746

AB Enzymes GmbH – Feldbergstrasse 78 , D-64293 Darmstadt

AB Enzymes
an ABF Ingredients company

November 8, 2017

Office of Food Additive Safety (HFS-255),
Center for Food Safety and Applied Nutrition,
Food and Drug Administration,
5100 Paint Branch Parkway, College Park, MD 20740.

RE: GRAS NOTICE for maltogenic amylase enzyme preparation from *Bacillus subtilis* strain expressing maltogenic amylase from *Geobacillus stearothermophilus*

AB Enzymes GmbH, we are submitting for FDA review, Form 3667, one paper copy, and the enclosed CD, free of viruses, containing a GRAS notification for Maltogenic amylase.

The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substances as discussed in GRAS final rule, 21 CFR Part 170.30 (a)(b), subpart E.

Please contact the undersigned by telephone or email if you have any questions or additional information is required.

We look forward to your feedback.

Candice Cryne
Regulatory Affairs Manager
1 647-919-3964
Candice.cryne@abenzymes.com

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
000746	RECEIVED NOV 13 2017 OFFICE OF FOOD ADDITIVE SAFETY
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*
   ☒ New ☐ Amendment to GRN No._____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Candice Cryne	Regulatory Affairs Manager
Organization *(if applicable)*	
AB Enzymes GmbH	
Mailing Address *(number and street)*	
Feldbergstr. 78	

City	State or Province	Zip Code/Postal Code	Country
Darmstadt		D-64293	Germany

Telephone Number	Fax Number	E-Mail Address
+49(0)6151/3680-100	+49(0)6151/3680-120	Candice.cryne@abenzymes.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Organization *(if applicable)*	
Mailing Address *(number and street)*	

City	State or Province	Zip Code/Postal Code	Country

Telephone Number	Fax Number	E-Mail Address

SECTION C GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

maltogenic amylase enzyme preparation from *Bacillus subtilis* strain expressing maltogenic amyla

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☒ Paper

If applicable give number and type of physical media

1 CD

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

The maltogenic amylase enzyme is to be used in baking processes. The enzyme preparation is used at minimum levels necessary to achieve the desired effect and according to requirements under current Good Manufacturing Practices. There are no maximal limits set, just suggested dosages.

The maltogenic amylase from *Bacillus subtilis* RF12029 object of this dossier is specifically intended to be used in **b**

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☒ Yes ☒ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E PARTS 2 7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that AB Enzymes GmbH
(name of notifier)

has concluded that the intended use*(s)* of maltogenic amylase enzyme preparation from Bacillus subtilis strain expressing maltogenic an
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. AB Enzymes GmbH
(name of notifier)
agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

Feldbergstrasse 78 Darmstadt, 64293 Germany
(address of notifier or other location)

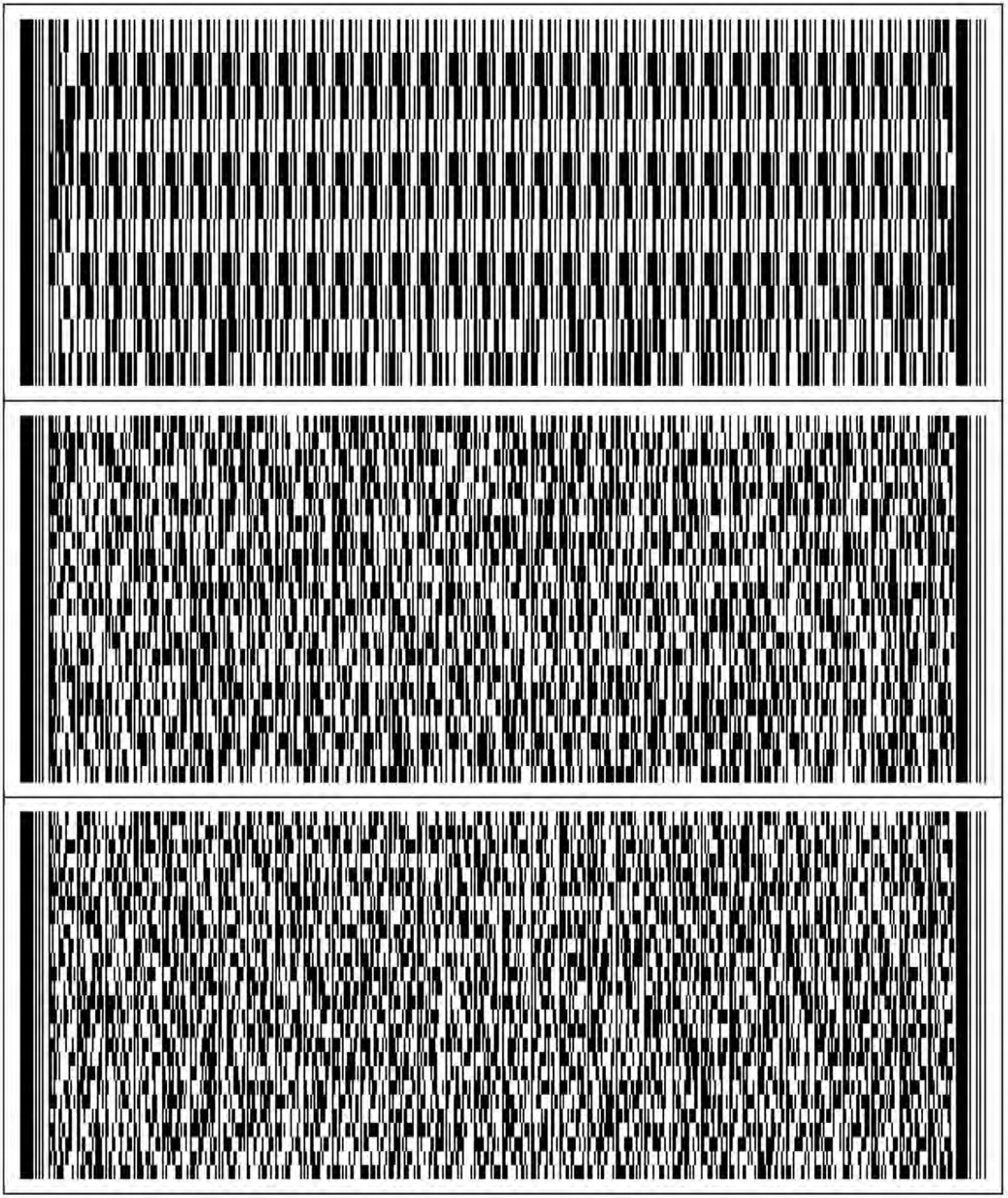
The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

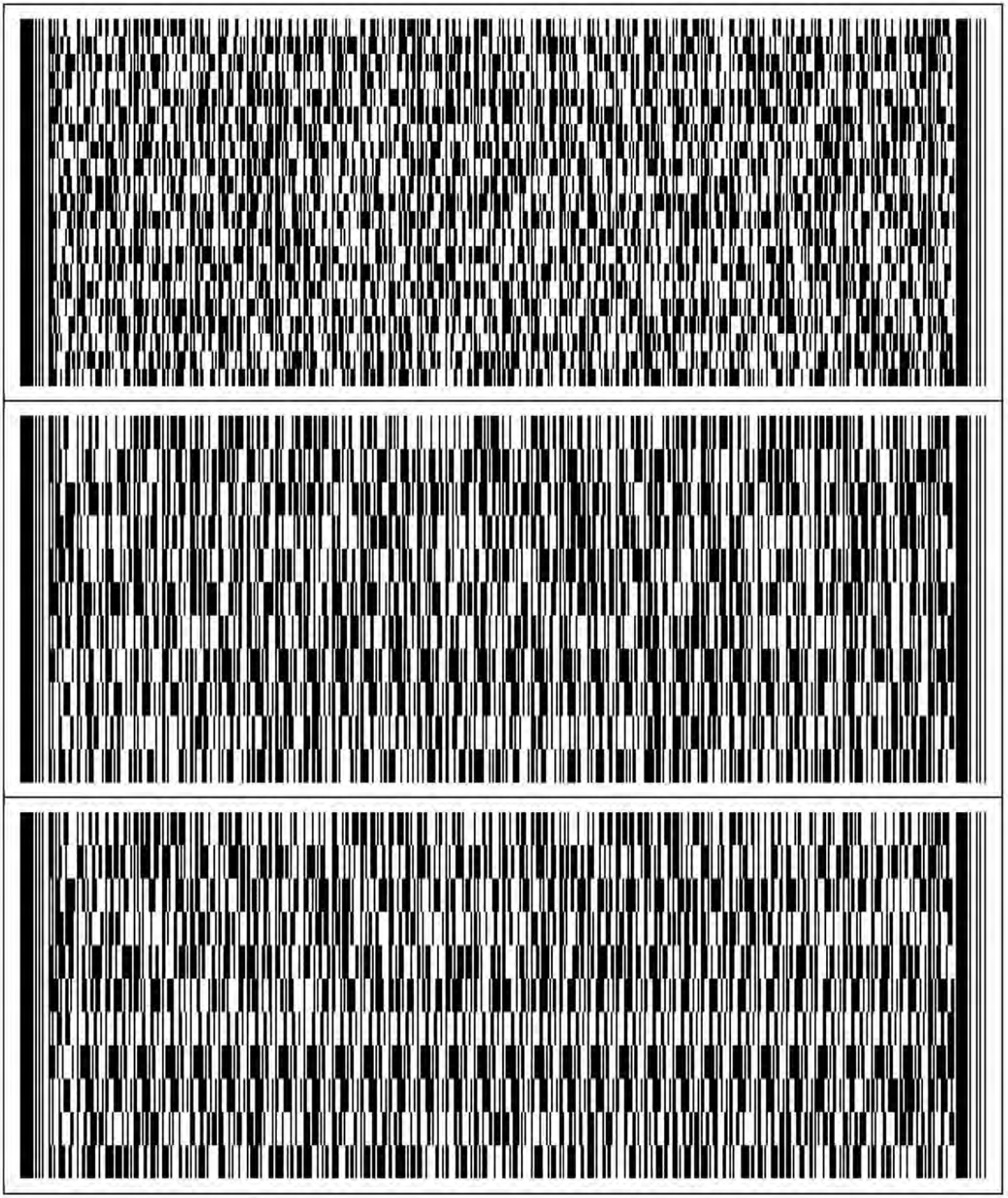
3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Candice Cryne Digitally signed by Candice Cryne Date: 2017.11.09 13:53:43 -05'00'	Candice Cryne, Manager of Regulatory Affairs	11/09/2017

SECTION G LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	1_Cover Lette	inistrativ
	2_GRAS Final Notic	issio
	Appendice	issio
	Reference	issio





AB Enzymes GmbH – Feldbergstrasse 78 , D-64293 Darmstadt

GRAS NOTIFICATION FOR MALTOGENIC AMYLASE FROM A GENETICALLY MODIFIED STRAIN OF *BACILLUS SUBTILIS*

AB ENZYMES GmbH

November 7, 2017

Contents

1 PART 1 §170.225 – SIGNED STATEMENTS AND CERTIFICATIONS

Pursuant to 21 C.F.R. Part 170, subpart E, AB Enzymes GmbH, submits a Generally Recognized as Safe (GRAS) notice and claims that the use of maltogenic amylase enzyme preparation from *Bacillus subtilis* strain expressing maltogenic amylase from *Geobacillus stearothermophilus* produced by submerged fermentation is Generally Recognized as Safe under the conditions of its intended use; therefore, they are exempt from statutory premarket approval requirements.

The name and address of the notifier:
AB Enzymes GmbH
Feldbergstr. 78
D-64293 Darmstadt, Germany

Appropriately descriptive term:
Maltogenic amylase enzyme preparation from *Bacillus subtilis* strain expressing maltogenic amylase from *Geobacillus stearothermophilus*.

Trade secret or confidential:
This notification does not contain any trade secret or confidential information.

Intended conditions of use:
The maltogenic amylase enzyme is to be used in baking processes. The enzyme preparation is used at minimum levels necessary to achieve the desired effect and according to requirements under current Good Manufacturing Practices. There are no maximal limits set, just suggested dosages.

Statutory basis for GRAS conclusion:
This GRAS determination is based upon scientific procedures.

Premarket approval:
The notified substance is not subject to the premarket approval requirements of the FD&C Act based on our conclusion that the substance is GRAS under the conditions of the intended use.

Availability of information:
A notification package providing a summary of the information which supports this GRAS determination is enclosed with this letter. The package includes a safety evaluation of the production strain, the enzyme, and the manufacturing process, as well as an evaluation of dietary exposure. Complete data and information that are the basis for this GRAS determination are

available to the Food and Drug Administration for review and copying at reasonable times (customary business hours) at a specific address set out in the notice or will be sent to FDA upon request (electronic format or on paper).

FOIA (Freedom of Information Act):

Parts 2 through 7 of this notification do not contain data or information that is exempt from disclosure under the FOIA (Freedom of Information Act).

Information included in the GRAS notification:

To the best of our knowledge, the information contained in this GRAS notification is complete, representative and balanced. It contains both favorable and unfavorable information, known to AB Enzymes and pertinent to the evaluation of the safety and GRAS status of the use of this substance.

(b) (6)

November 7, 2017

Candice Cryne — Date

Regulatory Affairs Manager

2 PART 2 §170.230 - IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS AND PHYSICAL OR TECHNICAL EFFECT OF THE NOTIFIED SUBSTANCE

2.1 Identity of the notified substance

The dossier concerns a **maltogenic amylase from a genetically modified *Bacillus subtilis*.**

2.1.1 Common name of the enzyme

Name of the enzyme protein:	Maltogenic amylase
Synonyms:	maltogenic alpha-amylase, 1,4-alpha-D-glucan alpha-maltohydrolase, glucan-1,4-alpha-maltohydrolase.
EC (IUBMB) number:	EC 3.2.1.133
Production strain:	*Bacillus subtilis RF12029*

2.1.2 Classification of the enzyme

IUBMB #	3.2.1.133
CAS number	160611-47-2

2.2 Identity of the Source

2.2.1 Recipient Strain

The recipient strain used for the genetic modifications in constructing RF12029 *Bacillus subtilis* is a genetically modified derivative of a classical *Bacillus subtilis* mutant strain.

The genus *Bacillus* is composed of rod-shaped, endospore forming bacteria that are members of the phylum Firmicutes. Owing largely to the fact that they are common inhabitants of soil and aquatic sediment, species within the genus are widespread in nature and are found in virtually

every environment. While their main roles appear to involve carbon and nitrogen cycling, some species are well known human and livestock pathogens (e.g. *Bacillus anthracis* and *Bacillus cereus*) and insect pathogens (e.g. *Bacillus thuringiensis*). However, the overwhelming majority of *Bacillus* species are non-pathogenic (as described in (Rooney et al. 2009)).

Together with *B. subtilis*, *B. amyloliquefaciens* and *B. licheniformis* these species form the "*B. subtilis* group" (Chun, Bae 2000), differing by few or no phenotypic characters and having high similarities of their 16S rRNA sequences.

As said above, the recipient strain used in the construction of the maltogenic amylase production strain RF12029 is a genetically modified derivative of a classical *Bacillus subtilis* mutant (parental strain). The original *Bacillus subtilis*, which has been isolated in year 1974 by the University of Osaka from soil and was characterized as *Bacillus subtilis* by the Deutsche Sammlung von Mikroorganismen (DSMZ). Which was further developed by conventional mutagenesis for better yield and the resulting mutant has been used in AB Enzymes since 2010 for the production of maltogenic amylase for food processing.

The identity of both the mutant parental strain and the genetically modified recipient strain was confirmed by Ribotyping in year 2009 and 2016 respectively, by the Deutsche Sammlung für Mikroorganismen (DSMZ) and both strains were classified as *Bacillus subtilis*.

Ribotyping is a method that can identify and classify bacteria based upon differences in rRNA. It generates a highly reproducible and precise fingerprint that can be used to classify bacteria from the genus through and beyond the species level. DNA is extracted from a colony of bacteria and then restricted into discrete-sized fragments. The DNA is then transferred to a membrane and probed with a region of the rRNA operon to reveal the pattern of rRNA genes. The pattern is recorded, digitized and stored in a database. The variations that exist among bacteria in both the position and intensity of generated rRNA bands can be used for classification and identification of bacteria.

Standardized, automated ribotyping was performed by DSMZ using the QualiconTM RiboPrinter system. The RiboPrinter system combines molecular processing steps of ribotyping in a stand-alone, automated instrument. Steps include cell lysis, digestion of chromosomal DNA with restriction enzymes (kits for EcoRI and PvuII), separation of fragments by electrophoresis, transfer of DNA fragments to a nylon membrane, hybridization to a probe generated from the rrnB operon from *E. coli*, chemiluminescent detection of the probe to the fragments containing rrnB operon sequences, image detection and computerized analysis of RiboPrint patterns (Bruce et al. 1995)

For further development, genetic modifications were introduced into the mutant (see steps 1-5 described in Section 2.3) to improve strain and product performance, resulting in the current recipient strain used for the construction of the maltogenic amylase production strain RF12029.

Both the mutant parental strain and the genetically modified recipient strains were identified by DSMZ by using the DuPont Identification Library with a similarity to DuPont ID DUP-12544 (*Bacillus subtilis*) of 1.00.

Therefore, the recipient can be described as followed:

Genus:	*Bacillus*
Group:	*Bacillus subtilis*
Species or subspecies:	*Bacillus subtilis*
Commercial name:	Not applicable: it is not sold as such.

2.2.2 Donor:

The strain RF12029 was constructed by a six steps transformation of the original Bacillus subtilis mutant (ie parental strain of the recipient). For further details, see section 2.3.

The synthetic maltogenic amylase gene was inserted into a plasmid consisting of backbone elements from well-known and defined cloning *Bacillus* vectors pBC16-1 (Kreft et al. 1978) and pUB110 (McKenzie et al. 1986) .The resulting expression plasmid pIK40 pyrF is devoid of any

transfer function (required for plasmid mobilization) or any sequence conferring antibiotic resistance. Vector selection is achieved using the *pyrF* gene encoding the native orotidine 5-phosphate decarboxylase from parental *B. subtilis*.

The plasmid pIK40 pyrF contains no genes conferring antibiotic resistance.

Other DNA fragments included in the expression cassette of the maltogenic amylase:

The signal sequence and the **promoter** for the maltogenic amylase are from *Bacillus amyloliquefaciens* ((Palva et al. 1981; Hofemeister et al. 1994).

Genus:	*Bacillus*
Group:	*Bacillus subtilis*
Species or Subspecies:	*Bacillus amyloliquefaciens*

The transcription terminator [is from *Thermoactinomyces vulgaris* 94-2A (Hofemeister et al. 1994).

Genus:	*Thermoactinomyces*
Species or Subspecies:	*Thermoactinomyces vulgaris*

Synthetic DNA

Maltogenic amylase gene: the maltogenic amylase gene (*amyM*) was synthesised based on the sequence published by Diderichsen, Christiansen (1988) and in patent US 6,162,628

Genus:	*Bacillus*
Species or Subspecies:	*Geobacillus stearothermophilus*

The synthetised mature maltogenic amylase sequence for expression in *B. subtilis* shows 100% identity to the original maltogenic amylase sequence from *G. stearothermophilus* (swissprot:AMYM_GEOSE P19531 *Geobacillus stearothermophilus* recname: full=maltogenic alpha-amylase; ec=3.2.1.133). The maltogenic amylase protein overproduced by RF12029 is identical in its sequence and properties to maltogenic amylase produced by the wild-type *Geobacillus*.

2.3 Genetic modification

Bacillus subtilis RF12029 was constructed for maltogenic amylase production. This was achieved by cloning the synthetic maltogenic amylase gene from *Geobacillus stearothermophilus* into plasmid pIK40 pyrF and transformation of the *B. subtilis* recipient strain.

The resulting production strain RF12029 secretes high amounts of maltogenic amylase into its culture supernatant, resulting in high maltogenic amylase activity in the cultivation broth.

The strain RF12029 was constructed by a six steps transformation of the original *Bacillus subtilis* mutant (ie parental strain of the recipient).

The transformed production strain containing the maltogenic amylase gene is *Bacillus subtilis* strain RF12029 which is deposited in the "Centraalbureau voor Schimmelcultures" (CBS) in the Netherlands with the deposit number CBS141004.

The plasmid pIK40 pyrF contains no genes conferring antibiotic resistance.

The strain **RF12029** was constructed in six genetic modification steps, as described below.

STEP 1-5: Markerless deletions in the genome of the recipient strain

The *B. subtilis* recipient strain was constructed according to the well described methods for markerless deletions in the genome of *Bacillus species* (Vehmaanperä et al. 1991; Iordănescu 1975;

Rachinger et al. 2013) to arrive at a strain with improved product stability and performance having lost its ability to sporulate.

The deletions from the genome of the original *Bacillus subtilis* mutant (ie parental strain of the recipient) and its derivatives were confirmed by PCR and sequencing and resulted in *Bacillus subtilis* recipient strain.

The recipient strain was constructed in five genetic modification steps:

After each of the five consecutive deletions it was verified that **no DNA-fragments of the deletion vector remained in the cell**).

The deletion vectors were only used for targeted deletion of native genes and are not present any more in the final recipient strain.

STEP 6: Construction of production strain RF12029 – Introduction of pIK40 pyrF into the *Bacillus subtilis* recipient strain

In the sixth step, plasmid pIK40 pyrF containing the expression cassette for the maltogenic amylase was introduced into the recipient strain by protoplast transformation according to the method of Chang, Cohen (1979). Transformants were plated on minimal agar without uracil to select for pIK40 pyrF carrying cells expressing the episomally encoded essential *pyrF*.

2.3.1 Stability of the Transformed Genetic Sequence

In practice, the fermentation process always starts from identical replicas of the RF12029 seed ampoule. Production preserves from the "Master Cell Bank" are used to start the fermentation process.

A Master Cell Bank is a collection of ampoules containing a pure culture. The cell line history and the production of a Cell Bank, propagation, preservation and storage is monitored and controlled. The MCB is prepared from a selected strain. A MCB ampoule is only accepted for production runs if its quality meets the required standards. This is determined by checking identity, viability,

microbial purity and productivity of the MCB ampoule. The accepted MCB ampoule is used as seed material for the inoculum.

The annual production starts from "Master Cell Bank" preserves. A Petri dish is inoculated from the culture collection preserve in such a way that single colonies can be selected. Altogether individual colonies are picked up from plates and inoculated into shake flasks. Care is taken to select only those colonies which present the familiar picture (same phenotype). Colonies are used for inoculating 2 rounds of shake flask cultivation. Subsequently these are combined for the inoculation of the first process bioreactor.

Mutations do not normally occur, and if so only in the vegetative state during cell division. Owing to the above-described procedure, this vegetative state of the cultures is reduced to an inevitable minimum during production.

Potential changes in the genome of the production strain or rearrangements of the plasmid could theoretically occur during the production process. Analysis via PCR-based genotypic fingerprinting performed from different independent batches of pilot-scale fermentation of RF12029 revealed that the strain stays genetically stable and the plasmid is stable over necessary time needed for industrial fermentation process of RF12029 production strain.

Additionally, testimony to the stability of the strain is also given by comparable levels of maltogenic amylase activity in a number of fermentation batches performed for the RF12029 strain. The activity measurements from parallel fermentations showed that the productivity of the RF12029 strain remains similar.

The data of the analysis of enzyme activities from preparations from different fermentation batches of the recombinant RF12029 strain is presented in Appendix #1.

2.3.2 Structure and amount of vector and/or nucleic acid remaining in the GMM

pIK40 pyrF consists of :

- Defined elements derived from plasmids pBC16-1 (Kreft et al. 1978 and pUB110. pUB110 was isolated the first time by Gryczan et al. (1978). Ever since it has been used worldwide for the cloning in *Bacilli*. pUB110 is known to be able to be maintained in *B. subtilis*, but also in *B. stearothermophilus*, *B. licheniformis, B. megaterium* and *B. pumilus*.
- The *Geobacillus stearothermophilus* maltogenic α-amylase gene coding for the mature protein fused with an appropriate signal sequence from *B. amyloliquefaciens,* a transcription terminator from *Thermoactinomyces vulgaris* 94-2A (Hofemeister et al. 1994) and preceded by a promoter from *Bacillus amyloliquefaciens* (Amsler et al. 2010; Hofemeister et al. 1994; Palva et al. 1981)
- The native *Bacillus subtilis pyrF* gene

pBC16-1 and pUB110 can be regarded as safe vectors, because of their fully known nucleotide sequence and the known biological functions of the open reading frames, which reveal no potential hazards.

No genes conferring antibiotic resistance or encoding any transfer functions are present in pIK40 pyrF.

Plasmid instabilities (e.g., structural or segregational vector instabilities) could theoretically occur and could potentially cause changes of the production strain during propagation in the production process. Structural and segregational plasmid stability of pIK40 pyrF has been demonstrated over about 200 generations. Fermentations at production level (counting from the production pre-culture onwards) typically last for maximally 150 generations.

2.3.3 Demonstration of the absence of the GMM in the product

The down-stream process following the fermentation includes unit operations to separate the production strain. The procedures are executed by trained staff according to documented standard operating procedures complying with the requirements of the quality system.

The RF12029 production strain is recovered from the fermentation broth by a widely used process that results in a cell-free enzyme concentrate. The absence of the production strain is confirmed for every production batch (Appendix #1), using an internal Roal method. This method has been validated in-house. The sensitivity of the method is 1 cfu/20 ml in liquid and 1 cfu/0,2 gram in dried semifinals.

2.3.4 Inactivation of the GMM and evaluation of the presence of remaining physically intact cells

The RF12029 enzyme preparation is free from detectable, viable production organism. As the absence of the production strain is confirmed for every production batch, no additional information regarding the inactivation of the GMM cells is required.

2.3.5 Information on the possible presence of recombinant DNA

RF12029 enzyme preparation is produced by an aerobic submerged microbial fermentation using a genetically modified *Bacillus subtilis* strain. All viable cells of the production strain, RF12029, are removed during the down-stream processing: the fermentation broth is filtered with pressure filters and subsequent sheet filters, concentrated with ultra-filtration, and optionally followed by sheet filtration(s).

After this the final product does not contain any detectable number of fungal colony forming units or recombinant DNA. Two separate food enzyme samples (concentrates from industrial scale production) were tested for the presence of recombinant DNA using highly sensitive and specific PCR techniques. No recombinant DNA (recDNA) of the production strain was shown to be present above the detection limits (Appendix #1).

2.3.6 Absence of Antibiotic Genes and Toxic Compounds

As noted above, the transformed DNA does not contain any antibiotic resistance genes. Further, the production of known mycotoxins according to the specifications elaborated by the General Specifications for Enzyme Preparations Used in Food Processing Joint FAO/WHO Expert Committee on Food Additives, Compendium of Food Additive Specifications, FAO Food and Nutrition Paper *(Food and Agriculture Organization of the United Nations 2006)* has been also tested from the fermentation products. Adherence to specifications of microbial counts is

routinely analysed. Two production batches produced by the production strain *B. subtilis* RF12029 (concentrates) were analyzed and no antibiotic or toxic compounds were detected (Appendix #1).

2.4 ENZYME PRODUCTION PROCESS

2.4.1 Overview

The food enzyme is produced by ROAL Oy[1] by submerged fermentation of *Bacillus subtilis* RF12029 in accordance with current Good Manufacturing Practices for Food (GMP) and the principles of Hazard Analysis of Critical Control Points (HACCP). As it is run in the EU, it is also subject to the Food Hygiene Regulation (852/2004).

The enzyme preparation described herein is produced by controlled fed-batch submerged fermentation. The production process involves the fermentation process, recovery (downstream processing) and formulation and packaging. Finally, measures are taken to comply with cGMPs and HACCP. The manufacturing flow-chart is presented in Appendix #2.

It should be noted that the fermentation process of microbial food enzymes is substantially equivalent across the world. This is also true for the recovery process: in a vast majority of cases, the enzyme protein in question is only partially separated from the other organic material present in the food enzyme.

2.4.2 Fermentation

The production of food enzymes from microbial sources follows the process involving fermentation as described below. Fermentation is a well-known process that occurs in food and has been used for the production of food enzymes for decades. The main fermentation steps are:

- Inoculum
- Seed fermentation
- Main fermentation

[1] See footnote 1

2.4.3 Raw materials

The raw materials used in the fermentation and recovery processes are standard ingredients that meet predefined quality standards controlled by Quality Assurance for ROAL OY. The safety is further confirmed by toxicology studies. The raw materials conform to either specifications set out in the Food Chemical Codex, 10th edition, 2016 or The Council Regulation 93/315/EEC, setting the basic principles of EU legislation on contaminants and food, and Commission Regulation (EC) No 1881/2006 setting maximum limits for certain contaminants in food. The maximum use levels of antifoam and flocculants are ≤0.15% and ≤1.5% respectively.

2.4.4 Materials used in the fermentation process (inoculum, seed and main fermentation)

- Potable water
- A carbon source
- A nitrogen source
- Salts and minerals
- pH adjustment agents
- Foam control agents

2.4.5 Inoculum

A suspension of a pure culture of RF12029 is aseptically transferred to a shake flask (1 liter) containing fermentation medium.

In order to have sufficient amount of biomass, the process is repeated several times. When a sufficient amount of biomass is obtained the shake flasks are combined to be used to inoculate the seed fermentor.

2.4.6 Seed fermentation

The inoculum is aseptically transferred to a pilot fermentor and then to the seed fermentor. The seed fermentation is run at a constant temperature and a fixed pH. At the end of fermentation, the inoculum is aseptically transferred to the main fermentation.

2.4.7 Main fermentation

Biosynthesis of the enzyme by the production strain occurs during the main fermentation.

The fermentation in the main fermentor is run as normal submerged fed-batch fermentation. The content of the seed fermentor is aseptically transferred to the main fermentor containing fermentation medium.

As in all processes, additional fermentation medium is added during the fermentation. In order to control the growth of the production organism and the enzyme production, the feed-rate of this medium is based upon a predetermined profile or on deviation from defined set points.

The fermentation process is continued for a predetermined time or until laboratory test data show that the desired enzyme production has been obtained or that the rate of enzyme production has decreased below a predetermined production rate. When these conditions are met, the fermentation is completed.

2.4.8 Recovery

The purpose of the recovery process is:

- to separate the fermentation broth into biomass and fermentation medium containing the desired enzyme protein,
- to concentrate the desired enzyme protein and to improve the ratio enzyme activity/Total Organic Substance (TOS).

During fermentation, the enzyme protein is excreted by the producing microorganism into the fermentation medium. During recovery, the enzyme-containing fermentation medium is separated from the biomass.

This Section first describes the materials used during recovery (downstream processing), followed by a description of the different recovery process steps:

- Pre-treatment
- Primary solid/ liquid separation
- Concentration
- Polish and germ filtration

The nature, number and sequence of the different types of unit operations described below may vary, depending on the specific enzyme production plant.

2.4.9 Materials

Materials used, if necessary, during recovery of the food enzyme include:

- Flocculants
- Filter aids
- pH adjustment agents

Potable water can also be used in addition to the above mentioned materials during recovery.

2.4.10 Pre-Treatment

Flocculants and/or filter aids are added to the fermentation broth, in order to get clear filtrates, and to facilitate the primary solid/liquid separation. Typical amount of filter aids is 2.5 %.

2.4.11 Primary solid/liquid separation

The purpose of the primary separation is to remove the solids from the enzyme containing fermentation medium. The primary separation is performed at a defined pH and a specific temperature range in order to minimize loss of enzyme activity.

The separation process may vary, depending on the specific enzyme production plant. This can be achieved by different operations like centrifugation or filtration.

2.4.12 Concentration

The liquid containing the enzyme protein needs to be concentrated in order to achieve the desired enzyme activity and/or to increase the ratio enzyme activity/TOS before formulation. Temperature and pH are controlled during the concentration step, which is performed until the desired concentration has been obtained. The filtrate containing the enzyme protein is collected for further recovery and formulation.

2.4.13 Polish and germ filtration

After concentration, for removal of residual cells of the production strain and as a general precaution against microbial contamination, filtration on dedicated germ filters is applied at various stages during the recovery process. Pre-filtration (polish filtration) is included if needed to

remove insoluble substances and facilitate the germ filtration. The final polish and germ filtration at the end of the recovery process results in a concentrated enzyme solution free of the production strain and insoluble substances.

2.4.14 Formulation and Packaging

Subsequently, the food enzyme is formulated. The resulting product is defined as a 'food enzyme preparation'.

The maltogenic amylase preparations from *B.subtilis* RF12029 are sold mainly as solid preparations. For all kinds of food enzyme preparations, the food enzyme is adjusted to a declared activity, standardized and preserved with food ingredients or food additives (food grade quality).

The food enzyme preparation is tested by Quality Control for all quality related aspects, like expected enzyme activity and the general JECFA Specification for Food Enzyme Preparations, and released by Quality Assurance. The final product is packed in suitable food packaging material before storage. Warehousing and transportation are performed according to specified conditions mentioned on the accordant product label for food enzyme preparations.

2.4.15 General Production Controls and Specifications

In order to comply with cGMPs and HACCP principles for food production, the following potential hazards in food enzyme production are taken into account and controlled during production as described below:

Identity and purity of the producing microorganism:

The assurance that the production microorganism efficiently produces the desired enzyme protein is of utmost importance to the food enzyme producer. Therefore, it is essential that the identity and purity of the microorganism is controlled.

Production of the required enzyme protein is based on a well-defined Master (MCB) and Working Cell Bank (WCB). A Cell Bank is a collection of ampoules containing a pure culture. The cell line history and the production of a Cell Bank, propagation, preservation and storage is monitored and controlled. The MCB is prepared from a selected strain. The WCB is derived by sub-culturing

of one or more ampoules of the MCB. A WCB is only accepted for production runs if its quality meets the required standards. This is determined by checking identity, viability, microbial purity and productivity of the WCB. The accepted WCB is used as seed material for the inoculum.

Microbiological hygiene:

For optimal enzyme production, it is important that hygienic conditions are maintained throughout the entire fermentation process. Microbial contamination would immediately result in decreased growth of the production organism, and consequently, in a low yield of the desired enzyme protein, resulting in a rejected product.

Measures utilized by ROAL OY to guarantee microbiological hygiene and prevent contamination with microorganisms ubiquitously present in the environment (water, air, raw materials) are as follows:

- Hygienic design of equipment:
    - all equipment is designed, constructed and used to prevent contamination by foreign micro-organisms
- Cleaning and sterilization:
    - Validated standard cleaning and sterilization procedures of the production area and equipment: all fermentors, vessels and pipelines are washed after use with a CIP-system (Cleaning in Place), where hot caustic soda and nitric acid are used as cleaning agents. After cleaning, the vessels are inspected manually; all valves and connections not in use for the fermentation are sealed by steam at more than 120°C; critical parts of down-stream equipment are sanitized with disinfectants approved for food industry
- Sterilization of all fermentation media:
    - all the media are sterilized with steam injection in fermentors or media tanks (at 121°C for at least 20 min at pH 4.3 – 4.8.).
- Use of sterile air for aeration of the fermentors:
    - Air and ammonia water are sterilized with filtration (by passing a sterile filter).

- Hygienic processing:
    - Aseptical transfer of the content of the WCB ampoule, inoculum flask or seed fermentor
    - Maintaining a positive pressure in the fermentor
- Germ filtration

In parallel, hygienic conditions in production are furthermore ensured by:

- Training of staff:
    - all the procedures are executed by trained staff according to documented procedures complying with the requirements of the quality system.
- Procedures for the control of personal hygiene
- pest control
- Inspection and release by independent quality organization according to version-controlled specifications
- Procedures for cleaning of equipment including procedures for check of cleaning efficiency (inspections, flush water samples etc.) and master cleaning schedules for the areas where production take place
- Procedures for identification and implementation of applicable legal requirements
- Control of labelling
- Requirements to storage and transportation

Chemical contaminants:

It is also important that the raw materials used during fermentation are of suitable quality and do not contain contaminants which might affect the product safety of the food enzyme and/or the optimal growth of the production organism and thus enzyme yield.

It is ensured that all raw materials used in production of food enzymes are of food grade quality or have been assessed to be fit for their intended use and comply with agreed specifications.

In addition to these control measures in-process testing and monitoring is performed to guarantee an optimal and efficient enzyme production process and a high quality product (cGMPs). The whole process is controlled with a computer control system which reduces the probability of human errors in critical process steps.

These in-process controls comprise:

Microbial controls:

Absence of significant microbial contamination is analyzed by microscopy or plate counts before inoculation of both the seed and main fermentation and at regular intervals and at critical process steps during fermentation and recovery.

Monitoring of fermentation parameters may include:

- pH
- Temperature
- Aeration conditions

The measured values of these parameters are constantly monitored during the fermentation process. The values indicate whether sufficient biomass or enzyme protein has been developed and the fermentation process evolves according to plan.

Deviations from the pre-defined values lead to adjustment, ensuring an optimal and consistent process.

Enzyme activity and other relevant analyses (like dry matter, refraction index or viscosity):

This is monitored at regular intervals and at critical steps during the whole food enzyme production process.

2.4.16 Stability of the enzyme during storage and prior to use

Food enzymes are formulated into various enzyme preparations in order to obtain standardized and stable products. The stability thus depends on the type of formulation, not on the food enzyme as such.

The date of minimum durability or use-by-date is indicated on the label of the food enzyme preparation. If necessary, special conditions of storage and/or use will also be mentioned on the label.

2.5 Composition and specifications

2.5.1 Characteristics of the enzyme preparation

The characteristics of the enzyme preparation are:

Property	Requirement	
Activity	min.	10500
Appearance	Solid, light beige	
Density	1.0 – 1.1 g/ml	

2.5.2 Formulation of a typical enzyme preparation

Composition	
Constituent	**%**
Maltogenic amylase	13.2%
Sunflower oil	0.4
Maltodextrin	80.8%
Sodium chloride	5.6%

2.5.3 Purity and identity specifications of the enzyme preparation

It is proposed that the food enzyme maltogenic amylase should comply with the internationally accepted JECFA specifications for chemical and microbiological purity of food enzymes (Food and Agriculture Organization of the United Nations 2006):

Lead: Not more than 5 mg/kg

Salmonella sp.: Absent in 25 g of sample

Total coliforms:	Not more than 30 per gram
Escherichia coli:	Absent in 25 g of sample
Antimicrobial activity:	Not detected
Mycotoxins:	No significant levels[2]

The proof that the food enzyme maltogenic amylase complies with these specifications is shown by the analyses on 2 different batches (see Appendix #1) and summarised below:

Batch #	***161140422***	***161132676***
Lead (mg/kg)	<0.05	<0.05
Salmonella sp. (per 25 g)	Not detected	Not detected
Total coliforms (per g)	<10	<10
Escherichia coli (per 25 g)	Not detected	Not detected
Antimicrobial activity	Not detected*	Not detected*

2.5.4 Composition of the enzyme preparation

Batch Number	***161140422***	***161132676***	**Mean**
Ash (%)	1.17	1.62	1.4
Water (%)	90.1	86.7	88.4
Protein (%)	2.67	2.95	2.81
TOS (%)	8.7	11.7	10.2
Activity (MAZ/g concentrate)	5,550	5,620	5,585
Activity/mg TOS	63,793	48,034	55,913

[2] See JECFA specifications, ftp://ftp.fao.org/docrep/fao/009/a0675e/a0675e00.pdf, page 64: Although nonpathogenic and nontoxigenic microorganisms are normally used in the production of enzymes used in food processing, several fungal species traditionally used as sources of enzymes are known to include strains capable of producing low levels of certain mycotoxins under fermentation conditions conducive to mycotoxin synthesis. Enzyme preparations derived from such fungal species should not contain toxicologically significant levels of mycotoxins that could be produced by these species. Also see Section 3.2.1.2.4.

The typical batch sizes range from 1 000 L to 150 000 L and are deeply depending on the market demand. Therefore, the frequency and the volume of production of the food enzyme vary. AB Enzymes is a small to mid-size company and this enzyme has only recently been developed. So far, the current market demand for this specific enzyme has not justified more than 2 full-scale fermentations and AB Enzymes is unfortunately not in the situation to have a wider range of batches available for comparisons. This explains the reduced number of samples that have been analysed for the purpose of this dossier.

TOS values were calculated using the following formula: % TOS = 100 % - (% Ash + % Moisture + % Diluents) as recommended by JECFA. The 2 samples do not contain any diluents.

2.6 Enzymatic Activity

The main activity of the enzyme preparation is maltogenic amylase (IUB 3.2.1.133).

Maltogenic amylase catalyses the hydrolysis of α (1-4) glycosic bonds in polysaccharides so as to remove successive α-maltose residues from the non-reducing ends of the chains.

The substrates for maltogenic amylase are starch and related polysaccharides and oligosaccharides.

The starch molecule consists of a large number of glucose units joined by glycosidic bonds. It is produced by all vegetables as an energy store. Starch is a carbohydrate extracted from agricultural raw materials which is widely present in literally thousands of everyday food (and non-food) applications. It is the most important carbohydrate in the human diet. For this purpose, starch is used chemically and enzymatically processed into a variety of different products such as starch hydrolysates, glucose syrups, fructose, maltodextrin derivatives or cyclodextrins, used in food industry.

Consequently, the substrates for maltogenic amylase occurs naturally in nature and is therefore a natural part of the human diet.

Reaction products: as a result of the catalytic activity of maltogenic amylase, maltose (disaccharide formed from 2 units of glucose joined with an α (1→4) bond) is formed (Outtrup, Norman 1984).

Maltose is naturally present in spelt, kamut and sweet potatoes and in general is found in germinating cereal seeds (e.g. wheat, barley, rye, oat, triticale) as they break down their starch stores to use for food, which is why it was named after malt.

When starchy foods such as cereal grains, corn, potatoes, legumes, nuts and some fruits and vegetables are digested, maltose results. Maltose is as well created in the malting process when making beer and when distilling malt alcohol. During beer production, grains such as barley are germinated and dried to encourage the breakdown of starch into sugars, including maltose. The use of malted cereal products (e.g. malt flour) is a common practice for the production of certain bakery products.

Consequently, adverse effects are not to be expected.

The method to analyse the activity of the enzyme is company specific and is capable of quantifying maltogenic amylase activity as defined by its IUBMB classification. The enzyme activity is usually reported in MANU/g or MAZ/g.

2.6.1 Side activities of the enzyme protein which might cause adverse effects

Food enzymes are biological concentrates containing – apart from the desired enzyme protein (expressing the activity intended to perform a technological purpose in a certain food process, also called 'main enzyme activity') - also some other substances. This is the reason why JECFA developed the TOS concept for food enzymes and why it is important that the source of a food enzyme is safe.

These other substances may include various enzyme activities (defined as 'side activities') derived from the producing microorganism. Like all living cells, microorganisms produce a variety of enzymes responsible for the hundreds of metabolic processes that sustain their life. As

microorganisms do not possess a digestive system, many enzymes are excreted to digest the material on which the microorganisms grow. Most of these enzymes are hydrolases that digest carbohydrates, proteins and lipids (fats). These are the very same activities that play a role in the production of fermented food and in the digestion of food by – amongst others – the intestinal micro flora in the human body. In addition, if a food raw material contains a certain substrate (e.g. carbohydrate, protein or lipid), then, by nature, it also contains the very same enzymatic activities that break down such a substrate; e.g. to avoid its accumulation. Consequently, the presence in food of such enzyme activities and of the potential reaction products is not new and should not be of any safety concern. In addition, it is generally accepted that the enzyme proteins themselves do not pose any safety concern either.

Apart from maltogenic amylase, the food enzyme also contains other enzymatic side activities in small amount which are naturally and typically produced by the production organism *Bacillus subtilis*, mainly xylanase, lipase, protease and endo-glucanase.

As far as AB Enzymes is aware, the maltogenic amylase described in this dossier does not possess any enzymatic side activities which might cause adverse effects.

2.7 Allergenicity

As some enzymes manufactured for use in food have been reported to cause inhalation allergy in workers exposed to enzyme dust in manufacturing facilities, maltogenic amylase may also cause such occupational allergy in sensitive individuals. However, the possibility of an allergic reaction to the maltogenic amylase residues in food seems remote. In order to address allergenicity by ingestion, it may be taken into account that:

- The allergenic potential of enzymes was studied by Bindslev-Jensen et al. (2006) and reported in the publication: "*Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry*". The investigation comprised enzymes produced by wild-type and genetically modified strains as well as wild-type enzymes and

Protein Engineered variants and comprised 400 patients with a diagnosed allergy to inhalation allergens, food allergens, bee or wasp. It was concluded from this study that ingestion of food enzymes in general is not likely to be a concern with regard to food allergy.

- Previously, the AMFEP Working Group on Consumer Allergy Risk from Enzyme Residues in Food performed an in-depth analysis of the allergenicity of enzyme products (Daurvin et al. 1998). The overall conclusion was that – as opposed to exposure by inhalation – there are no scientific indications that the small amounts of enzymes in food can sensitize or induce allergy reactions in consumers.
- Enzymes when used as digestive (Abad et al. 2010) aids are ingested daily, over many years, at much higher amounts when compared to enzymes present in food (up to 1 million times more).

Thus, there are no scientific indications that small amounts of enzymes in food can sensitize or induce allergic reactions in consumers.

Additional considerations supporting the assumptions that the ingestion of an enzyme protein is not a concern for food allergy should also be taken into account:

- The majority of proteins are not food allergens and based on previous experience, the enzyme industry is not aware of enzyme proteins used in food being homologous to known food allergens[3].
- The food enzyme is used in small amounts during food processing resulting in very small amounts of the enzyme protein in the final food. A high concentration generally equals a higher risk of sensitization, whereas a low level in the final food equals a lower risk (Goodman et al. 2008).
- In the case where proteins are denatured which is the case for this enzyme due to the food process conditions, the tertiary conformation of the enzyme molecule is destroyed.

[3] The only enzyme protein used in food an known to have a weak allergenic potential is egg lysozyme

In general, these alterations in conformation are associated with decrease in the antigenic reactivity in humans: in the vast majority of investigated cases, denatured proteins are much less immunogenic than the corresponding native proteins (Valenta 2002; Valenta, Kraft 2002; Takai et al. 1997; Takai et al. 2000; Nakazawa et al. 2005; Kikuchi et al. 2006).

- In addition, residual enzyme proteins still present in the final food will be subjected to digestion in the gastro-intestinal system, which reduces further the risk of enzyme allergenicity. While stability to digestion is considered as a potential risk factor of allergenicity, it is believed that small protein fragments resulting from digestion are less likely to be allergenic (Food and Agriculture Organization of the United Nations January/2001; Goodman et al. 2008).
- Finally, enzymes have a long history of safe use in food processing, with no indication of adverse effects or reactions. Moreover, a wide variety of enzyme classes (and structures) are naturally present in food. This is in contrast with most known food allergens, which are naturally present in a narrow range of foods.

2.7.1 Allergenicity Search

In order to specifically evaluate the risk that the maltogenic amylase enzyme would cross react with known allergens and induce a reaction in an already sensitized individual, sequence homology testing to known allergens was performed. This test used a 80 amino acid (aa) sliding window search as well as conventional FASTA alignment (overall homology), with the threshold of 35% homology as recommended in the most recent literature (Food and Agriculture Organization of the United Nations January/2001; Ladics et al. 2007; Goodman et al. 2008).

A sequence homology comparison test was then performed using a database of allergens from the Food Allergy Research and Resource Program (FARRP), University of Nebraska, Allergen Database (Version 17, January 18, 2017), which contains the amino acid sequences of known and putative allergenic proteins.

The resulting alignments of the mature maltogenic amylase protein to any allergenic proteins in the allergen database showed an identity of 28.4% with Taka-amylase A precursor (Taa-G 1; gi166531) produced by the fungal species Aspergillus oryzae. Aalberse suggested "cross-reactivity is rare below 50% amino acid identity and in most situations requires more than 70% identity" (Aalberse 2000) making it unlikely that the maltogenic amylase in question can be presumed to be allergenic based on full-length sequence relatedness to known allergens.

Using the 80-mer sliding window analysis the maltogenic amylase protein sequence, identity matches higher than 35% were found for 4 proteins: Alpha amylase A type-1/2 precursor from Aspergillus oryzae (gi94706935), Taka-amylase A (Taa-G1) precursor from Aspergillus oryzae (gi166531), glycoside hydrolase family 1 from Schizophyllum commune (gi302681819) and probable maltase from Aedes aegypti (gi126713). The highest identity (42.54%) was for a 80-mer amino acid stretch from Aspergillus oryzae amylase. As recommended by the FAO/WHO, a possible cross-reactivity has to be considered, when there is more than 35% identity in the amino acid sequence of the expressed protein using an 80 amino acids window and a suitable gap penalty (Food and Agriculture Organization of the United Nations January/2001). This recommendation was challenged however recently. According to Ladics et al. (2007) by comparing the predictive value of a full-length (conventional) FASTA search to the 80-mer analysis "a conventional FASTA search provides more relevant identity to the query protein and better reflects the functional similarities between proteins. It is recommended that the conventional FASTA analysis be conducted to compare identities of proteins to allergens". This judgement on the predictive inferiority of the 80-mer (35% threshold) approach was supported recently by Goodman, Tetteh (2011) who suggested "because the purpose of the bioinformatics search is to identify matches that may require further evaluation by IgE binding, full-length sequence evaluation or an increase in the threshold from 35% identity toward 50% for the 80 amino acid alignment should be considered" (Goodman, Tetteh 2011). Using the latter recommendation the maltogenic amylase in question would be below threshold even using the 80-mer sliding window approach.

