected animals

*/**/- : Fisher's Exact Test significant at 5% (*), 1% (**) or not significant (-)

OPHTHALMOSCOPY
ACCLIMATIZATION, Day 2
FEMALES

	Group 1		Group 2		Group 3		Group 4	
	0 mg/kg		50 mg/kg		200 mg/kg		1000 mg/kg	
Animals observed	10		10		10		10	
	Mean	%	Mean	%	Mean	%	Mean	%
Unscheduled Findings								
CORNEA								
- CORNEAL OPACITY (3)								
LEFT EYE	1.0	10%	1.0	30% -	1.0	20% -	1.0	40% -
RIGHT EYE	1.0	20%	1.0	20% -	1.0	20% -	1.0	30% -
Total:	2.0		2.0		2.0		2.0	
LENS								
- LENTICULAR VACUOLE (3)								
RIGHT EYE	-	0%	-	0% -	-	0% -	1.0	10% -
Total:							1.0	
VITREOUS BODY								
- PERSISTENT HYALOID VESSEL (1)								
LEFT EYE	1.0	10%	-	0% -	-	0% -	1.0	20% -
RIGHT EYE	-	0%	-	0% -	1.0	10% -	-	0% -
Total:	1.0				1.0		1.0	

% : Percentage of affected animals

*/**/- : Fisher's Exact Test significant at 5% (*), 1% (**) or not significant (-)

OPHTHALMOSCOPY
GR. 1 AND GR. 4
TREATMENT, Day 89
MALES

	Group 1		Group 4	
	0 mg/kg		1000 mg/kg	
Animals observed	10		10	
	Mean	%	Mean	%
Unscheduled Findings				
CORNEA				
- CORNEAL OPACITY (3)				
LEFT EYE	1.0	40%	1.0	50% -
RIGHT EYE	1.0	40%	1.0	50% -
Total:	2.0		2.0	
VITREOUS BODY				
- PERSISTENT HYALOID VESSEL (1)				
LEFT EYE	-	0%	-	0% -
RIGHT EYE	-	0%	-	0% -
IRIS				
- PERSISTENT PUPILLARY MEMB (1)				
RIGHT EYE	-	0%	-	0% -

% : Percentage of affected animals

*/**/- : Fisher's Exact Test significant at 5% (*), 1% (**) or not significant (-)

OPHTHALMOSCOPY
GR. 1 AND GR. 4
TREATMENT, Day 89
FEMALES

	Group 1		Group 4	
	0 mg/kg		1000 mg/kg	
Animals observed	10		10	
	Mean	%	Mean	%
Unscheduled Findings				
CORNEA				
- CORNEAL OPACITY (3)				
LEFT EYE	1.0	20%	1.0	30% -
RIGHT EYE	1.0	40%	1.0	30% -
Total:	2.0		2.0	
LENS				
- LENTICULAR VACUOLE (3)				
RIGHT EYE	-	0%	-	0% -
VITREOUS BODY				
- PERSISTENT HYALOID VESSEL (1)				
LEFT EYE	-	0%	-	0% -

% : Percentage of affected animals

*/**/- : Fisher's Exact Test significant at 5% (*), 1% (**) or not significant (-)

Hematology - SUMMARY

Data excluded from Summary Report

Not Reported

All Measurements

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Reported Parameter

Parameter

Statistical Testing

After 13 Weeks

RBC	ERYTHROCYTES (RBC)	DUNNETT
HB	HEMOGLOBIN (HB)	DUNNETT
HCT	HEMATOCRIT (HCT)	DUNNETT
MCV	MEAN CORPUSCULAR VOLUME (MCV)	DUNNETT
RDW	RED CELL VOL. DISTR. WIDTH (RDW)	DUNNETT
MCH	MEAN CORPUSCULAR HEMOGLOBIN (MCH)	DUNNETT
MCHC	MEAN CORPUSCULAR HEMOGLOBIN CONC. (MCHC)	DUNNETT
HDW	HEMOGLOBIN CONC. DISTR. WIDTH	DUNNETT
RETI	RETICULOCYTE (REL)	STEEL
RETI	RETICULOCYTE (ABS)	DUNNETT
L RETI	MATURITY INDEX (L-RETI)	STEEL
M RETI	MATURITY INDEX (M-RETI)	STEEL
H RETI	MATURITY INDEX (H-RETI)	STEEL
WBC	LEUKOCYTES, TOTAL (WBC)	DUNNETT
NEUT	NEUTROPHILS (NEUT)	STEEL
EOS	EOSINOPHILS (EOS)	STEEL
BASO	BASOPHILS (BASO)	STEEL
LYMPH	LYMPHOCYTES (LYMPH)	STEEL
MONO	MONOCYTES (MONO)	STEEL
LUC	LARGE UNSTAINED CELLS (LUC)	STEEL
NEUT	NEUTROPHILS (NEUT)	DUNNETT
EOS	EOSINOPHILS (EOS)	DUNNETT
BASO	BASOPHILS (BASO)	DUNNETT
LYMPH	LYMPHOCYTES (LYMPH)	DUNNETT
MONO	MONOCYTES (MONO)	DUNNETT
LUC	LARGE UNSTAINED CELLS (LUC)	DUNNETT
PLATELETS	THROMBOCYTES (PLATELETS)	DUNNETT
MET-HB	METHEMOGLOBIN (MET-HB)	STEEL
PT	PROTHROMBIN TIME (PT)	STEEL
PTT	PARTIAL THROMBOPLASTIN TIME (PTT)	STEEL

Hematology - SUMMARY**Statistical Methods**

DUNNETT DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not significant (-)

STEEL STEEL-Test sig. at 5% (*), 1% (**) or not significant (-)

Hematology - SUMMARY

MALES

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. 1	fl	rel. 1	fmol	mmol/l
After 13 Weeks							
Group 1	9.13	10.0	0.48	52.3	0.146	1.10	21.00
Group 2	9.24 -	9.9 -	0.48 -	51.7 -	0.145 -	1.08 -	20.80 -
Group 3	8.96 -	10.1 -	0.48 -	53.0 -	0.145 -	1.13 -	21.23 -
Group 4	9.10 -	9.9 -	0.47 -	51.9 -	0.131 -	1.10 -	21.09 -

	GENERAL		RETICULOCYTE COUNT				GENERAL
	HDW	RETI	RETI	L RETI	M RETI	H RETI	WBC
	mmol/l	rel. 1	G/l	rel. 1	rel. 1	rel. 1	G/l
After 13 Weeks							
Group 1	1.88	0.022	210	0.626	0.316	0.064	7.06
Group 2	1.74 -	0.023 -	214 -	0.634 -	0.318 -	0.046 -	6.74 -
Group 3	1.77 -	0.022 -	200 -	0.629 -	0.315 -	0.055 -	6.51 -
Group 4	1.59 **	0.019 -	190 -	0.641 -	0.310 -	0.047 -	6.87 -

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
After 13 Weeks						
Group 1	0.168	0.021	0.003	0.787	0.020	0.004
Group 2	0.218 -	0.029 -	0.004 -	0.718 *	0.025 -	0.004 -
Group 3	0.243 *	0.024 -	0.004 -	0.699 *	0.023 -	0.004 -
Group 4	0.197 -	0.026 -	0.005 -	0.749 -	0.019 -	0.004 -

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

Hematology - SUMMARY

MALES

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT	EOS	BASO	LYMPH	MONO	LUC	PLATELETS
	G/l	G/l	G/l	G/l	G/l	G/l	G/l
After 13 Weeks							
Group 1	1.21	0.15	0.02	5.49	0.16	0.03	954
Group 2	1.52 -	0.19 *	0.03 -	4.80 -	0.18 -	0.03 -	1009 -
Group 3	1.77 -	0.14 -	0.02 -	4.40 -	0.14 -	0.03 -	924 -
Group 4	1.59 -	0.17 -	0.03 -	4.90 -	0.14 -	0.03 -	953 -

	GENERAL	COAGULATION	
	MET-HB	PT	PTT
	rel. 1	rel. 1	sec
After 13 Weeks			
Group 1	0.012	0.81	24.0
Group 2	0.013 -	0.80 -	23.2 -
Group 3	0.012 -	0.88 -	22.1 -
Group 4	0.013 -	0.89 **	23.4 -

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

Hematology - SUMMARY

FEMALES

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. 1	fl	rel. 1	fmol	mmol/l
After 13 Weeks							
Group 1	7.82	9.1	0.43	54.9	0.123	1.16	21.21
Group 2	7.84 -	9.1 -	0.43 -	55.0 -	0.116 -	1.16 -	21.09 -
Group 3	7.89 -	9.1 -	0.43 -	54.2 -	0.139 -	1.16 -	21.37 -
Group 4	7.88 -	9.2 -	0.43 -	55.0 -	0.119 -	1.18 -	21.37 -

	GENERAL	RETICULOCYTE COUNT					GENERAL
	HDW	RETI	RETI	L RETI	M RETI	H RETI	WBC
	mmol/l	rel. 1	G/l	rel. 1	rel. 1	rel. 1	G/l
After 13 Weeks							
Group 1	1.47	0.032	252	0.528	0.388	0.062	3.41
Group 2	1.38 -	0.031 -	246 -	0.555 -	0.384 -	0.061 -	2.76 -
Group 3	1.47 -	0.027 -	222 -	0.638 -	0.337 -	0.033 -	2.52 *
Group 4	1.43 -	0.027 -	234 -	0.645 -	0.323 -	0.034 -	3.34 -

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
After 13 Weeks						
Group 1	0.170	0.020	0.003	0.775	0.022	0.007
Group 2	0.208 -	0.029 -	0.003 -	0.721 -	0.023 -	0.005 -
Group 3	0.210 -	0.037 **	0.003 -	0.728 *	0.020 -	0.004 -
Group 4	0.241 -	0.032 -	0.002 -	0.704 -	0.023 -	0.004 -

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

Hematology - SUMMARY

FEMALES

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT	EOS	BASO	LYMPH	MONO	LUC	PLATELETS
	G/l	G/l	G/l	G/l	G/l	G/l	G/l
After 13 Weeks							
Group 1	0.58	0.07	0.01	2.66	0.07	0.02	1064
Group 2	0.54 -	0.08 -	0.01 -	2.06 -	0.06 -	0.01 -	1021 -
Group 3	0.54 -	0.09 -	0.01 -	1.82 *	0.05 -	0.01 -	974 -
Group 4	0.80 -	0.09 *	0.01 -	2.35 -	0.07 -	0.02 -	1083 -

	GENERAL	COAGULATION	
	MET-HB	PT	PTT
	rel. 1	rel. 1	sec
After 13 Weeks			
Group 1	0.015	0.79	31.6
Group 2	0.015 -	0.81 -	31.1 -
Group 3	0.015 -	0.80 -	32.7 -
Group 4	0.015 -	0.87 *	28.4 -

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

Biochemistry - SUMMARY**Data excluded from Summary Report**

Not Reported

All Measurements

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Reported Parameter

Parameter		Statistical Testing
After 13 Weeks		
GLUCOSE	GLUCOSE	DUNNETT
UREA	UREA	DUNNETT
CREAT	CREATININE	DUNNETT
BILI-T	BILIRUBIN, TOTAL	DUNNETT
BILE AC	BILE ACIDS	DUNNETT
CHOLEST	CHOLESTEROL, TOTAL	DUNNETT
TRIGLY	TRIGLYCERIDES	DUNNETT
PHOS-LIP	PHOSPHOLIPIDS	DUNNETT
ASAT	ASPARTATE AMINOTRANSFERASE (ASAT)	DUNNETT
ALAT	ALANINE AMINOTRANSFERASE (ALAT)	DUNNETT
LDH	LACTATE DEHYDROGENASE (LDH)	DUNNETT
ALP	ALKALINE PHOSPHATASE (ALP)	DUNNETT
GGT	GAMMA-GLUTAMYLTRANSFERASE (GGT)	STEEL
CK	CREATINE KINASE (CK)	DUNNETT
SODIUM	SODIUM	DUNNETT
POTASSIUM	POTASSIUM	DUNNETT
CHLORIDE	CHLORIDE	DUNNETT
CALCIUM	CALCIUM	DUNNETT
PHOSPHORUS	PHOSPHORUS	DUNNETT
PROTEIN	PROTEIN, TOTAL	DUNNETT
ALBUMIN	ALBUMIN	DUNNETT
GLOBULIN	GLOBULIN	DUNNETT
A/G RATIO	A/G RATIO	STEEL

Statistical Methods

DUNNETT	DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not significant (-)
STEEL	STEEL-Test sig. at 5% (*), 1% (**) or not significant (-)

Biochemistry - SUMMARY

MALES

	GENERAL						
	GLUCOSE	UREA	CREAT	BILI-T	BILE AC	CHOLEST	TRIGLY
	mmol/l	mmol/l	µmol/l	µmol/l	µmol/l	mmol/l	mmol/l
After 13 Weeks							
Group 1	7.11	5.26	25.5	1.17	16.59	2.62	0.47
Group 2	6.70 -	5.73 -	27.7 -	0.56 -	10.08 -	2.73 -	0.71 -
Group 3	7.83 -	5.55 -	27.1 -	0.39 -	10.69 -	2.68 -	0.53 -
Group 4	8.20 *	5.03 -	24.9 -	0.00 **	9.25 -	2.62 -	0.54 -

	GENERAL						
	PHOS-LIP	ASAT	ALAT	LDH	ALP	GGT	CK
	mmol/l	U/l	U/l	U/l	U/l	U/l	U/l
After 13 Weeks							
Group 1	2.02	64.3	23.9	117.3	52.3	0.0	122.0
Group 2	2.07 -	65.2 -	23.4 -	108.5 -	52.9 -	0.0 -	116.9 -
Group 3	2.10 -	83.3 -	31.3 -	229.3 -	49.2 -	0.0 -	143.8 -
Group 4	2.07 -	65.9 -	27.8 -	125.4 -	55.4 -	0.0 -	104.4 -

	GENERAL						
	SODIUM	POTASSIUM	CHLORIDE	CALCIUM	PHOSPHORUS	PROTEIN	ALBUMIN
	mmol/l	mmol/l	mmol/l	mmol/l	mmol/l	g/l	g/l
After 13 Weeks							
Group 1	144.0	4.23	101.3	2.74	1.72	69.14	43.06
Group 2	144.7 -	4.23 -	102.3 -	2.72 -	1.59 -	68.01 -	42.16 -
Group 3	145.3 -	4.29 -	103.0 *	2.67 -	1.60 -	67.64 -	42.49 -
Group 4	144.7 -	4.68 *	103.0 *	2.64 *	1.57 -	66.91 -	41.80 -

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

Biochemistry - SUMMARY
MALES

GENERAL		
.....		
	GLOBULIN	A/G RATIO
	g/l	

After 13 Weeks

Group 1	26.08	1.65
Group 2	25.84 -	1.67 -
Group 3	25.14 -	1.70 -
Group 4	25.11 -	1.73 -

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

Biochemistry - SUMMARY

FEMALES

	GENERAL						
	GLUCOSE	UREA	CREAT	BILI-T	BILE AC	CHOLEST	TRIGLY
	mmol/l	mmol/l	µmol/l	µmol/l	µmol/l	mmol/l	mmol/l
After 13 Weeks							
Group 1	6.55	6.18	27.1	2.11	7.59	2.31	0.43
Group 2	6.62 -	5.53 -	28.4 -	1.65 -	9.53 -	2.26 -	0.48 -
Group 3	6.35 -	6.48 -	27.7 -	2.02 -	10.12 -	2.07 -	0.42 -
Group 4	6.48 -	6.76 -	29.4 -	2.20 -	10.21 -	2.09 -	0.37 -

	GENERAL						
	PHOS-LIP	ASAT	ALAT	LDH	ALP	GGT	CK
	mmol/l	U/l	U/l	U/l	U/l	U/l	U/l
After 13 Weeks							
Group 1	2.56	64.7	18.1	86.0	19.0	0.0	92.3
Group 2	2.54 -	69.9 -	18.5 -	107.0 -	20.2 -	0.0 -	105.3 -
Group 3	2.48 -	66.9 -	16.6 -	101.9 -	19.6 -	0.0 -	99.0 -
Group 4	2.45 -	69.3 -	20.8 -	96.4 -	22.5 -	0.0 -	139.3 -

	GENERAL						
	SODIUM	POTASSIUM	CHLORIDE	CALCIUM	PHOSPHORUS	PROTEIN	ALBUMIN
	mmol/l	mmol/l	mmol/l	mmol/l	mmol/l	g/l	g/l
After 13 Weeks							
Group 1	142.2	4.07	101.1	2.46	1.34	72.33	51.84
Group 2	143.3 -	4.16 -	101.3 -	2.73 -	1.32 -	72.50 -	51.55 -
Group 3	144.9 **	3.92 -	103.0 -	2.76 -	1.26 -	74.79 -	53.59 -
Group 4	145.5 **	4.13 -	102.8 -	2.80 -	1.42 -	74.83 -	53.41 -

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

Biochemistry - SUMMARY
FEMALES

GENERAL		
.....		
	GLOBULIN	A/G RATIO
	g/l	

After 13 Weeks

Group 1	20.50	2.62
Group 2	20.94 -	2.47 -
Group 3	21.20 -	2.56 -
Group 4	21.42 -	2.63 -

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

Urinalysis - SUMMARY**Data excluded from Summary Report**

Not Reported

All Measurements

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Reported Parameter

Parameter	Statistical Testing
-----------	---------------------

After 13 Weeks

VOLUME/18h	VOLUME/18h	STEEL
REL DENS	RELATIVE DENSITY	STEEL
pH	pH	STEEL
NITRITE	NITRITE	
PROTEIN	PROTEIN	STEEL
GLUCOSE	GLUCOSE	STEEL
KETONES	KETONES	STEEL
UROBILI	UROBILINOGEN	STEEL
BILIRUBIN	BILIRUBIN	STEEL
ERY	ERYTHROCYTES	STEEL
LEU	LEUCOCYTES	STEEL

Statistical Methods

STEEL	STEEL-Test sig. at 5% (*), 1% (**) or not significant (-)
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Urinalysis - SUMMARY

MALES

GENERAL							
	VOLUME/18h ml	REL DENS rel. 1	pH	NITRITE SCORE 0/1	PROTEIN g/l	GLUCOSE mmol/l	KETONES mmol/l
After 13 Weeks							
Group 1	5.5	1.038	6.5	1	0.25	0	1.5
Group 2	4.3 -	1.054 -	6.5 -	1	0.25 -	0 -	1.5 -
Group 3	5.2 -	1.044 -	6.0 -	1	0.25 -	0 -	1.5 -
Group 4	5.9 -	1.044 -	6.5 -	1	0.25 -	0 -	1.5 -

GENERAL				
	UROBILI μmol/l	BILIRUBIN μmol/l	ERY per μl	LEU per μl
After 13 Weeks				
Group 1	0	9	10	25
Group 2	0 -	17 -	10 -	25 -
Group 3	0 -	17 -	10 -	25 -
Group 4	0 -	17 -	10 -	25 -

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

Urinalysis - SUMMARY

FEMALES

	GENERAL						
	VOLUME/18h ml	REL DENS rel. 1	pH	NITRITE SCORE 0/1	PROTEIN g/l	GLUCOSE mmol/l	KETONES mmol/l
After 13 Weeks							
Group 1	7.7	1.024	6.3	0	0.00	0	0.3
Group 2	6.4 -	1.027 -	6.3 -	1	0.00 -	0 -	0.5 -
Group 3	3.2 -	1.050 -	6.0 -	1	0.25 -	0 -	1.0 -
Group 4	3.8 -	1.050 -	5.5 -	1	0.25 -	0 -	1.0 -

	GENERAL			
	UROBILI μmol/l	BILIRUBIN μmol/l	ERY per μl	LEU per μl
After 13 Weeks				
Group 1	0	0	0	0
Group 2	0 -	0 -	0 -	0 -
Group 3	0 -	17 -	0 -	0 -
Group 4	0 -	17 -	5 -	0 -

*/**/- : Significant at 5% (*), 1% (**) or not significant (-)

ORGAN WEIGHTS (GRAM) - SUMMARY**Exclusions from Summary**

Not Reported

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Selection of Organs

All organs reported

ORGAN WEIGHTS (GRAM) - SUMMARY
AFTER 13 WEEKS
MALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
BODY W.	MEAN	412.2	419.0 -	427.3 -	414.4 -
	ST.DEV.	26.8	48.2	53.3	34.2
	MINIMUM	360.6	344.9	355.8	355.8
	MAXIMUM	443.2	491.3	501.1	473.6
	N	10	10	10	10
BRAIN	MEAN	2.08	2.07 -	2.14 -	2.08 -
	ST.DEV.	0.09	0.08	0.09	0.13
	MINIMUM	1.87	1.90	1.99	1.86
	MAXIMUM	2.19	2.18	2.24	2.30
	N	10	10	10	10
HEART	MEAN	1.05	1.05 -	1.11 -	1.06 -
	ST.DEV.	0.10	0.11	0.14	0.11
	MINIMUM	0.81	0.85	0.93	0.89
	MAXIMUM	1.16	1.18	1.32	1.20
	N	10	10	10	10
LIVER	MEAN	10.34	11.08 -	11.65 -	11.05 -
	ST.DEV.	1.30	1.25	1.60	2.01
	MINIMUM	7.87	8.42	9.12	8.46
	MAXIMUM	12.25	12.67	13.98	14.63
	N	10	10	10	10
THYMUS	MEAN	0.360	0.337 -	0.351 -	0.338 -
	ST.DEV.	0.066	0.064	0.097	0.040
	MINIMUM	0.269	0.248	0.208	0.258
	MAXIMUM	0.482	0.451	0.518	0.386
	N	10	10	10	10
KIDNEYS	MEAN	2.15	2.06 -	2.32 -	2.25 -
	ST.DEV.	0.19	0.13	0.22	0.22
	MINIMUM	1.72	1.85	1.88	1.80
	MAXIMUM	2.37	2.22	2.63	2.52
	N	10	10	10	10
ADRENALS	MEAN	0.068	0.068 -	0.067 -	0.064 -
	ST.DEV.	0.009	0.009	0.005	0.014
	MINIMUM	0.046	0.055	0.059	0.046
	MAXIMUM	0.082	0.084	0.074	0.090
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN WEIGHTS (GRAM) - SUMMARY
AFTER 13 WEEKS
MALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
SPLEEN	MEAN	0.68	0.75 -	0.70 -	0.69 -
	ST.DEV.	0.11	0.09	0.10	0.06
	MINIMUM	0.51	0.60	0.56	0.61
	MAXIMUM	0.86	0.94	0.86	0.79
	N	10	10	10	10
TESTES	MEAN	4.11	3.87 -	4.04 -	3.99 -
	ST.DEV.	0.41	0.30	0.32	0.29
	MINIMUM	3.15	3.57	3.33	3.62
	MAXIMUM	4.51	4.33	4.49	4.62
	N	10	10	10	10
EPIDIDYDYMID	MEAN	1.626	1.601 -	1.649 -	1.512 -
	ST.DEV.	0.169	0.115	0.191	0.104
	MINIMUM	1.384	1.460	1.432	1.336
	MAXIMUM	1.859	1.776	1.984	1.655
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN/BODY WEIGHT RATIOS (%) - SUMMARY
AFTER 13 WEEKS
MALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
BODY W.	MEAN	412.2	419.0 -	427.3 -	414.4 -
	ST.DEV.	26.8	48.2	53.3	34.2
	MINIMUM	360.6	344.9	355.8	355.8
	MAXIMUM	443.2	491.3	501.1	473.6
	N	10	10	10	10
BRAIN	MEAN	0.51	0.50 -	0.51 -	0.50 -
	ST.DEV.	0.03	0.06	0.06	0.03
	MINIMUM	0.46	0.43	0.43	0.46
	MAXIMUM	0.54	0.62	0.57	0.55
	N	10	10	10	10
HEART	MEAN	0.25	0.25 -	0.26 -	0.26 -
	ST.DEV.	0.02	0.02	0.02	0.02
	MINIMUM	0.22	0.23	0.22	0.23
	MAXIMUM	0.28	0.28	0.29	0.28
	N	10	10	10	10
LIVER	MEAN	2.51	2.65 -	2.73 -	2.65 -
	ST.DEV.	0.26	0.16	0.20	0.30
	MINIMUM	2.00	2.44	2.47	2.22
	MAXIMUM	2.78	2.89	3.07	3.13
	N	10	10	10	10
THYMUS	MEAN	0.088	0.081 -	0.082 -	0.082 -
	ST.DEV.	0.016	0.015	0.020	0.008
	MINIMUM	0.064	0.057	0.054	0.071
	MAXIMUM	0.109	0.110	0.113	0.092
	N	10	10	10	10
KIDNEYS	MEAN	0.52	0.50 -	0.55 -	0.54 -
	ST.DEV.	0.04	0.05	0.06	0.03
	MINIMUM	0.44	0.44	0.47	0.50
	MAXIMUM	0.60	0.57	0.66	0.60
	N	10	10	10	10
ADRENALS	MEAN	0.017	0.016 -	0.016 -	0.016 -
	ST.DEV.	0.002	0.003	0.002	0.003
	MINIMUM	0.013	0.011	0.013	0.012
	MAXIMUM	0.019	0.020	0.018	0.021
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN/BODY WEIGHT RATIOS (%) - SUMMARY
AFTER 13 WEEKS
MALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
SPLEEN	MEAN	0.17	0.18 -	0.16 -	0.17 -
	ST.DEV.	0.02	0.03	0.01	0.01
	MINIMUM	0.13	0.15	0.14	0.14
	MAXIMUM	0.20	0.27	0.18	0.19
	N	10	10	10	10
TESTES	MEAN	1.00	0.93 -	0.95 -	0.97 -
	ST.DEV.	0.08	0.08	0.11	0.06
	MINIMUM	0.87	0.84	0.80	0.89
	MAXIMUM	1.15	1.04	1.09	1.06
	N	10	10	10	10
EPIDIDYDYMID	MEAN	0.395	0.385 -	0.389 -	0.365 -
	ST.DEV.	0.032	0.036	0.047	0.020
	MINIMUM	0.333	0.330	0.305	0.333
	MAXIMUM	0.444	0.437	0.435	0.384
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN/BRAIN WEIGHT RATIOS (%) - SUMMARY
AFTER 13 WEEKS
MALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
BRAIN	MEAN	2.08	2.07 -	2.14 -	2.08 -
	ST.DEV.	0.09	0.08	0.09	0.13
	MINIMUM	1.87	1.90	1.99	1.86
	MAXIMUM	2.19	2.18	2.24	2.30
	N	10	10	10	10
HEART	MEAN	50.37	50.68 -	51.65 -	50.76 -
	ST.DEV.	3.77	6.26	5.51	4.16
	MINIMUM	43.32	39.72	46.27	46.36
	MAXIMUM	54.55	58.42	61.11	57.42
	N	10	10	10	10
LIVER	MEAN	497.29	535.71 -	544.39 -	529.79 -
	ST.DEV.	52.27	62.65	68.24	81.59
	MINIMUM	420.86	393.46	448.88	431.86
	MAXIMUM	586.12	597.64	656.34	671.10
	N	10	10	10	10
THYMUS	MEAN	17.409	16.310 -	16.323 -	16.195 -
	ST.DEV.	3.374	3.189	4.127	1.412
	MINIMUM	12.570	11.589	10.452	13.871
	MAXIMUM	23.861	21.893	23.229	17.706
	N	10	10	10	10
KIDNEYS	MEAN	103.44	99.58 -	108.53 -	107.94 -
	ST.DEV.	6.27	7.54	8.70	7.20
	MINIMUM	91.98	86.45	93.53	96.77
	MAXIMUM	112.56	108.46	121.76	117.71
	N	10	10	10	10
ADRENALS	MEAN	3.290	3.281 -	3.140 -	3.071 -
	ST.DEV.	0.420	0.504	0.217	0.649
	MINIMUM	2.460	2.523	2.813	2.255
	MAXIMUM	4.059	4.138	3.380	4.306
	N	10	10	10	10
SPLEEN	MEAN	32.87	36.07 -	32.82 -	33.12 -
	ST.DEV.	4.65	3.67	4.72	2.98
	MINIMUM	26.64	31.58	25.11	29.55
	MAXIMUM	40.69	43.93	40.38	37.81
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN/BRAIN WEIGHT RATIOS (%) - SUMMARY
AFTER 13 WEEKS
MALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
TESTES	MEAN	197.50	187.15 -	188.53 -	192.36 -
	ST.DEV.	15.59	14.90	10.76	15.45
	MINIMUM	168.45	166.82	167.34	170.87
	MAXIMUM	215.79	215.42	205.09	223.44
	N	10	10	10	10
EPIDIDYMI	MEAN	78.356	77.342 -	76.998 -	72.713 -
	ST.DEV.	7.615	4.944	7.430	4.095
	MINIMUM	67.184	69.299	71.171	66.468
	MAXIMUM	89.265	83.774	91.620	79.583
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN WEIGHTS (GRAM) - SUMMARY
AFTER 13 WEEKS
FEMALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
BODY W.	MEAN	218.3	228.2 -	224.8 -	224.6 -
	ST.DEV.	15.1	12.9	19.9	13.8
	MINIMUM	199.5	212.9	206.0	204.4
	MAXIMUM	241.6	249.0	259.9	244.8
	N	10	10	10	10
BRAIN	MEAN	1.87	1.90 -	1.89 -	1.90 -
	ST.DEV.	0.14	0.12	0.12	0.08
	MINIMUM	1.63	1.80	1.72	1.79
	MAXIMUM	2.04	2.22	2.09	2.06
	N	10	10	10	10
HEART	MEAN	0.75	0.73 -	0.74 -	0.76 -
	ST.DEV.	0.08	0.07	0.06	0.05
	MINIMUM	0.64	0.63	0.67	0.68
	MAXIMUM	0.85	0.85	0.84	0.83
	N	10	10	10	10
LIVER	MEAN	6.66	7.00 -	6.47 -	6.62 -
	ST.DEV.	0.85	0.87	0.83	0.53
	MINIMUM	5.34	5.96	4.74	5.57
	MAXIMUM	8.04	8.54	7.77	7.35
	N	10	10	10	10
THYMUS	MEAN	0.286	0.262 -	0.314 -	0.294 -
	ST.DEV.	0.072	0.049	0.047	0.042
	MINIMUM	0.162	0.185	0.272	0.249
	MAXIMUM	0.374	0.338	0.414	0.365
	N	10	10	10	10
KIDNEYS	MEAN	1.48	1.49 -	1.43 -	1.44 -
	ST.DEV.	0.14	0.15	0.13	0.14
	MINIMUM	1.23	1.28	1.23	1.21
	MAXIMUM	1.61	1.75	1.59	1.73
	N	10	10	10	10
ADRENALS	MEAN	0.077	0.073 -	0.069 -	0.075 -
	ST.DEV.	0.012	0.008	0.007	0.010
	MINIMUM	0.058	0.060	0.056	0.058
	MAXIMUM	0.097	0.092	0.077	0.088
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN WEIGHTS (GRAM) - SUMMARY
AFTER 13 WEEKS
FEMALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
SPLEEN	MEAN	0.47	0.49 -	0.51 -	0.52 -
	ST.DEV.	0.08	0.08	0.06	0.09
	MINIMUM	0.36	0.38	0.44	0.41
	MAXIMUM	0.62	0.63	0.60	0.70
	N	10	10	10	10
OVARIES	MEAN	0.108	0.110 -	0.097 -	0.108 -
	ST.DEV.	0.017	0.025	0.017	0.015
	MINIMUM	0.087	0.080	0.077	0.084
	MAXIMUM	0.136	0.151	0.130	0.130
	N	10	10	10	10
UTERUS	MEAN	1.15	1.16 -	1.13 -	0.97 -
	ST.DEV.	0.33	0.50	0.45	0.28
	MINIMUM	0.68	0.67	0.66	0.71
	MAXIMUM	1.58	2.02	2.04	1.63
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN/BODY WEIGHT RATIOS (%) - SUMMARY
AFTER 13 WEEKS
FEMALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
BODY W.	MEAN	218.3	228.2 -	224.8 -	224.6 -
	ST.DEV.	15.1	12.9	19.9	13.8
	MINIMUM	199.5	212.9	206.0	204.4
	MAXIMUM	241.6	249.0	259.9	244.8
	N	10	10	10	10
BRAIN	MEAN	0.86	0.83 -	0.84 -	0.85 -
	ST.DEV.	0.06	0.05	0.07	0.04
	MINIMUM	0.77	0.74	0.73	0.79
	MAXIMUM	0.96	0.92	0.96	0.92
	N	10	10	10	10
HEART	MEAN	0.34	0.32 -	0.33 -	0.34 -
	ST.DEV.	0.02	0.02	0.02	0.03
	MINIMUM	0.31	0.29	0.29	0.29
	MAXIMUM	0.38	0.38	0.37	0.40
	N	10	10	10	10
LIVER	MEAN	3.06	3.07 -	2.88 -	2.95 -
	ST.DEV.	0.38	0.34	0.29	0.11
	MINIMUM	2.46	2.71	2.27	2.73
	MAXIMUM	3.69	3.86	3.35	3.08
	N	10	10	10	10
THYMUS	MEAN	0.130	0.115 -	0.140 -	0.131 -
	ST.DEV.	0.030	0.023	0.016	0.019
	MINIMUM	0.081	0.083	0.123	0.108
	MAXIMUM	0.163	0.151	0.178	0.162
	N	10	10	10	10
KIDNEYS	MEAN	0.68	0.65 -	0.64 -	0.64 -
	ST.DEV.	0.06	0.05	0.05	0.05
	MINIMUM	0.58	0.57	0.54	0.52
	MAXIMUM	0.76	0.72	0.69	0.71
	N	10	10	10	10
ADRENALS	MEAN	0.035	0.032 -	0.031 -	0.033 -
	ST.DEV.	0.004	0.004	0.003	0.004
	MINIMUM	0.029	0.027	0.027	0.026
	MAXIMUM	0.041	0.038	0.037	0.039
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN/BODY WEIGHT RATIOS (%) - SUMMARY
AFTER 13 WEEKS
FEMALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
SPLEEN	MEAN	0.22	0.21 -	0.23 -	0.23 -
	ST.DEV.	0.03	0.04	0.02	0.03
	MINIMUM	0.17	0.15	0.20	0.19
	MAXIMUM	0.26	0.28	0.28	0.30
	N	10	10	10	10
OVARIES	MEAN	0.050	0.048 -	0.043 -	0.049 -
	ST.DEV.	0.008	0.011	0.005	0.008
	MINIMUM	0.040	0.033	0.035	0.037
	MAXIMUM	0.066	0.068	0.050	0.059
	N	10	10	10	10
UTERUS	MEAN	0.53	0.51 -	0.50 -	0.43 -
	ST.DEV.	0.16	0.21	0.17	0.11
	MINIMUM	0.31	0.31	0.28	0.33
	MAXIMUM	0.79	0.90	0.86	0.68
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN/BRAIN WEIGHT RATIOS (%) - SUMMARY
AFTER 13 WEEKS
FEMALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
BRAIN	MEAN	1.87	1.90 -	1.89 -	1.90 -
	ST.DEV.	0.14	0.12	0.12	0.08
	MINIMUM	1.63	1.80	1.72	1.79
	MAXIMUM	2.04	2.22	2.09	2.06
	N	10	10	10	10
HEART	MEAN	40.00	38.35 -	39.41 -	40.19 -
	ST.DEV.	3.53	4.10	4.51	3.28
	MINIMUM	34.54	32.14	33.50	35.23
	MAXIMUM	46.01	45.70	47.19	46.37
	N	10	10	10	10
LIVER	MEAN	359.17	369.71 -	343.74 -	348.64 -
	ST.DEV.	57.28	51.75	43.20	23.35
	MINIMUM	275.26	295.05	237.00	296.28
	MAXIMUM	444.20	459.14	390.45	378.87
	N	10	10	10	10
THYMUS	MEAN	15.297	13.857 -	16.605 -	15.550 -
	ST.DEV.	3.690	2.794	1.962	2.458
	MINIMUM	9.050	10.221	14.241	12.476
	MAXIMUM	20.184	18.370	20.000	19.415
	N	10	10	10	10
KIDNEYS	MEAN	79.22	78.56 -	75.97 -	76.19 -
	ST.DEV.	6.38	5.77	7.00	8.12
	MINIMUM	65.46	70.05	67.21	58.74
	MAXIMUM	86.74	84.97	89.33	89.18
	N	10	10	10	10
ADRENALS	MEAN	4.116	3.823 -	3.694 -	3.923 -
	ST.DEV.	0.528	0.243	0.465	0.503
	MINIMUM	3.351	3.333	3.060	3.069
	MAXIMUM	4.755	4.144	4.477	4.525
	N	10	10	10	10
SPLEEN	MEAN	25.12	25.76 -	27.15 -	27.08 -
	ST.DEV.	3.52	5.19	3.62	4.44
	MINIMUM	21.13	19.69	22.50	21.69
	MAXIMUM	32.29	35.00	34.88	36.27
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN/BRAIN WEIGHT RATIOS (%) - SUMMARY
AFTER 13 WEEKS
FEMALES

		Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
OVARIES	MEAN	5.784	5.754 -	5.136 -	5.695 -
	ST.DEV.	0.941	1.202	0.908	0.719
	MINIMUM	4.608	4.249	3.850	4.468
	MAXIMUM	7.607	8.207	6.878	6.436
	N	10	10	10	10
UTERUS	MEAN	61.62	60.86 -	60.37 -	50.85 -
	ST.DEV.	17.72	25.50	25.24	13.98
	MINIMUM	35.78	35.83	31.58	37.77
	MAXIMUM	88.27	106.99	114.61	83.59
	N	10	10	10	10

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

MACROSCOPICAL FINDINGS - SUMMARY
AFTER 13 WEEKS
ALL NECROPSIES

Not Reported

Animal 100	Male	Group 1	Reserve Removed
Animal 101	Male	Group 1	Reserve Removed
Animal 200	Female	Group 1	Reserve Removed
Animal 201	Female	Group 1	Reserve Removed

MACROSCOPICAL FINDINGS - SUMMARY
AFTER 13 WEEKS
ALL NECROPSIES
MALES

	Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
ANIMALS EXAMINED	10	10	10	10
ANIMALS COMPLETED	10	10	10	10
ANIMALS WITHOUT FINDINGS	10	10	10	10

*/**/- : Fisher's Exact Test significant at 5% (*), 1% (**) or not significant (-)

MACROSCOPICAL FINDINGS - SUMMARY
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

	Group 1 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
ANIMALS EXAMINED	10	10	10	10
ANIMALS COMPLETED	10	10	10	10
ANIMALS WITHOUT FINDINGS	9	10	9	10
ANIMALS AFFECTED				
LYMPH NODES				
- DISCOLORATION	1 (10 %)	0 (0 %) -	0 (0 %) -	0 (0 %) -
EYES				
- DESICCATED	0 (0 %)	0 (0 %) -	1 (10 %) -	0 (0 %) -

*/**/- : Fisher's Exact Test significant at 5% (*), 1% (**) or not significant (-)

9 INDIVIDUAL TABLES

MORTALITY DATA
ALL NECROPSIES**Animal(s) without death date / death status**

Not Reported

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

MORTALITY DATA
ALL NECROPSIES
MALES

Group 1 (0 mg/kg)

TREATMENT (Days 1 to 94)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
1	18-DEC-13	94	X				
2	18-DEC-13	94	X				
3	18-DEC-13	94	X				
4	18-DEC-13	94	X				
5	18-DEC-13	94	X				
6	18-DEC-13	94	X				
7	18-DEC-13	94	X				
8	18-DEC-13	94	X				
9	18-DEC-13	94	X				
10	18-DEC-13	94	X				
Total:			10	0	0	0	

P = PLANNED NECROPSY , K = KILLED IN EXTR. , S = SPONTAN. DEATH , O = OTHER

MORTALITY DATA
ALL NECROPSIES
MALES

Group 2 (50 mg/kg)

TREATMENT (Days 1 to 94)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
11	18-DEC-13	94	X				
12	18-DEC-13	94	X				
13	18-DEC-13	94	X				
14	18-DEC-13	94	X				
15	18-DEC-13	94	X				
16	18-DEC-13	94	X				
17	18-DEC-13	94	X				
18	18-DEC-13	94	X				
19	18-DEC-13	94	X				
20	18-DEC-13	94	X				
Total:			10	0	0	0	

P = PLANNED NECROPSY , K = KILLED IN EXTR. , S = SPONTAN. DEATH , O = OTHER

MORTALITY DATA
ALL NECROPSIES
MALES

Group 3 (200 mg/kg)

TREATMENT (Days 1 to 94)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
21	18-DEC-13	94	X				
22	18-DEC-13	94	X				
23	18-DEC-13	94	X				
24	18-DEC-13	94	X				
25	18-DEC-13	94	X				
26	18-DEC-13	94	X				
27	18-DEC-13	94	X				
28	18-DEC-13	94	X				
29	18-DEC-13	94	X				
30	18-DEC-13	94	X				
Total:			10	0	0	0	

P = PLANNED NECROPSY , K = KILLED IN EXTR. , S = SPONTAN. DEATH , O = OTHER

**MORTALITY DATA
ALL NECROPSIES
MALES**

Group 4 (1000 mg/kg)

TREATMENT (Days 1 to 94)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
31	18-DEC-13	94	X				
32	18-DEC-13	94	X				
33	18-DEC-13	94	X				
34	18-DEC-13	94	X				
35	18-DEC-13	94	X				
36	18-DEC-13	94	X				
37	18-DEC-13	94	X				
38	18-DEC-13	94	X				
39	18-DEC-13	94	X				
40	18-DEC-13	94	X				
Total:			10	0	0	0	

P = PLANNED NECROPSY , K = KILLED IN EXTR. , S = SPONTAN. DEATH , O = OTHER

**MORTALITY DATA
ALL NECROPSIES
FEMALES**

Group 1 (0 mg/kg)

TREATMENT (Days 1 to 94)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
41	17-DEC-13	93	X				
42	17-DEC-13	93	X				
43	17-DEC-13	93	X				
44	17-DEC-13	93	X				
45	17-DEC-13	93	X				
46	17-DEC-13	93	X				
47	17-DEC-13	93	X				
48	17-DEC-13	93	X				
49	17-DEC-13	93	X				
50	17-DEC-13	93	X				
Total:			10	0	0	0	

P = PLANNED NECROPSY , K = KILLED IN EXTR. , S = SPONTAN. DEATH , O = OTHER

**MORTALITY DATA
ALL NECROPSIES
FEMALES**

Group 2 (50 mg/kg)

TREATMENT (Days 1 to 94)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
51	17-DEC-13	93	X				
52	17-DEC-13	93	X				
53	17-DEC-13	93	X				
54	17-DEC-13	93	X				
55	17-DEC-13	93	X				
56	17-DEC-13	93	X				
57	17-DEC-13	93	X				
58	17-DEC-13	93	X				
59	17-DEC-13	93	X				
60	17-DEC-13	93	X				
Total:			10	0	0	0	

P = PLANNED NECROPSY , K = KILLED IN EXTR. , S = SPONTAN. DEATH , O = OTHER

**MORTALITY DATA
ALL NECROPSIES
FEMALES**

Group 3 (200 mg/kg)

TREATMENT (Days 1 to 94)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
61	17-DEC-13	93	X				
62	17-DEC-13	93	X				
63	17-DEC-13	93	X				
64	17-DEC-13	93	X				
65	17-DEC-13	93	X				
66	17-DEC-13	93	X				
67	17-DEC-13	93	X				
68	17-DEC-13	93	X				
69	17-DEC-13	93	X				
70	17-DEC-13	93	X				
Total:			10	0	0	0	

P = PLANNED NECROPSY , K = KILLED IN EXTR. , S = SPONTAN. DEATH , O = OTHER

**MORTALITY DATA
ALL NECROPSIES
FEMALES**

Group 4 (1000 mg/kg)

TREATMENT (Days 1 to 94)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
71	17-DEC-13	93	X				
72	17-DEC-13	93	X				
73	17-DEC-13	93	X				
74	17-DEC-13	93	X				
75	17-DEC-13	93	X				
76	17-DEC-13	93	X				
77	17-DEC-13	93	X				
78	17-DEC-13	93	X				
79	17-DEC-13	93	X				
80	17-DEC-13	93	X				
Total:			10	0	0	0	

P = PLANNED NECROPSY , K = KILLED IN EXTR. , S = SPONTAN. DEATH , O = OTHER

DAILY OBSERVATIONS**Comments**

Data excluded from Summary Report

Not Reported

All Study Phases

Animal	100	Male	Group 10	Reserve Removed
Animal	101	Male	Group 10	Reserve Removed
Animal	200	Female	Group 10	Reserve Removed
Animal	201	Female	Group 10	Reserve Removed

Incomplete Recordings

Selection of Findings

All findings reported

DAILY OBSERVATIONS
MALES**ACCLIMATIZATION**

Weeks / Days

1

1 2 3 4 5

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)**Animal 17****APPEARANCE**

- FISSURES (3)

RIGHT EAR . . 1 1 1

No further abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)No abnormality recorded.

TREATMENT

1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7

No abnormality recorded.

RIGHT EAR 1

No further abnormality recorded.

No abnormality recorded.

NECK (CERVICAL) 1

NECK (CERVICAL) 1

No further abnormality recorded.

TREATMENT

7 8 9 10 11 12

1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7

RIGHT EYE 11111

```
ISSUES (S)
RIGHT EAR      1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
```

POSTERIOR DORSUM 2 2 2 2

NECK (CERVICAL) 11111111111111111111111111111111.....

DAILY OBSERVATIONS
MALES**TREATMENT**

Weeks / Days

7		8		9		10		11		12										
1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Group 4 (1000 mg/kg)**Animal 36**

APPEARANCE

- SCABS (3)

NECK (CERVICAL) 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

No further abnormality recorded.

DAILY OBSERVATIONS
MALES**TREATMENT**

Weeks / Days													
1 3							1 4						
1	2	3	4	5	6	7	1	2	3	4	5	6	7

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)**Animal 17**

APPEARANCE

- FISSURES (3)

RIGHT EAR 1 1 1 1 1 1 1 1 1

Animal 19

APPEARANCE

- HAIR LOSS (3)

POSTERIOR DORSUM 1 1 1 1 1 1 1 1 1

Animal 20

APPEARANCE

- NODULE (1)

LEFT AXILLARY REGION . . . 1 1 1 1 1 1

No further abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)No abnormality recorded.

DAILY OBSERVATIONS
FEMALES**ACCLIMATIZATION**

Weeks / Days

1

1 2 3 4 5

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)No abnormality recorded.

DAILY OBSERVATIONS**FEMALES****TREATMENT**

Weeks / Days

1							2							3							4							5							6						
1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7							

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)No abnormality recorded.

TREATMENT

7 8 9 10 11 12
1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7

No abnormality recorded.

No abnormality recorded.

No abnormality recorded.

No abnormality recorded.

DAILY OBSERVATIONS**FEMALES****TREATMENT**

Weeks / Days

1 3 1 4

1 2 3 4 5 6 7 1 2 -

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)No abnormality recorded.

WEEKLY BEHAVIORAL OBSERVATIONS**Comments**

Data excluded from Summary Report

Not Reported

All Study Phases

Animal	100	Male	Group 10	Reserve Removed
Animal	101	Male	Group 10	Reserve Removed
Animal	200	Female	Group 10	Reserve Removed
Animal	201	Female	Group 10	Reserve Removed

Incomplete Recordings

Selection of Findings

All findings reported

**WEEKLY BEHAVIORAL OBSERVATIONS
MALES****ACCLIMATIZATION**

Weeks

0

1

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

**WEEKLY BEHAVIORAL OBSERVATIONS
MALES****TREATMENT**

Weeks

0										1									
1	2	3	4	5	6	7	8	9	0	1	2	3	-						

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)No abnormality recorded.

**WEEKLY BEHAVIORAL OBSERVATIONS
FEMALES****ACCLIMATIZATION**

Weeks

0

1

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

**WEEKLY BEHAVIORAL OBSERVATIONS
FEMALES****TREATMENT**

Weeks

0										1									
1	2	3	4	5	6	7	8	9	0	1	2	3	-						

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (50 mg/kg)

No abnormality recorded.

Group 3 (200 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)No abnormality recorded.

GRIP STRENGTH**Comments**

Data excluded from Summary Report

Not Reported

All Measurements

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Reported Parameter

DURING WEEK 13

Grip Fore	GRIP FORELIMB
Grip Hind	GRIP HINDLIMB

DURING WEEK 13

Grip Fore	GRIP FORELIMB
Grip Hind	GRIP HINDLIMB

**GRIP STRENGTH
DURING WEEK 13
FEMALES**

Group 1 (0 mg/kg)

	GRIP STRENGTH	
	Grip Fore	Grip Hind
	KILOGRAM	KILOGRAM
<hr/>		
41	0.91	1.00
42	1.47	1.13
43	1.46	1.20
44	1.30	1.22
45	1.21	1.22
46	1.50	1.02
47	1.62	1.39
48	1.27	1.03
49	1.25	1.07
50	1.19	0.84

**GRIP STRENGTH
DURING WEEK 13
FEMALES**

Group 2 (50 mg/kg)

	GRIP STRENGTH	
	Grip Fore	Grip Hind
	KILOGRAM	KILOGRAM
<hr/>		
51	1.43	1.09
52	1.43	0.97
53	1.55	1.03
54	1.46	1.18
55	1.55	1.21
56	1.45	1.20
57	1.42	1.12
58	1.30	1.05
59	1.31	1.08
60	1.59	1.02

**GRIP STRENGTH
DURING WEEK 13
FEMALES**

Group 3 (200 mg/kg)

	GRIP STRENGTH	
	Grip Fore	Grip Hind
	KILOGRAM	KILOGRAM
<hr/>		
61	1.35	1.04
62	1.38	0.87
63	1.59	0.94
64	1.16	0.99
65	1.36	1.03
66	1.23	1.04
67	1.62	1.23
68	1.62	0.91
69	1.35	1.05
70	1.41	1.03

**GRIP STRENGTH
DURING WEEK 13
FEMALES**

Group 4 (1000 mg/kg)

	GRIP STRENGTH	
	Grip Fore	Grip Hind
	KILOGRAM	KILOGRAM
<hr/>		
71	1.51	1.10
72	1.62	1.07
73	1.27	0.86
74	1.54	1.14
75	1.44	1.16
76	1.83	1.17
77	1.59	1.13
78	1.32	1.03
79	1.37	1.07
80	1.71	1.00

**GRIP STRENGTH
DURING WEEK 13
MALES**

Group 1 (0 mg/kg)

	GRIP STRENGTH	
	Grip Fore	Grip Hind
	KILOGRAM	KILOGRAM
<hr/>		
1	1.91	1.28
2	2.02	1.41
3	2.13	1.40
4	1.76	1.28
5	1.82	1.30
6	1.89	1.30
7	1.31	1.27
8	1.64	1.21
9	2.16	1.43
10	1.92	1.43

**GRIP STRENGTH
DURING WEEK 13
MALES**

Group 2 (50 mg/kg)

	GRIP STRENGTH	
	Grip Fore	Grip Hind
	KILOGRAM	KILOGRAM
<hr/>		
11	2.12	1.40
12	1.99	1.21
13	1.95	1.42
14	1.88	1.24
15	1.96	1.17
16	1.88	1.43
17	2.00	1.38
18	1.90	1.40
19	2.00	1.33
20	1.92	1.44

**GRIP STRENGTH
DURING WEEK 13
MALES**

Group 3 (200 mg/kg)

	GRIP STRENGTH	
	Grip Fore	Grip Hind
	KILOGRAM	KILOGRAM
<hr/>		
21	1.84	1.30
22	2.21	1.42
23	2.14	1.44
24	2.09	1.49
25	1.84	1.17
26	1.91	1.33
27	1.72	1.26
28	2.21	1.46
29	2.01	1.38
30	1.94	1.21

**GRIP STRENGTH
DURING WEEK 13
MALES**

Group 4 (1000 mg/kg)

	GRIP STRENGTH	
	Grip Fore	Grip Hind
	KILOGRAM	KILOGRAM
<hr/>		
31	1.99	1.42
32	1.92	1.43
33	2.04	1.38
34	1.82	1.22
35	1.88	1.43
36	2.09	1.43
37	1.90	1.38
38	2.23	1.33
39	2.14	1.28
40	2.13	1.28

LOCOMOTOR ACTIVITY**Comments**

Data excluded from Summary Report

Not Reported

All Measurements

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Reported Parameter

DURING WEEK 13

0-10 MIN	LOCOMOTOR ACTIVITY
10-20 MIN	LOCOMOTOR ACTIVITY
20-30 MIN	LOCOMOTOR ACTIVITY
30-40 MIN	LOCOMOTOR ACTIVITY
40-50 MIN	LOCOMOTOR ACTIVITY
50-60 MIN	LOCOMOTOR ACTIVITY
Total	LOCOMOTOR ACTIVITY

DURING WEEK 13

0-10 MIN	LOCOMOTOR ACTIVITY
10-20 MIN	LOCOMOTOR ACTIVITY
20-30 MIN	LOCOMOTOR ACTIVITY
30-40 MIN	LOCOMOTOR ACTIVITY
40-50 MIN	LOCOMOTOR ACTIVITY
50-60 MIN	LOCOMOTOR ACTIVITY
Total	LOCOMOTOR ACTIVITY

**LOCOMOTOR ACTIVITY
DURING WEEK 13
FEMALES**

Group 1 (0 mg/kg)

	LOCOMOTOR ACTIVITY						
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	Total
41	616	301	532	574	303	257	2583
42	367	163	130	5	65	208	938
43	365	200	156	259	72	2	1054
44	376	198	315	253	101	26	1269
45	394	224	131	163	134	171	1217
46	430	271	172	76	337	44	1330
47	436	1	32	10	158	5	642
48	342	176	0	14	8	20	560
49	357	250	102	188	73	103	1073
50	425	204	46	270	205	148	1298

**LOCOMOTOR ACTIVITY
DURING WEEK 13
FEMALES**

Group 2 (50 mg/kg)

	LOCOMOTOR ACTIVITY						Total
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	
51	388	245	124	163	41	7	968
52	434	114	87	0	81	0	716
53	430	249	282	208	92	220	1481
54	469	298	237	26	0	0	1030
55	487	249	206	130	240	221	1533
56	604	344	165	303	140	197	1753
57	394	83	175	381	193	123	1349
58	220	11	0	51	24	40	346
59	565	94	0	232	132	2	1025
60	595	299	232	337	262	123	1848

**LOCOMOTOR ACTIVITY
DURING WEEK 13
FEMALES**

Group 3 (200 mg/kg)

	LOCOMOTOR ACTIVITY						Total
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	
61	325	146	80	125	5	0	681
62	260	122	140	141	152	20	835
63	268	186	183	124	59	37	857
64	490	225	35	4	221	323	1298
65	353	174	15	58	0	4	604
66	521	111	51	271	21	0	975
67	403	212	266	200	125	33	1239
68	393	156	220	106	114	59	1048
69	368	349	281	112	206	195	1511
70	564	252	86	6	167	4	1079

**LOCOMOTOR ACTIVITY
DURING WEEK 13
FEMALES**

Group 4 (1000 mg/kg)

	LOCOMOTOR ACTIVITY						Total
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	
71	378	24	140	196	2	3	743
72	498	225	274	176	195	156	1524
73	545	246	166	111	17	236	1321
74	616	222	247	208	28	73	1394
75	638	241	167	6	27	23	1102
76	410	180	19	258	139	0	1006
77	499	258	124	155	159	202	1397
78	375	183	124	18	6	109	815
79	422	231	167	84	214	159	1277
80	411	183	208	139	87	21	1049

**LOCOMOTOR ACTIVITY
DURING WEEK 13
MALES**

Group 1 (0 mg/kg)

	LOCOMOTOR ACTIVITY						Total
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	
1	509	344	179	148	14	0	1194
2	300	158	130	256	111	171	1126
3	354	298	211	93	87	33	1076
4	562	401	25	175	168	56	1387
5	559	355	107	264	202	186	1673
6	343	538	491	331	149	431	2283
7	529	316	150	169	40	199	1403
8	300	228	185	124	15	240	1092
9	453	125	250	48	19	8	903
10	769	366	265	288	176	338	2202

**LOCOMOTOR ACTIVITY
DURING WEEK 13
MALES**

Group 2 (50 mg/kg)

	LOCOMOTOR ACTIVITY						Total
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	
11	393	204	93	83	70	8	851
12	422	192	209	106	159	53	1141
13	382	215	70	136	129	100	1032
14	284	308	94	14	145	170	1015
15	411	239	99	171	119	79	1118
16	440	192	266	286	18	137	1339
17	578	267	177	209	254	8	1493
18	455	218	62	111	1	82	929
19	244	142	35	192	120	189	922
20	506	198	125	86	5	4	924

**LOCOMOTOR ACTIVITY
DURING WEEK 13
MALES**

Group 3 (200 mg/kg)

	LOCOMOTOR ACTIVITY						
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	Total
21	420	253	175	156	146	170	1320
22	376	32	376	19	51	147	1001
23	477	283	281	264	303	384	1992
24	598	384	194	170	364	126	1836
25	447	224	137	80	9	39	936
26	528	206	32	116	15	0	897
27	442	225	303	87	54	184	1295
28	481	230	338	97	0	1	1147
29	700	318	287	355	93	107	1860
30	621	423	68	102	31	18	1263

**LOCOMOTOR ACTIVITY
DURING WEEK 13
MALES**

Group 4 (1000 mg/kg)

	LOCOMOTOR ACTIVITY						Total
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	
31	399	116	62	0	23	396	996
32	394	327	237	157	193	14	1322
33	375	146	262	88	7	56	934
34	381	154	216	145	126	32	1054
35	430	351	173	136	35	111	1236
36	566	559	338	103	122	258	1946
37	514	213	78	24	189	38	1056
38	506	273	53	248	106	53	1239
39	462	334	42	30	266	234	1368
40	602	238	218	237	28	410	1733

FOOD CONSUMPTION (G/ANIMAL/DAY)**Comments**

Data excluded from Summary Report

Not Reported

All Study Phases

Cage	13	Male	Group 10	Reserve Removed
Cage	26	Female	Group 10	Reserve Removed

FOOD CONSUMPTION (G/ANIMAL/DAY)**MALES****Group 1 (0 mg/kg)**

CAGE	1	2	3
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ACCLIMATIZATION

Days 1-6	23.7	21.6	22.7
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CAGE	1	2	3
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TREATMENT

Days 1-8	26.7	23.6	23.9
8-15	27.8	25.0	24.9
15-22	27.8	24.1	25.0
22-29	27.3	24.1	24.8
29-36	26.2	22.8	24.1
36-43	25.2	22.3	23.9
43-50	25.2	22.5	23.9
50-57	25.8	22.7	23.9
57-64	24.3	22.5	24.2
64-71	23.7	22.1	23.8
71-78	23.8	21.9	23.9
78-85	25.7	19.5	23.9
85-92	21.9	19.7	21.9

FOOD CONSUMPTION (G/ANIMAL/DAY)**MALES****Group 2 (50 mg/kg)**

CAGE	4	5	6
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ACCLIMATIZATION

Days 1-6	21.7	22.3	22.9
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CAGE	4	5	6
------	---	---	---

TREATMENT

Days 1-8	23.1	23.5	24.5
8-15	24.5	24.9	26.3
15-22	24.8	23.9	25.6
22-29	24.1	23.8	25.5
29-36	23.7	24.0	24.7
36-43	24.0	23.6	24.1
43-50	24.4	23.5	24.3
50-57	24.4	23.3	24.4
57-64	23.0	23.1	23.9
64-71	20.4	23.4	23.8
71-78	22.2	23.5	24.3
78-85	22.0	22.8	25.0
85-92	23.0	22.2	24.3

FOOD CONSUMPTION (G/ANIMAL/DAY)**MALES****Group 3 (200 mg/kg)**

CAGE	7	8	9
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ACCLIMATIZATION

Days 1-6	23.6	24.2	21.1
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CAGE	7	8	9
------	---	---	---

TREATMENT

Days 1-8	25.1	25.2	23.2
8-15	27.0	26.6	25.7
15-22	27.0	25.7	25.0
22-29	26.6	25.8	25.1
29-36	25.9	25.3	24.5
36-43	25.5	24.1	23.7
43-50	25.4	24.5	24.0
50-57	25.3	24.4	23.6
57-64	25.0	24.3	23.2
64-71	24.8	24.2	22.3
71-78	24.8	24.3	23.4
78-85	25.0	23.3	22.8
85-92	24.3	23.3	22.4

FOOD CONSUMPTION (G/ANIMAL/DAY)
MALES**Group 4 (1000 mg/kg)**

CAGE	10	11	12
------	----	----	----

ACCLIMATIZATION

Days 1-6	24.4	21.5	23.0
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CAGE	10	11	12
------	----	----	----

TREATMENT

Days 1-8	26.1	22.4	23.9
8-15	27.2	24.1	25.4
15-22	26.0	24.9	25.3
22-29	26.6	23.7	25.1
29-36	26.1	21.9	24.1
36-43	24.4	20.9	23.6
43-50	24.0	19.9	23.5
50-57	24.1	21.2	23.4
57-64	23.8	20.6	23.6
64-71	23.7	20.7	23.1
71-78	24.2	21.6	24.5
78-85	23.7	21.6	24.5
85-92	22.7	20.7	23.5

FOOD CONSUMPTION (G/ANIMAL/DAY)
FEMALES**Group 1 (0 mg/kg)**

CAGE	14	15	16
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ACCLIMATIZATION

Days 1-6	16.1	15.3	15.5
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CAGE	14	15	16
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TREATMENT

Days 1-8	15.5	15.4	15.1
8-15	16.4	17.0	15.5
15-22	16.9	16.8	16.2
22-29	17.0	17.6	16.3
29-36	16.3	16.9	15.6
36-43	16.5	16.9	14.9
43-50	17.0	16.5	15.2
50-57	16.0	17.0	15.5
57-64	16.5	16.8	15.3
64-71	16.0	16.9	14.8
71-78	16.7	16.4	14.8
78-85	16.3	15.6	14.5
85-92	16.2	15.2	14.6

FOOD CONSUMPTION (G/ANIMAL/DAY)
FEMALES**Group 2 (50 mg/kg)**

CAGE	17	18	19
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ACCLIMATIZATION

Days 1-6	15.5	15.1	16.0
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CAGE	17	18	19
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TREATMENT

Days 1-8	15.2	15.8	14.9
8-15	17.1	16.8	15.8
15-22	17.7	16.7	16.3
22-29	17.7	17.3	16.9
29-36	16.5	17.6	16.9
36-43	16.6	17.4	16.2
43-50	16.8	17.8	16.0
50-57	16.9	17.8	16.8
57-64	17.4	18.0	16.3
64-71	16.6	17.5	16.2
71-78	17.5	17.5	16.8
78-85	16.2	16.9	16.2
85-92	16.3	16.3	15.6

FOOD CONSUMPTION (G/ANIMAL/DAY)
FEMALES**Group 3 (200 mg/kg)**

CAGE	20	21	22
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ACCLIMATIZATION

Days 1-6	16.4	17.3	15.2
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CAGE	20	21	22
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TREATMENT

Days 1-8	15.9	17.3	15.4
8-15	16.6	18.2	16.5
15-22	16.6	18.6	16.0
22-29	17.0	18.3	16.2
29-36	16.3	17.5	16.1
36-43	15.8	16.8	15.5
43-50	15.6	17.5	15.1
50-57	16.3	17.2	15.4
57-64	16.3	17.1	15.7
64-71	15.6	16.5	15.3
71-78	15.5	17.3	15.3
78-85	16.2	15.7	13.8
85-92	13.9	15.7	14.0

FOOD CONSUMPTION (G/ANIMAL/DAY)
FEMALES**Group 4 (1000 mg/kg)**

CAGE	23	24	25
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ACCLIMATIZATION

Days 1-6	16.3	16.0	16.0
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CAGE	23	24	25
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TREATMENT

Days 1-8	15.2	16.1	15.2
8-15	15.8	16.9	16.3
15-22	16.2	17.1	16.5
22-29	15.8	16.8	16.1
29-36	15.4	16.1	15.7
36-43	15.9	16.5	15.9
43-50	16.0	16.7	16.6
50-57	15.8	17.1	16.6
57-64	15.4	16.7	15.8
64-71	15.2	16.7	16.1
71-78	15.5	17.0	16.5
78-85	15.0	16.0	15.1
85-92	14.1	15.9	14.5

RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY)**Comments**

Data excluded from Summary Report

Not Reported

All Study Phases

Cage	13	Male	Group 10	Reserve Removed
Cage	26	Female	Group 10	Reserve Removed

RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY)
MALES**Group 1 (0 mg/kg)**

CAGE	1	2	3
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ACCLIMATIZATION

Days 1-6	113.0	107.2	104.1
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CAGE	1	2	3
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TREATMENT

Days 1-8	92.4	88.6	84.6
8-15	87.5	84.5	79.9
15-22	81.6	76.4	74.7
22-29	76.6	73.1	70.4
29-36	70.8	65.8	66.1
36-43	65.9	62.1	62.8
43-50	64.3	60.9	60.6
50-57	64.1	59.9	59.5
57-64	58.6	57.4	58.0
64-71	56.5	56.2	56.6
71-78	55.4	54.0	55.3
78-85	59.5	48.0	54.5
85-92	50.3	48.3	49.5

RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY)
MALES**Group 2 (50 mg/kg)**

CAGE	4	5	6
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ACCLIMATIZATION

Days 1-6	105.4	106.1	106.6
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CAGE	4	5	6
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TREATMENT

Days 1-8	84.9	86.3	86.1
8-15	81.3	82.9	83.3
15-22	76.7	75.2	75.7
22-29	71.7	70.6	72.0
29-36	67.1	68.6	67.3
36-43	65.5	64.5	63.0
43-50	64.4	62.9	61.0
50-57	62.2	60.4	59.9
57-64	58.5	58.1	57.0
64-71	51.3	58.0	55.5
71-78	54.7	57.7	55.3
78-85	53.4	55.5	56.0
85-92	54.9	53.2	53.8

RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY)
MALES**Group 3 (200 mg/kg)**

CAGE	7	8	9
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ACCLIMATIZATION

Days 1-6	109.8	107.0	104.3
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CAGE	7	8	9
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TREATMENT

Days 1-8	89.0	86.6	87.2
8-15	83.2	82.1	85.5
15-22	77.0	75.5	77.5
22-29	71.5	71.8	73.9
29-36	66.3	68.8	69.5
36-43	62.6	62.6	64.9
43-50	61.0	60.7	63.8
50-57	58.5	59.2	61.4
57-64	56.8	57.3	59.4
64-71	55.2	56.4	56.2
71-78	54.3	55.1	57.6
78-85	53.7	53.1	55.4
85-92	52.2	51.4	53.9

RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY)
MALES**Group 4 (1000 mg/kg)**

CAGE	10	11	12
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ACCLIMATIZATION

Days 1-6	118.8	106.9	104.7
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CAGE	10	11	12
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TREATMENT

Days 1-8	92.6	86.0	83.8
8-15	86.3	83.6	80.0
15-22	77.8	80.5	75.0
22-29	74.5	73.9	70.3
29-36	69.1	66.2	64.7
36-43	63.1	61.6	61.0
43-50	60.6	57.9	59.4
50-57	59.1	59.4	57.3
57-64	56.5	56.7	56.4
64-71	55.2	55.5	53.9
71-78	55.3	56.9	55.7
78-85	53.5	55.8	54.7
85-92	51.4	52.5	52.1

RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY)
FEMALES**Group 1 (0 mg/kg)**

CAGE	14	15	16
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ACCLIMATIZATION

Days 1-6	117.0	109.1	107.6
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CAGE	14	15	16
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TREATMENT

Days 1-8	94.3	91.7	88.0
8-15	94.3	91.3	84.3
15-22	92.2	86.1	85.1
22-29	87.5	86.0	84.1
29-36	80.5	81.3	77.9
36-43	82.1	77.1	72.1
43-50	82.7	74.5	72.8
50-57	75.8	74.3	74.1
57-64	76.5	73.4	70.8
64-71	74.7	72.1	67.9
71-78	76.0	67.6	66.7
78-85	75.3	66.5	65.6
85-92	72.0	63.0	64.0

RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY)
FEMALES**Group 2 (50 mg/kg)**

CAGE	17	18	19
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ACCLIMATIZATION

Days 1-6	109.2	109.9	110.7
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CAGE	17	18	19
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TREATMENT

Days 1-8	89.6	95.7	88.2
8-15	94.1	94.1	89.1
15-22	94.1	88.3	86.0
22-29	87.4	91.1	84.3
29-36	78.2	87.0	81.3
36-43	77.8	84.0	78.9
43-50	78.3	84.1	76.5
50-57	75.7	83.1	77.2
57-64	73.9	80.8	74.2
64-71	70.6	77.3	73.2
71-78	73.5	75.0	73.6
78-85	68.1	74.7	73.2
85-92	66.9	69.2	67.1

RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY)
FEMALES**Group 3 (200 mg/kg)**

CAGE	20	21	22
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ACCLIMATIZATION

Days 1-6	114.5	121.2	108.9
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CAGE	20	21	22
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TREATMENT

Days 1-8	92.2	99.4	92.0
8-15	87.0	97.5	89.2
15-22	84.6	94.1	82.8
22-29	81.8	88.0	81.2
29-36	76.9	81.9	78.9
36-43	72.3	78.5	73.6
43-50	70.5	80.1	70.8
50-57	71.3	76.0	70.5
57-64	69.8	74.1	70.9
64-71	65.2	72.0	67.4
71-78	64.4	73.7	66.1
78-85	66.9	69.1	61.3
85-92	57.2	68.0	60.9

RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY)
FEMALES**Group 4 (1000 mg/kg)**

CAGE	23	24	25
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ACCLIMATIZATION

Days 1-6	119.9	111.9	113.1
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CAGE	23	24	25
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TREATMENT

Days 1-8	91.3	92.0	90.1
8-15	89.9	90.9	91.4
15-22	88.6	85.5	87.3
22-29	81.0	79.1	80.2
29-36	77.2	74.5	77.0
36-43	78.5	77.2	77.3
43-50	77.7	74.9	76.8
50-57	73.3	74.3	74.7
57-64	70.5	71.6	70.3
64-71	69.6	71.3	71.9
71-78	68.5	71.2	71.3
78-85	67.2	67.7	66.4
85-92	62.8	65.6	62.1

BODY WEIGHTS (G)**Comments**

Data excluded from Summary Report

Not Reported

All Study Phases

Animal	100	Male	Group	10	Reserve	Removed
Animal	101	Male	Group	10	Reserve	Removed
Animal	200	Female	Group	10	Reserve	Removed
Animal	201	Female	Group	10	Reserve	Removed

BODY WEIGHTS (G)**MALES****Group 1 (0 mg/kg)**

Animal	1	2	3	4	5	6	7
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PRE-RANDOMIZATION

Day	1	205	175	191	197	178	186	210
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Animal	1	2	3	4	5	6	7
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ACCLIMATIZATION

Day	1	223	196	210	207	194	203	234
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Animal	1	2	3	4	5	6	7
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TREATMENT

Day	1	259	232	248	235	218	235	261
	8	296	276	294	272	248	279	289
	15	321	304	329	302	273	312	317
	22	342	328	352	321	289	337	334
	29	356	341	372	326	306	356	346
	36	370	350	389	356	315	368	363
	43	378	362	405	368	328	379	382
	50	383	377	414	376	342	389	393
	57	393	382	432	386	349	401	396
	64	407	394	442	403	361	411	416
	71	414	394	447	396	364	420	401
	78	423	402	465	414	371	433	420
	85	422	404	469	406	378	436	417
	92	424	410	472	418	375	434	430

Animal	8	9	10
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PRE-RANDOMIZATION

Day	1	201	214	185
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Animal	8	9	10
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ACCLIMATIZATION

Day	1	210	227	201
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Animal	8	9	10
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TREATMENT

Day	1	241	258	233
	8	271	297	272
	15	299	328	300
	22	322	355	329
	29	344	371	349
	36	354	383	361
	43	369	395	374
	50	383	409	394
	57	389	418	402
	64	404	430	418
	71	411	439	431
	78	415	455	440
	85	419	465	451

BODY WEIGHTS (G)**MALES****Group 1 (0 mg/kg)**

Animal	8	9	10
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TREATMENT

Day	92	425	461	454
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BODY WEIGHTS (G)**MALES****Group 2 (50 mg/kg)**

Animal	11	12	13	14	15	16	17
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PRE-RANDOMIZATION

Day	1	174	177	194	185	208	191	203
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Animal	11	12	13	14	15	16	17
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ACCLIMATIZATION

Day	1	217	195	207	196	223	210	218
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Animal	11	12	13	14	15	16	17
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TREATMENT

Day	1	250	224	237	220	249	251	255
	8	287	254	275	240	285	291	280
	15	324	272	307	267	310	322	308
	22	348	291	332	282	328	345	319
	29	365	300	344	295	345	370	323
	36	390	307	361	306	354	388	322
	43	401	320	379	322	368	406	342
	50	411	328	395	320	380	420	353
	57	428	340	406	338	387	433	362
	64	415	347	418	346	399	446	368
	71	422	348	425	349	403	457	381
	78	433	354	433	345	410	469	385
	85	440	354	439	352	414	469	387
	92	446	369	443	360	420	476	395

Animal	18	19	20
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PRE-RANDOMIZATION

Day	1	186	198	212
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Animal	18	19	20
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ACCLIMATIZATION

Day	1	201	214	228
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Animal	18	19	20
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TREATMENT

Day	1	229	247	270
	8	260	282	314
	15	285	321	348
	22	309	350	377
	29	316	376	400
	36	331	390	426
	43	347	405	437
	50	364	422	456
	57	374	431	466
	64	388	442	477
	71	395	456	483
	78	406	472	493
	85	419	477	503

BODY WEIGHTS (G)**MALES****Group 2 (50 mg/kg)**

Animal	18	19	20
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TREATMENT

Day	92	424	483	505
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BODY WEIGHTS (G)**MALES****Group 3 (200 mg/kg)**

Animal	21	22	23	24	25	26	27
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PRE-RANDOMIZATION

Day	1	178	202	208	211	190	232	197
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Animal	21	22	23	24	25	26	27
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ACCLIMATIZATION

Day	1	193	223	229	228	207	242	208
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Animal	21	22	23	24	25	26	27
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TREATMENT

Day	1	220	257	273	260	239	271	233
	8	237	286	321	295	279	299	257
	15	270	342	363	333	311	327	286
	22	283	374	394	346	334	341	299
	29	296	401	418	363	356	359	306
	36	310	426	438	363	372	369	313
	43	320	444	457	388	387	383	329
	50	319	461	471	403	413	394	338
	57	339	476	481	412	421	405	343
	64	341	489	493	416	444	414	346
	71	355	496	494	416	457	417	348
	78	357	508	507	425	473	426	351
	85	366	517	514	418	476	424	363
	92	359	521	514	439	487	430	370

Animal	28	29	30
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PRE-RANDOMIZATION

Day	1	186	177	194
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Animal	28	29	30
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ACCLIMATIZATION

Day	1	204	194	205
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Animal	28	29	30
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TREATMENT

Day	1	243	224	221
	8	287	256	265
	15	326	287	301
	22	356	312	323
	29	381	330	342
	36	396	343	356
	43	413	350	369
	50	425	362	378
	57	437	375	381
	64	448	384	387
	71	461	390	387
	78	470	405	401
	85	473	406	406

BODY WEIGHTS (G)**MALES****Group 3 (200 mg/kg)**

Animal	28	29	30
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TREATMENT

Day	92	475	409	410
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BODY WEIGHTS (G)**MALES****Group 4 (1000 mg/kg)**

Animal	31	32	33	34	35	36	37
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PRE-RANDOMIZATION

Day	1	202	177	206	178	190	193	186
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Animal	31	32	33	34	35	36	37
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ACCLIMATIZATION

Day	1	194	197	225	194	201	208	200
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Animal	31	32	33	34	35	36	37
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TREATMENT

Day	1	229	237	258	209	225	236	219
	8	274	275	298	251	261	269	248
	15	308	305	332	275	288	300	284
	22	327	324	353	291	312	323	292
	29	360	338	372	299	326	336	315
	36	382	357	392	313	336	342	335
	43	393	370	400	318	345	352	351
	50	397	382	410	318	354	360	362
	57	412	391	419	340	361	367	377
	64	427	402	435	341	370	377	382
	71	442	404	442	355	380	384	393
	78	436	422	453	348	392	397	400
	85	452	425	451	363	395	404	414
	92	443	429	454	369	397	416	409

Animal	38	39	40
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PRE-RANDOMIZATION

Day	1	218	211	197
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Animal	38	39	40
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ACCLIMATIZATION

Day	1	233	227	218
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Animal	38	39	40
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TREATMENT

Day	1	258	263	253
	8	289	304	299
	15	320	339	328
	22	340	364	355
	29	356	381	378
	36	373	385	396
	43	385	399	414
	50	394	403	427
	57	403	415	441
	64	413	426	453
	71	422	439	464
	78	433	447	477
	85	437	459	485

BODY WEIGHTS (G)**MALES****Group 4 (1000 mg/kg)**

Animal	38	39	40
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TREATMENT

Day	92	437	462	492
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BODY WEIGHTS (G)**FEMALES****Group 1 (0 mg/kg)**

Animal	41	42	43	44	45	46	47
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PRE-RANDOMIZATION

Day	1	120	128	121	131	126	130	136
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Animal	41	42	43	44	45	46	47
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ACCLIMATIZATION

Day	1	135	141	138	138	145	138	148
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Animal	41	42	43	44	45	46	47
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TREATMENT

Day	1	151	156	153	150	159	152	165
	8	152	170	171	163	174	167	183
	15	166	178	179	177	189	192	197
	22	180	191	180	186	196	204	205
	29	189	199	195	191	210	215	205
	36	200	204	202	195	218	212	208
	43	198	202	204	206	221	228	221
	50	205	210	203	210	219	235	222
	57	209	215	209	218	228	239	225
	64	218	215	215	215	239	233	233
	71	209	214	219	216	241	244	234
	78	218	226	216	227	247	254	243
	85	211	217	221	220	242	243	242
	92	219	228	226	233	249	241	256

Animal	48	49	50
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PRE-RANDOMIZATION

Day	1	124	145	133
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Animal	48	49	50
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ACCLIMATIZATION

Day	1	133	151	143
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Animal	48	49	50
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TREATMENT

Day	1	145	166	153
	8	164	177	165
	15	175	187	176
	22	184	190	184
	29	184	202	185
	36	192	208	194
	43	200	211	196
	50	202	213	196
	57	199	218	194
	64	208	222	199
	71	211	222	203
	78	213	227	206
	85	210	226	205

BODY WEIGHTS (G)**FEMALES****Group 1 (0 mg/kg)**

Animal	48	49	50
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TREATMENT

Day	92	214	229	209
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BODY WEIGHTS (G)**FEMALES****Group 2 (50 mg/kg)**

Animal	51	52	53	54	55	56	57
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PRE-RANDOMIZATION

Day	1	126	128	135	132	121	119	124
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Animal	51	52	53	54	55	56	57
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ACCLIMATIZATION

Day	1	138	140	148	144	137	130	137
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Animal	51	52	53	54	55	56	57
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TREATMENT

Day	1	143	154	162	147	148	140	148
	8	157	172	182	172	162	160	159
	15	162	187	195	194	169	172	172
	22	174	193	198	206	181	179	181
	29	180	211	218	195	192	181	190
	36	182	221	228	217	197	195	196
	43	181	226	235	221	197	203	195
	50	186	225	232	222	208	205	202
	57	190	238	243	221	216	204	210
	64	198	249	258	234	220	215	211
	71	200	247	258	239	217	223	215
	78	209	250	255	246	230	226	223
	85	210	247	255	235	221	221	213
	92	214	250	269	250	231	228	221

Animal	58	59	60
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PRE-RANDOMIZATION

Day	1	131	130	142
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Animal	58	59	60
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ACCLIMATIZATION

Day	1	142	139	160
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Animal	58	59	60
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TREATMENT

Day	1	159	158	175
	8	168	172	176
	15	173	186	179
	22	189	190	197
	29	200	207	205
	36	208	217	210
	43	207	218	203
	50	214	217	203
	57	223	228	209
	64	224	235	211
	71	226	229	216
	78	234	229	226
	85	226	228	220

BODY WEIGHTS (G)**FEMALES****Group 2 (50 mg/kg)**

Animal	58	59	60
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TREATMENT

Day	92	234	241	233
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BODY WEIGHTS (G)**FEMALES****Group 3 (200 mg/kg)**

Animal	61	62	63	64	65	66	67
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PRE-RANDOMIZATION

Day	1	129	128	134	121	124	142	131
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Animal	61	62	63	64	65	66	67
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ACCLIMATIZATION

Day	1	137	148	143	136	138	154	147
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Animal	61	62	63	64	65	66	67
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TREATMENT

Day	1	153	163	160	149	154	173	153
	8	158	181	179	155	172	194	173
	15	178	201	194	173	184	203	188
	22	184	207	198	187	185	222	184
	29	196	224	202	192	198	233	195
	36	193	230	213	199	203	240	204
	43	202	236	219	197	208	238	201
	50	203	237	224	202	205	250	199
	57	209	251	224	215	213	253	207
	64	208	258	234	214	218	258	222
	71	217	258	241	211	220	256	223
	78	221	259	244	220	218	268	217
	85	216	269	240	210	213	258	218
	92	210	271	248	208	218	267	228

Animal	68	69	70
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PRE-RANDOMIZATION

Day	1	118	132	125
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Animal	68	69	70
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ACCLIMATIZATION

Day	1	134	138	139
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Animal	68	69	70
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TREATMENT

Day	1	146	158	156
	8	158	172	167
	15	175	196	183
	22	188	207	194
	29	192	213	199
	36	193	217	201
	43	204	233	205
	50	212	236	207
	57	219	237	210
	64	217	236	212
	71	228	244	216
	78	236	252	219
	85	221	247	212

BODY WEIGHTS (G)**FEMALES****Group 3 (200 mg/kg)**

Animal	68	69	70
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TREATMENT

Day	92	237	240	217
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BODY WEIGHTS (G)**FEMALES****Group 4 (1000 mg/kg)**

Animal	71	72	73	74	75	76	77
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PRE-RANDOMIZATION

Day	1	125	118	129	128	131	140	132
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Animal	71	72	73	74	75	76	77
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ACCLIMATIZATION

Day	1	138	130	141	137	137	155	145
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Animal	71	72	73	74	75	76	77
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TREATMENT

Day	1	152	146	163	150	151	171	158
	8	166	156	177	168	172	187	170
	15	178	162	189	178	180	199	180
	22	179	177	192	199	194	209	193
	29	193	188	202	209	205	221	205
	36	197	193	210	216	208	226	205
	43	200	191	217	208	205	228	208
	50	197	204	219	228	214	226	220
	57	210	210	227	234	222	234	230
	64	214	211	231	231	228	240	230
	71	216	210	231	233	226	244	230
	78	217	219	241	239	236	240	237
	85	218	213	238	237	230	242	235
	92	213	214	244	244	233	251	240

Animal	78	79	80
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PRE-RANDOMIZATION

Day	1	120	124	133
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Animal	78	79	80
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ACCLIMATIZATION

Day	1	135	138	148
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Animal	78	79	80
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TREATMENT

Day	1	150	154	164
	8	163	169	173
	15	168	180	186
	22	180	192	192
	29	193	202	201
	36	194	209	207
	43	193	212	210
	50	209	222	211
	57	215	228	216
	64	216	231	220
	71	213	230	222
	78	223	242	223
	85	213	238	221

BODY WEIGHTS (G)**FEMALES****Group 4 (1000 mg/kg)**

Animal	78	79	80
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TREATMENT

Day	92	221	244	228
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BODY WEIGHT GAIN (%)**Comments**

Data excluded from Summary Report

Not Reported

All Study Phases

Animal	100	Male	Group 10	Reserve Removed
Animal	101	Male	Group 10	Reserve Removed
Animal	200	Female	Group 10	Reserve Removed
Animal	201	Female	Group 10	Reserve Removed

BODY WEIGHT GAIN (%)**MALES****Group 1 (0 mg/kg)**

Animal	1	2	3	4	5	6	7
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	14.7	18.9	18.6	15.8	13.7	18.5
	15	24.3	30.9	33.0	28.4	24.8	32.5
	22	32.3	41.4	42.3	36.9	32.2	43.1
	29	37.6	46.7	50.4	38.8	40.0	51.2
	36	42.9	50.8	57.3	51.4	44.3	56.3
	43	46.3	56.0	63.8	56.8	50.3	61.1
	50	48.3	62.2	67.4	60.0	56.7	65.7
	57	52.1	64.6	74.3	64.4	59.9	70.6
	64	57.5	69.4	78.5	71.5	65.2	74.9
	71	60.1	69.7	80.7	68.5	66.5	78.8
	78	63.6	73.1	87.8	76.3	69.7	84.4
	85	63.3	74.0	89.4	72.8	73.3	85.4
	92	64.0	76.6	90.6	78.1	71.6	84.5

Animal	8	9	10	
TREATMENT				
Day	1	0.0	0.0	0.0
	8	12.5	15.1	16.3
	15	24.3	27.4	28.6
	22	33.7	37.8	41.0
	29	42.9	43.8	49.5
	36	47.0	48.5	54.5
	43	53.2	53.0	60.1
	50	59.3	58.4	68.7
	57	61.9	62.0	72.3
	64	68.0	66.9	79.1
	71	71.0	70.3	84.5
	78	72.6	76.4	88.6
	85	74.1	80.2	93.4
	92	76.6	78.7	94.6

BODY WEIGHT GAIN (%)**MALES****Group 2 (50 mg/kg)**

Animal	11	12	13	14	15	16	17
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TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	14.7	13.5	16.0	9.1	14.3	16.1
	15	29.5	21.7	29.7	21.3	24.6	28.6
	22	39.1	29.8	40.3	28.3	31.8	37.6
	29	45.9	34.1	45.4	34.4	38.4	47.5
	36	55.9	37.2	52.3	39.3	42.3	55.0
	43	60.5	42.8	60.2	46.5	47.9	61.8
	50	64.4	46.8	66.7	45.7	52.5	67.7
	57	71.3	52.1	71.6	54.0	55.4	72.9
	64	65.9	55.2	76.6	57.5	60.2	78.0
	71	68.6	55.4	79.6	59.0	61.7	82.3
	78	73.3	58.3	82.6	57.1	64.7	87.2
	85	76.0	58.2	85.4	60.0	66.2	87.1
	92	78.4	65.0	86.9	63.8	68.4	89.7

Animal	18	19	20
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TREATMENT

Day	1	0.0	0.0	0.0
	8	13.6	14.3	16.5
	15	24.6	30.0	29.1
	22	35.0	41.8	39.8
	29	38.1	52.5	48.4
	36	44.5	58.0	57.9
	43	51.4	64.3	62.1
	50	58.8	70.9	69.0
	57	63.3	74.9	72.5
	64	69.3	79.1	76.8
	71	72.7	84.7	79.0
	78	77.4	91.5	82.8
	85	82.9	93.3	86.5
	92	85.2	95.9	87.3

BODY WEIGHT GAIN (%)**MALES****Group 3 (200 mg/kg)**

Animal	21	22	23	24	25	26	27
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TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	8.0	11.5	17.7	13.5	16.7	10.2
	15	22.7	33.2	32.8	28.0	30.2	22.8
	22	29.0	45.8	44.5	33.2	40.0	28.1
	29	34.6	56.1	53.0	39.6	49.3	31.3
	36	41.3	66.0	60.3	39.6	56.0	34.2
	43	45.7	73.0	67.5	49.1	62.1	41.0
	50	45.0	79.8	72.6	55.2	73.1	45.1
	57	54.2	85.3	76.3	58.5	76.5	47.2
	64	55.0	90.4	80.6	60.1	86.0	48.4
	71	61.6	93.2	81.1	59.9	91.2	49.2
	78	62.4	98.1	85.8	63.6	98.0	50.5
	85	66.4	101.6	88.2	60.7	99.4	55.7
	92	63.2	103.1	88.2	69.1	104.0	58.6