In addition, the maltogenic amylase protein sequence showed no perfect match to any known allergen when searching for a straight stretch of eight amino acids that could serve as potential IgE binding sites.

In summary therefore the bioinformatics approach to estimate potential allergenicity based on relatedness to known allergens and taking into account the most recent scientific recommendations on the interpretation of such data leads us to conclude that the maltogenic amylase produced by *Bacillus subtilis* RF12029 is of no concern.

Conclusion:

Based on the results obtained from the bioinformatics approach to estimate potential allergenicity on relatedness to known allergens and taking into account the most recent scientific recommendations on the interpretation of such data, and based on the fact that the enzyme is typically denatured during the food manufacturing process and that any residual enzyme still present in the final food will be subject to digestion in the gastro-intestinal system, it is not likely that the maltogenic amylase produced by *Bacillus subtilis* RF12029 under evaluation will cause allergic reactions after ingestion of food containing the residues of these enzymes.

Furthermore, the final enzyme preparation does not contain any major food allergen from the fermentation media.

2.8 Technological purpose and mechanism of action of the enzyme in food

Like any other enzyme, maltogenic amylase acts as a biocatalyst: with the help of the enzyme, a certain substrate is converted into a certain reaction product. It is not the food enzyme itself, but the result of this conversion that determines the effect in the food or food ingredient. After the conversion has taken place, the enzyme no longer performs a technological function.

The **substrates** for maltogenic amylase are starch and related polysaccharides and oligosachharides. The starch molecule consists of a large number of glucose units joined by

glycosidic bonds. It is produced by all vegetables as an energy store. Starch is a carbohydrate extracted from agricultural raw materials which is widely present in literally thousands of everyday food (and non-food) applications. It is the most important carbohydrate in the human diet. For this purpose, starch is used chemically and enzymatically processed into a variety of different products such as starch hydrolysates, glucose syrups, fructose, maltodextrin derivatives or cyclodextrins, used in food industry.

Consequently, the substrate for maltogenic amylase occurs naturally in nature and is therefore a natural part of the human diet.

The **function** of maltogenic amylase is to catalyse the hydrolysis of α (1→4) glycosidic bonds in polysaccharides so as to remove successive α-maltose residues from the non-reducing ends of the chains.

Reaction products: as a result of the catalytic activity of maltogenic amylase, maltose (disaccharide formed from 2 units of glucose joined with an α (1→4) bond) is formed (Outtrup, Norman 1984). Maltose is naturally present in spelt, kamut and sweet potatoes and in general is found in germinating cereal seeds (e.g. wheat, barley, rye, oat, triticale) as they break down their starch stores to use for food, which is why it was named after malt.

When starchy foods such as cereal grains, corn, potatoes, legumes, nuts and some fruits and vegetables are digested, maltose results. Maltose is as well created in the malting process when making beer and when distilling malt alcohol. During beer production, grains such as barley are germinated and dried to encourage the breakdown of starch into sugars, including maltose. The use of malted cereal products (e.g. malt flour) is a common practice for the production of certain bakery products.

Consequently, adverse effects on nutrients are not to be expected.

The maltogenic amylase from *Bacillus subtilis* RF12029 object of this dossier is specifically intended to be used in **baking** (e.g. bread, bread buns, tortillas, crackers, sweat bake potatoes)**.**

In this processes, the maltogenic amylase is used as a processing aid in food manufacturing and is not added directly to final foodstuffs.

The baking industry is a large consumer of starch and starch-modifying enzymes. Amylases have been used in baking cereal based processes for decades (especially alpha-amylases) and their use in the bakery industry is continuously increasing. In the late eighties, maltogenic amylases, as well as other enzymes active on starch, have been suggested to act on bread staling.

Since alpha-amylases cause stickiness of baked goods, especially when overdosed, it was suggested that these problems could be solved using an exoamylase, since they do not produce the branched maltooligosaccharides of DP20-100. Such enzymes produce linear oligosaccharides of 2–6 glucose residues. In particular, maltogenic amylases produces maltose and modifies starch at a temperature when most of the starch starts to gelatinize, therefore delaying retrogradation of the starch compound ((Diderichsen, Christiansen 1988).

This application has been specifically approved for a number of years in Denmark and France, which – together with its use for a significant number of years in a number of EU countries- demonstrates the technological need of maltogenic amylase in this food process.

Below, the benefits of the use of industrial maltogenic amylase in baking are described.

The beneficial effects are of value to the food chain because they lead to better and/or more consistent product characteristics by reducing the rate of staling during storage. Moreover, the application leads to more effective production processes, resulting in better production economy. The reduced staling rate results in less waste bread which results in environmental benefits such as more efficient use of agricultural raw materials, and the reduction of green-house gas emissions by savings in energy consumption in milling and baking and by reduced transportation (Ulber, Sell 2007).

BAKING PROCESS

Maltogenic amylase can be used in the manufacturing of bakery products such as, but not limited to bread, steamed bread, bread buns, tortillas, cakes, pancakes and waffles.

Bread baking starts with dough preparation by mixing flour, water, yeast and salt and possibly additives. Flour consists mainly of gluten, starch, non-starch polysaccharides and lipids.

Immediately after dough preparation, the yeast starts to ferment the available sugars into alcohols and carbon dioxide, which causes rising of the dough. Amylases can be added to the dough to degrade the damaged starch in the flour into smaller dextrins, which are subsequently fermented by the yeast.

After rising, the dough is baked. When the bread is removed from the oven, a series of changes start. These changes include increase of crumb firmness, loss of crispness of the crust, decrease in moisture content of the crumb and loss of bread flavor. All undesirable changes that do occur upon storage together are called staling. Staling is of considerable economic importance for the baking industry since it limits the shelf life of baked products.

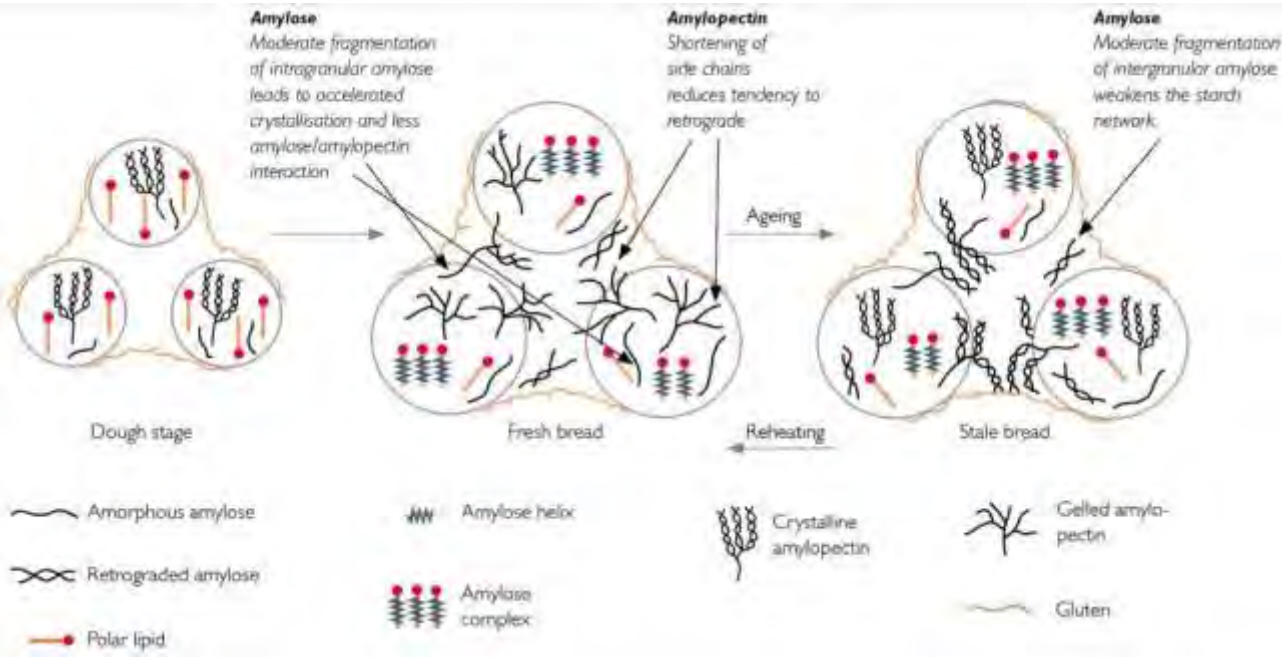
Staling is a highly complex phenomenon with firming being the most well-known and important symptom (Gray, Bemiller 2003).

During the dough stages of baking, most of the starch in the flour is in semi-crystalline granules. As higher temperatures are reached in the oven the granular starch begins to gelatinize – to absorb water, swell and lose crystallinity. As the granules begin to rupture, much of the highly soluble amylose is leached out of the granule into the open matrix of the bread.

After baking, as the bread cools, the solubilized amylose retrogrades or recrystallizes within few hours. This is an intermolecular association in which the long, linear amylose chain hydrogen-bond to form an ordered, very stable array. At the same time, the amylose will complex with polar lipids (either naturally occurring or adjunct added). Together, these restructurings are responsible for the oven set of the bread.

After this initial rapid retrogradation of the amylose, a much slower rate of retrogradation of the amylopectin occurs. During storage, an extensive, partially crystalline, permanent amylopectin network is formed, with junction zones formed by intermolecular recrystallization of amylopectin branches. This network further matures during storage, thereby increasing size and number of both inter- and intramolecular crystalline zones and, hence contributes to increased crumb firmness (Goesaert et al. 2009b).

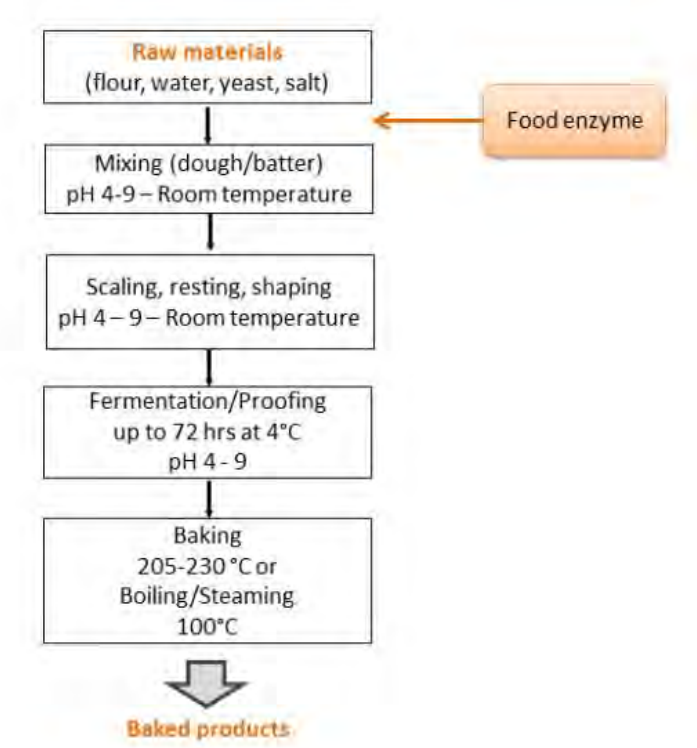
Thus, retrogradation (recrystallization) of the starch fraction in bread is considered very important in staling (Kulp et al. 2009). Especially the extent of amylopectin retrogradation correlates strongly with the firming rate of bread.



By degrading the outer amylopectin branches to a large extent, and releasing malto-oligosaccharides (maltose) during baking, maltogenic amylase forms a high level of very short amylopectin chains. Short amylopectin chains are correlated with reduced amylopectin retrogradation. Due to the action of maltogenic amylase the outer chains of amylopectin becomes too short to crystallize, and crystalline junction zone formation is inhibited. As a consequence, the formation of a permanent amylopectin network during storage is largely prevented, and the

networks of soft, freshly bread is retained, and the bread staling is reduced (Goesaert et al. 2009a; Goesaert et al. 2009b).

The process flows of the baking process is presented below:



Therefore, **the benefits of the conversion of starch with the help of maltogenic amylase in baking** can be summarised as follows:

- Reduce capability of amylopectin retrogradation, by shortening the amylopectin chain (down to the branch points) during the baking process.

Beside the main intention to modify the starch structure of the dough (shortening the amylopectin chain structure), some beneficial effects may be associated with effects on the final food, which are however not exclusively obtainable by means of enzyme treatment: they can be achieved without the use of enzymes through e.g. modified, maybe more expensive, production processes, the use of chemicals or recipe changes:

- Ensure an improved / uniformed/ softer / more elastic and less gummy-sticky crumb structure of the bakery product, which might otherwise be impaired by fluctuating processing of the bakery products;
- Possible effects are less product variation, ensuring uniform/standardised quality products;
- All this leading to improved eating quality that would ensure a better consumer acceptability of the final products.

Use of maltogenic amylase in baking ensures a maximum compatibility with modern industrial processes (also leading to less product variations, hereby ensuring standardised quality products). The enzyme is technologically justified and has been demonstrated to be effective in achieving its stated purpose. Adequate assurance is also provided that the enzyme in the form and amounts prescribed are consistent with achieving its technological function.

Maltogenic amylase performs its technological function during the first steps of the baking process (when temperatures rise in the oven). The maltogenic amylase is denatured by heat during baking (when higher temperatures above 80°C are raised) and has no further technological effect after baking.

2.9 Use Levels

Commercial food enzyme preparations are generally used following the *Quantum Satis* (QS) principle, i.e. at a level not higher than the necessary dosage to achieve the desired enzymatic reaction – according to Good Manufacturing Practice. The amount of enzyme activity added to the raw material by the individual food manufacturer has to be determined case by case, based on the desired effect and process conditions.

Therefore, the enzyme manufacturer can only issue a recommended enzyme dosage range. Such a dosage range is the starting point for the individual food producer to fine-tune his process and

determine the amount of enzyme that will provide the desired effect and nothing more. Consequently, from a technological point of view, there are no 'normal or maximal use levels' and maltogenic amylase is used according to the QS principle. A food producer who would add much higher doses than the needed ones would experience untenable costs as well as negative technological consequences.

The dosage of a food enzyme depends on the activity of the enzyme protein (in this case maltogenic amylase) present in the final food enzyme preparation (i.e. the formulated food enzyme). However, the activity Units as such do not give an indication of the amount of food enzyme actually added.

Microbial food enzymes contain, apart from the enzyme protein in question, also some substances derived from the producing microorganism and the fermentation medium. The presence of all organic materials is expressed as Total Organic Solids (TOS). From a safety point of view, the dosage on basis of TOS is more relevant. It must also be noted that the methods of analysis and the expression of the Units are company specific. Consequently, in contrast to when the amount is expressed in TOS the activity Units of a certain enzyme cannot be compared when coming from different companies. Because of these reasons, the use levels are expressed in TOS in the Table below.

The Table below shows the range of recommended use levels for each application where the maltogenic amylase is used:

Food Application	**Raw material (RM)**	**Suggested recommended use levels (mg TOS/kg RM)**
Baking and other cereal based processes	Flour	20

2.10 Effect of the presence of (residues of) the food enzyme on the final food

It is not the food enzyme itself, but the result of the enzymatic conversion that determines the effect in the food or food ingredient (including raw materials). This effect remains, irrespective of whether the food enzyme is still present or removed from the final food.

Maltogenic amylase performs its technological function during food processing. In some cases, the enzyme may no longer be present in the final food. In other cases, where the enzyme protein is still present in the final food, it does not perform any technological function in the final food, just like the endogenous maltogenic amylase present in the cereal-based raw materials and ingredients.

In order to be able to perform a technological function in the final food, a number of conditions have to be fulfilled at the same time:

- the enzyme protein must be in its 'native' (non-denatured) form, AND
- the substrate must still be present, AND
- the enzyme must be free to move (able to reach the substrate), AND
- conditions like pH, temperature and water content must be favourable

In baking, maltogenic amylase performs its technological function during the first steps of the baking process (when temperatures rise in the oven). The maltogenic amylase is denatured by heat during baking (when higher temperatures above 80°C are raised) and has no further technological effect after baking.

Based on the conditions of use and the activity of maltogenic amylase under such conditions, it can be concluded the presence of (residues of) enzyme maltogenic amylase in the final food does not lead to an effect in or on the final foods.

3 PART 3 § 170.325 - DIETARY EXPOSURE

The most appropriate way to estimate the human consumption in the case of food enzymes is using the so-called Budget Method *(Hansen 1966; Douglass et al. 1997)*. This method enables to calculate a Theoretical Maximum Daily Intake (TMDI) based on conservative assumptions regarding physiological requirements for energy from food and the energy density of food rather than on food consumption survey data.

The Budget Method was originally developed for determining food additive use limits and is known to result in conservative estimations of the daily intake.

The Budget Method is based on the following assumed consumption of important foodstuffs and beverages (for less important foodstuffs, e.g. snacks, lower consumption levels are assumed):

Average consumption over the course of a lifetime/kg body weight/day 0.025	Total solid food (kg)	Total non-milk beverages (l)	Processed food (50% of total solid food) (kg)	Soft drinks (25% of total beverages) (l)
	0.025	0.1	0.0125	0.025

For the calculation of the TMDI, the maximum use levels are chosen. Furthermore, the calculation takes into account how much food or beverage is obtained per kg raw material (see below the table) and it is assumed that all the TOS will end up in the final product.

Applications		Raw material (RM)	Suggested recommended use level (mg TOS/kg RM)	Final food (FF)	Ratio RM/FF*	Suggested level in final food (mg TOS/kg food)
SOLID FOODS	Baking	Flour	20	Baked products, Pastas and noodles…	0.71	14.2

* *Assumptions behind ratios of raw material to final food:*

Baking:

Bakery products fall in the category of solid foods.

Flour is the raw material for bakery product and the yield will vary depending on the type of final food produced.

From 1 kg of flour you would have 4 kg of cakes, 1.4 kg of bread or 1.1 kg of cracker. Cracker may represent the most conservative input from the bakery processes. However, consumption of bread is higher than that of cracker, this is why bread is used as the assumption for the calculation of dietary exposure from bakery processes.

The yield of 1.4 kg of bread per 1 kg of flour correspond to a RM/FF ratio of 0.71 kg of flour per kg bakery product is used.

The Total TMDI can be calculated on basis of the maximal values found in food and beverage (in this case, the enzyme won't be used in a process leading to liquid food, so the maximal value is found in baked products) multiplied by the average consumption of food and beverage/kg body weight/day.

The Total TMDI will consequently be calculated as follows:

TMDI in food (mg TOS/kg body weight/day)	TMDI in beverage (mg TOS/kg body weight/day)	Total TMDI (mg TOS/kg body weight/day)
14.2 x 0.0125 = 0.178	0 x 0,025 = 0	**0.178**

It should be stressed that this Total TMDI is based on conservative assumptions and represents a highly exaggerated value because of the following reasons:

- It is assumed that ALL producers of the above mentioned foodstuffs use the specific enzyme maltogenic amylase from *Bacillus subtilis* RF12029;
- It is assumed that ALL producers apply the HIGHEST use level per application;
- For the calculation of the TMDI's in food and in beverages, only THOSE foodstuffs and beverages were selected containing the highest theoretical amount of TOS.
- Thus, foodstuffs and beverages containing lower theoretical amounts were not taken into account;
- It is assumed that the amount of TOS does not decrease as a result of the food production process;
- It is assumed that the final food containing the calculated theoretical amount of TOS is consumed DAILY over the course of a lifetime;
- Assumptions regarding food and beverage intake of the general population are overestimates of the actual average levels (Douglass et al. 1997).

The Margin of Safety (MoS) for human consumption can be calculated by dividing the NOAEL by the Total Theoretical Maximal Daily Intake (TMDI). Total TMDI of the food enzyme is 0.178 mg TOS/kg body weight/day. Consequently, the MoS is:

MoS = 1,000 / 0.178 = 5,634**.**

Total TMDI is highly exaggerated. Moreover, the NOAEL was based on the highest dose administered, and is therefore to be considered as a minimum value. Therefore, the actual Margin of Safety in practice will be some magnitudes higher. Consequently, there are no safety reasons for laying down maximum levels of use.

Conclusion:

The overall conclusion is that the use of the food enzyme maltogenic amylase from *Bacillus subtilis* RF12029 in the production of food is absolutely safe. Considering the high safety factor – even when calculated by means of an overestimation of the intake via the Budget method – there is no need to restrict the use of the enzyme in food processing. The suggested dosage for food manufacturers is not a restrictive value and could be higher or lower depending on usage.

4 PART 4 §170.240 – Self-limiting levels of use

This part is not applicable to this notified substance, see **Section 2.9** for further details regarding use levels.

5 PART 5 § 170.245 – Experience based on common use in food before 1958

This part is not applicable to this notified substance

6 PART 6 § 170.250 – Part 6 of a GRAS notice: Narrative

The data and information contained in this GRAS notice provides a basis that the notified substance is safe under the conditions of its intended use described herein. In the following sub-sections, the safety of the enzyme, the genetic modification and toxicological studies are presented. The information is generally available and PART 6 § 170.250 does not contain any confidential information. This section provides the basis that the notified substance is generally recognized, among qualified experts, and study data, to be safe under the conditions of its intended use.

All available known information has been reviewed and AB Enzymes GmbH is not aware of any data or information that is, or may appear to be, consistent with our conclusion of the notified substance GRAS status.

6.1 Safety of the production strain

The safety of *Bacillus subtilis* as an enzyme producer has been reviewed by de Boer Sietske, A. and Diderichsen, B. (1991) Schallmey et al. (2004) and Olempska-Beer et al. (2006).

Bacillus subtilis is among the most widely used bacteria for the production of enzymes and specialty chemicals. Industrial applications include (but are not restricted to) production of amylase, protease, glucanase, xylanase, etc.

In addition to *Bacillus licheniformis*, *B. subtilis* has become one of the most well-established cell factories in biotechnology especially for the production of exo-proteins like proteases and alpha-amylases (Westers et al. 2004) (Pohl, Harwood 2010) (van Dijl, Hecker 2013).

One of the oldest recorded uses of *Bacillus* is the fermentation of soybeans into Natto, a Tempe-like fermentation that uses a strain of *Bacillus* now recognized as *Bacillus subtilis* (natto). The production of Natto dates back more than a thousand years and was first practiced in Japan. Some $6x10^6$ kg of Natto are consumed annually in Japan.

While *B. subtilis* produces many enzymes, including amylases and cellulases, the most important enzymes in the production of Natto are proteases. The proteases are responsible for creating its

main flavor, through hydrolysis of soybean protein. Natto or the underlying microbial culture of *B. subtilis* (natto), is reported to have a number of beneficial health effects.

Furthermore *Bacillus subtilis* has been used in the food industry and biotechnology since many years for e.g., the production of amylases and glucanases for the baking and beverages markets, as well as for desizing of textiles and for starch modification for sizing of paper (Ferrari et al. 1993), the production of proteases for protein modification of e.g. milk or soybean protein or in the brewing industry (Schallmey et al. 2004), for use in detergent products and for de-hairing and batting in the leather industry, and for the production of xylanases as bread improver (Harbak, Thygesen 2002).

Food use safety:

B. subtilis-like organisms are ubiquitous in the environment (soil, water, plants and animals) and as a result can be also found in food (de Boer Sietske, A. and Diderichsen, B. 1991). *B. subtilis* has already been used for decades for the production of food enzymes with no known reports of adverse effects to human health or the environment (de Boer Sietske, A. and Diderichsen, B. 1991). Alpha-amylase enzyme preparation from *B. subtilis* has been used commercially since 1929, when it was used in the manufacture of chocolate syrup to reduce viscosity.

Recently the US Food and Drug Administration reviewed the safe use of food-processing enzymes from recombinant microorganisms, including *B. subtilis* (Olempska-Beer et al. 2006). An extensive risk assessment of *B. subtilis*, including its history of commercial use has been published by the US EPA (US EPA, 1997[4]). It was concluded that *B. subtilis* is not a human pathogen nor is it toxigenic.

Food enzymes derived from *B. subtilis* strains (including recombinant strains) have been evaluated by JECFA and many countries which regulate the use of food enzymes, such as the USA, France, Denmark, Australia/New Zealand and Canada, resulting in the approval of the use of food

[4] https://www.epa.gov/sites/production/files/2015-09/documents/fra009.pdf

enzymes from *B. subtilis* in the production of various foods, such as baking, brewing, juice production, wine production, distillation, starch industry, protein processing, etc.

For an extensive overview of countries that accepted *B. subtilis* as safe production organisms for a broad range of food enzymes.

Tab. 1 - Non-exhaustive list of authorised food enzymes (other than maltogenic amylase) produced by *Bacillus subtilis*

Authority	Food enzyme	Reference
JECFA	α-acetolactate decarboxylase α-amylase Mixed microbial carbohydrase and protease from *B. subtilis* Xylanase	Combined Compendium of FA specifications
Australia/NZ	α-amylase β-amylase β-glucanase Hemicellulase, endo-1,4-β-xylanase Hemicellulase, multicomponent Metalloproteinase Pullulanase Serine proteinase	Standard 1.3.3 processing aids
Canada	α-acetolactate decarboxylase amylase Asparaginase Glucanase Hemicellulase Pentosanase Protease Pullulanase Xylanase	B.16.100, Table V
France	α-acetolactate decarboxylase α-amylase Asparaginase endo-1,4-β-xylanase Hemicellulase Metalloproteases Serine protease Pullulanase Xylanase	Arrêté du 19 octobre 2006

USA[5]	β-galactosidase	GRN 649
	β-glucanase	GRAS Notices GRN592
	Lactase	GRAS Notices GRN579
	Asparaginase	GRAS Notices GRN476
	Pullulanase	GRAS Notices GRN 205
	Pectate lyase	GRAS Notices GRN114

At Roal Oy and AB Enzymes GmbH, *Bacillus subtilis* has been used as enzyme producer for many years without any safety problems. *Bacillus subtilis* strains have been cultivated in the production plant of Alko Oy/Roal Oy starting from year 1993 and the parental strain from which the production strain described here is derived has been used since 2010.

Bacillus subtilis strains are non-pathogenic for healthy humans and animals (Boer, Diderichsen 1991). Apart from the well-established pathogenicity of *B. anthracis*, a pathogen of humans and some animals, *B. cereus*, which causes gastroenteritis, and the group of insect pathogens related *to B. thuringiensis*, most other species of *Bacillus* are regarded as nonpathogenic or cause only opportunistic infections, often in compromised patients. The lack of pathogenicity among strains of *B. subtilis* or any of its close relatives has resulted in the Food and Drug Administration granting the organism GRAS (generally regarded as safe) status.

Pathogenicity

Pathogenic *B. subtilis* strains are not described in the Bergey's Manual or in the ATCC and other catalogues. The species *B. subtilis* does not appear on the list of pathogens in Annex III of Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agent at work.

[5] GRAS affirmations and GRAS notifications

Bacillus subtilis is a microorganism regarded as safe globally:

- In Canada, *B. subtilis* as per CEPA (Canadian Environmental Protection Act), does not meet the criteria of section 64 of the act – dangerous substances and no further regulatory action is required for its use[6]
- In the USA, *B. subtilis* is exempted as a host of certified host-vector systems under the NIH Guidelines in the USA since 1994 (NIH, 1996[7]). The US EPA has added *B. subtilis* to the list of exempted organisms in 1997 (US EPA, 1997[8]).
- In Europe, *B. subtilis* is classified as a low-risk-class microorganism, as exemplified by being listed as Risk Group 1 in the microorganism classification lists of the German Federal Institute for Occupational Safety and Health (BAuA, 2002[9]) and the Federal Office of Consumer Protection and Food Safety (BVL, 2013), and not appearing on the list of pathogens from Belgium (Belgian Biosafety Server, 2010[10]).

B. subtilis is therefore generally accepted as a non-pathogenic organism.

Secondary metabolites:

A review of the literature by the US EPA (1997) failed to reveal the production of metabolites of toxicological concern by *B. subtilis*. Although *B. subtilis* has been associated with outbreaks of food poisoning (Gilbert *et al.*, 1981 and Kramer *et al.*, 1982 as cited by Logan (1988)), the exact nature of its involvement has not been established. Unlike the case in these outbreaks of food poisoning, where apparently *B. subtilis* was isolated from a food source, the strains used for food enzyme production are not present in the processed food; instead, only the enzyme preparation is used in the food process.

[6] http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&n=5AE12597-1&offset=2&toc=show

[7] http://ehs.uky.edu/docs/pdf/bio_rna_nih_exempt_host_vector_systems_0001.pdf

[8] https://www.epa.gov/sites/production/files/2015-09/documents/fra009.pdf

[9] https://www.baua.de/EN/Topics/Work-design/Biological-agents/pdf/TRBA-466.pdf?__blob=publicationFile&v=2

[10] http://www.biosafety.be/

B. subtilis, like other closely related species in the genus, *B. licheniformis*, *B. pumilus*, and *B. megaterium*, has been shown to be capable of producing lecithinase, an enzyme which disrupts membranes of mammalian cells. However, there has been no correlation between lecithinase production and human disease in *B. subtilis*.

Concern about possible involvement of *B. cereus*-like enterotoxins in the rare cases where some *Bacillus* strains have been associated with food poisoning caused the Scientific Committee on Animal Nutrition to require specific testing of industrially used *Bacillus* strains. Subsequent testing showed the absence of *B. cereus*-like enterotoxins (Pedersen et al. 2002) and the current view is that the very few reports of *B. cereus*-like enterotoxins occurring in other species of *Bacillus* are likely to have resulted from misidentification of the strain involved (From et al. 2005).

Metabolites of human toxicological concern are usually produced by microorganisms for their own protection. Microbes in natural environments are affected by several and highly variable abiotic (e.g. availability of nutrients, temperature and moisture) and biotic factors (e.g. competitors and predators). Their ever changing environments put a constant pressure on microbes as they are prompted by various environmental signals of different amplitude over time. In nature, this results in continuous adaptation of the microbes through induction of different biochemical systems; e.g. adjusting metabolic activity to current availability of nutrients and carbon source(s), or activation of stress or defence mechanisms to produce secondary metabolites as 'counter stimuli' to external signals (Klein, Paschke 2004; Earl et al. 2008). However, the 'environmental' conditions of microbial production strains during industrial scale fermentation have been optimized and 'customized' to the biological requirements of the strain in question (see e.g. review by Parekh et al. (2000)). Thus, the metabolic activity and growth of a particular microbial production strain during the fermentation process (primarily the 'exponential growth phase') will efficiently lead to building of cell biomass, which in turn produces the molecule of interest. Industrial fermentations are run as monocultures (i.e. no external competitors or predators) with optimal abiotic conditions. Hence, there are no strong environmental signals that would induce stress (e.g. starvation, competitive environment or low/high temperature) or defence mechanisms

(e.g. production of antibiotic, antiviral or neurotoxic molecules). Biosynthesis of stress and/or defence secondary metabolites of toxicological relevance by industrial microbial production organisms during the fermentation process is thus highly unexpected (Sanchez, Demain 2002) and is furthermore avoided from an economical perspective to optimize enzyme production.

Finally, most industrial *B. subtilis* strains are from safe strain lineages that have been repeatedly tested according to the criteria laid out in the Pariza & Johnson publication (Pariza & Johnson, 2001). See Appendix #3 for Decision Tree.

In Conclusion:

B. subtilis has a long history of safe use in industrial-scale enzyme production. The long industrial use and wide distribution of *B. subtilis*-like organisms in nature has never led to any symptoms of pathogenicity. Moreover, no case demonstrating invasive properties of the species has been found in the literature.

During recent years, genetic engineering techniques have been used to improve the industrial production strains of *B. subtilis* and considerable experience on the safe use of recombinant *B. subtilis* strains at industrial scale has accumulated.

Secondary metabolites are not a safety concern in fermentation products derived from industrial *B. subtilis* strains. In addition, food enzymes *B. subtilis* have been subjected to a significant number of toxicological tests (including 90-day toxicological tests), as part of their safety assessment for use in food product manufacturing processes. These studies demonstrate that there are no concerns for fermentation products as produced using *B. subtilis*.

Therefore, *B. subtilis* can be considered generally safe not only as production organisms of its natural enzymes, but also as safe hosts for other safe gene products.

6.1.1 Safety of the genetic modification

The genetic modification, i.e., transformation of the vector pIK40 pyrF into the recipient strain *B. subtilis* results in the recombinant strain RF12029. As laid down before, the recipient belongs to a non-pathogenic species. The strain-line has been used since 2010 for safe food enzyme production.

The production strain (RF12029) differs from its original parental strain in expressing maltogenic amylase and featuring a set of defined genomic deletions.

Furthermore, we have noticed no differences in the production strain RF12029 as compared to the parental strain.

Maltogenic amylase:

Maltogenic amylase (EC 3.2.1.133) catalyses the hydrolysis of a (1-4) glycosidic bonds in polysaccharides so as to remove successively α-maltose residues from the non-reducing ends of the chains.

Amylases in general have been used in the food industry, particularly in baking processes, for decades (especially alpha-amylases) and their use in the bakery industry is continuously increasing. Maltogenic amylases, as well as other enzymes active on starch, have been suggested to prevent bread staling, by modifying starch at a temperature when most of the starch starts to gelatinize, therefore delaying retrogradation of the starch components which is the mean reason for bread staling.

Commercial maltogenic amylase enzyme preparations from various microorganisms (including genetically modified ones) are widely accepted and *Bacillus subtilis* - whether or not genetically

modified[11] - is widely accepted as a safe production organism for a broad range of enzymes that have been used e.g., as processing aids i*n food industry for several decades.*

Tab 2 - Non-exhaustive list of authorised maltogenic amylases from similar production organisms

Authority	Food enzyme	
Australia/NZ	*Bacillus subtilis* containing the gene for maltogenic α-amylase isolated from *Geobacillus stearothermophilus*12	Standard 1.3.3 processing aids
Canada	Maltogenic amylase from *Bacillus subtilis* BRG-1 (pBRG1); *Bacillus subtilis* DN1413 (pDN1413); *Bacillus subtilis* LFA 63 (pLFA63); *Bacillus subtilis* RB-147 (pRB147)	B.16.100, Table V
France	Maltogenic amylase from *B. stearothermophilus* expressed in *B. subtilis* Maltogenic amylase from *B. stearothermophilus* expressed in *B. subtilis* strain SM, SO, OC, DS67348	Arrêté du 19 octobre 2006
JECFA	Maltogenic amylase from *B. stearothermophilus* expressed in *B. subtilis*	FAS 372

[11] *Overproduction of chosen enzymes and/or modification of enzyme- (e.g. cellulase) profiles has not been observed to convey harmful properties to the host organism or its products (-animal tests- Huuskonen 1990).*

[12] *Geobacillus stearothermophilus* – former name *Bacillus stearothermophilus*

The maltogenic amylase protein overproduced by RF12029 originates from *Geobacillus sterothermophilus* and is identical in its sequence and properties to maltogenic amylase produced by the wild-type *Geobacillus stearothermophilus*.

As the maltogenic amylase protein is not toxic our evaluation of the genetically modified *B. subtilis* strain is comparable to that of the recipient strain. Based on the available information, it would be reasonable to conclude that the use of *G. stearothermophilus* maltogenic amylase gene for the production of maltogenic amylase in *Bacillus subtilis* RF12029 does not lead to any particular safety concern.

Plasmid pUB110 and pBC16-1

The well-known and characterized vectors pUB110 and pBC16-1 are naturally not present in *Bacillus subtilis*. The full nucleotide sequence of pUB110 and pBC16-1 and the biological functions of their open reading frames are known (http://www.ncbi.nlm.nih.gov/nuccore/NC_001384.1 & http://www.ncbi.nlm.nih.gov/nuccore/NC_001705.1). There is no transfer function present any more. ORFb (*mobB*) is essential for plasmid mobilization and was destroyed (Selinger et al. 1990; Kreft et al. 1978). By deleting the EcoRI-fragment of the pBC16 vector resulting in vector pBC16-1, the mobilization function is destroyed (Bernhard et al. 1978).

The antibiotic resistance genes of both pBC16-1 and pUB110 were destroyed or deleted constructing plasmid pIK40 pyrF - it contains no genes conferring antibiotic resistance.

The *repU* gene present on plasmid pIK40 pyrF is coding for the Rep protein, the replication initiation protein responsible for replication of the plasmid, but no intact genes required for mobilization are present (Selinger et al. 1990).

pUB110 is stably maintained in *B. subtilis* for more than 80 generations in the absence of selective pressure at a copy number estimated at about 50 per chromosome equivalent (Keggins et al. 1978).

The production strain RF12029 does not carry any genes conferring antimicrobial resistance.

The vector itself is fully characterized and free from potential hazards. It has been shown to be genetically stable.

The transformation does not increase the natural mutation frequency. If there were any mutations happening to the genes affecting the relevant characters of the bacterium, this would be noticed in the growth characteristics in the fermentation and / or in the product obtained. This has not happened. In addition, the possibility of mutations is decreased to its minimum by inoculating the seed culture for the fermentation with controlled stocks in "Master Cell Bank".

No additional growth/mutagenesis cycles have been performed after the RF12029 strain deposition to the culture collection. Therefore no reason can be seen that this genetic modification should have a negative effect on the safety properties.

The safety of the maltogenic amylase produced by the genetically modified *Bacillus subtilis* is supported by a standard package of genotoxicity and toxicological testing as herein.

In toxicological tests that have been performed, including a 90-days repeated dose study, no toxicity was detected. There was no indicator for toxicity in any of the dose levels tested. Therefore, a NOAEL of 1000 mg/kg/day was established. Additionally, the strain was shown not to be cytotoxic (appendix #4).

We consider that the colonization capacity of RF12029 in the environment must be considered rather low because of its adaptation to artificial fermentation conditions, deletion of nutrient mobilizing secreted hydrolases and inability to form spores to withstand unfavorable conditions.

The recipient has been adapted by conventional mutagenesis and has targeted gene deletions in the genome to meet production conditions in the fermenter. Such conditions, e.g., no competitive microorganisms, optimal provision of nutrients and aeration are not present in the environment.

In addition, the fitness of the strain to survive is very likely to be reduced by its high secretion performance characteristic. Most of its energy is needed for the maintenance of the plasmid and the production of maltogenic amylase and this will be of no advantage in a natural environment.

The inability of *B. subtilis* RF12029 to form spores and the deletion of relevant secreted hydrolases further greatly reduces its fitness to survive in nature, because there is no protection against common environmental stresses like extremes of pH or temperature, lack of oxygen or poor nutrient supply. In the presence of a well-adapted competing wild-type flora as found ubiquitously in soil or water, the fitness and therefore the colonization capacity of *B. subtilis* RF12029 must be considered rather low or zero.

As demonstrated above, the maltogenic amylase food enzyme from *Bacillus subtilis* RF12029 does not contain viable GMMs or their recombinant DNA. Consequently, environmental exposure of the GMM is negligible.

6.2 DATA FOR RISK ASSESSMENT

6.2.1 Toxicological testing

The following studies were performed:

— *In vitro* Bacterial reverse mutation test Appendix #5

— *In vitro* Chromosomal aberration test Appendix #6

— 13-week oral toxicity study in rats Appendix #7

Additionally, the strain has shown not to produce any cytotoxicity, when tested as recommended by the updated EFSA Guidance on *Bacillus* safety[13].

The original maltogenic alpha-amylase preparation produced with *Bacillus subtilis* has been subjected to several tests as part of its safety assessment for the production of food products. In

[13] http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2014.3665/epdf

toxicological tests that have been performed, including a 90-days repeated dose rat feeding study, no toxicity was detected.

For further development of the original *B. subtilis* host, genetically well-defined modifications were introduced to improve strain and product performance. No changes occurred in the gene coding for the mature alpha-amylase (amyM). Changes have been considered minor as they dealt only with well-documented deletions and minor changes to the plasmid backbone. Changes in the process were as well considered minor. Additionally, a derivative of the original host carrying most of the mutations was shown not to produce any cytotoxic effects when tested as recommended by the updated EFSA Guidance on Bacillus safety.

Because:

- the original host organism is safe,
- all the genetic modifications carried out (for original host improvement) are well characterized and specifically utilizing well-known plasmids for vector construction,
- the introduced genetic material does not encode and express any toxic substances,

It is concluded that the use of the maltogenic amylase produced with the current genetically modified *Bacillus subtilis* RF12029 as a processing aid in food processes does not pose any significant risk to human health.

Because the host organism is safe and because the genetic modifications are well characterized and specific utilizing well-known plasmids for vector constructs, and the introduced genetic material does not encode and express any toxic substances, it is concluded that the use of the maltogenic amylase from genetically modified *Bacillus subtilis* RF12029 as a processing aid in food processes would pose no significant risk to human health.

7 PART 7 §170.255 – LIST OF SUPPORTING DATA AND INFORMATION

This section contains a list of all the data and literature discussed in this dossier to provide a basis that the notified substance is safe under the conditions of its intended use as described in accordance with §170.250 (a)(1). All information presented in this section are publically available.

Appendices

1. Certificate of Analysis
2. Manufacturing Flow Chart
3. Decision Tree
4. Cytotoxicity Test
5. Reverse Mutation Test
6. Chromosome Aberration Test
7. 13 Week Oral Toxicity

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Analytical report
RH11662
09.11.2017

Objective: **Chemical composition analysis of Maltogenic amylase from *Bacillus subtilis* strain RH11662**

Sample: **1. Fermentation concentrate 161140422, LIMS ID 2016-336-51**
2. Fermentation concentrate 161132676, LIMS ID 2016-2311-53

Table 1. Main activity

	161140422	161132676
Maltogenic amylase (MAZ/g)	5550	5620

MANU: Assay of maltogenic amylase activity, Roal internal method B073

Table 2. Antibiotic activity, presence of production strain, recombinant DNA and microbiological quality

	161140422	161132676
Antibiotic activity	not detected	not detected
Presence of production strain	not detected	not detected
Recombinant DNA	not detected	not detected
Escherichia coli (/25 g)	not detected[1)]	not detected[2)]
Salmonella (/25 g)	not detected	not detected
Total coliforms (cfu*/g)	<1	<1
Staphylococcus aureus (/25 g)	not detected	not detected
Sulphite reducing anaerobes (cfu*/g)	not detected	not detected

Antibiotic activity: Specifications for Identity and Purity of Certain food Additives, FAO Food and Nutrition Paper 65 (2006), Rome, Vol.4, p. 122.
Production strain: Roal internal method M041 (In Finnish: Tuotantokannan toteaminen bakteeriamylaasivalmisteesta)
rDNA: Qualitative PCR for rDNA in food and feed enzymes, validated for the specific sequences
E. coli: [1)] SFS 4089:1998, mod. [2)] ISO 16649-3:2015, mod.
Salmonella: NMKL 71:1999, mod.
Total coliforms: ISO 4832:2006, mod.
S.aureus: Eur. Pharmac. 6.3.
Sulphite reducing anaerobes: NMKL 56:2008
*cfu: colony forming units

Table 3. Nutritional analysis

	161140422	161132676
Protein %	2.67	2.95
Moisture %	90.1	86.7
Ash %	1.17	1.62
TOS %[1)]	8.7	11.7

Protein: NMKL 6, Kjeldahl
Moisture: NMKL 23
Ash: NMKL 173
1) Total organic solids TOS%= [100-(moisture % + ash %)]

Table 4. Heavy metals

	161140422	161132676
Arsenic, As (mg/kg)	<0.5	<0.5
Cadmium, Cd (mg/kg)	<0.05	<0.05
Lead, Pb (mg/kg)	<0.05	<0.05
Mercury, Hg (mg/kg)	<0.05	<0.05

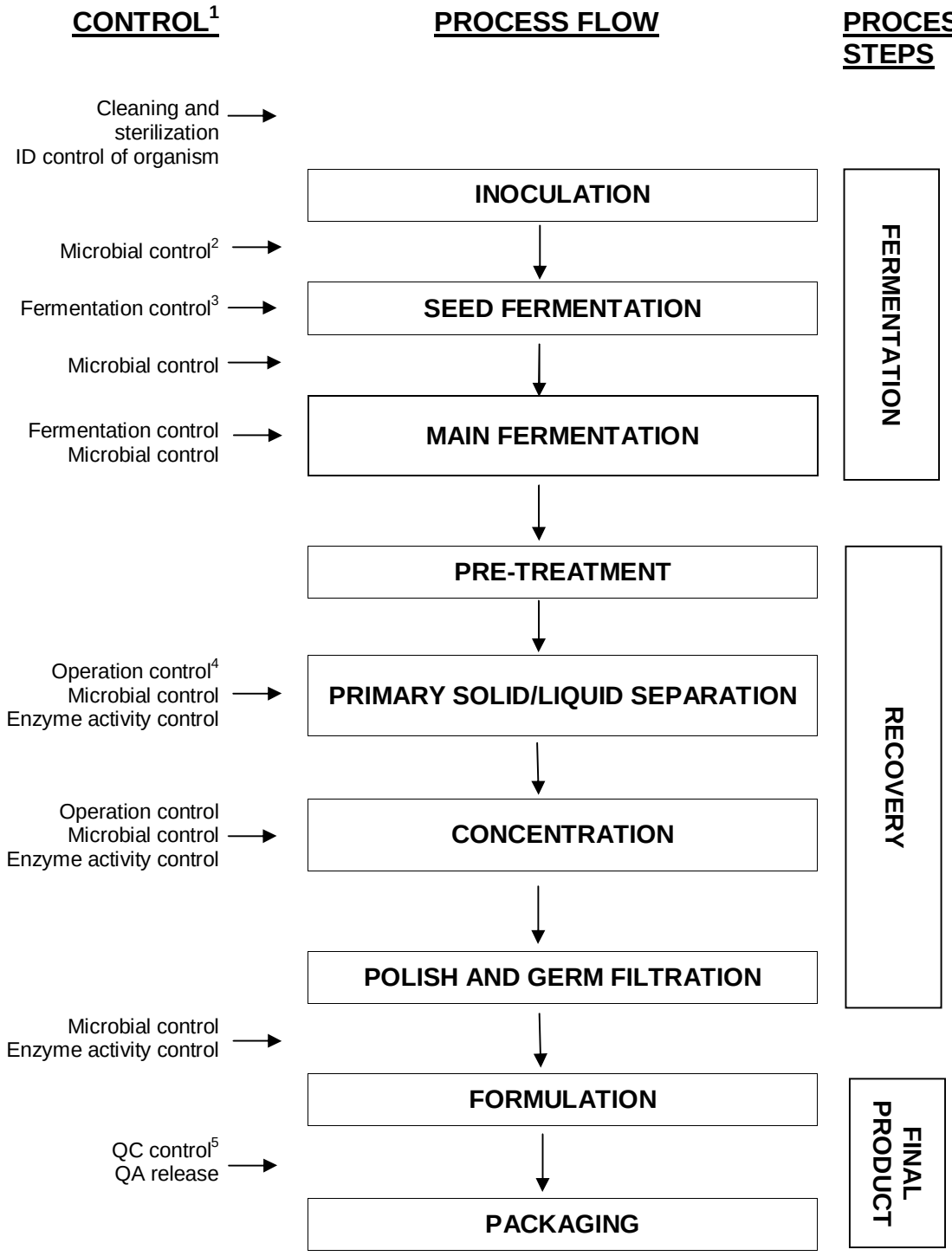
Heavy metals: ISO 17294-2:2003

Rajamäki 09.11.2017

(b) (6)

Hanna-Riikka Pirttilahti
Quality Management Coordinator
Roal Oy

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, CO_2, 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.

REPORT

RH 11923:
Detection of cytotoxicity of *Bacillus subtilis* RH 11293 supernatant using CaCo-2 cells

Study Director:	M. Sc. Claudia Steinert
Test Facility:	**Harlan Cytotest Cell Research GmbH** In den Leppsteinswiesen 19 64380 Rossdorf Germany
Sponsor:	**AB Enzymes GmbH** Feldbergstr. 78 64293 Darmstadt Deutschland
Study Monitor:	Dr. Hans-Jürgen Schepers
Harlan Study Number:	**1688900**
Study Completion Date:	**31 March 2015**
Version	**Final**

CONTENTS

LIST OF TABLES

PROJECT STAFF SIGNATURE

Harlan Cytotest Cell Research GmbH, In den Leppsteinswiesen 19, 64380 Rossdorf, Germany.

Harlan Study Number:	1688900
Study Title:	RH 11293: Detection of cytotoxicity of *Bacillus subtilis* RH 11293 supernatant using CaCo-2 cells
Study Director:	M. Sc. Claudia Steinert

(b) (6)

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Date: 31 March 2015

SUMMARY

The supernatant of from a fermentation broth generated with *Bacillus subtilis* RH 11293 was tested for its cytotoxic potential on CaCo-2 cells.

The supernatant was incubated with a protease inhibitor cocktail 30 minutes prior to the treatment of the CaCo-2 cells to block enzymatic activity. The CaCo-2 cells were incubated with 10%, 50%, and 90% of supernatant for 2 h at 37 °C/5% CO_2.

Pure *Bacillus* medium served as negative control and Triton® X-100 as positive control.

The cytotoxicity was determined by fluorescence detection of LDH release, whereat the values of the positive control (Triton® X-100) were considered representative for 100% cytotoxicity and the values of the negative control for 0% cytotoxicity.

The samples of the positive control showed a high LDH release, representing a high cytotoxicity. The negative control and the test item showed significantly lower fluorescence values – whereat the values of the test item were slightly lower than the ones of the negative control – both indicating no cytotoxicity.

In conclusion it can be stated that in this study and under the experimental conditions reported the supernatant from a fermentation broth generated with *Bacillus subtilis* RH 11293 did not exhibit cytotoxic potential on CaCo-2 cells.

GENERAL INFORMATION

Schedule

Experimental Starting Date:	25 March 2015
Experimental Completion Date:	25 March 2015

Additional Responsibilities

Deputy Study Director:	Dr. Hans-Eric Wollny Harlan Cytotest Cell Research GmbH

Deviations from Study Plan

There were no deviations from the study plan noted.

Storage

Unless instructed otherwise by the Sponsor, the study plan, all raw data (paper and electronic) and the final report will be stored at the Harlan Cytotest Cell Research GmbH for 6 months.

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

1 INTRODUCTION AND PURPOSE

Bacillus species are used in animal production directly as microbial feed additives or as the source of other feed and food additives, notably enzymes. The principal safety concern for consumers and, to a lesser extent, livestock associated with *Bacillus* is a capacity for toxin production. Therefore, a cytotoxicity test was performed on CaCo-2 cells with the supernatant from a fermentation broth generated with *Bacillus subtilis* RH 11293.

2 TEST AND REFERENCE ITEMS

Information as provided by the Sponsor.

Identification:	*Bacillus* supernatant RH 11293
Purity:	Not applicable for *Bacillus* supernatant
Physical state/Appearance:	*Bacillus* supernatant (yellow liquid)
Expiry Date:	Not indicated
Storage Conditions:	- 80 °C
Stability in Solvent:	Not indicated

3 MATERIALS AND METHODS

3.1 Definitions and Abbreviations

Bacillus medium	Pure culture medium of the *Bacillus* strain, sterilized (not inoculated with *Bacillus*)
Bacillus supernatant	Supernatant from the fermentation broth generated with the *Bacillus* strain
DMEM	Dulbecco's Modified Eagle's Medium
Pefabloc® SC	(4-(-2-Aminoethyl)benzenesulfonyl fluoride hydrochloride, AEBSF)
Triton® X-100	4-octylphenol polyethoxylate; positive control 1

3.2 Test System

The test system consisted of a human intestinal epithelial cell line originally isolated from a human colorectal cancer of a 72 year old man. The subclone HTB 37 used was provided from ATCC (American Type Culture Collection) cell bank and further propagated in-house.

3.3 Protease Inhibitor

A 5 mM stock solution of the protease inhibitor Pefabloc® SC (Fluka) was prepared by dissolving 5.30 mg in 4.420 mL *Bacillus* medium. This stock solution was diluted 20fold (50 µL of the stock solution in 1 mL matrix) to give a final concentration in the assay of 0.25 mM (according to the manufacturer's specification).

3.4 Test Item Preparation

One part of the *Bacillus* supernatant (TI 10% = 300 µL, TI 50% = 1500 µL, and TI 90% = 2700 µL) was pre-incubated with protease inhibitor (150 µL, respectively) for 30 min at room temperature. The other part was pre-incubated with *Bacillus* medium (150 µL, respectively) instead. The pre-incubated *Bacillus* supernatants were combined with DME medium (TI 10% = + 2700 µL, TI 50% = + 1500 µL, and TI 90% = + 300 µL) to give the final test item concentrations of 10 %, 50 %, and 90 %.

3.5 Culture of CaCo-2 cells

The CaCo-2 cells had been propagated in DME medium (Gibco) supplemented with 10% fetal calf serum (FBS, GE Healthcare), 1% non-essential amino acids (Sigma) and 1 % Gentamycin (Lonza) and 1% Amphotericin (Gibco) and 1% Na-Pyruvat (Biochrom). The cells had been cultivated at 37 °C/5% CO_2 in a humidified incubator. Cells had been seeded into a 24-well plate and grown until 80-100% confluent monolayers, which had been inspected visually and microscopically prior to treatment.

3.6 Controls

3.6.1 Positive control

Triton® X-100 (Promega) was used as positive control in a 1:5 dilution (20 µL Triton® X-100 + 80 µL DME medium).

3.6.2 Negative control

As negative control for the test item cells were treated with 10%, 50%, and 90% *Bacillus* medium, pre-incubated for 30 min with or without protease inhibitor. This was done equivalently to the procedure described in section 3.4.

3.7 Treatment of seeded cells

Prior to the treatment the cells were washed with 0.5 mL DME medium. Then 150 µL, 550 µL, or 950 µL of the pre-incubated *Bacillus* supernatant with or without protease inhibitor were added to the wells. For the negative control 150 µL, 550 µL, or 950 µL of the pre-incubated *Bacillus* medium with or without protease inhibitor were added to the wells. For the positive control 10 µL of Triton® X-100 and 540 µL of *Bacillus* medium were added to the wells. Each well was filled up to 1 mL with DME medium. The cells were incubated for 2 h ± 15 min at 37 °C/5% CO_2 in a humidified incubator. Each sample was performed in triplicate.

3.8 LDH release

For determination of the LDH release the CytoTox-ONE™ Homogenous Membrane Integrity Assay Kit from Promega was used.

After the incubation time 3 x 50 µL of the supernatant were transferred to a fluorescence microtiter plate and an equal volume of CytoTox-ONE™ Reagent (Promega) was added. After shaking for approx. 30 sec the incubation was continued at RT for 10 min.

Afterwards 30 µL of stop solution (Promega) were added to each well. The fluorescence was determined with an excitation wavelength of 560 nm and an emission wavelength of 590 nm.

3.9 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 and the controls.

3.10 Evaluation of Results

A decrease in number of living cells results in an increase of LDH release.

The cytotoxicity values were determined according to the formula:

$$Cytotoxicity\ [\%] = \frac{(Test\ Item - Negative\ Control)}{(Positive\ Control\ 1 - \ Negative\ Control)} * 100$$

The values of the positive control = maximum LDH release represent 100% cytotoxicity. The values of the test item are expressed as a percentage of the positive control. Negative values were set as 0.

4 RESULTS AND DISCUSSION

Table 1 Determination of the cytotoxicity in relation to the positive control (= 100 % cytotoxicity)

		Mean of triplicates			[value] -	neg. values	%
		neg. control	test item	pos. control	[neg. control]	set 0	pos. control
with	10%	12599	10799		-1799	0	0
inhibitor	50%	10671	9488		-1183	0	0
	90%	9925	9176		-749	0	0
without	10%	12705	11061		-1644	0	0
inhibitor	50%	10899	9955		-944	0	0
	90%	10343	9256		-1086	0	0
				39418	28519*	28519	100

* The value of the negative control 50% without inhibitor was used for calculation since it has the same solvent composition.

The fluorescence values of the positive control represent 100% cytotoxicity, the values of the negative control 0% cytotoxicity. The values of the test item were lower than the values of the negative control, indicating that the test item is not cytotoxic. Microscopic evaluation of these wells did not show any cytotoxic effects.

As expected, the values of the positive control were significantly higher than the ones of the test item and the negative control representing a high cytotoxicity.

5 CONCLUSION

In conclusion it can be stated that in this study and under the experimental conditions reported the supernatant from a fermentation broth generated with *Bacillus subtilis* RH 11293 did not exhibit cytotoxic potential on CaCo-2 cells.

6 APPENDIX

Table 2 Fluorescence values of the negative control

		Triplicate 1				Triplicate 2				Triplicate 3			
		1	2	3	mean				mean				mean
with	10%	13375	12905	12114	12798	13166	12983	12257	12802	12676	11986	11928	12197
inhibitor	50%	11981	10803	11088	11291	10872	9803	9935	10203	11482	10192	9881	10518
	90%	10124	9742	10450	10105	9887	9932	9613	9811	9829	9753	9994	9859
without	10%	12406	12364	11513	12094	13656	13351	13222	13410	13101	12657	12079	12612
inhibitor	50%	10582	10228	10508	10439	11203	11042	10965	11070	11518	11019	11024	11187
	90%	10454	9891	10375	10240	10158	10716	10305	10393	9759	10646	10780	10395

Table 3 Fluorescence values of the test item

		Triplicate 1				Triplicate 2				Triplicate 3			
		1	2	3	mean				mean				mean
with	10%	11769	11146	10968	11294	10672	10536	10138	10449	11021	10559	10386	10655
inhibitor	50%	10757	9834	9730	10107	9626	8545	9016	9062	9632	9252	8999	9294
	90%	9471	8969	9676	9372	9160	9327	9429	9305	8635	8548	9370	8851
without	10%	11034	12074	10367	11158	10990	11021	10830	10947	10829	11617	10787	11078
inhibitor	50%	10101	9681	9873	9885	10334	9767	9996	10032	10312	9774	9757	9948
	90%	9288	9076	9590	9318	8796	9485	9002	9094	8747	9702	9621	9357

Table 4 Fluorescence values of the positive control

	Triplicate 1				Triplicate 2				Triplicate 3			
	1	2	3	mean				mean				mean
positive control	38625	35197	42316	38713	33066	39268	42432	38255	36615	48454	38790	41286

harlan™

STUDY NUMBER 1283801

SALMONELLA TYPHIMURIUM REVERSE MUTATION ASSAY

WITH

Maltogenic amylase from *Bacillus stearothermophilus* expressed in *Bacillus subtilis* (EL 2009083)

REPORT
(FIRST ORIGINAL OF TWO)

STUDY COMPLETION DATE:
NOVEMBER 10, 2009

1 COPY OF GLP CERTIFICATE

[redacted]

HESSEN

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
Harlan Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

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
6 Prüfungen zur Bestimmung von Rückständen	**6** Residues
8 Analytische Prüfungen an biologischen Materialien	**8** Analytical studies on biological materials

15.08. und 27. – 29.10.2008
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.

(b) (6)

Th. Zimmermann, Referent, Wiesbaden, den **30. März 2009**
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

HESSISCHES MINISTERIUM FÜR UMWELT, LÄNDLICHEN RAUM UND VERBRAUCHERSCHUTZ 2

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

2 CONTENTS

3 PREFACE

3.1 General

Title:	Salmonella typhimurium Reverse Mutation Assay with Maltogenic amylase from *Bacillus stearothermophilus* expressed in *Bacillus subtilis* (EL 2009083)
Sponsor:	AB Enzymes GmbH Feldbergstr. 78 64293 Darmstadt Germany
Study Monitor:	Dr. H.-J. Schepers
Test Facility:	Harlan Cytotest Cell Research GmbH (Harlan CCR) In den Leppsteinswiesen 19 64380 Rossdorf/Germany

3.2 Responsibilities

Study Director:	Dipl. Biol. Andrea Sokolowski
Deputy Study Director:	Dr. Hans-Eric Wollny
Management:	Dr. Wolfgang Völkner
Head of Quality Assurance Unit:	Frauke Hermann

3.3 Schedule

Experimental Starting Date:	September 01, 2009
Experimental Completion Date:	September 28, 2009

3.4 Project Staff Signatures

Study Director — Dipl. Biol. Andrea Sokolowski

(b) (6)

.....

Date: November 10, 2009

Management — Dr. Wolfgang Völkner

(b) (6)

.....

Date: November 10, 2009

3.5 Good Laboratory Practice

The study was performed in compliance with:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1) dated July 25, 1994 („BGBl. I 1994", pp. 1703), last revision dated June 27, 2002.

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final].

3.6 Guidelines

This study followed 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

3.7 Archiving

Harlan CCR will archive the following data for 15 years:

Raw data, study plan, report, and a sample of the test item.

No data will be discarded without the sponsor´s consent.

3.8 Deviations from the Study Plan

5.1 Test Item

None.

4 STATEMENT OF COMPLIANCE

Study Number:	1283801
Test Item:	EL 2009 083
Study Director:	Dipl. Biol. Andrea Sokolowski
Title:	Salmonella Typhimurium Reverse Mutation Assay with Maltogenic amylase from *Bacillus stearothermophilus* expressed in *Bacillus subtilis* (EL 2009083)

This study performed in the test facility of Harlan CCR was conducted in compliance with Good Laboratory Practice Regulations:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1) dated July 25, 1994 („BGBl. I 1994", pp. 1703), last revision dated June 27, 2002.

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final].

There were no circumstances that may have affected the quality or integrity of the study.

Study Director

Harlan C C R
Dipl. Biol. Andrea Sokolowski

(b) (6)

.....

Date: November 10, 2009

5 STATEMENT OF QUALITY ASSURANCE UNIT

Study Number: 1283801

Test Item: EL 200983

Study Director: Dipl. Biol. Andrea Sokolowski

Title: Salmonella Typhimurium Reverse Mutation Assay with Maltogenic amylase from *Bacillus stearothermophilus* expressed in *Bacillus subtilis* (EL 2009083)

The general facilities and activities of Harlan CCR are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were inspected periodically. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Phases and Dates of QAU Inspections/ Audits		Dates of Reports to the Study Director and to Management
Study Plan:	August 20, 2009	August 20, 2009
1st Amendment to the Study Plan:	October 29, 2009	October 29, 2009
Process Inspection Preparation for Application: Evaluation: Raw Data:	 September 01, 2009 September 14, 2009 September 22, 2009	 September 01, 2009 September 14, 2009 September 22, 2009
Report	October 29, 2009	October 29, 2009

This statement is to confirm that the present report reflects the raw data.

Head of Quality Assurance Unit

Frauke Hermann

(b) (6)

a Rudolph

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Date: November 10, 2009

6 SUMMARY OF RESULTS

This study was performed to investigate the potential of EL 2009083 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. 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

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.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with EL 2009083 at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

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

6.1 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, EL 2009083 is considered to be non-mutagenic in this Salmonella typhimurium reverse mutation assay.

7 OBJECTIVE

7.1 Aims of the Study

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.

7.2 Reasons for the Study

The most widely used assays for detecting gene mutations are those using bacteria (3). 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 with which an agent reverses 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 growth 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 $his^- \rightarrow his^+$ 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 and 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 at least six dose levels with adequately spaced concentrations were tested. The maximum dose level was 5000 µg/plate.

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

8 MATERIALS AND METHODS

8.1 Test Item

Internal Test Item Number: S 1043111

The test item and the information concerning the test item were provided by the sponsor.

Identity:	EL 2009083
Batch No.:	AA09087A3
Purity:	Not applicable (UVCB substance)
Stability in Solvent:	1 day at room temperature, 5 days in the refrigerator and 1 year in the freezer in water and saline
Storage:	At room temperature, moisture protected, light protected
Expiration Date:	August 2011

On the day of the experiment, the test item EL 2009083 was dissolved in deionised water. The stock solution was sterile filtrated before use. The solvent was chosen because of its solubility properties (4).

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

8.2 Controls

8.2.1 Negative Controls

Concurrent untreated and solvent controls were performed.

8.2.2 Positive Control Substances

Without metabolic activation

Strains:	TA 1535, TA 100
Name:	sodium azide, NaN_3
Supplier:	SERVA, D-69042 Heidelberg
Catalogue No.:	30175
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
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	N 9504
Purity:	> 99.9 %
Dissolved in:	DMSO (purity >99 %, MERCK, D-64293 Darmstadt)
Concentration:	10 µg/plate in TA 98, 50 µg/plate in TA 1537

Strain:	TA 102
Name:	methyl methane sulfonate, MMS
Supplier:	MERCK-SCHUCHARDT, D-85662 Hohenbrunn
Catalogue No.:	820775
Purity:	> 99.0 %
Dissolved in:	deionised water
Concentration:	3.0 µL/plate

With metabolic activation

Strains:	TA 1535, TA 1537, TA 98, TA 100, TA 102
Name:	2-aminoanthracene, 2-AA
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	A 1381
Purity:	97.5 %
Dissolved in:	DMSO (purity >99 %, MERCK, D-64293 Darmstadt)
Concentration:	2.5 µg/plate (10.0 µg/plate in TA 102)

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

8.3 Test System

8.3.1 Characterisation of the Salmonella typhimurium Strains

The histidine dependent strains are derived from S. typhimurium strain LT2 through a mutation 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 and is excision repair proficient. Additionally, TA 102 contains the multicopy plasmid pAQ1 carrying the hisG428 mutation (ochre mutation in the hisG gene) and a tetracycline resistance gene (5).

In summary, the mutations of the TA strains used in this study can be described as follows:

Salmonella typhimurium		
Strains	**Genotype**	**Type of mutations indicated**
TA 1537	his C 3076; rfa$^-$; uvrB$^-$:	frame shift mutations
TA 98	his D 3052; rfa$^-$; uvrB$^-$; R-factor	" "
TA 1535	his G 46; rfa$^-$; uvrB$^-$:	base-pair substitutions
TA 102	his G 428; rfa$^-$; uvrB$^+$; R-factor	" "
TA 100	his G 46; rfa$^-$; uvrB$^-$; R-factor	" "

Regular checking of the properties of the strains regarding the membrane permeability, ampicillin- and tetracycline-resistance as well as spontaneous mutation rates is performed in the laboratory of Harlan CCR according to B. Ames et al. (1) and D. Maron and B. Ames (5). In this way it was ensured that the experimental conditions set down by Ames were fulfilled.

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

8.3.2 Storage

The strain cultures were stored as stock cultures in ampoules with nutrient broth + 5 % DMSO (MERCK, D-64293 Darmstadt) in liquid nitrogen.

8.3.3 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 Merck Nutrient Broth (MERCK, D-64293 Darmstadt)
5 g NaCl (MERCK, D-64293 Darmstadt)

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).

8.3.4 Selective Agar

The plates with the minimal agar were obtained from E. Merck, D-64293 Darmstadt.

8.3.5 Overlay Agar

The overlay agar contains per litre:

6.0 g Agar Agar*
6.0 g NaCl*
10.5 mg L-Histidine x HCl x H_2O*
12.2 mg Biotin*

* (MERCK, D-64293 Darmstadt)

Sterilisations were performed at 121 °C in an autoclave.

8.4 Mammalian Microsomal Fraction S9 Mix

The bacteria used in this assay do not possess the enzyme systems which, in mammals, are known to convert promutagens into active DNA damaging metabolites. In order to overcome this major drawback an exogenous metabolic system is added in form of mammalian microsome enzyme activation mixture.

8.4.1 S9 (Preparation by Harlan C C R)

Phenobarbital/β-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar rats (Hsd Cpb: WU, Harlan Laboratories GmbH, 33178 Borchen, Germany), weight approx. 220 - 320 g induced by applications of 80 mg/kg b.w. Phenobarbital i.p. (Desitin; D-22335 Hamburg) and β-Naphthoflavone p.o. (Aldrich, D-89555 Steinheim) each on three consecutive days. The livers are prepared 24 hours after the last treatment. The S9 fractions are produced by dilution of the liver homogenate with a KCl solution (1+3) followed by centrifugation at 9000 g. Aliquots of the supernatant are frozen and stored in ampoules at -80 °C. Small numbers of the ampoules can be kept at -20 °C for up to one week. Each batch of S9 mix is routinely tested with 2-aminoanthracene as well as benzo(a)pyrene.

The protein concentration in the S9 preparation was 31.6 mg/mL (lot no. R 130309) in both experiments.

8.4.2 S9 Mix

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution. The amount of S9 supernatant was 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 was stored in an ice bath. The S9 mix preparation was performed according to Ames et al.(1).

8.5 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.

8.6 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 toxic effects were observed 5000 µg/plate were chosen as maximal concentration.

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

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

8.7 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:

- 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

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 (2).

8.8 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. The counter was connected to an IBM AT compatible PC with printer which printed out both, the individual 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).

8.9 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

8.10 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 (3).

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration (2).

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.

8.11 Biometry

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

9 DISCUSSION OF RESULTS

The test item EL 2009083 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. Due to contamination the pre-experiment/experiment I had to be terminated and no data were obtained. An additional pre-experiment/experiment I was performed according to the plate incorporation method. This additional experiment is reported as pre-experiment/experiment I. Each concentration and the controls, were 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

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.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with EL 2009083 at any concentration level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

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

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.

10 REFERENCES

1. 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

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Recommendations on data production and analysis using the Salmonella/microsome mutagenicity assay
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3. Hollstein,M., J. McCann, F.A. Angelosanto and W.W. Nichols (1979)
Short-term tests for carcinogens and mutagens
Mutation Res. 65, 133-226

4. Maron D.M., J. Katzenellenbogen and B.N. Ames (1981)
Compatibility of organic solvents with the Salmonella/Microsome Test
Mutation Res. 88, 343-350

5. Maron, D.M., Ames, B.N. (1983)
Revised methods for the Salmonella mutagenicity test
Mutation Res. 113, 173-215

11 DISTRIBUTION OF THE REPORT

Sponsor	3x (original, copy and electronic copy (pdf))
Study Director	1x (original)

12 SUMMARY OF RESULTS

12.1 Summary of Results Pre-Experiment and Experiment I

Study Name: 1283801 Study Code: Harlan CCR 1283801
Experiment: 1283801 VVa Plate Date Plated: 15/09/2009
Assay Conditions: Date Counted: 18/09/2009

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	Deionised water		14 ± 1	9 ± 1	27 ± 4	129 ± 11	390 ± 16
	Untreated		19 ± 1	8 ± 1	26 ± 8	126 ± 9	383 ± 21
	EL 2009083	3 µg	16 ± 7	10 ± 5	26 ± 2	111 ± 3	396 ± 35
		10 µg	12 ± 3	10 ± 3	26 ± 4	123 ± 7	410 ± 30
		33 µg	14 ± 1	6 ± 1	26 ± 3	135 ± 9	352 ± 30
		100 µg	13 ± 1	9 ± 1	27 ± 2	139 ± 6	379 ± 26
		333 µg	8 ± 1	10 ± 2	29 ± 1	138 ± 15	412 ± 17
		1000 µg	14 ± 1	10 ± 1	30 ± 1	135 ± 8	430 ± 17
		2500 µg	16 ± 4	9 ± 3	28 ± 7	139 ± 5	401 ± 33
		5000 µg	22 ± 1	10 ± 1	36 ± 8	170 ± 1	334 ± 31
	NaN3	10 µg	1879 ± 54			1926 ± 34	
	4-NOPD	10 µg			270 ± 46		
	4-NOPD	50 µg		70 ± 3			
	MMS	3.0 µL					2747 ± 312
With Activation	Deionised water		16 ± 1	15 ± 2	32 ± 3	138 ± 21	511 ± 27
	Untreated		21 ± 3	15 ± 1	31 ± 1	148 ± 14	547 ± 17
	EL 2009083	3 µg	21 ± 0	13 ± 3	32 ± 2	126 ± 4	503 ± 57
		10 µg	17 ± 4	12 ± 2	35 ± 3	142 ± 15	530 ± 63
		33 µg	18 ± 1	12 ± 3	32 ± 3	134 ± 3	507 ± 48
		100 µg	18 ± 1	13 ± 3	29 ± 1	139 ± 15	515 ± 69
		333 µg	19 ± 1	15 ± 1	32 ± 4	130 ± 6	489 ± 40
		1000 µg	18 ± 2	13 ± 4	31 ± 4	162 ± 9	503 ± 60
		2500 µg	22 ± 1	16 ± 4	32 ± 2	182 ± 3	559 ± 84
		5000 µg	21 ± 2	20 ± 1	40 ± 3	224 ± 9	511 ± 1
	2-AA	2.5 µg	292 ± 63	265 ± 25	1689 ± 92	1852 ± 363	
	2-AA	10.0 µg					1496 ± 24

Key to Positive Controls

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

12.2 Summary of Results Experiment II

Study Name: 1283801
Experiment: 1283801 HV2 pre
Assay Conditions:

Study Code: Harlan CCR 1283801
Date Plated: 23/09/2009
Date Counted: 28/09/2009

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	Deionised water		17 ± 3	17 ± 5	38 ± 9	131 ± 8	396 ± 27
	Untreated		16 ± 3	14 ± 7	28 ± 2	131 ± 21	354 ± 16
	EL 2009083	33 µg	19 ± 4	17 ± 8	26 ± 2	128 ± 1	371 ± 28
		100 µg	18 ± 4	15 ± 5	38 ± 4	138 ± 8	392 ± 41
		333 µg	18 ± 9	17 ± 1	38 ± 8	144 ± 3	407 ± 35
		1000 µg	16 ± 3	17 ± 4	37 ± 2	159 ± 4	391 ± 21
		2500 µg	18 ± 3	18 ± 4	38 ± 6	185 ± 24	366 ± 16
		5000 µg	21 ± 6	19 ± 2	39 ± 3	213 ± 18	326 ± 12
	NaN3	10 µg	1528 ± 75			1777 ± 61	
	4-NOPD	10 µg			408 ± 16		
	4-NOPD	50 µg		86 ± 7			
	MMS	3.0 µL					1110 ± 70
With Activation	Deionised water		21 ± 4	20 ± 4	45 ± 15	164 ± 8	532 ± 33
	Untreated		17 ± 4	19 ± 4	44 ± 6	177 ± 10	538 ± 34
	EL 2009083	33 µg	26 ± 4	26 ± 4	34 ± 4	176 ± 4	539 ± 86
		100 µg	24 ± 9	22 ± 4	51 ± 7	176 ± 2	544 ± 40
		333 µg	21 ± 6	21 ± 1	48 ± 11	177 ± 13	575 ± 37
		1000 µg	22 ± 3	28 ± 3	49 ± 6	204 ± 8	561 ± 39
		2500 µg	28 ± 2	24 ± 8	44 ± 7	227 ± 15	400 ± 44
		5000 µg	34 ± 5	30 ± 5	56 ± 10	212 ± 14	282 ± 31
	2-AA	2.5 µg	249 ± 11	204 ± 14	1718 ± 106	2157 ± 52	
	2-AA	10.0 µg					1859 ± 71

Key to Positive Controls

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

13 HISTORICAL CONTROL DATA

These data represent the laboratory´s historical control data from January 2008 until October 2008 representing approx. 600 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	17	5.17	9	39	21	5.82	8	41
	Negative control	17	5.33	9	38	20	6.23	10	46
	Positive control	2024	315.78	1041	3138	294	140.02	102	945
TA1537	Solvent control	13	3.12	6	25	17	3.90	9	35
	Negative control	13	3.38	5	26	18	4.05	8	31
	Positive control	116	30.52	68	407	204	69.54	72	454
TA 98	Solvent control	30	5.59	13	59	39	6.34	20	60
	Negative control	31	5.45	16	55	39	6.53	19	59
	Positive control	489	169.76	211	1694	1455	463.01	200	3553
TA 100	Solvent control	130	18.79	89	224	155	22.54	92	218
	Negative control	139	17.30	93	205	147	21.78	92	234
	Positive control	2160	342.67	588	3379	1839	621.27	404	3868
TA 102	Solvent control	439	45.63	299	538	545	83.52	282	689
	Negative control	426	45.16	301	537	549	78.37	351	693
	Positive control	3087	281.95	1039	6082	2224	541.31	937	3956

Mean = mean value of revertants/plate
SD = standard deviation Min = minimal value/Max = maximal value

14 ANNEX: TABLES OF RESULTS (8 PAGES)

Pre-Experiment and Experiment I: 1283801 VVa Plate Incorporation (4 pages)

Experiment II: 1283801 HV2 Pre-Incubation (4 pages)

Study Name: 1283801
Experiment: 1283801 VVa Plate
Assay Conditions:

Study Code: Harlan CCR 1283801
Date Plated: 15/09/2009
Date Counted: 18/09/2009

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**EL 2009083**	3 µg	*16.3*	*6.5*	*1.2*	16, 23, 10
		10 µg	*11.7*	*2.9*	*0.9*	10, 10, 15
		33 µg	*14.3*	*0.6*	*1.0*	14, 15, 14
		100 µg	*13.0*	*1.0*	*1.0*	12, 14, 13
		333 µg	*8.3*	*0.6*	*0.6*	8, 8, 9
		1000 µg	*14.3*	*0.6*	*1.0*	14, 15, 14
		2500 µg	*16.0*	*3.6*	*1.2*	19, 12, 17
		5000 µg	*21.7*	*0.6*	*1.6*	22, 22, 21
	Deionised water		*13.7*	*0.6*		13, 14, 14
	Untreated Control		*19.3*	*0.6*		20, 19, 19
TA 1537	**EL 2009083**	3 µg	*10.3*	*4.6*	*1.2*	13, 5, 13
		10 µg	*10.0*	*2.6*	*1.2*	8, 9, 13
		33 µg	*6.3*	*0.6*	*0.7*	6, 7, 6
		100 µg	*9.0*	*1.0*	*1.0*	8, 9, 10
		333 µg	*10.3*	*1.5*	*1.2*	10, 12, 9
		1000 µg	*9.7*	*0.6*	*1.1*	10, 10, 9
		2500 µg	*9.0*	*2.6*	*1.0*	12, 8, 7
		5000 µg	*9.7*	*0.6*	*1.1*	10, 10, 9
	Deionised water		*8.7*	*1.2*		8, 8, 10
	Untreated Control		*7.7*	*0.6*		8, 7, 8
TA 98	**EL 2009083**	3 µg	*26.3*	*2.1*	*1.0*	28, 27, 24
		10 µg	*26.3*	*4.0*	*1.0*	30, 27, 22
		33 µg	*25.7*	*2.9*	*0.9*	24, 29, 24
		100 µg	*27.3*	*1.5*	*1.0*	27, 29, 26
		333 µg	*28.7*	*1.2*	*1.0*	30, 28, 28
		1000 µg	*30.0*	*1.0*	*1.1*	30, 29, 31
		2500 µg	*28.3*	*6.8*	*1.0*	26, 23, 36
		5000 µg	*35.7*	*8.0*	*1.3*	35, 28, 44
	Deionised water		*27.3*	*3.5*		31, 24, 27
	Untreated Control		*26.0*	*7.8*		30, 31, 17
TA 100	**EL 2009083**	3 µg	*111.3*	*2.9*	*0.9*	108, 113, 113
		10 µg	*123.0*	*7.2*	*1.0*	129, 115, 125
		33 µg	*135.0*	*8.7*	*1.0*	125, 140, 140
		100 µg	*138.7*	*6.0*	*1.1*	145, 133, 138
		333 µg	*138.0*	*15.4*	*1.1*	134, 125, 155
		1000 µg	*135.0*	*7.9*	*1.0*	132, 144, 129
		2500 µg	*138.7*	*4.6*	*1.1*	136, 136, 144
		5000 µg	*170.3*	*1.2*	*1.3*	171, 171, 169
	Deionised water		*128.7*	*11.0*		134, 116, 136
	Untreated Control		*126.0*	*8.9*		129, 116, 133

Study Name: 1283801
Experiment: 1283801 VVa Plate
Assay Conditions:

Study Code: Harlan CCR 1283801
Date Plated: 15/09/2009
Date Counted: 18/09/2009

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 102	**EL 2009083**	3 µg	*396.3*	*34.6*	*1.0*	410, 357, 422
		10 µg	*409.7*	*29.7*	*1.0*	432, 376, 421
		33 µg	*352.3*	*29.9*	*0.9*	342, 329, 386
		100 µg	*379.3*	*26.4*	*1.0*	392, 397, 349
		333 µg	*412.0*	*16.8*	*1.1*	425, 418, 393
		1000 µg	*430.0*	*16.5*	*1.1*	441, 411, 438
		2500 µg	*401.0*	*32.8*	*1.0*	436, 396, 371
		5000 µg	*334.3*	*31.3*	*0.9*	368, 306, 329
	Deionised water		*390.3*	*15.6*		392, 405, 374
	Untreated Control		*383.3*	*20.8*		390, 400, 360
TA 1535	**NaN3**	10 µg	*1879.3*	*54.3*	*137.5*	1849, 1847, 1942
TA 1537	**4-NOPD**	50 µg	*69.7*	*2.5*	*8.0*	67, 70, 72
TA 98	**4-NOPD**	10 µg	*269.7*	*46.1*	*9.9*	222, 273, 314
TA 100	**NaN3**	10 µg	*1925.7*	*34.0*	*15.0*	1935, 1888, 1954
TA 102	**MMS**	3.0 µL	*2747.3*	*311.8*	*7.0*	2582, 2553, 3107

Key to Positive Controls

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

Study Name: 1283801
Experiment: 1283801 VVa Plate
Assay Conditions:

Study Code: Harlan CCR 1283801
Date Plated: 15/09/2009
Date Counted: 18/09/2009

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**EL 2009083**	3 µg	*21.0*	*0.0*	*1.3*	21, 21, 21
		10 µg	*17.3*	*4.2*	*1.1*	22, 14, 16
		33 µg	*18.3*	*1.2*	*1.1*	19, 19, 17
		100 µg	*18.3*	*1.2*	*1.1*	19, 19, 17
		333 µg	*19.3*	*0.6*	*1.2*	20, 19, 19
		1000 µg	*17.7*	*2.3*	*1.1*	15, 19, 19
		2500 µg	*22.0*	*1.0*	*1.4*	22, 23, 21
		5000 µg	*21.0*	*1.7*	*1.3*	19, 22, 22
	Deionised water		*16.0*	*1.0*		17, 16, 15
	Untreated Control		*20.7*	*2.9*		24, 19, 19
TA 1537	**EL 2009083**	3 µg	*13.3*	*3.1*	*0.9*	14, 10, 16
		10 µg	*11.7*	*1.5*	*0.8*	13, 10, 12
		33 µg	*12.3*	*3.1*	*0.8*	9, 15, 13
		100 µg	*12.7*	*2.5*	*0.9*	13, 10, 15
		333 µg	*15.3*	*1.2*	*1.0*	16, 16, 14
		1000 µg	*12.7*	*3.5*	*0.9*	9, 16, 13
		2500 µg	*15.7*	*4.0*	*1.1*	15, 12, 20
		5000 µg	*20.0*	*1.0*	*1.4*	19, 20, 21
	Deionised water		*14.7*	*1.5*		15, 16, 13
	Untreated Control		*15.3*	*0.6*		16, 15, 15
TA 98	**EL 2009083**	3 µg	*32.0*	*1.7*	*1.0*	31, 31, 34
		10 µg	*35.0*	*2.6*	*1.1*	38, 34, 33
		33 µg	*32.0*	*3.5*	*1.0*	36, 30, 30
		100 µg	*29.0*	*1.0*	*0.9*	29, 28, 30
		333 µg	*32.0*	*3.6*	*1.0*	28, 35, 33
		1000 µg	*31.0*	*3.6*	*1.0*	35, 28, 30
		2500 µg	*32.0*	*1.7*	*1.0*	33, 30, 33
		5000 µg	*39.7*	*2.5*	*1.2*	37, 40, 42
	Deionised water		*32.3*	*3.1*		29, 33, 35
	Untreated Control		*30.7*	*0.6*		31, 30, 31
TA 100	**EL 2009083**	3 µg	*126.0*	*4.4*	*0.9*	128, 129, 121
		10 µg	*141.7*	*14.6*	*1.0*	125, 152, 148
		33 µg	*134.0*	*2.6*	*1.0*	133, 132, 137
		100 µg	*139.0*	*15.1*	*1.0*	144, 151, 122
		333 µg	*130.0*	*6.1*	*0.9*	137, 126, 127
		1000 µg	*161.7*	*8.7*	*1.2*	152, 164, 169
		2500 µg	*182.3*	*3.1*	*1.3*	183, 185, 179
		5000 µg	*223.7*	*9.3*	*1.6*	234, 221, 216
	Deionised water		*137.7*	*20.5*		155, 143, 115
	Untreated Control		*147.7*	*14.2*		164, 141, 138

Study Name: 1283801
Experiment: 1283801 VVa Plate
Assay Conditions:

Study Code: Harlan CCR 1283801
Date Plated: 15/09/2009
Date Counted: 18/09/2009

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 102	**EL 2009083**	3 µg	*503.3*	*56.5*	*1.0*	545, 439, 526
		10 µg	*529.7*	*62.5*	*1.0*	581, 460, 548
		33 µg	*507.0*	*47.6*	*1.0*	534, 535, 452
		100 µg	*514.7*	*69.4*	*1.0*	547, 435, 562
		333 µg	*488.7*	*39.6*	*1.0*	443, 513, 510
		1000 µg	*502.7*	*59.8*	*1.0*	486, 569, 453
		2500 µg	*558.7*	*84.1*	*1.1*	463, 592, 621
		5000 µg	*511.0*	*1.0*	*1.0*	510, 512, 511
	Deionised water		*511.3*	*26.5*		490, 541, 503
	Untreated Control		*547.0*	*17.1*		552, 528, 561
TA 1535	**2-AA**	2.5 µg	*291.7*	*62.7*	*18.2*	346, 306, 223
TA 1537	**2-AA**	2.5 µg	*265.0*	*25.2*	*18.1*	294, 253, 248
TA 98	**2-AA**	2.5 µg	*1689.0*	*92.2*	*52.2*	1751, 1733, 1583
TA 100	**2-AA**	2.5 µg	*1852.3*	*363.4*	*13.5*	1687, 1601, 2269
TA 102	**2-AA**	10.0 µg	*1496.0*	*23.5*	*2.9*	1469, 1512, 1507

Key to Positive Controls

2-AA 2-aminoanthracene

Study Name: 1283801
Experiment: 1283801 HV2 pre
Assay Conditions:

Study Code: Harlan CCR 1283801
Date Plated: 23/09/2009
Date Counted: 28/09/2009

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**EL 2009083**	33 µg	*18.7*	*3.5*	*1.1*	22, 19, 15
		100 µg	*18.0*	*4.4*	*1.1*	15, 16, 23
		333 µg	*18.0*	*8.5*	*1.1*	17, 10, 27
		1000 µg	*16.3*	*3.2*	*1.0*	14, 15, 20
		2500 µg	*18.3*	*2.9*	*1.1*	15, 20, 20
		5000 µg	*21.0*	*6.0*	*1.2*	15, 21, 27
	Deionised water		*17.0*	*3.0*		14, 17, 20
	Untreated Control		*16.3*	*2.5*		16, 19, 14
TA 1537	**EL 2009083**	33 µg	*16.7*	*8.3*	*1.0*	26, 10, 14
		100 µg	*15.3*	*4.7*	*0.9*	17, 10, 19
		333 µg	*16.7*	*0.6*	*1.0*	17, 16, 17
		1000 µg	*16.7*	*3.5*	*1.0*	13, 17, 20
		2500 µg	*18.0*	*3.6*	*1.1*	14, 19, 21
		5000 µg	*19.3*	*2.1*	*1.2*	21, 17, 20
	Deionised water		*16.7*	*4.7*		13, 22, 15
	Untreated Control		*14.3*	*6.5*		21, 8, 14
TA 98	**EL 2009083**	33 µg	*26.3*	*2.1*	*0.7*	28, 24, 27
		100 µg	*37.7*	*4.0*	*1.0*	40, 33, 40
		333 µg	*37.7*	*8.4*	*1.0*	43, 42, 28
		1000 µg	*36.7*	*1.5*	*1.0*	37, 38, 35
		2500 µg	*38.0*	*5.6*	*1.0*	33, 44, 37
		5000 µg	*39.3*	*3.2*	*1.0*	43, 38, 37
	Deionised water		*38.0*	*8.9*		31, 48, 35
	Untreated Control		*27.7*	*2.1*		27, 30, 26
TA 100	**EL 2009083**	33 µg	*127.7*	*1.2*	*1.0*	127, 129, 127
		100 µg	*138.3*	*7.6*	*1.1*	130, 145, 140
		333 µg	*144.0*	*2.6*	*1.1*	147, 142, 143
		1000 µg	*158.7*	*4.0*	*1.2*	154, 161, 161
		2500 µg	*185.3*	*23.9*	*1.4*	158, 202, 196
		5000 µg	*212.7*	*18.1*	*1.6*	192, 226, 220
	Deionised water		*130.7*	*8.1*		138, 132, 122
	Untreated Control		*131.0*	*20.8*		118, 155, 120
TA 102	**EL 2009083**	33 µg	*370.7*	*28.1*	*0.9*	344, 368, 400
		100 µg	*392.0*	*40.8*	*1.0*	378, 360, 438
		333 µg	*406.7*	*34.7*	*1.0*	417, 435, 368
		1000 µg	*391.3*	*21.0*	*1.0*	392, 412, 370
		2500 µg	*366.3*	*15.9*	*0.9*	376, 348, 375
		5000 µg	*325.7*	*12.4*	*0.8*	319, 340, 318
	Deionised water		*396.3*	*26.8*		392, 372, 425
	Untreated Control		*353.7*	*16.1*		342, 347, 372

Study Name: 1283801 Study Code: Harlan CCR 1283801
Experiment: 1283801 HV2 pre Date Plated: 23/09/2009
Assay Conditions: Date Counted: 28/09/2009

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**NaN3**	10 µg	*1527.7*	*74.8*	*89.9*	1442, 1580, 1561
TA 1537	**4-NOPD**	50 µg	*85.7*	*7.0*	*5.1*	85, 93, 79
TA 98	**4-NOPD**	10 µg	*408.3*	*15.9*	*10.7*	390, 418, 417
TA 100	**NaN3**	10 µg	*1777.0*	*61.2*	*13.6*	1835, 1713, 1783
TA 102	**MMS**	3.0 µL	*1110.3*	*70.4*	*2.8*	1029, 1150, 1152

Key to Positive Controls

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

Study Name: 1283801
Experiment: 1283801 HV2 pre
Assay Conditions:

Study Code: Harlan CCR 1283801
Date Plated: 23/09/2009
Date Counted: 28/09/2009

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**EL 2009083**	33 µg	*26.0*	*3.6*	*1.2*	22, 29, 27
		100 µg	*24.0*	*8.7*	*1.1*	14, 29, 29
		333 µg	*21.0*	*6.2*	*1.0*	19, 28, 16
		1000 µg	*22.3*	*2.9*	*1.0*	24, 24, 19
		2500 µg	*28.3*	*1.5*	*1.3*	30, 27, 28
		5000 µg	*34.3*	*5.1*	*1.6*	40, 30, 33
	Deionised water		*21.3*	*3.8*		17, 24, 23
	Untreated Control		*16.7*	*3.8*		21, 15, 14
TA 1537	**EL 2009083**	33 µg	*25.7*	*3.5*	*1.3*	22, 26, 29
		100 µg	*22.0*	*4.4*	*1.1*	20, 27, 19
		333 µg	*20.7*	*1.2*	*1.0*	20, 22, 20
		1000 µg	*27.7*	*2.9*	*1.4*	31, 26, 26
		2500 µg	*23.7*	*7.6*	*1.2*	15, 27, 29
		5000 µg	*29.7*	*5.1*	*1.5*	34, 24, 31
	Deionised water		*20.3*	*3.5*		17, 20, 24
	Untreated Control		*19.3*	*4.0*		17, 17, 24
TA 98	**EL 2009083**	33 µg	*34.3*	*3.5*	*0.8*	34, 31, 38
		100 µg	*51.3*	*7.1*	*1.1*	59, 50, 45
		333 µg	*47.7*	*10.7*	*1.1*	57, 36, 50
		1000 µg	*49.3*	*6.4*	*1.1*	42, 52, 54
		2500 µg	*44.3*	*6.5*	*1.0*	38, 44, 51
		5000 µg	*56.0*	*10.1*	*1.2*	47, 67, 54
	Deionised water		*45.0*	*14.7*		62, 36, 37
	Untreated Control		*44.3*	*5.8*		51, 41, 41
TA 100	**EL 2009083**	33 µg	*176.0*	*4.0*	*1.1*	176, 180, 172
		100 µg	*176.3*	*1.5*	*1.1*	176, 178, 175
		333 µg	*177.0*	*13.2*	*1.1*	182, 162, 187
		1000 µg	*203.7*	*8.1*	*1.2*	199, 213, 199
		2500 µg	*227.3*	*15.0*	*1.4*	212, 242, 228
		5000 µg	*212.0*	*14.0*	*1.3*	206, 228, 202
	Deionised water		*164.0*	*7.9*		158, 161, 173
	Untreated Control		*177.3*	*10.3*		180, 166, 186
TA 102	**EL 2009083**	33 µg	*539.3*	*86.5*	*1.0*	484, 495, 639
		100 µg	*544.0*	*40.0*	*1.0*	583, 546, 503
		333 µg	*575.0*	*37.2*	*1.1*	532, 596, 597
		1000 µg	*560.7*	*39.0*	*1.1*	578, 516, 588
		2500 µg	*399.7*	*43.9*	*0.8*	349, 424, 426
		5000 µg	*282.3*	*31.1*	*0.5*	268, 318, 261
	Deionised water		*531.7*	*32.5*		564, 499, 532
	Untreated Control		*538.3*	*34.2*		502, 570, 543

Study Name: 1283801
Experiment: 1283801 HV2 pre
Assay Conditions:

Study Code: Harlan CCR 1283801
Date Plated: 23/09/2009
Date Counted: 28/09/2009

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**2-AA**	2.5 µg	*249.0*	*11.3*	*11.7*	255, 256, 236
TA 1537	**2-AA**	2.5 µg	*204.3*	*14.4*	*10.0*	221, 196, 196
TA 98	**2-AA**	2.5 µg	*1718.0*	*106.4*	*38.2*	1607, 1728, 1819
TA 100	**2-AA**	2.5 µg	*2157.3*	*51.5*	*13.2*	2208, 2105, 2159
TA 102	**2-AA**	10.0 µg	*1858.7*	*71.0*	*3.5*	1849, 1793, 1934

Key to Positive Controls

2-AA 2-aminoanthracene

harlan™

STUDY NUMBER 1283802

IN VITRO CHROMOSOME ABERRATION TEST IN CHINESE HAMSTER *V79* CELLS WITH Maltogenic amylase from *Bacillus stearothermophilus* expressed in *Bacillus subtilis* (EL 2009083)

REPORT

1st Original of 2

Study Completion Date:

October 30, 2009

1 COPY OF THE GLP CERTIFICATE

[redacted]

HESSEN

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
Harlan Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

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
6 Prüfungen zur Bestimmung von Rückständen	**6** Residues
8 Analytische Prüfungen an biologischen Materialien	**8** Analytical studies on biological materials

15.08. und 27. – 29.10.2008
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

(b) (6)

Th. Zimmermann, Referent, Wiesbaden, den **30. März 2009**
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

HESSISCHES MINISTERIUM FÜR UMWELT, LÄNDLICHEN RAUM UND VERBRAUCHERSCHUTZ

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

2 CONTENTS

3 PREFACE

3.1 General

Title:	*In vitro* Chromosome Aberration Test in Chinese Hamster *V79* Cells with Maltogenic amylase from *Bacillus stearothermophilus* expressed in *Bacillus subtilis* (EL 2009083)
Sponsor:	AB Enzymes GmbH Feldbergstr. 78 64293 Darmstadt Germany
Study Monitor:	Dr. H.-J. Schepers
Test Facility:	Harlan Cytotest Cell Research GmbH (Harlan CCR) In den Leppsteinswiesen 19 64380 Rossdorf Germany

3.2 Responsibilities

Study Director:	Dr. Caroline Hall
Deputy Study Director:	Dr. Susanne Bohnenberger
Management:	Dr. Wolfgang Völkner
Head of Quality Assurance Unit:	Frauke Hermann

3.3 Schedule

Experimental Starting Date: August 26, 2009

Experimental Completion Date: October 02, 2009

3.4 Project Staff Signatures

Study Director Dr. Caroline Hall

(b) (6)

.....

Date: October 30, 2009

Management Dr. Wolfgang Völkner

(b) (6)

Dr. A. Poth

.....

Date: October 30, 2009

3.5 Good Laboratory Practice

The study was performed in compliance with:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1), dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002, and amended version dated July 02, 2008 ("BGBl.", p. 1146).

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final].

3.6 Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

Ninth Addendum to the OECD Guidelines for Testing of Chemicals, February 1998, adopted July 21, 1997, Guideline No. 473 "*In vitro* Mammalian Chromosome Aberration Test".

Commission Regulation (EC) No. 440/2008, B10: "Mutagenicity – *In vitro* Mammalian Chromosome Aberration Test", dated May 30, 2008.