Animal	28	29	30
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TREATMENT

Day	1	0.0	0.0	0.0
	8	18.3	14.3	19.6
	15	34.5	28.0	36.1
	22	46.7	39.2	46.0
	29	57.2	47.4	54.2
	36	63.3	53.2	60.8
	43	70.4	56.2	66.6
	50	75.1	61.6	70.7
	57	80.0	67.1	72.1
	64	84.9	71.4	75.0
	71	89.8	74.1	75.0
	78	93.7	80.7	81.1
	85	95.0	81.1	83.4
	92	95.7	82.4	85.0

BODY WEIGHT GAIN (%)**MALES****Group 4 (1000 mg/kg)**

Animal	31	32	33	34	35	36	37
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	19.8	16.3	15.5	20.2	16.0	13.2
	15	34.8	28.8	28.7	31.9	28.1	29.3
	22	43.0	36.8	37.0	39.3	38.6	33.2
	29	57.4	43.1	44.3	43.2	45.0	42.4
	36	67.1	51.0	52.0	49.9	49.2	44.8
	43	71.8	56.3	55.3	52.6	53.2	49.2
	50	73.5	61.6	59.1	52.3	57.1	52.4
	57	80.2	65.3	62.6	62.9	60.5	55.5
	64	86.6	69.8	68.8	63.2	64.3	59.6
	71	93.5	70.8	71.4	70.0	68.9	62.7
	78	90.6	78.6	76.0	66.6	74.0	68.3
	85	97.6	79.5	75.2	73.8	75.4	71.1
	92	93.8	81.4	76.2	76.8	76.6	86.6
Animal	38	39	40				
TREATMENT							
Day	1	0.0	0.0	0.0			
	8	11.9	15.4	17.9			
	15	23.9	28.8	29.7			
	22	31.8	38.4	40.0			
	29	38.0	44.9	49.2			
	36	44.3	46.4	56.4			
	43	49.1	51.6	63.3			
	50	52.7	53.2	68.7			
	57	56.0	57.9	74.0			
	64	60.0	62.1	78.8			
	71	63.3	66.8	83.1			
	78	67.6	70.1	88.5			
	85	69.4	74.5	91.6			
	92	69.4	75.5	94.2			

BODY WEIGHT GAIN (%)
FEMALES
Group 1 (0 mg/kg)

Animal	41	42	43	44	45	46	47
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.9	9.2	11.5	8.8	9.6	11.3
	15	9.9	14.2	16.8	18.4	19.2	19.8
	22	19.6	22.6	17.4	24.2	23.5	24.2
	29	25.5	27.9	27.0	27.2	32.2	24.6
	36	32.6	31.0	31.6	30.1	37.4	26.5
	43	31.7	29.7	32.8	37.7	39.6	34.4
	50	35.8	34.8	32.2	40.2	38.3	34.8
	57	38.4	37.8	36.5	45.3	43.8	36.8
	64	44.4	38.2	40.0	43.2	50.8	41.5
	71	39.0	37.4	42.7	44.4	52.2	42.0
	78	44.8	45.5	40.5	51.5	55.6	47.2
	85	40.3	39.1	44.3	47.0	52.5	46.8
	92	45.5	46.7	47.1	55.7	57.1	55.6
Animal	48	49	50				
TREATMENT							
Day	1	0.0	0.0	0.0			
	8	12.8	6.5	8.1			
	15	20.8	12.7	15.1			
	22	26.7	14.5	20.0			
	29	27.1	21.8	21.1			
	36	32.7	25.6	26.8			
	43	37.6	27.4	28.4			
	50	39.2	28.4	28.3			
	57	37.6	31.8	27.0			
	64	43.6	34.1	30.2			
	71	45.7	34.0	32.7			
	78	47.0	37.1	34.8			
	85	44.8	36.4	33.9			
	92	47.8	38.0	36.9			

BODY WEIGHT GAIN (%)
FEMALES
Group 2 (50 mg/kg)

Animal	51	52	53	54	55	56	57
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	9.3	11.4	12.3	17.0	9.5	7.5
	15	13.3	21.2	20.2	32.4	14.1	15.8
	22	21.1	25.5	22.1	40.3	22.3	22.2
	29	25.3	36.9	34.4	33.0	29.7	28.2
	36	26.7	43.7	40.9	47.8	33.1	32.4
	43	26.5	46.7	45.0	50.5	33.1	31.2
	50	30.0	46.0	43.0	51.2	40.5	36.5
	57	32.7	54.3	49.8	50.9	45.8	41.8
	64	38.1	61.5	59.1	59.7	48.8	42.1
	71	39.3	60.5	59.6	62.9	46.4	45.0
	78	45.8	62.0	57.2	67.7	55.3	50.6
	85	46.1	60.6	57.4	60.3	49.1	43.6
	92	49.4	62.3	65.8	70.0	55.9	48.7
Animal	58	59	60				
TREATMENT							
Day	1	0.0	0.0	0.0			
	8	5.8	9.0	0.6			
	15	8.9	18.0	2.4			
	22	19.2	20.5	12.5			
	29	25.9	31.5	17.6			
	36	31.3	37.9	20.0			
	43	30.2	38.6	16.0			
	50	34.8	38.0	15.9			
	57	40.5	44.7	19.3			
	64	41.2	48.9	21.0			
	71	42.4	45.6	23.9			
	78	47.5	45.3	29.6			
	85	42.6	44.5	25.9			
	92	47.2	53.3	33.3			

BODY WEIGHT GAIN (%)
FEMALES
Group 3 (200 mg/kg)

Animal	61	62	63	64	65	66	67
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.4	10.5	11.8	4.5	12.0	12.7
	15	16.3	22.8	20.9	16.5	19.8	22.2
	22	19.9	26.8	23.4	25.4	20.7	28.3
	29	27.9	37.2	26.2	29.2	28.7	35.1
	36	26.2	40.8	32.7	33.9	31.9	39.2
	43	31.9	44.4	36.5	32.3	35.7	37.7
	50	32.4	45.3	39.5	35.7	33.3	44.6
	57	36.4	53.9	39.7	44.5	38.7	46.7
	64	36.0	57.9	46.0	44.1	42.0	49.6
	71	41.6	58.2	50.3	41.9	43.2	48.3
	78	44.2	58.5	52.2	48.2	42.0	55.2
	85	40.9	64.6	49.8	40.9	38.6	49.1
	92	37.4	65.9	54.7	39.8	42.1	48.5
Animal	68	69	70				
TREATMENT							
Day	1	0.0	0.0	0.0			
	8	8.1	9.2	7.2			
	15	19.8	24.3	17.2			
	22	28.5	31.2	24.3			
	29	31.1	34.7	27.8			
	36	31.8	37.3	29.1			
	43	39.8	47.5	31.7			
	50	45.0	49.6	33.1			
	57	49.6	50.3	34.8			
	64	48.6	49.6	36.3			
	71	55.8	54.7	38.5			
	78	61.7	59.7	40.7			
	85	51.4	56.7	36.0			
	92	61.9	52.3	39.6			

BODY WEIGHT GAIN (%)
FEMALES
Group 4 (1000 mg/kg)

Animal	71	72	73	74	75	76	77
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	9.1	7.3	8.4	11.5	13.6	7.4
	15	17.0	11.3	15.7	18.2	18.9	13.9
	22	17.9	21.5	17.6	32.2	28.0	22.4
	29	27.0	29.0	23.9	38.9	35.5	30.0
	36	29.8	32.6	28.5	43.7	37.2	29.9
	43	31.3	31.2	33.0	38.1	35.2	31.4
	50	29.4	40.3	33.9	51.5	41.5	39.2
	57	38.1	44.3	39.2	55.5	46.7	45.8
	64	41.0	44.9	41.5	54.0	50.7	45.8
	71	41.8	44.1	41.2	55.0	49.2	45.9
	78	42.4	50.7	47.6	59.3	55.7	50.3
	85	43.6	46.4	45.9	57.4	51.7	49.0
	92	40.1	47.0	49.6	62.5	53.8	52.0
Animal	78	79	80				
TREATMENT							
Day	1	0.0	0.0	0.0			
	8	8.9	10.4	5.3			
	15	12.0	17.0	13.2			
	22	20.6	25.0	16.7			
	29	29.0	31.9	22.5			
	36	30.0	36.3	25.9			
	43	28.7	38.3	27.7			
	50	39.6	44.7	28.7			
	57	43.4	48.7	31.5			
	64	44.2	50.2	34.1			
	71	42.5	49.9	35.3			
	78	49.3	57.8	35.9			
	85	42.2	55.3	34.4			
	92	47.8	59.0	38.7			

OPHTHALMOSCOPY**Comments**

Data excluded from Summary Report

Not Reported

All Study Phases

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

[illegible]

[illegible]

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[illegible]

[illegible]

OPHTHALMOSCOPY
ACCLIMATIZATION, Day 2
FEMALES
Group 3 (200 mg/kg)

	Animal	61	62	63	64	65	66	67	68	69	70
Unscheduled Findings											
CORNEA											
- CORNEAL OPACITY (3)											
(LEFT EYE)		0	0	0	0	0	0	1	0	0	1
(RIGHT EYE)		0	0	0	0	0	1	1	0	0	0
LENS											
- LENTICULAR VACUOLE (3)											
(RIGHT EYE)		0	0	0	0	0	0	0	0	0	0
VITREOUS BODY											
- PERSISTENT HYALOID VESSEL (1)											
(LEFT EYE)		0	0	0	0	0	0	0	0	0	0
(RIGHT EYE)		0	0	0	0	0	1	0	0	0	0

[illegible]

OPHTHALMOSCOPY

GR. 1 AND GR. 4

TREATMENT, Day 89

FEMALES

Group 1 (0 mg/kg)

[illegible]

OPHTHALMOSCOPY

GR. 1 AND GR. 4

TREATMENT, Day 89

FEMALES

Group 4 (1000 mg/kg)

[illegible]

REPORT (PART II OF II)

Lipase produced with *Trichoderma reesei*: 90-Day Oral Toxicity (Gavage) Study in the Wistar Rat

Study Director:	W. H. Braun
Test Facility:	Harlan Laboratories Ltd. Zelgliweg 1 4452 Itingen / Switzerland
Sponsor:	AB Enzymes Feldbergstrasse 78 64293 Darmstadt / Germany
Study Monitor:	Dr. H.-J. Schepers
Harlan Study Number:	D80691
Study Completion Date:	30-Apr-2014

Hematology

Comments

a not enough sample
b coagulated sample

Data excluded from Summary Report

Not Reported

All Measurements

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Reported Parameter

After 13 Weeks

RBC	ERYTHROCYTES (RBC)
HB	HEMOGLOBIN (HB)
HCT	HEMATOCRIT (HCT)
MCV	MEAN CORPUSCULAR VOLUME (MCV)
RDW	RED CELL VOL. DISTR. WIDTH (RDW)
MCH	MEAN CORPUSCULAR HEMOGLOBIN (MCH)
MCHC	MEAN CORPUSCULAR HEMOGLOBIN CONC. (MCHC)
HDW	HEMOGLOBIN CONC. DISTR. WIDTH
RETI	RETICULOCYTE (REL)
RETI	RETICULOCYTE (ABS)
L RETI	MATURITY INDEX (L-RETI)
M RETI	MATURITY INDEX (M-RETI)
H RETI	MATURITY INDEX (H-RETI)
WBC	LEUKOCYTES, TOTAL (WBC)
NEUT	NEUTROPHILS (NEUT)
EOS	EOSINOPHILS (EOS)
BASO	BASOPHILS (BASO)
LYMPH	LYMPHOCYTES (LYMPH)
MONO	MONOCYTES (MONO)
LUC	LARGE UNSTAINED CELLS (LUC)
NEUT	NEUTROPHILS (NEUT)
EOS	EOSINOPHILS (EOS)
BASO	BASOPHILS (BASO)
LYMPH	LYMPHOCYTES (LYMPH)
MONO	MONOCYTES (MONO)
LUC	LARGE UNSTAINED CELLS (LUC)
PLATELETS	THROMBOCYTES (PLATELETS)
MET-HB	METHEMOGLOBIN (MET-HB)
PT	PROTHROMBIN TIME (PT)
PTT	PARTIAL THROMBOPLASTIN TIME (PTT)

Hematology
After 13 Weeks
MALES

Group 1 (0 mg/kg)

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. 1	fl	rel. 1	fmol	mmol/l
1	9.29	10.1	0.48	51.9	0.168	1.09	21.00
2	9.03	10.0	0.47	51.8	0.140	1.10	21.30
3	8.81	10.3	0.48	54.7	0.219	1.17	21.43
4	9.03	9.8	0.46	51.0	0.140	1.09	21.30
5	9.28	10.0	0.48	52.1	0.137	1.07	20.60
6	9.56	10.2	0.50	52.5	0.121	1.07	20.40
7	9.24	10.2	0.49	53.4	0.124	1.10	20.64
8	9.25	9.9	0.46	50.2	0.133	1.07	21.23
9	---	a	---	---	---	---	---
10	8.64	9.7	0.46	53.2	0.135	1.12	21.13

	GENERAL		RETICULOCYTE COUNT				GENERAL
	HDW	RETI	RETI	L RETI	M RETI	H RETI	WBC
	mmol/l	rel. 1	G/l	rel. 1	rel. 1	rel. 1	G/l
1	1.89	0.017	155	0.754	0.220	0.026	6.79
2	2.01	0.029	264	0.596	0.328	0.076	7.87
3	1.96	0.021	185	0.652	0.293	0.055	7.94
4	1.89	0.021	187	0.672	0.295	0.033	8.12
5	2.05	0.022	208	0.605	0.327	0.069	4.86
6	1.66	0.023	219	0.577	0.359	0.064	6.68
7	1.64	0.025	229	0.617	0.316	0.067	5.66
8	1.97	0.028	256	0.626	0.329	0.045	8.37
9	---	---	---	---	---	---	---
10	1.81	0.022	191	0.632	0.298	0.070	7.29

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
1	0.135	0.023	0.005	0.815	0.019	0.003
2	0.135	0.021	0.004	0.817	0.019	0.004
3	0.149	0.017	0.003	0.804	0.023	0.004
4	0.165	0.024	0.005	0.766	0.029	0.010
5	0.173	0.019	0.003	0.788	0.013	0.004
6	0.182	0.020	0.003	0.771	0.020	0.004
7	0.211	0.021	0.002	0.741	0.022	0.004
8	0.231	0.022	0.005	0.707	0.032	0.003
9	---	---	---	---	---	---
10	0.168	0.019	0.003	0.787	0.019	0.004

a: See explanation on section cover page

Hematology
After 13 Weeks
MALES

Group 1 (0 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT	EOS	BASO	LYMPH	MONO	LUC	PLATELETS
	G/1	G/1	G/1	G/1	G/1	G/1	G/1
1	0.92	0.15	0.03	5.54	0.13	0.02	788
2	1.06	0.17	0.03	6.43	0.15	0.03	1102
3	1.18	0.14	0.02	6.38	0.18	0.04	925
4	1.34	0.19	0.04	6.22	0.24	0.08	1003
5	0.84	0.09	0.01	3.83	0.06	0.02	981
6	1.22	0.14	0.02	5.15	0.13	0.03	1007
7	1.19	0.12	0.01	4.19	0.12	0.02	984
8	1.93	0.18	0.04	5.92	0.27	0.03	934
9	---	---	---	---	---	---	---
10	1.23	0.14	0.02	5.74	0.14	0.03	865

	GENERAL	COAGULATION	
	MET-HB	PT	PTT
	rel. 1	rel. 1	sec
1	0.012	0.81	25.9
2	0.012	0.78	24.2
3	0.012	0.81	25.6
4	0.012	0.78	24.0
5	0.011	0.75	23.9
6	0.012	0.83	23.4
7	0.014	0.84	23.5
8	0.012	--- b	--- b
9	0.012	0.80	24.0
10	0.013	0.82	22.4

b: See explanation on section cover page

Hematology
After 13 Weeks
MALES

Group 2 (50 mg/kg)

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. l	fl	rel. l	fmol	mmol/l
11	9.10	10.1	0.51	55.9	0.134	1.11	19.90
12	9.13	9.7	0.45	49.4	0.246	1.06	21.43
13	9.41	9.7	0.47	49.8	0.126	1.03	20.63
14	9.73	10.1	0.48	49.6	0.141	1.04	21.00
15	8.89	9.6	0.47	52.3	0.127	1.08	20.67
16	--- a	---	---	---	---	---	---
17	8.80	10.4	0.50	56.3	0.115	1.19	21.07
18	9.52	10.0	0.47	49.7	0.139	1.05	21.03
19	--- a	---	---	---	---	---	---
20	9.36	9.8	0.47	50.6	0.132	1.05	20.64

	GENERAL	RETICULOCYTE COUNT					GENERAL
	HDW	RETI	RETI	L RETI	M RETI	H RETI	WBC
	mmol/l	rel. l	G/l	rel. l	rel. l	rel. l	G/l
11	1.56	0.021	192	0.632	0.339	0.030	7.30
12	2.01	0.024	223	0.716	0.260	0.024	7.88
13	1.63	0.022	202	0.652	0.312	0.036	5.73
14	1.92	0.020	196	0.650	0.300	0.050	5.22
15	1.67	0.026	232	0.581	0.359	0.059	7.58
16	---	---	---	---	---	---	---
17	1.46	0.019	165	0.635	0.323	0.042	6.79
18	1.91	0.030	283	0.614	0.326	0.060	8.80
19	---	---	---	---	---	---	---
20	1.73	0.023	217	0.629	0.303	0.068	4.59

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. l	rel. l	rel. l	rel. l	rel. l	rel. l
11	0.325	0.033	0.004	0.615	0.021	0.004
12	0.192	0.019	0.003	0.745	0.037	0.004
13	0.194	0.023	0.003	0.751	0.026	0.003
14	0.194	0.036	0.004	0.743	0.020	0.003
15	0.149	0.025	0.006	0.787	0.029	0.004
16	---	---	---	---	---	---
17	0.243	0.036	0.004	0.692	0.021	0.004
18	0.256	0.021	0.006	0.689	0.024	0.004
19	---	---	---	---	---	---
20	0.241	0.044	0.006	0.674	0.031	0.003

a: See explanation on section cover page

Hematology
After 13 Weeks
MALES

Group 2 (50 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT	EOS	BASO	LYMPH	MONO	LUC	PLATELETS
	G/l	G/l	G/l	G/l	G/l	G/l	G/l
11	2.37	0.24	0.03	4.49	0.15	0.03	1195
12	1.51	0.15	0.03	5.87	0.29	0.04	970
13	1.11	0.13	0.02	4.30	0.15	0.02	1048
14	1.01	0.19	0.02	3.88	0.10	0.02	1053
15	1.13	0.19	0.05	5.97	0.22	0.03	1028
16	---	---	---	---	---	---	---
17	1.65	0.24	0.03	4.70	0.14	0.03	871
18	2.25	0.19	0.06	6.06	0.21	0.03	899
19	---	---	---	---	---	---	---
20	1.11	0.20	0.03	3.09	0.14	0.02	1006

	GENERAL	COAGULATION	
	MET-HB	PT	PTT
	rel. 1	rel. 1	sec
11	0.012	0.82	22.8
12	0.013	0.79	22.0
13	0.013	0.75	25.4
14	0.013	0.78	23.2
15	0.012	0.78	22.5
16	0.012	0.80	21.3
17	0.013	0.75	23.2
18	0.012	0.82	23.3
19	0.013	0.81	23.6
20	0.013	0.81	23.2

Hematology
After 13 Weeks
MALES

Group 3 (200 mg/kg)

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. l	fl	rel. l	fmol	mmol/l
21	9.24	10.3	0.48	52.0	0.151	1.12	21.52
22	9.27	10.3	0.50	53.5	0.125	1.11	20.72
23	8.49	9.6	0.44	51.7	0.140	1.13	21.83
24	9.64	10.2	0.49	50.3	0.128	1.06	20.99
25	9.01	10.4	0.48	53.8	0.146	1.16	21.51
26	8.89	9.9	0.47	52.8	0.126	1.11	21.05
27	8.55	10.3	0.49	57.2	0.125	1.20	21.05
28	9.36	9.8	0.47	50.5	0.129	1.05	20.80
29	8.98	9.7	0.47	51.9	0.129	1.08	20.77
30	8.21	10.1	0.46	55.8	0.247	1.23	22.06

	GENERAL	RETICULOCYTE COUNT					GENERAL
	HDW	RETI	RETI	L RETI	M RETI	H RETI	WBC
	mmol/l	rel. l	G/l	rel. l	rel. l	rel. l	G/l
21	1.98	0.020	185	0.664	0.281	0.055	8.89
22	1.57	0.022	204	0.655	0.301	0.044	4.05
23	1.95	0.029	242	0.607	0.333	0.060	6.64
24	1.55	0.020	189	0.703	0.256	0.041	6.01
25	1.90	0.024	215	0.607	0.327	0.066	6.92
26	1.89	0.027	236	0.626	0.310	0.064	6.60
27	1.67	0.023	193	0.632	0.314	0.055	6.61
28	1.59	0.021	198	0.632	0.322	0.047	4.74
29	1.62	0.021	188	0.603	0.315	0.082	5.94
30	2.02	0.018	149	0.607	0.350	0.044	8.66

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. l	rel. l	rel. l	rel. l	rel. l	rel. l
21	0.382	0.013	0.003	0.586	0.014	0.002
22	0.248	0.036	0.004	0.694	0.014	0.004
23	0.270	0.026	0.002	0.675	0.024	0.003
24	0.287	0.024	0.004	0.658	0.024	0.003
25	0.163	0.011	0.004	0.794	0.020	0.009
26	0.214	0.023	0.005	0.732	0.023	0.004
27	0.196	0.021	0.007	0.750	0.023	0.003
28	0.227	0.026	0.004	0.721	0.018	0.004
29	0.238	0.031	0.003	0.703	0.024	0.002
30	0.402	0.021	0.003	0.545	0.024	0.006

Hematology
After 13 Weeks
MALES

Group 3 (200 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT	EOS	BASO	LYMPH	MONO	LUC	PLATELETS
	G/l	G/l	G/l	G/l	G/l	G/l	G/l
21	3.39	0.11	0.03	5.21	0.13	0.01	1024
22	1.01	0.14	0.02	2.81	0.06	0.01	954
23	1.79	0.17	0.01	4.49	0.16	0.02	918
24	1.73	0.14	0.02	3.95	0.14	0.02	927
25	1.13	0.07	0.02	5.49	0.14	0.06	828
26	1.41	0.15	0.03	4.83	0.15	0.03	894
27	1.29	0.14	0.05	4.95	0.15	0.02	885
28	1.08	0.12	0.02	3.42	0.09	0.02	1000
29	1.41	0.18	0.02	4.17	0.14	0.01	975
30	3.49	0.18	0.02	4.72	0.21	0.05	834

	GENERAL	COAGULATION	
	MET-HB	PT	PTT
	rel. 1	rel. 1	sec
21	0.012	0.79	22.0
22	0.012	0.82	22.2
23	0.012	0.90	19.2
24	0.011	0.78	25.3
25	0.012	0.86	23.2
26	0.013	0.83	21.6
27	0.013	1.02	20.9
28	--- b	1.08	23.8
29	0.013	1.10	21.7
30	0.012	1.14	24.8

b: See explanation on section cover page

Hematology
After 13 Weeks
MALES

Group 4 (1000 mg/kg)

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. 1	fl	rel. 1	fmol	mmol/l
31	9.91	10.0	0.49	49.5	0.128	1.01	20.41
32	8.83	10.3	0.48	54.0	0.117	1.17	21.65
33	9.42	9.8	0.47	49.6	0.129	1.04	20.95
34	9.44	9.8	0.48	51.3	0.130	1.04	20.33
35	9.13	10.1	0.47	51.9	0.117	1.11	21.35
36	8.94	9.9	0.47	52.5	0.124	1.11	21.11
37	--- a	---	---	---	---	---	---
38	9.02	9.9	0.48	53.3	0.120	1.10	20.59
39	8.56	10.1	0.47	54.5	0.168	1.18	21.57
40	8.68	9.6	0.44	50.4	0.147	1.10	21.82

	GENERAL		RETICULOCYTE COUNT				GENERAL
	HDW	RETI	RETI	L RETI	M RETI	H RETI	WBC
	mmol/l	rel. 1	G/l	rel. 1	rel. 1	rel. 1	G/l
31	1.51	0.016	158	0.648	0.304	0.047	5.73
32	1.60	0.025	222	0.569	0.358	0.073	7.59
33	1.56	0.023	215	0.635	0.322	0.043	7.55
34	1.60	0.019	175	0.692	0.275	0.033	7.48
35	1.52	0.019	176	0.677	0.253	0.070	5.59
36	1.50	0.023	207	0.623	0.325	0.052	7.38
37	---	---	---	---	---	---	---
38	1.47	0.019	168	0.654	0.306	0.040	6.53
39	1.74	0.019	162	0.641	0.327	0.032	8.22
40	1.84	0.027	231	0.605	0.310	0.085	5.73

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
31	0.244	0.026	0.005	0.695	0.027	0.004
32	0.215	0.023	0.003	0.737	0.019	0.003
33	0.130	0.020	0.004	0.822	0.019	0.004
34	0.138	0.014	0.004	0.826	0.014	0.004
35	0.175	0.036	0.011	0.754	0.020	0.005
36	0.196	0.020	0.003	0.761	0.016	0.004
37	---	---	---	---	---	---
38	0.197	0.031	0.005	0.749	0.016	0.002
39	0.529	0.030	0.005	0.398	0.035	0.004
40	0.208	0.034	0.007	0.721	0.025	0.005

a: See explanation on section cover page

Hematology
After 13 Weeks
MALES

Group 4 (1000 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT	EOS	BASO	LYMPH	MONO	LUC	PLATELETS
	G/l	G/l	G/l	G/l	G/l	G/l	G/l
31	1.40	0.15	0.03	3.98	0.15	0.02	877
32	1.64	0.17	0.03	5.59	0.14	0.02	1146
33	0.98	0.15	0.03	6.20	0.15	0.03	1014
34	1.03	0.10	0.03	6.18	0.10	0.03	970
35	0.98	0.20	0.06	4.21	0.11	0.03	923
36	1.45	0.15	0.02	5.62	0.12	0.03	895
37	---	---	---	---	---	---	---
38	1.29	0.20	0.03	4.89	0.11	0.01	819
39	4.35	0.24	0.04	3.27	0.28	0.03	1015
40	1.19	0.19	0.04	4.13	0.14	0.03	917

	GENERAL	COAGULATION	
	MET-HB	PT	PTT
	rel. 1	rel. 1	sec
31	0.013	0.93	22.1
32	0.012	0.94	20.0
33	0.012	0.90	20.7
34	0.012	0.86	23.7
35	0.013	0.90	24.2
36	0.013	0.92	23.0
37	0.013	0.83	25.4
38	0.012	0.81	23.8
39	0.012	0.88	19.7
40	0.013	0.88	24.5

Hematology
After 13 Weeks
FEMALES

Group 1 (0 mg/kg)

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. 1	fl	rel. 1	fmol	mmol/l
41	7.47	9.0	0.41	55.1	0.133	1.20	21.79
42	8.22	9.3	0.45	55.2	0.109	1.13	20.45
43	7.49	9.0	0.42	56.0	0.115	1.20	21.33
44	7.82	9.4	0.43	55.1	0.141	1.20	21.82
45	7.20	9.0	0.42	58.1	0.135	1.24	21.44
46	7.91	9.3	0.43	54.2	0.138	1.17	21.61
47	7.58	8.7	0.41	54.7	0.115	1.15	21.00
48	8.27	9.2	0.45	54.1	0.110	1.11	20.59
49	7.82	9.0	0.42	53.7	0.116	1.15	21.35
50	8.42	9.1	0.44	52.3	0.117	1.08	20.73

	GENERAL	RETICULOCYTE COUNT					GENERAL
	HDW	RETI	RETI	L RETI	M RETI	H RETI	WBC
	mmol/l	rel. 1	G/l	rel. 1	rel. 1	rel. 1	G/l
41	1.54	0.030	224	0.526	0.422	0.052	5.79
42	1.32	0.027	221	0.497	0.425	0.079	2.29
43	1.47	0.038	288	0.439	0.424	0.138	2.79
44	1.62	0.035	277	0.595	0.370	0.035	3.39
45	1.42	0.036	258	0.508	0.418	0.074	3.32
46	1.53	0.031	245	0.684	0.297	0.019	2.78
47	1.43	0.035	265	0.464	0.377	0.159	3.91
48	1.37	0.028	232	0.529	0.399	0.072	3.93
49	1.55	0.031	242	0.645	0.319	0.036	3.30
50	1.43	0.032	271	0.597	0.361	0.043	2.61

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
41	0.148	0.012	0.003	0.823	0.011	0.003
42	0.206	0.016	0.004	0.717	0.050	0.007
43	0.121	0.017	0.002	0.832	0.020	0.008
44	0.214	0.020	0.002	0.742	0.015	0.006
45	0.152	0.026	0.003	0.795	0.018	0.006
46	0.168	0.030	0.005	0.764	0.024	0.008
47	0.175	0.026	0.003	0.775	0.018	0.004
48	0.158	0.020	0.004	0.782	0.024	0.013
49	0.172	0.014	0.003	0.774	0.031	0.007
50	0.213	0.026	0.006	0.722	0.030	0.003

Hematology
After 13 Weeks
FEMALES

Group 1 (0 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT	EOS	BASO	LYMPH	MONO	LUC	PLATELETS
	G/l	G/l	G/l	G/l	G/l	G/l	G/l
41	0.86	0.07	0.02	4.77	0.06	0.02	860
42	0.47	0.04	0.01	1.64	0.11	0.02	998
43	0.34	0.05	0.01	2.32	0.05	0.02	906
44	0.73	0.07	0.01	2.52	0.05	0.02	1126
45	0.50	0.09	0.01	2.64	0.06	0.02	972
46	0.47	0.08	0.01	2.12	0.07	0.02	965
47	0.68	0.10	0.01	3.03	0.07	0.01	1074
48	0.62	0.08	0.01	3.07	0.09	0.05	1259
49	0.57	0.05	0.01	2.56	0.10	0.02	1228
50	0.56	0.07	0.01	1.88	0.08	0.01	1256

	GENERAL	COAGULATION	
	MET-HB	PT	PTT
	rel. 1	rel. 1	sec
41	0.014	0.80	32.2
42	0.015	0.82	32.4
43	0.015	0.76	29.6
44	0.015	0.75	29.3
45	0.015	0.80	33.7
46	0.015	0.72	30.9
47	0.015	0.78	32.5
48	0.015	0.73	38.1
49	0.015	0.85	30.1
50	0.015	0.82	28.9

Hematology
After 13 Weeks
FEMALES

Group 2 (50 mg/kg)

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. 1	fl	rel. 1	fmol	mmol/l
51	8.06	9.6	0.45	56.1	0.111	1.19	21.26
52	7.65	8.9	0.43	56.2	0.109	1.17	20.82
53	7.34	8.8	0.42	57.3	0.119	1.19	20.83
54	7.67	8.6	0.41	53.2	0.114	1.12	20.97
55	7.63	9.1	0.43	56.8	0.108	1.20	21.07
56	8.22	9.5	0.45	54.6	0.112	1.15	21.07
57	8.27	9.3	0.44	53.6	0.108	1.13	21.03
58	7.62	9.2	0.42	55.5	0.136	1.21	21.79
59	8.15	9.2	0.43	53.1	0.114	1.13	21.24
60	7.77	8.7	0.42	53.9	0.127	1.12	20.81

	GENERAL	RETICULOCYTE COUNT					GENERAL
	HDW	RETI	RETI	L RETI	M RETI	H RETI	WBC
	mmol/l	rel. 1	G/l	rel. 1	rel. 1	rel. 1	G/l
51	1.31	0.025	203	0.436	0.400	0.163	3.81
52	1.34	0.032	241	0.579	0.363	0.057	2.39
53	1.37	0.035	259	0.450	0.408	0.142	1.64
54	1.33	0.034	257	0.576	0.367	0.057	2.08
55	1.37	0.028	214	0.502	0.391	0.107	3.32
56	1.41	0.028	228	0.639	0.330	0.031	3.37
57	1.34	0.033	273	0.646	0.329	0.025	2.41
58	1.54	0.029	221	0.533	0.403	0.064	3.29
59	1.37	0.027	221	0.587	0.376	0.038	1.92
60	1.46	0.044	344	0.512	0.416	0.072	3.34

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
51	0.203	0.032	0.006	0.731	0.023	0.006
52	0.148	0.036	0.001	0.794	0.017	0.004
53	0.210	0.049	0.002	0.712	0.017	0.009
54	0.206	0.034	0.005	0.727	0.026	0.002
55	0.120	0.017	0.004	0.842	0.013	0.005
56	0.276	0.025	0.001	0.671	0.024	0.003
57	0.230	0.023	0.003	0.714	0.028	0.003
58	0.242	0.020	0.002	0.710	0.023	0.004
59	0.226	0.041	0.003	0.700	0.023	0.006
60	0.110	0.022	0.004	0.841	0.017	0.006

Hematology
After 13 Weeks
FEMALES

Group 2 (50 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT	EOS	BASO	LYMPH	MONO	LUC	PLATELETS
	G/l	G/l	G/l	G/l	G/l	G/l	G/l
51	0.78	0.12	0.02	2.79	0.09	0.02	1009
52	0.35	0.09	0.00	1.90	0.04	0.01	835
53	0.34	0.08	0.00	1.16	0.03	0.02	1062
54	0.43	0.07	0.01	1.51	0.05	0.00	1045
55	0.40	0.06	0.01	2.79	0.04	0.02	935
56	0.93	0.08	0.00	2.26	0.08	0.01	1032
57	0.55	0.06	0.01	1.72	0.07	0.01	982
58	0.80	0.06	0.01	2.33	0.07	0.01	1066
59	0.43	0.08	0.01	1.34	0.04	0.01	930
60	0.37	0.07	0.01	2.81	0.06	0.02	1316

	GENERAL	COAGULATION	
	MET-HB	PT	PTT
	rel. 1	rel. 1	sec
51	0.015	0.86	28.0
52	0.015	0.81	28.3
53	0.016	0.90	25.4
54	0.015	0.71	31.1
55	0.015	0.79	31.4
56	0.016	0.71	36.2
57	0.016	0.81	35.8
58	0.014	0.78	32.1
59	0.015	0.81	31.1
60	0.015	0.87	27.4

Hematology
After 13 Weeks
FEMALES

Group 3 (200 mg/kg)

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. 1	fl	rel. 1	fmol	mmol/l
61	8.01	8.7	0.41	51.2	0.113	1.09	21.31
62	7.41	9.2	0.43	57.8	0.199	1.24	21.44
63	7.66	9.0	0.41	54.1	0.151	1.18	21.78
64	8.98	9.3	0.45	49.8	0.115	1.03	20.78
65	8.16	9.4	0.45	55.1	0.106	1.15	20.90
66	7.33	9.3	0.42	56.6	0.205	1.27	22.35
67	8.15	9.3	0.44	54.5	0.111	1.14	20.93
68	7.41	8.6	0.40	54.3	0.136	1.16	21.40
69	7.96	8.9	0.43	53.5	0.113	1.12	21.00
70	7.84	9.4	0.43	54.9	0.140	1.20	21.80

	GENERAL	RETICULOCYTE COUNT					GENERAL
	HDW	RETI	RETI	L RETI	M RETI	H RETI	WBC
	mmol/l	rel. 1	G/l	rel. 1	rel. 1	rel. 1	G/l
61	1.42	0.019	148	0.690	0.288	0.022	2.25
62	1.54	0.026	193	0.491	0.419	0.089	2.17
63	1.59	0.038	295	0.573	0.371	0.056	1.70
64	1.44	0.024	218	0.705	0.273	0.022	2.90
65	1.29	0.027	222	0.703	0.276	0.021	2.35
66	1.62	0.032	233	0.637	0.330	0.034	3.25
67	1.31	0.023	187	0.534	0.393	0.073	2.84
68	1.56	0.044	324	0.532	0.389	0.080	2.33
69	1.45	0.029	231	0.639	0.344	0.017	2.52
70	1.48	0.022	169	0.678	0.291	0.032	2.92

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
61	0.353	0.040	0.003	0.581	0.020	0.003
62	0.212	0.036	0.003	0.712	0.032	0.004
63	0.219	0.038	0.007	0.705	0.027	0.003
64	0.182	0.029	0.003	0.756	0.028	0.002
65	0.159	0.037	0.004	0.781	0.016	0.004
66	0.197	0.024	0.005	0.749	0.019	0.006
67	0.243	0.037	0.001	0.699	0.015	0.004
68	0.186	0.042	0.002	0.756	0.010	0.005
69	0.207	0.029	0.002	0.743	0.013	0.005
70	0.220	0.028	0.002	0.710	0.034	0.005

Hematology
After 13 Weeks
FEMALES

Group 3 (200 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT	EOS	BASO	LYMPH	MONO	LUC	PLATELETS
	G/l	G/l	G/l	G/l	G/l	G/l	G/l
61	0.79	0.09	0.01	1.31	0.05	0.01	1040
62	0.46	0.08	0.01	1.54	0.07	0.01	980
63	0.37	0.07	0.01	1.20	0.05	0.01	855
64	0.53	0.09	0.01	2.19	0.08	0.01	1129
65	0.37	0.09	0.01	1.84	0.04	0.01	1012
66	0.64	0.08	0.02	2.43	0.06	0.02	994
67	0.69	0.10	0.00	1.99	0.04	0.01	848
68	0.43	0.10	0.00	1.76	0.02	0.01	909
69	0.52	0.07	0.01	1.87	0.03	0.01	996
70	0.64	0.08	0.00	2.07	0.10	0.02	981

	GENERAL	COAGULATION	
	MET-HB	PT	PTT
	rel. 1	rel. 1	sec
61	0.016	0.76	32.7
62	0.015	0.79	32.7
63	0.015	0.81	36.5
64	0.015	0.76	32.8
65	0.015	0.79	33.1
66	0.016	0.84	30.7
67	0.015	0.82	26.3
68	0.016	0.80	29.0
69	0.017	0.84	28.7
70	0.015	0.73	33.3

Hematology
After 13 Weeks
FEMALES

Group 4 (1000 mg/kg)

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. 1	fl	rel. 1	fmol	mmol/l
71	8.18	9.5	0.44	54.2	0.124	1.16	21.48
72	7.55	9.1	0.42	56.0	0.123	1.21	21.61
73	7.55	9.3	0.43	56.9	0.150	1.23	21.53
74	7.71	9.1	0.43	56.3	0.118	1.18	20.99
75	8.13	9.5	0.45	55.1	0.109	1.17	21.30
76	7.88	9.4	0.44	55.7	0.110	1.20	21.47
77	7.92	9.1	0.43	53.8	0.115	1.15	21.35
78	8.03	9.0	0.42	52.3	0.117	1.13	21.55
79	---	a	---	---	---	---	---
80	7.98	9.2	0.44	55.0	0.108	1.16	21.06

	GENERAL		RETICULOCYTE COUNT				GENERAL
	HDW	RETI	RETI	L RETI	M RETI	H RETI	WBC
	mmol/l	rel. 1	G/l	rel. 1	rel. 1	rel. 1	G/l
71	1.46	0.022	177	0.706	0.273	0.021	3.49
72	1.43	0.026	200	0.652	0.316	0.033	4.00
73	1.64	0.042	317	0.586	0.369	0.046	4.74
74	1.43	0.040	309	0.481	0.422	0.096	4.11
75	1.34	0.022	182	0.689	0.276	0.034	2.63
76	1.37	0.027	215	0.567	0.373	0.059	3.15
77	1.40	0.032	251	0.577	0.359	0.065	2.66
78	1.46	0.031	253	0.692	0.282	0.027	2.53
79	---	---	---	---	---	---	---
80	1.32	0.025	202	0.645	0.323	0.031	2.77

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
71	0.159	0.037	0.002	0.781	0.017	0.004
72	0.175	0.016	0.004	0.789	0.013	0.004
73	0.241	0.024	0.003	0.704	0.023	0.006
74	0.321	0.022	0.005	0.619	0.027	0.006
75	0.313	0.039	0.001	0.626	0.019	0.002
76	0.176	0.027	0.004	0.759	0.030	0.004
77	0.286	0.032	0.002	0.651	0.023	0.006
78	0.327	0.033	0.002	0.607	0.027	0.004
79	---	---	---	---	---	---
80	0.175	0.033	0.002	0.765	0.019	0.006

a: See explanation on section cover page

Hematology
After 13 Weeks
FEMALES

Group 4 (1000 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT	EOS	BASO	LYMPH	MONO	LUC	PLATELETS
	G/l	G/l	G/l	G/l	G/l	G/l	G/l
71	0.56	0.13	0.01	2.72	0.06	0.01	885
72	0.70	0.06	0.02	3.15	0.05	0.02	1327
73	1.14	0.11	0.01	3.33	0.11	0.03	1053
74	1.32	0.09	0.02	2.54	0.11	0.03	1254
75	0.82	0.10	0.00	1.65	0.05	0.00	906
76	0.55	0.09	0.01	2.39	0.10	0.01	1224
77	0.76	0.09	0.00	1.73	0.06	0.02	1114
78	0.83	0.08	0.00	1.54	0.07	0.01	1086
79	---	---	---	---	---	---	---
80	0.49	0.09	0.00	2.12	0.05	0.02	897

	GENERAL	COAGULATION	
	MET-HB	PT	PTT
	rel. 1	rel. 1	sec
71	0.016	0.92	25.0
72	0.016	0.85	28.9
73	0.014	0.83	31.1
74	0.016	0.90	27.0
75	0.015	0.95	27.9
76	0.015	0.88	28.9
77	0.015	0.72	27.4
78	0.015	0.83	33.7
79	0.016	0.90	27.5
80	0.015	0.86	33.0

Biochemistry

Comments

Data excluded from Summary Report

Not Reported

All Measurements

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Reported Parameter

After 13 Weeks

GLUCOSE	GLUCOSE
UREA	UREA
CREAT	CREATININE
BILI-T	BILIRUBIN, TOTAL
BILE AC	BILE ACIDS
CHOLEST	CHOLESTEROL, TOTAL
TRIGLY	TRIGLYCERIDES
PHOS-LIP	PHOSPHOLIPIDS
ASAT	ASPARTATE AMINOTRANSFERASE (ASAT)
ALAT	ALANINE AMINOTRANSFERASE (ALAT)
LDH	LACTATE DEHYDROGENASE (LDH)
ALP	ALKALINE PHOSPHATASE (ALP)
GGT	GAMMA-GLUTAMYLTRANSFERASE (GGT)
CK	CREATINE KINASE (CK)
SODIUM	SODIUM
POTASSIUM	POTASSIUM
CHLORIDE	CHLORIDE
CALCIUM	CALCIUM
PHOSPHORUS	PHOSPHORUS
PROTEIN	PROTEIN, TOTAL
ALBUMIN	ALBUMIN
GLOBULIN	GLOBULIN
A/G RATIO	A/G RATIO

Biochemistry
After 13 Weeks
MALES

Group 1 (0 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT μmol/l	BILI-T μmol/l	BILE AC μmol/l	CHOLEST mmol/l	TRIGLY mmol/l
1	6.60	5.48	21.0	0.00	14.90	2.12	0.38
2	7.00	5.29	22.7	1.91	51.20	2.47	0.71
3	7.70	5.45	26.4	0.00	10.90	2.74	0.69
4	6.60	5.45	23.6	1.75	6.10	3.09	0.26
5	6.80	4.64	25.0	1.83	10.20	2.27	0.30
6	8.20	4.65	24.4	0.00	8.80	3.02	0.58
7	7.00	4.34	26.1	2.11	11.30	2.36	0.47
8	6.00	6.28	30.0	2.03	27.00	3.07	0.35
9	7.00	6.21	26.1	2.05	10.40	2.38	0.34
10	8.20	4.76	29.5	0.00	15.10	2.68	0.66

	GENERAL						
	PHOS-LIP mmol/l	ASAT U/l	ALAT U/l	LDH U/l	ALP U/l	GGT U/l	CK U/l
1	1.85	63.5	27.9	139.6	56.1	0.0	114.6
2	2.06	61.6	21.8	157.1	52.4	0.0	139.6
3	2.09	63.3	16.8	112.8	40.9	0.0	108.0
4	2.21	56.8	23.5	126.6	52.4	0.0	118.3
5	1.76	66.6	28.7	133.2	68.3	0.0	136.8
6	2.31	52.0	16.3	92.8	49.0	0.0	94.1
7	1.83	90.0	22.8	93.1	56.0	0.0	113.5
8	2.17	65.7	30.7	107.2	51.5	0.0	118.6
9	1.80	65.4	31.1	115.6	49.1	0.0	120.4
10	2.12	58.3	19.2	94.9	46.9	0.0	155.6

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
1	144.0	4.13	100.6	2.79	1.84	69.07	43.84
2	146.0	4.12	101.3	2.84	1.88	71.82	43.09
3	144.0	4.12	101.1	2.77	1.65	68.09	40.83
4	145.0	4.36	102.4	2.78	1.79	70.23	43.05
5	146.0	3.63	103.9	2.67	1.76	63.24	40.03
6	144.0	4.55	101.7	2.79	1.61	72.66	44.95
7	142.0	4.67	98.9	2.61	1.43	68.55	44.92
8	144.0	3.86	100.9	2.75	1.83	70.58	44.25
9	142.0	4.51	100.7	2.67	1.69	68.52	42.11
10	143.0	4.39	101.3	2.72	1.67	68.59	43.49

Biochemistry
After 13 Weeks
MALES

Group 1 (0 mg/kg)

GENERAL		
	GLOBULIN	A/G RATIO
	g/l	
<hr/>		
1	25.23	1.74
2	28.73	1.50
3	27.26	1.50
4	27.18	1.58
5	23.21	1.72
6	27.71	1.62
7	23.63	1.90
8	26.33	1.68
9	26.41	1.59
10	25.10	1.73

Biochemistry
After 13 Weeks
MALES

Group 2 (50 mg/kg)

	GENERAL						
	GLUCOSE	UREA	CREAT	BILI-T	BILE AC	CHOLEST	TRIGLY
	mmol/l	mmol/l	μmol/l	μmol/l	μmol/l	mmol/l	mmol/l
11	6.20	5.85	26.9	0.00	6.70	2.82	0.72
12	5.60	6.46	29.5	1.97	10.00	2.52	0.28
13	6.90	6.46	32.3	1.72	10.00	3.42	0.68
14	6.10	6.65	25.5	1.86	13.70	2.30	0.63
15	7.00	4.77	28.0	0.00	10.40	2.74	0.45
16	7.20	4.40	26.9	0.00	9.60	2.36	0.39
17	5.60	5.89	24.4	0.00	6.40	2.05	1.60
18	7.20	5.58	31.1	0.00	13.70	3.57	0.91
19	7.20	5.48	27.2	0.00	9.70	2.17	0.37
20	8.00	5.74	25.5	0.00	10.60	3.34	1.12

	GENERAL						
	PHOS-LIP	ASAT	ALAT	LDH	ALP	GGT	CK
	mmol/l	U/l	U/l	U/l	U/l	U/l	U/l
11	2.06	65.4	20.8	85.2	47.9	0.0	105.9
12	1.84	76.8	22.9	62.1	70.2	0.0	80.6
13	2.42	66.7	19.9	166.1	44.2	0.0	183.5
14	1.89	64.4	37.6	158.9	55.6	0.0	159.4
15	2.03	69.8	26.6	144.9	60.6	0.0	128.7
16	1.92	61.7	22.0	81.1	47.4	0.0	95.1
17	1.90	78.9	31.3	113.4	67.1	0.0	93.3
18	2.49	60.5	16.6	111.5	48.5	0.0	129.6
19	1.82	53.5	16.7	86.3	48.3	0.0	94.4
20	2.29	53.9	20.0	75.5	39.0	0.0	98.4

	GENERAL						
	SODIUM	POTASSIUM	CHLORIDE	CALCIUM	PHOSPHORUS	PROTEIN	ALBUMIN
	mmol/l	mmol/l	mmol/l	mmol/l	mmol/l	g/l	g/l
11	145.0	3.85	101.5	2.75	1.66	71.34	44.71
12	146.0	4.38	102.8	2.65	1.90	65.77	41.87
13	144.0	3.90	101.4	2.64	1.62	66.34	40.89
14	146.0	4.15	102.9	2.80	1.72	70.27	44.31
15	144.0	4.30	102.8	2.66	1.34	69.09	43.10
16	143.0	4.46	102.3	2.70	1.53	64.68	38.21
17	146.0	3.94	103.0	2.76	1.61	69.28	44.70
18	143.0	4.19	100.6	2.83	1.29	71.74	41.74
19	144.0	4.57	103.2	2.66	1.69	65.43	40.15
20	146.0	4.51	102.1	2.72	1.50	66.12	41.96

Biochemistry
After 13 Weeks
MALES

Group 2 (50 mg/kg)

	GENERAL	
	GLOBULIN	A/G RATIO
	g/l	
<hr/>		
11	26.63	1.68
12	23.90	1.75
13	25.45	1.61
14	25.96	1.71
15	25.99	1.66
16	26.47	1.44
17	24.58	1.82
18	30.00	1.39
19	25.28	1.59
20	24.16	1.74

Biochemistry
After 13 Weeks
MALES

Group 3 (200 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT μmol/l	BILI-T μmol/l	BILE AC μmol/l	CHOLEST mmol/l	TRIGLY mmol/l
21	5.80	4.78	31.1	1.91	10.10	1.95	0.41
22	7.70	5.54	26.9	0.00	8.50	2.63	0.51
23	8.30	5.83	25.2	0.00	9.60	2.38	0.68
24	7.00	6.61	26.9	0.00	8.40	2.21	0.32
25	8.20	5.60	28.0	2.00	13.80	3.24	0.45
26	8.00	4.67	23.3	0.00	9.00	2.98	0.60
27	7.30	6.40	24.4	0.00	12.50	3.63	0.68
28	9.00	5.36	33.9	0.00	10.10	2.34	0.58
29	9.10	6.37	27.8	0.00	11.80	2.18	0.39
30	7.90	4.35	23.6	0.00	13.10	3.23	0.72

	GENERAL						
	PHOS-LIP mmol/l	ASAT U/l	ALAT U/l	LDH U/l	ALP U/l	GGT U/l	CK U/l
21	1.80	88.0	35.8	201.4	39.8	0.0	197.1
22	2.06	68.3	16.5	99.2	54.6	0.0	100.8
23	1.99	66.4	28.5	97.4	56.1	0.0	118.9
24	1.97	69.9	22.9	115.8	44.9	0.0	137.3
25	2.40	68.0	24.7	118.3	53.4	0.0	109.7
26	2.37	71.9	15.9	100.7	53.4	0.0	93.0
27	2.30	198.0	97.2	401.7	67.1	0.0	117.6
28	1.89	75.0	21.8	936.2	30.9	0.0	339.0
29	1.81	61.6	22.3	101.1	57.2	0.0	114.3
30	2.39	65.4	27.8	120.7	34.9	0.0	109.9

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
21	145.0	3.97	102.4	2.64	1.53	66.16	41.62
22	148.0	4.24	105.6	2.76	1.39	71.08	41.87
23	146.0	4.12	103.4	2.76	1.46	69.96	43.25
24	145.0	3.95	102.8	2.65	1.57	67.92	43.64
25	144.0	4.06	100.9	2.73	1.51	71.63	45.16
26	144.0	4.50	102.7	2.70	1.58	66.03	43.15
27	146.0	3.88	102.7	2.72	1.62	64.71	40.57
28	144.0	5.43	103.3	2.50	1.83	64.69	40.85
29	146.0	4.27	103.5	2.56	1.59	66.69	42.65
30	145.0	4.50	102.3	2.67	1.96	67.50	42.18

Biochemistry
After 13 Weeks
MALES

Group 3 (200 mg/kg)

GENERAL		
	GLOBULIN	A/G RATIO
	g/l	
<hr/>		
21	24.54	1.70
22	29.21	1.43
23	26.71	1.62
24	24.28	1.80
25	26.47	1.71
26	22.88	1.89
27	24.14	1.68
28	23.84	1.71
29	24.04	1.77
30	25.32	1.67

Biochemistry
After 13 Weeks
MALES

Group 4 (1000 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT μmol/l	BILI-T μmol/l	BILE AC μmol/l	CHOLEST mmol/l	TRIGLY mmol/l
31	8.50	5.18	24.4	0.00	8.10	2.55	0.44
32	10.90	4.23	23.0	0.00	8.60	2.91	0.83
33	8.60	4.49	26.9	0.00	10.20	2.56	0.47
34	6.50	4.62	25.0	0.00	12.20	2.60	0.49
35	8.10	5.78	26.1	0.00	8.90	1.72	0.37
36	6.90	4.66	24.4	0.00	10.30	2.08	0.42
37	7.00	5.80	26.4	0.00	6.20	1.95	0.34
38	7.40	5.16	25.8	0.00	8.10	2.05	0.52
39	8.40	5.90	25.8	0.00	12.10	3.70	0.81
40	9.70	4.50	21.0	0.00	7.80	4.04	0.73

	GENERAL						
	PHOS-LIP mmol/l	ASAT U/l	ALAT U/l	LDH U/l	ALP U/l	GGT U/l	CK U/l
31	1.84	65.3	23.1	118.1	38.5	0.0	113.6
32	2.28	65.5	45.8	143.1	63.9	0.0	119.3
33	2.11	99.2	35.5	160.5	56.6	0.0	121.7
34	1.90	59.5	24.6	160.1	65.0	0.0	128.2
35	1.72	58.6	24.7	89.3	61.1	0.0	78.9
36	1.86	77.3	36.0	260.7	32.3	0.0	150.7
37	1.68	57.2	24.6	70.1	56.3	0.0	82.2
38	1.81	61.9	24.3	55.3	68.8	0.0	86.3
39	2.74	57.0	16.9	80.4	59.3	0.0	77.4
40	2.71	57.1	22.8	116.2	51.9	0.0	85.7

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
31	145.0	4.27	103.9	2.58	1.76	63.66	38.60
32	144.0	4.94	101.7	2.69	1.51	69.12	44.08
33	144.0	4.82	101.9	2.73	1.42	70.74	45.40
34	146.0	4.74	103.9	2.67	1.73	63.40	40.59
35	145.0	4.50	104.0	2.50	1.43	63.71	40.95
36	144.0	4.43	102.9	2.59	1.55	65.49	41.24
37	145.0	4.18	103.0	2.70	1.55	69.12	43.09
38	148.0	4.67	107.6	2.63	1.69	65.99	42.57
39	144.0	5.52	101.5	2.65	1.57	70.65	39.20
40	142.0	4.71	99.2	2.67	1.49	67.23	42.27

Biochemistry
After 13 Weeks
MALES

Group 4 (1000 mg/kg)

	GENERAL	
	GLOBULIN	A/G RATIO
	g/l	
<hr/>		
31	25.06	1.54
32	25.04	1.76
33	25.34	1.79
34	22.81	1.78
35	22.76	1.80
36	24.25	1.70
37	26.03	1.66
38	23.42	1.82
39	31.45	1.25
40	24.96	1.69

Biochemistry
After 13 Weeks
FEMALES

Group 1 (0 mg/kg)

	GENERAL						
	GLUCOSE	UREA	CREAT	BILI-T	BILE AC	CHOLEST	TRIGLY
	mmol/l	mmol/l	μmol/l	μmol/l	μmol/l	mmol/l	mmol/l
41	7.80	5.50	31.3	2.19	6.40	1.74	0.36
42	6.40	5.86	32.2	2.32	5.80	1.86	0.47
43	7.60	5.46	27.4	0.00	9.20	2.01	0.90
44	5.90	6.21	29.4	1.92	5.40	1.76	0.33
45	6.70	5.95	28.0	3.22	13.90	2.48	0.46
46	5.70	7.69	30.5	2.29	8.20	2.53	0.33
47	6.20	5.65	30.2	2.24	8.80	2.86	0.61
48	6.30	7.10	31.9	2.00	5.80	2.26	0.48
49	6.40	6.28	29.7	2.13	8.30	2.87	0.37
50	6.50	6.11	0.0	2.81	4.10	2.68	0.01

	GENERAL						
	PHOS-LIP	ASAT	ALAT	LDH	ALP	GGT	CK
	mmol/l	U/l	U/l	U/l	U/l	U/l	U/l
41	2.23	84.0	31.0	78.2	21.8	0.0	95.4
42	2.18	55.6	11.6	77.0	26.3	0.0	104.0
43	2.54	65.2	10.8	112.0	16.0	0.0	89.8
44	2.05	58.5	17.7	78.8	26.1	0.0	67.9
45	2.78	66.6	17.5	102.4	16.0	0.0	115.1
46	2.53	96.5	23.7	94.1	21.2	0.0	95.4
47	2.92	50.1	11.3	72.0	14.1	0.0	78.1
48	2.56	61.3	20.4	91.8	14.8	0.0	101.5
49	2.91	56.3	19.2	69.0	17.8	0.0	75.2
50	2.86	52.4	17.8	84.6	15.8	0.0	100.6

	GENERAL						
	SODIUM	POTASSIUM	CHLORIDE	CALCIUM	PHOSPHORUS	PROTEIN	ALBUMIN
	mmol/l	mmol/l	mmol/l	mmol/l	mmol/l	g/l	g/l
41	141.0	3.47	100.1	2.69	1.57	68.49	51.94
42	142.0	3.94	100.7	2.76	1.56	72.25	52.97
43	143.0	3.80	102.2	2.64	0.99	68.52	46.40
44	143.0	3.75	103.2	2.60	1.39	68.42	45.71
45	142.0	4.57	99.2	2.80	1.31	73.91	53.34
46	142.0	4.58	101.8	2.73	1.19	73.27	51.75
47	142.0	3.82	99.7	2.82	1.31	75.32	54.65
48	143.0	3.74	101.2	2.71	1.44	74.28	54.48
49	143.0	3.80	100.5	2.89	1.34	80.30	56.43
50	141.0	5.20	102.4	0.00	1.31	68.56	50.69

Biochemistry
After 13 Weeks
FEMALES

Group 1 (0 mg/kg)

	GENERAL	
	GLOBULIN	A/G RATIO
	g/l	
<hr/>		
41	16.55	3.14
42	19.28	2.75
43	22.12	2.10
44	22.71	2.01
45	20.57	2.59
46	21.52	2.40
47	20.67	2.64
48	19.80	2.75
49	23.87	2.36
50	17.87	2.84

Biochemistry
After 13 Weeks
FEMALES

Group 2 (50 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT μmol/l	BILI-T μmol/l	BILE AC μmol/l	CHOLEST mmol/l	TRIGLY mmol/l
51	6.40	5.77	32.2	1.78	7.30	1.42	0.41
52	6.60	4.38	30.2	0.00	6.20	2.96	0.57
53	8.80	6.45	26.0	1.97	22.90	3.85	0.64
54	6.00	5.44	29.7	1.83	6.60	1.66	0.51
55	7.60	7.07	28.5	2.51	8.20	1.81	0.53
56	7.40	4.25	24.3	2.32	8.40	1.55	0.57
57	5.90	3.92	26.3	2.21	6.30	1.77	0.31
58	5.40	5.38	30.2	1.75	9.10	2.20	0.36
59	5.20	5.86	26.0	2.11	6.40	2.08	0.42
60	6.90	6.75	30.2	0.00	13.90	3.27	0.43

	GENERAL						
	PHOS-LIP mmol/l	ASAT U/l	ALAT U/l	LDH U/l	ALP U/l	GGT U/l	CK U/l
51	1.84	114.0	28.2	90.1	20.1	0.0	92.5
52	2.73	63.0	15.6	62.8	18.8	0.0	74.1
53	3.60	52.0	14.2	77.8	24.1	0.0	87.3
54	2.03	71.5	16.8	112.9	16.3	0.0	107.0
55	2.38	69.9	18.8	139.2	23.6	0.0	186.9
56	2.25	66.4	10.4	164.2	25.2	0.0	116.3
57	2.21	58.5	19.6	94.7	18.5	0.0	99.2
58	2.63	70.6	19.9	99.6	17.8	0.0	94.2
59	2.50	57.2	20.0	108.9	24.0	0.0	107.8
60	3.25	75.7	21.0	119.7	13.9	0.0	87.9

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
51	143.0	4.16	101.4	2.67	1.47	68.24	47.41
52	142.0	3.60	98.5	2.78	1.27	74.42	50.59
53	143.0	4.09	100.6	2.79	1.13	74.63	50.28
54	143.0	3.98	101.1	2.74	1.60	69.46	50.25
55	143.0	4.56	102.3	2.75	1.24	74.28	55.65
56	140.0	4.96	101.0	2.58	1.21	69.77	49.56
57	145.0	4.48	103.1	2.72	1.05	75.61	53.93
58	145.0	3.63	100.8	2.73	1.57	72.15	52.29
59	145.0	4.10	102.4	2.76	1.51	73.25	54.62
60	144.0	4.06	101.7	2.79	1.16	73.16	50.96

Biochemistry
After 13 Weeks
FEMALES

Group 2 (50 mg/kg)

	GENERAL	
	GLOBULIN	A/G RATIO
	g/l	
<hr/>		
51	20.83	2.28
52	23.83	2.12
53	24.35	2.06
54	19.21	2.62
55	18.63	2.99
56	20.21	2.45
57	21.68	2.49
58	19.86	2.63
59	18.63	2.93
60	22.20	2.30

Biochemistry
After 13 Weeks
FEMALES

Group 3 (200 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT μmol/l	BILI-T μmol/l	BILE AC μmol/l	CHOLEST mmol/l	TRIGLY mmol/l
61	6.10	6.92	31.6	2.84	6.10	2.21	0.34
62	7.20	6.02	25.7	0.00	11.90	2.14	0.60
63	7.70	5.00	26.3	2.94	6.80	1.61	0.47
64	5.50	9.78	31.6	2.43	6.20	2.92	0.37
65	5.30	7.05	21.9	2.43	15.10	2.22	0.45
66	7.30	4.62	27.4	2.02	7.00	2.27	0.42
67	7.00	5.67	29.7	2.57	7.10	1.51	0.39
68	6.70	7.83	31.3	2.02	14.30	1.95	0.31
69	5.50	5.72	26.9	0.00	6.30	2.55	0.45
70	5.20	6.16	24.3	2.92	20.40	1.27	0.40

	GENERAL						
	PHOS-LIP mmol/l	ASAT U/l	ALAT U/l	LDH U/l	ALP U/l	GGT U/l	CK U/l
61	2.48	47.8	13.9	64.4	19.9	0.0	61.6
62	2.61	67.5	11.8	151.4	29.3	0.0	95.7
63	2.16	49.4	12.0	78.0	18.0	0.0	65.9
64	3.04	99.2	26.4	109.5	18.1	0.0	88.5
65	2.70	62.8	14.0	108.2	14.8	0.0	72.5
66	2.56	64.0	18.0	122.6	14.4	0.0	123.5
67	2.18	73.9	17.2	108.8	18.7	0.0	111.2
68	2.35	62.3	21.3	73.7	29.6	0.0	89.7
69	2.84	86.6	15.2	80.3	18.3	0.0	164.2
70	1.89	55.0	16.2	122.2	15.1	0.0	116.8

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
61	145.0	3.42	101.2	2.75	1.34	75.08	54.91
62	150.0	3.94	110.2	2.79	1.04	76.87	52.29
63	145.0	3.61	102.5	2.74	1.14	76.51	55.87
64	145.0	3.79	101.9	2.81	1.34	77.77	56.50
65	144.0	4.66	102.9	2.70	1.30	74.35	53.61
66	144.0	3.49	102.2	2.74	0.95	74.63	54.46
67	143.0	4.06	102.5	2.71	1.36	71.57	50.60
68	145.0	3.27	101.3	2.72	1.04	72.36	51.90
69	143.0	4.06	102.1	2.74	1.29	72.09	51.50
70	145.0	4.93	102.9	2.90	1.80	76.68	54.27

Biochemistry
After 13 Weeks
FEMALES

Group 3 (200 mg/kg)

	GENERAL	
	GLOBULIN	A/G RATIO
	g/l	
<hr/>		
61	20.17	2.72
62	24.58	2.13
63	20.64	2.71
64	21.27	2.66
65	20.74	2.58
66	20.17	2.70
67	20.97	2.41
68	20.46	2.54
69	20.59	2.50
70	22.41	2.42

Biochemistry
After 13 Weeks
FEMALES

Group 4 (1000 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT μmol/l	BILI-T μmol/l	BILE AC μmol/l	CHOLEST mmol/l	TRIGLY mmol/l
71	5.10	7.20	28.5	1.83	3.50	2.02	0.37
72	5.70	5.66	30.2	2.51	7.00	1.69	0.35
73	7.10	7.95	29.1	2.40	6.10	2.35	0.40
74	6.30	6.64	28.3	2.86	40.50	2.48	0.35
75	5.40	7.07	31.1	2.65	5.70	3.24	0.37
76	6.40	6.88	28.5	1.97	6.60	1.81	0.36
77	7.70	4.95	28.5	0.00	9.70	1.32	0.34
78	6.60	8.05	31.6	2.08	4.70	2.28	0.30
79	6.50	7.13	33.3	2.84	10.80	1.99	0.38
80	8.00	6.06	25.2	2.91	7.50	1.76	0.47

	GENERAL						
	PHOS-LIP mmol/l	ASAT U/l	ALAT U/l	LDH U/l	ALP U/l	GGT U/l	CK U/l
71	2.32	56.7	18.9	80.7	21.3	0.0	101.5
72	2.21	63.1	18.1	109.9	25.2	0.0	86.8
73	2.74	56.3	19.9	97.8	18.9	0.0	107.8
74	2.69	86.1	23.1	112.2	24.0	0.0	94.1
75	3.01	104.8	29.0	70.1	27.3	0.0	64.8
76	2.38	97.3	20.9	136.9	23.4	0.0	584.6
77	1.99	54.2	20.4	105.9	26.9	0.0	85.7
78	2.51	59.0	17.9	92.1	24.0	0.0	82.2
79	2.29	61.2	22.6	72.5	17.0	0.0	79.8
80	2.37	54.2	16.8	85.9	16.7	0.0	105.2

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
71	145.0	4.75	102.7	2.90	1.60	73.73	50.03
72	147.0	4.19	105.2	2.76	1.46	75.64	54.99
73	146.0	3.67	102.8	2.85	1.51	77.89	56.29
74	148.0	4.20	105.4	2.87	1.69	75.77	52.66
75	146.0	3.97	101.8	2.81	1.30	74.53	55.05
76	141.0	4.83	100.2	2.81	1.49	75.16	52.82
77	147.0	3.49	104.3	2.73	1.10	76.01	50.48
78	145.0	3.84	103.1	2.74	1.21	72.64	53.25
79	145.0	4.24	102.0	2.77	1.33	72.05	53.03
80	145.0	4.13	100.9	2.76	1.48	74.90	55.52

Biochemistry
After 13 Weeks
FEMALES

Group 4 (1000 mg/kg)

	GENERAL	
	GLOBULIN	A/G RATIO
	g/l	
<hr/>		
71	23.70	2.11
72	20.65	2.66
73	21.60	2.61
74	23.11	2.28
75	19.48	2.83
76	22.34	2.36
77	25.53	1.98
78	19.39	2.75
79	19.02	2.79
80	19.38	2.86

Urinalysis

Comments

Data excluded from Summary Report

Not Reported

All Measurements

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Reported Parameter

After 13 Weeks

VOLUME/18h	VOLUME/18h
REL DENS	RELATIVE DENSITY
COLOR	COLOR
APPEARANCE	APPEARANCE
pH	pH
NITRITE	NITRITE
PROTEIN	PROTEIN
GLUCOSE	GLUCOSE
KETONES	KETONES
UROBILI	UROBILINOGEN
BILIRUBIN	BILIRUBIN
ERY	ERYTHROCYTES
LEU	LEUCOCYTES

Urinalysis
After 13 Weeks
MALES

Group 1 (0 mg/kg)

GENERAL							
	VOLUME/18h ml	REL DENS rel. 1	COLOR	APPEARANCE	pH	NITRITE SCORE 0/1	PROTEIN g/l
1	7.0	1.033	yellow	cloudy	7.0	1	0.25
2	2.3	1.092	deep yell	cloudy	6.5	1	0.75
3	5.3	1.036	yellow	turbid	7.0	1	0.25
4	5.7	1.042	yellow	clear	6.0	0	0.25
5	7.4	1.033	yellow	clear	6.5	0	0.25
6	3.9	1.059	yellow	clear	5.0	1	0.25
7	27.0	1.010	light yel	clear	7.0	1	0.00
8	5.2	1.040	yellow	clear	6.5	1	0.25
9	5.3	1.041	yellow	cloudy	6.5	1	0.25
10	14.2	1.021	light yel	cloudy	7.0	1	0.25

GENERAL						
	GLUCOSE mmol/l	KETONES mmol/l	UROBILI μmol/l	BILIRUBIN μmol/l	ERY per μl	LEU per μl
1	0	1.5	0	0	0	25
2	0	5.0	17	17	10	25
3	0	1.5	0	0	10	25
4	0	0.5	0	17	10	100
5	0	1.5	0	0	10	25
6	0	1.5	0	17	10	25
7	0	0.5	0	0	0	25
8	0	1.5	0	17	10	25
9	0	1.5	0	17	10	25
10	0	0.5	0	0	25	25

Urinalysis
After 13 Weeks
MALES

Group 2 (50 mg/kg)

GENERAL							
	VOLUME/18h ml	REL DENS rel. 1	COLOR	APPEARANCE	pH	NITRITE SCORE 0/1	PROTEIN g/l
11	4.0	1.058	deep yell	cloudy	6.5	1	0.25
12	4.5	1.052	deep yell	cloudy	6.5	1	0.75
13	4.6	1.056	deep yell	clear	6.0	0	0.25
14	3.5	1.065	deep yell	cloudy	6.5	1	0.75
15	7.1	1.035	yellow	turbid	6.5	1	0.25
16	18.6	1.016	light yel	clear	7.0	1	0.00
17	3.1	1.080	deep yell	clear	6.0	1	0.25
18	7.4	1.032	yellow	turbid	7.0	1	0.25
19	3.8	1.040	yellow	clear	6.5	1	0.25
20	4.0	1.055	yellow	cloudy	6.0	1	0.75

GENERAL						
	GLUCOSE mmol/l	KETONES mmol/l	UROBILI μmol/l	BILIRUBIN μmol/l	ERY per μl	LEU per μl
11	0	1.5	0	17	10	25
12	0	1.5	0	17	10	100
13	0	1.5	0	17	10	25
14	0	1.5	0	17	25	100
15	0	0.5	0	0	10	25
16	0	0.0	0	0	0	25
17	0	5.0	0	17	10	25
18	0	1.5	0	0	10	100
19	0	1.5	0	17	10	25
20	0	1.5	0	17	10	100

Urinalysis
After 13 Weeks
MALES

Group 3 (200 mg/kg)

GENERAL							
	VOLUME/18h ml	REL DENS rel. 1	COLOR	APPEARANCE	pH	NITRITE SCORE 0/1	PROTEIN g/l
21	16.2	1.016	light yel	clear	7.0	1	0.00
22	9.1	1.037	yellow	cloudy	6.0	1	0.25
23	1.4	1.082	yellow	clear	6.0	1	0.75
24	6.2	1.035	yellow	clear	6.5	0	0.25
25	3.8	1.043	yellow	clear	6.0	0	0.25
26	3.9	1.055	deep yell	clear	6.5	1	0.75
27	4.5	1.048	deep yell	clear	6.0	0	0.25
28	4.1	1.059	deep yell	clear	6.0	1	0.25
29	5.9	1.044	yellow	clear	6.0	1	0.25
30	15.8	1.016	light yel	clear	6.5	1	0.00

GENERAL						
	GLUCOSE mmol/l	KETONES mmol/l	UROBILI μmol/l	BILIRUBIN μmol/l	ERY per μl	LEU per μl
21	0	0.5	0	0	0	25
22	0	0.5	0	0	10	25
23	0	1.5	0	17	25	25
24	0	0.5	0	0	10	25
25	0	1.5	0	17	10	25
26	0	1.5	0	17	10	25
27	0	1.5	0	17	10	25
28	0	1.5	0	17	10	25
29	0	1.5	0	17	10	25
30	0	1.5	0	0	0	25

Urinalysis
After 13 Weeks
MALES

Group 4 (1000 mg/kg)

GENERAL							
	VOLUME/18h ml	REL DENS rel. 1	COLOR	APPEARANCE	pH	NITRITE SCORE 0/1	PROTEIN g/l
31	4.4	1.061	deep yell	clear	6.0	0	0.25
32	4.0	1.058	yellow br	clear	6.0	1	0.25
33	19.6	1.017	light yel	cloudy	6.5	0	0.00
34	3.7	1.059	deep yell	clear	6.5	1	0.25
35	5.8	1.045	yellow	cloudy	6.5	1	0.25
36	19.3	1.015	light yel	cloudy	6.5	1	0.25
37	8.6	1.029	yellow	clear	6.5	0	0.25
38	6.1	1.042	yellow	cloudy	6.5	1	0.25
39	1.3	1.060	yellow	clear	6.5	0	0.25
40	7.4	1.038	yellow	clear	6.5	0	0.25

GENERAL						
	GLUCOSE mmol/l	KETONES mmol/l	UROBILI μmol/l	BILIRUBIN μmol/l	ERY per μl	LEU per μl
31	0	1.5	0	17	10	25
32	0	1.5	0	17	250	25
33	0	0.0	0	0	10	25
34	0	1.5	0	17	25	25
35	0	0.5	0	17	10	25
36	0	1.5	0	0	25	25
37	0	0.5	0	0	10	25
38	0	1.5	0	0	25	25
39	0	1.5	0	17	10	25
40	0	0.5	0	17	0	100

Urinalysis
After 13 Weeks
FEMALES

Group 1 (0 mg/kg)

GENERAL							
	VOLUME/18h ml	REL DENS rel. 1	COLOR	APPEARANCE	pH	NITRITE SCORE 0/1	PROTEIN g/l
41	20.2	1.011	light yel	clear	7.0	0	0.00
42	3.8	1.033	yellow	clear	6.0	0	0.25
43	5.9	1.036	yellow	clear	6.0	1	0.00
44	2.7	1.049	yellow	clear	6.0	0	0.25
45	9.1	1.022	light yel	clear	6.0	1	0.00
46	1.6	1.084	yellow	clear	5.0	1	0.25
47	12.1	1.019	light yel	clear	6.5	1	0.00
48	11.8	1.018	light yel	clear	7.0	0	0.00
49	6.3	1.026	yellow	clear	6.5	0	0.00
50	25.8	1.008	light yel	clear	6.5	0	0.00

GENERAL						
	GLUCOSE mmol/l	KETONES mmol/l	UROBILI μmol/l	BILIRUBIN μmol/l	ERY per μl	LEU per μl
41	0	0.0	0	0	0	0
42	0	0.5	0	0	0	0
43	0	1.5	0	0	0	0
44	0	1.5	0	17	0	25
45	0	0.0	0	0	0	25
46	0	1.5	17	17	10	25
47	0	0.0	0	0	0	0
48	0	0.0	0	0	0	0
49	0	0.5	0	0	0	25
50	0	0.0	0	0	0	0

Urinalysis
After 13 Weeks
FEMALES

Group 2 (50 mg/kg)

GENERAL							
	VOLUME/18h ml	REL DENS rel. 1	COLOR	APPEARANCE	pH	NITRITE SCORE 0/1	PROTEIN g/l
51	10.7	1.018	yellow	clear	6.5	1	0.00
52	6.9	1.026	yellow	clear	6.0	1	0.00
53	4.8	1.047	yellow	clear	6.0	1	0.25
54	5.8	1.039	yellow	clear	6.5	0	0.00
55	3.9	1.039	yellow	cloudy	6.0	1	0.25
56	9.4	1.021	yellow	clear	6.5	1	0.00
57	7.9	1.019	yellow	clear	6.5	1	0.00
58	9.4	1.014	light yel	clear	6.5	0	0.00
59	5.9	1.027	yellow	clear	6.0	0	0.00
60	5.1	1.037	yellow	clear	6.0	1	0.25

GENERAL						
	GLUCOSE mmol/l	KETONES mmol/l	UROBILI μmol/l	BILIRUBIN μmol/l	ERY per μl	LEU per μl
51	0	0.5	0	0	0	0
52	0	0.5	0	0	0	0
53	0	1.5	0	17	0	0
54	0	0.5	0	0	0	0
55	0	1.5	0	0	0	0
56	0	0.5	0	0	10	0
57	0	0.0	0	0	0	0
58	0	0.0	0	0	0	0
59	0	0.5	0	17	0	0
60	0	0.5	0	17	0	0

Urinalysis
After 13 Weeks
FEMALES

Group 3 (200 mg/kg)

GENERAL							
	VOLUME/18h ml	REL DENS rel. 1	COLOR	APPEARANCE	pH	NITRITE SCORE 0/1	PROTEIN g/l
61	2.3	1.068	yellow	clear	5.0	1	0.25
62	3.5	1.053	yellow	clear	5.0	1	0.25
63	2.4	1.076	yellow	clear	6.0	1	0.25
64	2.6	1.064	yellow	clear	5.0	1	0.25
65	1.0	1.062	yellow	clear	5.0	1	0.25
66	26.8	1.009	light yel	clear	6.5	0	0.00
67	3.0	1.046	yellow	clear	6.0	1	0.25
68	5.8	1.034	yellow	clear	6.0	1	0.00
69	12.6	1.011	light yel	clear	6.5	0	0.00
70	14.9	1.013	light yel	clear	7.0	0	0.00

GENERAL						
	GLUCOSE mmol/l	KETONES mmol/l	UROBILI μmol/l	BILIRUBIN μmol/l	ERY per μl	LEU per μl
61	0	1.5	0	17	0	25
62	0	1.5	0	17	10	0
63	0	1.5	17	17	10	25
64	0	0.5	0	17	0	0
65	0	1.5	0	17	10	25
66	0	0.0	0	0	0	0
67	0	1.5	0	17	0	0
68	0	0.5	0	0	250	0
69	0	0.0	0	0	0	0
70	0	0.0	0	0	0	0

Urinalysis
After 13 Weeks
FEMALES

Group 4 (1000 mg/kg)

GENERAL							
	VOLUME/18h ml	REL DENS rel. 1	COLOR	APPEARANCE	pH	NITRITE SCORE 0/1	PROTEIN g/l
71	2.7	1.060	yellow	clear	5.0	1	0.25
72	4.2	1.038	yellow	clear	6.0	1	0.00
73	4.9	1.044	yellow	clear	6.0	1	0.25
74	4.5	1.044	yellow	clear	6.0	1	0.25
75	0.7	1.178	deep yell	clear	5.0	1	1.50
76	3.3	1.056	yellow	clear	5.0	1	0.25
77	22.6	1.010	light yel	clear	7.0	0	0.00
78	2.6	1.060	yellow	clear	5.0	0	0.25
79	10.6	1.014	light yel	clear	6.5	0	0.00
80	2.7	1.062	yellow	clear	5.0	1	0.25

GENERAL						
	GLUCOSE mmol/l	KETONES mmol/l	UROBILI μmol/l	BILIRUBIN μmol/l	ERY per μl	LEU per μl
71	0	1.5	0	17	10	25
72	0	0.5	0	17	10	0
73	0	0.5	0	17	0	25
74	0	0.5	0	0	0	0
75	0	1.5	34	17	10	25
76	0	1.5	0	17	0	0
77	0	0.0	0	0	10	0
78	0	1.5	0	17	0	25
79	0	0.0	0	0	0	0
80	0	1.5	0	17	10	0

ORGAN WEIGHTS (GRAM)**Comments**

Exclusions

Not Reported

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

Selection of Organs

All organs reported

Animals without scheduled necropsy

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
MALES

Group 1 (0 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
1	398.1	2.13	1.02	9.59	0.401	2.13	0.063
2	387.3	2.07	1.02	10.41	0.296	2.33	0.071
3	443.2	2.02	1.09	8.87	0.482	1.97	0.082
4	403.2	2.15	1.13	10.66	0.439	2.23	0.076
5	360.6	1.87	0.81	7.87	0.369	1.72	0.046
6	418.8	2.14	1.00	11.44	0.269	2.11	0.068
7	415.0	2.06	1.00	10.13	0.305	2.23	0.067
8	410.1	2.04	1.10	11.40	0.346	2.17	0.072
9	442.9	2.19	1.16	10.79	0.336	2.37	0.068
10	442.7	2.09	1.14	12.25	0.361	2.24	0.071

Animal	SPLEEN	TESTES	EPIDIDYMI
1	0.70	4.14	1.508
2	0.64	4.45	1.630
3	0.59	4.19	1.661
4	0.74	3.86	1.529
5	0.51	3.15	1.440
6	0.57	4.24	1.591
7	0.68	3.82	1.384
8	0.83	4.19	1.821
9	0.86	4.50	1.859
10	0.72	4.51	1.839

ORGAN WEIGHTS (GRAM)**AFTER 13 WEEKS****MALES****Group 2 (50 mg/kg)**

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
11	438.2	2.15	1.02	12.22	0.250	2.14	0.067
12	344.9	2.14	0.85	8.42	0.248	1.85	0.070
13	427.4	2.03	1.12	11.24	0.358	2.00	0.084
14	350.9	1.90	0.98	10.09	0.286	2.01	0.068
15	407.2	2.18	0.96	10.47	0.377	1.99	0.055
16	462.5	2.02	1.18	11.72	0.390	2.15	0.068
17	391.6	2.10	1.09	10.47	0.334	2.22	0.062
18	409.3	2.06	0.94	11.84	0.451	1.89	0.073
19	466.4	2.01	1.16	11.64	0.353	2.18	0.075
20	491.3	2.12	1.17	12.67	0.323	2.16	0.055

Animal	SPLEEN	TESTES	EPIDIDYMI
11	0.73	4.09	1.768
12	0.94	3.57	1.483
13	0.83	3.61	1.615
14	0.60	3.61	1.460
15	0.77	3.76	1.528
16	0.73	3.87	1.526
17	0.72	3.99	1.711
18	0.71	3.58	1.542
19	0.68	4.33	1.603
20	0.77	4.31	1.776

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
MALES

Group 3 (200 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
21	355.8	2.01	0.93	9.12	0.286	1.88	0.059
22	501.1	2.13	1.14	13.98	0.326	2.36	0.072
23	498.8	2.16	1.32	13.52	0.418	2.63	0.066
24	428.8	2.13	1.19	10.58	0.462	2.11	0.068
25	471.7	2.05	1.05	11.96	0.257	2.21	0.068
26	412.1	2.22	1.08	12.29	0.401	2.43	0.074
27	360.4	1.99	0.93	10.26	0.208	2.38	0.066
28	457.4	2.23	1.32	12.71	0.518	2.51	0.072
29	392.1	2.23	1.05	10.01	0.334	2.27	0.063
30	394.4	2.24	1.05	12.09	0.300	2.44	0.063

Animal	SPLEEN	TESTES	EPIDIDYMI
21	0.65	3.88	1.432
22	0.86	4.00	1.530
23	0.86	4.43	1.979
24	0.62	4.06	1.610
25	0.74	3.91	1.459
26	0.71	4.49	1.653
27	0.62	3.33	1.568
28	0.71	4.07	1.984
29	0.56	4.05	1.597
30	0.68	4.13	1.673

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
MALES

Group 4 (1000 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
31	431.0	2.10	1.16	9.56	0.365	2.32	0.057
32	406.3	1.92	1.06	11.32	0.331	2.26	0.078
33	428.4	2.09	1.20	11.13	0.341	2.43	0.090
34	355.8	1.86	0.89	8.46	0.258	1.80	0.052
35	378.7	2.04	0.95	8.81	0.343	2.03	0.046
36	399.7	2.11	1.02	10.79	0.367	2.21	0.059
37	400.7	2.01	0.96	10.74	0.288	2.14	0.052
38	419.0	2.30	1.13	10.95	0.374	2.51	0.074
39	451.1	2.20	1.02	14.10	0.322	2.26	0.070
40	473.6	2.18	1.17	14.63	0.386	2.52	0.061

Animal	SPLEEN	TESTES	EPIDIDYMI
31	0.69	3.95	1.655
32	0.66	4.29	1.528
33	0.76	3.91	1.497
34	0.61	3.62	1.357
35	0.64	4.03	1.454
36	0.63	3.93	1.527
37	0.76	3.63	1.336
38	0.69	3.93	1.562
39	0.65	4.03	1.593
40	0.79	4.62	1.607

ORGAN/BODY WEIGHT RATIOS (%)**AFTER 13 WEEKS****MALES****Group 1 (0 mg/kg)**

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
1	398.1	0.54	0.26	2.41	0.101	0.54	0.016
2	387.3	0.53	0.26	2.69	0.076	0.60	0.018
3	443.2	0.46	0.25	2.00	0.109	0.44	0.019
4	403.2	0.53	0.28	2.64	0.109	0.55	0.019
5	360.6	0.52	0.22	2.18	0.102	0.48	0.013
6	418.8	0.51	0.24	2.73	0.064	0.50	0.016
7	415.0	0.50	0.24	2.44	0.073	0.54	0.016
8	410.1	0.50	0.27	2.78	0.084	0.53	0.018
9	442.9	0.49	0.26	2.44	0.076	0.54	0.015
10	442.7	0.47	0.26	2.77	0.082	0.51	0.016

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYDYMID (%)
1	0.18	1.04	0.379
2	0.17	1.15	0.421
3	0.13	0.95	0.375
4	0.18	0.96	0.379
5	0.14	0.87	0.399
6	0.14	1.01	0.380
7	0.16	0.92	0.333
8	0.20	1.02	0.444
9	0.19	1.02	0.420
10	0.16	1.02	0.415

ORGAN/BODY WEIGHT RATIOS (%)**AFTER 13 WEEKS****MALES****Group 2 (50 mg/kg)**

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
11	438.2	0.49	0.23	2.79	0.057	0.49	0.015
12	344.9	0.62	0.25	2.44	0.072	0.54	0.020
13	427.4	0.47	0.26	2.63	0.084	0.47	0.020
14	350.9	0.54	0.28	2.88	0.082	0.57	0.019
15	407.2	0.54	0.24	2.57	0.093	0.49	0.014
16	462.5	0.44	0.26	2.53	0.084	0.46	0.015
17	391.6	0.54	0.28	2.67	0.085	0.57	0.016
18	409.3	0.50	0.23	2.89	0.110	0.46	0.018
19	466.4	0.43	0.25	2.50	0.076	0.47	0.016
20	491.3	0.43	0.24	2.58	0.066	0.44	0.011

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYDYMID (%)
11	0.17	0.93	0.403
12	0.27	1.04	0.430
13	0.19	0.84	0.378
14	0.17	1.03	0.416
15	0.19	0.92	0.375
16	0.16	0.84	0.330
17	0.18	1.02	0.437
18	0.17	0.87	0.377
19	0.15	0.93	0.344
20	0.16	0.88	0.361

ORGAN/BODY WEIGHT RATIOS (%)**AFTER 13 WEEKS****MALES****Group 3 (200 mg/kg)**

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
21	355.8	0.56	0.26	2.56	0.080	0.53	0.017
22	501.1	0.43	0.23	2.79	0.065	0.47	0.014
23	498.8	0.43	0.26	2.71	0.084	0.53	0.013
24	428.8	0.50	0.28	2.47	0.108	0.49	0.016
25	471.7	0.43	0.22	2.54	0.054	0.47	0.014
26	412.1	0.54	0.26	2.98	0.097	0.59	0.018
27	360.4	0.55	0.26	2.85	0.058	0.66	0.018
28	457.4	0.49	0.29	2.78	0.113	0.55	0.016
29	392.1	0.57	0.27	2.55	0.085	0.58	0.016
30	394.4	0.57	0.27	3.07	0.076	0.62	0.016

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYDYMID (%)
21	0.18	1.09	0.402
22	0.17	0.80	0.305
23	0.17	0.89	0.397
24	0.14	0.95	0.375
25	0.16	0.83	0.309
26	0.17	1.09	0.401
27	0.17	0.92	0.435
28	0.16	0.89	0.434
29	0.14	1.03	0.407
30	0.17	1.05	0.424