3.7 Archiving

Harlan CCR will archive the following data for 15 years:

Raw data, study plan, report, and a sample of the test item.

Microscopic slides will be archived for at least 12 years.

No data will be discarded without the sponsor's prior written consent.

3.8 Deviations from the Study Plan

There were no deviations from the study plan.

4 STATEMENT OF COMPLIANCE

Study Number: 1283802

Test Item: EL 2009083

Study Director: Dr. Caroline Hall

Title: *In vitro* Chromosome Aberration Test
in Chinese Hamster *V79* Cells
with Maltogenic amylase from *Bacillus stearothermophilus*
expressed in *Bacillus subtilis* (EL 2009083)

This study performed in the test facility of Harlan CCR was conducted in compliance with Good Laboratory Practice Regulations:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1), dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002, and amended version dated July 02, 2008 ("BGBl.", p. 1146).

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final].

There were no circumstances that may have affected the quality or integrity of the study.

Study Director Harlan CCR
Dr. Caroline Hall

(b) (6)

.....

Date: October 30, 2009

5 STATEMENT OF QUALITY ASSURANCE UNIT

Study Number: 1283802

Test Item: EL 2009083

Study Director: Dr. Caroline Hall

Title: *In vitro* Chromosome Aberration Test
in Chinese Hamster *V79* Cells
with Maltogenic amylase from *Bacillus stearothermophilus*
expressed in *Bacillus subtilis* (EL 2009083)

The general facilities and activities of Harlan CCR are inspected periodically and the results are reported to the responsible person and the Management.

Study procedures were inspected periodically. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Phases and Dates of QAU Inspections/ Audits		Dates of Reports to the Study Director and to Management
Study Plan:	August 24, 2009	August 24, 2009
1st Amendment to Study Plan:	October 22, 2009	October 22, 2009
Process Inspection Test performance:	September 03, 2009	September 03, 2009
Report:	October 23, 2009	October 23, 2009

This statement is to confirm that the present report reflects the raw data.

Head of Quality Assurance Unit Frauke Hermann

(b) (6)

Rudolph

.....

Date: October 30, 2009

6 SUMMARY OF RESULTS

The test item EL 2009083, dissolved in deionised water, was assessed for its potential to induce structural chromosome aberrations in *V79* cells of the Chinese hamster *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 hours	18 hours	4 hours
Recovery	14 hours	-	14 hours
Preparation interval	18 hours	18 hours	18 hours

In each experimental group two parallel cultures were set up. At least 100 metaphases per culture were evaluated for structural chromosome aberrations, except for the positive control in Experiment I without metabolic activation, where only 50 metaphases were evaluated.

The highest applied concentration (5000 µg/mL) was chosen with respect to the current OECD Guideline 473.

Dose selection for the cytogenetic experiments was performed considering the toxicity data and the occurrence of precipitation. The chosen treatment concentrations are described in chapter 8.6 (page 16). The evaluated experimental points and the results are summarised in Table 1 (page 23).

In Experiment I in the absence and presence of S9 mix no cytotoxicity was observed up to the highest applied concentration. In Experiment II clear cytotoxicity was observed at the highest evaluated concentration after test item treatment for 18 hours without metabolic activation.

In Experiment I no clastogenicity was observed at the concentrations evaluated with metabolic activation. Without metabolic activation 5.0 % aberrant cells excluding gaps were determined at the highest concentration of 5000 µg/ml. This value exceeded the laboratory's historical control data range (0.0 – 4.0 % aberrant cellsexcluding gaps) but was not statistical significant higher than the solvent control (3.0 % aberrant cells excluding gaps). Presumably, this effect occurred partly due to a relatively high rate of aberrant cells in the solvent control.

In Experiment II no clastogenicity was observed at the concentrations evaluated without metabolic activation.

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 ($p < 0.05$) in cells with structural chromosome aberrations.

6.1 Conclusion

In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce structural chromosome aberrations in *V79* cells (Chinese hamster cell line) *in vitro*.

Therefore, EL 2009083 is considered to be non-clastogenic in this chromosome aberration test in the absence and presence of metabolic activation, when tested up to the highest required concentration.

7 INTRODUCTION

According to national and international acts chemicals have to be tested before introduction to the market for a possible hazard to humans and the environment. Genotoxicity studies provide important information for the assessment of the mutagenic potential of these substances (1, 4). The *in vitro* Chromosome Aberration Test performed in this study is an essential part of genotoxicity test batteries of substances.

This *in vitro* test is a test for the detection of structural chromosomal aberrations. Such aberrations are frequently lethal to the damaged cells (8, 10). However, cytogenetic damage in somatic cells is an indicator of a potential to induce subtle chromosomal damage that may be compatible with cell division. Similar damage induced in germ cells may lead to heritable cytogenetic abnormalities. Heritable cytogenetic abnormalities are known to have deleterious effects in man, e.g. induction of neoplastic events or birth defects. Also, chromosome abnormalities in somatic cells may become one of the reasons why a transformed cell population may develop into cancer.

Chromosome aberrations are generally evaluated in first post treatment mitoses. The majority of chemical mutagens induced structural aberrations are of the chromatid type, but chromosome type aberrations also occur.

For treatment, cell populations should be in exponential growth to guarantee that there are cells in all stages of the cell cycle (i.e. an asynchronous population). Since the normal cell cycle time is 14 hours (see page 14) and the guidelines require fixation times of about 1.5-fold of the normal cell cycle, a fixation time of around 18 hours is appropriate. Due to the limited capacity of the *V79* cells for metabolic activation of potential mutagens an exogenous metabolic activation system is included (7).

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

7.1 Aims of the Study

This *in vitro* test was performed to assess the potential of EL 2009083 to induce structural chromosome aberrations. Evaluation of cytogenetic damage induced in *V79* cells (cell line from the lung of the Chinese Hamster) in the absence and presence of metabolic activation was performed in two independent experiments at one preparation interval (18 hours).

8 MATERIALS AND METHODS

8.1 Test Item

Internal Test Item Number: S 1043111

The test item and the information concerning the test item were provided by the sponsor.

Identity:	EL 2009083
Batch No.:	AA09087A3
Molecular Weight:	Approx. 400 – 500 g/L (UVCB substance)
Purity:	Not applicable (UVCB substance)
Stability in Solvent:	1 day at room temperature, 5 days in the refrigerator and 1 year in the freezer in water and saline
Storage:	At room temperature, moisture protected, light protected
Expiration Date:	August 2011

On the day of the experiment (immediately before treatment), the test item was dissolved in deionised water. The final concentration of deionised water in the culture medium was 10 % (v/v). The solvent was chosen to its solubility properties and its relative non-toxicity to the cell cultures.

8.2 Controls

8.2.1 Solvent Controls

Concurrent solvent controls (deionised water) were performed.

8.2.2 Positive Control Substances

Without metabolic activation

Name:	EMS; Ethylmethane sulfonate
Supplier:	ACROS ORGANICS, 2440 Geel, Belgium
Purity:	≥ 98 %
Lot no.:	A0259466
Expiration Date:	March 2010
Dissolved in:	Nutrient medium
Final Concentration:	600.0 / 1000.0 µg/mL (4.8 / 8.0 mM)

Solutions were prepared on the day of experiment. The stability of the positive control substance in solution was proven by the mutagenic response in the expected range.

With metabolic activation

Name:	CPA; Cyclophosphamide
Supplier:	Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany
Purity:	≥ 98 %
Lot no.:	097K1311
Expiration Date:	March 2011
Dissolved in:	Saline (0.9 % [w/v])
Final Concentration:	1.4 µg/mL (5.0 µM)

The dilutions of the stock solutions were prepared on the day of experiment. The stability of CPA in solution at room temperature is good. At 25 °C only 3.5 % of its potency is lost after 24 hours (6).

8.3 Test System

8.3.1 Reasons for the Choice of the Cell Line *V79*

The *V79* cell line has been used successfully for many years in *in vitro* experiments. The high proliferation rate (doubling time of *V79* cells in stock cultures: 14 hours, determined on April 18, 2008) and a reasonable plating efficiency of untreated cells (as a rule more than 70 %) both necessary for the appropriate performance of the study, support the use of this cell line. The cells have a stable karyotype with a modal chromosome number of 22.

Lacking metabolic activities of cells under *in vitro* conditions are a disadvantage of tests with cell cultures as many chemicals only develop a mutagenic potential when they are metabolized by the mammalian organism. However, metabolic activation of chemicals can be achieved at least partially by supplementing the cell cultures with liver microsome preparations (S9 mix).

8.3.2 Cell Cultures

Large stocks of the *V79* cell line (supplied by Laboratory for Mutagenicity Testing, LMP, Technical University Darmstadt, 64287 Darmstadt, Germany) were stored in liquid nitrogen in the cell bank of Harlan CCR allowing the repeated use of the same cell culture batch in experiments. Before freezing each batch was screened for mycoplasma contamination and checked for karyotype stability. Consequently, the parameters of the experiments remain similar because of standardized characteristics of the cells.

Thawed stock cultures were propagated at 37 °C in 80 cm² plastic flasks (Greiner, 72632 Frickenhausen, Germany). About 5×10^5 cells per flask were seeded into 15 mL of MEM (Minimal Essential Medium; Invitrogen Gibco, 76131 Karlsruhe, Germany) supplemented with 10 % fetal calf serum (FCS; Invitrogen Gibco, 76131 Karlsruhe, Germany). Additionally, the medium was supplemented with 1 % 100x Penicillin/ Streptomycin solution (10.000 Units/mL Penicillin, 10 mg/mL Streptomycin; PAA Laboratories GmbH, 35091 Cölbe, Germany) and 1 % Amphotericin B-solution (250 µg/mL, PAA Laboratories GmbH, 35091 Cölbe, Germany). The cells were sub-cultured twice weekly. The cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % carbon dioxide (98.5 % air).

8.4 Mammalian Microsomal Fraction S9 Mix

8.4.1 S9 (Preparation by Harlan CCR)

Phenobarbital/β-Naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from 8 - 12 weeks old male Wistar rats (HsdCpb:WU, Harlan Laboratories GmbH, 33178 Borchen, Germany) weight approx. 220 - 320 g induced by intraperitoneal applications of 80 mg/kg b.w. Phenobarbital (Desitin; 22335 Hamburg, Germany) and by peroral administrations of 80 mg/kg b.w. β-Naphthoflavone (Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany) each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1:3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. Small numbers of the ampoules were kept at –20 °C for up to one week. Each batch of S9 mix was routinely tested with 2-aminoanthracene as well as benzo(a)pyrene.

The protein concentration was 32.3 mg/mL (Lot no. 020709).

8.4.2 S9 Mix

The S9 mix preparation was performed according to Ames et al. (1).

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. Cofactors were added to the S9 mix to reach the following concentrations:

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 was stored in an ice bath.

8.5 Pre-experiment

A pre-test on cell growth inhibition was performed in order to determine the toxicity of the test item (2), the solubility during exposure and changes in osmolarity and pH value at experimental conditions. In agreement with the OECD guideline no. 473 the test item was applied up to a maximum concentration of 5000 µg/mL. At the selected dose no influence on solubility, pH value, or osmolarity was detected.

The experimental conditions in this pre-experiment were identical to those required and described below for the mutagenicity assay.

8.6 Dose Selection

The highest concentration used in the cytogenetic experiments was chosen considering the current OECD Guideline for *in vitro* mammalian cytogenetic tests requesting for the top concentration clear toxicity with reduced cell numbers or mitotic indices below 50 % of control, whichever is the lowest concentration, and/or the occurrence of precipitation. In case of non-toxicity the maximum concentration should be 5 mg/mL, 5 µL/mL or 10 mM, whichever is the lowest, if formulation in an appropriate solvent is possible.

5000 µg/mL of EL 2009083 was applied as top concentration for treatment of the cultures. Test item concentrations between 19.5 and 5000 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. Precipitation of the test item was observed at 5000.0 µg/mL. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.

Dose selection of Experiment II was influenced by the results obtained in Experiment I. Since no relevant cytotoxicity indicated by reduced cell numbers or reduced mitotic indices below 50 % of control was observed 5000.0 µg/mL was chosen as top treatment concentration for continuous exposure in the absence of S9 mix in Experiment II.

Doses applied in the Chromosome aberration test with EL 2009083

Preparation interval	Exposure period	Exp.	Concentration in µg/mL								
			Without S9 mix								
18 hrs	4 hrs	I	19.5	39.1	78.1	156.3	312.5	625.0	**1250.0**	**2500.0**	**5000.0**[P]
18 hrs	18 hrs	II	19.5	39.1	78.1	156.3	312.5	625.0	**1250.0**	**2500.0**	**5000.0**
			With S9 mix								
18 hrs	4 hrs	I	19.5	39.1	78.1	156.3	312.5	625.0	**1250.0**	**2500.0**	**5000.0**[P]

Evaluated experimental points are shown in bold characters

[P] Precipitation occurred at the end of treatment

8.7 Experimental Performance

8.7.1 Seeding of the Cultures

Exponentially growing stock cultures more than 50 % confluent were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. Then the enzymatic treatment was stopped by adding complete culture medium and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.5 % (w/v) in Ca-Mg-free salt solution (Invitrogen Gibco, 76131 Karlsruhe, Germany).

Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution, which was composed as follows (per litre):

NaCl	8000 mg
KCl	200 mg
KH_2PO_4	200 mg
$Na_2HPO_4 \cdot 7\ H_2O$	2160 mg

The cells were seeded into Quadriperm dishes (Greiner, 72636 Frickenhausen, Germany) that contained microscopic slides (at least 2 chambers per dish and test group). In each chamber 1 x 10^4 - 6 x 10^4 cells were seeded with regard to the preparation time. The medium was MEM with 10 % FCS (complete medium), 1 % 100x Penicillin/ Streptomycin and 1 % Amphotericin B-solution.

8.7.2 Treatment

Exposure period 4 hours

The culture medium of exponentially growing cell cultures was replaced with serum-free medium with 1 % 100x Penicillin/-Streptomycin-solution and 1 % Amphotericin B-solution containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium were used. Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" and then the cells were cultured in complete medium for the remaining culture time.

The "Saline G" solution was composed as follows (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose $\cdot\ H_2O$	1100 mg
$Na_2HPO_4 \cdot 2\ H_2O$	192 mg
KH_2PO_4	150 mg

pH was adjusted to 7.2.

Exposure period 18 hours

The culture medium of exponentially growing cell cultures was replaced with complete medium (with 10 % FCS, 1 % 100x Penicillin/-Streptomycin-solution, 1 % Amphotericin B-solution) containing different concentrations of the test item without S9 mix. The medium was not changed until preparation of the cells.

All cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO_2 (98.5 % air).

8.7.3 Preparation of the Cultures

Colcemid was added (0.2 µg/mL culture medium) to the cultures 15.5 hours after the start of the treatment. The cells on the slides were treated 2.5 hours later, in the chambers with hypotonic solution (0.4 % KCl) for 20 min at 37 °C. After incubation in the hypotonic solution the cells were fixed with a mixture of methanol and glacial acetic acid (3:1 parts, respectively). Per experiment two slides per group were prepared. After preparation the cells were stained with Giemsa (E. MERCK, 64293 Darmstadt, Germany).

8.7.4 Evaluation of Cell Numbers

The evaluation of cytotoxicity indicated by reduced cell numbers was made after the preparation of the cultures on spread slides. The cell numbers were determined microscopically by counting 10 defined fields per coded slide. The cell number of the treatment groups is given in percentage compared to the respective solvent control.

8.7.5 Analysis of Metaphase Cells

Evaluation of the cultures was performed (according to standard protocol of the "Arbeits-gruppe der Industrie, Cytogenetik" [5]) using NIKON microscopes with 100x oil immersion objectives. Breaks, fragments, deletions, exchanges, and chromosome disintegrations were recorded as structural chromosome aberrations. Gaps were recorded as well but not included in the calculation of the aberration rates. At least 100 well spread metaphases per culture were evaluated for cytogenetic damage on coded slides, except for the positive control in Experiment I without metabolic activation, where only 50 metaphases were evaluated. Only metaphases with characteristic chromosome numbers of 22 ± 1 were included in the analysis. To describe a cytotoxic effect the mitotic index (% cells in mitosis) was determined.

8.8 Data Recording

The data generated were recorded in the raw data file. The results are presented in tabular form, including experimental groups with the test item, solvent and positive controls.

8.9 Acceptability of the Test

The chromosome aberration test performed in our laboratory is considered acceptable, if it meets the following criteria:

a) The number of structural aberrations found in the solvent controls falls within the range of the laboratory's historical control data (see ANNEX III).

b) The positive control substances produce significant increases in the number of cells with structural chromosome aberrations, which are within the range of the laboratory's historical control data (see ANNEX III).

8.10 Evaluation of Results

A test item is classified as non-clastogenic if:

- the number of induced structural chromosome aberrations in all evaluated dose groups is in the range of the laboratory's historical control data range (see ANNEX III).

and/or

- no significant increase of the number of structural chromosome aberrations is observed.

A test item is classified as clastogenic if:

- the number of induced structural chromosome aberrations is not in the range of the laboratory's historical control data range (see ANNEX III).

and

- either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed.

Statistical significance was confirmed by means of the Fisher's exact test (9) ($p < 0.05$). However, both biological and statistical significance should be considered together. If the criteria mentioned above for the test item are not clearly met, the classification with regard to the historical data and the biological relevance is discussed and/or a confirmatory experiment is performed.

Although the inclusion of the structural chromosome aberrations is the purpose of this study, it is important to include the polyploids and endoreduplications. The following criterion is valid:

A test item can be classified as aneugenic if:

- the number of induced numerical aberrations is not in the range of the laboratory's historical control data range (see ANNEX III).

9 RESULTS AND DISCUSSION

The test item EL 2009083, dissolved in deionised water, was assessed for its potential to induce structural chromosome aberrations in *V79* cells of the Chinese hamster *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 metabolic activation. In Experiment II the exposure period was 18 hours without S9 mix. The chromosomes were prepared 18 hours after start of treatment with the test item.

In each experimental group two parallel cultures were set up. At least 100 metaphases per culture were evaluated for structural chromosome aberrations, except for the positive control in Experiment I without metabolic activation, where only 50 metaphases were evaluated.

No relevant influence of the test item on pH value or osmolarity was observed (Experiment I: solvent control 289 mOsm, pH 7.2 versus 308 mOsm and pH 7.2 at 5000.0 µg/mL, Experiment II: solvent control 278 mOsm, pH 7.4 versus 309 mOsm and pH 7.3 at 5000.0 µg/mL).

In Experiment I in the absence and presence of S9 mix precipitation of the test item in culture medium was observed after treatment with 5000.0 µg/mL. In Experiment II no precipitation was observed up to the highest applied concentration.

No clear toxic effects indicated by reduced mitotic indices or reduced cell numbers were observed after treatment with the test item in Experiment I (see Table 2 and 3, pages 24, 25 and 26). In Experiment II in the absence of S9 mix clearly reduced cell numbers (35.8 % of control) were observed after treatment with 5000.0 µg/mL (see Table 2, page 24).

In Experiment I in the presence of S9 mix and in Experiment II in the absence of S9 mix no statistically significant or biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed (see Table 5 and 7, pages 28 and 30). The aberration rates of the cells after treatment with the test item (0.0 - 3.0 % aberrant cells, excluding gaps) corresponding to the range of the solvent control values (0.5 - 1.0 % aberrant cells, excluding gaps) were within the range of the laboratory's historical control data (see ANNEX III). In Experiment I in the absence of S9 mix a single increase in the number of aberrant cells excluding gaps (5.0 %) exceeding the laboratory's historical solvent control data range (see ANNEX III) was observed at the highest evaluated concentration (5000.0 µg/mL, see Table 4, page 27). This value was not statistically significantly higher than the solvent control and is therefore not regarded as biologically relevant.

No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the controls.

In both experiments, either EMS (600 or 1000 µg/mL) or CPA (1.4 µg/mL) were used as positive controls and showed distinct increases in the number of cells with structural chromosome aberrations.

In conclusion, it can be stated that under the experimental conditions reported, the test item EL 2009083 did not induce structural chromosome aberrations in *V79* cells (Chinese hamster cell line) when tested up to the highest required concentration.

10 DISTRIBUTION OF THE REPORT

Sponsor	1x (1x original, 1x electronic copy as PDF-file)
Study Director	1x (original)

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Mutagenesis, 2, 95-106

12 ANNEX I: TABLES OF RESULTS

12.1 Summary of Results

Table 1: Summary of results of the chromosome aberration study with EL 2009083

Exp.	Preparation interval	Test item concentration in µg/mL	Cell numbers in % of control	Mitotic indices in % of control	Aberrant cells in % incl. gaps*	excl. gaps*	with exchanges
Exposure period 4 hrs without S9 mix							
I	18 hrs	Solvent control[1]	100.0	100.0	3.0	3.0	1.5
		Positive control[#2]	n.t.	100.4	51.0	**51.0**[S]	40.0
		1250.0	104.6	110.6	3.0	1.5	0.5
		2500.0	77.5	94.7	3.0	3.0	0.0
		5000.0[##P]	83.3	96.8	5.5	**5.0**	0.8
Exposure period 18 hrs without S9 mix							
II	18 hrs	Solvent control[1]	100.0	100.0	1.5	1.0	0.0
		Positive control[3]	n.t.	92.4	23.0	**23.0**[S]	11.5
		1250.0	68.0	73.8	1.0	1.0	0.0
		2500.0	57.3	71.0	2.0	2.0	0.0
		5000.0	35.8	83.8	3.5	3.0	0.0
Exposure period 4 hrs with S9 mix							
I	18 hrs	Solvent control[1]	100.0	100.0	1.0	0.5	0.0
		Positive control[4]	n.t.	94.6	9.0	**9.0**[S]	1.5
		1250.0	104.5	98.3	2.0	1.5	1.5
		2500.0	97.6	99.3	0.5	0.5	0.0
		5000.0[P]	90.0	94.9	0.0	0.0	0.0

* Inclusive cells carrying exchanges
\# Evaluation of 50 metaphases per culture
\## Evaluation of 200 metaphases per culture
P Precipitation occurred at the end of treatment
S Aberration frequency statistically significant higher than corresponding control values
n.t. Not tested
1 Deionised water 10.0 % (v/v)
2 EMS 1000.0 µg/mL
3 EMS 600.0 µg/mL
4 CPA 1.4 µg/mL

12.2 Experiments I and II: Determination of Toxicity

The toxicity of the test item was examined using the determination of the cell number. Cell numbers of two cultures (10 coordinate defined fields per culture) were determined for each experimental group, except the positive control.

Table 2: Number of cells in % of solvent control

Without S9 mix							
Experiment I: 4 hrs exposure				Experiment II: continuous exposure			
Preparation interval	Concentration in µg/mL	Number of cells	Cells in % of solvent control	Preparation interval	Concentration in µg/mL	Number of cells	Cells in % of solvent control
18 hrs	Solvent control	608	100.0	18 hrs	Solvent control	923	100.0
"	19.5	627	103.1	"	19.5	n.d.	n.d.
"	39.1	663	109.1	"	39.1	n.d.	n.d.
"	78.1	612	100.7	"	78.1	n.d.	n.d.
"	156.3	639	105.1	"	156.3	n.d.	n.d.
"	312.5	646	106.3	"	312.5	774	83.8
"	625.0	646	106.3	"	625.0	809	87.6
"	**1250.0**	636	104.6	"	**1250.0**	627	68.0
"	**2500.0**	471	77.5	"	**2500.0**	529	57.3
"	**5000.0**[P]	506	83.3	"	**5000.0**	330	35.8

Experimental groups evaluated for cytogenetic damage are shown in bold characters

[P] Precipitation occurred at the end of treatment

n.d. Not determined

Table 2 cont.: Number of cells in % of solvent control

With S9 mix			
Experiment I: 4 hrs exposure			
Preparation interval	Concentration in µg/mL	Number of cells	Cells in % of solvent control
18 hrs	Solvent control	649	100.0
"	19.5	633	97.5
"	39.1	674	103.9
"	78.1	646	99.5
"	156.3	661	101.9
"	312.5	697	107.4
"	625.0	651	100.3
"	**1250.0**	678	104.5
"	**2500.0**	633	97.6
"	**5000.0**[P]	584	90.0

Experimental groups evaluated for cytogenetic damage are shown in bold characters

[P] Precipitation occurred at the end of treatment

12.3 Experiment I

Table 3: Mitotic index; preparation interval 18 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 / 14 hrs	13.3	15.0	14.2	100.0
Pos. control##	1000.0 µg	-	4 / 14 hrs	15.0	13.4	14.2	100.4
Test item	1250.0 µg	-	4 / 14 hrs	16.1	15.2	15.7	110.6
″	2500.0 µg	-	4 / 14 hrs	14.0	12.8	13.4	94.7
″	5000.0 µg	-	4 / 14 hrs	12.5	14.9	13.7	96.8
Solv. control#	10.0 %	+	4 / 14 hrs	15.8	13.6	14.7	100.0
Pos. control###	1.4 µg	+	4 / 14 hrs	14.7	13.1	13.9	94.6
Test item	1250.0 µg	+	4 / 14 hrs	15.6	13.3	14.5	98.3
″	2500.0 µg	+	4 / 14 hrs	13.9	15.3	14.6	99.3
″	5000.0 µg	+	4 / 14 hrs	14.8	13.1	14.0	94.9

* 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 4: Structural chromosome aberrations Experiment I;
preparation interval 18 hrs without S9 mix: exposure period 4 hrs

Slide no.	Cells scored	% Aberrant cells incl. gaps*	excl. gaps*	with ex-changes	Aberrations Gaps g	ig	Chromatid type b	f	d	ex	Chromosome type ib	if	id	cx	Other ma	cd
					Without S9 mix											
Solvent control: deionised water 10.0 %																
1	100				0	0	3	0	0	2	0	0	0	0	0	0
2	100				0	0	2	0	0	1	0	0	0	0	0	0
1 + 2	200	3.0	3.0	1.5	0	0	5	0	0	3	0	0	0	0	0	0
Positive control: EMS 1000.0 µg/mL**																
1	50				0	0	18	1	0	27	7	0	0	0	0	0
2	50				1	0	7	1	0	25	4	0	0	0	0	0
1 + 2	100	51.0	51.0	40.0	1	0	25	2	0	52	11	0	0	0	0	0
Test item: 1250.0 µg/mL																
1	100				2	0	2	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	1	0	0	0	0	0	0
1 + 2	200	3.0	1.5	0.5	3	0	2	0	0	1	0	0	0	0	0	0
Test item: 2500.0 µg/mL																
1	100				0	0	3	0	0	0	0	0	0	0	0	0
2	100				0	0	3	0	0	0	0	0	0	0	0	0
1 + 2	200	3.0	3.0	0.0	0	0	6	0	0	0	0	0	0	0	0	0
Test item: 5000.0 µg/mL***																
1	200				0	0	10	1	0	1	0	0	0	0	0	0
2	200				2	0	7	0	0	2	0	0	0	0	0	0
1 + 2	400	5.5	5.0	0.8	2	0	17	1	0	3	0	0	0	0	0	0

* Inclusive cells carrying exchanges

** 50 metaphases per culture were evaluated due to strong clastogenic effects

*** 200 metaphases per culture were evaluated due to inhomogeneous data

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 5: Structural chromosome aberrations Experiment I;
preparation interval 18 hrs with S9 mix: exposure period 4 hrs

Slide no.	Cells scored	% Aberrant cells incl. gaps*	excl. gaps*	with ex-changes	Aberrations Gaps g	ig	Chromatid type b	f	d	ex	Chromosome type ib	if	id	cx	Other ma	cd
					With S9 mix											
Solvent control: deionised water 10.0 %																
1	100				1	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	1.0	0.5	0.0	1	0	1	0	0	0	0	0	0	0	0	0
Positive control: CPA 1.4 µg/mL																
1	100				1	0	8	0	0	0	0	1	0	0	0	0
2	100				0	0	9	0	0	3	1	0	0	0	0	0
1 + 2	200	9.0	9.0	1.5	1	0	17	0	0	3	1	1	0	0	0	0
Test item: 1250.0 µg/mL																
1	100				1	0	0	0	0	2	0	0	0	0	0	0
2	100				0	0	0	0	0	1	0	0	0	0	0	0
1 + 2	200	2.0	1.5	1.5	1	0	0	0	0	3	0	0	0	0	0	0
Test item: 2500.0 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: 5000.0 µg/mL																
1	100				0	0	0	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	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0	0	0	0

* Inclusive cells carrying exchanges

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)

12.4 Experiment II

Table 6: Mitotic index;
preparation interval 18 hrs without S9 mix

Treatment group	Conc. per mL		S9 mix	Exposure period/ Recovery	Mitotic indices* Absolute 1	Absolute 2	Mean	%**
Solv. control#	10.0	%	-	18 / - hrs	8.4	12.6	10.5	100.0
Pos. control##	600.0	µg	-	18 / - hrs	9.6	9.8	9.7	92.4
Test item	1250.0	µg	-	18 / - hrs	7.6	7.9	7.8	73.8
″	2500.0	µg	-	18 / - hrs	7.8	7.1	7.5	71.0
″	5000.0	µg	-	18 / - hrs	10.2	7.4	8.8	83.8

* 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 7: Structural chromosome aberrations Experiment II;
preparation interval 18 hrs without S9 mix: exposure period 18 hrs

Slide no.	Cells scored	% Aberrant cells incl. gaps*	excl. gaps*	with ex-changes	Aberrations: Gaps g	ig	Chromatid type b	f	d	ex	Chromosome type ib	if	id	cx	Other ma	cd
					Without S9 mix											
Solvent control: Deionised water 10.0 %																
1	100				1	0	2	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	1.5	1.0	0.0	1	0	2	0	0	0	0	0	0	0	0	0
Positive control: EMS 600.0 µg/mL																
1	100				0	0	22	0	0	13	0	0	0	0	0	0
2	100				0	0	5	2	0	12	3	0	1	0	0	0
1 + 2	200	23.0	23.0	11.5	0	0	27	2	0	25	3	0	1	0	0	0
Test item: 1250.0 µg/mL																
1	100				0	0	0	0	0	0	1	0	0	0	0	0
2	100				0	0	0	0	0	0	1	0	0	0	0	0
1 + 2	200	1.0	1.0	0.0	0	0	0	0	0	0	2	0	0	0	0	0
Test item: 2500.0 µg/mL																
1	100				0	0	0	1	0	0	0	0	0	0	0	0
2	100				0	0	3	0	0	0	0	0	0	0	0	0
1 + 2	200	2.0	2.0	0.0	0	0	3	1	0	0	0	0	0	0	0	0
Test item: 5000.0 µg/mL																
1	100				0	0	2	0	0	0	0	0	0	0	0	0
2	100				1	0	4	0	0	0	0	0	0	0	0	0
1 + 2	200	3.5	3.0	0.0	1	0	6	0	0	0	0	0	0	0	0	0

* Inclusive cells carrying exchanges

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)

12.5 Biometry

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the Fisher´s exact test. Evaluation was performed only for cells carrying aberrations excluding gaps.

Table 8: Biometry of Experiment I

Test group versus solvent control		Preparation interval	Exposure period	S9 mix	p-value
Test group	1250.0 µg/mL	18 hrs	4 hrs	-	n.c.
"	2500.0 µg/mL	18 hrs	4 hrs	-	n.c.
"	5000.0 µg/mL	18 hrs	4 hrs	-	0.132
"	1250.0 µg/mL	18 hrs	4 hrs	+	0.187
"	2500.0 µg/mL	18 hrs	4 hrs	+	n.c.
"	5000.0 µg/mL	18 hrs	4 hrs	+	n.c.
Positive control versus solvent control					
EMS	1000.0 µg/mL	18 hrs	4 hrs	-	< 0.001[s]
CPA	1.4 µg/mL	18 hrs	4 hrs	+	< 0.001[s]

n.c. Not calculated as the aberration rate is equal or lower than the corresponding control rate
[s] Aberration rate is statistically significant higher than the control rate

Table 9: Biometry of Experiment II

Test group versus solvent control		Preparation interval	Exposure period	S9 mix	p-value
Test group	1250.0 µg/mL	18 hrs	18 hrs	-	n.c.
"	2500.0 µg/mL	18 hrs	18 hrs	-	0.225
"	5000.0 µg/mL	18 hrs	18 hrs	-	0.088
Positive control versus solvent control					
EMS	600.0 µg/mL	18 hrs	18 hrs	-	< 0.001[s]

n.c. Not calculated as the aberration rate is equal or lower than the corresponding control rate
[s] Aberration rate is statistically significant higher than the control rate

13 ANNEX II

13.1 Chromosome Aberrations: 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 evaluated 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 evaluated if the fragment is associated with a chromosome from which it was probably derived. However, fragments are often seen in isolation and are then evaluated as chromatid fragments (if). In addition, isodeletions can occur as a result of an 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 evaluated as part of the exchange.

5. **Multiple Aberrations**

   If many aberrations are present in one metaphase, the exact details may not be evaluable. 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 22 $\pm$ 1 then it is classified as a diploid cell and evaluated for aberrations. If less than 22 $\pm$ 1 chromosomes are counted then the cell is ignored under the assumption, that some chromosomes may have been lost for technical reasons. If greater than 22 $\pm$ 1 chromosomes are evaluated then the count is recorded and the cell classified as an aneuploid cell. If multiple copies of the haploid chromosome number (other than diploid) are evaluated 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 evaluated as endoreduplicated (e).

14 ANNEX III

14.1 Historical laboratory control data

14.1.1 Percentage of aberrant and polyploid cells in Chinese hamster *V79* cell cultures (January to December 2008)

Without S9 mix

Without S9 mix: preparation interval 18 hrs, treatment 4 hrs								
	Aberrant cells (%)				Polyploid cells (%)			
	No. of studies	Range	Mean	Calc. range[1]	No. of studies	Range	Mean	Calc. range[1]
Solvent controls								
Aqueous solv.[2]	29	0.0 – 3.5	1.5	0.6 – 2.3	23	1.2 – 3.8	2.4	1.8 – 3.1
Organic solv.[3]	33	0.0 – 4.0	1.7	0.7 – 2.7	23	1.2 – 3.7	2.4	1.7 – 3.1
Total	62	0.0 – 4.0	1.6	0.7 – 2.5	46	1.2 – 3.8	2.4	1.7 – 3.1
Positive control[4]								
EMS 400 - 1200 µg/ml	45	8.8 – 33.0	15.2	9.4 – 21.0	29	0.7 – 4.0	2.6	1.8 – 3.4

[1] Mean ± standard deviation

[2] Aqueous solvents: deionised water (10 % v/v), 0.9 % (w/v) saline (10 % v/v) and culture medium MEM; data obtained in the year 2007 and 2008

[3] Organic solvents: dimethyl sulfoxide, acetone, ethanol and tetrahydrofurane (0.5 % v/v)

[4] Positive control only for induction of structural aberrations

Without S9 mix: preparation interval 18 hrs, treatment 18 hrs								
	Aberrant cells (%)				Polyploid cells (%)			
	No. of studies	Range	Mean	Calc. range[1]	No. of studies	Range	Mean	Calc. range[1]
Solvent controls								
Aqueous solv.[2]	24	0.0 – 3.5	1.5	0.7 – 2.3	19	1.2 – 4.0	2.7	1.8 – 3.5
Organic solv.[3]	26	0.0 – 3.0	1.4	0.7 – 2.2	23	0.7 – 3.5	2.5	1.9 – 3.1
Total	50	0.0 – 3.5	1.5	0.7 – 2.2	42	0.7 – 4.0	2.6	1.9 – 3.3
Positive control[4]								
EMS 400 - 1200 µg/ml	36	8.5 – 37.0	15.7	9.2 – 22.2	28	0.5 – 4.1	2.4	1.6 – 3.2

[1] Mean ± standard deviation

[2] Aqueous solvents: deionised water (10 % v/v), 0.9 % (w/v) saline (10 % v/v) and culture medium MEM; data obtained in the year 2007 and 2008

[3] Organic solvents: dimethyl sulfoxide, acetone, ethanol and tetrahydrofurane (0.5 % v/v)

[4] Positive control only for induction of structural aberrations

With S9 mix

With S9 mix: preparation interval 18 hrs, treatment 4 hrs								
	Aberrant cells (%)				Polyploid cells (%)			
	No. of studies	Range	Mean	Calc. range[1]	No. of studies	Range	Mean	Calc. range[1]
Solvent controls								
Aqueous solv.[2]	36	0.0 – 3.5	2.0	1.1 – 2.9	27	1.1 – 4.1	2.7	1.9 – 3.4
Organic solv.[3]	38	0.0 – 4.0	1.8	0.5 – 3.0	25	0.7 – 4.0	2.5	1.6 – 3.5
Total	74	0.0 – 4.0	1.9	0.8 – 2.9	52	0.7 – 4.1	2.6	1.8 – 3.4
Positive control[4]								
CPA 1.0 – 2.0 µg/ml	57	8.0 – 38.0	13.2	8.7 – 17.6	36	1.3 – 5.1	2.6	1.8 – 3.3

1 Mean ± standard deviation

2 Aqueous solvents: deionised water (10 % v/v), 0.9 % (w/v) saline (10 % v/v) and culture medium MEM; data obtained in the year 2007 and 2008

3 Organic solvents: dimethyl sulfoxide, acetone, ethanol and tetrahydrofurane (0.5 % v/v)

4 Positive control only for induction of structural aberrations

REPORT (PART I OF II)

Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083)

13-Week 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 GmbH**
Feldbergstrasse 78
64293 Darmstadt / Germany

Study Identification: Harlan Laboratories Study **C68821**

Version: Final

Study Completion Date: 14-Dec-2010

CONTENTS

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

Harlan Laboratories Study:	C68821
Test Item:	Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083)
Study Director:	W. H. Braun
Study Title:	13-Week Oral Toxicity (Gavage) Study in the Wistar Rat

This study has been 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 and adopted on November 26th, 1997 by decision of the OECD Council [C (97)186/Final].

There were no circumstances that may have affected the quality or integrity of the data.

Study Director: W. H. Braun

(b) (6)

Date: 14 Dec 2010

QUALITY ASSURANCE STATEMENT

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

Harlan Laboratories Study:	C68821
Test Item:	Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083)
Study Director:	W. H. Braun
Study Title:	13-Week 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 responsible person and the management. Study procedures were periodically inspected. The study plan and this report were audited by the quality assurance. The dates are given below.

Dates and Types of QA Inspections		**Dates of Reports to the Study Director and Test Facility Management**
09-Dec-2009	Study plan	09-Dec-2009
07-Jan-2010	Study based (test item, dose preparation, test system, treatment, sample taking/handling)	07-Jan-2010
10-Feb-2010	Process based (formulation analysis - work up)	10-Feb-2010
30-Mar-2010	Study based (locomotor activity)	30-Mar-2010
08-Apr-2010	Study based (necropsy)	08-Apr-2010
17/18/21-Jun-2010	Formulation analysis appendix	21-Jun-2010
23-Jun-2010	Histopathology appendix	23-Jun-2010
18/21 to 23-Jun-2010	Report	23-Jun-2010

This statement also confirms that this final report reflects the raw data.

Quality Assurance: T. Frei

(b) (6)

Date: 14 December 2010

SIGNATURE(S) OF ADDITIONAL SCIENTIST(S)

Formulation Analysis: Dr. C. Bachmann

(b) (6)

.....

Date: 13-Dec-2010

Histopathology: Dr. T. Razinger

(b) (6)

.....

Date: 13-Dec-2010

PREFACE

General Information

Test Item:	Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083)
Study Title:	13-Week Oral Toxicity (Gavage) Study in the Wistar Rat
Sponsor:	AB Enzymes GmbH Feldbergstrasse 78 64293 Darmstadt / Germany
Sponsor Contact:	Dr. H.-J. Schepers
Test Facility:	Harlan Laboratories Ltd. Zelgliweg 1 4452 Itingen / Switzerland
QA:	Harlan Laboratories Ltd. Quality Assurance GLP Zelgliweg 1 4452 Itingen / Switzerland

Responsibilities

Study Director:	W. H. Braun
Deputy Study Director:	M. Sieber
Planning Coordinator:	A. Suter
Technical Coordinator:	P. Althaus Ravenstijn
Necropsy / Histotechnology:	Dr. K. Weber
Clinical Laboratory Investigations:	R. Draheim
Formulation Analysis:	C. Bachmann
Histopathology:	Dr. T. Razinger

Quality Assurance:

Head of QA:	T. Fink

Schedule

Experimental Starting Date:	14-Dec-2009
Delivery of Animals:	29-Dec-2009
Randomization:	30-Dec-2009
Acclimatization:	30-Dec 2009 to 06-Jan-2010
Administration / Treatment:	07-Jan to 07-Apr-2010
Termination (Necropsy):	08-Apr-2010
Experimental Completion Date:	13-Dec-2010

Accreditation

“Harlan Laboratories Ltd.” 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.

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.

Animal Welfare

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

Archiving

Harlan Laboratories Ltd. (4452 Itingen / Switzerland) will retain the study plan, all raw data, a sample of test item(s), specimens (as long as the quality permits evaluation) and the finalized report of the present study for at least ten years. No data will be discarded without the Sponsor’s written consent. Frozen samples were discarded upon issue of the final report.

1 SUMMARY

General

In this subacute toxicity study, Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083) was administered daily by oral gavage to SPF-bred Wistar rats of both sexes at dose levels of 100, 300 and 1000 mg/kg body weight/day for a period of 91 days. A control group was treated similarly with the vehicle, bidistilled water, only.

The groups comprised 10 animals per sex which were sacrificed after 91 days of treatment.

Clinical signs, outside cage observation, ophthalmoscopy, food consumption and body weights were recorded periodically during pretest and treatment periods. Functional observational battery, locomotor activity and grip strength were performed during week 12.

At the end of the dosing 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

All animals survived until scheduled necropsy.

Clinical Signs (Daily and Weekly)

There were no test item-related clinical signs noted during daily observations, and there were no test item-related clinical signs noted during weekly behavioral observations (weeks 1 – 11).

Functional Observational Battery

There were no test item-related clinical signs noted during the functional observational battery (week 12).

Grip Strength

There were no effects upon the fore- or hindlimb grip strength values of the test item-treated males or females when compared with the controls.

Locomotor Activity

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

Food Consumption

The mean daily food consumption of the test item-treated rats was unaffected when compared with that of the controls.

Body Weights

Mean body weights and mean body weight gain of the test item-treated males did not show changes of toxicological relevance when compared with those of the controls.

Ophthalmoscopic Examinations

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

Clinical Laboratory Investigations

Hematology

No test item-related changes in the hematology parameters were noted at any dose level when compared with the controls.

Clinical Biochemistry

Although multiple differences to the control values were noted in several parameters of clinical biochemistry, none were considered to be the result of systemic toxicity and therefore of no toxicological relevance.

Urinalysis

No test item-related changes in the urinalysis parameters were noted at any dose level when compared with the controls.

Organ Weights

There were no test item-related changes in the mean absolute or relative organ weights at any dose level. All changes were without a clearly dose-response relationship, were not associated with microscopic changes and were considered to be without toxicological relevance.

Macroscopic / Microscopic Findings

There were no test item-related macroscopic or microscopic changes at any dose level.

Conclusion

Based on the results of this study, 1000 mg/kg body weight/day of Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083) was established as the no-observed-effect-level (NOEL), and as the no-observed-adverse-effect-level (NOAEL).

2 PURPOSE

The purpose of this oral toxicity study was to assess the cumulative toxicity of Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083) when administered daily to rats by gavage for a period of 13 weeks (91 days).

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

3 MATERIALS AND METHODS

General Remark

Details of the materials and methods that are not specified in the subsequent sections of this report are contained in the appropriate standard operating procedures.

3.1 Test System

Animals:	Rat, RccHan™: 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 ordered:	42 males and 42 females
Age (at Delivery):	7 weeks
Body Weight Range (at Acclimatization):	Males: 187 to 211 g Females: 141 to 159 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 2 100 mg/kg/day**	**Group 3 300 mg/kg/day**	**Group 4 1000 mg/kg/day**
Males	01 - 10	11 - 20	21 - 30	31 - 40
Females	41 - 50	51 - 60	61 - 70	71 - 80

* Control animals were treated with the vehicle only

3.3 Husbandry

Room Numbers, Itingen:	Room no. 136
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%). On one occasion, the humidity was outside the lower range. This was considered to have no effect upon the study. These data are not reported but are retained at Harlan Laboratories Ltd. There was 12-hour fluorescent light/12-hour dark cycle with music during the light period.
Accommodation:	In groups of five 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 2014 and 2914C (batch nos. 58/09 and 82/09, resp.) 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. 320 .

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. 326 .

3.4 Test Item / Vehicle

3.4.1 Test Item

Identification:	Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083)
Description:	Light brown powder
Batch Number:	AA09087A3
Purity/Concentration as Supplied:	100% assumed (UVCB substance)
Purity/Concentration for Formulation:	100%
Correction for Purity:	No.
Salt/base Correction Factor:	None.
Expiry Date	AUG-2011 (defined as 31-Aug-2011 by Harlan)
Storage Conditions:	At room temperature (15 – 25 °C), away from moisture and protected from light.
Safety Precautions:	Routine hygienic procedures (gloves, goggles, face mask).

3.4.2 Vehicle and Control Item

Identification:	Bidistilled water

3.5 Dose Formulations

The test item was used as supplied by the Sponsor. The purity was not corrected.

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 C68810), 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.

A small amount of vehicle was added and the remaining 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.

These analyses were repeated under GLP for this study.

3.5.1 Storage of Dose Formulations

The dose formulations were stored in glass beakers in the refrigerator (5 ± 3 °C).

3.5.2 Analysis of Dose Formulations

The analysis was performed by Harlan Laboratories Ltd. using a photometric enzyme analytical method provided by the Sponsor.

After experimental start, on day 1, and at weeks 6 and 13, 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 at experimental start to confirm stability (4 hour and 7 days).

The samples were delivered to the analytical department (Harlan Laboratories Ltd., Analytics, Füllinsdorf / Switzerland) and stored there at 5 ± 3 °C until analysis.

The test item was used as analytical standard.

Dose formulation samples (primary samples or duplicates) were discarded upon written confirmation by the study director after acceptance of the draft report.

The results of analysis were summarized in an appendix and attached to the report (see Appendix III on p. 333).

3.6 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: 100 mg/kg/day Group 3: 300 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 non-GLP study C68810.
Dose Volume:	10 mL/kg body weight
Dose Concentrations:	Group 1: 0 mg/mL/day Group 2: 10 mg/mL/day Group 3: 30 mg/mL/day Group 4: 100 mg/mL/day
Duration of Pre-Randomization Phase:	1 day
Duration of Acclimatization Phase:	8 days
Duration of Treatment Phase:	91 days

3.7 Phase Designation

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

3.8 Activities and Observations

3.8.1 Viability / Mortality

Observations for viability / mortality were recorded twice daily.

3.8.2 Daily Observations

The animals were observed for clinical signs once daily before commencement of administration as well as daily on days 1 - 91 (twice daily during days 1 - 3) during the treatment period.

3.8.3 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 11) thereafter.

SUMMARY OF PARAMETERS (MINIMUM REQUIREMENT FOR EACH ANIMAL)
OBSERVATIONS: DAILY (D), PRETEST (P), WEEKLY (W) AND FOB (F)

	SCORE	PARAMETER	D	P	W1-11	F12
APPEARANCE	1 - 3	Piloerection	X	X	X	X
	1 - 3	Salivation	X	X	X	X
	1	Hunched posture	X	X	X	X
MOTOR	1 - 3	Ataxia	X	X	X	X
	1 - 3	Tremor/twitching	X	X	X	X
	1	Prostration	X	X	X	X
	1	Circling		X	X	X
	1 - 3	Spasm		X	X	X
BEHAVIOR	1 - 3	Hyperactivity	X	X	X	X
	1 - 3	Somnolence	X	X	X	X
	1 - 3	Increased exploration		X	X	X
	1 - 3	Reduced grooming		X	X	X
	1 - 3	Vocalisation		X	X	X
RESPIRATION	1	Dyspnea	X	X	X	X
	1	Tachypnea	X	X	X	X
	1	Bradypnea	X	X	X	X
REFLEXES	1	Blink		X	X	X
	1	Pinna		X	X	X
	1	Iridic light reflex		X	X	X
	1	Push-off (hind leg)		X	X	X
	1	Pain response		X	X	X
	1	Startle/hearing		X	X	X
	1	Righting reflex		X	X	X
MISCELLANEOUS	1 - 3	Lacrimation		X	X	X
	1	Limbs cyanotic		X	X	X
	1	Mydriasis		X	X	X
	1	Miosis		X	X	X
	1	Exophthalmos		X	X	X
	1 - 3	Reduced muscle tone		X	X	X

NB: Findings that were detected during daily observation of the standard parameters were tracked throughout the study and progression/regression in the afflicted animal, and are listed in the tables. Parameters marked with 'X' were specifically observed for presence or absence.