ORGAN/BODY WEIGHT RATIOS (%)**AFTER 13 WEEKS****MALES****Group 4 (1000 mg/kg)**

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
31	431.0	0.49	0.27	2.22	0.085	0.54	0.013
32	406.3	0.47	0.26	2.79	0.081	0.56	0.019
33	428.4	0.49	0.28	2.60	0.080	0.57	0.021
34	355.8	0.52	0.25	2.38	0.073	0.51	0.015
35	378.7	0.54	0.25	2.33	0.091	0.54	0.012
36	399.7	0.53	0.26	2.70	0.092	0.55	0.015
37	400.7	0.50	0.24	2.68	0.072	0.53	0.013
38	419.0	0.55	0.27	2.61	0.089	0.60	0.018
39	451.1	0.49	0.23	3.13	0.071	0.50	0.016
40	473.6	0.46	0.25	3.09	0.082	0.53	0.013

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYDYMID (%)
31	0.16	0.92	0.384
32	0.16	1.06	0.376
33	0.18	0.91	0.349
34	0.17	1.02	0.381
35	0.17	1.06	0.384
36	0.16	0.98	0.382
37	0.19	0.91	0.333
38	0.16	0.94	0.373
39	0.14	0.89	0.353
40	0.17	0.98	0.339

ORGAN/BRAIN WEIGHT RATIOS (%)**AFTER 13 WEEKS****MALES****Group 1 (0 mg/kg)**

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
1	2.13	47.89	450.23	18.826	100.00	2.958
2	2.07	49.28	502.90	14.300	112.56	3.430
3	2.02	53.96	439.11	23.861	97.52	4.059
4	2.15	52.56	495.81	20.419	103.72	3.535
5	1.87	43.32	420.86	19.733	91.98	2.460
6	2.14	46.73	534.58	12.570	98.60	3.178
7	2.06	48.54	491.75	14.806	108.25	3.252
8	2.04	53.92	558.82	16.961	106.37	3.529
9	2.19	52.97	492.69	15.342	108.22	3.105
10	2.09	54.55	586.12	17.273	107.18	3.397

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYDYMID (%)
1	32.86	194.37	70.798
2	30.92	214.98	78.744
3	29.21	207.43	82.228
4	34.42	179.53	71.116
5	27.27	168.45	77.005
6	26.64	198.13	74.346
7	33.01	185.44	67.184
8	40.69	205.39	89.265
9	39.27	205.48	84.886
10	34.45	215.79	87.990

ORGAN/BRAIN WEIGHT RATIOS (%)**AFTER 13 WEEKS****MALES****Group 2 (50 mg/kg)**

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
11	2.15	47.44	568.37	11.628	99.53	3.116
12	2.14	39.72	393.46	11.589	86.45	3.271
13	2.03	55.17	553.69	17.635	98.52	4.138
14	1.90	51.58	531.05	15.053	105.79	3.579
15	2.18	44.04	480.28	17.294	91.28	2.523
16	2.02	58.42	580.20	19.307	106.44	3.366
17	2.10	51.90	498.57	15.905	105.71	2.952
18	2.06	45.63	574.76	21.893	91.75	3.544
19	2.01	57.71	579.10	17.562	108.46	3.731
20	2.12	55.19	597.64	15.236	101.89	2.594

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYDYMID (%)
11	33.95	190.23	82.233
12	43.93	166.82	69.299
13	40.89	177.83	79.557
14	31.58	190.00	76.842
15	35.32	172.48	70.092
16	36.14	191.58	75.545
17	34.29	190.00	81.476
18	34.47	173.79	74.854
19	33.83	215.42	79.751
20	36.32	203.30	83.774

ORGAN/BRAIN WEIGHT RATIOS (%)**AFTER 13 WEEKS****MALES****Group 3 (200 mg/kg)**

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
21	2.01	46.27	453.73	14.229	93.53	2.935
22	2.13	53.52	656.34	15.305	110.80	3.380
23	2.16	61.11	625.93	19.352	121.76	3.056
24	2.13	55.87	496.71	21.690	99.06	3.192
25	2.05	51.22	583.41	12.537	107.80	3.317
26	2.22	48.65	553.60	18.063	109.46	3.333
27	1.99	46.73	515.58	10.452	119.60	3.317
28	2.23	59.19	569.96	23.229	112.56	3.229
29	2.23	47.09	448.88	14.978	101.79	2.825
30	2.24	46.88	539.73	13.393	108.93	2.813

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYDYMID (%)
21	32.34	193.03	71.244
22	40.38	187.79	71.831
23	39.81	205.09	91.620
24	29.11	190.61	75.587
25	36.10	190.73	71.171
26	31.98	202.25	74.459
27	31.16	167.34	78.794
28	31.84	182.51	88.969
29	25.11	181.61	71.614
30	30.36	184.38	74.688

ORGAN/BRAIN WEIGHT RATIOS (%)**AFTER 13 WEEKS****MALES****Group 4 (1000 mg/kg)**

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
31	2.10	55.24	455.24	17.381	110.48	2.714
32	1.92	55.21	589.58	17.240	117.71	4.063
33	2.09	57.42	532.54	16.316	116.27	4.306
34	1.86	47.85	454.84	13.871	96.77	2.796
35	2.04	46.57	431.86	16.814	99.51	2.255
36	2.11	48.34	511.37	17.393	104.74	2.796
37	2.01	47.76	534.33	14.328	106.47	2.587
38	2.30	49.13	476.09	16.261	109.13	3.217
39	2.20	46.36	640.91	14.636	102.73	3.182
40	2.18	53.67	671.10	17.706	115.60	2.798

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYDYMID (%)
31	32.86	188.10	78.810
32	34.38	223.44	79.583
33	36.36	187.08	71.627
34	32.80	194.62	72.957
35	31.37	197.55	71.275
36	29.86	186.26	72.370
37	37.81	180.60	66.468
38	30.00	170.87	67.913
39	29.55	183.18	72.409
40	36.24	211.93	73.716

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
FEMALES

Group 1 (0 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
41	211.2	1.63	0.75	6.97	0.329	1.37	0.077
42	217.1	1.94	0.67	5.34	0.270	1.27	0.065
43	205.8	1.97	0.79	6.45	0.332	1.57	0.080
44	218.0	1.97	0.78	5.92	0.356	1.46	0.084
45	236.6	1.94	0.82	6.29	0.336	1.61	0.082
46	235.3	2.04	0.82	6.68	0.245	1.61	0.097
47	241.6	1.92	0.85	8.03	0.374	1.58	0.076
48	199.5	1.66	0.65	6.23	0.190	1.23	0.058
49	217.9	1.81	0.69	8.04	0.265	1.57	0.086
50	200.0	1.79	0.64	6.65	0.162	1.50	0.064

Animal	SPLEEN	OVARIES	UTERUS
41	0.43	0.124	1.18
42	0.43	0.100	0.76
43	0.49	0.136	1.35
44	0.48	0.122	1.43
45	0.41	0.117	1.49
46	0.59	0.094	0.73
47	0.62	0.110	1.18
48	0.36	0.094	0.68
49	0.48	0.087	1.09
50	0.41	0.092	1.58

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
FEMALES

Group 2 (50 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
51	241.7	2.22	0.78	6.55	0.287	1.75	0.092
52	245.6	1.81	0.78	7.14	0.274	1.49	0.068
53	249.0	1.93	0.80	8.51	0.206	1.64	0.074
54	212.9	1.87	0.66	5.96	0.272	1.31	0.070
55	223.7	1.84	0.69	6.49	0.338	1.54	0.074
56	216.8	1.81	0.65	6.55	0.185	1.37	0.072
57	216.9	1.96	0.63	6.40	0.203	1.42	0.080
58	223.9	1.80	0.70	6.74	0.284	1.28	0.060
59	230.6	1.90	0.73	7.09	0.265	1.59	0.070
60	221.1	1.86	0.85	8.54	0.309	1.54	0.068

Animal	SPLEEN	OVARIES	UTERUS
51	0.47	0.149	1.42
52	0.61	0.111	0.75
53	0.38	0.082	2.02
54	0.53	0.102	0.67
55	0.46	0.151	0.90
56	0.46	0.112	0.70
57	0.41	0.101	1.16
58	0.63	0.080	1.09
59	0.45	0.119	0.92
60	0.46	0.088	1.99

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
FEMALES

Group 3 (200 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
61	206.8	1.83	0.67	5.96	0.292	1.34	0.056
62	259.9	1.89	0.76	6.77	0.378	1.59	0.076
63	237.1	1.78	0.84	6.95	0.313	1.59	0.069
64	208.7	1.83	0.71	5.87	0.274	1.23	0.077
65	208.7	2.00	0.67	4.74	0.321	1.45	0.070
66	253.6	2.01	0.78	7.21	0.311	1.36	0.075
67	218.4	1.91	0.74	6.69	0.272	1.44	0.061
68	216.7	1.72	0.81	6.48	0.286	1.39	0.077
69	232.1	2.09	0.74	7.77	0.414	1.59	0.067
70	206.0	1.80	0.68	6.26	0.276	1.32	0.066

Animal	SPLEEN	OVARIES	UTERUS
61	0.48	0.077	0.93
62	0.54	0.130	1.47
63	0.54	0.083	2.04
64	0.45	0.105	0.76
65	0.45	0.077	0.96
66	0.55	0.112	1.69
67	0.44	0.095	0.82
68	0.60	0.103	1.12
69	0.56	0.102	0.66
70	0.49	0.083	0.85

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
FEMALES

Group 4 (1000 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
71	209.5	1.84	0.72	6.27	0.321	1.35	0.067
72	204.4	1.88	0.76	5.57	0.249	1.33	0.065
73	226.9	1.83	0.81	6.66	0.341	1.45	0.080
74	238.4	1.95	0.79	7.05	0.288	1.53	0.088
75	226.0	1.88	0.77	6.25	0.365	1.51	0.071
76	244.8	1.94	0.76	7.35	0.265	1.73	0.068
77	232.9	2.06	0.81	7.05	0.257	1.21	0.087
78	207.7	1.79	0.83	6.40	0.278	1.46	0.081
79	234.8	1.93	0.68	7.11	0.329	1.39	0.080
80	220.4	1.89	0.69	6.51	0.251	1.48	0.058

Animal	SPLEEN	OVARIES	UTERUS
71	0.47	0.114	0.77
72	0.44	0.121	0.71
73	0.57	0.093	1.18
74	0.54	0.121	1.63
75	0.44	0.084	0.83
76	0.59	0.090	0.97
77	0.55	0.130	1.14
78	0.44	0.110	0.86
79	0.70	0.112	0.77
80	0.41	0.107	0.82

ORGAN/BODY WEIGHT RATIOS (%)**AFTER 13 WEEKS****FEMALES****Group 1 (0 mg/kg)**

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
41	211.2	0.77	0.36	3.30	0.156	0.65	0.036
42	217.1	0.89	0.31	2.46	0.124	0.58	0.030
43	205.8	0.96	0.38	3.13	0.161	0.76	0.039
44	218.0	0.90	0.36	2.72	0.163	0.67	0.039
45	236.6	0.82	0.35	2.66	0.142	0.68	0.035
46	235.3	0.87	0.35	2.84	0.104	0.68	0.041
47	241.6	0.79	0.35	3.32	0.155	0.65	0.031
48	199.5	0.83	0.33	3.12	0.095	0.62	0.029
49	217.9	0.83	0.32	3.69	0.122	0.72	0.039
50	200.0	0.90	0.32	3.33	0.081	0.75	0.032

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
41	0.20	0.059	0.56
42	0.20	0.046	0.35
43	0.24	0.066	0.66
44	0.22	0.056	0.66
45	0.17	0.049	0.63
46	0.25	0.040	0.31
47	0.26	0.046	0.49
48	0.18	0.047	0.34
49	0.22	0.040	0.50
50	0.21	0.046	0.79

ORGAN/BODY WEIGHT RATIOS (%)**AFTER 13 WEEKS****FEMALES****Group 2 (50 mg/kg)**

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
51	241.7	0.92	0.32	2.71	0.119	0.72	0.038
52	245.6	0.74	0.32	2.91	0.112	0.61	0.028
53	249.0	0.78	0.32	3.42	0.083	0.66	0.030
54	212.9	0.88	0.31	2.80	0.128	0.62	0.033
55	223.7	0.82	0.31	2.90	0.151	0.69	0.033
56	216.8	0.83	0.30	3.02	0.085	0.63	0.033
57	216.9	0.90	0.29	2.95	0.094	0.65	0.037
58	223.9	0.80	0.31	3.01	0.127	0.57	0.027
59	230.6	0.82	0.32	3.07	0.115	0.69	0.030
60	221.1	0.84	0.38	3.86	0.140	0.70	0.031

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
51	0.19	0.062	0.59
52	0.25	0.045	0.31
53	0.15	0.033	0.81
54	0.25	0.048	0.31
55	0.21	0.068	0.40
56	0.21	0.052	0.32
57	0.19	0.047	0.53
58	0.28	0.036	0.49
59	0.20	0.052	0.40
60	0.21	0.040	0.90

ORGAN/BODY WEIGHT RATIOS (%)**AFTER 13 WEEKS****FEMALES****Group 3 (200 mg/kg)**

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
61	206.8	0.88	0.32	2.88	0.141	0.65	0.027
62	259.9	0.73	0.29	2.60	0.145	0.61	0.029
63	237.1	0.75	0.35	2.93	0.132	0.67	0.029
64	208.7	0.88	0.34	2.81	0.131	0.59	0.037
65	208.7	0.96	0.32	2.27	0.154	0.69	0.034
66	253.6	0.79	0.31	2.84	0.123	0.54	0.030
67	218.4	0.87	0.34	3.06	0.125	0.66	0.028
68	216.7	0.79	0.37	2.99	0.132	0.64	0.036
69	232.1	0.90	0.32	3.35	0.178	0.69	0.029
70	206.0	0.87	0.33	3.04	0.134	0.64	0.032

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
61	0.23	0.037	0.45
62	0.21	0.050	0.57
63	0.23	0.035	0.86
64	0.22	0.050	0.36
65	0.22	0.037	0.46
66	0.22	0.044	0.67
67	0.20	0.043	0.38
68	0.28	0.048	0.52
69	0.24	0.044	0.28
70	0.24	0.040	0.41

ORGAN/BODY WEIGHT RATIOS (%)**AFTER 13 WEEKS****FEMALES****Group 4 (1000 mg/kg)**

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
71	209.5	0.88	0.34	2.99	0.153	0.64	0.032
72	204.4	0.92	0.37	2.73	0.122	0.65	0.032
73	226.9	0.81	0.36	2.94	0.150	0.64	0.035
74	238.4	0.82	0.33	2.96	0.121	0.64	0.037
75	226.0	0.83	0.34	2.77	0.162	0.67	0.031
76	244.8	0.79	0.31	3.00	0.108	0.71	0.028
77	232.9	0.88	0.35	3.03	0.110	0.52	0.037
78	207.7	0.86	0.40	3.08	0.134	0.70	0.039
79	234.8	0.82	0.29	3.03	0.140	0.59	0.034
80	220.4	0.86	0.31	2.95	0.114	0.67	0.026

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
71	0.22	0.054	0.37
72	0.22	0.059	0.35
73	0.25	0.041	0.52
74	0.23	0.051	0.68
75	0.19	0.037	0.37
76	0.24	0.037	0.40
77	0.24	0.056	0.49
78	0.21	0.053	0.41
79	0.30	0.048	0.33
80	0.19	0.049	0.37

ORGAN/BRAIN WEIGHT RATIOS (%)**AFTER 13 WEEKS****FEMALES****Group 1 (0 mg/kg)**

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
41	1.63	46.01	427.61	20.184	84.05	4.724
42	1.94	34.54	275.26	13.918	65.46	3.351
43	1.97	40.10	327.41	16.853	79.70	4.061
44	1.97	39.59	300.51	18.071	74.11	4.264
45	1.94	42.27	324.23	17.320	82.99	4.227
46	2.04	40.20	327.45	12.010	78.92	4.755
47	1.92	44.27	418.23	19.479	82.29	3.958
48	1.66	39.16	375.30	11.446	74.10	3.494
49	1.81	38.12	444.20	14.641	86.74	4.751
50	1.79	35.75	371.51	9.050	83.80	3.575

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
41	26.38	7.607	72.39
42	22.16	5.155	39.18
43	24.87	6.904	68.53
44	24.37	6.193	72.59
45	21.13	6.031	76.80
46	28.92	4.608	35.78
47	32.29	5.729	61.46
48	21.69	5.663	40.96
49	26.52	4.807	60.22
50	22.91	5.140	88.27

ORGAN/BRAIN WEIGHT RATIOS (%)**AFTER 13 WEEKS****FEMALES****Group 2 (50 mg/kg)**

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
51	2.22	35.14	295.05	12.928	78.83	4.144
52	1.81	43.09	394.48	15.138	82.32	3.757
53	1.93	41.45	440.93	10.674	84.97	3.834
54	1.87	35.29	318.72	14.545	70.05	3.743
55	1.84	37.50	352.72	18.370	83.70	4.022
56	1.81	35.91	361.88	10.221	75.69	3.978
57	1.96	32.14	326.53	10.357	72.45	4.082
58	1.80	38.89	374.44	15.778	71.11	3.333
59	1.90	38.42	373.16	13.947	83.68	3.684
60	1.86	45.70	459.14	16.613	82.80	3.656

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
51	21.17	6.712	63.96
52	33.70	6.133	41.44
53	19.69	4.249	104.66
54	28.34	5.455	35.83
55	25.00	8.207	48.91
56	25.41	6.188	38.67
57	20.92	5.153	59.18
58	35.00	4.444	60.56
59	23.68	6.263	48.42
60	24.73	4.731	106.99

ORGAN/BRAIN WEIGHT RATIOS (%)**AFTER 13 WEEKS****FEMALES****Group 3 (200 mg/kg)**

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
61	1.83	36.61	325.68	15.956	73.22	3.060
62	1.89	40.21	358.20	20.000	84.13	4.021
63	1.78	47.19	390.45	17.584	89.33	3.876
64	1.83	38.80	320.77	14.973	67.21	4.208
65	2.00	33.50	237.00	16.050	72.50	3.500
66	2.01	38.81	358.71	15.473	67.66	3.731
67	1.91	38.74	350.26	14.241	75.39	3.194
68	1.72	47.09	376.74	16.628	80.81	4.477
69	2.09	35.41	371.77	19.809	76.08	3.206
70	1.80	37.78	347.78	15.333	73.33	3.667

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
61	26.23	4.208	50.82
62	28.57	6.878	77.78
63	30.34	4.663	114.61
64	24.59	5.738	41.53
65	22.50	3.850	48.00
66	27.36	5.572	84.08
67	23.04	4.974	42.93
68	34.88	5.988	65.12
69	26.79	4.880	31.58
70	27.22	4.611	47.22

ORGAN/BRAIN WEIGHT RATIOS (%)**AFTER 13 WEEKS****FEMALES****Group 4 (1000 mg/kg)**

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
71	1.84	39.13	340.76	17.446	73.37	3.641
72	1.88	40.43	296.28	13.245	70.74	3.457
73	1.83	44.26	363.93	18.634	79.23	4.372
74	1.95	40.51	361.54	14.769	78.46	4.513
75	1.88	40.96	332.45	19.415	80.32	3.777
76	1.94	39.18	378.87	13.660	89.18	3.505
77	2.06	39.32	342.23	12.476	58.74	4.223
78	1.79	46.37	357.54	15.531	81.56	4.525
79	1.93	35.23	368.39	17.047	72.02	4.145
80	1.89	36.51	344.44	13.280	78.31	3.069

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
71	25.54	6.196	41.85
72	23.40	6.436	37.77
73	31.15	5.082	64.48
74	27.69	6.205	83.59
75	23.40	4.468	44.15
76	30.41	4.639	50.00
77	26.70	6.311	55.34
78	24.58	6.145	48.04
79	36.27	5.803	39.90
80	21.69	5.661	43.39

**MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES**

Animals without necropsy

Animals not recorded

Animals not completed

Animals with not translated finding

Not Reported

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 200	Female	Group 10	Reserve Removed
Animal 201	Female	Group 10	Reserve Removed

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 1 (0 mg/kg)

Animal 1 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 2 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 3 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 4 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 5 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 6 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 7 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 8 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 9 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

**MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES**

Group 1 (0 mg/kg)

Animal 10 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 2 (50 mg/kg)

Animal 11 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 12 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 13 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 14 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 15 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 16 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 17 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 18 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 19 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 2 (50 mg/kg)

Animal 20 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 3 (200 mg/kg)

Animal 21 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 22 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 23 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 24 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 25 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 26 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 27 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 28 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 29 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

**MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES**

Group 3 (200 mg/kg)

Animal 30 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 4 (1000 mg/kg)

Animal 31 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 32 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 33 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 34 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 35 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 36 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 37 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 38 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

Animal 39 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 4 (1000 mg/kg)

Animal 40 PLANNED NECROPSY , 18-DEC-2013

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

Group 1 (0 mg/kg)

Animal 41 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 42 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 43 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 44 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 45 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 46 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 47 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 48 PLANNED NECROPSY , 17-DEC-2013

LYMPH NODES MANDIBULAR: DISCOLORATION, DARK RED.

Animal 49 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

**MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES**

Group 1 (0 mg/kg)

Animal 50 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

Group 2 (50 mg/kg)

Animal 51 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 52 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 53 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 54 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 55 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 56 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 57 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 58 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 59 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

**MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES**

Group 2 (50 mg/kg)

Animal 60 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

Group 3 (200 mg/kg)

Animal 61 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 62 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 63 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 64 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 65 PLANNED NECROPSY , 17-DEC-2013

EYES VITREOUS HUMOR, RIGHT SIDE: DESICCATED.

Animal 66 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 67 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 68 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 69 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

**MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES**

Group 3 (200 mg/kg)

Animal 70 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

Group 4 (1000 mg/kg)

Animal 71 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 72 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 73 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 74 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 75 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 76 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 77 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 78 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

Animal 79 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

**MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES**

Group 4 (1000 mg/kg)

Animal 80 PLANNED NECROPSY , 17-DEC-2013

NO FINDINGS NOTED

APPENDIX I

Chemical Analysis of Feed

LUFA-ITL GmbH

Dr.-Hell-Str. 6, 24107 Kiel, Germany
 Fax: +49(0431)1228-498
 eMail: zentrale@lufa-iti.de www.agrolab.de



LUFA - ITL Dr.-Hell-Str. 6, 24107 Kiel

PROVIMI KLIBA AG
 RINAUSTRASSE
 4303 KAISERAUGST / SCHWEIZ
 SCHWEIZ

Date 25.06.2013

Customer no. 1209835

Page 1 of 3

REPORT**Order nr. 1140430**

Sample no. 470699
 Order GLP Schadstoffuntersuchung
 Sample acceptance 12.06.2013
 Date of sampling not specified
 Sample code TEKLAD GLOBAL RODENT 2914C - Pel. 10 mm eckig
 Alleinfuttermittel für Mäuse und Ratten
 Rezeptur 3255 - GLP-Batch: 29/13
 Fabr.-Code: 1306804 - Hergestellt: 06.06.2013 - MHD: 06.03.2014
 Packaging plastic bag

		limits acc. GV-SOLAS			
	Unit	Result	A-08-2001	Substance	Method
Trace-elements / Heavy metals					
Copper	mg/kg	10,7		OM	VDLUFA VII 2.2.2.6
Selenium	mg/kg	0,10		OM	VDLUFA VII 2.2.2.5; ICPMS
Cadmium	mg/kg	0,06	0,4	OM	VDLUFA VII 2.2.2.5; ICPMS
Lead	mg/kg	<0,10	1,5	OM	VDLUFA VII 2.2.2.5; ICPMS
Mercury	mg/kg	<0,02	0,1	OM	\$64 LFGB L00.00-19
Arsenic	mg/kg	<0,10	1	OM	VDLUFA VII 2.2.2.5; ICPMS
Mycotoxins					
Aflatoxine B1	µg/kg	<1,00	10	OM	Inhousemethod HPLC-MS/MS
Aflatoxine B2	µg/kg	<1,00	5	OM	Inhousemethod HPLC-MS/MS
Aflatoxine G1	µg/kg	<1,00	5	OM	Inhousemethod HPLC-MS/MS
Aflatoxine G2	µg/kg	<1,00	5	OM	Inhousemethod HPLC-MS/MS
Sum Aflatoxines	µg/kg	<1,0 ^{x)}		OM	Calculated
Non-dioxinlike PCB (ndl-PCB)					
PCB 28	mg/kg	<0,00080 ^{a)}		OM	\$64 LFGB L00.00-34
PCB 52	mg/kg	<0,00080 ^{a)}		OM	\$64 LFGB L00.00-34
PCB 101	mg/kg	<0,00080 ^{a)}		OM	\$64 LFGB L00.00-34
PCB 118	mg/kg	<0,00080 ^{a)}		OM	\$64 LFGB L00.00-34
PCB 138	mg/kg	<0,00080 ^{a)}		OM	\$64 LFGB L00.00-34
PCB 153	mg/kg	<0,00080		OM	\$64 LFGB L00.00-34
PCB 180	mg/kg	<0,00080 ^{a)}		OM	\$64 LFGB L00.00-34
sum PCB	mg/kg	<0,002 ^{x)}	0,05	OM	Calculated
Organochlorous-Pesticides GC-Multiresidueanalysis					
Dieldrin	mg/kg	<0,002		OM	\$64 LFGB L00.00-34
HCH-gamma (Lindan)	mg/kg	<0,002	0,1	OM	\$64 LFGB L00.00-34
Heptachlor	mg/kg	<0,00200		OM	\$64 LFGB L00.00-34



1) Durch die DAkkS wurde die
 ISO/IEC 17025 akkreditiert
 Professionsbereich:
 Die Akkreditierung gilt für die in
 der Tabelle aufgeführten
 Prüfverfahren.
 DAkkS
 Deutsche
 Akkreditierungsstelle
 D-PL 14082-01-00

LUFA-ITL GmbH

Dr.-Hell-Str. 6, 24107 Kiel, Germany
 Fax: +49(0431)1228-498
 eMail: zentrale@lufa-iti.de www.agrolab.de



Date 25.06.2013
 Customer no. 1209835
 Page 2 of 3

Order nr. 1140430 Sample no. 470699

	Unit	limits acc. Result GV-SOLAS A-08-2001	Substance	Method
Heptachlorepoxide-cis	mg/kg	<0,00200	OM	\$64 LFGB L00.00-34
Heptachlorepoxide-trans	mg/kg	<0,00200	OM	\$64 LFGB L00.00-34
o,p-DDD	mg/kg	<0,00200	OM	\$64 LFGB L00.00-34
o,p-DDE	mg/kg	<0,00200	OM	\$64 LFGB L00.00-34
o,p-DDT	mg/kg	<0,002	OM	\$64 LFGB L00.00-34
p,p-DDD	mg/kg	<0,00200	OM	\$64 LFGB L00.00-34
p,p-DDE	mg/kg	<0,00200	OM	\$64 LFGB L00.00-34
p,p-DDT	mg/kg	<0,00200	OM	\$64 LFGB L00.00-34
Sum DDTs	mg/kg	<0,005 ^{x)} 0,05	OM	Calculated
Sum Heptachlor	mg/kg	<0,005 ^{x)} 0,01	OM	Calculated
Organo-Phosphorous Pesticides GC-Multiresidueanalysis				
Malathion	mg/kg	<0,010 1	OM	\$64 LFGB L00.00-34
Estrogens				
dienestrol	µg/kg	<0,500	OM	(TG) ^{v)}
diethyl stilbestrol	µg/kg	<0,500	OM	(TG) ^{v)}
hexestrol	µg/kg	<0,500	OM	(TG) ^{v)}
Sum Estrogens	µg/kg	n.q.	OM	Calculated(TG) ^{v)}
Nitrosamines				
N-Nitrosodiethylamin	µg/kg	<5,00 10	OM	GC-Inhousemethod(HH) ^{v)}
N-Nitrosodimethylamin	µg/kg	<5,00 10	OM	GC-Inhousemethod(HH) ^{v)}
Sum Nitrosamines	µg/kg	n.q.	OM	Calculated(HH) ^{v)}

x) The sum calculation is done without taking into account the report limits.

a) See note

Explanation: "<" or "n.q." represent the fact that the concentration of the analyte is below the limit of quantification (LOQ).

Explanation: OM = on original matter; DM = on dry matter base

v) Forwarded to an accredited laboratory

According to the extent of the analysis the sample complies with the requirements of limits acc. GV-SOLAS A-08-2001

LUFA - ITL Frau Dr. Verena Gonzalez Lopez, Tel. 0431/1228-316

Customer Relations Management feed

This electronically transmitted report was checked and released. It is in accordance with the requirements of DIN EN ISO/IEC 17025:2005 for simplified reports and valid without signature.

Copies

PROVIMI KLIBA AG

Subcontractors

Analysed by

(HH) Zentrale Analytik - Organische Henkel KGaA, Henkelstrasse 67 4, Gebäude Z43, 40589 Düsseldorf

Methods

Calculated; GC-Inhousemethod

(TG) TIERGESUNDHEITSDIENST, for the cited method accredited according to ISO/IEC 17025:2005, certificate of Accreditation: DAP-PL-2963.00

Methods

Calculated



LUFA-ITL GmbH

Dr.-Hell-Str. 6, 24107 Kiel, Germany
Fax: +49(0431)1228-498
eMail: zentrale@lufa-iti.de www.agrolab.de



Date 25.06.2013
Customer no. 1209835
Page 3 of 3

Order nr. 1140430 Sample no. 470699

Start of testing: 12.06.13

End of testing: 25.06.13

The analytical results are only valid for the delivered sample material. A plausibility check is hardly possible for samples of unknown origin. Duplication of this document or of parts of it requires the authorization from laboratory.

LUFA-ITL GmbH

Dr.-Hell-Str. 6, 24107 Kiel, Germany
 Fax: +49(0)431)1228-498
 eMail: zentrale@lufa-iti.de www.agrolab.de



LUFA - ITL Dr.-Hell-Str. 6, 24107 Kiel

PROVIMI KLIBA AG
 RINAUSTRASSE
 4303 KAISERAUGST / SCHWEIZ
 SCHWEIZ

Date 05.08.2013

Customer no. 1209835

Page 1 of 3

REPORT 1158435 - 510043

Order 1158435 GLP Schadstoffuntersuchung
 Sample no. 510043
 Sample acceptance 17.07.2013
 Date of sampling not specified
 Sample code TEKLAD GLOBAL RODENT 2914C - Pell. 10 mm eckig
 Alleinfuttermittel für Mäuse und Ratten
 Rezeptur 3255 - GLP-Batch: 35/13
 Fabr.-Code: 1307804 - Hergestellt: 09.07.2013 - MHD: 09.03.2014
 Packaging plastic bag

		limits acc. GV-SOLAS Result A-08-2001		Substance Method	
Trace-elements / Heavy metals					
Copper	mg/kg	8,38		OM	VDLUFA VII 2.2.2.6
Selenium	mg/kg	<0,10		OM	VDLUFA VII 2.2.2.5; ICPMS
Cadmium	mg/kg	0,05	0,4	OM	VDLUFA VII 2.2.2.5; ICPMS
Lead	mg/kg	<0,10	1,5	OM	VDLUFA VII 2.2.2.5; ICPMS
Mercury	mg/kg	<0,02	0,1	OM	§64 LFGB L00.00-19
Arsenic (As)	mg/kg	<0,10	1	OM	VDLUFA VII 2.2.2.5; ICPMS
Mycotoxins					
Aflatoxine B1	µg/kg	<1,00	10	OM	Inhousemethod HPLC-MS/MS
Aflatoxine B2	µg/kg	<1,00	5	OM	Inhousemethod HPLC-MS/MS
Aflatoxine G1	µg/kg	<1,00	5	OM	Inhousemethod HPLC-MS/MS
Aflatoxine G2	µg/kg	<1,00	5	OM	Inhousemethod HPLC-MS/MS
Sum Aflatoxines	µg/kg	<1,0	10	OM	Calculated
Non-dioxinlike PCB (ndl-PCB)					
PCB 28	mg/kg	<0,00080	10	OM	§64 LFGB L00.00-34
PCB 52	mg/kg	<0,00080		OM	§64 LFGB L00.00-34
PCB 101	mg/kg	<0,00080		OM	§64 LFGB L00.00-34
PCB 118	mg/kg	<0,00080	10	OM	§64 LFGB L00.00-34
PCB 138	mg/kg	<0,00080		OM	§64 LFGB L00.00-34
PCB 153	mg/kg	<0,00080		OM	§64 LFGB L00.00-34
PCB 180	mg/kg	<0,00080		OM	§64 LFGB L00.00-34
sum PCB	mg/kg	<0,002	0,05	OM	Calculated
Organochlorous-Pesticides GC-Multiresidueanalysis					
Dieldrin	mg/kg	<0,002		OM	§64 LFGB L00.00-34
HCH-gamma (Lindan)	mg/kg	<0,002	0,1	OM	§64 LFGB L00.00-34
Heptachlor	mg/kg	<0,00200		OM	§64 LFGB L00.00-34
Heptachlorepoxide-cis	mg/kg	<0,00200		OM	§64 LFGB L00.00-34
Heptachlorepoxide-trans	mg/kg	<0,00200		OM	§64 LFGB L00.00-34



1. Schritt der Güte nach DIN EN
 15189:2003 (ISO 9001:2008)
 2. Schritt der Güte nach DIN EN
 15189:2003 (ISO 9001:2008)
 3. Schritt der Güte nach DIN EN
 15189:2003 (ISO 9001:2008)

LUFA-ITL GmbH

Dr.-Hell-Str. 6, 24107 Kiel, Germany
 Fax: +49(0)431)1228-498
 eMail: zentrale@lufa-iti.de www.agrolab.de



Date 05.08.2013

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Unit	Result	limits acc. GV-SOLAS A-08-2001		Substance	Method
<i>o,p</i> -DDD	mg/kg	<0,00200	^{a)}	OM	§64 LFGB L00.00-34
<i>o,p</i> -DDE	mg/kg	<0,00200	^{a)}	OM	§64 LFGB L00.00-34
<i>o,p</i> -DDT	mg/kg	<0,002	^{a)}	OM	§64 LFGB L00.00-34
<i>p,p</i> -DDD	mg/kg	<0,00200	^{a)}	OM	§64 LFGB L00.00-34
<i>p,p</i> -DDE	mg/kg	<0,00200	^{a)}	OM	§64 LFGB L00.00-34
<i>p,p</i> -DDT	mg/kg	<0,00200	^{a)}	OM	§64 LFGB L00.00-34
Sum DDTs	mg/kg	<0,005	^{a)} 0,05	OM	Calculated
Sum Heptachlor	mg/kg	<0,005	^{a)} 0,01	OM	Calculated

Organo-Phosphorous Pesticides GC-Multiresidueanalysis

Malathion	mg/kg	<0,010	1	OM	§64 LFGB L00.00-34
-----------	-------	--------	---	----	--------------------

Estrogenes

dienestrol	µg/kg	<0,500		OM	(TG) ^{v)}
diethyl stilbestrol	µg/kg	<2,00		OM	(TG) ^{v)}
hexestrol	µg/kg	<1,00		OM	(TG) ^{v)}
Sum Estrogenes	µg/kg	n.q.		OM	Calculated(TG) ^{v)}

nitrosamines

N-Nitrosodiethylamin	µg/kg	<5,00	10	OM	GC-Inhousemethod(HH) ^{v)}
N-Nitrosodimethylamin	µg/kg	<5,00	10	OM	GC-Inhousemethod(HH) ^{v)}
Sum Nitrosamines	µg/kg	n.q.		OM	Calculated(HH) ^{v)}

x) The sum calculation is done without taking into account the report limits.

a) See note

Explanation: "<" or "n.q." represent the fact that the concentration of the analyte is below the limit of quantification (LOQ).

Explanation: OM = on original matter; DM = on dry matter base

v) Forwarded to an accredited laboratory

According to the extent of the analysis the sample complies with the requirements of limits

This electronically transmitted report was checked and released. It is in accordance with the requirements of DIN EN ISO/IEC 17025:2005 for simplified reports and is valid with the digital signature.

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17025:2005
 DIN EN ISO/IEC 17025:2005
 Zertifizierung
 für die Konformität mit
 den Anforderungen
 der ISO/IEC 17025:2005

LUFA-ITL GmbH

Dr.-Hell-Str. 6, 24107 Kiel, Germany
Fax: +49(0)431)1228-498
eMail: zentrale@lufa-iti.de www.agrolab.de



Date 05.08.2013
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REPORT 1158435 - 510043**Subcontractors****Analysed by**

(HH) Zentrale Analytik - Organische Henkel KGaA, Henkelstrasse 67 ½ Gebäude Z43, 40589 Düsseldorf

Methods

Calculated; GC-Inhousemethod

(TG) TIERGESUNDHEITSDIENST, SENATOR-GERAUER STR 23, 85586 POING, for the cited method accredited according to ISO/IEC 17025:2005, certificate of Accreditation: DAP-PL-2963.00

Methods

Calculated

Start of testing: 17.07.13

End of testing: 05.08.13

The analytical results are only valid for the delivered sample material. A plausibility check is hardly possible for samples of unknown origin. Duplication of this document or of parts of it requires the authorization from laboratory.



Tiergesundheitsdienst
Senateur-Gerauer Str. 23
85586 Poing
Tel: 089 24000-1
Fax: 089 24000-20
E-Mail: info@tdg.de

APPENDIX II

Drinking Water Analysis



BACTERIOLOGICAL ASSAY OF DRINKING WATER, ITINGEN

Official Laboratory Kanton Basel-Landschaft:

Liestal, 30 July 2013

Reference No.:

100052789

Sampling Point:

Netwater, Harlan Laboratories Ltd., Itingen,
1st basement floor, room no. 10

Date and Time of Sampling:

26 July 2013, 8:00 a.m.

Sample ID:

200122533

Water Temperature (°C)	15.9
Aerobic mesophilic bacteria/mL	1
E. coli/100 mL	0
Enterococci/100 mL	0

Assessment:

At the time of sampling, the tested bacteriological parameters met the requirements for drinking water according to "Artikel 3 der Verordnung über Trink-, Quell-, und Mineralwasser (SR 817.022.102) [Article 3 of Regulation on drinking water, spring and mineral water (SR 817.022.102)].

Issued by:

CHEMICAL ANALYSIS OF DRINKING WATER, ITINGEN**Official Laboratory Kanton Basel-****Landschaft:**

Liestal, 13 August 2013

Reference No.

100052790

Sampling Point:Netwater, Harlan Laboratories Ltd., Itingen,
1st basement floor, room no. 10**Date and Time of Sampling:**

26 July 2013, 8:00 a.m.

Sample ID:

200122535

Water temperature [°C]	15.9
UV-absorption [at 254 nm/100 cm]	1.18
Oxygen demand (KMnO ₄ consumption) [mg/L]	2.07
Conductivity [µS/cm]	714
pH-Value	7.72
Turbidity [FNU]	0.04
Nitrate [mg/L]	17.8
Sulfate [mg/L]	104
Chloride [mg/L]	31.2
Phosphate as P [mg/L]	0.01
Fluoride [mg/L]	0.18
Total hardness [fr.H°]	41.0
Alkaline hardness [fr.H°]	27.6
Non-carbonate hardness [fr.H°]	13.4
Sodium [mg/L]	19.6
Potassium [mg/L]	3.25
Calcium [mg/L]	142
Magnesium [mg/L]	13.4
Color	Colorless
Odour	Odourless

Assessment:

At the time of sampling, the tested chemical parameters met the requirements for drinking water according to article "Artikel 3 der Verordnung über Trink-, Quell-, und Mineralwasser (SR 817.022.102) [Article 3 of Regulation on drinking water, spring and mineral water (SR 817.022.102)].



CONTAMINANT ASSAY OF DRINKING WATER, ITINGEN

Harlan Laboratories

Study: D72725

Sampling Point: Harlan Laboratories Ltd., Itingen, 1st basement floor, room no. 10

Date of Sampling: 26 July 2013, 8:00 a.m

	Result	Limit*
	[µg/L]	[µg/L]
Copper	<4	1500
Cadmium	<0.5	5
Lead	<3	50
Arsenic	<3	50
Selenium	<3	10
Mercury	<1	1

* According to "Schweizer Lebensmittelbuch" (Swiss Food Manual)

Issued by:



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auf die untersuchten Proben.
STS-Nr. 524



Test Report: Contaminant Assay of Drinking Water

Zürich, 21. August 2013

Order number: 213-0568 (Harlan Laboratories, CH-4452 Itingen)

Sampling: Customer

Sample receipt: 30.07.2013

Handling time: 30.07.2013 – 21.08.2013

Analyte	Result Unit	Method
---------	-------------	--------

Sample number: 213-0568/2

Water Itingen, UG, Room 10, Box re., Sampling Week 31

Lindane	<0.05 µg/l	GC/MS/MS
Heptachlor	<0.05 µg/l	GC/MS/MS
Malathion	<0.05 µg/l	GC/MS/MS
DDT (total)	<0.05 µg/l	GC/MS/MS
Dieldrin	<0.05 µg/l	GC/MS/MS
PCB 28	<0.02 µg/l	AP0_SAV_244
PCB 52	<0.02 µg/l	AP0_SAV_244
PCB 101	<0.02 µg/l	AP0_SAV_244
PCB 138	<0.02 µg/l	AP0_SAV_244
PCB 153	<0.02 µg/l	AP0_SAV_244
PCB 180	<0.02 µg/l	AP0_SAV_244
PCB (Sum)	<0.12 µg/l	AP0_SAV_244
NDMA (Nitrosodimethylamine)	<2 µg/l	GC/TEA
NDEA (Nitrosodiethylamine)	<2 µg/l	GC/TEA
NPIP (Nitrosopiperidine)	<2 µg/l	GC/TEA
NMOR (Nitrosomorpholine)	<2 µg/l	GC/TEA

Abbreviations and symbols: nd = not detectable, na = not analyzed, < = smaller,
> = greater

Measuring uncertainty: Measuring results always contain an element of uncertainty. This fact could be of great importance when testing for adherence to specifications or limiting values. If the client really needs an estimation of the degree of measuring uncertainty, the relevant information can be requested from Labor Veritas.

APPENDIX III

Formulation Analysis

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1 MATERIALS AND METHODS

1.1 Reference Item

The test item as described in the main report was used as reference item.

1.2 Study Samples and Storage

Representative samples were dispatched to the analytical laboratories internally (at room temperature) and stored frozen at -20 ± 5 °C until analysis.

1.3 Reagents and Materials

Purified water:	In-house prepared by ELGA water purification system (Ultra Bio No. UBH 279651)
-----------------	--

1.4 Analytical Procedure

1.4.1 Preparation of Calibration Solutions

Stock solutions of Lipase produced with *Trichoderma reesei* in purified water were prepared for external calibration. For example, 5.360 mg of Lipase produced with *Trichoderma reesei* was weighed into a 50 mL volumetric flask and filled to about 75% of final volume with purified water. The mixture was well shaken until everything was solved and brought to volume with purified water to yield a solution with a concentration of 101.1 µg/mL (a correction factor of 1.06 was taken into account). Aliquots of this stock calibration solution were diluted with purified water to obtain calibration solutions with nominal concentrations ranging from 1.011 to 5.057 µg/mL. On each occasion calibration solutions derived from two stock solutions were used for calibration.

1.4.2 Work up of Samples

1.4.2.1 Samples of Group 1 and 2

Each sample was dissolved with 5 mL purified water by shaken until everything was solved and then further diluted with purified water into the calibration range.

1.4.2.2 Samples of Group 3 and 4

Each sample was quantitatively transferred into an appropriate volumetric flask. The latter was successively rinsed with at least two portions of purified water and the rinsings were combined in the volumetric flask. The flask was filled to about 75% of the target volume with purified water and dissolution was achieved by shaken until everything was solved. The flask was filled to the mark with purified water. Sample solutions were further diluted with purified water into the calibration range.

1.4.3 TOC-Method Determination

All glass vessels were cleaned very accurately using ELGA-water. The total organic carbon (TOC) was determined as the difference between total carbon (TC) and total inorganic carbon (IC). A calibration curve was prepared for both values separately. After using 2M hydrochloric acid to remove all CO₂ the IC was determined. Subsequently, the TC was determined. Therefore, all organic compounds were being oxidized to CO₂ using a catalyst at 680 °C.

TOC-device:	TOC-V _{CPH} Total Organic Carbon Analyzer, Shimadzu
Range of sample calibration curve:	1 - 5 µg/mL
Pressure:	5 bar
Carrier gas:	Artificial air (80% pure N ₂ and 20% pure O ₂ ; free of hydrocarbons)
Syringe volume:	2500 µL

1.4.4 Evaluation of Results

Injected samples were quantified by comparing peak areas of Lipase produced with *Trichoderma reesei* with reference to the calibration curve. The latter was obtained by correlation of the peak areas of the calibration solutions with their corresponding concentrations [µg/mL], using the linear regression model following equation 1:

$$y = a + b \cdot x \quad (1)$$

where

y	=	Response for Lipase produced with <i>Trichoderma reesei</i>
a	=	Intercept derived from linear regression of calibration data
b	=	Slope derived from linear regression of calibration data
x	=	Actual concentration of Lipase produced with <i>Trichoderma reesei</i> in sample aliquot [µg/mL]

Sample aliquot concentrations were corrected for density of the application formulation and for dilution using equation 2:

$$C_{act} = \frac{x \cdot V \cdot D}{W \cdot 1000} \quad (2)$$

where

C_{act}	=	Actual sample concentration [mg/mL]
x	=	Actual concentration of Lipase produced with <i>Trichoderma reesei</i> in sample aliquot according to equation 1 [μ g/mL]
V	=	Dilution volume [mL]
D	=	Density of application formulation [set to 1 g/mL]
W	=	Sample weight [g]

The sample recovery was determined as follows:

$$R = \frac{C_{act}}{C_{nom}} \cdot 100 \quad (3)$$

where

R	=	Sample recovery [%]
C_{act}	=	Actual sample concentration [mg/mL]
C_{nom}	=	Nominal sample concentration [mg/mL]

2 RESULTS

The linearity of the analytical system used for sample analyses was demonstrated with a good relationship between peak areas measured and calibration solution concentrations. All calibration points used met the acceptance limit of $\pm 20\%$ variation from the calibration curve derived by linear regression analysis. The coefficients of determination (R^2) calculated were found to be better than 0.99. An example is presented in [Table 2](#).

The application formulations investigated during the study were found to comprise Lipase produced with *Trichoderma reesei* in the range of 86.5% to 114.2%, thus, the required content limit of $\pm 20\%$ with reference to the nominal content was met.

The homogeneous distribution of Lipase produced with *Trichoderma reesei* in the preparations was approved because single results found did not deviate more than 5.5% (acceptance criterion: $<15\%$) from the corresponding mean.

In addition, the test item was found to be stable with reference to the TOC method in application formulations when kept up to eight days at room temperature due to recoveries which met the variation limit of 10% from the time-zero (homogeneity) mean.

Detailed results are shown in [Table 1](#).

In conclusion, the results indicate the accurate preparation and storage of the test item Lipase produced with *Trichoderma reesei* in vehicle during this study.

TABLES

Table 1 Detailed Results of Application Formulation Analysis

(Rounded results presented are based on calculations with exact data)

Dose Group	Sample taken from/after	Date of Analysis	Nominal Conc. [mg/mL]	Actual Conc. [mg/mL]	Recovery	Mean Recovery	Coeff. of Variation	Variation from Mean Recovery
Date of Preparation: 16-Sep-2013								
1	vehicle	18-Sep-13	0	--- ¹	---	---	---	---
2	top	18-Sep-13	5	4.840	96.8%	99.9%	4.0%	---
	middle	18-Sep-13	5	4.931	98.6%			
	bottom	18-Sep-13	5	5.221	104.4%			
	4 hours/20±5°C	18-Sep-13	5	4.782	95.6%	---	---	4.3%
	8 days/20±5°C	04-Oct-13	5	4.614 ²	92.3%	---	---	7.7%
3	top	18-Sep-13	20	19.83	99.2%	103.0%	3.6%	---
	middle	18-Sep-13	20	20.69	103.4%			
	bottom	18-Sep-13	20	21.29	106.5%			
	4 hours/20±5°C	18-Sep-13	20	21.11	105.6%	---	---	2.5%
	8 days/20±5°C	18-Oct-13	20	20.27	101.3%	---	---	1.6%
4	top	18-Sep-13	100	86.50	86.5%	89.8%	3.7%	---
	middle	18-Sep-13	100	89.91	89.9%			
	bottom	18-Sep-13	100	93.10	93.1%			
	4 hours/20±5°C	18-Sep-13	100	91.37	91.4%	---	---	1.7%
	8 days/20±5°C	18-Oct-13	100	96.68	96.7%	---	---	7.6%
Date of Preparation: 21-Oct-2013								
1	vehicle	22-Oct-13	0	--- ¹	---	---	---	---
2	top	22-Oct-13	5	5.198	104.0%	105.1%	1.1%	---
	middle	22-Oct-13	5	5.318	106.4%			
	bottom	22-Oct-13	5	5.253	105.1%			
3	top	22-Oct-13	20	21.35	106.8%	104.2%	3.1%	---
	middle	22-Oct-13	20	21.07	105.4%			
	bottom	22-Oct-13	20	20.10	100.5%			
4	top	22-Oct-13	100	102.5	102.5%	109.1%	5.5%	---
	middle	22-Oct-13	100	110.7	110.7%			
	bottom	22-Oct-13	100	114.2	114.2%			
Date of Preparation: 02-Dec-2013								
1	vehicle	03-Dec-13	0	--- ¹	---	---	---	---
2	top	03-Dec-13	5	4.909	98.2%	98.5%	0.3%	---
	middle	03-Dec-13	5	4.934	98.7%			
	bottom	03-Dec-13	5	4.928	98.6%			
3	top	03-Dec-13	20	21.75	108.7%	102.4%	5.4%	---
	middle	03-Dec-13	20	19.82	99.1%			
	bottom	03-Dec-13	20	19.85	99.3%			
4	top	03-Dec-13	100	97.53	97.5%	97.2%	0.5%	---
	middle	03-Dec-13	100	96.65	96.7%			
	bottom	03-Dec-13	100	97.43	97.4%			

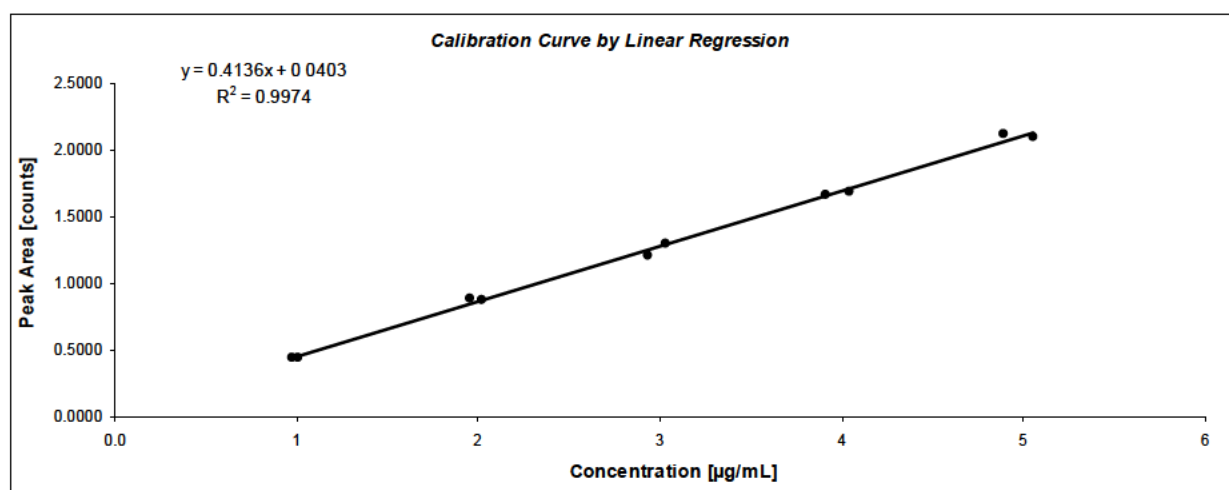
¹ Signal detected was below the lowest calibration point

² Result of back up sample (mean of duplicate analysis)

Table 2 Example of Calibration Curve

Date of analysis: 18 September 2013

Standard Concentration [µg/mL]	Peak Area [counts]	Variation of Peak Area
1.011	0.4461	-2.7%
2.023	0.8746	-0.3%
3.034	1.303	0.6%
4.046	1.691	-1.3%
5.057	2.100	-1.5%
0.978	0.4483	0.8%
1.956	0.8921	5.1%
2.933	1.208	-3.6%
3.911	1.662	0.2%
4.889	2.119	2.7%



APPENDIX IV

Clinical Laboratory Investigations

CLINICAL LABORATORY INVESTIGATIONS

Blood and Urine Sampling:

After 13 Weeks:

17/18-Dec-2013

Blood samples were drawn sublingually from all animals under light isoflurane anesthesia. The animals were fasted in metabolism cages for approximately 18 hours before blood sampling but allowed access to water *ad libitum*. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms. Urine was collected during the fasting period into a specimen vial.

In the summary and individual tables the names of some parameters have been abbreviated. Any abbreviation has been defined in this section. Clinical laboratory data are expressed, with a few exceptions, in general accordance with the International System of Units (SI).

Key to abbreviations of units:

L	liter	g	gram	m	milli (10^{-3})
mol	mole	G	giga (10^9)	μ	micro (10^{-6})
sec	seconds	T	tera (10^{12})	f	femto (10^{-15})
U	Unit				

Hematology

The following anticoagulants were used during blood collection:

Complete Blood Cell Count:	Tri-potassium-EDTA
Methemoglobin:	Lithium heparin
Coagulation:	Sodium citrate, 3.2% (1 part anticoagulant to 9 parts blood)

Complete Blood Cell Count

Parameter	Abbreviation	Unit	Instrumentation
Erythrocyte count	RBC	T/L	Advia 120 ¹
Hemoglobin	HB	mmol/L	Advia 120
Hematocrit	HCT	rel.1	Advia 120
Mean corpuscular volume	MCV	fL	Advia 120
Red cell volume distribution width	RDW	rel.1	Advia 120

¹ ADVIA 120 hematology system (Siemens)

Parameter	Abbreviation	Unit		Instrumentation
Mean corpuscular hemoglobin	MCH	fmol		Advia 120
Mean corpuscular hemoglobin concentration	MCHC	mmol/L		Advia 120
Hemoglobin concentration	HDW	mmol/L		Advia 120
				Advia 120
Reticulocyte count	RETI	relative rel.1	absolute G/L	Advia 120
Reticulocyte maturity index (low, medium, high fluorescence)	L RETI M RETI H RETI	rel.1 rel.1 rel.1		Advia 120
				Advia 120
Leukocyte count, total	WBC	G/L		Advia 120
				Advia 120
Differential leukocyte count		relative	absolute	Advia 120
Neutrophils	NEUT	rel.1	G/L	Advia 120
Eosinophils	EOS	rel.1	G/L	Advia 120
Basophils	BASO	rel.1	G/L	Advia 120
Lymphocytes	LYMPH	rel.1	G/L	Advia 120
Monocytes	MONO	rel.1	G/L	Advia 120
Large unstained cells	LUC	rel.1	G/L	Advia 120
				Advia 120
Platelet count	PLATELETS	G/L		Advia 120

Hemoglobin Derivatives

Parameter	Abbreviation	Unit	Method	Instrumentation
Methemoglobin	MET-HB	rel. 1	Spectrometry, results given as ratio of total hemoglobin	ABL80 FLEX ²
Heinz bodies	HEINZ BOD	rel.1	Microscopic examination of New Methylene Blue stained films, results given as ratio of total RBC	Microscope (blood smears prepared, but not evaluated)

² ABL80 FLEX, Radiometer

Coagulation

Parameter	Abbreviation	Unit	Method	Instrumentation
Prothrombin time (=Thromboplastin time)	PT	rel. 1	Clotting assay, thromboplastin from rabbit brain tissue, results as ratio of normal activity	STA ³
Activated partial thromboplastin time	PTT	sec	Clotting assay, cephalin from rabbit cerebral tissue, silica surface activator	STA

³ STA-compact analyzer (Roche Diagnostics)

Clinical Biochemistry

Lithium heparin was used as anticoagulant during blood collection.

Parameter	Abbreviation	Unit	Method	Instrumentation
Glucose		mmol/L	Hexokinase/G6P-DH	Cobas 6000/501 ⁴
Urea		mmol/L	Urease/GLDH	Cobas 6000/501
Creatinine	CREAT	μmol/L	Enzymatic colorimetric test	Cobas 6000/501
Bilirubin, total *	BILI-T	μmol/L	Reaction with 3,5-Dichlorophenyl-diazonium salt	Cobas 6000/501
Bile acids	BILE AC	μmol/L	Colorimetric, enzymatic (3α-HSD, Diaphorase)	Cobas 6000/501
Cholesterol, total	CHOLEST	mmol/L	Enzymatic, CHOD/PAP	Cobas 6000/501
Triglycerides	TRIGLY	mmol/L	Glycerol-Kinase GPO/PAP method	Cobas 6000/501
Phospholipids	PHOS-LIP	mmol/L	Phospholipase-Cholinoxidase-Peroxidase-reaction	Cobas 6000/501
Aspartate aminotransferase EC 2.6.1.1 ⁵	ASAT	U/L 37 °C	AST/MDH	Cobas 6000/501
Alanine aminotransferase EC 2.6.1.2	ALAT	U/L 37 °C	ALT/LDH	Cobas 6000/501
Lactate dehydrogenase EC 1.1.1.27	LDH	U/L 37 °C	NADH/LDH coupled reaction using pyruvate as substrate	Cobas 6000/501
Alkaline phosphatase EC 3.1.3.1	ALP	U/L 37 °C	p-Nitrophenyl-phosphate as substrate	Cobas 6000/501
Gamma-glutamyl transferase EC 2.3.2.2	GGT	U/L 37 °C	Substrate: L-gamma-glutamyl-3-carboxy-4-nitroanilide	Cobas 6000/501
Creatine kinase EC 2.7.3.2	CK	U/L 37 °C	HK/ATP and G6P-DH/NADPH coupled reaction method	Cobas 6000/501

⁴ Cobas 6000/501 analyzer, Roche Diagnostics

⁵ Identification of enzymes with EC-Number (Enzyme Commission) according to Enzyme Nomenclature, Recommendations (1972) of the IUPAC and IUB, Elsevier Scient. Publ. Comp., Amsterdam, 1973

Parameter	Abbreviation	Unit	Method	Instrumentation
Sodium		mmol/L	Ion selective electrode	Cobas 6000/501
Potassium		mmol/L	Ion selective electrode	Cobas 6000/501
Chloride		mmol/L	Ion selective electrode	Cobas 6000/501
Calcium		mmol/L	o-Cresolphthalein complexone method	Cobas 6000/501
Phosphorus		mmol/L	Phosphomolybdate reaction	Cobas 6000/501
Protein, total	PROTEIN	g/L	Biuret reaction	Cobas 6000/501
Albumin		g/L	Bromocresol green method	Cobas 6000/501
Globulin		g/L	Calculated value (total protein minus albumin)	
Albumin / Globulin Ratio	A/G RATIO		Calculated value (albumin / globulin)	

* Remark:

Values under the lower limit of quantification (LLOQ, 1.7 µmol/L) were expressed as 0.00 µmol/L.

Urinalysis

Physical Examination

Parameter	Abbreviation	Unit	Method / Instrumentation
Urine volume (18-hour)		mL	Volumetric ⁶
Relative density (= Specific gravity)	REL DENS	rel.1	Refractometer ⁷
Color			Visual inspection
Appearance			Visual inspection

The following urine components were investigated using a semi-automated test strip analyzer Cobas U411 (Roche Diagnostics) applying reflectance spectroscopy. Results are given as discrete values representing a concentration range (semi-quantitative results).

Chemical Examination

Parameter	Abbreviation	Unit	Set Points	Instrumentation
pH-value	pH		5.0, 6.0, 6.5, 7.0, 8.0, 9.0	Cobas U411 ⁸
Nitrite		score	0 (negative), 1 (positive)	Cobas U411
Protein		g/L	0, 0.25, 0.75, 1.50, 5.00	Cobas U411
Glucose		mmol/L	0, 3, 6, 17, 56	Cobas U411
Ketones		mmol/L	0, 0.5, 1.5, 5.0, 15.0	Cobas U411
Urobilinogen	UROBILI	μmol/L	0, 17, 68, 135, 203	Cobas U411
Bilirubin		μmol /L	0, 17, 50, 100	Cobas U411
Erythrocytes	ERY	per μL	0, 10, 25, 50, 150, 250	Cobas U411
Leukocytes	LEU	per μL	0, 25, 100, 500	Cobas U411

⁶ Volume calculated with a density of 1 g/mL using a Mettler balance

⁷ Clinical Refractometer SU-202, Kernco

⁸ Cobas U411 semi-automated urine chemistry analyzer and reagent test strips, Roche Diagnostics

REFERENCE VALUES - HEMATOLOGY

STRAIN: RAT / HanRcc: WI ST (MALES)

AGE: FROM 19 TO 40 WEEKS

DATA COLLECTION PERIOD: 29-JAN-02 TO 23-AUG-05

PARAMETER	UNIT	N	MEAN	STAND. DEV	95% TOLERANCE LIMITS	
ERYTHROCYTES (RBC)	T/I	813	8.81	0.42	7.94	9.56
HEMOGLOBIN (HB)	mmol /l	813	9.9	0.4	9.1	10.6
HEMATOCRIT (HCT)	rel. 1	813	0.45	0.02	0.42	0.49
MEAN CELL VOLUME (MCV)	fl	813	51.6	2.2	47.8	56.0
RED CELL VOL. DISTR. WIDTH (RDW)	rel. 1	783	0.144	0.039	0.116	0.274
MEAN CELL HEMOGLOBIN (MCH)	fmol	813	1.12	0.05	1.04	1.25
MEAN CELL HEMOGLOBIN CONC. (MCHC)	mmol /l	813	21.77	0.84	20.33	23.48
HEMOGLOBIN CONC. DISTR. WIDTH	mmol /l	783	1.71	0.20	1.35	2.09
RETICULOCYTE COUNT						
RETICULOCYTE (REL)	rel. 1	813	0.021	0.004	0.014	0.029
RETICULOCYTE (ABS)	G/I	813	182	34	124	256
MATURITY INDEX (L-RETI)	rel. 1	783	0.538	0.126	0.359	0.802
MATURITY INDEX (M-RETI)	rel. 1	783	0.324	0.053	0.189	0.400
MATURITY INDEX (H-RETI)	rel. 1	783	0.138	0.101	0.010	0.340
LEUKOCYTES, TOTAL (WBC)	G/I	813	6.14	1.44	3.75	9.23
DIFF. WBC COUNT (REL)						
NEUTROPHILS (NEUT)	rel. 1	30	0.215	0.047	---	---
EOSINOPHILS (EOS)	rel. 1	813	0.020	0.007	0.010	0.035
BASOPHILS (BASO)	rel. 1	813	0.004	0.003	0.001	0.013
LYMPHOCYTES (LYMPH)	rel. 1	813	0.749	0.058	0.631	0.846
MONOCYTES (MONO)	rel. 1	813	0.022	0.008	0.011	0.038
LARGE UNSTAINED CELLS (LUC)	rel. 1	813	0.008	0.004	0.002	0.020
DIFF. WBC COUNT (ABS)						
NEUTROPHILS (NEUT)	G/I	30	1.46	0.43	---	---
EOSINOPHILS (EOS)	G/I	813	0.12	0.04	0.06	0.23
BASOPHILS (BASO)	G/I	813	0.02	0.02	0.00	0.09
LYMPHOCYTES (LYMPH)	G/I	813	4.61	1.20	2.62	7.26
MONOCYTES (MONO)	G/I	813	0.14	0.05	0.06	0.25
LARGE UNSTAINED CELLS (LUC)	G/I	813	0.05	0.03	0.01	0.12
THROMBOCYTES (PLATELETS)	G/I	813	885	107	700	1104
COAGULATION						
PROTHROMBIN TIME (PT)	rel. 1	823	0.79	0.07	0.68	0.94
PARTIAL THROMBOPLASTIN TIME (PTT)	sec	822	20.1	4.0	13.3	31.2

REFERENCE VALUES - HEMATOLOGY

STRAIN: RAT / HanRcc: WI ST (FEMALES)

AGE: FROM 19 TO 40 WEEKS

DATA COLLECTION PERIOD: 29-JAN-02 TO 23-AUG-05

PARAMETER	UNIT	N	MEAN	STAND. DEV	95% TOLERANCE LIMITS	
ERYTHROCYTES (RBC)	T/I	827	8.03	0.40	7.22	8.79
HEMOGLOBIN (HB)	mmol /l	827	9.6	0.4	8.8	10.4
HEMATOCRIT (HCT)	rel. 1	827	0.44	0.02	0.40	0.47
MEAN CELL VOLUME (MCV)	fl	827	54.5	2.2	50.3	58.9
RED CELL VOL. DISTR. WIDTH (RDW)	rel. 1	795	0.125	0.030	0.105	0.221
MEAN CELL HEMOGLOBIN (MCH)	fmol	827	1.19	0.05	1.10	1.29
MEAN CELL HEMOGLOBIN CONC. (MCHC)	mmol /l	827	21.89	0.77	20.48	23.43
HEMOGLOBIN CONC. DISTR. WIDTH	mmol /l	795	1.39	0.14	1.14	1.68
RETICULOCYTE COUNT						
RETICULOCYTE (REL)	rel. 1	827	0.024	0.006	0.014	0.035
RETICULOCYTE (ABS)	G/l	827	192	44	114	276
MATURITY INDEX (L-RETI)	rel. 1	795	0.516	0.132	0.317	0.785
MATURITY INDEX (M-RETI)	rel. 1	795	0.316	0.052	0.198	0.408
MATURITY INDEX (H-RETI)	rel. 1	795	0.167	0.128	0.010	0.421
LEUKOCYTES, TOTAL (WBC)	G/l	827	3.68	1.09	1.93	6.09
DIFF. WBC COUNT (REL)						
NEUTROPHILS (NEUT)	rel. 1	30	0.190	0.046	---	---
EOSINOPHILS (EOS)	rel. 1	827	0.021	0.011	0.009	0.046
BASOPHILS (BASO)	rel. 1	827	0.003	0.003	0.000	0.012
LYMPHOCYTES (LYMPH)	rel. 1	827	0.759	0.069	0.598	0.861
MONOCYTES (MONO)	rel. 1	827	0.020	0.007	0.010	0.036
LARGE UNSTAINED CELLS (LUC)	rel. 1	827	0.007	0.004	0.002	0.017
DIFF. WBC COUNT (ABS)						
NEUTROPHILS (NEUT)	G/l	30	0.70	0.28	---	---
EOSINOPHILS (EOS)	G/l	827	0.08	0.04	0.03	0.16
BASOPHILS (BASO)	G/l	827	0.01	0.01	0.00	0.05
LYMPHOCYTES (LYMPH)	G/l	827	2.81	0.91	1.30	4.83
MONOCYTES (MONO)	G/l	827	0.07	0.03	0.03	0.15
LARGE UNSTAINED CELLS (LUC)	G/l	827	0.03	0.02	0.01	0.08
THROMBOCYTES (PLATELETS)	G/l	827	948	126	730	1210
METHEMOGLOBIN (MET-HB)	rel. 1	232	0.008	0.002	0.001	0.012
COAGULATION						
PROTHROMBIN TIME (PT)	rel. 1	835	0.83	0.08	0.69	1.00
PARTIAL THROMBOPLASTIN TIME (PTT)	sec	825	19.9	4.8	12.5	32.7

REFERENCE VALUES - CLINICAL BIOCHEMISTRY

STRAIN: RAT / HanRcc: WIST (MALES)

AGE: FROM 19 TO 40 WEEKS

DATA COLLECTION PERIOD: 29-JAN-02 TO 23-AUG-05

PARAMETER	UNIT	N	MEAN	STAND. DEV	95% TOLERANCE LIMITS	
GLUCOSE	mmol /l	826	5.68	1.20	3.83	8.68
UREA	mmol /l	825	5.36	0.78	4.04	7.03
CREATININE	μmol /l	825	27.1	2.9	21.9	33.4
BILIRUBIN, TOTAL	μmol /l	825	1.66	0.34	1.02	2.37
CHOLESTEROL, TOTAL	mmol /l	825	1.84	0.37	1.19	2.67
TRIGLYCERIDES	mmol /l	825	0.50	0.27	0.19	1.17
PHOSPHOLIPIDS	mmol /l	813	1.57	0.25	1.11	2.10
ASPARTATE AMINOTRANSFERASE (ASAT)	U/l	826	75.2	10.5	58.6	100.1
ALANINE AMINOTRANSFERASE (ALAT)	U/l	826	33.1	6.4	22.2	48.2
LACTATE DEHYDROGENASE (LDH)	U/l	706	151.0	67.7	80.3	335.3
ALKALINE PHOSPHATASE (ALP)	U/l	825	58.4	13.2	37.5	87.1
GAMMA-GLUTAMYLTRANSFERASE (GGT)	U/l	803	0.0	0.2	0.0	0.0
CREATINE KINASE (CK)	U/l	773	147.0	92.3	78.8	343.8
SODIUM	mmol /l	826	143.6	3.8	136.7	155.1
POTASSIUM	mmol /l	826	3.79	0.33	3.23	4.54
CHLORIDE	mmol /l	826	104.2	2.9	99.1	109.3
CALCIUM	mmol /l	826	2.76	0.11	2.55	2.94
PHOSPHORUS	mmol /l	825	1.75	0.19	1.37	2.14
PROTEIN, TOTAL	g/l	825	67.33	2.75	61.87	72.93
ALBUMIN	g/l	670	41.89	1.79	38.36	45.53
GLOBULIN	g/l	670	25.41	2.27	21.13	29.98
A/G RATIO	-	670	1.66	0.17	1.35	2.05

REFERENCE VALUES - CLINICAL BIOCHEMISTRY

STRAIN: RAT / HanRcc: WIST (FEMALES)

AGE: FROM 19 TO 40 WEEKS

DATA COLLECTION PERIOD: 29-JAN-02 TO 23-AUG-05

PARAMETER	UNIT	N	MEAN	STAND. DEV	95% TOLERANCE LIMITS	
GLUCOSE	mmol /l	841	5.37	1.04	3.70	7.61
UREA	mmol /l	841	6.73	0.93	5.17	8.73
CREATININE	μmol /l	841	31.5	3.9	24.6	39.9
BILIRUBIN, TOTAL	μmol /l	841	2.11	0.56	1.22	3.43
CHOLESTEROL, TOTAL	mmol /l	841	1.65	0.44	0.91	2.57
TRIGLYCERIDES	mmol /l	841	0.34	0.12	0.18	0.61
PHOSPHOLIPIDS	mmol /l	829	1.75	0.39	1.09	2.62
ASPARTATE AMINOTRANSFERASE (ASAT)	U/l	841	80.0	28.5	56.1	154.9
ALANINE AMINOTRANSFERASE (ALAT)	U/l	841	30.2	14.0	16.6	68.4
LACTATE DEHYDROGENASE (LDH)	U/l	711	153.0	81.8	78.7	348.8
ALKALINE PHOSPHATASE (ALP)	U/l	840	20.8	5.9	11.9	35.1
GAMMA-GLUTAMYLTRANSFERASE (GGT)	U/l	808	0.0	0.2	0.0	0.0
CREATINE KINASE (CK)	U/l	789	131.7	80.6	68.3	325.0
SODIUM	mmol /l	841	142.5	2.8	136.7	147.6
POTASSIUM	mmol /l	841	3.33	0.35	2.74	3.98
CHLORIDE	mmol /l	841	105.2	2.7	99.8	110.8
CALCIUM	mmol /l	841	2.77	0.11	2.56	2.98
PHOSPHORUS	mmol /l	841	1.38	0.25	0.86	1.84
PROTEIN, TOTAL	g/l	841	71.01	3.84	63.68	78.79
ALBUMIN	g/l	680	48.78	3.14	42.78	54.92
GLOBULIN	g/l	680	22.34	2.24	18.41	26.99
A/G RATIO	-	680	2.21	0.26	1.77	2.81

REFERENCE VALUES - URINALYSIS

STRAIN: RAT / HanRcc: WIST (MALES)

AGE: FROM 19 TO 40 WEEKS

DATA COLLECTION PERIOD: 29-JAN-02 TO 23-AUG-05

PARAMETER	UNIT	N	MEAN	STAND. DEV	95% TOLERANCE LIMITS	
VOLUME/18h	ml	814	7.3	3.2	2.2	14.0
RELATIVE DENSITY	rel. 1	30	1.032	0.012	---	---
pH	-	814	6.6	0.5	6.0	7.0
PROTEIN	g/l	814	0.34	0.24	0.00	0.75
GLUCOSE	mmol /l	814	0	0	0	0
KETONES	mmol /l	814	0.6	0.6	0.0	1.5
UROBILINOGEN	μmol /l	814	0	2	0	0
BILIRUBIN	μmol /l	814	1	4	0	17
ERYTHROCYTES	per μl	814	12	19	0	25
LEUCOCYTES	per μl	814	31	47	0	100

REFERENCE VALUES - URINALYSIS

STRAIN: RAT / HanRcc: WIST (FEMALES)

AGE: FROM 19 TO 40 WEEKS

DATA COLLECTION PERIOD: 29-JAN-02 TO 23-AUG-05

PARAMETER	UNIT	N	MEAN	STAND. DEV	95% TOLERANCE	LIMITS

VOLUME/18h	ml	830	5.8	3.9	1.5	16.2
RELATIVE DENSITY	rel. 1	30	1.031	0.016	---	---
pH	-	831	6.0	0.4	5.0	7.0
PROTEIN	g/l	831	0.23	0.18	0.00	0.75
GLUCOSE	mmol /l	831	0	0	0	0
KETONES	mmol /l	831	0.3	0.3	0.0	1.5
UROBILINOGEN	μmol /l	831	0	4	0	0
BILIRUBIN	μmol /l	831	2	5	0	17
ERYTHROCYTES	per μl	831	3	14	0	25
LEUCOCYTES	per μl	831	7	23	0	25

APPENDIX V

Histopathology

ProPath GmbH

Pathology Report

Lipase produced with *Trichoderma reesei*:

90-Day Oral (Gavage) Toxicity Study

in the Wistar Rat

Harlan Study Number: D80691

This report contains 101 pages

Dr. J.Th. Wilson
Muttenerstr. 30
Pratteln 4133
Switzerland

tel: +41 61 811 72 13
jwilson@propath.ch

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

Harlan Project no. : D80691
Propath no. : 14001
Date : 29.Apr.2014

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Propath no. : 14001
Date : 29.Apr.2014

Statement of Compliance

The undersigned hereby declares, that the histopathology data in this report were compiled by him, and that they reflect accurately the primary data records. This report, consisting of 101 pages, was created by the computer system of Propath GmbH.

This study phase was conducted in compliance with:

Swiss Ordinance relating to Good Laboratory Practice, adopted May 18th, 2005 [SR 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

Date: 29.04.2014

Propath GmbH
CH-4133 Pratteln, Switzerland

PATHOLOGY REPORT

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Date : 29.Apr.2014

Summary

Pathomorphologic examination was performed on 80 Wistar Rats (40 males, 40 females) which had been subjected to a 90-day oral (gavage) toxicity study with the test item **Lipase produced with *Trichoderma reesei***. The In-Life part of this study was conducted at Harlan Laboratories Ltd., Itingen, Switzerland during September - December 2013.

The rats were assigned to four dose groups each containing ten animals of each sex. The test item was administered by oral gavage at doses of 50, 200 and 1000 mg/kg bw/day (dose groups 2, 3 and 4 respectively). The rats of the control group 1 (0 mg/kg bw/day) received the vehicle, bidistilled water.

All rats were necropsied. Histopathologic examination was performed on an extensive list of organs and tissues from all group 1 and 4 rats.

There were no unscheduled deaths.

There were no treatment related macroscopic or microscopic findings.

The NOAEL may be considered as 1000 mg/kg bw/day.

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
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 Propath no. : 14001
 Date : 29.Apr.2014

Materials and Methods*Study Design for Histopathology Evaluation*

Dose group	Dose mg/kg bw/day Lipase produced with <i>Trichoderma reesei</i>	Number of rats		Animal numbers	
		males	females	males	females
1	0	10	10	1 - 10	41 - 50
2	50	10	10	11 - 20	51 - 60
3	200	10	10	21 - 30	61 - 70
4	1000	10	10	31 - 40	71 - 80

Administration of the Test Item

The test item was administered once daily by gavage for 92 or 93 days. Rats of the control group 1 (0 mg/kg bw/day) received the vehicle, bidistilled water.

Necropsy and Histopathology

At the end of the assigned study period, the rats were killed by exsanguination following anesthesia by an intraperitoneal injection of pentobarbitone. Complete necropsies were performed on all rats. The terminal body weight and the weights of the adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus, and uterus including oviducts, cervix and vagina were recorded at necropsy. Paired organs were weighed separately. Body and organ weights are reported in the main toxicology report.

Representative tissue samples of the following organs from all animals were preserved in 4% phosphate buffered neutral formaldehyde solution (10% formalin). Eyes with optic nerve and Harderian gland were initially fixed in Davidson's solution and testes and epididymides in modified Davidson's solution.

Adrenal glands (2), aorta (1), [bone - sternum and femur including joint], bone marrow - sternal (1), brain - medulla oblongata, pons, cerebellum and cerebrum (4); eyes (2) with optic nerve (2) [and Harderian gland]; epididymides (2), esophagus (1), heart (1), kidneys (2), [lacrima glands - exorbital], large intestine - cecum, colon and rectum (3);

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Materials and Methods

larynx (1), liver (2), lungs (2), lymph nodes – mesenteric (1) and mandibular (1); mammary gland area (1), [nasal cavity], [pharynx], ovaries (2), oviducts (2), pancreas (1), pituitary gland (1), prostate gland (1), salivary glands – mandibular (2) and sublingual (2); sciatic nerve (2), seminal vesicles (2) with coagulating glands [skeletal muscle], skin (1), small intestine - duodenum, jejunum and ileum (3) with Peyer's patches (2); spinal cord - cervical, thoracic and lumbar (3); spleen (1), stomach (2); testes (2), thymus (2), thyroid glands (2) with parathyroid glands (2), [tongue], trachea (1), [ureters], urinary bladder (1), uterus with uterine cervix (3), vagina (1) and all organs or tissues with macroscopic abnormalities.

Organs listed in brackets were fixed but not further processed. Following fixation, the above listed organs from all group 1 and 4 main study animals, were trimmed, processed and embedded in paraffin wax. Sections (numbers given in parentheses) were cut at a thickness of 2-4 micrometers and stained with hematoxylin and eosin.

The sections were examined by light microscopy in February 2014.

Data Compilation

The animal data and macroscopic findings were electronically transferred from the necropsy raw data files of Harlan Laboratories Ltd., Itingen, Switzerland into the computer system of Propath GmbH where the microscopic findings were recorded by the undersigned pathologist using on-line input.

Macroscopic findings are presented in summary in Table 1 - Incidence Macroscopic Findings and in full descriptive terms in Table 5 - Individual Animal Data Records. Wherever possible, macroscopic findings were correlated with a microscopic finding.

Microscopic findings are listed for each animal along with severity grades in Table 3 - Individual Animal Microscopic Findings and summarized in Table 2 - Incidence All Microscopic Findings. They are further given in full descriptive terms in Table 5 - Individual Animal Data Records. Histologic changes were described according to their distribution and morphologic character and were graded for severity on a scale of 1 – 5 (see key Table 3 - Individual Animal Microscopic Findings).

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Test item : Lipase produced with *Trichoderma reesei*
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Date : 29.Apr.2014

Materials and Methods*Archiving*

The original final pathology report, all slides, dispatch list(s) and raw data provided will be sent to the test facility.

Peer Review

A peer review was conducted at the Test Facility by: Wendy Henderson BVM&S MRCVS, Global Head of Pathology, Harlan Laboratories Ltd. Any diagnostic discrepancies were resolved by discussion, and the diagnoses presented in this report represent the consensus opinion.

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
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Date : 29.Apr.2014

Results*Mortality*

There were no unscheduled deaths.

Macroscopic Findings

The few macroscopic findings were unremarkable.

Microscopic Findings

There were no treatment related morphological alterations. All recorded microscopic findings were within the range of background pathology encountered in Wistar rats of this age and occurred at similar incidences and severity in both control and treated rats.

PATHOLOGY REPORT

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Sponsor : AB Enzymes

Harlan Project no. : D80691
Propath no. : 14001
Date : 29.Apr.2014

Conclusions

The administration of **Lipase produced with *Trichoderma reesei*** by once daily oral gavage to Wistar rats at doses up to 1000 mg/kg bw for 92 or 93 days, did not result in any findings indicative of toxicity.

The NOAEL may be considered as 1000 mg/kg bw/day.

PATHOLOGY REPORT

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 Date : 29.Apr.2014

Table 1 Incidence Macroscopic Findings

	SEX: MALE				SEX: FEMALE			
	1	2	3	4	1	2	3	4
DOSE GROUP: number of animals	10	10	10	10	10	10	10	10
Lymph nodes								
Discoloration mandibular	0	0	0	0	1	0	0	0
Eyes								
Desiccated vitreous humor	0	0	0	0	0	0	1	0

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Test item : Lipase produced with *Trichoderma reesei*
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Table 2 Incidence All Microscopic Findings

	SEX: DOSE GROUP: number of animals	MALE				FEMALE			
		1	2	3	4	1	2	3	4
		10	10	10	10	10	10	10	10
Brain - cerebrum									
number examined		10			10	10			10
Brain - midbrain									
number examined		10			10	10			10
Brain - cerebellum									
number examined		10			10	10			10
Brain - pons									
number examined		10			10	10			10
Spinal Cord - cervical									
number examined		9			10	10			10
Spinal Cord - midthoracic									
number examined		10			10	10			10
Spinal Cord - lumbar									
number examined		10			10	10			10
Sciatic Nerve									
number examined		10			10	10			10
Axonal fragmentation		0			2	0			0
Heart									
number examined		10			10	10			10
Cardiomyopathy		1			2	0			0

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 Sponsor : AB Enzymes

Harlan Project no. : D80691
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Table 2 Incidence All Microscopic Findings

	SEX: DOSE GROUP: number of animals	MALE				FEMALE			
		1	2	3	4	1	2	3	4
		10	10	10	10	10	10	10	10
Aorta									
number examined		10			10	10			10
Larynx									
number examined		10			10	10			10
Inflammatory infiltrate, lymphoid		4			2	2			2
Trachea									
number examined		10			10	10			10
Lungs									
number examined		10			10	10			10
Vascular mineralisation, focal		6			5	2			1
Peri- vascular/bronchial, inflammatory cell foci		1			1	1			0
Alveolar inflammation, lymphocytic		0			1	0			0
Microgranuloma		0			1	0			0
Osseous metaplasia		1			1	3			1
Lymphoid hyperplasia (BALT)		0			1	2			1
Esophagus									
number examined		10			10	10			10
Myodegeneration, focal		1			1	1			1

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Test item : Lipase produced with *Trichoderma reesei*
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 Sponsor : AB Enzymes

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Table 2 Incidence All Microscopic Findings

	SEX: DOSE GROUP: number of animals	MALE				FEMALE			
		1	2	3	4	1	2	3	4
		10	10	10	10	10	10	10	10
Stomach									
number examined		10			10	10			10
Glandular inflammatory infiltrate, granulolymphocy		2			0	0			0
Duodenum									
number examined		10			10	10			10
Jejunum									
number examined		10			10	10			10
Ileum									
number examined		10			10	10			10
Peyer's Patches (GALT)									
number examined		9			10	10			8
Cecum									
number examined		10			10	10			10
Colon									
number examined		9			9	9			9
Rectum									
number examined		10			9	10			10
Liver									
number examined		10			10	10			10
Inflammatory infiltrate, lymphoid		10			9	5			8

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Table 2 Incidence All Microscopic Findings

	SEX: DOSE GROUP: number of animals	MALE				FEMALE			
		1	2	3	4	1	2	3	4
		10	10	10	10	10	10	10	10
Liver									
Hepatocellular pigment, yellow-brown		0			1	4			3
Hepatocellular vacuolation		1			0	0			1
Pancreas									
number examined		10			10	10			10
Reduced zymogen, acinar cell basophilia		1			0	0			0
Inflammatory infiltrate, granulolymphocytic		0			0	0			1
Exocrine hypertrophy, focal		0			1	0			0
Exocrine atrophy, focal		1			0	0			0
Kidneys									
number examined		10			10	10			10
Pelvic dilation		0			0	1			1
Pelvic/papillary mineralization		0			0	3			0
Tubular mineralisation		1			0	2			6
Inflammatory infiltrate, lymphoid		0			1	0			0
Hyaline cast(s)		0			0	0			1
Tubular basophilia, corticomedullary		1			0	0			1

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Table 2 Incidence All Microscopic Findings

	SEX: DOSE GROUP: number of animals	MALE				FEMALE			
		1	2	3	4	1	2	3	4
		10	10	10	10	10	10	10	10
Urinary Bladder									
number examined		10			10	10			10
Testes									
number examined		10			10				
Seminiferous cell debris, intratubular		0			1				
Epididymides									
number examined		10			10				
Inflammatory infiltrate, lymphoid		1			0				
Prostate Gland									
number examined		10			10				
Seminal Vesicles									
number examined		10			10				
Coagulating Glands									
number examined		10			10				
Ovaries									
number examined						10			10
Corpora hemorrhagica						0			1
Oviducts									
number examined						10			10

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Table 2 Incidence All Microscopic Findings

	SEX:	MALE				FEMALE			
	DOSE GROUP:	1	2	3	4	1	2	3	4
	number of animals	10	10	10	10	10	10	10	10
Uterus									
number examined						10			10
Uterus - cervix									
number examined						10			10
Vagina									
number examined						10			10
Proestrus epithelium						2			1
Estrus epithelium						1			0
Metestrus epithelium						1			5
Diestrus epithelium						5			3
Epithelial mucification						1			1
Pituitary Gland									
number examined		10			10	10			10
Microcyst		0			2	0			2
Thyroid Glands									
number examined		10			10	10			10
Inflammatory infiltrate, lymphoid		0			1	0			0
Follicular hypertrophy/hyperplasia, diffuse		1			1	0			0

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Table 2 Incidence All Microscopic Findings

	SEX: DOSE GROUP: number of animals	MALE				FEMALE			
		1	2	3	4	1	2	3	4
		10	10	10	10	10	10	10	10
Parathyroid Glands									
number examined		9			10	10			10
Adrenal Glands									
number examined		10			10	10			10
Extracapsular nodule		1			0	0			2
Inflammatory infiltrate, lymphoid		0			0	4			3
Vacuolation, multifocal in z. fasciculata		0			1	0			0
Hypertrophy, cortical diffuse		0			0	1			0
Spleen									
number examined		10			10	10			10
Hemosiderin pigment		9			10	10			10
Hemopoietic foci, primarily erythroid		7			4	8			7
Bone Marrow - sternal									
number examined		10			10	10			10
Adipocytes		10			10	9			8
Thymus									
number examined		10			10	10			10
Lymphoid atrophy - involution		5			5	6			7

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Table 2 Incidence All Microscopic Findings

	SEX: DOSE GROUP: number of animals	MALE				FEMALE			
		1	2	3	4	1	2	3	4
		10	10	10	10	10	10	10	10
Mesenteric Lymph Node									
number examined		10			10	10			10
Macrophage foci		5			4	4			4
Lymphoid hyperplasia		0			0	1			0
Mandibular Lymph Node									
number examined		10			10	8			9
Congestion/erythrophagocytosis		5			2	3			2
Plasmacytosis		9			6	5			9
Lymphoid hyperplasia		3			1	0			1
Sublingual Salivary Glands									
number examined		10			10	10			10
Acinar atrophy, diffuse		1			0	0			0
Submandibular Salivary Glands									
number examined		10			10	10			10
Mammary Gland Area									
number examined		10			10	10			10
Skin									
number examined		10			10	10			10

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Table 2 Incidence All Microscopic Findings