3.8.4 Functional Observational Battery (Screen)

During week 12, relevant parameters (presented in Section 3.8.3) 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 12.

Grip Strength

Forelimb and hind limb 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 12th 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.

3.8.5 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.

3.8.6 Body Weights

Body weights were recorded once 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.

3.8.7 Ophthalmoscopy

Acclimatization:	All animals
During Week 13:	High dose and control group animals

The ophthalmoscopic examinations of both eyes of all animals were performed after the application of a mydriatic solution (Ciba Vision AG, 3172 Niederwangen / Switzerland) using a direct ophthalmoscope. A description of any abnormality was recorded. For unilateral findings unless otherwise indicated in the tables, the contralateral eye was without abnormalities.

3.9 Clinical Laboratory Investigations

Blood and Urine Sampling:

After 13 Weeks:	08-Apr-2010

Blood samples were drawn 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. Blood samples were drawn from the retro-orbital plexus using a micro-hematocrit glass capillary tube.

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. 342 .

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

3.9.1 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
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Hemoglobin concentration distribution width
Reticulocyte count
Reticulocyte maturity index (low, medium, high fluorescence)
Leukocyte count, total
Lymphocytes
Monocytes
Large unstained cells
Platelet count

Hemoglobin Derivatives

Methemoglobin
Heinz bodies (slides were prepared but not evaluated)

Coagulation

Prothrombin time (= Thromboplastin time)
Activated partial Thromboplastin time

3.9.2 Clinical Biochemistry

The following clinical biochemistry parameters were determined:

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

3.9.3 Urinalysis

The following urine parameters were determined:

Physical Examination

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

Chemical Examination

pH value
Nitrite
Protein
Glucose
Ketones
Urobilinogen
Bilirubin
Erythrocytes
Leukocytes

3.10 Pathology

3.10.1 Necropsy

Sacrifice:

After 13 Weeks: 08-Apr-2010

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	X
Bone marrow (femur)		X	X
Brain - including section of medulla/pons, cerebral and cerebellar cortex	X	X	X
Cecum		X	X
Colon		X	X

Tissues / Organs	Weight	Collect	Examine
Duodenum		X	X
Epididymides (fixed in Bouin'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	X
Heart including auricles	X	X	X
Ileum, with Peyer's patches		X	X
Jejunum with Peyer's patches		X	X
Kidneys	X	X	X
Larynx		X	X
Lacrimal gland, exorbital		X	X
Liver	X	X	X
Lungs, filled w/formalin at necropsy		X	X
Lymph nodes – mesenteric and mandibular		X	X
Mammary gland area		X	X
Nasal cavity		X	X
Ovaries	X	X	X
Pancreas		X	X
Pharynx		X	X
Pituitary gland		X	X
Prostate gland incl. coagulating glands		X	X
Rectum		X	X
Salivary glands - mandibular, sublingual		X	X
Sciatic nerve		X	X
Seminal vesicles		X	X
Skeletal muscle		X	X
Skin		X	X
Spinal cord - cervical, midthoracic, lumbar		X	X
Spleen	X	X	X
Stomach		X	X
Testes (fixed in Bouin's solution)	X	X	X
Thymus	X	X	X
Thyroid (incl. parathyroid gland, if possible)		X	X

Tissues / Organs	Weight	Collect	Examine
Tongue		X	X
Trachea		X	X
Urinary bladder, filled w/formalin at necropsy		X	X
Uterus with Vagina	X	X	X
All gross lesions		X	X

3.10.2 Organ Weights

The organs from animals listed in the table in Section 3.10.1 were weighed before fixation and recorded on the scheduled date 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.

3.10.3 Histotechnique

All organ and tissue samples, as defined under Histopathology (see Section 3.10.4), were processed, embedded and cut at an approximate thickness of 2 to 4 micrometers and stained with hematoxylin and eosin.

3.10.4 Histopathology

Slides of all organs and tissues listed in the table in Section 3.10.1 collected at scheduled sacrifice 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. 355). Attempts were made to correlate gross observations with microscopic findings.

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

3.11 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 a magnetic medium.

Individual values were rounded before printing. All derived values that appear in the tables represent the rounded results of calculations that used the exact raw data value.

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.

3.12 Statistical Analysis

The following statistical methods were used to analyze body weight, 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

4.1 Analysis of Dose Formulations

(See Appendix III on p. 333)

Stability

After storage at room temperature for 4 hours, accuracies of 102.3 to 103.6% were observed for dose formulations. In dose formulations prepared on 14-Dec-2009 and stored for 7 days at 2 - 8 °C, accuracies of 85.3 to 99.3% were found. The test item was considered to be sufficiently stable in the vehicle for at least 7 days.

Concentration and Homogeneity

All results obtained fulfilled the requirements of acceptance criteria. Accuracies of 84.0 to 120.1% (group 2), 85.2 to 107.8% (group 3) and 82.8 to 121.2% (group 4) were observed. No test item was detected in formulation of the control group.

4.2 Observations

4.2.1 Viability / Mortality

(See Individual Tables on p. 131)

All animals survived until scheduled necropsy.

4.2.2 Daily Observations

(See Summary Tables on p. 49 , Individual Tables on p. 140)

There were no test item-related clinical signs noted during daily observations.

At 1000 mg/kg/day, transient, localized hair loss was noted in male no. 32 and, whereas localized hair loss was noted in a second male (no. 37) beginning in week 12 and persisting until necropsy. The latter male also had unilateral scabbing of the left eye from weeks 10 - 12. In a single female, cervical hair loss was noted during week 1 only.

There were no findings evident at 100 mg/kg/day or 300 mg/kg/day.

4.2.3 Weekly Behavioral Observations

(See Summary Tables on p. 58 , Individual Tables on p. 149)

There were no test item-related clinical signs noted during weekly behavioral observations (weeks 1 - 11).

4.2.4 Functional Observational Battery (Screen)

There were no test item-related clinical signs noted during the functional observational battery (week 12).

Grip Strength

(See Summary Tables on p. 63 , Individual Tables on p. 154)

There were no effects upon the fore- or hindlimb grip strength values of the test item-treated males or females when compared with the controls.

Locomotor Activity

(See Summary Tables on p. 66 , Individual Tables on p. 163)

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

At 1000 mg/kg/day, the males and females treated with 1000 mg/kg/day were significantly more active ($p<0.01$ and $p<0.05$, respectively) during the first measurement interval (0-10 minutes), when compared with their respective controls. The females remained significantly more active ($p<0.05$) during the second measurement interval (10 - 20 minutes) when compared with the controls.

No differences were noted between rats treated with 100 mg/kg/day or 300 mg/kg/days and the control rats.

4.2.5 Food Consumption

(See Figures on p. 36 , Summary Tables on p. 71 , Individual Tables on p. 172)

The mean daily food consumption of the test item-treated rats was unaffected when compared with that of the controls.

4.2.6 Body Weights

(See Figures on p. 42 , Summary Tables on p. 81 , Individual Tables on p. 190)

Mean body weights and mean body weight gain of the test item-treated males did not show changes of toxicological relevance when compared with those of the controls.

In females treated with 1000 mg/kg/day, significantly elevated mean body weights were noted on day 15 ($p<0.05$), day 43 ($p<0.05$), day 64 ($p<0.05$), day 78 ($p<0.05$) and day 91 ($p<0.05$), when compared with the controls. At 100 mg/kg/day, females showed significantly higher mean absolute body weights on day 91 only ($p<0.05$), when compared with the controls. These findings were largely reflected in the data values for body weight gain: females at 1000 mg/kg/day showed significantly greater mean body weight gain ($p<0.05$) on days 15, 22, 43, 50, 64, 78 and 91 of treatment, whereas females at 100 mg/kg/day showed greater mean body weight gain ($p<0.05$) on days 64, 71 and 91, when compared with controls. In the females treated with 300 mg/kg/day, marginally higher body weights and mean body weight gain values were noted but these differences did not attain statistical significance.

In the absence of any dose response relationship, the minimal differences noted in the females were considered to be incidental.

4.2.7 Ophthalmoscopy

(See Summary Tables on p. 95 , Individual Tables on p. 216)

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

During week 13, unilateral corneal opacities were noted in two males (nos. 5 and 6) and one female (no 46), and bilateral opacities were noted in one male (no. 7) of the control group. In rats treated with 1000 mg/kg/day, unilateral corneal opacities were noted in four males (nos. 32, 33, 37 and 39). One female (no. 79) showed a persistent hyaloid vessel (considered to be a typical juvenile finding), whereas a second female (no. 80) showed bilateral corneal opacities. These findings were considered to be background findings and within the range of typical biological variation.

4.3 Clinical Laboratory Investigations

4.3.1 Hematology

(See Summary Tables on p. 100 , Individual Tables on p. 233)

No test item-related changes in the hematology parameters were noted at any dose level when compared with the controls.

At 1000 mg/kg/day, males showed significantly elevated mean relative eosinophil counts ($p<0.05$) and significantly reduced mean absolute lymphocyte counts ($p<0.05$) when compared with the controls. In females, the mean hemoglobin distribution width was significantly decreased ($p<0.05$). All differences remained within the ranges of the historical control values and were considered to be incidental.

At 300 mg/kg/day, no differences to the control values were seen.

At 100 mg/kg/day, only a slight and dose-unrelated reduction in the mean absolute leukocyte count was noted in females. The difference, when compared with the control values, was statistically significant ($p<0.05$) but considered unrelated to the treatment with the test item.

4.3.2 Clinical Biochemistry

(See Summary Tables on p. 106 , Individual Tables on p. 250)

Although multiple differences to the control values were noted in several parameters of clinical biochemistry, none were considered to be the result of systemic toxicity and therefore of no toxicological relevance.

In males and females treated with 100, 300 and 1000 mg/kg/day, significantly higher mean chloride levels were noted when compared with the controls (in males all $p<0.01$, in females $p<0.05$ at 100 and 300 mg/kg/day and $p<0.01$ at 1000 mg/kg/day). The differences, however, remained within the ranges of the historical control data.

In males treated with 100, 300 and 1000 mg/kg/day, the mean glucose levels were significantly elevated ($p<0.05$, $p<0.05$ and $p<0.01$, respectively) when compared with the controls. Since all remained within the historical control ranges and no such differences were noted in females, these differences were not considered to be related to the treatment with the test item.

The mean total bilirubin level in the treated males at all dose levels were significantly reduced ($p<0.01$ or $p<0.05$), these differences were considered to be the result of a high control value and were of no toxicological relevance.

At 1000 mg/kg/day, significantly elevated sodium levels seen in females ($p<0.01$) which were above the limits of the historical control values. The results of the sodium analyses were

generally elevated (including the controls) and therefore no toxicological relevance was associated with these changes.

At 300 mg/kg/day, significantly elevated sodium levels were noted in males ($p<0.05$) and elevated alanine aminotransferase activity was noted in females ($p<0.05$) when compared with their respective controls, but were without dose dependence and therefore considered to be incidental.

4.3.3 Urinalysis

(See Summary Tables on p. 111 , Individual Tables on p. 267)

No test item-related changes in the urinalysis parameters were noted at any dose level when compared with the controls.

In males treated with 300 mg/kg/day, the mean concentration of urinary bilirubin was significantly higher than that of the controls ($p<0.05$). This difference was not reflected in the males at the higher dose level of 1000 mg/kg/day and was therefore considered to be incidental.

4.4 Pathology

4.4.1 Organ Weights

(See Summary Tables on p. 114 , Individual Tables on p. 276)

There were no test item-related changes in the mean absolute or relative organ weights at any dose level. All changes were without a clearly dose-response relationship, were not associated with microscopic changes and were considered to be without toxicological relevance.

At 1000 mg/kg/day, the mean absolute weights of the epididymides were significantly elevated ($p<0.05$) when compared with the controls. In females, the mean heart-to-body weight ratio was significantly reduced ($p<0.01$), but this was considered to be the result of significantly elevated mean terminal body weight ($p<0.05$) of these females rather than a systemic effect.

At 300 mg/kg/day, significantly higher mean liver-to-body weight ratios ($p<0.05$) were noted in the males when compared with the controls. In the absence of any similar change in these values at higher doses, this was considered to be incidental.

At 100 mg/kg/day, the mean absolute spleen weights of the males were significantly reduced ($p<0.05$) when compared with the controls. The mean heart-to-brain weight ratio of males was significantly lower ($p<0.05$) when compared with the controls, as was the spleen-to-brain weight ratio ($p<0.05$). In the females at this dose level, the mean absolute liver weights were significantly higher ($p<0.05$) than those of the controls.

4.4.2 Macroscopic Findings

(See Summary Tables on p. 127 , Individual Tables on p. 301)

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

At 1000 mg/kg/day, unilateral renal pelvis dilation was noted in one male (no. 36) and dark red foci on the thymus were noted on a second male (no. 31). The right eye of one female (no. 72) was damaged during terminal blood sampling.

At 300 mg/kg/day, discoloration of the pancreas was noted in one male (no. 23). Females were without macroscopic findings.

At 100 mg/kg/day, bilateral size reduction of the testes was noted in one male (no.11). This finding was not seen at higher dose levels and therefore considered to be incidental. Dark red foci were noted on the mandibular lymph nodes and thymus of females 53 and 57, respectively.

4.4.3 Microscopic Findings

(See Appendix V on p. 355)

All findings recorded were within the range of normal background lesions which may be recorded in animals of this strain and age.

5 DISCUSSION AND CONCLUSION

Oral administration of Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083) to Wistar rats at doses of 100, 300 and 1000 mg/kg/day, for 91 days resulted in no deaths, no clinical signs of toxicological relevance seen during daily or weekly observation, no clinical signs in the functional observational battery (including no effects upon mean fore- or hindlimb grip strength, or locomotor activity), no changes in mean daily food consumption, no toxicologically relevant differences in mean body weight development, no ocular changes, no toxicologically relevant differences in the parameters of hematology, clinical biochemistry or urinalysis, no differences in mean absolute and relative organ weights between test item-treated and control animals, no relevant macroscopic or microscopic changes.

Based on the results of this study, 1000 mg/kg body weight/day of Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083) 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

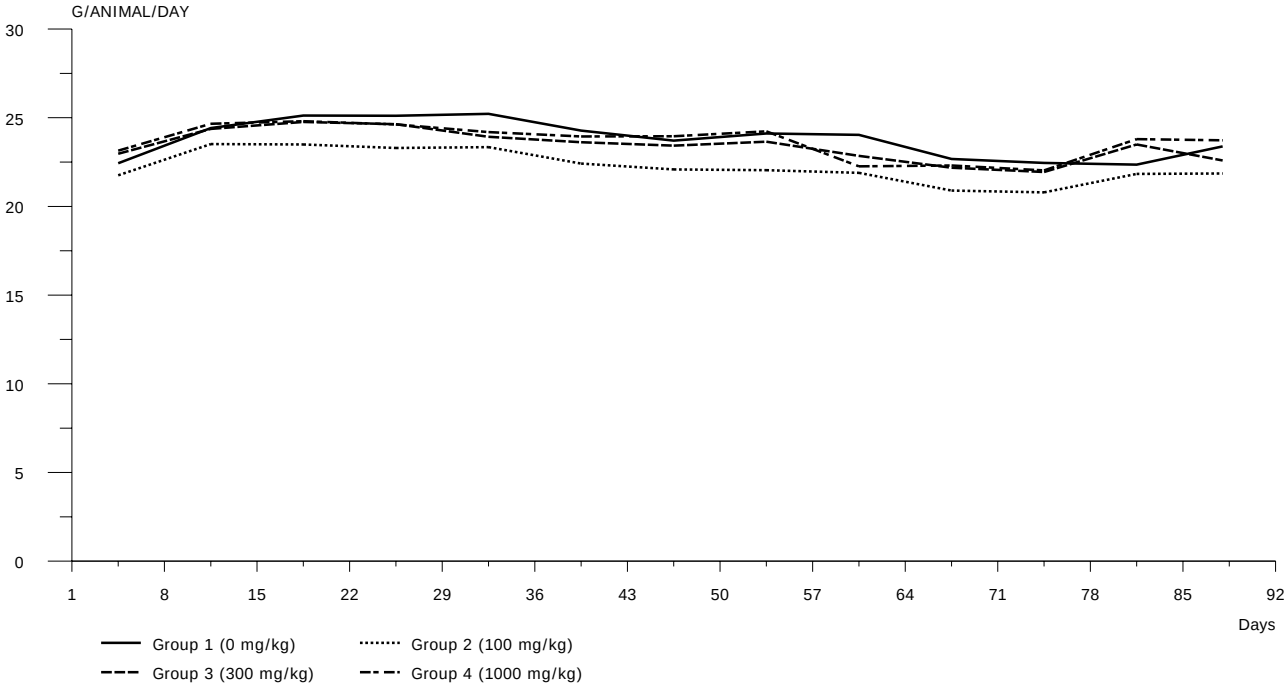
Not Reported

All Study Phases

Cage 17 Male Group 10 Reserve Removed
Cage 18 Female Group 10 Reserve Removed

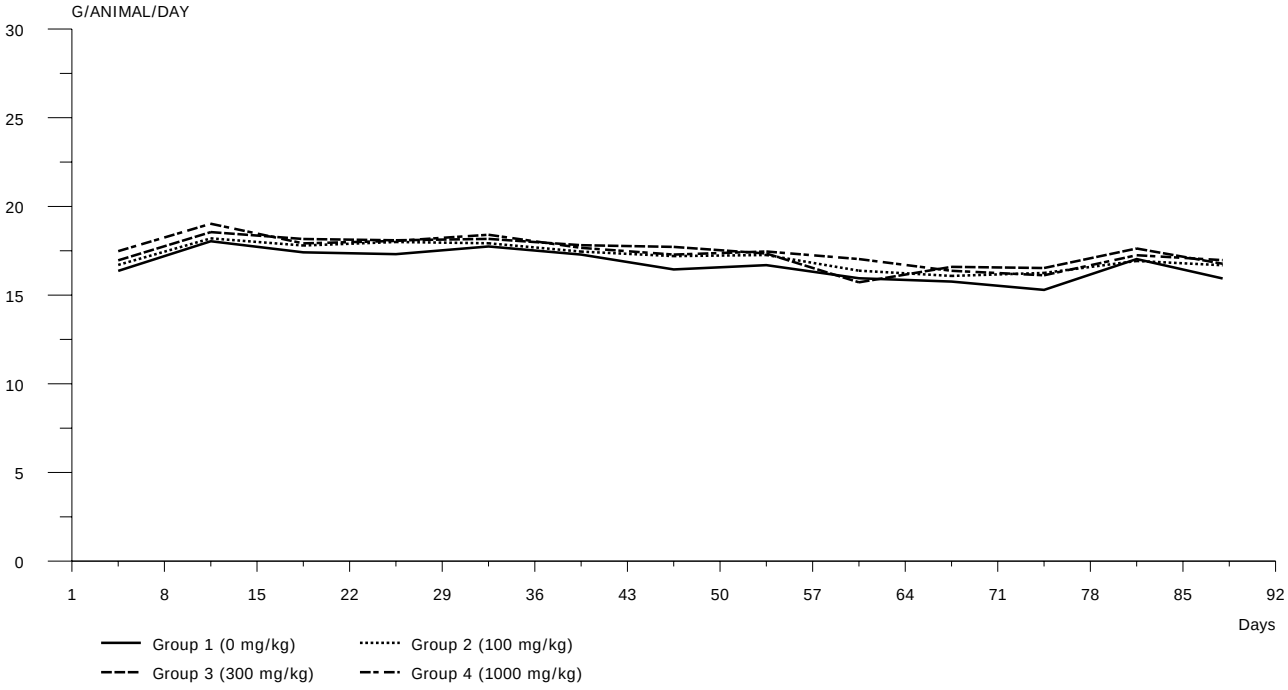
FOOD CONSUMPTION (G/ANIMAL/DAY) - GRAPHICS
MALES

TREATMENT



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

TREATMENT



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

Data excluded from Summary Report

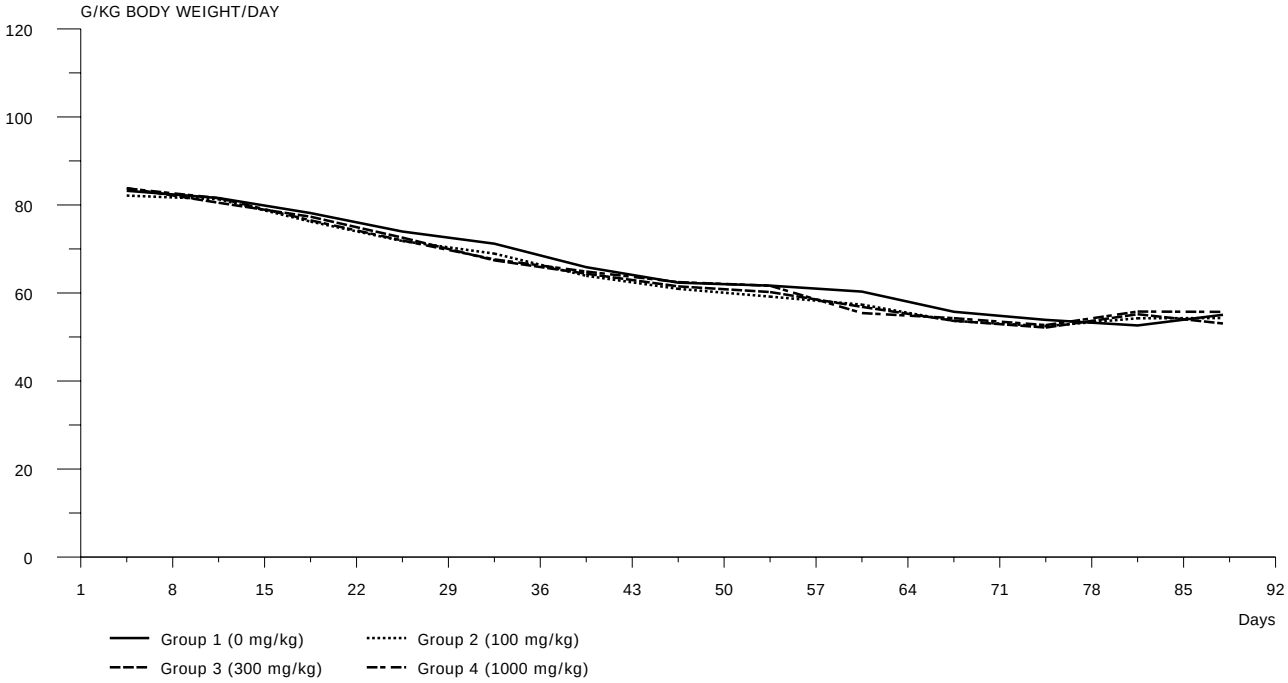
Not Reported

All Study Phases

Cage 17 Male Group 10 Reserve Removed
Cage 18 Female Group 10 Reserve Removed

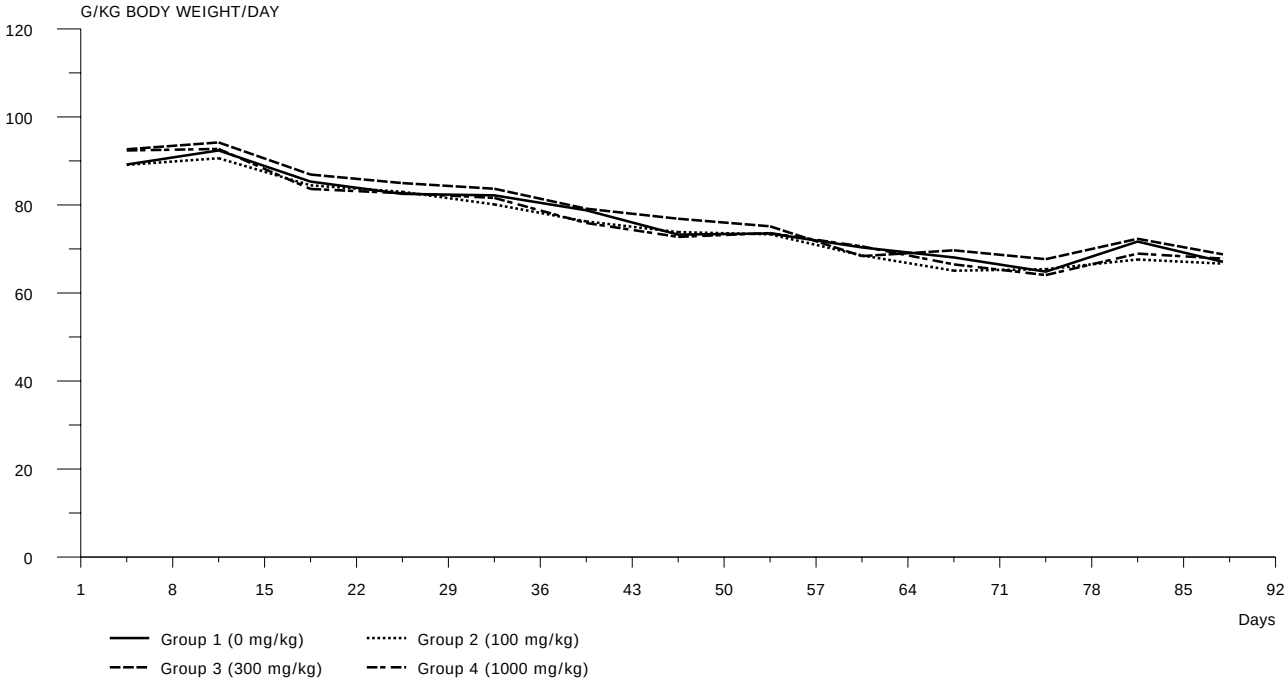
RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) - GRAPHICS MALES

TREATMENT



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

TREATMENT



BODY WEIGHTS (G) - GRAPHICS

Data excluded from Summary Report

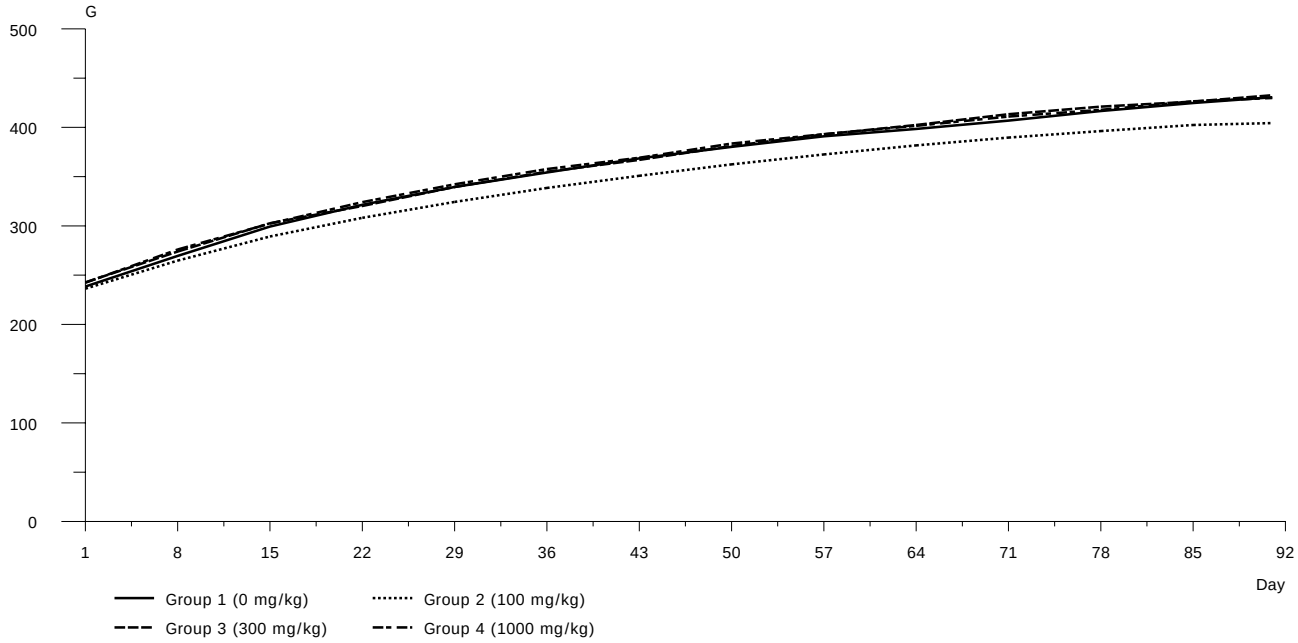
Not Reported

All Study Phases

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 102	Female	Group 10	Reserve Removed
Animal 103	Female	Group 10	Reserve Removed

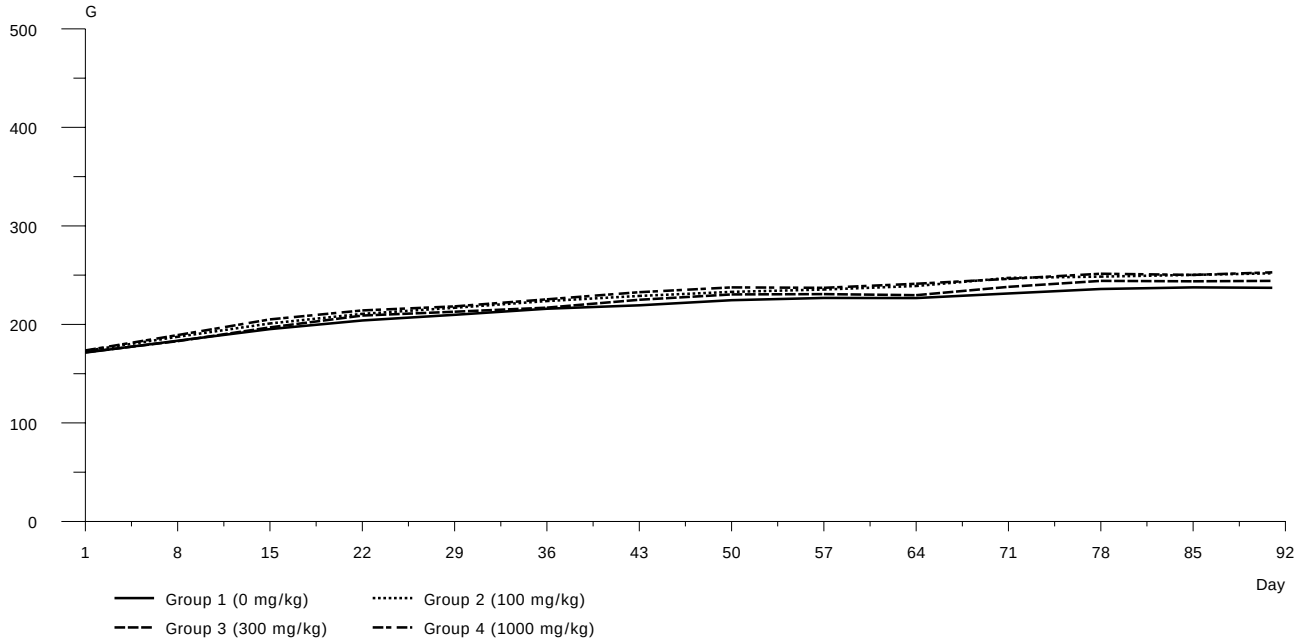
BODY WEIGHTS (G) - GRAPHICS
MALES

TREATMENT



BODY WEIGHTS (G) - GRAPHICS
FEMALES

TREATMENT



BODY WEIGHT GAIN (%) - GRAPHICS

Data excluded from Summary Report

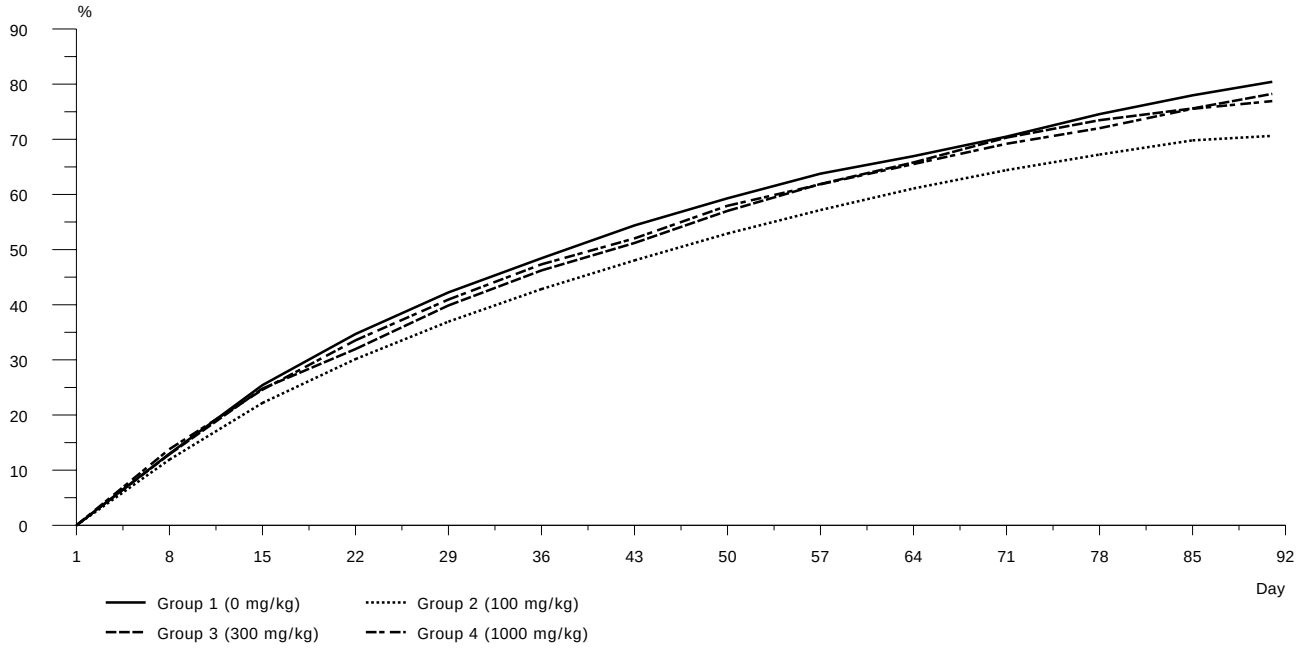
Not Reported

All Study Phases

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 102	Female	Group 10	Reserve Removed
Animal 103	Female	Group 10	Reserve Removed

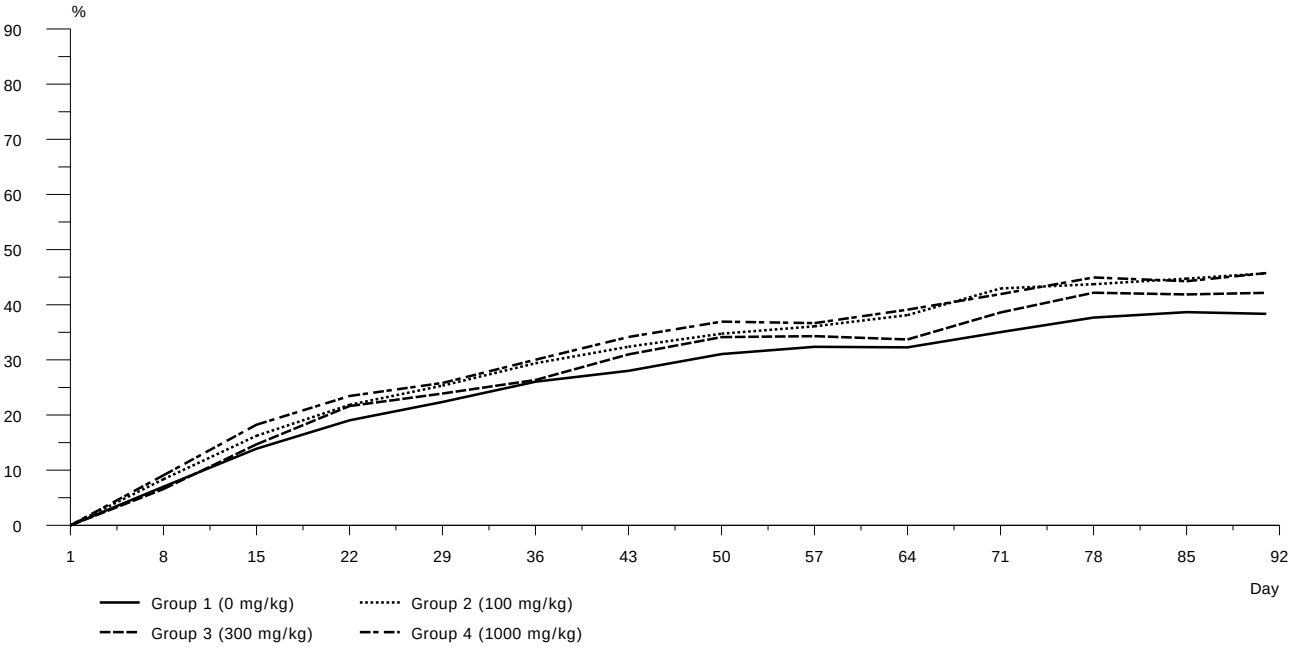
BODY WEIGHT GAIN (%) - GRAPHICS
MALES

TREATMENT



BODY WEIGHT GAIN (%) - GRAPHICS
FEMALES

TREATMENT



8 SUMMARY TABLES

CLINICAL SIGNS - 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 102 Female Group 10 Reserve Removed
Animal 103 Female Group 10 Reserve Removed

Incomplete Recordings

Selection of Findings

All findings reported

CLINICAL SIGNS - SUMMARY
MALES

ACCLIMATIZATION

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

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 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)

CLINICAL SIGNS - SUMMARY
MALES

TREATMENT

	Weeks / Days					
	1	2	3	4	5	6
	1234567	1234567	1234567	1234567	1234567	1234567

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 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)

CLINICAL SIGNS - SUMMARY
MALES

TREATMENT

```
                                Weeks / Days
                                7       8       9       10      11      12
                                1234567123456712345671234567123456712345671234567
```

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

```
APPEARANCE
- HAIR LOSS (3)
   RIGHT CHEEK              G .........................11111111.........
                            % .........................11111111.........
   LEFT EYE                 G ...................................111111
                            % ...................................111111
- SCABS (3)
   LEFT EYE                 G .......................1111111111111111
                            % .......................1111111111111111
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)

CLINICAL SIGNS - SUMMARY
MALES

TREATMENT

	Weeks / Days
	13 14
	1234567-

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

APPEARANCE

-HAIR LOSS (3)		
LEFT EYE	G	1111111
	%	1111111
-SCABS (3)		
LEFT EYE	G	1.....
	%	1.....

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)

CLINICAL SIGNS - SUMMARY
FEMALES

ACCLIMATIZATION

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

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

APPEARANCE

- HAIR LOSS (3)		
NECK (CERVICAL)	G	. . . . . 1 1
	%	. . . . . 1 1

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)

CLINICAL SIGNS - SUMMARY
FEMALES

TREATMENT

	Weeks / Days					
	1	2	3	4	5	6
	1234567123456712345671234567123456712345671234567

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

APPEARANCE

- HAIR LOSS (3)
  NECK (CERVICAL) G 111.....
  % 111.....

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)

CLINICAL SIGNS - 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 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 (100 mg/kg)

No abnormality recorded.

Group 3 (300 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)

CLINICAL SIGNS - SUMMARY
FEMALES

TREATMENT

	Weeks / Days
	13 14
	1234567-

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 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 DETAILED 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 102	Female	Group 10	Reserve Removed
Animal 103	Female	Group 10	Reserve Removed

Incomplete Recordings

Selection of Findings

All findings reported

WEEKLY DETAILED OBSERVATIONS - SUMMARY
MALES

ACCLIMATIZATION

	Weeks
	0
	1-

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 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 DETAILED OBSERVATIONS - SUMMARY
MALES

TREATMENT

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

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 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 DETAILED OBSERVATIONS - SUMMARY
FEMALES

ACCLIMATIZATION

	Weeks
	0
	1-

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 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 DETAILED OBSERVATIONS - SUMMARY
FEMALES

TREATMENT

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

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 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 102	Female	Group 10	Reserve Removed
Animal 103	Female	Group 10	Reserve Removed

Reported Parameter

Parameter		Statistical Testing
AT WEEK 12		
Grip Fore	GRIP FORELIMB	DUNNETT, MEAN
Grip Hind	GRIP HINDLIMB	DUNNETT, MEAN
AT WEEK 12		
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 (-)

GRIP STRENGTH - SUMMARY
AT WEEK 12
MALES

		Group 1 **0 mg/kg**	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
GRIP STRENGTH					
Grip Fore KILOGRAM	MEAN	1.52	1.51 -	1.54 -	1.52 -
	ST.DEV.	0.06	0.07	0.05	0.06
	MINIMUM	1.41	1.41	1.46	1.42
	MAXIMUM	1.59	1.62	1.61	1.60
	N	10	10	10	10
Grip Hind KILOGRAM	MEAN	1.09	1.07 -	1.08 -	1.05 -
	ST.DEV.	0.05	0.05	0.05	0.06
	MINIMUM	1.02	0.99	1.01	0.98
	MAXIMUM	1.16	1.15	1.16	1.18
	N	10	10	10	10

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

GRIP STRENGTH - SUMMARY
AT WEEK 12
FEMALES

		Group 1 **0 mg/kg**	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
GRIP STRENGTH					
Grip Fore KILOGRAM	MEAN	1.27	1.28 -	1.29 -	1.31 -
	ST.DEV.	0.05	0.03	0.05	0.06
	MINIMUM	1.20	1.21	1.21	1.24
	MAXIMUM	1.36	1.33	1.39	1.41
	N	10	10	10	10
Grip Hind KILOGRAM	MEAN	0.78	0.77 -	0.78 -	0.78 -
	ST.DEV.	0.03	0.05	0.04	0.04
	MINIMUM	0.76	0.67	0.74	0.69
	MAXIMUM	0.83	0.81	0.86	0.84
	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 102	Female	Group 10	Reserve Removed
Animal 103	Female	Group 10	Reserve Removed

Reported Parameter

Parameter		Statistical Testing
AT WEEK 12		
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
AT WEEK 12		
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 (-)

LOCOMOTOR ACTIVITY - SUMMARY
AT WEEK 12
MALES

		Group 1 **0 mg/kg**	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
LOCOMOTOR ACTIVITY					
0-10 MIN	MEAN	489	552 -	595 -	658 **
	ST.DEV.	99	68	158	121
	MINIMUM	348	436	425	406
	MAXIMUM	706	651	932	774
	N	10	10	10	10
10-20 MIN	MEAN	291	314 -	327 -	365 -
	ST.DEV.	68	62	148	96
	MINIMUM	236	236	116	236
	MAXIMUM	410	416	657	512
	N	10	10	10	10
20-30 MIN	MEAN	240	205 -	231 -	193 -
	ST.DEV.	103	89	101	68
	MINIMUM	128	87	127	88
	MAXIMUM	483	375	486	311
	N	10	10	10	10
30-40 MIN	MEAN	185	181 -	167 -	134 -
	ST.DEV.	132	51	143	142
	MINIMUM	29	81	14	4
	MAXIMUM	477	235	472	439
	N	10	10	10	10
40-50 MIN	MEAN	200	173 -	165 -	195 -
	ST.DEV.	122	97	168	172
	MINIMUM	24	10	0	2
	MAXIMUM	445	351	433	547
	N	10	10	10	10
50-60 MIN	MEAN	140	146 -	132 -	122 -
	ST.DEV.	85	111	151	125
	MINIMUM	8	13	11	8
	MAXIMUM	272	383	507	434
	N	10	10	10	10

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

LOCOMOTOR ACTIVITY - SUMMARY
AT WEEK 12
MALES

		Group 1 **0 mg/kg**	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
LOCOMOTOR ACTIVITY					
Total	MEAN	1545	1571 -	1617 -	1667 -
	ST.DEV.	401	292	492	384
	MINIMUM	958	1083	1232	1157
	MAXIMUM	2439	2014	2913	2520
	N	10	10	10	10

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

LOCOMOTOR ACTIVITY - SUMMARY
AT WEEK 12
FEMALES

		Group 1 **0 mg/kg**	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
LOCOMOTOR ACTIVITY					
0-10 MIN	MEAN	422	454 -	492 -	561 *
	ST.DEV.	78	86	151	140
	MINIMUM	306	335	296	363
	MAXIMUM	534	603	808	813
	N	10	10	10	10
10-20 MIN	MEAN	213	297 -	295 -	337 *
	ST.DEV.	78	81	102	119
	MINIMUM	54	199	178	223
	MAXIMUM	308	485	493	632
	N	10	10	10	10
20-30 MIN	MEAN	211	253 -	199 -	244 -
	ST.DEV.	61	143	94	137
	MINIMUM	138	67	9	62
	MAXIMUM	308	496	356	500
	N	10	10	10	10
30-40 MIN	MEAN	188	180 -	261 -	213 -
	ST.DEV.	93	106	79	139
	MINIMUM	4	7	147	0
	MAXIMUM	303	327	404	383
	N	10	10	10	10
40-50 MIN	MEAN	109	172 -	211 -	122 -
	ST.DEV.	84	78	125	98
	MINIMUM	0	77	7	2
	MAXIMUM	222	277	408	298
	N	10	10	10	10
50-60 MIN	MEAN	126	151 -	117 -	99 -
	ST.DEV.	78	39	98	108
	MINIMUM	13	90	10	0
	MAXIMUM	281	199	293	310
	N	10	10	10	10

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

LOCOMOTOR ACTIVITY - SUMMARY
AT WEEK 12
FEMALES

		Group 1 **0 mg/kg**	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
LOCOMOTOR ACTIVITY					
Total	MEAN	1268	1507 -	1575 -	1575 -
	ST.DEV.	263	309	336	569
	MINIMUM	800	1229	974	880
	MAXIMUM	1667	2055	2102	2738
	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 17 Male Group 10 Reserve Removed
Cage 18 Female Group 10 Reserve Removed

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

		Group 1 0 mg/kg	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
ACCLIMATIZATION					
Days 1-9	MEAN	21.3	21.6	22.6	22.4
	ST.DEV.	0.3	1.1	0.4	1.5
	N	2	2	2	2
TREATMENT					
Days 1-8	MEAN	22.4	21.8	23.0	23.1
	ST.DEV.	0.7	1.1	0.6	1.9
	N	2	2	2	2
Days 8-15	MEAN	24.4	23.5	24.4	24.7
	ST.DEV.	1.1	0.7	0.1	1.3
	N	2	2	2	2
Days 15-22	MEAN	25.1	23.5	24.8	24.8
	ST.DEV.	0.6	0.5	0.4	1.6
	N	2	2	2	2
Days 22-29	MEAN	25.1	23.3	24.6	24.6
	ST.DEV.	0.8	0.7	0.3	1.8
	N	2	2	2	2
Days 29-36	MEAN	25.2	23.3	23.9	24.2
	ST.DEV.	0.5	1.0	0.4	1.2
	N	2	2	2	2
Days 36-43	MEAN	24.3	22.4	23.6	23.9
	ST.DEV.	0.2	0.2	0.2	1.0
	N	2	2	2	2
Days 43-50	MEAN	23.7	22.1	23.4	24.0
	ST.DEV.	0.7	0.3	0.1	1.1
	N	2	2	2	2
Days 50-57	MEAN	24.1	22.0	23.6	24.2
	ST.DEV.	0.4	0.6	0.0	1.1
	N	2	2	2	2
Days 57-64	MEAN	24.0	21.9	22.9	22.3
	ST.DEV.	0.4	0.9	0.8	0.9
	N	2	2	2	2
Days 64-71	MEAN	22.7	20.9	22.2	22.3
	ST.DEV.	0.0	0.2	0.8	1.3
	N	2	2	2	2

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

		Group 1 0 mg/kg	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Days 71-78	MEAN	22.5	20.8	21.9	22.0
	ST.DEV.	0.1	0.3	0.1	1.6
	N	2	2	2	2
Days 78-85	MEAN	22.4	21.8	23.5	23.8
	ST.DEV.	0.2	0.3	0.3	1.5
	N	2	2	2	2
Days 85-91	MEAN	23.4	21.9	22.6	23.7
	ST.DEV.	0.4	0.9	0.2	0.3
	N	2	2	2	2
MEAN OF MEANS Over TREATMENT		23.8	22.2	23.4	23.7

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

		Group 1 0 mg/kg	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
ACCLIMATIZATION					
Days 1-9	MEAN	16.2	16.1	15.7	16.2
	ST.DEV.	0.4	0.5	0.1	0.2
	N	2	2	2	2
TREATMENT					
Days 1-8	MEAN	16.4	16.7	17.0	17.5
	ST.DEV.	0.9	0.0	0.5	0.2
	N	2	2	2	2
Days 8-15	MEAN	18.0	18.2	18.6	19.0
	ST.DEV.	0.4	0.3	0.4	0.3
	N	2	2	2	2
Days 15-22	MEAN	17.4	17.8	18.2	17.9
	ST.DEV.	0.7	0.2	0.4	0.4
	N	2	2	2	2
Days 22-29	MEAN	17.3	18.0	18.1	18.1
	ST.DEV.	0.1	0.0	0.2	0.6
	N	2	2	2	2
Days 29-36	MEAN	17.7	17.9	18.2	18.4
	ST.DEV.	0.5	0.0	0.5	0.5
	N	2	2	2	2
Days 36-43	MEAN	17.3	17.5	17.8	17.7
	ST.DEV.	0.6	0.1	0.2	0.5
	N	2	2	2	2
Days 43-50	MEAN	16.5	17.2	17.7	17.3
	ST.DEV.	0.5	0.5	0.0	0.5
	N	2	2	2	2
Days 50-57	MEAN	16.7	17.3	17.3	17.5
	ST.DEV.	0.8	0.1	0.1	0.9
	N	2	2	2	2
Days 57-64	MEAN	16.0	16.4	15.7	17.0
	ST.DEV.	0.6	0.2	1.8	0.5
	N	2	2	2	2
Days 64-71	MEAN	15.8	16.1	16.6	16.4
	ST.DEV.	0.9	0.1	0.4	0.5
	N	2	2	2	2

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

		Group 1 0 mg/kg	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Days 71-78	MEAN	15.3	16.3	16.5	16.1
	ST.DEV.	0.4	0.4	0.7	0.8
	N	2	2	2	2
Days 78-85	MEAN	17.0	16.9	17.6	17.3
	ST.DEV.	0.4	0.2	1.0	0.5
	N	2	2	2	2
Days 85-91	MEAN	15.9	16.7	16.8	17.0
	ST.DEV.	0.7	0.3	0.8	0.7
	N	2	2	2	2
MEAN OF MEANS Over TREATMENT		16.7	17.1	17.4	17.5

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

Data excluded from Summary Report

Not Reported

All Study Phases

Cage 17 Male Group 10 Reserve Removed
Cage 18 Female Group 10 Reserve Removed

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

		Group 1 0 mg/kg	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
ACCLIMATIZATION					
Days 1-9	MEAN	106.8	109.9	114.3	113.8
	ST.DEV.	2.0	5.1	0.6	5.6
	N	2	2	2	2
TREATMENT					
Days 1-8	MEAN	83.2	82.1	83.8	83.8
	ST.DEV.	1.7	2.0	0.3	3.2
	N	2	2	2	2
Days 8-15	MEAN	81.6	81.3	80.5	81.6
	ST.DEV.	2.7	1.0	0.1	0.5
	N	2	2	2	2
Days 15-22	MEAN	78.2	76.2	77.3	76.5
	ST.DEV.	1.2	0.7	2.7	0.8
	N	2	2	2	2
Days 22-29	MEAN	74.0	71.8	72.6	71.9
	ST.DEV.	1.4	1.2	0.8	1.3
	N	2	2	2	2
Days 29-36	MEAN	71.2	68.9	67.4	67.6
	ST.DEV.	0.7	2.0	1.0	0.1
	N	2	2	2	2
Days 36-43	MEAN	65.9	63.9	64.4	64.9
	ST.DEV.	0.3	1.0	1.3	0.5
	N	2	2	2	2
Days 43-50	MEAN	62.4	61.0	61.5	62.5
	ST.DEV.	1.2	1.0	1.5	0.1
	N	2	2	2	2
Days 50-57	MEAN	61.7	59.2	60.2	61.7
	ST.DEV.	0.2	2.1	1.6	0.4
	N	2	2	2	2
Days 57-64	MEAN	60.3	57.4	56.8	55.4
	ST.DEV.	0.1	2.6	3.4	0.4
	N	2	2	2	2
Days 64-71	MEAN	55.7	53.6	53.7	54.3
	ST.DEV.	0.8	1.1	3.5	0.2
	N	2	2	2	2

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

		Group 1 0 mg/kg	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Days 71-78	MEAN	53.9	52.5	52.1	52.7
	ST.DEV.	0.7	1.0	1.5	0.6
	N	2	2	2	2
Days 78-85	MEAN	52.6	54.3	55.2	55.8
	ST.DEV.	1.2	0.9	2.7	0.0
	N	2	2	2	2
Days 85-91	MEAN	55.1	54.3	53.1	55.7
	ST.DEV.	0.2	2.5	2.4	2.6
	N	2	2	2	2
MEAN OF MEANS Over TREATMENT		65.8	64.3	64.5	64.9

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

		Group 1 0 mg/kg	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
ACCLIMATIZATION					
Days 1-9	MEAN	107.7	106.9	107.1	108.5
	ST.DEV.	1.7	2.1	0.1	0.2
	N	2	2	2	2
TREATMENT					
Days 1-8	MEAN	89.2	89.1	92.7	92.4
	ST.DEV.	1.8	1.4	1.9	2.3
	N	2	2	2	2
Days 8-15	MEAN	92.4	90.6	94.2	92.7
	ST.DEV.	0.2	4.7	0.3	0.6
	N	2	2	2	2
Days 15-22	MEAN	85.3	84.5	86.9	83.7
	ST.DEV.	1.4	2.8	0.5	0.4
	N	2	2	2	2
Days 22-29	MEAN	82.5	83.0	85.0	82.7
	ST.DEV.	0.8	1.1	1.0	1.4
	N	2	2	2	2
Days 29-36	MEAN	82.2	80.1	83.7	81.6
	ST.DEV.	1.1	1.7	1.8	0.2
	N	2	2	2	2
Days 36-43	MEAN	78.8	76.3	79.1	75.9
	ST.DEV.	1.0	2.1	0.2	0.2
	N	2	2	2	2
Days 43-50	MEAN	73.3	73.8	76.9	72.8
	ST.DEV.	0.2	3.2	0.6	0.0
	N	2	2	2	2
Days 50-57	MEAN	73.5	73.3	75.1	73.6
	ST.DEV.	1.2	0.5	0.4	1.5
	N	2	2	2	2
Days 57-64	MEAN	70.4	68.5	68.4	70.6
	ST.DEV.	0.8	0.2	6.4	0.0
	N	2	2	2	2
Days 64-71	MEAN	68.1	65.1	69.7	66.5
	ST.DEV.	1.9	1.6	2.3	1.0
	N	2	2	2	2

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

		Group 1 0 mg/kg	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Days 71-78	MEAN	64.8	65.4	67.7	64.1
	ST.DEV.	0.6	2.7	2.5	0.7
	N	2	2	2	2
Days 78-85	MEAN	71.7	67.6	72.3	69.0
	ST.DEV.	0.1	1.4	3.1	0.8
	N	2	2	2	2
Days 85-91	MEAN	67.1	66.7	68.8	67.8
	ST.DEV.	1.3	1.8	2.3	0.3
	N	2	2	2	2
MEAN OF MEANS Over TREATMENT		76.9	75.7	78.5	76.4

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 102	Female	Group 10	Reserve Removed
Animal 103	Female	Group 10	Reserve Removed

BODY WEIGHTS (G) - SUMMARY
MALES

		Group 1 0 mg/kg	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
PRE-RANDOM PHASE					
Day 1	MEAN	187	186 -	184 -	186 -
	ST.DEV.	7	6	5	5
	MINIMUM	177	176	174	176
	MAXIMUM	202	195	190	195
	N	10	10	10	10
ACCLIMATIZATION					
Day 1	MEAN	199	197 -	197 -	197 -
	ST.DEV.	5	5	4	4
	MINIMUM	189	190	187	190
	MAXIMUM	211	205	202	203
	N	10	10	10	10
TREATMENT					
Day 1	MEAN	238	236 -	243 -	242 -
	ST.DEV.	9	10	8	12
	MINIMUM	222	217	226	228
	MAXIMUM	253	251	253	269
	N	10	10	10	10
Day 8	MEAN	270	265 -	274 -	276 -
	ST.DEV.	13	19	11	18
	MINIMUM	242	229	252	255
	MAXIMUM	288	295	288	315
	N	10	10	10	10
Day 15	MEAN	299	289 -	303 -	302 -
	ST.DEV.	18	25	9	23
	MINIMUM	260	244	288	275
	MAXIMUM	324	321	315	347
	N	10	10	10	10
Day 22	MEAN	321	308 -	320 -	324 -
	ST.DEV.	20	31	14	27
	MINIMUM	277	251	292	291
	MAXIMUM	344	347	341	372
	N	10	10	10	10
Day 29	MEAN	339	324 -	340 -	342 -
	ST.DEV.	24	35	17	31
	MINIMUM	287	261	307	306
	MAXIMUM	363	369	363	397
	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 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 36	MEAN	354	339 -	355 -	358 -
	ST.DEV.	26	39	19	34
	MINIMUM	296	268	315	328
	MAXIMUM	382	397	380	422
	N	10	10	10	10
Day 43	MEAN	368	351 -	367 -	369 -
	ST.DEV.	27	41	20	37
	MINIMUM	307	279	324	335
	MAXIMUM	395	412	395	434
	N	10	10	10	10
Day 50	MEAN	380	362 -	381 -	384 -
	ST.DEV.	29	43	22	40
	MINIMUM	318	286	335	346
	MAXIMUM	408	426	417	455
	N	10	10	10	10
Day 57	MEAN	391	373 -	393 -	393 -
	ST.DEV.	30	44	23	42
	MINIMUM	324	297	349	352
	MAXIMUM	422	439	436	471
	N	10	10	10	10
Day 64	MEAN	398	382 -	402 -	402 -
	ST.DEV.	32	44	25	42
	MINIMUM	330	309	357	362
	MAXIMUM	433	449	452	474
	N	10	10	10	10
Day 71	MEAN	407	390 -	413 -	411 -
	ST.DEV.	33	45	27	44
	MINIMUM	340	313	364	369
	MAXIMUM	443	460	468	487
	N	10	10	10	10
Day 78	MEAN	417	396 -	421 -	418 -
	ST.DEV.	36	46	29	46
	MINIMUM	344	320	371	373
	MAXIMUM	455	470	484	496
	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 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 85	MEAN	425	402 -	426 -	427 -
	ST.DEV.	35	47	28	49
	MINIMUM	353	323	382	384
	MAXIMUM	464	477	486	510
	N	10	10	10	10
Day 91	MEAN	431	404 -	433 -	430 -
	ST.DEV.	37	49	28	48
	MINIMUM	356	325	383	384
	MAXIMUM	469	482	492	511
	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 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
PRE-RANDOM PHASE					
Day 1	MEAN	142	142 -	141 -	142 -
	ST.DEV.	4	4	3	3
	MINIMUM	137	138	136	136
	MAXIMUM	149	152	146	148
	N	10	10	10	10
ACCLIMATIZATION					
Day 1	MEAN	150	150 -	147 -	149 -
	ST.DEV.	5	4	4	4
	MINIMUM	142	145	141	143
	MAXIMUM	157	157	154	159
	N	10	10	10	10
TREATMENT					
Day 1	MEAN	171	173 -	172 -	174 -
	ST.DEV.	6	9	6	7
	MINIMUM	162	160	166	163
	MAXIMUM	178	190	186	188
	N	10	10	10	10
Day 8	MEAN	183	187 -	183 -	189 -
	ST.DEV.	7	9	9	9
	MINIMUM	171	175	171	177
	MAXIMUM	193	204	198	201
	N	10	10	10	10
Day 15	MEAN	195	201 -	197 -	205 *
	ST.DEV.	6	10	11	8
	MINIMUM	185	187	184	194
	MAXIMUM	203	218	219	218
	N	10	10	10	10
Day 22	MEAN	204	211 -	209 -	214 -
	ST.DEV.	8	11	13	8
	MINIMUM	191	195	189	202
	MAXIMUM	215	226	230	225
	N	10	10	10	10
Day 29	MEAN	210	217 -	213 -	218 -
	ST.DEV.	9	13	14	10
	MINIMUM	199	199	198	205
	MAXIMUM	223	236	241	232
	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 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 36	MEAN	216	224 -	217 -	226 -
	ST.DEV.	8	11	11	11
	MINIMUM	205	211	204	210
	MAXIMUM	230	242	241	244
	N	10	10	10	10
Day 43	MEAN	219	229 -	225 -	233 *
	ST.DEV.	9	12	15	11
	MINIMUM	201	214	201	221
	MAXIMUM	230	244	254	252
	N	10	10	10	10
Day 50	MEAN	225	233 -	230 -	238 -
	ST.DEV.	9	12	15	11
	MINIMUM	208	219	211	224
	MAXIMUM	237	252	260	255
	N	10	10	10	10
Day 57	MEAN	227	235 -	231 -	237 -
	ST.DEV.	10	14	15	12
	MINIMUM	208	214	208	222
	MAXIMUM	242	259	261	254
	N	10	10	10	10
Day 64	MEAN	227	239 -	230 -	241 *
	ST.DEV.	9	14	15	12
	MINIMUM	213	216	207	223
	MAXIMUM	241	261	255	263
	N	10	10	10	10
Day 71	MEAN	231	247 -	238 -	246 -
	ST.DEV.	11	15	16	17
	MINIMUM	211	223	209	212
	MAXIMUM	251	265	268	269
	N	10	10	10	10
Day 78	MEAN	236	249 -	244 -	251 *
	ST.DEV.	11	15	15	13
	MINIMUM	215	227	214	235
	MAXIMUM	251	276	272	271
	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 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 85	MEAN	238	250 -	244 -	250 -
	ST.DEV.	10	14	15	13
	MINIMUM	218	229	214	227
	MAXIMUM	256	277	270	269
	N	10	10	10	10
Day 91	MEAN	237	252 *	244 -	253 *
	ST.DEV.	11	13	13	15
	MINIMUM	214	235	222	233
	MAXIMUM	251	273	273	280
	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 102	Female	Group 10	Reserve Removed
Animal 103	Female	Group 10	Reserve Removed

BODY WEIGHT GAIN (%) - SUMMARY
MALES

		Group 1 0 mg/kg	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
ACCLIMATIZATION					
Day 1	MEAN	0.0	0.0	0.0	0.0
	ST.DEV.	0.0	0.0	0.0	0.0
	MINIMUM	0.0	0.0	0.0	0.0
	MAXIMUM	0.0	0.0	0.0	0.0
	N	10	10	10	10
TREATMENT					
Day 1	MEAN	0.0	0.0	0.0	0.0
	ST.DEV.	0.0	0.0	0.0	0.0
	MINIMUM	0.0	0.0	0.0	0.0
	MAXIMUM	0.0	0.0	0.0	0.0
	N	10	10	10	10
Day 8	MEAN	13.0	11.9 -	12.9 -	13.8 -
	ST.DEV.	2.3	3.6	1.6	2.4
	MINIMUM	8.9	5.8	10.4	10.7
	MAXIMUM	16.4	17.6	15.4	17.1
	N	10	10	10	10
Day 15	MEAN	25.4	22.2 -	24.8 -	24.6 -
	ST.DEV.	4.2	5.7	4.0	4.2
	MINIMUM	17.0	12.8	20.6	20.0
	MAXIMUM	30.5	28.5	34.1	30.7
	N	10	10	10	10
Day 22	MEAN	34.7	30.2 -	32.0 -	33.5 -
	ST.DEV.	5.1	7.8	3.2	5.8
	MINIMUM	24.6	15.9	27.7	27.4
	MAXIMUM	40.9	38.9	37.9	42.3
	N	10	10	10	10
Day 29	MEAN	42.2	36.9 -	39.9 -	40.9 -
	ST.DEV.	6.6	9.3	3.8	7.1
	MINIMUM	29.0	20.4	33.8	33.8
	MAXIMUM	51.3	47.3	46.6	51.6
	N	10	10	10	10
Day 36	MEAN	48.4	42.8 -	46.2 -	47.3 -
	ST.DEV.	7.6	10.8	4.6	8.2
	MINIMUM	33.3	23.9	39.6	39.2
	MAXIMUM	57.9	58.6	53.4	60.6
	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 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 43	MEAN	54.4	48.0 -	51.2 -	52.0 -
	ST.DEV.	8.0	11.4	5.6	9.2
	MINIMUM	38.0	28.6	43.6	42.5
	MAXIMUM	64.0	64.4	59.5	66.0
	N	10	10	10	10
Day 50	MEAN	59.3	52.9 -	57.0 -	57.9 -
	ST.DEV.	8.6	12.2	6.6	10.2
	MINIMUM	43.3	32.1	48.4	47.0
	MAXIMUM	69.0	69.9	68.5	73.5
	N	10	10	10	10
Day 57	MEAN	63.8	57.2 -	61.9 -	61.8 -
	ST.DEV.	9.2	12.4	7.1	10.8
	MINIMUM	45.8	37.1	54.6	49.6
	MAXIMUM	74.6	75.2	75.8	77.3
	N	10	10	10	10
Day 64	MEAN	66.9	61.1 -	65.8 -	65.5 -
	ST.DEV.	10.1	12.2	8.2	13.4
	MINIMUM	48.7	42.6	56.8	50.2
	MAXIMUM	79.2	79.3	82.4	89.8
	N	10	10	10	10
Day 71	MEAN	70.5	64.4 -	70.3 -	69.2 -
	ST.DEV.	10.7	12.5	8.9	11.6
	MINIMUM	53.0	44.4	61.2	56.9
	MAXIMUM	83.7	83.4	89.1	87.4
	N	10	10	10	10
Day 78	MEAN	74.6	67.2 -	73.5 -	72.0 -
	ST.DEV.	12.0	12.9	9.8	12.0
	MINIMUM	54.9	47.6	64.0	58.7
	MAXIMUM	90.0	87.7	95.5	90.5
	N	10	10	10	10
Day 85	MEAN	78.0	69.8 -	75.6 -	75.5 -
	ST.DEV.	11.5	13.4	9.7	13.3
	MINIMUM	58.9	49.3	65.4	60.5
	MAXIMUM	92.3	90.2	96.2	96.2
	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 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 91	MEAN	80.4	70.6 -	78.2 -	76.9 -
	ST.DEV.	12.4	14.0	9.4	12.9
	MINIMUM	60.3	50.2	67.0	62.7
	MAXIMUM	97.7	92.4	98.6	97.6
	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 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
ACCLIMATIZATION					
Day 1	MEAN	0.0	0.0	0.0	0.0
	ST.DEV.	0.0	0.0	0.0	0.0
	MINIMUM	0.0	0.0	0.0	0.0
	MAXIMUM	0.0	0.0	0.0	0.0
	N	10	10	10	10
TREATMENT					
Day 1	MEAN	0.0	0.0	0.0	0.0
	ST.DEV.	0.0	0.0	0.0	0.0
	MINIMUM	0.0	0.0	0.0	0.0
	MAXIMUM	0.0	0.0	0.0	0.0
	N	10	10	10	10
Day 8	MEAN	7.0	8.4 -	6.6 -	9.1 -
	ST.DEV.	2.7	2.3	3.2	3.7
	MINIMUM	2.5	4.4	1.9	4.2
	MAXIMUM	11.2	13.2	11.3	14.5
	N	10	10	10	10
Day 15	MEAN	13.9	16.2 -	14.7 -	18.2 *
	ST.DEV.	2.3	3.8	3.7	2.6
	MINIMUM	9.5	9.3	9.8	14.2
	MAXIMUM	16.2	22.8	21.1	21.3
	N	10	10	10	10
Day 22	MEAN	19.0	21.8 -	21.6 -	23.4 *
	ST.DEV.	3.0	4.5	4.6	3.3
	MINIMUM	12.5	13.4	12.4	19.0
	MAXIMUM	22.2	27.4	29.2	27.7
	N	10	10	10	10
Day 29	MEAN	22.4	25.3 -	23.9 -	25.8 -
	ST.DEV.	3.8	3.9	3.9	2.8
	MINIMUM	15.0	20.1	18.1	21.4
	MAXIMUM	26.2	32.0	29.9	30.4
	N	10	10	10	10
Day 36	MEAN	26.0	29.4 -	26.3 -	30.0 -
	ST.DEV.	5.2	3.6	2.9	6.5
	MINIMUM	16.7	24.8	21.3	19.9
	MAXIMUM	31.8	37.6	30.0	38.7
	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 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 43	MEAN	28.0	32.4 -	31.0 -	34.1 *
	ST.DEV.	5.0	5.1	5.2	4.9
	MINIMUM	16.2	26.8	20.1	27.0
	MAXIMUM	32.5	41.2	39.3	43.1
	N	10	10	10	10
Day 50	MEAN	31.0	34.8 -	34.1 -	36.9 *
	ST.DEV.	4.7	5.3	4.9	5.2
	MINIMUM	20.2	25.9	26.1	27.2
	MAXIMUM	35.7	42.0	41.8	45.2
	N	10	10	10	10
Day 57	MEAN	32.4	36.1 -	34.3 -	36.7 -
	ST.DEV.	5.0	5.2	4.9	4.6
	MINIMUM	20.5	28.9	24.4	26.3
	MAXIMUM	37.1	44.5	41.0	44.3
	N	10	10	10	10
Day 64	MEAN	32.3	38.1 *	33.7 -	39.1 *
	ST.DEV.	4.4	3.6	5.6	6.9
	MINIMUM	23.1	34.1	23.1	26.5
	MAXIMUM	38.1	45.0	41.9	49.7
	N	10	10	10	10
Day 71	MEAN	35.0	42.9 *	38.6 -	41.9 -
	ST.DEV.	5.8	7.0	6.2	8.4
	MINIMUM	22.0	35.8	24.8	25.7
	MAXIMUM	42.5	54.7	46.8	53.1
	N	10	10	10	10
Day 78	MEAN	37.7	43.7 -	42.2 -	45.0 *
	ST.DEV.	5.3	7.2	5.4	6.3
	MINIMUM	24.2	34.2	28.3	33.4
	MAXIMUM	42.8	57.3	46.8	54.1
	N	10	10	10	10
Day 85	MEAN	38.7	44.7 -	41.9 -	44.3 -
	ST.DEV.	5.8	6.3	5.5	6.8
	MINIMUM	26.0	36.2	28.1	29.2
	MAXIMUM	44.9	57.5	47.0	53.0
	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 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
TREATMENT					
Day 91	MEAN	38.3	45.7 *	42.2 -	45.7 *
	ST.DEV.	5.8	6.2	4.9	7.1
	MINIMUM	24.0	38.0	32.6	32.5
	MAXIMUM	43.3	59.4	48.0	59.4
	N	10	10	10	10

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

OPHTHALMOSCOPY - 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 102	Female	Group 10	Reserve Removed
Animal 103	Female	Group 10	Reserve Removed

Reported Grades

No grade conversion defined

OPHTHALMOSCOPY - SUMMARY
ACCLIMATIZATION, Day 6, OPHTHALMOSCOPY
MALES

	Group 1 0 mg/kg		Group 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
Animals observed	10		10		10		10	
	Mean	%	Mean	%	Mean	%	Mean	%
Unscheduled Findings								
CORNEA								
- CORNEAL OPACITY (3)								
LEFT EYE	-	0%	1.0	30%-	-	0%-	1.0	30%-
RIGHT EYE	1.0	10%	-	0%-	1.0	10%-	-	0%-
VITREOUS BODY								
- PERSISTENT HYALOID VESSEL (1)								
LEFT EYE	-	0%	-	0%-	-	0%-	1.0	20%-
RIGHT EYE	-	0%	-	0%-	1.0	10%-	1.0	10%-

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

OPHTHALMOSCOPY - SUMMARY
ACCLIMATIZATION, Day 6, OPHTHALMOSCOPY
FEMALES

	Group 1 0 mg/kg		Group 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
Animals observed	10		10		10		10	
	Mean	%	Mean	%	Mean	%	Mean	%
Unscheduled Findings								
CORNEA								
- CORNEAL OPACITY (3)								
LEFT EYE	1.0	30%	1.0	10% -	1.0	10% -	1.0	10% -
RIGHT EYE	1.0	30%	1.0	10% -	-	0% -	1.0	10% -
VITREOUS BODY								
- PERSISTENT HYALOID VESSEL (1)								
LEFT EYE	-	0%	1.0	10% -	1.0	10% -	1.0	10% -
RIGHT EYE	1.0	10%	1.0	10% -	1.0	10% -	1.0	10% -

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

OPHTHALMOSCOPY - SUMMARY
TREATMENT, Day 90, OPHTHALMOSCOPY
MALES

	Group 1 0 mg/kg		Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg	
Animals observed	10		0	0	10	
	Mean	%			Mean	%
Unscheduled Findings						
CORNEA						
- CORNEAL OPACITY (3)						
LEFT EYE	1.0	10%	-	-	1.0	40% -
RIGHT EYE	1.0	30%	-	-	-	0% -
VITREOUS BODY						
- PERSISTENT HYALOID VESSEL (1)						
LEFT EYE	-	0%	-	-	-	0% -
RIGHT EYE	-	0%	-	-	-	0% -

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

OPHTHALMOSCOPY - SUMMARY
TREATMENT, Day 90, OPHTHALMOSCOPY
FEMALES

	Group 1 0 mg/kg		Group 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
Animals observed	10		0		0		10	
	Mean	%				Mean	%	
Unscheduled Findings								
CORNEA								
- CORNEAL OPACITY (3)								
LEFT EYE	-	0%	-		-	1.0	10%	-
RIGHT EYE	1.0	10%	-		-	1.0	10%	-
VITREOUS BODY								
- PERSISTENT HYALOID VESSEL (1)								
LEFT EYE	-	0%	-		-	1.0	10%	-
RIGHT 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 102 Female Group 10 Reserve Removed
Animal 103 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 T/l	HB mmol/l	HCT rel. 1	MCV fl	RDW rel. 1	MCH fmol	MCHC mmol/l
After 13 Weeks							
Group 1	8.64	9.5	0.44	50.4	0.167	1.10	21.87
Group 2	8.82 -	9.5 -	0.43 -	49.1 -	0.153 -	1.08 -	22.04 -
Group 3	8.74 -	9.6 -	0.44 -	50.0 -	0.155 -	1.10 -	21.92 -
Group 4	8.91 -	9.5 -	0.44 -	49.3 -	0.165 -	1.07 -	21.64 -

	GENERAL	RETICULOCYTE COUNT					GENERAL
	HDW mmol/l	RETI rel. 1	RETI G/l	L RETI rel. 1	M RETI rel. 1	H RETI rel. 1	WBC G/l
After 13 Weeks							
Group 1	1.88	0.020	181	0.679	0.290	0.039	6.33
Group 2	1.84 -	0.020 -	179 -	0.673 -	0.286 -	0.047 -	5.09 *
Group 3	1.77 -	0.020 -	168 -	0.667 -	0.289 -	0.036 -	5.16 -
Group 4	1.74 -	0.023 -	220 -	0.648 -	0.305 -	0.044 -	5.25 -

	DIFF.WBC COUNT (REL)					
	NEUT rel. 1	EOS rel. 1	BASO rel. 1	LYMPH rel. 1	MONO rel. 1	LUC rel. 1
After 13 Weeks						
Group 1	0.228	0.015	0.002	0.725	0.027	0.006
Group 2	0.212 -	0.016 -	0.003 -	0.738 -	0.025 -	0.005 -
Group 3	0.224 -	0.017 -	0.002 -	0.725 -	0.028 -	0.006 -
Group 4	0.259 -	0.021 *	0.002 -	0.680 -	0.031 -	0.005 -

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

Hematology - SUMMARY
MALES

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT G/l	EOS G/l	BASO G/l	LYMPH G/l	MONO G/l	LUC G/l	PLATELETS G/l
After 13 Weeks							
Group 1	1.46	0.09	0.01	4.58	0.16	0.04	1057
Group 2	1.12 -	0.08 -	0.01 -	3.71 -	0.13 -	0.03 -	1014 -
Group 3	1.14 -	0.09 -	0.01 -	3.74 -	0.14 -	0.03 -	988 -
Group 4	1.40 -	0.12 -	0.01 -	3.53 *	0.16 -	0.03 -	1026 -

	GENERAL	COAGULATION	
	MET-HB rel. 1	PT rel. 1	PTT sec
After 13 Weeks			
Group 1	0.014	0.81	24.1
Group 2	0.014 -	0.82 -	24.7 -
Group 3	0.014 -	0.82 -	24.1 -
Group 4	0.014 -	0.83 -	24.0 -

*/**/- : 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	7.71	9.0	0.41	52.7	0.152	1.17	22.19
Group 2	7.79 -	9.0 -	0.41 -	52.6 -	0.127 -	1.16 -	22.09 -
Group 3	7.80 -	8.9 -	0.40 -	51.9 -	0.164 -	1.14 -	22.05 -
Group 4	7.70 -	9.0 -	0.41 -	53.0 -	0.143 -	1.17 -	22.10 -

	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	1.50	0.027	210	0.630	0.342	0.031	2.93
Group 2	1.44 -	0.025 -	194 -	0.640 -	0.319 -	0.037 -	2.66 -
Group 3	1.48 -	0.026 -	202 -	0.643 -	0.322 -	0.037 -	2.57 -
Group 4	1.42 *	0.027 -	206 -	0.644 -	0.325 -	0.034 -	2.91 -

	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	0.232	0.022	0.002	0.708	0.021	0.006
Group 2	0.242 -	0.022 -	0.002 -	0.682 -	0.020 -	0.005 -
Group 3	0.228 -	0.013 -	0.003 -	0.705 -	0.022 -	0.006 -
Group 4	0.220 -	0.018 -	0.002 -	0.739 -	0.019 -	0.006 -

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

Hematology - SUMMARY
FEMALES

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT G/l	EOS G/l	BASO G/l	LYMPH G/l	MONO G/l	LUC G/l	PLATELETS G/l
After 13 Weeks							
Group 1	0.65	0.07	0.01	2.12	0.06	0.02	1103
Group 2	0.73 -	0.07 -	0.01 -	1.78 -	0.06 -	0.01 -	1078 -
Group 3	0.64 -	0.04 -	0.01 -	1.81 -	0.06 -	0.02 -	1081 -
Group 4	0.67 -	0.06 -	0.01 -	2.10 -	0.05 -	0.02 -	1180 -

	GENERAL	COAGULATION	
	MET-HB rel. 1	PT rel. 1	PTT sec
After 13 Weeks			
Group 1	0.014	0.83	35.2
Group 2	0.014 -	0.83 -	34.9 -
Group 3	0.015 -	0.83 -	32.2 -
Group 4	0.014 -	0.85 -	32.1 -

*/**/- : 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 102	Female	Group 10	Reserve Removed
Animal 103	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	STEEL
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
GLDH	GLUTAMATE-DEHYDROGENASE (GLDH)	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 mmol/l	UREA mmol/l	CREAT µmol/l	BILI-T µmol/l	CHOLEST mmol/l	TRIGLY mmol/l	PHOS-LIP mmol/l
After 13 Weeks							
Group 1	5.38	5.43	26.3	2.07	2.40	0.38	1.81
Group 2	6.39 *	5.05 -	24.8 -	1.61 **	2.15 -	0.41 -	1.72 -
Group 3	6.63 *	4.95 -	25.7 -	1.56 **	2.34 -	0.44 -	1.83 -
Group 4	6.71 **	5.11 -	26.5 -	1.67 *	2.32 -	0.53 -	1.83 -

	GENERAL						
	ASAT U/l	ALAT U/l	LDH U/l	GLDH U/l	ALP U/l	GGT U/l	CK U/l
After 13 Weeks							
Group 1	75.1	31.0	113.3	8.4	47.6	0.0	179.3
Group 2	73.0 -	30.7 -	95.4 -	8.8 -	51.8 -	0.0 -	124.4 -
Group 3	75.8 -	30.7 -	101.3 -	9.9 -	49.2 -	0.0 -	183.1 -
Group 4	73.2 -	30.5 -	96.6 -	10.0 -	49.5 -	0.0 -	159.9 -

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
After 13 Weeks							
Group 1	146.6	3.77	105.2	2.72	1.78	72.29	44.06
Group 2	147.5 -	3.74 -	107.0 **	2.68 -	1.64 -	71.51 -	43.58 -
Group 3	148.0 *	3.85 -	107.2 **	2.73 -	1.77 -	71.79 -	43.54 -
Group 4	147.7 -	3.83 -	107.2 **	2.74 -	1.75 -	72.29 -	43.57 -

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

Biochemistry - SUMMARY
MALES

	GENERAL	
	GLOBULIN g/l	A/G RATIO
After 13 Weeks		
Group 1	28.23	1.49
Group 2	27.93 -	1.56 -
Group 3	28.25 -	1.53 -
Group 4	28.73 -	1.54 -

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

Biochemistry - SUMMARY
FEMALES

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT µmol/l	BILI-T µmol/l	CHOLEST mmol/l	TRIGLY mmol/l	PHOS-LIP mmol/l
After 13 Weeks							
Group 1	5.54	6.05	28.6	2.30	2.21	0.38	2.27
Group 2	5.51 -	6.20 -	28.9 -	1.94 -	2.08 -	0.29 *	2.10 -
Group 3	5.66 -	6.34 -	29.9 -	2.01 -	1.92 -	0.34 -	2.07 -
Group 4	6.07 -	6.26 -	29.4 -	1.96 -	2.20 -	0.32 -	2.18 -

	GENERAL						
	ASAT U/l	ALAT U/l	LDH U/l	GLDH U/l	ALP U/l	GGT U/l	CK U/l
After 13 Weeks							
Group 1	66.8	22.9	108.1	5.9	21.1	0.0	141.4
Group 2	73.5 -	22.4 -	105.4 -	9.2 -	22.6 -	0.0 -	129.2 -
Group 3	80.5 -	28.7 *	116.7 -	8.4 -	23.6 -	0.0 -	151.3 -
Group 4	70.9 -	21.1 -	105.5 -	6.0 -	20.9 -	0.0 -	169.3 -

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
After 13 Weeks							
Group 1	147.2	3.14	106.4	2.80	1.33	81.24	55.12
Group 2	148.3 -	3.21 -	108.6 *	2.74 -	1.35 -	77.78 -	52.39 -
Group 3	148.4 -	3.23 -	108.6 *	2.75 -	1.29 -	78.57 -	54.67 -
Group 4	149.7 **	3.29 -	109.6 **	2.79 -	1.35 -	78.84 -	52.97 -

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

Biochemistry - SUMMARY
FEMALES

	GENERAL	
	GLOBULIN g/l	A/G RATIO
After 13 Weeks		
Group 1	26.12	2.12
Group 2	25.39 -	2.11 -
Group 3	23.90 -	2.20 -
Group 4	25.87 -	2.00 -

*/**/- : 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 102	Female	Group 10	Reserve Removed
Animal 103	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	ERYTHROCYES	STEEL
LEU	LEUCOCYTES	STEEL

Statistical Methods

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

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	9.6	1.031	7.0	1	0.25	0	0.5
Group 2	9.1 -	1.031 -	7.0 -	0	0.25 -	0 -	0.5 -
Group 3	6.1 -	1.039 -	6.5 -	1	0.25 -	0 -	1.5 -
Group 4	8.5 -	1.031 -	6.8 -	1	0.25 -	0 -	0.5 -

	GENERAL			
	UROBILI µmol/l	BILIRUBIN µmol/l	ERY per µl	LEU per µl
After 13 Weeks				
Group 1	0	0	0	25
Group 2	0 -	0 -	0 -	25 -
Group 3	0 -	9 *	0 -	25 -
Group 4	0 -	0 -	0 -	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	6.2	1.031	6.3	0	0.25	0	0.3
Group 2	7.2 -	1.028 -	6.0 -	0	0.25 -	0 -	0.0 -
Group 3	7.0 -	1.030 -	6.0 -	0	0.25 -	0 -	0.0 -
Group 4	8.3 -	1.023 -	6.0 -	0	0.25 -	0 -	0.0 -

	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 -	0 -	0 -	0 -
Group 4	0 -	0 -	0 -	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 102	Female	Group 10	Reserve Removed
Animal 103	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 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
BODY W.	MEAN	415.3	391.9	-	410.5	-	413.1	-
	ST.DEV.	35.1	47.4		36.1		45.4	
	MINIMUM	347.7	311.0		353.0		371.0	
	MAXIMUM	458.3	468.2		476.5		484.6	
	N	10	10		10		10	
BRAIN	MEAN	2.04	2.06	-	2.02	-	2.09	-
	ST.DEV.	0.12	0.07		0.09		0.12	
	MINIMUM	1.87	1.95		1.89		1.95	
	MAXIMUM	2.26	2.16		2.19		2.36	
	N	10	10		10		10	
HEART	MEAN	1.07	0.98	-	1.07	-	1.04	-
	ST.DEV.	0.10	0.09		0.08		0.11	
	MINIMUM	0.98	0.85		0.97		0.84	
	MAXIMUM	1.32	1.17		1.20		1.18	
	N	10	10		10		10	
LIVER	MEAN	10.78	10.47	-	11.71	-	10.82	-
	ST.DEV.	1.01	1.48		1.14		1.22	
	MINIMUM	9.70	7.84		10.05		8.80	
	MAXIMUM	12.73	13.22		13.66		12.76	
	N	10	10		10		10	
THYMUS	MEAN	0.296	0.282	-	0.306	-	0.295	-
	ST.DEV.	0.042	0.052		0.066		0.051	
	MINIMUM	0.223	0.175		0.188		0.204	
	MAXIMUM	0.370	0.357		0.380		0.372	
	N	10	10		10		10	
KIDNEYS	MEAN	2.09	2.04	-	2.19	-	2.03	-
	ST.DEV.	0.13	0.28		0.22		0.23	
	MINIMUM	1.92	1.53		1.81		1.74	
	MAXIMUM	2.28	2.39		2.62		2.50	
	N	10	10		10		10	
ADRENALS	MEAN	0.062	0.060	-	0.060	-	0.066	-
	ST.DEV.	0.009	0.007		0.006		0.009	
	MINIMUM	0.044	0.049		0.047		0.055	
	MAXIMUM	0.076	0.070		0.067		0.080	
	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 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
SPLEEN	MEAN	0.82	0.70	*	0.72	-	0.75	-
	ST.DEV.	0.09	0.12		0.07		0.12	
	MINIMUM	0.68	0.55		0.60		0.58	
	MAXIMUM	0.94	0.93		0.83		0.94	
	N	10	10		10		10	
TESTES	MEAN	3.72	3.73	-	3.76	-	3.94	-
	ST.DEV.	0.28	0.37		0.42		0.22	
	MINIMUM	3.20	3.00		3.34		3.50	
	MAXIMUM	4.09	4.34		4.71		4.25	
	N	10	10		10		10	
EPIDIDYMID	MEAN	1.420	1.429	-	1.486	-	1.568	*
	ST.DEV.	0.080	0.155		0.164		0.082	
	MINIMUM	1.285	1.164		1.308		1.450	
	MAXIMUM	1.541	1.659		1.742		1.702	
	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 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
BODY W.	MEAN	415.3	391.9	-	410.5	-	413.1	-
	ST.DEV.	35.1	47.4		36.1		45.4	
	MINIMUM	347.7	311.0		353.0		371.0	
	MAXIMUM	458.3	468.2		476.5		484.6	
	N	10	10		10		10	
BRAIN	MEAN	0.49	0.53	-	0.49	-	0.51	-
	ST.DEV.	0.04	0.06		0.03		0.03	
	MINIMUM	0.42	0.45		0.43		0.46	
	MAXIMUM	0.57	0.64		0.55		0.54	
	N	10	10		10		10	
HEART	MEAN	0.26	0.25	-	0.26	-	0.25	-
	ST.DEV.	0.02	0.02		0.02		0.02	
	MINIMUM	0.23	0.22		0.23		0.22	
	MAXIMUM	0.30	0.29		0.30		0.29	
	N	10	10		10		10	
LIVER	MEAN	2.60	2.67	-	2.86	*	2.62	-
	ST.DEV.	0.19	0.20		0.22		0.13	
	MINIMUM	2.30	2.44		2.62		2.34	
	MAXIMUM	2.87	2.97		3.33		2.80	
	N	10	10		10		10	
THYMUS	MEAN	0.072	0.073	-	0.075	-	0.072	-
	ST.DEV.	0.010	0.017		0.018		0.012	
	MINIMUM	0.051	0.041		0.045		0.052	
	MAXIMUM	0.085	0.103		0.105		0.089	
	N	10	10		10		10	
KIDNEYS	MEAN	0.51	0.52	-	0.53	-	0.49	-
	ST.DEV.	0.04	0.04		0.05		0.03	
	MINIMUM	0.46	0.48		0.46		0.45	
	MAXIMUM	0.57	0.58		0.62		0.54	
	N	10	10		10		10	
ADRENALS	MEAN	0.015	0.016	-	0.015	-	0.016	-
	ST.DEV.	0.002	0.002		0.001		0.002	
	MINIMUM	0.012	0.012		0.013		0.014	
	MAXIMUM	0.019	0.018		0.016		0.020	
	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 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
SPLEEN	MEAN	0.20	0.18	-	0.18	-	0.18	-
	ST.DEV.	0.02	0.02		0.02		0.02	
	MINIMUM	0.16	0.14		0.15		0.15	
	MAXIMUM	0.23	0.22		0.21		0.22	
	N	10	10		10		10	
TESTES	MEAN	0.90	0.96	-	0.92	-	0.96	-
	ST.DEV.	0.12	0.07		0.09		0.07	
	MINIMUM	0.73	0.86		0.83		0.85	
	MAXIMUM	1.14	1.11		1.10		1.05	
	N	10	10		10		10	
EPIDIDYMID	MEAN	0.344	0.367	-	0.363	-	0.383	-
	ST.DEV.	0.033	0.040		0.033		0.037	
	MINIMUM	0.295	0.301		0.323		0.312	
	MAXIMUM	0.417	0.456		0.417		0.443	
	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 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
BRAIN	MEAN	2.04	2.06	-	2.02	-	2.09	-
	ST.DEV.	0.12	0.07		0.09		0.12	
	MINIMUM	1.87	1.95		1.89		1.95	
	MAXIMUM	2.26	2.16		2.19		2.36	
	N	10	10		10		10	
HEART	MEAN	52.65	47.58	*	53.00	-	49.64	-
	ST.DEV.	4.39	3.97		3.07		4.90	
	MINIMUM	48.23	41.26		48.36		42.00	
	MAXIMUM	61.40	56.25		57.97		56.46	
	N	10	10		10		10	
LIVER	MEAN	531.03	509.19	-	579.43	-	517.06	-
	ST.DEV.	65.91	67.75		55.28		42.23	
	MINIMUM	473.58	395.96		520.49		451.28	
	MAXIMUM	666.49	623.58		659.90		596.26	
	N	10	10		10		10	
THYMUS	MEAN	14.543	13.717	-	15.146	-	14.125	-
	ST.DEV.	1.885	2.315		3.269		2.362	
	MINIMUM	10.519	8.578		9.543		9.533	
	MAXIMUM	16.943	16.840		20.106		17.799	
	N	10	10		10		10	
KIDNEYS	MEAN	102.93	99.20	-	108.20	-	96.91	-
	ST.DEV.	9.54	12.20		11.47		7.34	
	MINIMUM	91.59	77.18		89.50		85.78	
	MAXIMUM	115.71	112.74		126.57		105.93	
	N	10	10		10		10	
ADRENALS	MEAN	3.046	2.942	-	2.943	-	3.144	-
	ST.DEV.	0.452	0.369		0.212		0.302	
	MINIMUM	2.353	2.513		2.487		2.750	
	MAXIMUM	3.938	3.590		3.249		3.645	
	N	10	10		10		10	
SPLEEN	MEAN	40.06	34.09	*	35.63	-	35.85	-
	ST.DEV.	4.39	5.27		3.48		5.70	
	MINIMUM	33.50	26.70		30.15		28.43	
	MAXIMUM	48.70	43.87		40.10		44.98	
	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 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
TESTES	MEAN	183.30	181.56	-	185.98	-	188.56	-
	ST.DEV.	18.26	17.01		20.05		7.65	
	MINIMUM	150.94	151.52		161.19		175.00	
	MAXIMUM	200.52	208.65		227.54		199.53	
	N	10	10		10		10	
EPIDIDYMID	MEAN	69.896	69.551	-	73.516	-	75.160	-
	ST.DEV.	5.693	7.055		7.964		4.803	
	MINIMUM	60.613	58.429		62.535		64.110	
	MAXIMUM	77.202	79.760		85.392		81.716	
	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 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
BODY W.	MEAN	224.2	241.1	*	232.0	-	242.0	*
	ST.DEV.	10.2	13.7		12.9		14.0	
	MINIMUM	201.6	225.1		206.1		220.9	
	MAXIMUM	237.6	268.2		255.9		262.2	
	N	10	10		10		10	
BRAIN	MEAN	1.90	1.92	-	1.89	-	1.95	-
	ST.DEV.	0.10	0.07		0.08		0.09	
	MINIMUM	1.72	1.80		1.79		1.78	
	MAXIMUM	2.02	2.01		2.05		2.10	
	N	10	10		10		10	
HEART	MEAN	0.72	0.73	-	0.69	-	0.69	-
	ST.DEV.	0.02	0.06		0.03		0.06	
	MINIMUM	0.68	0.66		0.67		0.62	
	MAXIMUM	0.75	0.85		0.75		0.81	
	N	10	10		10		10	
LIVER	MEAN	6.56	7.17	*	6.96	-	7.09	-
	ST.DEV.	0.50	0.46		0.51		0.60	
	MINIMUM	5.79	6.23		6.06		6.20	
	MAXIMUM	7.27	7.80		7.68		7.79	
	N	10	10		10		10	
THYMUS	MEAN	0.234	0.282	-	0.220	-	0.269	-
	ST.DEV.	0.058	0.064		0.032		0.035	
	MINIMUM	0.143	0.188		0.178		0.210	
	MAXIMUM	0.316	0.399		0.287		0.322	
	N	10	10		10		10	
KIDNEYS	MEAN	1.41	1.45	-	1.37	-	1.44	-
	ST.DEV.	0.14	0.12		0.08		0.18	
	MINIMUM	1.24	1.21		1.28		1.21	
	MAXIMUM	1.74	1.62		1.48		1.83	
	N	10	10		10		10	
ADRENALS	MEAN	0.071	0.075	-	0.072	-	0.075	-
	ST.DEV.	0.008	0.008		0.011		0.008	
	MINIMUM	0.058	0.064		0.056		0.062	
	MAXIMUM	0.085	0.092		0.097		0.086	
	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 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
SPLEEN	MEAN	0.53	0.55	-	0.55	-	0.54	-
	ST.DEV.	0.06	0.09		0.09		0.09	
	MINIMUM	0.38	0.41		0.44		0.42	
	MAXIMUM	0.62	0.69		0.75		0.70	
	N	10	10		10		10	
OVARIES	MEAN	0.085	0.101	-	0.099	-	0.103	-
	ST.DEV.	0.018	0.020		0.017		0.022	
	MINIMUM	0.049	0.069		0.074		0.072	
	MAXIMUM	0.103	0.128		0.133		0.142	
	N	10	10		10		10	
UTERUS	MEAN	0.91	0.93	-	0.96	-	1.05	-
	ST.DEV.	0.26	0.17		0.22		0.19	
	MINIMUM	0.66	0.65		0.67		0.73	
	MAXIMUM	1.56	1.24		1.35		1.37	
	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 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
BODY W.	MEAN	224.2	241.1	*	232.0	-	242.0	*
	ST.DEV.	10.2	13.7		12.9		14.0	
	MINIMUM	201.6	225.1		206.1		220.9	
	MAXIMUM	237.6	268.2		255.9		262.2	
	N	10	10		10		10	
BRAIN	MEAN	0.85	0.80	-	0.82	-	0.81	-
	ST.DEV.	0.06	0.04		0.04		0.07	
	MINIMUM	0.75	0.74		0.76		0.69	
	MAXIMUM	0.97	0.86		0.91		0.87	
	N	10	10		10		10	
HEART	MEAN	0.32	0.30	-	0.30	-	0.29	**
	ST.DEV.	0.01	0.03		0.02		0.02	
	MINIMUM	0.31	0.26		0.26		0.25	
	MAXIMUM	0.35	0.35		0.34		0.32	
	N	10	10		10		10	
LIVER	MEAN	2.93	2.98	-	3.01	-	2.94	-
	ST.DEV.	0.19	0.17		0.21		0.27	
	MINIMUM	2.60	2.69		2.72		2.46	
	MAXIMUM	3.21	3.20		3.45		3.32	
	N	10	10		10		10	
THYMUS	MEAN	0.104	0.117	-	0.094	-	0.111	-
	ST.DEV.	0.024	0.026		0.010		0.014	
	MINIMUM	0.065	0.082		0.079		0.087	
	MAXIMUM	0.137	0.163		0.112		0.129	
	N	10	10		10		10	
KIDNEYS	MEAN	0.63	0.60	-	0.59	-	0.60	-
	ST.DEV.	0.07	0.05		0.04		0.08	
	MINIMUM	0.56	0.53		0.54		0.47	
	MAXIMUM	0.79	0.66		0.66		0.76	
	N	10	10		10		10	
ADRENALS	MEAN	0.031	0.031	-	0.031	-	0.031	-
	ST.DEV.	0.003	0.004		0.005		0.004	
	MINIMUM	0.028	0.026		0.024		0.024	
	MAXIMUM	0.038	0.038		0.042		0.036	
	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 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
SPLEEN	MEAN	0.23	0.23	-	0.24	-	0.23	-
	ST.DEV.	0.03	0.03		0.03		0.04	
	MINIMUM	0.17	0.18		0.20		0.19	
	MAXIMUM	0.27	0.28		0.29		0.30	
	N	10	10		10		10	
OVARIES	MEAN	0.038	0.042	-	0.043	-	0.043	-
	ST.DEV.	0.008	0.008		0.008		0.009	
	MINIMUM	0.022	0.028		0.033		0.032	
	MAXIMUM	0.047	0.052		0.058		0.061	
	N	10	10		10		10	
UTERUS	MEAN	0.40	0.39	-	0.42	-	0.44	-
	ST.DEV.	0.11	0.07		0.10		0.08	
	MINIMUM	0.30	0.28		0.28		0.33	
	MAXIMUM	0.68	0.50		0.58		0.56	
	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 2 100 mg/kg		Group 3 300 mg/kg		Group 4 1000 mg/kg	
BRAIN	MEAN	1.90	1.92	-	1.89	-	1.95	-
	ST.DEV.	0.10	0.07		0.08		0.09	
	MINIMUM	1.72	1.80		1.79		1.78	
	MAXIMUM	2.02	2.01		2.05		2.10	
	N	10	10		10		10	
HEART	MEAN	38.08	37.93	-	36.66	-	35.63	-
	ST.DEV.	2.23	2.49		1.97		3.35	
	MINIMUM	34.69	35.08		32.68		31.96	
	MAXIMUM	41.86	42.71		39.11		42.86	
	N	10	10		10		10	
LIVER	MEAN	346.24	373.60	-	368.16	-	364.41	-
	ST.DEV.	32.84	20.18		27.08		29.88	
	MINIMUM	302.55	346.11		322.34		314.43	
	MAXIMUM	392.97	405.35		407.82		410.58	
	N	10	10		10		10	
THYMUS	MEAN	12.381	14.684	-	11.588	-	13.860	-
	ST.DEV.	3.086	3.231		1.415		1.934	
	MINIMUM	7.114	9.543		9.674		10.000	
	MAXIMUM	16.545	19.851		14.000		17.037	
	N	10	10		10		10	
KIDNEYS	MEAN	74.23	75.51	-	72.34	-	73.90	-
	ST.DEV.	7.02	5.54		3.38		6.74	
	MINIMUM	65.31	67.22		67.19		64.43	
	MAXIMUM	86.57	83.96		79.14		87.14	
	N	10	10		10		10	
ADRENALS	MEAN	3.728	3.895	-	3.817	-	3.832	-
	ST.DEV.	0.524	0.436		0.567		0.306	
	MINIMUM	2.959	3.184		3.094		3.196	
	MAXIMUM	4.696	4.623		5.079		4.180	
	N	10	10		10		10	
SPLEEN	MEAN	27.87	28.42	-	28.86	-	27.96	-
	ST.DEV.	3.86	4.51		3.57		4.40	
	MINIMUM	18.91	22.04		23.40		21.65	
	MAXIMUM	33.51	36.51		36.59		34.83	
	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 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
OVARIES	MEAN	4.510	5.281 -	5.236 -	5.273 -
	ST.DEV.	0.976	1.038	0.950	1.091
	MINIMUM	2.438	3.467	3.756	3.667
	MAXIMUM	5.568	6.772	6.963	7.065
	N	10	10	10	10
UTERUS	MEAN	48.07	48.33 -	50.64 -	54.13 -
	ST.DEV.	14.53	8.84	10.42	9.57
	MINIMUM	33.50	36.11	36.65	37.63
	MAXIMUM	82.54	65.61	65.85	68.84
	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 10   Reserve Removed
Animal 101  Male   Group 10   Reserve Removed
Animal 102  Female Group 10   Reserve Removed
Animal 103  Female Group 10   Reserve Removed
```

MACROSCOPICAL FINDINGS - SUMMARY
AFTER 13 WEEKS
ALL NECROPSIES
MALES

	Group 1 **0 mg/kg**	Group 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
ANIMALS EXAMINED	10	10	10	10
ANIMALS COMPLETED	10	10	10	10
ANIMALS WITHOUT FINDINGS	10	9	9	8
ANIMALS AFFECTED				
PANCREAS				
- DISCOLORATION	0 (0%)	0 (0%) -	1 (10%) -	0 (0%) -
KIDNEYS				
- PELVIC DILATION	0 (0%)	0 (0%) -	0 (0%) -	1 (10%) -
TESTES				
- REDUCED IN SIZE	0 (0%)	1 (10%) -	0 (0%) -	0 (0%) -
THYMUS				
- FOCUS/FOCI	0 (0%)	0 (0%) -	0 (0%) -	1 (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 2 100 mg/kg	Group 3 300 mg/kg	Group 4 1000 mg/kg
ANIMALS EXAMINED	10	10	10	10
ANIMALS COMPLETED	10	10	10	10
ANIMALS WITHOUT FINDINGS	8	8	10	9
ANIMALS AFFECTED				
THYMUS				
- FOCUS/FOCI	1 (10%)	1 (10%) -	0 (0%) -	0 (0%) -
MANDIBULAR L.NODE				
- FOCUS/FOCI	1 (10%)	1 (10%) -	0 (0%) -	0 (0%) -
EYES				
- DESTROYED DURING BLOOD COLLECTION	0 (0%)	0 (0%) -	0 (0%) -	1 (10%) -

*/**/- : 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 1	Reserve	Removed
Animal 101	Male	Group 1	Reserve	Removed
Animal 102	Female	Group 1	Reserve	Removed
Animal 103	Female	Group 1	Reserve	Removed

MORTALITY DATA
ALL NECROPSIES
MALES

Group 1 (0 mg/kg)

PRE-RANDOM PHASE (Days 1 to 1)

No mortality data recorded

ACCLIMATIZATION (Days 1 to 8)

No mortality data recorded

TREATMENT (Days 1 to 92)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
1	08-APR-10	92	X				
2	08-APR-10	92	X				
3	08-APR-10	92	X				
4	08-APR-10	92	X				
5	08-APR-10	92	X				
6	08-APR-10	92	X				
7	08-APR-10	92	X				
8	08-APR-10	92	X				
9	08-APR-10	92	X				
10	08-APR-10	92	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 (100 mg/kg)

PRE-RANDOM PHASE (Days 1 to 1)

No mortality data recorded

ACCLIMATIZATION (Days 1 to 8)

No mortality data recorded

TREATMENT (Days 1 to 92)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
11	08-APR-10	92	X				
12	08-APR-10	92	X				
13	08-APR-10	92	X				
14	08-APR-10	92	X				
15	08-APR-10	92	X				
16	08-APR-10	92	X				
17	08-APR-10	92	X				
18	08-APR-10	92	X				
19	08-APR-10	92	X				
20	08-APR-10	92	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 (300 mg/kg)

PRE-RANDOM PHASE (Days 1 to 1)

No mortality data recorded

ACCLIMATIZATION (Days 1 to 8)

No mortality data recorded

TREATMENT (Days 1 to 92)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
21	08-APR-10	92	X				
22	08-APR-10	92	X				
23	08-APR-10	92	X				
24	08-APR-10	92	X				
25	08-APR-10	92	X				
26	08-APR-10	92	X				
27	08-APR-10	92	X				
28	08-APR-10	92	X				
29	08-APR-10	92	X				
30	08-APR-10	92	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)

PRE-RANDOM PHASE (Days 1 to 1)

No mortality data recorded

ACCLIMATIZATION (Days 1 to 8)

No mortality data recorded

TREATMENT (Days 1 to 92)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
31	08-APR-10	92	X				
32	08-APR-10	92	X				
33	08-APR-10	92	X				
34	08-APR-10	92	X				
35	08-APR-10	92	X				
36	08-APR-10	92	X				
37	08-APR-10	92	X				
38	08-APR-10	92	X				
39	08-APR-10	92	X				
40	08-APR-10	92	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)

PRE-RANDOM PHASE (Days 1 to 1)

No mortality data recorded

ACCLIMATIZATION (Days 1 to 8)

No mortality data recorded

TREATMENT (Days 1 to 92)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
41	08-APR-10	92	X				
42	08-APR-10	92	X				
43	08-APR-10	92	X				
44	08-APR-10	92	X				
45	08-APR-10	92	X				
46	08-APR-10	92	X				
47	08-APR-10	92	X				
48	08-APR-10	92	X				
49	08-APR-10	92	X				
50	08-APR-10	92	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 (100 mg/kg)

PRE-RANDOM PHASE (Days 1 to 1)

No mortality data recorded

ACCLIMATIZATION (Days 1 to 8)

No mortality data recorded

TREATMENT (Days 1 to 92)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
51	08-APR-10	92	X				
52	08-APR-10	92	X				
53	08-APR-10	92	X				
54	08-APR-10	92	X				
55	08-APR-10	92	X				
56	08-APR-10	92	X				
57	08-APR-10	92	X				
58	08-APR-10	92	X				
59	08-APR-10	92	X				
60	08-APR-10	92	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 (300 mg/kg)

PRE-RANDOM PHASE (Days 1 to 1)

No mortality data recorded

ACCLIMATIZATION (Days 1 to 8)

No mortality data recorded

TREATMENT (Days 1 to 92)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
61	08-APR-10	92	X				
62	08-APR-10	92	X				
63	08-APR-10	92	X				
64	08-APR-10	92	X				
65	08-APR-10	92	X				
66	08-APR-10	92	X				
67	08-APR-10	92	X				
68	08-APR-10	92	X				
69	08-APR-10	92	X				
70	08-APR-10	92	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)

PRE-RANDOM PHASE (Days 1 to 1)

No mortality data recorded

ACCLIMATIZATION (Days 1 to 8)

No mortality data recorded

TREATMENT (Days 1 to 92)

ANIMAL	DEATH DATE	DAY	P	K	S	O	COMMENT
71	08-APR-10	92	X				
72	08-APR-10	92	X				
73	08-APR-10	92	X				
74	08-APR-10	92	X				
75	08-APR-10	92	X				
76	08-APR-10	92	X				
77	08-APR-10	92	X				
78	08-APR-10	92	X				
79	08-APR-10	92	X				
80	08-APR-10	92	X				
		Total:	10	0	0	0	

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

CLINICAL SIGNS

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 102 Female Group 10 Reserve Removed
Animal 103 Female Group 10 Reserve Removed

Incomplete Recordings

Selection of Findings

All findings reported

CLINICAL SIGNS
MALES

ACCLIMATIZATION

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

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

CLINICAL SIGNS
MALES

TREATMENT

	Weeks / Days					
	1	2	3	4	5	6
	1234567	1234567	1234567	1234567	1234567	1234567
Group 1 (0 mg/kg)						
No abnormality recorded.						
Group 2 (100 mg/kg)						
No abnormality recorded.						
Group 3 (300 mg/kg)						
No abnormality recorded.						
Group 4 (1000 mg/kg)						
No abnormality recorded.						

CLINICAL SIGNS
MALES

TREATMENT

	Weeks / Days 7 8 9 10 11 12 1234567123456712345671234567123456712345671234567
Group 1 (0 mg/kg)	
No abnormality recorded.	
Group 2 (100 mg/kg)	
No abnormality recorded.	
Group 3 (300 mg/kg)	
No abnormality recorded.	
Group 4 (1000 mg/kg)	
Animal 32	
APPEARANCE	
- HAIR LOSS (3)	
RIGHT CHEEK	.....11111111.....
Animal 37	
APPEARANCE	
- HAIR LOSS (3)	
LEFT EYE	111111
- SCABS (3)	
LEFT EYE	1111111111111111
No further abnormality recorded.	

CLINICAL SIGNS
MALES

TREATMENT

	Weeks / Days 13 14 1234567-

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

Animal 37

APPEARANCE	
- HAIR LOSS (3)	
LEFT EYE	1111111
- SCABS (3)	
LEFT EYE	1.....

No further abnormality recorded.

CLINICAL SIGNS
FEMALES

ACCLIMATIZATION

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

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

Animal 75

APPEARANCE	
- HAIR LOSS (3)	
NECK (CERVICAL)	. . . . . 1 1

No further abnormality recorded.

CLINICAL SIGNS
FEMALES

TREATMENT

	Weeks / Days 1 2 3 4 5 6 1234567123456712345671234567123456712345671234567
Group 1 (0 mg/kg)	
No abnormality recorded.	
Group 2 (100 mg/kg)	
No abnormality recorded.	
Group 3 (300 mg/kg)	
No abnormality recorded.	
Group 4 (1000 mg/kg)	
Animal 75	
APPEARANCE	
- HAIR LOSS (3)	
NECK (CERVICAL)	111.....
No further abnormality recorded.	

CLINICAL SIGNS
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	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 (100 mg/kg)

No abnormality recorded.

Group 3 (300 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

CLINICAL SIGNS
FEMALES

TREATMENT

	Weeks / Days 1 3 1 4 1 2 3 4 5 6 7 -
Group 1 (0 mg/kg)	
No abnormality recorded.	
Group 2 (100 mg/kg)	
No abnormality recorded.	
Group 3 (300 mg/kg)	
No abnormality recorded.	
Group 4 (1000 mg/kg)	
No abnormality recorded.	

WEEKLY DETAILED 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 102 Female Group 10 Reserve Removed
Animal 103 Female Group 10 Reserve Removed

Incomplete Recordings

Selection of Findings

All findings reported

WEEKLY DETAILED OBSERVATIONS
MALES

ACCLIMATIZATION

	Weeks
	0
	1 -

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

WEEKLY DETAILED OBSERVATIONS
MALES

TREATMENT

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

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

WEEKLY DETAILED OBSERVATIONS
FEMALES

ACCLIMATIZATION

	Weeks
	0
	1 -

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 mg/kg)

No abnormality recorded.

Group 4 (1000 mg/kg)

No abnormality recorded.

WEEKLY DETAILED OBSERVATIONS
FEMALES

TREATMENT

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

Group 1 (0 mg/kg)

No abnormality recorded.

Group 2 (100 mg/kg)

No abnormality recorded.

Group 3 (300 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 102  Female Group 10   Reserve Removed
Animal 103  Female Group 10   Reserve Removed
```

Reported Parameter

AT WEEK 12

```
Grip Fore    GRIP FORELIMB
Grip Hind    GRIP HINDLIMB
```

AT WEEK 12

```
Grip Fore    GRIP FORELIMB
Grip Hind    GRIP HINDLIMB
```

GRIP STRENGTH
AT WEEK 12
MALES

Group 1 (0 mg/kg)

	GRIP STRENGTH	
	Grip Fore KILOGRAM	Grip Hind KILOGRAM
1	1.41	1.03
2	1.46	1.04
3	1.57	1.16
4	1.58	1.14
5	1.56	1.06
6	1.57	1.15
7	1.45	1.02
8	1.48	1.12
9	1.59	1.11
10	1.48	1.11

GRIP STRENGTH
AT WEEK 12
MALES

Group 2 (100 mg/kg)

	GRIP STRENGTH	
	Grip Fore KILOGRAM	Grip Hind KILOGRAM
11	1.54	1.02
12	1.62	1.09
13	1.41	1.07
14	1.58	1.09
15	1.51	1.15
16	1.52	1.07
17	1.47	0.99
18	1.45	1.08
19	1.46	0.99
20	1.57	1.12

GRIP STRENGTH
AT WEEK 12
MALES

Group 3 (300 mg/kg)

	GRIP STRENGTH	
	Grip Fore KILOGRAM	Grip Hind KILOGRAM
21	1.61	1.13
22	1.55	1.01
23	1.60	1.15
24	1.46	1.08
25	1.58	1.03
26	1.50	1.10
27	1.51	1.04
28	1.49	1.03
29	1.59	1.16
30	1.50	1.05

GRIP STRENGTH
AT WEEK 12
MALES

Group 4 (1000 mg/kg)

	GRIP STRENGTH	
	Grip Fore KILOGRAM	Grip Hind KILOGRAM
31	1.56	1.09
32	1.53	1.05
33	1.56	1.02
34	1.45	1.09
35	1.57	0.99
36	1.47	1.02
37	1.60	1.18
38	1.42	0.98
39	1.55	1.03
40	1.45	1.03

GRIP STRENGTH
AT WEEK 12
FEMALES

Group 1 (0 mg/kg)

	GRIP STRENGTH	
	Grip Fore KILOGRAM	Grip Hind KILOGRAM
41	1.24	0.77
42	1.28	0.77
43	1.23	0.76
44	1.31	0.78
45	1.36	0.80
46	1.29	0.77
47	1.33	0.83
48	1.26	0.83
49	1.22	0.76
50	1.20	0.77

GRIP STRENGTH
AT WEEK 12
FEMALES

Group 2 (100 mg/kg)

	GRIP STRENGTH	
	Grip Fore KILOGRAM	Grip Hind KILOGRAM
51	1.27	0.75
52	1.28	0.79
53	1.26	0.72
54	1.32	0.81
55	1.31	0.81
56	1.21	0.67
57	1.26	0.75
58	1.27	0.81
59	1.33	0.76
60	1.27	0.78

GRIP STRENGTH
AT WEEK 12
FEMALES

Group 3 (300 mg/kg)

	GRIP STRENGTH	
	Grip Fore KILOGRAM	Grip Hind KILOGRAM
61	1.29	0.83
62	1.21	0.74
63	1.23	0.77
64	1.39	0.86
65	1.36	0.78
66	1.26	0.77
67	1.30	0.75
68	1.28	0.78
69	1.26	0.78
70	1.31	0.78

GRIP STRENGTH
AT WEEK 12
FEMALES

Group 4 (1000 mg/kg)

	GRIP STRENGTH	
	Grip Fore KILOGRAM	Grip Hind KILOGRAM
71	1.30	0.79
72	1.38	0.84
73	1.29	0.81
74	1.33	0.76
75	1.33	0.76
76	1.41	0.81
77	1.26	0.77
78	1.24	0.69
79	1.26	0.76
80	1.27	0.76

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 102 Female Group 10 Reserve Removed
Animal 103 Female Group 10 Reserve Removed

Reported Parameter

AT WEEK 12

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

AT WEEK 12

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
AT WEEK 12
MALES

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
1	348	290	130	116	63	11	958
2	422	236	198	169	207	106	1338
3	408	275	272	214	445	192	1806
4	494	240	180	259	167	158	1498
5	536	243	301	227	277	142	1726
6	706	385	483	477	219	169	2439
7	542	238	128	29	292	107	1336
8	480	410	254	36	24	8	1212
9	431	238	247	235	195	272	1618
10	520	356	208	87	115	230	1516

LOCOMOTOR ACTIVITY
AT WEEK 12
MALES

Group 2 (100 mg/kg)

	LOCOMOTOR ACTIVITY						
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	Total
11	635	314	180	205	196	149	1679
12	577	327	268	199	185	13	1569
13	486	289	231	235	195	194	1630
14	515	338	202	227	235	94	1611
15	651	245	292	178	180	383	1929
16	601	347	145	182	10	59	1344
17	550	239	168	233	227	226	1643
18	506	416	103	81	87	16	1209
19	436	236	87	119	67	138	1083
20	561	390	375	153	351	184	2014

LOCOMOTOR ACTIVITY
AT WEEK 12
MALES

Group 3 (300 mg/kg)

	LOCOMOTOR ACTIVITY						
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	Total
21	425	474	232	178	386	58	1753
22	464	275	251	14	0	228	1232
23	508	253	205	56	433	29	1484
24	544	116	159	208	148	200	1375
25	731	281	285	45	370	121	1833
26	680	369	216	82	14	60	1421
27	685	321	127	68	78	20	1299
28	488	262	154	472	22	11	1409
29	494	259	195	271	142	85	1446
30	932	657	486	275	56	507	2913

LOCOMOTOR ACTIVITY
AT WEEK 12
MALES

Group 4 (1000 mg/kg)

	LOCOMOTOR ACTIVITY						
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	Total
31	406	236	218	228	7	62	1157
32	767	458	121	4	6	8	1364
33	498	350	88	200	547	17	1700
34	710	336	311	101	258	54	1770
35	717	319	192	247	179	116	1770
36	774	512	287	439	305	203	2520
37	691	472	209	30	111	434	1947
38	723	294	181	69	239	130	1636
39	594	250	158	12	293	138	1445
40	697	420	165	14	2	60	1358

LOCOMOTOR ACTIVITY
AT WEEK 12
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	516	308	192	100	2	100	1218
42	393	133	144	4	0	126	800
43	413	236	308	242	197	165	1561
44	306	205	138	152	112	87	1000
45	310	278	175	230	222	114	1329
46	444	196	239	303	89	31	1302
47	534	54	160	291	12	13	1064
48	390	264	299	237	196	281	1667
49	488	279	245	193	112	142	1459
50	427	172	206	128	148	200	1281

LOCOMOTOR ACTIVITY
AT WEEK 12
FEMALES

Group 2 (100 mg/kg)

	LOCOMOTOR ACTIVITY						
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	Total
51	444	270	259	238	81	196	1488
52	455	288	175	189	277	172	1556
53	389	224	191	110	225	90	1229
54	347	199	148	193	175	199	1261
55	603	485	496	248	92	131	2055
56	502	310	116	164	77	132	1301
57	335	287	338	293	107	192	1552
58	497	249	67	29	276	167	1285
59	416	281	281	7	207	109	1301
60	549	373	460	327	203	125	2037

LOCOMOTOR ACTIVITY
AT WEEK 12
FEMALES

Group 3 (300 mg/kg)

	LOCOMOTOR ACTIVITY						
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	Total
61	463	324	164	226	289	81	1547
62	513	178	9	316	160	248	1424
63	470	322	201	243	185	15	1436
64	475	327	356	358	408	85	2009
65	440	493	281	404	367	117	2102
66	296	200	203	203	201	293	1396
67	690	400	277	147	7	186	1707
68	808	298	184	282	205	20	1797
69	397	226	187	197	238	115	1360
70	371	182	129	234	48	10	974

LOCOMOTOR ACTIVITY
AT WEEK 12
FEMALES

Group 4 (1000 mg/kg)

	LOCOMOTOR ACTIVITY						
	0-10 MIN	10-20 MIN	20-30 MIN	30-40 MIN	40-50 MIN	50-60 MIN	Total
71	363	238	207	130	66	46	1050
72	472	223	267	14	35	0	1011
73	541	304	62	172	158	139	1376
74	623	293	127	347	298	193	1881
75	727	403	360	341	97	0	1928
76	655	315	398	383	212	4	1967
77	477	246	155	0	2	0	880
78	444	377	209	173	2	102	1307
79	493	336	151	243	198	193	1614
80	813	632	500	331	152	310	2738

FOOD CONSUMPTION (G/ANIMAL/DAY)

Comments

Data excluded from Summary Report

Not Reported

All Study Phases

Cage 17 Male Group 10 Reserve Removed
Cage 18 Female Group 10 Reserve Removed

FOOD CONSUMPTION (G/ANIMAL/DAY)
MALES

Group 1 (0 mg/kg)

CAGE		1	2
ACCLIMATIZATION			
Days	1-9	21.1	21.5

CAGE		1	2
TREATMENT			
Days	1-8	22.9	22.0
	8-15	25.2	23.7
	15-22	25.6	24.7
	22-29	25.7	24.6
	29-36	25.6	24.9
	36-43	24.4	24.1
	43-50	24.2	23.2
	50-57	24.4	23.8
	57-64	24.3	23.8
	64-71	22.6	22.7
	71-78	22.5	22.4
	78-85	22.2	22.5
	85-91	23.7	23.1

FOOD CONSUMPTION (G/ANIMAL/DAY)
MALES

Group 2 (100 mg/kg)

CAGE		3	4
ACCLIMATIZATION			
Days	1-9	20.9	22.4

CAGE		3	4
TREATMENT			
Days	1-8	21.0	22.5
	8-15	23.0	24.0
	15-22	23.2	23.8
	22-29	22.8	23.8
	29-36	22.7	24.0
	36-43	22.5	22.3
	43-50	22.3	21.9
	50-57	22.5	21.6
	57-64	22.5	21.3
	64-71	21.1	20.7
	71-78	21.0	20.6
	78-85	22.1	21.6
	85-91	22.5	21.2

FOOD CONSUMPTION (G/ANIMAL/DAY)
MALES

Group 3 (300 mg/kg)

CAGE		5	6
ACCLIMATIZATION			
Days	1-9	22.3	22.9

CAGE		5	6
TREATMENT			
Days	1-8	22.5	23.4
	8-15	24.3	24.5
	15-22	25.0	24.5
	22-29	24.4	24.8
	29-36	23.7	24.2
	36-43	23.5	23.8
	43-50	23.4	23.5
	50-57	23.6	23.7
	57-64	23.4	22.3
	64-71	22.8	21.6
	71-78	21.9	22.0
	78-85	23.7	23.3
	85-91	22.7	22.5

FOOD CONSUMPTION (G/ANIMAL/DAY)
MALES

Group 4 (1000 mg/kg)

CAGE		7	8
ACCLIMATIZATION			
Days	1-9	23.5	21.3

CAGE		7	8
TREATMENT			
Days	1-8	24.5	21.8
	8-15	25.6	23.7
	15-22	25.9	23.7
	22-29	25.9	23.3
	29-36	25.0	23.3
	36-43	24.6	23.2
	43-50	24.7	23.2
	50-57	25.0	23.5
	57-64	22.9	21.6
	64-71	23.3	21.4
	71-78	23.1	20.9
	78-85	24.8	22.8
	85-91	24.0	23.5

FOOD CONSUMPTION (G/ANIMAL/DAY)
FEMALES

Group 1 (0 mg/kg)

CAGE		9	10
ACCLIMATIZATION			
Days	1-9	15.9	16.5

CAGE		9	10
TREATMENT			
Days	1-8	15.7	17.0
	8-15	17.8	18.3
	15-22	16.9	17.9
	22-29	17.2	17.4
	29-36	17.4	18.1
	36-43	16.8	17.7
	43-50	16.1	16.8
	50-57	16.1	17.3
	57-64	15.5	16.4
	64-71	15.1	16.4
	71-78	15.0	15.6
	78-85	16.7	17.3
	85-91	15.5	16.4

FOOD CONSUMPTION (G/ANIMAL/DAY)
FEMALES

Group 2 (100 mg/kg)

CAGE		11	12
ACCLIMATIZATION			
Days	1-9	16.4	15.7

CAGE		11	12
TREATMENT			
Days	1-8	16.7	16.7
	8-15	18.0	18.4
	15-22	17.6	18.0
	22-29	18.0	18.0
	29-36	17.9	17.9
	36-43	17.5	17.4
	43-50	16.8	17.6
	50-57	17.4	17.2
	57-64	16.5	16.2
	64-71	16.0	16.2
	71-78	16.0	16.5
	78-85	16.8	17.1
	85-91	16.5	16.9

FOOD CONSUMPTION (G/ANIMAL/DAY)
FEMALES

Group 3 (300 mg/kg)

CAGE		13	14
ACCLIMATIZATION			
Days	1-9	15.7	15.8

CAGE		13	14
TREATMENT			
Days	1-8	17.3	16.6
	8-15	18.9	18.3
	15-22	18.5	17.9
	22-29	18.2	17.9
	29-36	18.5	17.8
	36-43	17.9	17.7
	43-50	17.7	17.7
	50-57	17.2	17.4
	57-64	17.0	14.4
	64-71	16.3	16.9
	71-78	16.0	17.0
	78-85	16.9	18.3
	85-91	16.2	17.3

FOOD CONSUMPTION (G/ANIMAL/DAY)
FEMALES

Group 4 (1000 mg/kg)

CAGE		15	16
ACCLIMATIZATION			
Days	1-9	16.4	16.1

CAGE		15	16
TREATMENT			
Days	1-8	17.3	17.6
	8-15	19.3	18.8
	15-22	18.2	17.7
	22-29	18.5	17.6
	29-36	18.7	18.1
	36-43	18.0	17.3
	43-50	17.6	17.0
	50-57	18.1	16.8
	57-64	17.4	16.7
	64-71	16.7	16.0
	71-78	16.7	15.6
	78-85	17.6	16.9
	85-91	17.5	16.5

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

Comments

Data excluded from Summary Report

Not Reported

All Study Phases

Cage 17 Male Group 10 Reserve Removed
Cage 18 Female Group 10 Reserve Removed

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

Group 1 (0 mg/kg)

CAGE		1	2
ACCLIMATIZATION			
Days	1-9	105.4	108.3

CAGE		1	2
TREATMENT			
Days	1-8	84.4	82.0
	8-15	83.5	79.7
	15-22	79.0	77.3
	22-29	75.0	73.0
	29-36	71.7	70.7
	36-43	65.6	66.1
	43-50	63.2	61.5
	50-57	61.8	61.5
	57-64	60.4	60.3
	64-71	55.1	56.3
	71-78	53.4	54.4
	78-85	51.8	53.5
	85-91	55.2	54.9

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

Group 2 (100 mg/kg)

CAGE		3	4
ACCLIMATIZATION			
Days	1-9	106.3	113.5

CAGE		3	4
TREATMENT			
Days	1-8	80.7	83.5
	8-15	80.6	82.0
	15-22	75.7	76.7
	22-29	71.0	72.6
	29-36	67.5	70.4
	36-43	64.6	63.2
	43-50	61.7	60.2
	50-57	60.6	57.7
	57-64	59.2	55.5
	64-71	54.4	52.9
	71-78	53.2	51.8
	78-85	54.9	53.6
	85-91	56.1	52.6

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

Group 3 (300 mg/kg)

CAGE		5	6
ACCLIMATIZATION			
Days	1-9	113.9	114.7

CAGE		5	6
TREATMENT			
Days	1-8	83.6	84.0
	8-15	80.6	80.4
	15-22	79.3	75.4
	22-29	73.1	72.0
	29-36	68.1	66.7
	36-43	65.3	63.4
	43-50	62.5	60.5
	50-57	61.4	59.0
	57-64	59.3	54.4
	64-71	56.2	51.3
	71-78	53.2	51.1
	78-85	57.1	53.3
	85-91	54.7	51.4

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

Group 4 (1000 mg/kg)

CAGE		7	8
ACCLIMATIZATION			
Days	1-9	117.7	109.8

CAGE		7	8
TREATMENT			
Days	1-8	86.0	81.5
	8-15	81.9	81.2
	15-22	77.1	75.9
	22-29	72.8	71.0
	29-36	67.5	67.7
	36-43	64.5	65.3
	43-50	62.4	62.5
	50-57	61.4	61.9
	57-64	55.2	55.7
	64-71	54.4	54.2
	71-78	53.1	52.3
	78-85	55.8	55.8
	85-91	53.8	57.6

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

Group 1 (0 mg/kg)

CAGE		9	10
ACCLIMATIZATION			
Days	1-9	106.5	108.9

CAGE		9	10
TREATMENT			
Days	1-8	87.9	90.4
	8-15	92.3	92.5
	15-22	84.3	86.3
	22-29	83.1	82.0
	29-36	81.4	82.9
	36-43	78.1	79.5
	43-50	73.1	73.4
	50-57	72.7	74.4
	57-64	69.8	70.9
	64-71	66.8	69.4
	71-78	65.3	64.4
	78-85	71.6	71.8
	85-91	66.2	68.0

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

Group 2 (100 mg/kg)

CAGE		11	12
ACCLIMATIZATION			
Days	1-9	108.4	105.4

CAGE		11	12
TREATMENT			
Days	1-8	88.1	90.1
	8-15	87.3	94.0
	15-22	82.5	86.5
	22-29	82.2	83.7
	29-36	78.9	81.3
	36-43	74.8	77.7
	43-50	71.6	76.1
	50-57	73.0	73.7
	57-64	68.4	68.7
	64-71	63.9	66.2
	71-78	63.5	67.3
	78-85	66.7	68.6
	85-91	65.4	67.9

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

Group 3 (300 mg/kg)

CAGE	13	14
ACCLIMATIZATION		
Days 1-9	107.2	107.0

CAGE	13	14
TREATMENT		
Days 1-8	94.0	91.3
8-15	94.4	94.0
15-22	87.3	86.5
22-29	85.7	84.2
29-36	85.0	82.4
36-43	79.3	79.0
43-50	76.5	77.3
50-57	74.9	75.4
57-64	72.9	63.9
64-71	68.1	71.3
71-78	65.9	69.5
78-85	70.1	74.5
85-91	67.2	70.4

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

Group 4 (1000 mg/kg)

CAGE		15	16
ACCLIMATIZATION			
Days	1-9	108.4	108.7

CAGE		15	16
TREATMENT			
Days	1-8	90.8	94.0
	8-15	93.1	92.3
	15-22	84.0	83.3
	22-29	83.7	81.7
	29-36	81.8	81.5
	36-43	76.1	75.7
	43-50	72.7	72.8
	50-57	74.6	72.6
	57-64	70.6	70.6
	64-71	65.8	67.2
	71-78	64.6	63.6
	78-85	68.4	69.5
	85-91	68.0	67.6

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 102	Female	Group 10	Reserve Removed
Animal 103	Female	Group 10	Reserve Removed

BODY WEIGHTS (G)
MALES

Group 1 (0 mg/kg)

Animal		1	2	3	4	5	6	7
PRE-RANDOM PHASE								
Day	1	184	186	191	180	202	187	177
ACCLIMATIZATION								
Day	1	196	199	199	195	211	201	189
TREATMENT								
Day	1	231	232	237	239	253	242	222
	8	269	256	265	278	288	275	242
	15	302	278	296	309	324	312	260
	22	326	299	316	335	344	337	277
	29	350	314	332	353	363	360	287
	36	362	325	350	367	377	382	296
	43	379	342	365	387	389	395	307
	50	391	346	374	402	403	408	318
	57	402	357	384	413	417	422	324
	64	413	362	393	421	424	433	330
	71	425	368	398	427	434	443	340
	78	440	374	409	444	445	455	344
	85	445	381	416	449	453	464	353
	91	457	386	426	455	456	469	356

Animal		8	9	10
PRE-RANDOM PHASE				
Day	1	189	185	184
ACCLIMATIZATION				
Day	1	201	202	200
TREATMENT				
Day	1	242	242	246
	8	273	273	277
	15	304	302	307
	22	330	320	333
	29	348	337	350
	36	369	348	366
	43	381	362	380
	50	398	373	389
	57	407	382	402
	64	418	384	407
	71	430	385	418
	78	438	394	423
	85	446	408	432

BODY WEIGHTS (G)
MALES

Group 1 (0 mg/kg)

Animal		8	9	10
TREATMENT				
Day	91	454	409	438

BODY WEIGHTS (G)
MALES

Group 2 (100 mg/kg)

Animal		11	12	13	14	15	16	17
PRE-RANDOM PHASE								
Day	1	190	186	195	182	188	185	176
ACCLIMATIZATION								
Day	1	192	194	205	193	197	196	190
TREATMENT								
Day	1	217	241	244	232	241	232	225
	8	229	274	270	254	274	260	243
	15	244	306	293	275	310	284	258
	22	251	328	317	299	335	299	266
	29	261	346	333	313	354	314	277
	36	268	364	347	329	370	325	286
	43	279	379	361	344	383	334	295
	50	286	389	372	360	399	341	304
	57	297	402	382	364	409	349	314
	64	309	409	392	376	417	356	322
	71	313	418	400	381	425	360	333
	78	320	425	408	385	434	365	339
	85	323	433	414	393	444	369	344
	91	325	438	415	395	443	368	341

Animal		18	19	20
PRE-RANDOM PHASE				
Day	1	184	179	194
ACCLIMATIZATION				
Day	1	202	197	202
TREATMENT				
Day	1	243	239	251
	8	282	268	295
	15	309	291	321
	22	330	310	347
	29	353	325	369
	36	361	338	397
	43	375	348	412
	50	389	359	426
	57	402	368	439
	64	411	376	449
	71	422	386	460
	78	426	390	470
	85	432	393	477

BODY WEIGHTS (G)
MALES

Group 2 (100 mg/kg)

Animal		18	19	20
TREATMENT				
Day	91	436	399	482

BODY WEIGHTS (G)
MALES

Group 3 (300 mg/kg)

Animal		21	22	23	24	25	26	27
PRE-RANDOM PHASE								
Day	1	181	190	185	178	174	184	185
Animal		21	22	23	24	25	26	27
ACCLIMATIZATION								
Day	1	197	202	198	194	187	196	201
Animal		21	22	23	24	25	26	27
TREATMENT								
Day	1	243	246	245	238	226	235	253
	8	275	272	283	266	252	264	288
	15	299	297	312	294	303	288	314
	22	317	318	334	317	292	308	331
	29	335	340	355	334	307	328	353
	36	347	354	374	348	315	347	368
	43	360	361	391	361	324	366	377
	50	368	383	403	379	335	378	383
	57	381	391	415	389	349	391	394
	64	392	396	427	400	357	401	402
	71	402	411	439	410	364	413	412
	78	410	416	444	414	371	424	421
	85	402	420	451	421	382	431	424
	91	415	426	457	424	383	432	423

Animal		28	29	30
PRE-RANDOM PHASE				
Day	1	186	185	187
Animal		28	29	30
ACCLIMATIZATION				
Day	1	202	199	199
Animal		28	29	30
TREATMENT				
Day	1	249	248	244
	8	281	286	274
	15	310	315	295
	22	331	341	312
	29	354	363	327
	36	372	380	345
	43	380	395	355
	50	395	417	368
	57	404	436	379
	64	412	452	383
	71	420	468	394
	78	426	484	401
	85	435	486	410

BODY WEIGHTS (G)
MALES

Group 3 (300 mg/kg)

Animal		28	29	30
TREATMENT				
Day	91	438	492	436

BODY WEIGHTS (G)
MALES

Group 4 (1000 mg/kg)

Animal		31	32	33	34	35	36	37
PRE-RANDOM PHASE								
Day	1	187	185	195	191	184	188	176
Animal		31	32	33	34	35	36	37
ACCLIMATIZATION								
Day	1	200	193	203	203	197	196	193
Animal		31	32	33	34	35	36	37
TREATMENT								
Day	1	249	242	269	246	233	235	248
	8	288	272	315	285	263	260	290
	15	315	293	347	322	287	283	322
	22	340	311	372	351	308	300	350
	29	360	326	397	374	323	318	373
	36	371	338	422	396	328	328	390
	43	384	348	434	407	337	335	411
	50	399	357	455	423	347	346	430
	57	411	367	471	433	355	352	439
	64	419	371	474	450	362	446	372
	71	432	382	487	462	374	369	457
	78	444	388	496	470	380	373	464
	85	458	389	510	483	385	384	474
	91	453	394	511	487	384	387	479

Animal		38	39	40
PRE-RANDOM PHASE				
Day	1	186	183	180
Animal		38	39	40
ACCLIMATIZATION				
Day	1	197	195	190
Animal		38	39	40
TREATMENT				
Day	1	237	236	228
	8	263	271	255
	15	285	296	275
	22	304	314	291
	29	317	328	306
	36	332	342	330
	43	342	349	344
	50	356	365	357
	57	365	371	366
	64	369	381	371
	71	377	390	378
	78	385	398	381
	85	391	403	387

BODY WEIGHTS (G)
MALES

Group 4 (1000 mg/kg)

Animal		38	39	40
TREATMENT				
Day	91	399	408	395

BODY WEIGHTS (G)
FEMALES

Group 1 (0 mg/kg)

Animal		41	42	43	44	45	46	47
PRE-RANDOM PHASE								
Day	1	137	146	138	144	141	140	139

Animal		41	42	43	44	45	46	47
ACCLIMATIZATION								
Day	1	148	154	144	148	150	153	151

Animal		41	42	43	44	45	46	47
TREATMENT								
Day	1	164	173	162	170	175	174	176
	8	177	184	171	182	180	190	193
	15	190	189	185	195	203	193	199
	22	199	194	191	206	214	207	215
	29	206	199	199	213	220	215	223
	36	216	205	207	221	219	219	230
	43	217	201	211	220	229	218	228
	50	222	208	215	226	232	226	237
	57	223	208	215	229	234	228	242
	64	219	213	217	234	231	229	241
	71	229	211	223	228	241	229	251
	78	228	215	225	238	245	239	247
	85	236	218	229	239	247	234	256
	91	233	214	224	243	245	236	251

Animal		48	49	50
PRE-RANDOM PHASE				
Day	1	143	149	142

Animal		48	49	50
ACCLIMATIZATION				
Day	1	153	157	142

Animal		48	49	50
TREATMENT				
Day	1	176	178	166
	8	189	184	185
	15	202	201	193
	22	208	208	197
	29	214	209	200
	36	219	208	216
	43	230	220	220
	50	234	224	222
	57	235	228	227
	64	234	226	223
	71	243	233	227
	78	251	238	233
	85	244	238	237

BODY WEIGHTS (G)
FEMALES

Group 1 (0 mg/kg)

Animal		48	49	50
TREATMENT				
Day	91	246	241	237

BODY WEIGHTS (G)
FEMALES

Group 2 (100 mg/kg)

Animal		51	52	53	54	55	56	57
PRE-RANDOM PHASE								
Day	1	138	139	141	152	146	141	138

Animal		51	52	53	54	55	56	57
ACCLIMATIZATION								
Day	1	151	148	146	157	155	146	145

Animal		51	52	53	54	55	56	57
TREATMENT								
Day	1	176	162	168	174	190	172	160
	8	193	183	183	186	204	179	175
	15	212	199	196	204	218	202	187
	22	224	205	201	217	221	213	195
	29	232	206	203	224	229	218	199
	36	233	223	214	224	242	217	211
	43	244	229	217	237	244	229	214
	50	250	224	221	241	241	234	221
	57	249	234	216	241	249	234	214
	64	247	235	225	243	257	238	216
	71	259	246	232	258	259	265	223
	78	261	255	232	257	256	249	227
	85	252	255	229	259	264	247	234
	91	260	258	239	259	263	249	235

Animal		58	59	60
PRE-RANDOM PHASE				
Day	1	144	142	143

Animal		58	59	60
ACCLIMATIZATION				
Day	1	156	150	149

Animal		58	59	60
TREATMENT				
Day	1	185	170	174
	8	201	184	186
	15	210	192	190
	22	226	206	197
	29	236	213	209
	36	239	218	217
	43	237	217	221
	50	252	227	219
	57	259	231	227
	64	261	232	236
	71	263	232	237
	78	276	239	235
	85	277	243	243

BODY WEIGHTS (G)
FEMALES

Group 2 (100 mg/kg)

Animal		58	59	60
TREATMENT				
Day	91	273	240	242

BODY WEIGHTS (G)
FEMALES

Group 3 (300 mg/kg)

Animal		61	62	63	64	65	66	67
PRE-RANDOM PHASE								
Day	1	143	141	141	146	139	141	145
ACCLIMATIZATION								
Day	1	146	143	148	154	141	150	153
TREATMENT								
Day	1	173	167	168	186	166	174	178
	8	188	179	171	198	183	180	198
	15	207	187	184	219	201	199	201
	22	223	202	195	230	207	213	219
	29	222	199	198	241	203	215	226
	36	224	207	204	241	213	217	229
	43	241	201	216	254	219	233	227
	50	245	211	221	260	223	237	238
	57	244	208	221	261	218	237	239
	64	245	215	221	255	229	233	243
	71	254	209	234	268	235	246	241
	78	253	214	238	272	239	252	250
	85	254	214	235	270	234	253	251
	91	248	222	238	273	241	255	244

Animal		68	69	70
PRE-RANDOM PHASE				
Day	1	142	136	138
ACCLIMATIZATION				
Day	1	147	143	146
TREATMENT				
Day	1	168	171	167
	8	182	176	175
	15	188	194	189
	22	189	208	203
	29	202	213	209
	36	214	213	209
	43	218	223	219
	50	215	227	228
	57	223	227	230
	64	207	222	227
	71	227	232	235
	78	238	242	245
	85	241	240	246

BODY WEIGHTS (G)
FEMALES

Group 3 (300 mg/kg)

Animal		68	69	70
TREATMENT				
Day	91	235	240	248

BODY WEIGHTS (G)
FEMALES

Group 4 (1000 mg/kg)

Animal		71	72	73	74	75	76	77
PRE-RANDOM PHASE								
Day	1	139	148	145	141	138	142	144

Animal		71	72	73	74	75	76	77
ACCLIMATIZATION								
Day	1	147	159	152	149	147	148	146

Animal		71	72	73	74	75	76	77
TREATMENT								
Day	1	181	188	170	163	176	166	168
	8	197	199	177	182	201	186	178
	15	218	214	194	198	210	202	198
	22	224	225	202	207	224	212	206
	29	232	231	209	205	229	213	213
	36	238	233	210	220	244	231	215
	43	248	241	221	224	252	229	222
	50	252	248	228	227	255	236	227
	57	253	251	232	223	254	230	230
	64	253	250	231	235	263	243	230
	71	262	260	240	241	269	246	212
	78	270	262	251	237	271	252	235
	85	267	263	249	238	269	251	239
	91	269	264	251	245	280	238	240

Animal		78	79	80
PRE-RANDOM PHASE				
Day	1	143	136	140

Animal		78	79	80
ACCLIMATIZATION				
Day	1	152	143	149

Animal		78	79	80
TREATMENT				
Day	1	176	171	176
	8	188	196	189
	15	208	205	205
	22	220	212	210
	29	223	215	213
	36	224	230	211
	43	235	232	223
	50	239	239	224
	57	240	236	222
	64	239	246	223
	71	247	254	231
	78	251	251	235
	85	248	253	227

BODY WEIGHTS (G)
FEMALES

Group 4 (1000 mg/kg)

Animal		78	79	80
TREATMENT				
Day	91	252	256	233

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 102	Female	Group 10	Reserve Removed
Animal 103	Female	Group 10	Reserve Removed

BODY WEIGHT GAIN (%)
MALES

Group 1 (0 mg/kg)

Animal		1	2	3	4	5	6	7
ACCLIMATIZATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	16.2	10.5	12.0	16.4	13.9	13.8	8.9
	15	30.5	20.0	25.2	29.2	28.0	29.2	17.0
	22	40.9	28.9	33.4	39.9	36.1	39.3	24.6
	29	51.3	35.4	40.5	47.5	43.6	49.1	29.0
	36	56.7	40.2	48.1	53.6	49.3	57.9	33.3
	43	64.0	47.6	54.2	61.6	53.8	63.3	38.0
	50	69.0	49.5	58.0	68.1	59.3	69.0	43.3
	57	73.6	54.2	62.4	72.6	64.8	74.6	45.8
	64	78.5	56.1	66.4	76.1	67.7	79.2	48.7
	71	83.7	59.0	68.4	78.8	71.7	83.3	53.0
	78	90.0	61.6	72.7	85.8	76.0	88.5	54.9
	85	92.3	64.7	75.9	87.9	79.1	92.2	58.9
	91	97.7	66.6	80.2	90.2	80.3	94.2	60.3

Animal		8	9	10
ACCLIMATIZATION				
Day	1	0.0	0.0	0.0
TREATMENT				
Day	1	0.0	0.0	0.0
	8	12.9	12.7	12.6
	15	25.9	24.7	24.7
	22	36.3	32.2	35.3
	29	44.1	39.3	42.4
	36	52.6	43.8	48.7
	43	57.5	49.5	54.5
	50	64.4	54.3	58.1
	57	68.3	57.9	63.6
	64	72.7	58.8	65.3
	71	77.8	59.2	69.9
	78	81.2	62.8	72.2
	85	84.3	68.8	75.7
	91	87.8	68.9	78.0

BODY WEIGHT GAIN (%)
MALES

Group 2 (100 mg/kg)

Animal		11	12	13	14	15	16	17
ACCLIMATIZATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	5.8	13.6	10.4	9.6	13.6	12.0	8.0
	15	12.8	27.1	20.0	18.6	28.5	22.5	14.7
	22	15.9	36.1	29.9	29.0	38.9	28.9	18.6
	29	20.4	43.6	36.4	35.1	46.5	35.6	23.2
	36	23.9	50.9	42.0	42.2	53.3	40.1	27.6
	43	28.6	57.3	47.7	48.6	58.5	44.0	31.5
	50	32.1	61.3	52.4	55.5	65.1	47.1	35.5
	57	37.1	66.8	56.3	57.1	69.3	50.6	39.9
	64	42.6	69.9	60.4	62.1	72.7	53.7	43.6
	71	44.4	73.3	63.7	64.7	75.8	55.3	48.2
	78	47.6	76.6	67.2	66.2	79.8	57.6	51.0
	85	49.3	79.9	69.7	69.8	83.9	59.3	53.2
	91	50.2	81.7	70.1	70.7	83.6	58.7	51.9

Animal		18	19	20
ACCLIMATIZATION				
Day	1	0.0	0.0	0.0
TREATMENT				
Day	1	0.0	0.0	0.0
	8	16.3	12.4	17.6
	15	27.3	22.1	28.2
	22	35.8	30.0	38.6
	29	45.2	36.0	47.3
	36	48.5	41.4	58.6
	43	54.4	45.6	64.4
	50	60.0	50.3	69.9
	57	65.4	54.2	75.2
	64	69.3	57.4	79.3
	71	73.6	61.5	83.4
	78	75.4	63.3	87.7
	85	77.9	64.8	90.2
	91	79.8	67.1	92.4

BODY WEIGHT GAIN (%)
MALES

Group 3 (300 mg/kg)

Animal		21	22	23	24	25	26	27
ACCLIMATIZATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	13.2	10.4	15.4	12.1	11.7	12.1	13.8
	15	23.3	20.6	27.5	23.9	34.1	22.2	23.9
	22	30.4	29.1	36.4	33.7	29.6	31.1	30.7
	29	38.1	38.0	44.7	40.5	36.0	39.5	39.3
	36	42.9	43.5	52.6	46.3	39.6	47.6	45.3
	43	48.5	46.7	59.5	51.9	43.6	55.4	48.9
	50	51.6	55.5	64.6	59.6	48.4	60.7	51.3
	57	57.0	58.8	69.3	63.9	54.6	66.3	55.5
	64	61.5	60.8	74.3	68.6	58.4	70.6	58.9
	71	65.6	66.9	79.0	72.7	61.2	75.7	62.6
	78	69.0	68.8	81.1	74.2	64.6	80.2	66.0
	85	65.4	70.5	84.1	77.1	69.4	83.0	67.3
	91	71.0	73.1	86.5	78.4	69.9	83.5	67.0

Animal		28	29	30
ACCLIMATIZATION				
Day	1	0.0	0.0	0.0
TREATMENT				
Day	1	0.0	0.0	0.0
	8	12.8	15.3	12.1
	15	24.7	27.0	20.9
	22	33.0	37.9	27.7
	29	42.2	46.6	33.8
	36	49.7	53.4	41.4
	43	52.7	59.5	45.4
	50	59.1	68.5	50.8
	57	62.5	75.8	55.0
	64	65.9	82.4	56.8
	71	68.9	89.1	61.5
	78	71.2	95.5	64.0
	85	75.1	96.2	67.8
	91	76.0	98.6	78.5

BODY WEIGHT GAIN (%)
MALES

Group 4 (1000 mg/kg)

Animal		31	32	33	34	35	36	37
ACCLIMATIZATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	15.5	12.2	16.9	15.6	12.7	10.7	17.1
	15	26.5	20.7	28.8	30.7	23.2	20.4	30.0
	22	36.7	28.2	38.3	42.3	32.3	27.7	41.3
	29	44.8	34.3	47.6	51.6	38.5	35.1	50.3
	36	49.1	39.2	56.7	60.6	41.0	39.6	57.4
	43	54.5	43.4	61.3	65.3	44.5	42.5	66.0
	50	60.3	47.4	69.2	71.7	49.1	47.0	73.5
	57	65.0	51.2	75.2	75.7	52.5	49.6	77.3
	64	68.3	53.2	76.1	82.7	55.3	89.8	50.2
	71	73.7	57.4	81.2	87.4	60.6	56.9	84.6
	78	78.4	60.0	84.2	90.5	63.1	58.7	87.1
	85	84.0	60.5	89.5	96.2	65.4	63.5	91.3
	91	82.0	62.7	89.8	97.6	65.0	64.7	93.4

Animal		38	39	40
ACCLIMATIZATION				
Day	1	0.0	0.0	0.0
TREATMENT				
Day	1	0.0	0.0	0.0
	8	10.8	14.7	11.8
	15	20.0	25.4	20.3
	22	28.3	33.0	27.4
	29	33.8	39.2	34.0
	36	40.0	45.2	44.6
	43	44.1	48.1	50.7
	50	50.2	54.7	56.2
	57	54.1	57.3	60.4
	64	55.7	61.3	62.5
	71	59.1	65.5	65.6
	78	62.4	68.9	66.9
	85	64.8	71.0	69.4
	91	68.3	72.8	73.1

BODY WEIGHT GAIN (%)
FEMALES

Group 1 (0 mg/kg)

Animal		41	42	43	44	45	46	47
ACCLIMATIZATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	8.0	6.7	5.4	7.3	2.5	8.7	9.3
	15	16.2	9.5	14.2	15.1	16.0	10.7	13.0
	22	21.7	12.5	17.8	21.6	22.2	18.7	22.0
	29	26.0	15.0	22.6	25.6	25.6	23.0	26.2
	36	31.8	18.4	27.9	30.3	25.1	25.6	30.2
	43	32.5	16.2	30.4	29.5	30.5	25.1	29.4
	50	35.7	20.2	32.6	33.2	32.5	29.3	34.4
	57	36.1	20.5	32.9	34.9	33.6	30.7	37.1
	64	33.8	23.1	33.8	38.1	31.6	31.4	36.6
	71	39.7	22.0	37.8	34.2	37.2	31.2	42.5
	78	39.6	24.2	38.7	40.4	39.9	37.3	40.1
	85	44.1	26.0	41.1	41.0	40.7	34.3	44.9
	91	42.6	24.0	38.1	43.3	39.9	35.2	42.4

Animal		48	49	50
ACCLIMATIZATION				
Day	1	0.0	0.0	0.0
TREATMENT				
Day	1	0.0	0.0	0.0
	8	7.8	3.1	11.2
	15	15.1	13.0	16.1
	22	18.4	17.0	18.6
	29	21.5	17.7	20.4
	36	24.3	16.7	30.0
	43	30.6	23.7	32.1
	50	33.4	26.0	33.3
	57	33.8	27.9	36.2
	64	33.2	27.0	34.1
	71	38.5	31.0	36.4
	78	42.8	33.9	40.0
	85	38.7	33.6	42.2
	91	39.9	35.5	42.5

BODY WEIGHT GAIN (%)
FEMALES

Group 2 (100 mg/kg)

Animal		51	52	53	54	55	56	57
ACCLIMATIZATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	9.8	13.2	9.0	7.2	7.0	4.4	9.0
	15	20.6	22.8	17.0	17.2	14.3	17.5	16.9
	22	27.4	26.6	20.1	25.1	16.0	24.3	21.9
	29	32.0	26.9	20.9	28.8	20.4	27.2	23.9
	36	32.3	37.6	27.7	28.9	27.0	26.8	31.4
	43	38.9	41.2	29.4	36.6	28.2	33.5	33.3
	50	42.0	38.4	31.7	38.8	26.3	36.2	38.0
	57	41.5	44.5	28.9	38.8	30.8	36.5	33.6
	64	40.6	45.0	34.1	40.0	34.8	38.7	34.7
	71	47.1	51.9	38.1	48.3	35.8	54.7	39.1
	78	48.2	57.3	38.0	47.6	34.2	45.0	41.8
	85	43.0	57.5	36.2	49.3	38.6	44.3	45.9
	91	47.7	59.4	42.7	49.2	38.0	45.2	46.7

Animal		58	59	60
ACCLIMATIZATION				
Day	1	0.0	0.0	0.0
TREATMENT				
Day	1	0.0	0.0	0.0
	8	8.6	8.5	6.8
	15	13.6	13.2	9.3
	22	22.3	21.4	13.4
	29	27.6	25.5	20.1
	36	29.5	28.0	24.8
	43	28.6	27.4	26.8
	50	36.4	33.7	25.9
	57	40.2	35.8	30.4
	64	41.3	36.6	35.4
	71	42.5	36.3	35.8
	78	49.5	40.9	34.9
	85	49.8	42.9	39.7
	91	48.0	41.0	39.1

BODY WEIGHT GAIN (%)
FEMALES

Group 3 (300 mg/kg)

Animal		61	62	63	64	65	66	67
ACCLIMATIZATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	8.7	7.2	1.9	6.8	10.4	3.8	11.3
	15	19.9	12.0	9.8	17.9	21.1	14.6	13.2
	22	29.2	20.7	16.2	23.9	25.1	22.5	23.1
	29	28.4	19.3	18.1	29.9	22.6	23.9	27.2
	36	29.6	23.7	21.3	30.0	28.6	25.0	28.9
	43	39.3	20.1	28.9	36.6	32.3	33.9	27.8
	50	41.8	26.1	31.6	39.9	34.8	36.5	33.8
	57	41.0	24.4	31.7	40.5	31.6	36.6	34.8
	64	41.9	28.3	31.6	37.6	38.4	34.3	36.5
	71	46.8	24.8	39.2	44.4	41.8	41.9	35.5
	78	46.1	28.3	42.0	46.8	44.1	45.3	40.5
	85	46.8	28.1	39.9	45.2	41.1	45.7	41.2
	91	43.2	32.6	41.6	47.0	45.2	46.9	37.1

Animal		68	69	70
ACCLIMATIZATION				
Day	1	0.0	0.0	0.0
TREATMENT				
Day	1	0.0	0.0	0.0
	8	8.3	3.2	4.3
	15	12.0	13.3	13.1
	22	12.4	21.9	21.4
	29	20.3	24.6	24.6
	36	27.0	24.5	25.1
	43	29.9	30.2	30.9
	50	27.9	33.1	36.0
	57	32.5	32.9	37.2
	64	23.1	29.8	35.4
	71	35.3	35.9	40.5
	78	41.4	41.3	46.0
	85	43.3	40.3	47.0
	91	39.6	40.4	48.0

BODY WEIGHT GAIN (%)
FEMALES

Group 4 (1000 mg/kg)

Animal		71	72	73	74	75	76	77
ACCLIMATIZATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Animal		71	72	73	74	75	76	77
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	8.8	5.9	4.2	11.4	14.4	11.6	5.8
	15	20.3	14.2	14.2	21.1	19.5	21.3	17.7
	22	24.0	19.6	19.0	26.8	27.4	27.7	22.0
	29	28.1	23.0	22.9	25.6	30.4	28.1	26.4
	36	31.6	24.0	23.9	34.8	38.5	38.7	27.6
	43	36.9	28.6	30.0	37.4	43.1	37.6	31.8
	50	39.4	32.1	34.4	39.1	45.2	42.1	34.9
	57	40.1	33.8	36.7	36.6	44.3	38.2	36.7
	64	39.9	33.2	36.0	44.1	49.7	45.9	36.6
	71	44.6	38.6	41.4	47.4	53.1	48.2	25.7
	78	49.0	39.6	48.2	45.1	54.1	51.4	39.4
	85	47.4	40.1	46.5	45.6	53.0	50.7	41.7
	91	48.5	40.5	47.8	49.9	59.4	43.2	42.6

Animal		78	79	80
ACCLIMATIZATION				
Day	1	0.0	0.0	0.0

Animal		78	79	80
TREATMENT				
Day	1	0.0	0.0	0.0
	8	6.5	14.5	7.7
	15	18.0	19.6	16.5
	22	24.7	23.6	19.5
	29	26.5	25.9	21.4
	36	27.2	34.1	19.9
	43	33.6	35.6	27.0
	50	35.7	39.4	27.2
	57	36.1	37.8	26.3
	64	35.6	43.5	26.5
	71	40.1	48.7	31.5
	78	42.5	46.8	33.4
	85	40.5	47.9	29.2
	91	43.2	49.8	32.5

OPHTHALMOSCOPY

Comments

Data excluded from Summary Report

Not Reported

All Study Phases

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Animal 100  Male   Group 10   Reserve Removed
Animal 101  Male   Group 10   Reserve Removed
Animal 102  Female Group 10   Reserve Removed
Animal 103  Female Group 10   Reserve Removed
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OPHTHALMOSCOPY
ACCLIMATIZATION, Day 6, OPHTHALMOSCOPY
MALES
Group 1 (0 mg/kg)

Animal	1	2	3	4	5	6	7	8	9	10
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	0	0	0	0	0	0	0	0	0	0
(RIGHT EYE)	0	0	0	0	1	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	0	0	0	0	0

OPHTHALMOSCOPY
ACCLIMATIZATION, Day 6, OPHTHALMOSCOPY
MALES
Group 2 (100 mg/kg)

Animal	11	12	13	14	15	16	17	18	19	20
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	1	0	0	0	0	1	0	0	1	0
(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	0	0	0	0	0

OPHTHALMOSCOPY
ACCLIMATIZATION, Day 6, OPHTHALMOSCOPY
MALES
Group 3 (300 mg/kg)

Animal	21	22	23	24	25	26	27	28	29	30
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	0	0	0	0	0	0	0	0	0	0
(RIGHT EYE)	1	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	0	0	0	1	0

OPHTHALMOSCOPY
ACCLIMATIZATION, Day 6, OPHTHALMOSCOPY
MALES
Group 4 (1000 mg/kg)

Animal	31	32	33	34	35	36	37	38	39	40
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	0	1	1	0	0	0	0	0	1	0
(RIGHT EYE)	0	0	0	0	0	0	0	0	0	0
VITREOUS BODY										
- PERSISTENT HYALOID VESSEL (1)										
(LEFT EYE)	1	0	0	0	1	0	0	0	0	0
(RIGHT EYE)	1	0	0	0	0	0	0	0	0	0

OPHTHALMOSCOPY
ACCLIMATIZATION, Day 6, OPHTHALMOSCOPY
FEMALES
Group 1 (0 mg/kg)

Animal	41	42	43	44	45	46	47	48	49	50
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	0	0	1	0	1	0	1	0	0	0
(RIGHT EYE)	0	0	0	0	1	1	1	0	0	0
VITREOUS BODY										
- PERSISTENT HYALOID VESSEL (1)										
(LEFT EYE)	0	0	0	0	0	0	0	0	0	0
(RIGHT EYE)	1	0	0	0	0	0	0	0	0	0

OPHTHALMOSCOPY
ACCLIMATIZATION, Day 6, OPHTHALMOSCOPY
FEMALES
Group 2 (100 mg/kg)

Animal	51	52	53	54	55	56	57	58	59	60
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	0	0	0	0	1	0	0	0	0	0
(RIGHT EYE)	0	0	0	0	1	0	0	0	0	0
VITREOUS BODY										
- PERSISTENT HYALOID VESSEL (1)										
(LEFT EYE)	0	1	0	0	0	0	0	0	0	0
(RIGHT EYE)	0	0	0	0	0	0	0	0	0	1

OPHTHALMOSCOPY
ACCLIMATIZATION, Day 6, OPHTHALMOSCOPY
FEMALES
Group 3 (300 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	0	0	1	0
(RIGHT EYE)	0	0	0	0	0	0	0	0	0	0
VITREOUS BODY										
- PERSISTENT HYALOID VESSEL (1)										
(LEFT EYE)	0	0	0	0	1	0	0	0	0	0
(RIGHT EYE)	0	0	0	0	0	0	1	0	0	0

OPHTHALMOSCOPY
ACCLIMATIZATION, Day 6, OPHTHALMOSCOPY
FEMALES
Group 4 (1000 mg/kg)

Animal	71	72	73	74	75	76	77	78	79	80
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	0	0	0	0	0	0	0	0	0	1
(RIGHT EYE)	0	0	0	0	0	0	0	0	0	1
VITREOUS BODY										
- PERSISTENT HYALOID VESSEL (1)										
(LEFT EYE)	0	0	0	0	0	0	0	0	1	0
(RIGHT EYE)	0	0	0	0	0	0	0	1	0	0

OPHTHALMOSCOPY
TREATMENT, Day 90, OPHTHALMOSCOPY
MALES
Group 1 (0 mg/kg)

Animal	1	2	3	4	5	6	7	8	9	10
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	0	0	0	0	0	0	1	0	0	0
(RIGHT EYE)	0	0	0	0	1	1	1	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	0	0	0	0	0

OPHTHALMOSCOPY
TREATMENT, Day 90, OPHTHALMOSCOPY
MALES
Group 2 (100 mg/kg)

Animal	11	12	13	14	15	16	17	18	19	20
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	-	-	-	-	-	-	-	-	-	-
(RIGHT EYE)	-	-	-	-	-	-	-	-	-	-
VITREOUS BODY										
- PERSISTENT HYALOID VESSEL (1)										
(LEFT EYE)	-	-	-	-	-	-	-	-	-	-
(RIGHT EYE)	-	-	-	-	-	-	-	-	-	-

OPHTHALMOSCOPY
TREATMENT, Day 90, OPHTHALMOSCOPY
MALES
Group 3 (300 mg/kg)

Animal	21	22	23	24	25	26	27	28	29	30
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	-	-	-	-	-	-	-	-	-	-
(RIGHT EYE)	-	-	-	-	-	-	-	-	-	-
VITREOUS BODY										
- PERSISTENT HYALOID VESSEL (1)										
(LEFT EYE)	-	-	-	-	-	-	-	-	-	-
(RIGHT EYE)	-	-	-	-	-	-	-	-	-	-

OPHTHALMOSCOPY
TREATMENT, Day 90, OPHTHALMOSCOPY
MALES
Group 4 (1000 mg/kg)

Animal	31	32	33	34	35	36	37	38	39	40
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	0	1	1	0	0	0	1	0	1	0
(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	0	0	0	0	0

OPHTHALMOSCOPY
TREATMENT, Day 90, OPHTHALMOSCOPY
FEMALES
Group 1 (0 mg/kg)

Animal	41	42	43	44	45	46	47	48	49	50
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	0	0	0	0	0	0	0	0	0	0
(RIGHT EYE)	0	0	0	0	0	1	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	0	0	0	0	0

OPHTHALMOSCOPY
TREATMENT, Day 90, OPHTHALMOSCOPY
FEMALES
Group 2 (100 mg/kg)

Animal	51	52	53	54	55	56	57	58	59	60
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	-	-	-	-	-	-	-	-	-	-
(RIGHT EYE)	-	-	-	-	-	-	-	-	-	-
VITREOUS BODY										
- PERSISTENT HYALOID VESSEL (1)										
(LEFT EYE)	-	-	-	-	-	-	-	-	-	-
(RIGHT EYE)	-	-	-	-	-	-	-	-	-	-

OPHTHALMOSCOPY
TREATMENT, Day 90, OPHTHALMOSCOPY
FEMALES
Group 3 (300 mg/kg)

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

OPHTHALMOSCOPY
TREATMENT, Day 90, OPHTHALMOSCOPY
FEMALES
Group 4 (1000 mg/kg)

Animal	71	72	73	74	75	76	77	78	79	80
Unscheduled Findings										
CORNEA										
- CORNEAL OPACITY (3)										
(LEFT EYE)	0	0	0	0	0	0	0	0	0	1
(RIGHT EYE)	0	0	0	0	0	0	0	0	0	1
VITREOUS BODY										
- PERSISTENT HYALOID VESSEL (1)										
(LEFT EYE)	0	0	0	0	0	0	0	0	1	0
(RIGHT EYE)	0	0	0	0	0	0	0	0	0	0

Hematology

Comments

a 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 102	Female	Group 10	Reserve Removed
Animal 103	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	8.20	9.3	0.41	50.3	0.139	1.13	22.43
2	8.48	9.4	0.43	50.4	0.132	1.10	21.91
3	8.92	9.0	0.42	46.9	0.132	1.00	21.43
4	8.57	9.6	0.44	51.8	0.180	1.12	21.63
5	8.43	9.4	0.43	50.6	0.167	1.11	21.93
6	8.68	9.7	0.44	51.0	0.121	1.11	21.84
7	9.37	10.3	0.48	50.9	0.130	1.09	21.50
8	8.40	9.1	0.41	49.3	0.214	1.08	21.91
9	9.05	10.0	0.46	50.3	0.124	1.11	22.01
10	8.29	9.6	0.43	52.5	0.328	1.16	22.10

	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	1.83	0.019	155	0.694	0.276	0.030	6.85
2	1.83	0.025	216	0.614	0.323	0.063	9.21
3	1.82	0.019	173	0.700	0.276	0.024	6.29
4	1.96	0.020	167	0.631	0.301	0.068	4.00
5	1.90	0.021	174	0.698	0.264	0.038	4.72
6	1.67	0.019	167	0.664	0.291	0.044	5.93
7	1.76	0.027	255	0.597	0.342	0.061	6.57
8	2.17	0.023	198	0.658	0.302	0.040	7.79
9	1.74	0.016	149	0.695	0.289	0.016	6.19
10	2.07	0.019	153	0.721	0.250	0.029	5.79

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
1	0.238	0.011	0.004	0.724	0.019	0.005
2	0.194	0.014	0.003	0.762	0.018	0.009
3	0.184	0.012	0.001	0.771	0.027	0.006
4	0.236	0.021	0.001	0.704	0.030	0.007
5	0.219	0.016	0.002	0.725	0.032	0.006
6	0.269	0.015	0.003	0.682	0.027	0.005
7	0.169	0.008	0.002	0.791	0.022	0.007
8	0.204	0.017	0.002	0.751	0.023	0.003
9	0.301	0.012	0.002	0.653	0.027	0.004
10	0.319	0.021	0.002	0.616	0.035	0.006

Hematology
After 13 Weeks
MALES

Group 1 (0 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT G/l	EOS G/l	BASO G/l	LYMPH G/l	MONO G/l	LUC G/l	PLATELETS G/l
1	1.63	0.07	0.02	4.96	0.13	0.04	1043
2	1.78	0.13	0.03	7.01	0.17	0.09	1228
3	1.16	0.07	0.01	4.85	0.17	0.04	1207
4	0.95	0.09	0.00	2.82	0.12	0.03	966
5	1.03	0.08	0.01	3.42	0.15	0.03	1083
6	1.59	0.09	0.01	4.04	0.16	0.03	982
7	1.11	0.05	0.02	5.20	0.15	0.04	959
8	1.58	0.13	0.01	5.85	0.18	0.03	976
9	1.87	0.08	0.01	4.04	0.17	0.03	1061
10	1.85	0.12	0.01	3.57	0.20	0.04	1061

	GENERAL	COAGULATION	
	MET-HB rel. 1	PT rel. 1	PTT sec
1	0.015	0.89	20.8
2	0.014	0.94	24.0
3	0.013	0.78	23.4
4	0.014	0.82	25.8
5	0.013	0.81	25.2
6	0.014	0.80	23.3
7	0.014	0.80	23.5
8	0.013	0.77	25.1
9	0.013	0.89	25.7
10	0.013	0.80	24.2

Hematology
After 13 Weeks
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Group 2 (100 mg/kg)

	GENERAL						
	RBC T/l	HB mmol/l	HCT rel. 1	MCV fl	RDW rel. 1	MCH fmol	MCHC mmol/l
11	9.07	9.4	0.43	47.2	0.125	1.03	21.91
12	8.40	9.2	0.42	50.0	0.187	1.09	21.87
13	8.78	9.2	0.42	48.4	0.133	1.05	21.72
14	9.49	10.1	0.47	49.3	0.127	1.07	21.71
15	8.98	9.8	0.44	49.0	0.186	1.09	22.19
16	8.45	9.5	0.43	50.5	0.133	1.13	22.34
17	9.32	9.6	0.44	47.6	0.134	1.03	21.74
18	8.69	9.6	0.43	49.9	0.157	1.11	22.18
19	8.54	9.3	0.42	48.9	0.186	1.09	22.24
20	8.46	9.5	0.42	50.0	0.164	1.13	22.50

	GENERAL	RETICULOCYTE COUNT					GENERAL
	HDW mmol/l	RETI rel. 1	RETI G/l	L RETI rel. 1	M RETI rel. 1	H RETI rel. 1	WBC G/l
11	1.73	0.018	160	0.695	0.275	0.030	3.75
12	1.83	0.019	162	0.674	0.276	0.050	4.86
13	1.76	0.022	192	0.602	0.341	0.057	5.24
14	1.68	0.021	200	0.639	0.314	0.048	5.67
15	2.07	0.024	215	0.656	0.296	0.048	5.93
16	1.73	0.020	170	0.672	0.291	0.037	3.69
17	1.96	0.020	184	0.696	0.265	0.040	6.68
18	1.81	0.018	160	0.675	0.281	0.045	5.10
19	1.99	0.020	168	0.627	0.301	0.072	4.50
20	1.81	0.021	181	0.691	0.266	0.043	5.44

	DIFF.WBC COUNT (REL)					
	NEUT rel. 1	EOS rel. 1	BASO rel. 1	LYMPH rel. 1	MONO rel. 1	LUC rel. 1
11	0.204	0.014	0.007	0.755	0.016	0.004
12	0.155	0.013	0.002	0.787	0.032	0.009
13	0.283	0.018	0.001	0.654	0.038	0.005
14	0.256	0.015	0.003	0.703	0.018	0.005
15	0.207	0.016	0.002	0.754	0.016	0.005
16	0.217	0.013	0.003	0.734	0.024	0.009
17	0.249	0.016	0.003	0.700	0.029	0.004
18	0.200	0.018	0.004	0.742	0.027	0.008
19	0.245	0.016	0.001	0.712	0.025	0.001
20	0.180	0.014	0.002	0.770	0.025	0.008

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Group 2 (100 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT G/l	EOS G/l	BASO G/l	LYMPH G/l	MONO G/l	LUC G/l	PLATELETS G/l
11	0.77	0.05	0.03	2.83	0.06	0.02	912
12	0.75	0.06	0.01	3.82	0.16	0.05	1046
13	1.48	0.09	0.01	3.43	0.20	0.03	979
14	1.45	0.08	0.02	3.99	0.10	0.03	1208
15	1.23	0.09	0.01	4.47	0.10	0.03	1038
16	0.80	0.05	0.01	2.71	0.09	0.03	1168
17	1.66	0.11	0.02	4.67	0.19	0.03	1022
18	1.02	0.09	0.02	3.78	0.14	0.04	961
19	1.10	0.07	0.00	3.21	0.11	0.00	923
20	0.98	0.08	0.01	4.19	0.14	0.04	878

	GENERAL	COAGULATION	
	MET-HB rel. 1	PT rel. 1	PTT sec
11	0.013	0.81	24.3
12	0.014	0.87	25.7
13	0.015	0.78	22.8
14	0.013	0.82	23.5
15	0.015	0.82	25.0
16	0.014	0.83	25.5
17	0.014	0.88	24.2
18	0.014	0.78	26.9
19	0.014	0.83	23.3
20	0.015	0.77	26.8

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Group 3 (300 mg/kg)

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. 1	fl	rel. 1	fmol	mmol/l
21	8.74	9.3	0.42	48.5	0.125	1.07	21.99
22	9.07	9.8	0.45	49.9	0.133	1.08	21.69
23	8.54	9.8	0.45	52.2	0.171	1.15	22.07
24	8.97	9.6	0.44	48.6	0.165	1.07	22.14
25	9.05	9.7	0.45	49.2	0.126	1.08	21.83
26	9.01	9.5	0.45	49.7	0.132	1.06	21.32
27	7.92	9.5	0.42	53.2	0.192	1.20	22.49
28	8.39	9.3	0.42	50.6	0.178	1.10	21.82
29	9.00	9.2	0.43	47.5	0.135	1.02	21.56
30	8.74	9.8	0.44	50.2	0.192	1.12	22.31

	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
21	1.69	0.018	160	0.652	0.296	0.051	4.95
22	1.74	0.017	155	0.714	0.261	0.025	6.32
23	1.77	0.023	196	0.563	0.388	0.048	5.08
24	1.77	0.016	143	0.710	0.258	0.032	5.13
25	1.63	0.018	159	0.666	0.306	0.028	5.04
26	1.82	0.022	194	0.649	0.311	0.040	5.32
27	1.72	0.021	169	0.646	0.308	0.046	4.15
28	1.81	0.020	163	0.667	0.280	0.053	3.67
29	1.86	0.020	177	0.710	0.266	0.024	6.98
30	1.87	0.019	162	0.688	0.281	0.032	4.94

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
21	0.308	0.017	0.004	0.627	0.041	0.004
22	0.157	0.015	0.002	0.802	0.019	0.005
23	0.218	0.022	0.003	0.732	0.019	0.007
24	0.237	0.022	0.002	0.699	0.035	0.005
25	0.137	0.015	0.002	0.830	0.013	0.003
26	0.288	0.023	0.002	0.656	0.027	0.005
27	0.194	0.009	0.002	0.752	0.030	0.014
28	0.191	0.014	0.003	0.759	0.027	0.006
29	0.230	0.016	0.003	0.717	0.028	0.006
30	0.254	0.017	0.002	0.688	0.032	0.008

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Group 3 (300 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT G/l	EOS G/l	BASO G/l	LYMPH G/l	MONO G/l	LUC G/l	PLATELETS G/l
21	1.53	0.08	0.02	3.10	0.20	0.02	908
22	0.99	0.09	0.02	5.07	0.12	0.03	1063
23	1.11	0.11	0.02	3.72	0.09	0.04	871
24	1.21	0.11	0.01	3.58	0.18	0.03	860
25	0.69	0.08	0.01	4.18	0.07	0.02	975
26	1.53	0.12	0.01	3.49	0.15	0.03	1315
27	0.80	0.04	0.01	3.12	0.12	0.06	797
28	0.70	0.05	0.01	2.78	0.10	0.02	1000
29	1.61	0.11	0.02	5.00	0.20	0.04	1016
30	1.25	0.08	0.01	3.40	0.16	0.04	1078

	GENERAL	COAGULATION	
	MET-HB rel. 1	PT rel. 1	PTT sec
21	0.014	--- a	--- a
22	0.014	0.82	22.0
23	0.013	0.91	21.0
24	0.014	0.81	25.2
25	0.015	0.82	24.1
26	0.015	0.90	22.6
27	0.014	0.79	25.3
28	0.014	0.78	26.1
29	0.014	0.82	23.5
30	0.014	0.83	25.3

a: See explanation on section cover page

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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	9.10	9.3	0.44	48.2	0.126	1.03	21.31
32	9.16	9.4	0.44	47.7	0.129	1.03	21.59
33	8.30	8.9	0.42	50.8	0.159	1.08	21.20
34	9.42	9.5	0.44	46.8	0.131	1.01	21.57
35	9.49	9.9	0.46	48.4	0.133	1.04	21.50
36	8.51	9.5	0.43	50.6	0.195	1.12	22.15
37	9.11	9.6	0.44	48.4	0.195	1.05	21.79
38	8.45	9.4	0.43	50.4	0.181	1.11	22.04
39	9.36	9.7	0.46	48.8	0.129	1.03	21.18
40	8.21	9.6	0.43	52.7	0.270	1.16	22.11

	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	1.62	0.022	198	0.659	0.303	0.038	3.38
32	1.58	0.019	177	0.650	0.306	0.044	5.85
33	1.67	0.056	461	0.589	0.344	0.067	5.90
34	1.64	0.023	214	0.687	0.278	0.035	6.41
35	1.68	0.019	184	0.645	0.302	0.052	6.40
36	1.93	0.025	213	0.661	0.294	0.044	4.68
37	1.88	0.017	156	0.771	0.208	0.020	5.72
38	1.82	0.024	203	0.641	0.316	0.043	5.11
39	1.70	0.023	211	0.637	0.311	0.052	4.10
40	1.91	0.022	179	0.627	0.314	0.059	4.97

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
31	0.199	0.023	0.004	0.735	0.035	0.005
32	0.306	0.019	0.004	0.624	0.041	0.007
33	0.166	0.010	0.001	0.784	0.032	0.007
34	0.380	0.021	0.002	0.560	0.032	0.005
35	0.205	0.030	0.002	0.727	0.032	0.004
36	0.249	0.026	0.002	0.682	0.029	0.013
37	0.268	0.020	0.002	0.677	0.026	0.006
38	0.331	0.021	0.003	0.619	0.022	0.004
39	0.199	0.017	0.002	0.753	0.025	0.003
40	0.329	0.030	0.003	0.604	0.030	0.005

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Group 4 (1000 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT G/l	EOS G/l	BASO G/l	LYMPH G/l	MONO G/l	LUC G/l	PLATELETS G/l
31	0.67	0.08	0.01	2.48	0.12	0.02	916
32	1.79	0.11	0.02	3.65	0.24	0.04	1157
33	0.98	0.06	0.01	4.62	0.19	0.04	1252
34	2.43	0.14	0.01	3.59	0.21	0.03	1087
35	1.31	0.19	0.01	4.65	0.20	0.03	810
36	1.16	0.12	0.01	3.19	0.13	0.06	823
37	1.54	0.12	0.01	3.87	0.15	0.04	1409
38	1.69	0.11	0.02	3.17	0.11	0.02	811
39	0.82	0.07	0.01	3.08	0.10	0.01	911
40	1.63	0.15	0.02	3.00	0.15	0.03	1082

	GENERAL	COAGULATION	
	MET-HB rel. 1	PT rel. 1	PTT sec
31	0.015	0.75	27.0
32	0.014	0.83	26.2
33	0.014	0.82	23.3
34	0.013	0.83	24.6
35	0.014	0.96	21.5
36	0.015	0.83	23.7
37	0.014	0.84	26.8
38	0.014	0.82	24.3
39	0.014	0.83	23.7
40	0.014	0.86	22.5

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After 13 Weeks
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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	7.36	8.8	0.39	52.9	0.153	1.19	22.49
42	8.52	9.5	0.44	51.1	0.141	1.11	21.72
43	8.06	9.2	0.42	52.3	0.121	1.14	21.75
44	8.05	9.0	0.41	51.3	0.119	1.12	21.88
45	7.44	8.7	0.39	52.9	0.146	1.18	22.23
46	7.51	8.8	0.39	51.8	0.168	1.17	22.63
47	7.67	9.2	0.41	53.9	0.160	1.20	22.18
48	7.90	9.1	0.41	51.7	0.115	1.15	22.17
49	7.26	8.7	0.39	53.8	0.149	1.19	22.18
50	7.28	9.2	0.40	55.5	0.252	1.26	22.67

	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	1.52	0.027	199	0.647	0.321	0.032	2.13
42	1.52	0.023	197	0.688	0.290	0.022	2.71
43	1.46	0.023	188	0.718	0.261	0.021	2.92
44	1.42	0.027	215	0.625	0.346	0.029	3.53
45	1.52	0.029	217	0.610	0.351	0.039	2.19
46	1.59	0.026	198	0.659	0.316	0.024	3.35
47	1.51	0.030	228	0.574	0.365	0.060	2.44
48	1.40	0.021	164	0.594	0.347	0.060	2.79
49	1.52	0.039	281	0.535	0.413	0.051	2.57
50	1.54	0.029	213	0.635	0.338	0.028	4.68

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
41	0.222	0.049	0.001	0.702	0.021	0.005
42	0.240	0.022	0.001	0.713	0.016	0.007
43	0.223	0.029	0.003	0.717	0.023	0.005
44	0.154	0.035	0.003	0.784	0.017	0.007
45	0.261	0.022	0.002	0.688	0.020	0.007
46	0.207	0.014	0.001	0.750	0.022	0.006
47	0.273	0.018	0.002	0.682	0.021	0.004
48	0.259	0.012	0.004	0.691	0.028	0.006
49	0.245	0.045	0.004	0.678	0.018	0.010
50	0.186	0.015	0.001	0.772	0.020	0.005

Hematology
After 13 Weeks
FEMALES

Group 1 (0 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT G/l	EOS G/l	BASO G/l	LYMPH G/l	MONO G/l	LUC G/l	PLATELETS G/l
41	0.47	0.10	0.00	1.50	0.04	0.01	1043
42	0.65	0.06	0.00	1.93	0.04	0.02	1275
43	0.65	0.09	0.01	2.09	0.07	0.01	815
44	0.55	0.12	0.01	2.77	0.06	0.02	1292
45	0.57	0.05	0.00	1.50	0.04	0.02	1159
46	0.69	0.05	0.00	2.51	0.07	0.02	1096
47	0.67	0.04	0.00	1.66	0.05	0.01	1220
48	0.72	0.03	0.01	1.93	0.08	0.02	909
49	0.63	0.11	0.01	1.74	0.05	0.02	929
50	0.87	0.07	0.01	3.61	0.09	0.03	1294

	GENERAL	COAGULATION	
	MET-HB rel. 1	PT rel. 1	PTT sec
41	0.014	0.79	34.5
42	0.013	0.85	27.3
43	0.016	0.82	32.2
44	0.015	0.88	28.2
45	0.016	0.82	31.0
46	0.014	0.85	35.9
47	0.015	0.82	37.5
48	0.014	0.86	39.8
49	0.010	0.82	37.2
50	0.010	0.83	42.8

Hematology
After 13 Weeks
FEMALES

Group 2 (100 mg/kg)

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. 1	fl	rel. 1	fmol	mmol/l
51	8.37	9.7	0.43	51.8	0.114	1.16	22.42
52	7.28	8.7	0.38	52.7	0.125	1.20	22.69
53	7.26	8.4	0.39	53.7	0.119	1.16	21.57
54	7.62	9.1	0.42	54.6	0.133	1.20	21.96
55	7.95	9.2	0.41	52.1	0.118	1.16	22.19
56	7.81	8.9	0.40	50.7	0.149	1.14	22.40
57	7.60	8.6	0.40	52.2	0.119	1.13	21.73
58	7.68	9.4	0.42	54.6	0.154	1.22	22.40
59	8.61	9.3	0.43	49.9	0.124	1.07	21.51
60	7.67	9.1	0.41	53.7	0.116	1.18	22.04

	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	1.44	0.024	203	0.670	0.303	0.027	2.73
52	1.54	0.026	191	0.629	0.334	0.037	3.12
53	1.39	0.030	216	0.585	0.376	0.039	2.34
54	1.44	0.035	266	0.621	0.344	0.035	2.74
55	1.42	0.024	190	0.647	0.310	0.043	2.15
56	1.54	0.022	170	0.694	0.270	0.036	2.76
57	1.39	0.027	202	0.570	0.378	0.052	2.79
58	1.39	0.022	171	0.706	0.266	0.027	2.30
59	1.49	0.017	145	0.769	0.214	0.016	2.87
60	1.36	0.025	188	0.632	0.327	0.041	2.78

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
51	0.373	0.044	0.003	0.562	0.015	0.003
52	0.208	0.026	0.005	0.739	0.017	0.007
53	0.299	0.046	0.003	0.627	0.021	0.003
54	0.231	0.016	0.002	0.732	0.014	0.005
55	0.226	0.065	0.001	0.679	0.023	0.007
56	0.302	0.013	0.002	0.656	0.023	0.005
57	0.223	0.016	0.002	0.733	0.017	0.009
58	0.253	0.018	0.002	0.684	0.037	0.005
59	0.228	0.029	0.001	0.714	0.023	0.005
60	0.403	0.018	0.002	0.556	0.018	0.003

Hematology
After 13 Weeks
FEMALES

Group 2 (100 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT G/l	EOS G/l	BASO G/l	LYMPH G/l	MONO G/l	LUC G/l	PLATELETS G/l
51	1.02	0.12	0.01	1.53	0.04	0.01	728
52	0.65	0.08	0.01	2.31	0.05	0.02	1060
53	0.70	0.11	0.01	1.47	0.05	0.01	1048
54	0.63	0.04	0.01	2.01	0.04	0.01	1072
55	0.49	0.14	0.00	1.46	0.05	0.01	854
56	0.83	0.04	0.00	1.81	0.06	0.01	1558
57	0.62	0.04	0.01	2.04	0.05	0.03	1285
58	0.58	0.04	0.00	1.58	0.09	0.01	1140
59	0.65	0.08	0.00	2.05	0.07	0.01	881
60	1.12	0.05	0.00	1.55	0.05	0.01	1151

	GENERAL	COAGULATION	
	MET-HB rel. 1	PT rel. 1	PTT sec
51	0.016	0.84	34.3
52	0.015	0.77	39.8
53	0.015	0.82	39.2
54	0.014	0.79	35.4
55	0.014	0.92	30.3
56	0.014	0.80	31.0
57	0.014	0.83	33.5
58	0.014	0.78	35.9
59	0.015	0.90	36.0
60	0.014	0.83	31.6

Hematology
After 13 Weeks
FEMALES

Group 3 (300 mg/kg)

	GENERAL						
	RBC	HB	HCT	MCV	RDW	MCH	MCHC
	T/l	mmol/l	rel. 1	fl	rel. 1	fmol	mmol/l
61	7.81	8.5	0.39	50.0	0.138	1.09	21.81
62	8.04	9.1	0.41	51.1	0.169	1.13	22.20
63	7.92	8.9	0.41	51.4	0.137	1.13	21.96
64	7.47	9.0	0.41	54.9	0.215	1.21	22.03
65	7.87	8.7	0.40	51.0	0.116	1.11	21.77
66	7.53	8.6	0.38	50.8	0.165	1.14	22.51
67	7.49	8.8	0.40	53.3	0.189	1.18	22.14
68	8.09	9.7	0.44	54.0	0.187	1.20	22.18
69	8.08	8.8	0.40	49.9	0.161	1.09	21.87
70	7.65	8.8	0.40	52.2	0.162	1.15	22.05

	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	1.45	0.021	165	0.656	0.308	0.036	1.86
62	1.46	0.019	153	0.706	0.257	0.037	2.89
63	1.39	0.029	231	0.558	0.394	0.048	2.43
64	1.56	0.032	239	0.509	0.433	0.058	2.95
65	1.35	0.021	165	0.635	0.331	0.034	2.50
66	1.57	0.034	259	0.651	0.312	0.037	2.86
67	1.51	0.023	176	0.722	0.265	0.014	2.24
68	1.41	0.020	163	0.743	0.234	0.023	2.55
69	1.59	0.029	235	0.606	0.354	0.040	3.05
70	1.52	0.031	236	0.592	0.360	0.048	2.37

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
61	0.236	0.031	0.002	0.702	0.022	0.008
62	0.263	0.016	0.003	0.692	0.019	0.006
63	0.274	0.014	0.002	0.673	0.030	0.007
64	0.212	0.012	0.003	0.748	0.016	0.009
65	0.277	0.023	0.001	0.666	0.026	0.005
66	0.391	0.007	0.001	0.575	0.021	0.005
67	0.212	0.036	0.003	0.707	0.025	0.016
68	0.212	0.012	0.003	0.743	0.023	0.006
69	0.183	0.009	0.002	0.786	0.017	0.002
70	0.220	0.011	0.003	0.747	0.018	0.002

Hematology
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FEMALES

Group 3 (300 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT G/l	EOS G/l	BASO G/l	LYMPH G/l	MONO G/l	LUC G/l	PLATELETS G/l
61	0.44	0.06	0.00	1.31	0.04	0.01	900
62	0.76	0.05	0.01	2.00	0.06	0.02	1296
63	0.67	0.03	0.00	1.64	0.07	0.02	1009
64	0.63	0.04	0.01	2.21	0.05	0.03	1041
65	0.69	0.06	0.00	1.66	0.07	0.01	1090
66	1.12	0.02	0.00	1.64	0.06	0.01	1037
67	0.48	0.08	0.01	1.59	0.06	0.04	946
68	0.54	0.03	0.01	1.89	0.06	0.02	1070
69	0.56	0.03	0.01	2.40	0.05	0.01	1390
70	0.52	0.03	0.01	1.77	0.04	0.00	1034

	GENERAL	COAGULATION	
	MET-HB rel. 1	PT rel. 1	PTT sec
61	0.015	0.83	40.5
62	0.014	0.82	28.9
63	0.014	0.87	29.5
64	0.014	0.87	30.7
65	0.015	0.82	32.1
66	0.015	0.81	36.8
67	0.015	0.86	32.3
68	0.015	0.85	26.9
69	0.014	0.77	40.1
70	0.015	0.78	40.9

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	7.17	8.5	0.38	53.1	0.154	1.19	22.35
72	7.48	8.8	0.40	52.9	0.146	1.18	22.30
73	7.06	8.7	0.40	56.7	0.144	1.23	21.76
74	7.48	9.3	0.42	55.9	0.157	1.24	22.14
75	7.58	8.8	0.39	51.9	0.139	1.15	22.25
76	7.61	9.0	0.40	52.9	0.153	1.19	22.45
77	8.25	9.0	0.42	51.0	0.121	1.09	21.46
78	8.57	9.5	0.43	50.1	0.110	1.11	22.10
79	8.13	9.1	0.42	51.7	0.114	1.12	21.63
80	7.64	9.2	0.41	53.4	0.193	1.21	22.59

	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	1.44	0.022	159	0.735	0.251	0.014	2.80
72	1.41	0.033	248	0.672	0.296	0.032	2.03
73	1.36	0.032	223	0.525	0.401	0.075	2.11
74	1.43	0.027	199	0.577	0.377	0.047	2.93
75	1.44	0.030	225	0.541	0.403	0.056	2.84
76	1.46	0.025	188	0.709	0.258	0.033	3.65
77	1.46	0.026	215	0.588	0.366	0.046	2.76
78	1.39	0.023	199	0.724	0.258	0.018	3.68
79	1.33	0.022	180	0.650	0.321	0.029	3.69
80	1.50	0.030	228	0.638	0.328	0.034	2.60

	DIFF.WBC COUNT (REL)					
	NEUT	EOS	BASO	LYMPH	MONO	LUC
	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1	rel. 1
71	0.221	0.021	0.000	0.732	0.017	0.008
72	0.210	0.000	0.000	0.770	0.020	0.000
73	0.309	0.012	0.001	0.647	0.021	0.009
74	0.165	0.011	0.004	0.802	0.014	0.004
75	0.177	0.018	0.003	0.778	0.020	0.004
76	0.218	0.018	0.002	0.746	0.012	0.004
77	0.287	0.048	0.003	0.644	0.014	0.004
78	0.318	0.018	0.002	0.633	0.021	0.008
79	0.171	0.012	0.001	0.792	0.017	0.007
80	0.254	0.033	0.004	0.675	0.023	0.012

Hematology
After 13 Weeks
FEMALES

Group 4 (1000 mg/kg)

	DIFF.WBC COUNT (ABS)						GENERAL
	NEUT G/l	EOS G/l	BASO G/l	LYMPH G/l	MONO G/l	LUC G/l	PLATELETS G/l
71	0.62	0.06	0.00	2.05	0.05	0.02	1165
72	0.43	0.00	0.00	1.56	0.04	0.00	1251
73	0.65	0.03	0.00	1.36	0.04	0.02	1009
74	0.48	0.03	0.01	2.35	0.04	0.01	997
75	0.50	0.05	0.01	2.21	0.06	0.01	1238
76	0.80	0.07	0.01	2.72	0.05	0.02	1004
77	0.79	0.13	0.01	1.78	0.04	0.01	1057
78	1.17	0.06	0.01	2.33	0.08	0.03	1458
79	0.63	0.04	0.00	2.92	0.06	0.02	1583
80	0.66	0.09	0.01	1.75	0.06	0.03	1038

	GENERAL	COAGULATION	
	MET-HB rel. 1	PT rel. 1	PTT sec
71	0.015	0.91	28.6
72	0.014	0.86	33.5
73	0.015	0.80	31.8
74	0.014	0.80	31.7
75	0.014	0.79	39.4
76	0.014	0.85	32.4
77	0.013	0.88	27.7
78	0.014	0.82	33.5
79	0.014	0.91	36.2
80	0.014	0.84	30.9

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 102	Female	Group 10	Reserve Removed
Animal 103	Female	Group 10	Reserve Removed

Reported Parameter

After 13 Weeks

GLUCOSE	GLUCOSE
UREA	UREA
CREAT	CREATININE
BILI-T	BILIRUBIN, TOTAL
CHOLEST	CHOLESTEROL, TOTAL
TRIGLY	TRIGLYCERIDES
PHOS-LIP	PHOSPHOLIPIDS
ASAT	ASPARTATE AMINOTRANSFERASE (ASAT)
ALAT	ALANINE AMINOTRANSFERASE (ALAT)
LDH	LACTATE DEHYDROGENASE (LDH)
GLDH	GLUTAMATE-DEHYDROGENASE (GLDH)
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	CHOLEST mmol/l	TRIGLY mmol/l	PHOS-LIP mmol/l
1	5.08	5.61	28.0	2.30	2.57	0.46	1.91
2	5.58	4.63	20.7	1.84	2.40	0.23	1.90
3	6.93	5.57	25.6	2.27	2.23	0.27	1.66
4	6.09	5.71	26.9	1.87	2.78	0.50	2.03
5	5.43	5.88	26.0	1.55	3.12	0.51	2.14
6	3.75	6.69	31.5	2.02	2.13	0.27	1.39
7	4.88	4.52	23.2	2.12	2.09	0.45	1.64
8	6.34	4.51	25.8	2.05	2.77	0.38	2.12
9	4.50	5.96	31.1	2.21	2.00	0.36	1.68
10	5.17	5.24	24.2	2.08	1.95	0.39	1.62

	GENERAL						
	ASAT U/l	ALAT U/l	LDH U/l	GLDH U/l	ALP U/l	GGT U/l	CK U/l
1	61.7	23.4	114.7	5.9	58.2	0.0	112.8
2	57.9	27.2	120.3	6.7	37.3	0.0	119.3
3	74.5	39.7	139.8	10.7	43.5	0.0	140.5
4	79.6	24.6	129.4	8.1	54.9	0.0	275.8
5	83.0	32.9	72.0	10.3	56.9	0.0	84.7
6	72.9	27.4	110.6	8.0	37.2	0.0	99.3
7	