	SEX: DOSE GROUP: number of animals	MALE				FEMALE			
		1	2	3	4	1	2	3	4
		10	10	10	10	10	10	10	10
Eyes									
number examined		10			10	10		1	10
Retinal rosette(s) - dysplasia		0			0	1		0	2
Optic Nerves									
number examined		10			10	10			10

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Table 3 Individual Animal Microscopic Findings

Codes and symbols in table heading:

m Males

f Females

Codes and symbols in animal lines:

p Planned terminal

Grading system used in finding lines:

0 finding not present

1 minimal

2 slight

3 moderate

4 severe

5 very severe

x present

Only organs/groups **with findings** are listed in the table

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Table 3 Individual Animal Microscopic Findings*Group 1 Males*

ANIMAL NUMBER	1	2	3	4	5	6	7	8	9	10
SEX	m	m	m	m	m	m	m	m	m	m
necropsy status	p	p	p	p	p	p	p	p	p	p
Heart										
Cardiomyopathy	0	0	1	0	0	0	0	0	0	0
Larynx										
Inflammatory infiltrate, lymphoid	2	0	0	1	0	2	0	2	0	0
Lungs										
Vascular mineralisation, focal	1	0	1	0	2	1	1	2	0	0
Peri- vascular/bronchial, inflammatory cell foci	0	0	1	0	0	0	0	0	0	0
Osseous metaplasia	0	0	0	0	1	0	0	0	0	0
Esophagus										
Myodegeneration, focal	1	0	0	0	0	0	0	0	0	0
Stomach										
Glandular inflammatory infiltrate, granulolymphocy	1	1	0	0	0	0	0	0	0	0
Liver										
Inflammatory infiltrate, lymphoid	2	1	2	2	1	1	1	1	2	1
Hepatocellular vacuolation	0	0	0	0	1	0	0	0	0	0
Pancreas										
Reduced zymogen, acinar cell basophilia	0	0	0	0	0	0	0	2	0	0
Exocrine atrophy, focal	0	0	2	0	0	0	0	0	0	0

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Table 3 Individual Animal Microscopic Findings*Group 1 Males*

ANIMAL NUMBER	1	2	3	4	5	6	7	8	9	10
SEX	m	m	m	m	m	m	m	m	m	m
necropsy status	p	p	p	p	p	p	p	p	p	p
Kidneys										
Tubular mineralisation	0	0	0	0	0	1	0	0	0	0
Tubular basophilia, corticomedullary	0	0	0	0	0	0	1	0	0	0
Epididymides										
Inflammatory infiltrate, lymphoid	1	0	0	0	0	0	0	0	0	0
Thyroid Glands										
Follicular hypertrophy/hyperplasia, diffuse	1	0	0	0	0	0	0	0	0	0
Adrenal Glands										
Extracapsular nodule	0	0	0	0	0	x	0	0	0	0
Spleen										
Hemosiderin pigment	0	2	2	1	2	2	2	2	2	2
Hemopoietic foci, primarily erythroid	0	1	1	0	1	1	1	2	0	1
Bone Marrow - sternal										
Adipocytes	3	2	3	2	3	2	2	2	2	2
Thymus										
Lymphoid atrophy - involution	0	1	0	1	2	1	0	2	0	0
Mesenteric Lymph Node										
Macrophage foci	1	0	1	1	1	0	1	0	0	0

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Table 3 Individual Animal Microscopic Findings*Group 1 Males*

ANIMAL NUMBER	1	2	3	4	5	6	7	8	9	10
SEX	m	m	m	m	m	m	m	m	m	m
necropsy status	p	p	p	p	p	p	p	p	p	p
Mandibular Lymph Node										
Congestion/erythrophagocytosis	0	1	0	0	0	2	3	2	0	2
Plasmacytosis	2	1	2	1	0	1	1	1	1	1
Lymphoid hyperplasia	1	0	0	2	0	0	0	1	0	0
Sublingual Salivary Glands										
Acinar atrophy, diffuse	0	0	0	0	0	0	3	0	0	0

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Table 3 Individual Animal Microscopic Findings*Group 4 Males*

ANIMAL NUMBER	31	32	33	34	35	36	37	38	39	40
SEX	m	m	m	m	m	m	m	m	m	m
necropsy status	p	p	p	p	p	p	p	p	p	p
Sciatic Nerve										
Axonal fragmentation	1	0	1	0	0	0	0	0	0	0
Heart										
Cardiomyopathy	0	0	2	0	0	0	0	1	0	0
Larynx										
Inflammatory infiltrate, lymphoid	2	0	0	0	0	1	0	0	0	0
Lungs										
Vascular mineralisation, focal	1	1	0	1	0	0	0	1	0	1
Peri- vascular/bronchial, inflammatory cell foci	0	0	0	1	0	0	0	0	0	0
Alveolar inflammation, lymphocytic	0	0	1	0	0	0	0	0	0	0
Microgranuloma	0	0	0	1	0	0	0	0	0	0
Osseous metaplasia	1	0	0	0	0	0	0	0	0	0
Lymphoid hyperplasia (BALT)	0	0	0	0	0	0	0	0	0	1
Esophagus										
Myodegeneration, focal	2	0	0	0	0	0	0	0	0	0
Liver										
Inflammatory infiltrate, lymphoid	2	2	1	1	2	2	1	1	0	2
Hepatocellular pigment, yellow-brown	0	2	0	0	0	0	0	0	0	0

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Table 3 Individual Animal Microscopic Findings*Group 4 Males*

ANIMAL NUMBER	31	32	33	34	35	36	37	38	39	40
SEX	m	m	m	m	m	m	m	m	m	m
necropsy status	p	p	p	p	p	p	p	p	p	p
Pancreas										
Exocrine hypertrophy, focal	0	0	2	0	0	0	0	0	0	0
Kidneys										
Inflammatory infiltrate, lymphoid	0	0	0	0	0	0	0	0	1	0
Testes										
Seminiferous cell debris, intratubular	0	0	0	0	0	0	1	0	0	0
Pituitary Gland										
Microcyst	x	0	0	0	0	x	0	0	0	0
Thyroid Glands										
Inflammatory infiltrate, lymphoid	0	0	0	0	1	0	0	0	0	0
Follicular hypertrophy/hyperplasia, diffuse	0	0	0	0	0	0	0	0	0	1
Adrenal Glands										
Vacuolation, multifocal in z. fasiculata	0	1	0	0	0	0	0	0	0	0
Spleen										
Hemosiderin pigment	2	2	2	2	1	2	1	2	2	2
Hemopoietic foci, primarily erythroid	0	1	1	0	0	1	1	0	0	0

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Table 3 Individual Animal Microscopic Findings*Group 1 Females*

ANIMAL NUMBER	41	42	43	44	45	46	47	48	49	50
SEX	f	f	f	f	f	f	f	f	f	f
necropsy status	p	p	p	p	p	p	p	p	p	p
Larynx										
Inflammatory infiltrate, lymphoid	0	0	0	0	1	0	0	2	0	0
Lungs										
Vascular mineralisation, focal	1	0	0	0	0	0	1	0	0	0
Peri- vascular/bronchial, inflammatory cell foci	0	0	0	0	0	1	0	0	0	0
Osseous metaplasia	0	0	0	0	1	0	0	0	1	1
Lymphoid hyperplasia (BALT)	2	0	0	0	0	1	0	0	0	0
Esophagus										
Myodegeneration, focal	0	0	0	0	0	0	0	0	0	2
Liver										
Inflammatory infiltrate, lymphoid	1	0	1	0	0	0	0	1	1	1
Hepatocellular pigment, yellow-brown	0	0	0	1	0	1	0	0	3	3
Kidneys										
Pelvic dilation	0	0	0	0	x	0	0	0	0	0
Pelvic/papillary mineralization	0	1	0	0	0	0	0	1	1	0
Tubular mineralisation	0	2	0	0	0	0	0	0	2	0

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Table 3 Individual Animal Microscopic Findings*Group 1 Females*

ANIMAL NUMBER	41	42	43	44	45	46	47	48	49	50
SEX	f	f	f	f	f	f	f	f	f	f
necropsy status	p	p	p	p	p	p	p	p	p	p
Vagina										
Proestrus epithelium	0	0	0	0	x	0	0	0	0	x
Estrus epithelium	0	0	0	x	0	0	0	0	0	0
Metestrus epithelium	x	0	0	0	0	0	0	0	0	0
Diestrus epithelium	0	x	0	0	0	x	x	x	x	0
Epithelial mucification	0	0	2	0	0	0	0	0	0	0
Adrenal Glands										
Inflammatory infiltrate, lymphoid	0	0	0	0	1	1	1	0	1	0
Hypertrophy, cortical diffuse	0	0	2	0	0	0	0	0	0	0
Spleen										
Hemosiderin pigment	2	3	2	1	2	2	3	2	2	2
Hemopoietic foci, primarily erythroid	2	1	1	1	1	0	3	1	1	0
Bone Marrow - sternal										
Adipocytes	1	1	1	1	2	0	1	1	2	2
Thymus										
Lymphoid atrophy - involution	1	0	0	0	0	1	2	2	2	2
Mesenteric Lymph Node										
Macrophage foci	0	1	0	1	1	0	0	0	0	2
Lymphoid hyperplasia	0	0	0	1	0	0	0	0	0	0

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Table 3 Individual Animal Microscopic Findings*Group 1 Females*

ANIMAL NUMBER	41	42	43	44	45	46	47	48	49	50
SEX	f	f	f	f	f	f	f	f	f	f
necropsy status	p	p	p	p	p	p	p	p	p	p
Mandibular Lymph Node										
Congestion/erythrophagocytosis	1	0	2	0	-	0	0	2	-	0
Plasmacytosis	1	2	0	0	-	2	2	1	-	0
Eyes										
Retinal rosette(s) - dysplasia	0	2	0	0	0	0	0	0	0	0

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Table 3 Individual Animal Microscopic Findings

Group 4 Females

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Table 3 Individual Animal Microscopic Findings*Group 4 Females*

ANIMAL NUMBER	71	72	73	74	75	76	77	78	79	80
SEX	f	f	f	f	f	f	f	f	f	f
necropsy status	p	p	p	p	p	p	p	p	p	p
Kidneys										
Tubular basophilia, corticomedullary	0	0	0	0	1	0	0	0	0	0
Ovaries										
Corpora hemorrhagica	0	0	0	0	0	0	0	0	3	0
Vagina										
Proestrus epithelium	0	0	0	x	0	0	0	0	0	0
Metestrus epithelium	0	x	0	0	x	0	x	x	0	x
Diestrus epithelium	x	0	0	0	0	x	0	0	x	0
Epithelial mucification	0	0	3	0	0	0	0	0	0	0
Pituitary Gland										
Microcyst	x	0	0	0	0	0	0	x	0	0
Adrenal Glands										
Extracapsular nodule	0	0	0	x	0	x	0	0	0	0
Inflammatory infiltrate, lymphoid	0	0	2	2	0	1	0	0	0	0
Spleen										
Hemosiderin pigment	2	2	3	2	2	2	2	2	2	1
Hemopoietic foci, primarily erythroid	0	1	2	2	1	1	1	0	1	0
Bone Marrow - sternal										
Adipocytes	2	2	0	0	1	3	2	2	1	3

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Table 3 Individual Animal Microscopic Findings*Group 4 Females*

ANIMAL NUMBER	71	72	73	74	75	76	77	78	79	80
SEX	f	f	f	f	f	f	f	f	f	f
necropsy status	p	p	p	p	p	p	p	p	p	p
Thymus										
Lymphoid atrophy - involution	1	1	0	0	1	1	2	1	0	1
Mesenteric Lymph Node										
Macrophage foci	0	1	0	1	0	0	0	1	1	0
Mandibular Lymph Node										
Congestion/erythrophagocytosis	0	0	-	0	0	0	1	0	2	0
Plasmacytosis	1	1	-	2	1	1	2	1	1	1
Lymphoid hyperplasia	0	0	-	0	0	0	2	0	0	0
Eyes										
Retinal rosette(s) - dysplasia	0	0	0	0	2	0	1	0	0	0

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 Date : 29.Apr.2014

Table 4 Animal Data List**Males**

ANIMAL NUMBER	SEX M/F	FINAL STATE	TEST DAYS	FIRST DAY	LAST DAY	DATE NECROPSY
DOSE GROUP 1						
1	m	p	93	16/09/13	17/12/13	18/12/13
2	m	p	93	16/09/13	17/12/13	18/12/13
3	m	p	93	16/09/13	17/12/13	18/12/13
4	m	p	93	16/09/13	17/12/13	18/12/13
5	m	p	93	16/09/13	17/12/13	18/12/13
6	m	p	93	16/09/13	17/12/13	18/12/13
7	m	p	93	16/09/13	17/12/13	18/12/13
8	m	p	93	16/09/13	17/12/13	18/12/13
9	m	p	93	16/09/13	17/12/13	18/12/13
10	m	p	93	16/09/13	17/12/13	18/12/13
DOSE GROUP 2						
11	m	p	93	16/09/13	17/12/13	18/12/13
12	m	p	93	16/09/13	17/12/13	18/12/13
13	m	p	93	16/09/13	17/12/13	18/12/13
14	m	p	93	16/09/13	17/12/13	18/12/13
15	m	p	93	16/09/13	17/12/13	18/12/13
16	m	p	93	16/09/13	17/12/13	18/12/13
17	m	p	93	16/09/13	17/12/13	18/12/13
18	m	p	93	16/09/13	17/12/13	18/12/13
19	m	p	93	16/09/13	17/12/13	18/12/13
20	m	p	93	16/09/13	17/12/13	18/12/13
DOSE GROUP 3						
21	m	p	93	16/09/13	17/12/13	18/12/13
22	m	p	93	16/09/13	17/12/13	18/12/13
23	m	p	93	16/09/13	17/12/13	18/12/13
24	m	p	93	16/09/13	17/12/13	18/12/13
25	m	p	93	16/09/13	17/12/13	18/12/13
26	m	p	93	16/09/13	17/12/13	18/12/13
27	m	p	93	16/09/13	17/12/13	18/12/13
28	m	p	93	16/09/13	17/12/13	18/12/13
29	m	p	93	16/09/13	17/12/13	18/12/13
30	m	p	93	16/09/13	17/12/13	18/12/13
DOSE GROUP 4						
31	m	p	93	16/09/13	17/12/13	18/12/13
32	m	p	93	16/09/13	17/12/13	18/12/13
33	m	p	93	16/09/13	17/12/13	18/12/13
34	m	p	93	16/09/13	17/12/13	18/12/13
35	m	p	93	16/09/13	17/12/13	18/12/13
36	m	p	93	16/09/13	17/12/13	18/12/13
37	m	p	93	16/09/13	17/12/13	18/12/13
38	m	p	93	16/09/13	17/12/13	18/12/13
39	m	p	93	16/09/13	17/12/13	18/12/13
40	m	p	93	16/09/13	17/12/13	18/12/13

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 4 Animal Data List**Females**

ANIMAL NUMBER	SEX M/F	FINAL STATE	TEST DAYS	FIRST DAY	LAST DAY	DATE NECROPSY
DOSE GROUP 1						
41	f	p	92	16/09/13	16/12/13	17/12/13
42	f	p	92	16/09/13	16/12/13	17/12/13
43	f	p	92	16/09/13	16/12/13	17/12/13
44	f	p	92	16/09/13	16/12/13	17/12/13
45	f	p	92	16/09/13	16/12/13	17/12/13
46	f	p	92	16/09/13	16/12/13	17/12/13
47	f	p	92	16/09/13	16/12/13	17/12/13
48	f	p	92	16/09/13	16/12/13	17/12/13
49	f	p	92	16/09/13	16/12/13	17/12/13
50	f	p	92	16/09/13	16/12/13	17/12/13
DOSE GROUP 2						
51	f	p	92	16/09/13	16/12/13	17/12/13
52	f	p	92	16/09/13	16/12/13	17/12/13
53	f	p	92	16/09/13	16/12/13	17/12/13
54	f	p	92	16/09/13	16/12/13	17/12/13
55	f	p	92	16/09/13	16/12/13	17/12/13
56	f	p	92	16/09/13	16/12/13	17/12/13
57	f	p	92	16/09/13	16/12/13	17/12/13
58	f	p	92	16/09/13	16/12/13	17/12/13
59	f	p	92	16/09/13	16/12/13	17/12/13
60	f	p	92	16/09/13	16/12/13	17/12/13
DOSE GROUP 3						
61	f	p	92	16/09/13	16/12/13	17/12/13
62	f	p	92	16/09/13	16/12/13	17/12/13
63	f	p	92	16/09/13	16/12/13	17/12/13
64	f	p	92	16/09/13	16/12/13	17/12/13
65	f	p	92	16/09/13	16/12/13	17/12/13
66	f	p	92	16/09/13	16/12/13	17/12/13
67	f	p	92	16/09/13	16/12/13	17/12/13
68	f	p	92	16/09/13	16/12/13	17/12/13
69	f	p	92	16/09/13	16/12/13	17/12/13
70	f	p	92	16/09/13	16/12/13	17/12/13
DOSE GROUP 4						
71	f	p	92	16/09/13	16/12/13	17/12/13
72	f	p	92	16/09/13	16/12/13	17/12/13
73	f	p	92	16/09/13	16/12/13	17/12/13
74	f	p	92	16/09/13	16/12/13	17/12/13
75	f	p	92	16/09/13	16/12/13	17/12/13
76	f	p	92	16/09/13	16/12/13	17/12/13
77	f	p	92	16/09/13	16/12/13	17/12/13
78	f	p	92	16/09/13	16/12/13	17/12/13
79	f	p	92	16/09/13	16/12/13	17/12/13
80	f	p	92	16/09/13	16/12/13	17/12/13

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 1
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal	Adipocytes (moderate)
Epididymis/1	Inflammatory infiltrate, lymphoid (minimal)
Esophagus	Myodegeneration, focal (minimal)
Larynx	Inflammatory infiltrate, lymphoid (slight)
Liver	Inflammatory infiltrate, lymphoid (slight)
Lungs	Vascular mineralisation, focal (minimal)
Mammary Gland Area	No mammary tissue in section
Mandibular Lymph Node	Plasmacytosis (slight) Lymphoid hyperplasia (minimal)
Mesenteric Lymph Node	Macrophage foci (minimal)
Stomach	Glandular inflammatory infiltrate, granulolymphocy (minimal)
Thyroid Gland/1	Follicular hypertrophy/hyperplasia, diffuse (minimal)

Number of Sections less than protocol for Optic Nerve/2 (0).

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 1
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/2, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Optic Nerve/1, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Spleen, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thymus, Thyroid Gland/2, Trachea, Urinary Bladder.

Animal No: 2
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal	Adipocytes (slight)
Liver	Inflammatory infiltrate, lymphoid (minimal)
Mandibular Lymph Node	Congestion/erythrophagocytosis (minimal) Plasmacytosis (minimal)
Spleen	Hemosiderin pigment (slight) Hemopoietic foci, primarily erythroid (minimal)
Stomach	Glandular inflammatory infiltrate, granulolymphocy (minimal)
Thymus	Lymphoid atrophy - involution (minimal)

Number of Sections less than protocol for Colon (0), Parathyroid Gland/1 (0).

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

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 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 2
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Lungs, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

Animal No: 3
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal	Adipocytes (moderate)
Heart	Cardiomyopathy (minimal)
Liver	Inflammatory infiltrate, lymphoid (slight)
Lungs	Vascular mineralisation, focal (minimal) Peri- vascular/bronchial, inflammatory cell foci (minimal)
Mandibular Lymph Node	Plasmacytosis (slight)
Mesenteric Lymph Node	Macrophage foci (minimal)
Pancreas	Exocrine atrophy, focal (slight)

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
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 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 3
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (minimal)

Sublingual Salivary Gland/2 Parotid rests

Number of Sections less than protocol for Optic Nerve/1 (0), Parathyroid Gland/1 (0), Parathyroid Gland/2 (0), Peyer's Patches (GALT) (0), Sublingual Salivary Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Mammary Gland Area, Optic Nerve/2, Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

Animal No: 4
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (slight)

Larynx Inflammatory infiltrate, lymphoid (minimal)

Liver Inflammatory infiltrate, lymphoid (slight)

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
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 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 4
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

Mandibular Lymph Node Plasmacytosis (minimal)
 Lymphoid hyperplasia (slight)

Mesenteric Lymph Node Macrophage foci (minimal)

Spleen Hemosiderin pigment (minimal)

Thymus Lymphoid atrophy - involution (minimal)

Number of Sections less than protocol for Parathyroid Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Lungs, Mammary Gland Area, Optic Nerve/1, Optic Nerve/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

Animal No: 5
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
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 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 5
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (moderate)
Liver Inflammatory infiltrate, lymphoid (minimal)
 Hepatocellular vacuolation (minimal)
Lungs Vascular mineralisation, focal (slight)
 Osseous metaplasia (minimal)
Mesenteric Lymph Node Macrophage foci (minimal)
Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (minimal)
Thymus Lymphoid atrophy - involution (slight)

Number of Sections less than protocol for Optic Nerve/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Mammary Gland Area, Mandibular Lymph Node, Optic Nerve/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 6
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Adrenal Gland/1	Extracapsular nodule present
Bone Marrow - sternal	Adipocytes (slight)
Kidney/1	Tubular mineralisation (minimal)
Kidney/2	Tubular mineralisation (minimal)
Larynx	Inflammatory infiltrate, lymphoid (slight)
Liver	Inflammatory infiltrate, lymphoid (minimal)
Lungs	Vascular mineralisation, focal (minimal)
Mandibular Lymph Node	Congestion/erythrophagocytosis (slight) Plasmacytosis (minimal)
Spleen	Hemosiderin pigment (slight) Hemopoietic foci, primarily erythroid (minimal)
Thymus	Lymphoid atrophy - involution (minimal)

Number of Sections less than protocol for Optic Nerve/1 (0), Parathyroid Gland/1 (0), Spinal Cord - cervical (0).

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 6
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

No abnormalities found in: Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

Animal No: 7
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal	Adipocytes (slight)
Kidney/2	Tubular basophilia, corticomedullary (minimal)
Liver	Inflammatory infiltrate, lymphoid (minimal)
Lungs	Vascular mineralisation, focal (minimal)
Mammary Gland Area	No mammary tissue in section
Mandibular Lymph Node	Congestion/erythrophagocytosis (moderate) Plasmacytosis (minimal)
Mesenteric Lymph Node	Macrophage foci (minimal)

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 7
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (minimal)

Sublingual Salivary Gland/1 Acinar atrophy, diffuse (moderate)

Number of Sections less than protocol for Parathyroid Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Larynx, Optic Nerve/1, Optic Nerve/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

Animal No: 8
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (slight)

Larynx Inflammatory infiltrate, lymphoid (slight)

Liver Inflammatory infiltrate, lymphoid (minimal)

Lungs Vascular mineralisation, focal (slight)

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 8
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

Mammary Gland Area No mammary tissue in section

Mandibular Lymph Node Congestion/erythrophagocytosis (slight)
 Plasmacytosis (minimal)
 Lymphoid hyperplasia (minimal)

Pancreas Reduced zymogen, acinar cell basophilia (slight)

Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (slight)

Thymus Lymphoid atrophy - involution (slight)

Number of Sections less than protocol for Coagulating Gland/1 (0), Peyer's Patches (GALT) (1).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

Animal No: 9
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 9
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (slight)
Liver Inflammatory infiltrate, lymphoid (slight)
Mandibular Lymph Node Plasmacytosis (minimal)
Spleen Hemosiderin pigment (slight)

Number of Sections less than protocol for Sublingual Salivary Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Lungs, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

Animal No: 10
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 10
SEX: Male
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (slight)
Liver Inflammatory infiltrate, lymphoid (minimal)
Mandibular Lymph Node Congestion/erythrophagocytosis (slight)
 Plasmacytosis (minimal)
Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (minimal)

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Lungs, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

Animal No: 41
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
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Table 5 Individual Animal Data Records

Animal No: 41
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

Bone Marrow - sternal	Adipocytes (minimal)
Liver	Inflammatory infiltrate, lymphoid (minimal)
Lungs	Vascular mineralisation, focal (minimal) Lymphoid hyperplasia (BALT) (slight)
Mandibular Lymph Node	Congestion/erythrophagocytosis (minimal) Plasmacytosis (minimal)
Spleen	Hemosiderin pigment (slight) Hemopoietic foci, primarily erythroid (slight)
Thymus	Lymphoid atrophy - involution (minimal)
Vagina	Metestrus epithelium present

Number of Sections less than protocol for Parathyroid Gland/1 (0), Peyer's Patches (GALT) (1), Sublingual Salivary Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

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Test item : Lipase produced with *Trichoderma reesei*
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 Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 42
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (minimal)
Eye/1 Retinal rosette(s) - dysplasia (slight)
Eye/2 Retinal rosette(s) - dysplasia (minimal)
Kidney/1 Pelvic/papillary mineralization (minimal)
 Tubular mineralisation (slight)
Kidney/2 Tubular mineralisation (minimal)
Mandibular Lymph Node Plasmacytosis (slight)
Mesenteric Lymph Node Macrophage foci (minimal)
Spleen Hemosiderin pigment (moderate)
 Hemopoietic foci, primarily erythroid (minimal)
Vagina Diestrus epithelium present

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Heart, Ileum, Jejunum, Larynx, Liver, Lungs, Mammary Gland Area, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 43
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Adrenal Gland/1 Hypertrophy, cortical diffuse (slight)
Bone Marrow - sternal Adipocytes (minimal)
Liver Inflammatory infiltrate, lymphoid (minimal)
Mandibular Lymph Node Congestion/erythrophagocytosis (slight)
Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (minimal)
Vagina Epithelial mucification (slight)

Number of Sections less than protocol for Parathyroid Gland/1 (0).

No abnormalities found in: Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Lungs, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
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 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 44
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (minimal)
Liver Hepatocellular pigment, yellow-brown (minimal)
Mesenteric Lymph Node Macrophage foci (minimal)
 Lymphoid hyperplasia (minimal)
Spleen Hemosiderin pigment (minimal)
 Hemopoietic foci, primarily erythroid (minimal)
Vagina Estrus epithelium present

Number of Sections less than protocol for Parathyroid Gland/1 (0), Peyer's Patches (GALT) (1).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Lungs, Mammary Gland Area, Mandibular Lymph Node, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 45
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Adrenal Gland/1 Inflammatory infiltrate, lymphoid (minimal)

Bone Marrow - sternal Adipocytes (slight)

Kidney/1 Pelvic dilation present

Kidney/2 Pelvic dilation present

Larynx Inflammatory infiltrate, lymphoid (minimal)

Lungs Osseous metaplasia (minimal)

Mesenteric Lymph Node Macrophage foci (minimal)

Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (minimal)

Vagina Proestrus epithelium present

Number of Sections less than protocol for Mandibular Lymph Node (0), Optic Nerve/2 (0),
 Sublingual Salivary Gland/1 (0).

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

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 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 45
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

No abnormalities found in: Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Liver, Mammary Gland Area, Optic Nerve/1, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

Animal No: 46
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Adrenal Gland/1	Inflammatory infiltrate, lymphoid (minimal)
Adrenal Gland/2	Inflammatory infiltrate, lymphoid (minimal)
Liver	Hepatocellular pigment, yellow-brown (minimal)
Lungs	Peri- vascular/bronchial, inflammatory cell foci (minimal) Lymphoid hyperplasia (BALT) (minimal)
Mandibular Lymph Node	Plasmacytosis (slight)
Spleen	Hemosiderin pigment (slight)
Thymus	Lymphoid atrophy - involution (minimal)
Vagina	Diestrus epithelium present

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 46
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

Number of Sections less than protocol for Peyer's Patches (GALT) (1).

No abnormalities found in: Aorta, Bone Marrow - sternal, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

Animal No: 47
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Adrenal Gland/1	Inflammatory infiltrate, lymphoid (minimal)
Adrenal Gland/2	Inflammatory infiltrate, lymphoid (minimal)
Bone Marrow - sternal	Adipocytes (minimal)
Lungs	Vascular mineralisation, focal (minimal)
Mandibular Lymph Node	Plasmacytosis (slight)
Spleen	Hemosiderin pigment (moderate) Hemopoietic foci, primarily erythroid (moderate)

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

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 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 47
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

Thymus Lymphoid atrophy - involution (slight)

Vagina Diestrus epithelium present

Number of Sections less than protocol for Optic Nerve/2 (0).

No abnormalities found in: Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Liver, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

Animal No: 48
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

Lymph nodes mandibular: Discoloration, DARK RED.

No abnormalities were found in any of the other tissues examined

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (minimal)

Kidney/1 Pelvic/papillary mineralization (minimal)

Larynx Inflammatory infiltrate, lymphoid (slight)

Liver Inflammatory infiltrate, lymphoid (minimal)

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 48
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

Mandibular Lymph Node	Congestion/erythrophagocytosis (slight) (correlates to GROSS finding) Plasmacytosis (minimal)
Spleen	Hemosiderin pigment (slight) Hemopoietic foci, primarily erythroid (minimal)
Thymus	Lymphoid atrophy - involution (slight)
Vagina	Diestrus epithelium present

Number of Sections less than protocol for Colon (0), Optic Nerve/2 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/2, Lungs, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

Animal No: 49
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
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Table 5 Individual Animal Data Records

Animal No: 49
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MICROSCOPIC FINDINGS

Adrenal Gland/1	Inflammatory infiltrate, lymphoid (minimal)
Bone Marrow - sternal	Adipocytes (slight)
Kidney/1	Pelvic/papillary mineralization (minimal) Tubular mineralisation (slight)
Kidney/2	Tubular mineralisation (minimal)
Liver	Inflammatory infiltrate, lymphoid (minimal) Hepatocellular pigment, yellow-brown (moderate)
Lungs	Osseous metaplasia (minimal)
Spleen	Hemosiderin pigment (slight) Hemopoietic foci, primarily erythroid (minimal)
Thymus	Lymphoid atrophy - involution (slight)
Vagina	Diestrus epithelium present

Number of Sections less than protocol for Mandibular Lymph Node (0), Parathyroid Gland/1 (0), Peyer's Patches (GALT) (1).

No abnormalities found in: Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Larynx, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 50
SEX: Female
DOSE GROUP: Group 1, 0 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (slight)
Esophagus Myodegeneration, focal (slight)
Liver Inflammatory infiltrate, lymphoid (minimal)
 Hepatocellular pigment, yellow-brown (moderate)
Lungs Osseous metaplasia (minimal)
Mesenteric Lymph Node Macrophage foci (slight)
Spleen Hemosiderin pigment (slight)
Thymus Lymphoid atrophy - involution (slight)
Vagina Proestrus epithelium present

Number of Sections less than protocol for Peyer's Patches (GALT) (1).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Mammary Gland Area, Mandibular Lymph Node, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 11
SEX: Male
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 12
SEX: Male
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

Harlan Project no. : D80691
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Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 13
SEX: Male
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 14
SEX: Male
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 15
SEX: Male
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 16
SEX: Male
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 17
SEX: Male
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 18
SEX: Male
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

Harlan Project no. : D80691
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Table 5 Individual Animal Data Records

Animal No: 19
SEX: Male
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 20
SEX: Male
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

Harlan Project no. : D80691
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Table 5 Individual Animal Data Records

Animal No: 51
SEX: Female
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 52
SEX: Female
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

Harlan Project no. : D80691
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Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 53
SEX: Female
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 54
SEX: Female
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

Harlan Project no. : D80691
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Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 55
SEX: Female
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 56
SEX: Female
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

Harlan Project no. : D80691
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Table 5 Individual Animal Data Records

Animal No: 57
SEX: Female
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 58
SEX: Female
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

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Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 59
SEX: Female
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 60
SEX: Female
DOSE GROUP: Group 2, 50 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 21
SEX: Male
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 22
SEX: Male
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 23
SEX: Male
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 24
SEX: Male
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 25
SEX: Male
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 26
SEX: Male
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 27
SEX: Male
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 28
SEX: Male
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 29
SEX: Male
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 30
SEX: Male
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 17/12/13
date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 61
SEX: Female
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 62
SEX: Female
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 63
SEX: Female
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 64
SEX: Female
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
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Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 65
SEX: Female
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

Eyes vitreous humor: right side Desiccated.

No abnormalities were found in any of the other tissues examined

MICROSCOPIC FINDINGS

Eye/1 Trauma (correlates to GROSS finding)

No abnormalities found in: Eye/2.

Animal No: 66
SEX: Female
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
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Table 5 Individual Animal Data Records

Animal No: 67
SEX: Female
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 68
SEX: Female
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
Test System : 90-Day Oral Toxicity Study in the Wistar Rat
Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 69
SEX: Female
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

Animal No: 70
SEX: Female
DOSE GROUP: Group 3, 200 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
sacrifice group: terminal sacrifice
necropsy status: planned terminal
date of start of treatment: 16/09/13
date of end of treatment: 16/12/13
date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

No tissues examined

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 31
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal	Adipocytes (slight)
Esophagus	Myodegeneration, focal (slight)
Larynx	Inflammatory infiltrate, lymphoid (slight)
Liver	Inflammatory infiltrate, lymphoid (slight)
Lungs	Vascular mineralisation, focal (minimal) Osseous metaplasia (minimal)
Pituitary Gland	Microcyst present
Sciatic Nerve	Axonal fragmentation (minimal)
Spleen	Hemosiderin pigment (slight)
Thymus	Lymphoid atrophy - involution (minimal)

Number of Sections less than protocol for Parathyroid Gland/1 (0), Rectum (0), Sublingual Salivary Gland/1 (0).

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Test item : Lipase produced with *Trichoderma reesei*
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 Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 31
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MICROSCOPIC FINDINGS

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Mammary Gland Area, Mandibular Lymph Node, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Prostate Gland, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

Animal No: 32
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Adrenal Gland/1	Vacuolation, multifocal in z. fasciculata (minimal)
Adrenal Gland/2	Vacuolation, multifocal in z. fasciculata (minimal)
Bone Marrow - sternal	Adipocytes (slight)
Liver	Inflammatory infiltrate, lymphoid (slight) Hepatocellular pigment, yellow-brown (slight)
Lungs	Vascular mineralisation, focal (minimal)
Mandibular Lymph Node	Plasmacytosis (minimal)

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Table 5 Individual Animal Data Records

Animal No: 32
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MICROSCOPIC FINDINGS

Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (minimal)

Thymus Lymphoid atrophy - involution (slight)

Number of Sections less than protocol for Optic Nerve/1 (0), Peyer's Patches (GALT) (1).

No abnormalities found in: Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

Animal No: 33
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

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 Sponsor : AB Enzymes

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Table 5 Individual Animal Data Records

Animal No: 33
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MICROSCOPIC FINDINGS

Bone Marrow - sternal	Adipocytes (minimal)
Heart	Cardiomyopathy (slight)
Liver	Inflammatory infiltrate, lymphoid (minimal)
Lungs	Alveolar inflammation, lymphocytic (minimal)
Mandibular Lymph Node	Plasmacytosis (minimal)
Pancreas	Exocrine hypertrophy, focal (slight)
Sciatic Nerve	Axonal fragmentation (minimal)
Spleen	Hemosiderin pigment (slight) Hemopoietic foci, primarily erythroid (minimal)

Number of Sections less than protocol for Peyer's Patches (GALT) (1).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

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Table 5 Individual Animal Data Records

Animal No: 34
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS**Bone Marrow - sternal**

Adipocytes (moderate)

Liver

Inflammatory infiltrate, lymphoid (minimal)

Lungs

Vascular mineralisation, focal (minimal)
 Peri- vascular/bronchial, inflammatory cell foci (minimal)
 Microgranuloma (minimal)

Mesenteric Lymph Node

Macrophage foci (minimal)

Spleen

Hemosiderin pigment (slight)

Number of Sections less than protocol for Colon (0), Parathyroid Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Mammary Gland Area, Mandibular Lymph Node, Optic Nerve/1, Optic Nerve/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

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Table 5 Individual Animal Data Records

Animal No: 35
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (slight)
Liver Inflammatory infiltrate, lymphoid (slight)
Mammary Gland Area No mammary tissue in section
Mandibular Lymph Node Congestion/erythrophagocytosis (slight)
 Plasmacytosis (minimal)
Spleen Hemosiderin pigment (minimal)
Thyroid Gland/1 Inflammatory infiltrate, lymphoid (minimal)

Number of Sections less than protocol for Parathyroid Gland/1 (0), Sublingual Salivary Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Lungs, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thymus, Thyroid Gland/2, Trachea, Urinary Bladder.

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Table 5 Individual Animal Data Records

Animal No: 36
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (slight)
Larynx Inflammatory infiltrate, lymphoid (minimal)
Liver Inflammatory infiltrate, lymphoid (slight)
Mandibular Lymph Node Plasmacytosis (minimal)
Mesenteric Lymph Node Macrophage foci (minimal)
Pituitary Gland Microcyst present
Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (minimal)

Number of Sections less than protocol for Parathyroid Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Lungs, Mammary Gland Area, Optic Nerve/1, Optic Nerve/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

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Table 5 Individual Animal Data Records

Animal No: 37
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (minimal)
Liver Inflammatory infiltrate, lymphoid (minimal)
Mesenteric Lymph Node Macrophage foci (minimal)
Spleen Hemosiderin pigment (minimal)
 Hemopoietic foci, primarily erythroid (minimal)
Testes/1 Seminiferous cell debris, intratubular (minimal)
Thymus Lymphoid atrophy - involution (minimal)

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Lungs, Mammary Gland Area, Mandibular Lymph Node, Optic Nerve/1, Optic Nerve/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

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Table 5 Individual Animal Data Records

Animal No: 38
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (moderate)
Heart Cardiomyopathy (minimal)
Liver Inflammatory infiltrate, lymphoid (minimal)
Lungs Vascular mineralisation, focal (minimal)
Spleen Hemosiderin pigment (slight)
Thymus Lymphoid atrophy - involution (minimal)

Number of Sections less than protocol for Optic Nerve/2 (0), Parathyroid Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Mammary Gland Area, Mandibular Lymph Node, Mesenteric Lymph Node, Optic Nerve/1, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

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Table 5 Individual Animal Data Records

Animal No: 39
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (slight)
Kidney/1 Inflammatory infiltrate, lymphoid (minimal)
Mandibular Lymph Node Congestion/erythrophagocytosis (minimal)
 Plasmacytosis (minimal)
 Lymphoid hyperplasia (minimal)
Mesenteric Lymph Node Macrophage foci (minimal)
Spleen Hemosiderin pigment (slight)
Thymus Lymphoid atrophy - involution (minimal)

Number of Sections less than protocol for Parathyroid Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/2, Larynx, Liver, Lungs, Mammary Gland Area, Optic Nerve/1, Optic Nerve/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder.

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Table 5 Individual Animal Data Records

Animal No: 40
SEX: Male
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 93
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 17/12/13
 date of necropsy: 18/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (minimal)
Liver Inflammatory infiltrate, lymphoid (slight)
Lungs Vascular mineralisation, focal (minimal)
 Lymphoid hyperplasia (BALT) (minimal)
Mandibular Lymph Node Plasmacytosis (minimal)
Spleen Hemosiderin pigment (slight)
Thyroid Gland/1 Follicular hypertrophy/hyperplasia, diffuse (minimal)
Thyroid Gland/2 Follicular hypertrophy/hyperplasia, diffuse (minimal)

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Coagulating Gland/1, Coagulating Gland/2, Colon, Duodenum, Epididymis/1, Epididymis/2, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Prostate Gland, Rectum, Sciatic Nerve, Seminal Vesicle/1, Seminal Vesicle/2, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Testes/1, Testes/2, Thymus, Trachea, Urinary Bladder.

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Table 5 Individual Animal Data Records

Animal No: 71
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (slight)
Liver Hepatocellular pigment, yellow-brown (moderate)
Mandibular Lymph Node Plasmacytosis (minimal)
Pituitary Gland Microcyst present
Spleen Hemosiderin pigment (slight)
Thymus Lymphoid atrophy - involution (minimal)
Vagina Diestrus epithelium present

Number of Sections less than protocol for Parathyroid Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Larynx, Lungs, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

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Table 5 Individual Animal Data Records

Animal No: 72
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (slight)
Kidney/1 Tubular mineralisation (minimal)
Liver Inflammatory infiltrate, lymphoid (minimal)
Mandibular Lymph Node Plasmacytosis (minimal)
Mesenteric Lymph Node Macrophage foci (minimal)
Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (minimal)
Thymus Lymphoid atrophy - involution (minimal)
Vagina Metestrus epithelium present

Number of Sections less than protocol for Parathyroid Gland/1 (0), Peyer's Patches (GALT) (0), Thyroid Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/2, Larynx, Lungs, Mammary Gland Area, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/2, Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

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 Sponsor : AB Enzymes

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 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 73
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Adrenal Gland/1 Inflammatory infiltrate, lymphoid (slight)
Adrenal Gland/2 Inflammatory infiltrate, lymphoid (slight)
Larynx Inflammatory infiltrate, lymphoid (minimal)
Liver Inflammatory infiltrate, lymphoid (minimal)
 Hepatocellular vacuolation (minimal)
Lungs Lymphoid hyperplasia (BALT) (minimal)
Spleen Hemosiderin pigment (moderate)
 Hemopoietic foci, primarily erythroid (slight)
Vagina Epithelial mucification (moderate)

Number of Sections less than protocol for Mandibular Lymph Node (0), Parathyroid Gland/1 (0), Peyer's Patches (GALT) (0).

No abnormalities found in: Aorta, Bone Marrow - sternal, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/1, Kidney/2, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/2, Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

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Table 5 Individual Animal Data Records

Animal No: 74
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Adrenal Gland/1	Extracapsular nodule present Inflammatory infiltrate, lymphoid (slight)
Adrenal Gland/2	Inflammatory infiltrate, lymphoid (slight)
Kidney/1	Tubular mineralisation (minimal)
Liver	Inflammatory infiltrate, lymphoid (minimal)
Mandibular Lymph Node	Plasmacytosis (slight)
Mesenteric Lymph Node	Macrophage foci (minimal)
Pancreas	Inflammatory infiltrate, granulolymphocytic (minimal)
Spleen	Hemosiderin pigment (slight) Hemopoietic foci, primarily erythroid (slight)
Vagina	Proestrus epithelium present

Number of Sections less than protocol for Parathyroid Gland/1 (0), Peyer's Patches (GALT) (1), Sublingual Salivary Gland/1 (0).

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Table 5 Individual Animal Data Records

Animal No: 74
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MICROSCOPIC FINDINGS

No abnormalities found in: Aorta, Bone Marrow - sternal, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/2, Larynx, Lungs, Mammary Gland Area, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thymus, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

Animal No: 75
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal	Adipocytes (minimal)
Eye/1	Retinal rosette(s) - dysplasia (slight)
Kidney/1	Tubular mineralisation (slight)
Kidney/2	Tubular mineralisation (moderate) Tubular basophilia, corticomedullary (minimal)
Liver	Inflammatory infiltrate, lymphoid (minimal) Hepatocellular pigment, yellow-brown (slight)
Mandibular Lymph Node	Plasmacytosis (minimal)

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 75
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MICROSCOPIC FINDINGS

Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (minimal)

Thymus Lymphoid atrophy - involution (minimal)

Vagina Metestrus epithelium present

Number of Sections less than protocol for Parathyroid Gland/1 (0), Peyer's Patches (GALT) (1).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/2, Heart, Ileum, Jejunum, Larynx, Lungs, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

Animal No: 76
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 76
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MICROSCOPIC FINDINGS

Adrenal Gland/1 Inflammatory infiltrate, lymphoid (minimal)

Adrenal Gland/2 Extracapsular nodule present

Bone Marrow - sternal Adipocytes (moderate)

Kidney/1 Tubular mineralisation (minimal)

Kidney/2 Tubular mineralisation (minimal)

Liver Inflammatory infiltrate, lymphoid (minimal)

Mandibular Lymph Node Plasmacytosis (minimal)

Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (minimal)

Thymus Lymphoid atrophy - involution (minimal)

Vagina Diestrus epithelium present

Number of Sections less than protocol for Parathyroid Gland/1 (0).

No abnormalities found in: Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Larynx, Lungs, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 77
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal	Adipocytes (slight)
Esophagus	Myodegeneration, focal (minimal)
Eye/1	Retinal rosette(s) - dysplasia (minimal)
Kidney/1	Tubular mineralisation (minimal)
Kidney/2	Tubular mineralisation (slight)
Liver	Inflammatory infiltrate, lymphoid (minimal)
Lungs	Vascular mineralisation, focal (slight)
Mandibular Lymph Node	Congestion/erythrophagocytosis (minimal) Plasmacytosis (slight) Lymphoid hyperplasia (slight)
Spleen	Hemosiderin pigment (slight) Hemopoietic foci, primarily erythroid (minimal)
Thymus	Lymphoid atrophy - involution (slight)
Vagina	Metestrus epithelium present

Number of Sections less than protocol for Parathyroid Gland/1 (0).

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 77
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MICROSCOPIC FINDINGS

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Eye/2, Heart, Ileum, Jejunum, Larynx, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Optic Nerve/2, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

Animal No: 78
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS

Bone Marrow - sternal	Adipocytes (slight)
Kidney/1	Pelvic dilation present
Lungs	Osseous metaplasia (minimal)
Mandibular Lymph Node	Plasmacytosis (minimal)
Mesenteric Lymph Node	Macrophage foci (minimal)
Pituitary Gland	Microcyst present
Spleen	Hemosiderin pigment (slight)
Thymus	Lymphoid atrophy - involution (minimal)

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 78
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MICROSCOPIC FINDINGS**Vagina**

Metestrus epithelium present

Number of Sections less than protocol for Optic Nerve/2 (0), Peyer's Patches (GALT) (1).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/2, Larynx, Liver, Mammary Gland Area, Optic Nerve/1, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/1, Parathyroid Gland/2, Peyer's Patches (GALT), Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

Animal No: 79
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

MICROSCOPIC FINDINGS**Bone Marrow - sternal**

Adipocytes (minimal)

Kidney/1

Tubular mineralisation (minimal)

Larynx

Inflammatory infiltrate, lymphoid (minimal)

Liver

Inflammatory infiltrate, lymphoid (minimal)
 Hepatocellular pigment, yellow-brown (minimal)

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 79
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MICROSCOPIC FINDINGS

Mandibular Lymph Node Congestion/erythrophagocytosis (slight)
 Plasmacytosis (minimal)

Mesenteric Lymph Node Macrophage foci (minimal)

Ovary/1 Corpora hemorrhagica (slight)

Ovary/2 Corpora hemorrhagica (moderate)

Spleen Hemosiderin pigment (slight)
 Hemopoietic foci, primarily erythroid (minimal)

Vagina Diestrus epithelium present

Number of Sections less than protocol for Optic Nerve/1 (0), Parathyroid Gland/1 (0), Peyer's Patches (GALT) (1), Thyroid Gland/1 (0).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Colon, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/2, Lungs, Mammary Gland Area, Optic Nerve/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thymus, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

Animal No: 80
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MACROSCOPIC FINDINGS

days of treatment: 92
 sacrifice group: terminal sacrifice
 necropsy status: planned terminal
 date of start of treatment: 16/09/13
 date of end of treatment: 16/12/13
 date of necropsy: 17/12/13

No abnormality detected

PATHOLOGY REPORT

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Test item : Lipase produced with *Trichoderma reesei*
 Test System : 90-Day Oral Toxicity Study in the Wistar Rat
 Sponsor : AB Enzymes

Harlan Project no. : D80691
 Propath no. : 14001
 Date : 29.Apr.2014

Table 5 Individual Animal Data Records

Animal No: 80
SEX: Female
DOSE GROUP: Group 4, 1000 mg/kg bw/day

MICROSCOPIC FINDINGS

Bone Marrow - sternal Adipocytes (moderate)
Kidney/1 Hyaline cast(s) (minimal)
Liver Inflammatory infiltrate, lymphoid (minimal)
Mandibular Lymph Node Plasmacytosis (minimal)
Spleen Hemosiderin pigment (minimal)
Thymus Lymphoid atrophy - involution (minimal)
Vagina Metestrus epithelium present

Number of Sections less than protocol for Colon (0), Optic Nerve/2 (0), Parathyroid Gland/1 (0), Peyer's Patches (GALT) (1).

No abnormalities found in: Adrenal Gland/1, Adrenal Gland/2, Aorta, Brain - cerebellum, Brain - cerebrum, Brain - midbrain, Brain - pons, Cecum, Duodenum, Esophagus, Eye/1, Eye/2, Heart, Ileum, Jejunum, Kidney/2, Larynx, Lungs, Mammary Gland Area, Mesenteric Lymph Node, Optic Nerve/1, Ovary/1, Ovary/2, Oviduct/1, Oviduct/2, Pancreas, Parathyroid Gland/2, Peyer's Patches (GALT), Pituitary Gland, Rectum, Sciatic Nerve, Skin, Spinal Cord - cervical, Spinal Cord - lumbar, Spinal Cord - midthoracic, Stomach, Sublingual Salivary Gland/1, Sublingual Salivary Gland/2, Submandibular Salivary Gland/1, Submandibular Salivary Gland/2, Thyroid Gland/1, Thyroid Gland/2, Trachea, Urinary Bladder, Uterus, Uterus - cervix.

REPORT



Lipase produced with *Trichoderma reesei*: *In vitro* Chromosome Aberration Test in Human Lymphocytes

Study Director:	Dr. Susanne Bohnenberger
Test Facility:	Harlan Cytotest Cell Research GmbH (Harlan CCR) In den Leppsteinswiesen 19 64380 Rossdorf Germany
Sponsor:	AB Enzymes GmbH Feldbergstr. 78 64293 Darmstadt Germany
Study Monitor:	Dr. H.-J. Schepers
Harlan Study Number:	1544901
Study Completion Date:	02 September 2013
Version	Final

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STUDY DIRECTOR STATEMENT OF GLP COMPLIANCE

Harlan Cytotest Cell Research GmbH (Harlan CCR)
In den Leppsteinswiesen 19
64380 Rossdorf
Germany

Harlan Study Number: 1544901
Study Title: Lipase produced with *Trichoderma reesei*:
In vitro Chromosome Aberration Test
in Human Lymphocytes

This study was performed in compliance with “Chemikaliengesetz” (Chemicals Act) of the Federal Republic of Germany, “Anhang 1” (Annex 1); in its currently valid version. These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI).

This report fully and accurately reflects the procedures used and data generated. There were no circumstances considered to have affected the integrity of the study or the validity of the data.

Study Director: Dr. Susanne Bohnenberger



.....
Date: 02 September 2013

QUALITY ASSURANCE STATEMENT

Harlan Study Number: 1544901
Study Title: Lipase produced with *Trichoderma reesei*:
In vitro Chromosome Aberration Test
in Human Lymphocytes

The general facilities and activities are inspected at least once a year and the results are reported to the relevant responsible person and management.

Study-related procedures conducted at the test facility were audited and inspected. The details of these audits and inspections are given below.

Dates and Types of QA Inspections			Reported to the relevant Study Director and Test Facility Management
Date of Inspection	Type of Inspection	Phase Inspected	Report Date
12 April 2013	Study Plan Verification	N/A	12 April 2013
17 April 2013	1 st Amendment to Study Plan Verification	N/A	17 April 2013
15 May 2013	2 nd Amendment to Study Plan Verification	N/A	15 May 2013
08 May 2013	Process – based	Test Performance	08 May 2013
05 June 2013	Process – based	Test Item Preparation	05 June 2013
30 August 2013	Report Audit	N/A	30 August 2013

This statement confirms that this report reflects the raw data and the procedures followed.

Quality Assurance:

L. Wilck



.....
Date: 02 September 2013

SUMMARY

The test item Lipase produced with *Trichoderma reesei*, dissolved in deionised water, was assessed for its potential to induce structural chromosomal aberrations in human lymphocytes *in vitro* in two independent experiments. The following study design was performed:

	Without S9 mix		With S9 mix
	Exp. I	Exp. II	Exp. I
Exposure period	4 hrs	22 hrs	4 hrs
Recovery	18 hrs	—	18 hrs
Preparation interval	22 hrs	22 hrs	22 hrs

In each experimental group two parallel cultures were analysed. Per culture at least 100 metaphases were evaluated for structural chromosomal aberrations.

The highest applied concentration in this study (5300.0 µg/mL of the test item) was chosen with regard to the TOS (94.38 %) of the test item and with respect to the current OECD Guideline 473.

Dose selection of the cytogenetic experiment was performed considering the toxicity data in accordance with OECD Guideline 473. The rationale for the dose selection is reported in section 3.5.1 (page 11). The evaluated experimental points and the results are summarised in Table 2 (page 19).

In both cytogenetic experiments, in the absence and presence of S9 mix, at the highest evaluated concentrations the mitotic indices were clearly reduced.

Either with or without metabolic activation, no clastogenicity was observed at the concentrations evaluated. However, in the presence of S9 mix, one increase in chromosomal aberrations (3.3 % aberrant cells, excluding gaps) slightly above the laboratory historical solvent control data (0.0 – 3.0 % aberrant cells, excluding gaps) was observed after treatment with 322.9 µg/mL. Since the value is not statistically significant this finding has to be regarded as being biologically irrelevant.

No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.

Appropriate mutagens were used as positive controls. They induced statistically significant increases in cells with structural chromosome aberrations.

Conclusion

In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce structural chromosomal aberrations in human lymphocytes *in vitro*.

Therefore, Lipase produced with *Trichoderma reesei* is considered to be non-clastogenic in this chromosome aberration test, when tested up to cytotoxic concentrations.

GENERAL INFORMATION

Schedule

Experimental Starting Date: 22 April 2013

Experimental Completion Date: 11 June 2013

Additional Responsibilities

Deputy Study Director: Dipl. Biol. Andrea Sokolowski
Harlan Cytotest Cell Research GmbH (Harlan CCR)

Deviations from Study Plan

There were no deviations (unplanned changes) from the study plan.

Archiving

Unless instructed otherwise by the Sponsor, the study plan, all raw data, specimens (if any) and the final report will be retained in the Harlan Cytotest Cell Research GmbH archive for at least 3 years. Thereafter, the material will be transferred to the GLP archive of Harlan Laboratories Ltd. in Füllinsdorf, Switzerland, for further archiving up to a total archiving period of 15 years.

No data will be discarded without contacting the Sponsor to obtain their written consent.

A sample of the test item will be archived two years after the expiration date provided by the sponsor. If no expiration date is given, the archiving period will be the required 15 years. Thereafter the samples will be discarded without further notice.

1 INTRODUCTION AND PURPOSE

Chromosomal aberrations and potentially resulting mutations are related to many human genetic diseases. As well, chromosomal mutations causing alterations in oncogenes and tumor suppressor genes of somatic cells are involved in cancer induction in humans and experimental animals. The purpose of the *in vitro* chromosomal aberration test is to identify agents that cause structural chromosomal aberrations in cultured human lymphocytes. Structural aberrations may be of the chromosome or chromatid type. An increase in polyploidy may indicate that a chemical has the potential to induce numerical aberrations.

The induction of cytogenetic damage in human lymphocytes was assessed in two independent experiments with one preparation interval (22 hours).

Treatments started after a 72 hour stimulation period with phytohemagglutinine (PHA) when cells were actively proliferating and metaphases were prepared at approx. 1.5 fold of the normal cell cycle time.

For validation of the test, control mutagens were tested in parallel to the test item.

1.1 Guidelines / Regulations

This test methods described are designed to be compatible with the procedures indicated by the following internationally accepted guidelines and recommendations:

- OECD Guidelines for Testing of Chemicals No. 473 “*In vitro* Mammalian Chromosome Aberration Test” (adopted July 21, 1997)
- Commission Regulation (EC) 440/2008 B10 “Mutagenicity – *In vitro* Mammalian Chromosome Aberration Test”, dated May 30, 2008

The following alterations from the guidelines were planned:

- The treatment of lymphocytes started approx. 72 hours after mitogenic stimulation.

2 TEST AND REFERENCE ITEM

2.1 Test Item

Information as provided by the Sponsor.

Identification:	Lipase produced with <i>Trichoderma reesei</i>
Batch:	LP 12136B3; RF 10625
Purity:	TOS value: 94.38 % (=Total Organic Substance)
Expiry / Retest Date:	November 2014
Storage Conditions:	At room temperature, moisture protected
Stability in Solvent:	1 day in water at room temperature

The test item concentrations were administered on TOS level = Proteine, peptides, carbohydrates, and fat.

3 MATERIALS AND METHODS

3.1 Test System

3.1.1 Human lymphocytes

Blood samples were drawn from healthy non-smoking donors not receiving medication. Blood was collected from one single donor for each experiment, i.e. human lymphocytes in Experiment I and Experiment II originated from different donors. For this study, blood was collected from a male donor (31 years old) for the first experiment and from a 35 year-old female donor for Experiment II. The lymphocytes of the respective donors have been shown to respond well to stimulation of proliferation with PHA and to positive control substances. All donors had a previously established low incidence of chromosomal aberrations in their peripheral blood lymphocytes.

Human lymphocytes were stimulated for proliferation by the addition of the mitogen PHA to the culture medium for a period of 72 hours. The cell harvest time point was approximately 1.5 x AGT. Any specific cell cycle time delay induced by the test item was not accounted for directly.

3.1.2 Culture conditions

Blood cultures were established by preparing a 11 % mixture of whole blood in medium within 30 hrs after blood collection. The culture medium was Dulbecco's Modified Eagles Medium/Ham's F12 (DMEM/F12, mixture 1:1) already supplemented with 200 mM GlutaMAXTM. Additionally, the medium was supplemented with penicillin/streptomycin

(100 U/mL/100 µg/mL), the mitogen PHA (3 µg/mL), 10 % FBS (fetal bovine serum), 10 mM HEPES and the anticoagulant heparin (125 U.S.P.-U/mL).

All incubations were done at 37 °C with 5.5 % CO₂ in humidified air.

3.2 Test Item Preparation

Stock solutions of the test item and a serial dilution were formulated in deionised water. The final concentration of deionised water in the culture medium was 10 %. The solvent was chosen due to its solubility properties and its relative non-toxicity to the cell cultures.

The effect of the test item on pH and osmolarity in the medium was measured. All formulations were prepared freshly before treatment and used within two hours of preparation. The formulation was assumed to be stable for this period unless specified otherwise by the Sponsor.

The osmolarity and pH-value were determined in the solvent control and the maximum concentration without metabolic activation:

		Concentration [µg/mL]	Solvent control	Test item
Exp. I	Osmolarity [mOsm]	5300.0	286	292
	pH-value		7.6	7.4
Exp. II	Osmolarity [mOsm]	3508.9	284	290
	pH-value		7.6	7.6

3.3 Controls

3.3.1 Solvent control

Concurrent solvent controls deionised water (local tap water deionised at Harlan CCR) were performed.

3.3.2 Positive controls

Without metabolic activation

Name: EMS; ethylmethane sulfonate
Purity: ≥ 98 %
Dissolved in: Nutrient medium
Concentration: 550.0 – 660.0 µg/mL

With metabolic activation

Name: CPA; cyclophosphamide
Purity: ≥ 98 %
Dissolved in: Saline (0.9 % NaCl [w/v])
Concentration: 2.5 µg/mL

The dilutions of the stock solutions were prepared on the day of the experiment. The stability of the positive control substance in solution is unknown but a mutagenic response in the expected range is a sufficient biological evidence for chemical stability.

3.4 Mammalian Microsomal Fraction S9 Mix

Due to the limited capacity for metabolic activation of potential mutagens in *in vitro* methods an exogenous metabolic activation system was used.

Phenobarbital/ β -naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 was prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 was routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test.

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/mL in the cultures. S9 mix contained MgCl₂ (8 mM), KCl (33 mM), glucose-6-phosphate (5 mM) and NADP (4 mM) in sodium-ortho-phosphate-buffer (100 mM, pH 7.4).

The protein concentration of the S9 preparation used for this study was 31.4 mg/mL (Lot no. 080313).

3.5 Experimental Design and Procedures

3.5.1 Dose Selection

Dose selection was performed according to the current OECD Guideline for chromosomal aberration studies. The highest test item concentration should be 5000 μ g/mL, 5 μ L/mL or 10 mM, whichever is the lowest. At least three test item concentrations should be evaluated for cytogenetic damage.

With regard to the TOS (94.38 %) of the test item, 5300.0 μ g/mL of Lipase produced with *Trichoderma reesei* were applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations between 34.4 and 5300.0 μ g/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. In the pre-test for toxicity, no precipitation of the test item was observed. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.

Using reduced mitotic indices as an indicator for toxicity in Experiment I, clear toxic effects were observed after 4 hours treatment with 5300.0 μ g/mL and above in the absence of S9 mix. Therefore, 5300.0 μ g/mL should have been chosen as top concentration in Experiment II. Due to a technical problem 3508.9 μ g/mL was the top concentration in Experiment II.

The cytogenetic evaluation of concentrations in Experiment I (with S9 mix) and Experiment II (without S9 mix) higher than indicated in Table 1, page 18 was impossible due to strong test item-induced toxic effects (low metaphase numbers).

3.5.2 Pre-experiment

A preliminary cytotoxicity test was performed to determine the concentrations to be used in the main experiment. Cytotoxicity is characterized by the percentages of mitotic suppression in comparison to the controls by counting 1000 cells per culture in duplicate. The experimental conditions in this pre-test phase were identical to those required and described below for the main experiment.

The pre-test was performed with 10 concentrations of the test item separated by no more than a factor of $\sqrt{10}$ and a solvent and positive control. All cell cultures were set up in duplicate. Exposure time was 4 hrs (with and without S9 mix). The preparation interval was 22 hrs after start of the exposure.

3.5.3 Cytogenetic Experiment

Pulse exposure

About 72 hrs after seeding 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL culture medium was added. After 4 hrs the cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were resuspended in and washed with "saline G" (pH 7.2, containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose · H₂O, 192 mg/L Na₂HPO₄ · 2 H₂O and 150 mg/L KH₂PO₄). The washing procedure was repeated once as described. After washing the cells were resuspended in complete culture medium (with 10 % FBS) and cultured until preparation of the cells.

Continuous exposure (without S9 mix)

About 72 hrs after seeding 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with complete medium (with 10 % FBS) containing the test item. The culture medium was not changed until preparation of the cells.

3.5.4 Preparation of metaphases

Cultures were treated with the metaphase-arresting substance colcemid (final concentration: 0.2 µg/mL) approximately three hours before the requested harvest time. The cultures were harvested by centrifugation 22 hrs after beginning of treatment. The supernatant was discarded and the cells were resuspended in hypotonic solution (0.0375 M KCl). Then the cell suspension was allowed to stand at 37 °C for 20 minutes. After removal of the hypotonic solution by centrifugation (approx. 900 x g) the cells were fixed with a mixture of methanol and glacial acetic acid (3+1 parts, respectively). A small amount of cell suspension was then dropped onto clean, wet microscope slides and allowed to dry. The slides were stained with Giemsa, mounted after drying and covered with a slid. All slides were labelled with a computer-generated random code to prevent scorer bias.

3.5.5 Evaluation of cytotoxicity and cytogenetic damage

Evaluation of the slides was performed according to the standard protocol of the "Arbeitsgruppe der Industrie, Cytogenetik" using microscopes with 100 x oil immersion objectives.

Cytotoxicity is characterized by the percentages of mitotic suppression in comparison with the controls by counting 1000 cells per culture in duplicate.

At least 100 well-spread metaphases were evaluated per culture for structural aberrations. Only metaphases containing a number of centromeres equal to a number of 46 ± 1 were included in the analysis. Breaks, fragments, deletions, exchanges and chromosomal disintegrations are recorded as structural chromosomal aberrations. Gaps were recorded as well, but they are not included in the calculation of the aberration rates since gaps are achromatic lesions of unknown biological relevance for which a clear relationship to treatment cannot be established.

3.6 Data Recording

The data generated were recorded in the laboratory protocol. The results are presented in tabular form, including experimental groups with the test item, solvent, and positive controls.

3.7 Interpretation of Results

Many experiments with human lymphocytes have established a range of aberration frequencies acceptable for control cultures in normal volunteer donors. The current historical data range together with the statistical significance, confirmed by the Fisher's exact test ($p < 0.05$), should be considered for classification of the test item.

The chromosomal aberration assay will be considered acceptable if it meets the following criteria:

- a) The rate of chromosomal aberrations in the solvent controls falls within the historical laboratory control data range.
- b) The rate of chromosomal aberrations in the positive controls is statistically significant increased.

A test item can be classified as non-clastogenic if:

- the number of induced structural chromosomal aberrations in all evaluated dose groups is in the range of the historical laboratory control data and
- no statistically significant increase of the rate of structural chromosomal aberrations is observed in comparison to the respective solvent control.

A test item can be classified as clastogenic if:

- the number of induced structural chromosomal aberrations is not in the range of the historical laboratory control data and
- either a concentration-related or a statistically significant increase in the number of cells carrying structural chromosomal aberrations is observed.

If the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

4 RESULTS AND DISCUSSION

The test item Lipase produced with *Trichoderma reesei*, dissolved in deionised water, was assessed for its potential to induce chromosomal aberrations in human lymphocytes *in vitro* in the absence and presence of metabolic activation by S9 mix.

Two independent experiments were performed. In Experiment I the exposure period was 4 hours with and without S9 mix. In Experiment II the exposure period was 22 hours without S9 mix. The chromosomes were prepared 22 hours (Exp. I & II) after the start of treatment with the test item.

In each experimental group two parallel cultures were analysed. At least 100 metaphases per culture were scored for structural chromosomal aberrations. 1000 cells were counted per culture for determination of the mitotic index.

The highest treatment concentration in this study, 5300.0 µg/mL was chosen with regard to the TOS (94.38 %) of the test item and with respect to the OECD Guideline for *in vitro* mammalian cytogenetic tests.

No visible precipitation of the test item in the culture medium was observed.

No relevant influence on osmolarity or pH value was observed.

In both cytogenetic experiments, in the absence and presence of S9 mix, at the highest evaluated concentrations the mitotic indices were clearly reduced (51.7, 37.5 and 39.3 % of control) (see Table 3 and 4, page 20 and 21).

Either with or without metabolic activation, no clastogenicity was observed at the concentrations evaluated (see Table 6, 7 and 9, page 23, 24 and 26). The aberration rates of the cells after treatment with the test item (0.5 – 3.3 % aberrant cells, excluding gaps) were above the range of the solvent control values (0.5 – 1.5 % aberrant cells, excluding gaps) but within the range of the laboratory historical solvent control data (see Appendix 2). However, in the presence of S9 mix, one increase in chromosomal aberrations (3.3 % aberrant cells, excluding gaps) slightly above the laboratory historical solvent control data (0.0 – 3.0 % aberrant cells, excluding gaps) was observed after treatment with 322.9 µg/mL. Since the value is not statistically significant this finding has to be regarded as being biologically irrelevant.

No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.

In both experiments, either EMS (550 or 660 µg/mL) or CPA (2.5 µg/mL) were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

5 CONCLUSION

In conclusion, it can be stated that under the experimental conditions reported, the test item Lipase produced with *Trichoderma reesei* did not induce structural chromosomal aberrations in human lymphocytes *in vitro*, when tested up to cytotoxic concentrations.

6 REFERENCES

ENVIRONMENT DIRECTORATE, ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT (OECD) (1997) *No. 473 In vitro Mammalian Chromosome Aberration Test*. Paris: OECD Environmental Health and Safety Publications Series on Testing and Assessment.

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE (ICH) (2011) *Guideline S2 (R1) Genotoxicity testing and data interpretation for pharmaceuticals intended for human use*.

ARMSTRONG M.J., BEAN C.L. and GALLOWAY, S.M. (1992) A quantitative assessment of the cytotoxicity associated with chromosomal aberration detection in Chinese hamster ovary cells. *Mutation Research*, 265, 45-60.

BEAN C.L., ARMSTRONG M.J. and GALLOWAY S.M. (1992) Effect of sampling time on chromosome aberration yield for 7 chemicals in Chinese hamster ovary cells. *Mutation Research*, 265, 31-44.

EASTERBROOK J., LU C., SAKAI Y. and LI A.P. (2001) Effects of organic solvents on the activities of cytochrome P450 isoforms, UDP-dependent glucuronyl transferase, and phenol sulfotransferase in human hepatocytes. *Drug Metabolism and Disposition*, 29, 141-144.

ENGELHARDT G. (1987) "Arbeitsgruppe der Industrie, Cytogenetik". Standard-Protokoll zur cytogenetischen Auswertung von Mitose- und Meiosechromosomen bei der Routineuntersuchung.

EVANS H.J. and O'RIORDAN M.L. (1975) Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. *Mutation Research*, 31, 135-148.

KIRKLAND D.J. (1992) Chromosomal aberration tests *in vitro*: problems with protocol design and interpretation of results. *Mutagenesis*, 2, 95-106.

MITCHELL A.D., AULETTA A.E., CLIVE D., KIRBY P.E., MOORE M.M. and MYHR B.C. (1997) The L5178Y/*tk*^{+/-} mouse lymphoma specific gene and chromosomal mutation assay - A phase III report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutation Research*, 394, 177-303.

OBE G. and BEEK B. (1982) The human leukocyte test system. In: Chemical mutagens, principles and methods for their detection. Vol. 7 (De Serres, F.J., Hollander, A., eds.) Plenum Press, N.Y., London, 337-400.

PRESTON R.J.J., SAN SEBASTIAN J.R. and MC FEE A.F. (1987) The *in vitro* human lymphocyte assay for assessing the clastogenicity of chemical agents. *Mutation Research*, 189, 175-183.

SWIERENGA S.H.H., HEDDLE J.A., SIGAL E.A., GILMAN J.P.W., BRILLINGER R.L., DOUGLAS G.R. and NESTMANN E.R. (1991) Recommended protocols based on a survey of current practice in genotoxicity testing laboratories. IV: Chromosome aberration and sister chromatid exchange in Chinese hamster ovary, V79 Chinese hamster lung and human lymphocyte cultures. *Mutation Research*, 246, 301-322.

TABLES

Table 1 Concentrations applied

Exp.	Prep. interval	Exposure period	Concentrations in µg/mL									
Without S9 mix												
I	22 hrs	4 hrs	34.4	60.3	105.4	184.5	322.9	565.1	988.9	1730.6	3028.6	5300.0
II	22 hrs	22 hrs	22.8	39.9	69.8	122.2	213.8	374.1	654.7	1145.8	2005.1	3508.9
With S9 mix												
I	22 hrs	4 hrs	34.4	60.3	105.4	184.5	322.9	565.1	988.9	1730.6	3028.6	5300.0

Evaluated experimental points are shown in bold characters

Table 2 Summary of results

Summary of results of the chromosomal aberration study with
Lipase produced with *Trichoderma reesei*

Exp.	Preparation	Test item	Mitotic indices	Aberrant cells		
	interval	concentration	in %	in %		
		in µg/mL	of control	incl. gaps*	excl. gaps*	carrying exchanges
Exposure period 4 hrs without S9 mix						
I	22 hrs	Solvent control ¹	100.0	1.5	1.0	0.0
		Positive control ²	72.6	8.5	8.0 ^S	0.5
		1730.6	86.1	1.0	1.0	0.0
		3028.6	86.1	1.0	1.0	0.0
		5300.0	51.7	1.0	1.0	0.0
Exposure period 22 hrs without S9 mix						
II	22 hrs	Solvent control ¹	100.0	0.5	0.5	0.0
		Positive control ³	45.5	19.5	19.0 ^S	5.0
		374.1	85.4	1.0	1.0	0.0
		654.7	80.5	0.5	0.5	0.0
		1145.8	39.3	0.5	0.5	0.0
Exposure period 4 hrs with S9 mix						
I	22 hrs	Solvent control ¹	100.0	1.5	1.5	0.5
		Positive control ⁴	95.8	12.0	11.5 ^S	0.5
		105.4	81.5	1.0	1.0	0.5
		184.5	56.8	1.0	1.0	0.0
		322.9 [#]	37.5	3.5	3.3	0.3

* Including cells carrying exchanges

Evaluation of 200 metaphases per culture

^S Aberration frequency statistically significant higher than corresponding control values

¹ Deionised water 10.0 % (v/v)

² EMS 660.0 µg/mL

³ EMS 550.0 µg/mL

⁴ CPA 2.5 µg/mL

Table 3 Toxicity – Experiment I

Concentration (µg/mL)	Exposure time	Preparation interval	Mitotic cells per 1000 cells*	% of solvent control
Without S9 mix				
Solvent control	4 hrs	22 hrs	14.4	100.0
34.4	4 hrs	22 hrs	14.2	98.3
60.3	4 hrs	22 hrs	11.9	82.3
105.4	4 hrs	22 hrs	12.1	84.0
184.5	4 hrs	22 hrs	11.8	81.6
322.9	4 hrs	22 hrs	10.8	75.0
565.1	4 hrs	22 hrs	9.9	68.4
988.9	4 hrs	22 hrs	9.2	63.9
1730.6	4 hrs	22 hrs	12.4	86.1
3028.6	4 hrs	22 hrs	12.4	86.1
5300.0	4 hrs	22 hrs	7.5	51.7
With S9 mix				
Solvent control	4 hrs	22 hrs	13.0	100.0
34.4	4 hrs	22 hrs	11.9	91.5
60.3	4 hrs	22 hrs	10.0	76.8
105.4	4 hrs	22 hrs	10.6	81.5
184.5	4 hrs	22 hrs	7.4	56.8
322.9	4 hrs	22 hrs	4.9	37.5
565.1	4 hrs	22 hrs	0.0	0.0
988.9	4 hrs	22 hrs	n.e.	n.e.
1730.6	4 hrs	22 hrs	n.e.	n.e.
3028.6	4 hrs	22 hrs	n.e.	n.e.
5300.0	4 hrs	22 hrs	n.e.	n.e.

Experimental groups evaluated for cytogenetic damage are shown in bold characters

* Mean value of two cultures in %

n.e. Not evaluable due to strong cytotoxic effects

Table 4 Toxicity – Experiment II

Concentration (µg/mL)	Exposure time	Preparation interval	Mitotic cells per 1000 cells*	% of solvent control
Without S9 mix				
Solvent control	22 hrs	22 hrs	15.4	100.0
22.8	22 hrs	22 hrs	15.0	97.1
39.9	22 hrs	22 hrs	14.0	90.6
69.8	22 hrs	22 hrs	14.9	96.4
122.2	22 hrs	22 hrs	15.3	99.4
213.8	22 hrs	22 hrs	14.2	92.2
374.1	22 hrs	22 hrs	13.2	85.4
654.7	22 hrs	22 hrs	12.4	80.5
1145.8	22 hrs	22 hrs	6.1	39.3
2005.1	22 hrs	22 hrs	5.0	32.5
3508.9	22 hrs	22 hrs	0.0	0.0

Experimental groups evaluated for cytogenetic damage are shown in bold characters

* Mean value of two cultures in %

Table 5: Mitotic index; preparation interval 22 hrs with and without S9 mix

Treatment group	Conc. per mL	S9 mix	Exposure period/ Recovery	Mitotic indices*			
				Absolute 1	Absolute 2	Mean	%**
Solv. control [#]	10.0 %	-	4 / 18 hrs	14.1	14.7	14.4	100.0
Pos. control ^{##}	660.0 µg	-	4 / 18 hrs	9.9	11.0	10.5	72.6
Test item	1730.6 µg	-	4 / 18 hrs	11.8	13.0	12.4	86.1
"	3028.6 µg	-	4 / 18 hrs	11.1	13.7	12.4	86.1
"	5300.0 µg	-	4 / 18 hrs	8.9	6.0	7.5	51.7
Solv. control [#]	10.0 %	+	4 / 18 hrs	14.2	11.7	13.0	100.0
Pos. control ^{###}	2.5 µg	+	4 / 18 hrs	13.2	11.6	12.4	95.8
Test item	105.4 µg	+	4 / 18 hrs	8.7	12.4	10.6	81.5
"	184.5 µg	+	4 / 18 hrs	9.3	5.4	7.4	56.8
"	322.9 µg	+	4 / 18 hrs	4.8	4.9	4.9	37.5

* The mitotic index was determined in a sample of 1000 cells per culture of each test group in %

** For the positive control groups and the test item groups, the relative values of the mitotic index are related to the solvent controls

Deionised water

EMS

CPA

Table 6: Structural chromosome aberrations Experiment I; preparation interval 22 hrs without S9 mix: exposure period 4 hrs

Slide no.	Cells scored	% Aberrant cells			Aberrations**											
		incl.	excl.	carrying ex-changes	Gaps		Chromatid type				Chromosome type				Other	
		gaps*	gaps*		g	ig	b	f	d	ex	ib	if	id	cx	ma	cd
					Without S9 mix											
Solvent control: Deionised water 10.0 %																
1	100				0	0	0	1	0	0	0	0	0	0	0	0
2	100				1	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	1.5	1.0	0.0	1	0	1	1	0	0	0	0	0	0	0	0
Positive control: EMS 660.0 µg / mL																
1	100				0	0	7	2	0	1	0	0	0	0	0	0
2	100				1	0	6	2	0	0	0	0	0	0	0	0
1 + 2	200	8.5	8.0	0.5	1	0	13	4	0	1	0	0	0	0	0	0
Test item: 1730.6 µg / mL																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	2	0	0	0	0	0	0	0	0	0
1 + 2	200	1.0	1.0	0.0	0	0	2	0	0	0	0	0	0	0	0	0
Test item: 3028.6 µg / mL																
1	100				0	0	1	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	1	0	0	0	0
1 + 2	200	1.0	1.0	0.0	0	0	1	0	0	0	0	1	0	0	0	0
Test item: 5300.0 µg / mL																
1	100				0	0	1	1	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	1.0	1.0	0.0	0	0	1	1	0	0	0	0	0	0	0	0

* Including cells carrying exchanges

** Note: multiple aberrations may occur in a single cell, therefore, the numbers in these columns may not (nor are they intended to) correlate with the number in the columns of % Aberrant cells.

Abbreviations

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 7: Structural chromosome aberrations Experiment I; preparation interval 22 hrs with S9 mix: exposure period 4 hrs

Slide no.	Cells scored	% Aberrant cells			Aberrations**											
		incl. gaps*	excl. gaps*	carrying ex-changes	Gaps		Chromatid type				Chromosome type				Other	
					g	ig	b	f	d	ex	ib	if	id	cx	ma	cd
					With S9 mix											
Solvent control: Deionised water 10.0 %																
1	100				0	0	0	0	0	1	0	0	0	0	0	0
2	100				0	0	2	0	0	0	0	0	0	0	0	0
1 + 2	200	1.5	1.5	0.5	0	0	2	0	0	1	0	0	0	0	0	0
Positive control: CPA 2.5 µg / mL																
1	100				2	0	7	2	0	0	0	0	0	0	0	0
2	100				0	0	8	0	0	1	2	3	0	0	0	0
1 + 2	200	12.0	11.5	0.5	2	0	15	2	0	1	2	3	0	0	0	0
Test item: 105.4 µg / mL																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	1	0	0	1	0	0	0	0	0	0
1 + 2	200	1.0	1.0	0.5	0	0	1	0	0	1	0	0	0	0	0	0
Test item: 184.5 µg / mL																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	2	0	0	0	0	0	0	0	0	0
1 + 2	200	1.0	1.0	0.0	0	0	2	0	0	0	0	0	0	0	0	0
Test item: 322.9 µg / mL																
1	200				1	0	3	2	0	1	0	1	0	0	0	0
2	200				0	0	2	2	0	0	0	2	0	0	0	0
1 + 2	400	3.5	3.3	0.3	1	0	5	4	0	1	0	3	0	0	0	0

* Including cells carrying exchanges

** Note: multiple aberrations may occur in a single cell, therefore, the numbers in these columns may not (nor are they intended to) correlate with the number in the columns of % Aberrant cells.

Abbreviations

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 8: Mitotic index; preparation interval 22 hrs with and without S9 mix

Treatment group	Conc. per mL	S9 mix	Exposure period/ Recovery	Mitotic indices*			
				Absolute 1	Absolute 2	Mean	%**
Solv. control [#]	10.0 %	-	22 / 0 hrs	15.3	15.5	15.4	100.0
Pos. control ^{##}	550.0 µg	-	22 / 0 hrs	6.7	7.3	7.0	45.5
Test item	374.1 µg	-	22 / 0 hrs	12.7	13.6	13.2	85.4
"	654.7 µg	-	22 / 0 hrs	13.2	11.6	12.4	80.5
"	1145.8 µg	-	22 / 0 hrs	6.4	5.7	6.1	39.3

* The mitotic index was determined in a sample of 1000 cells per culture of each test group in %

** For the positive control groups and the test item groups, the relative values of the mitotic index are related to the solvent controls

Deionised water

EMS

Table 9: Structural chromosome aberrations Experiment II; preparation interval 22 hrs without S9 mix: exposure period 22 hrs

Slide no.	Cells scored	% Aberrant cells			Aberrations**											
		incl. gaps*	excl. gaps*	carrying ex-changes	Gaps		Chromatid type				Chromosome type				Other	
					g	ig	b	f	d	ex	ib	if	id	cx	ma	cd
					Without S9 mix											
Solvent control: Deionised water 10.0 %																
1	100				0	0	1	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	0.5	0.5	0.0	0	0	1	0	0	0	0	0	0	0	0	0
Positive control: EMS 550.0 µg / mL																
1	100				2	0	20	2	0	5	1	0	0	0	0	0
2	100				0	0	20	2	0	5	2	0	0	0	0	0
1 + 2	200	19.5	19.0	5.0	2	0	40	4	0	10	3	0	0	0	0	0
Test item: 374.1 µg / mL																
1	100				0	0	1	0	0	0	0	0	0	0	0	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	1.0	1.0	0.0	0	0	2	0	0	0	0	0	0	0	0	0
Test item: 654.7 µg / mL																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	0.5	0.5	0.0	0	0	1	0	0	0	0	0	0	0	0	0
Test item: 1145.8 µg / mL																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	0.5	0.5	0.0	0	0	1	0	0	0	0	0	0	0	0	0

* Including cells carrying exchanges

** Note: multiple aberrations may occur in a single cell, therefore, the numbers in these columns may not (nor are they intended to) correlate with the number in the columns of % Aberrant cells.

Abbreviations

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 10 Biometry – Experiment I

	Test item versus solvent control	Preparation interval	Exposure period	S9 mix	p-value
Test item	1730.6 µg/mL	22 hrs	4 hrs	-	n.c.
"	3028.6 µg/mL	22 hrs	4 hrs	-	n.c.
"	5300.0 µg/mL	22 hrs	4 hrs	-	n.c.
"	105.4 µg/mL	22 hrs	4 hrs	+	n.c.
"	184.5 µg/mL	22 hrs	4 hrs	+	n.c.
"	322.9 µg/mL	22 hrs	4 hrs	+	0.110
Positive control versus solvent control					
EMS	660.0 µg/mL	22 hrs	4 hrs	-	< 0.001 ^s
CPA	2.5 µg/mL	22 hrs	4 hrs	+	< 0.001 ^s

n.c. Not calculated as the aberration rate is equal or lower than the control rate

^s Aberration rate is statistically significantly higher than the control rate

Table 11 Biometry – Experiment II

	Test item versus solvent control	Preparation interval	Exposure period	S9 mix	p-value
Test item	374.1 µg/mL	22 hrs	22 hrs	-	0.312
"	654.7 µg/mL	22 hrs	22 hrs	-	n.c.
"	1145.8 µg/mL	22 hrs	22 hrs	-	n.c.
Positive control versus solvent control					
EMS	550.0 µg/mL	22 hrs	22 hrs	-	< 0.001 ^s

n.c. Not calculated as the aberration rate is equal or lower than the control rate

^s Aberration rate is statistically significantly higher than the control rate

APPENDICES

Appendix 1 Chromosome Abberations: Classification and Criteria

1. Gaps

Gaps are small areas of the chromosome which are unstained. The chromatids remain aligned as normal and the gap does not extend along the chromatid for a distance greater than the width of a chromatid. If the gap occurs on one chromatid only it is a chromatid gap (g).

2. Chromatid Breaks

Chromatid breaks (b) vary in appearance. The chromatid may remain aligned but show a gap which is too large to classify as a gap. Alternatively, the chromatid may be broken so that the broken fragment is displaced. In some cases, the fragment is not seen at all. A chromatid fragment (f) should be scored if the chromosome of origin cannot be identified. In addition, deletions can occur as a result of a break. The missing terminal end of a chromatid in the assessed metaphase is classified as deletion (d).

3. Chromosome breaks

Chromosome breaks (ib) are breaks in both chromatids of the chromosome. A fragment with two chromatids is formed and this may be displaced by varying degrees. Breaks are distinguished from gaps by the size of the unstained region. A chromosome break is scored if the fragment is associated with a chromosome from which it was probably derived. However, fragments are often seen in isolation and are then scored as chromosome fragments (if). In addition, isodeletions can occur as a result of a isobreak. The missing terminal end of a chromosome in the assessed metaphase is classified as isodeletion (id).

4. Exchanges

Exchanges are formed by faulty rejoining of broken chromosomes and may be of the chromosome or chromatid type. Chromatid exchanges (ex) have numerous different forms but are generally not further classified. Where multiple exchanges have occurred each exchange point is counted as one chromatid exchange. Chromosome exchanges (cx) generally appear as either a dicentric or a ring form, either of which can be associated with a fragment, which if possible should be scored as part of the exchange.

5. Multiple Aberrations

If many aberrations are present in one metaphase, the exact details may not be scorable. This is particularly the case when chromosome pulverisation (cd) occurs. If the number of aberrations is greater than 4 then the cell is classified as multiple aberrant (ma).

6. Chromosome Number

If the chromosome (centromere) number is 46 ± 1 then it is classified as a diploid cell and scored for aberrations. If less than 46 ± 1 chromosomes are counted then the cell is ignored under the assumption that some chromosomes may have been lost for technical reasons. If multiple copies of the haploid chromosome number (other than diploid) are scored then the count is recorded and the cell classified as polyploid. If the chromosomes are arranged in closely apposed pairs, i.e. 4 chromatids instead of 2, the cell is scored as endoreduplicated (e).

Appendix 2 Historical laboratory control data

Percentage of aberrant cells in human lymphocyte cultures (2010-2012)

Without S9 mix: preparation interval 22 hrs, treatment 4 hrs					Without S9 mix: preparation interval 22 hrs, treatment 22 hrs					With S9 mix: preparation interval 22 hrs, treatment 4 hrs				
	Aberrant cells (%)					Aberrant cells (%)					Aberrant cells (%)			
	No. of exp.	Range	Mean	Standard deviation		No. of exp.	Range	Mean	Standard deviation		No. of exp.	Range	Mean	Standard deviation
Solvent control					Solvent control					Solvent control				
Aqueous solv. ¹	12	0.0 – 2.5	1.1	±0.6	Aqueous solv. ¹	12	0.0 – 2.0	1.0	±0.5	Aqueous solv. ¹	19	0.0 – 3.5	1.2	±0.7
Organic solv. ²	40	0.0 – 3.0	1.3	±0.6	Organic solv. ²	36	0.0 – 2.5	1.1	±0.6	Organic solv. ²	67	0.0 – 2.5	1.1	±0.5
Total	52	0.0 – 3.0	1.2	±0.6	Total	48	0.0 – 2.5	1.1	±0.6	Total	86	0.0 – 3.5	1.1	±0.6
Positive control					Positive control					Positive control				
EMS 550-825 µg/ml	51	7.5 – 14.0	9.6	±1.3	EMS 550-825 µg/ml	48	8.0 – 40.0	17.3	±5.3	CPA 2.5-20.0 µg/ml	84	7.5 – 37.0	13.6	±3.7

¹ Aqueous solvents: deionised water (10 % v/v) and culture medium DMEM:F12

² Organic solvents: dimethyl sulfoxide (0.5 or 1.0 % v/v), acetone, ethanol and tetrahydrofurane (0.5 % v/v)

Appendix 3 Monitoring Authority Statement of GLP Compliance**Gute Laborpraxis/Good Laboratory Practice****GLP-Bescheinigung/Statement of GLP Compliance**

(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

HESSEN



Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Harlan Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according chemVwV-GLP Nr. 5.3/OECD guidance)

2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften

2 Toxicity studies

3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)

3 Mutagenicity studies

8 Analytische Prüfungen an biologischen Materialien

8 Analytical studies on biological materials

25. April, 23./25. und 26. Juli 2012

Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

**Hess. Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz,
Mainzer Straße 80 D65189 Wiesbaden**

(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority)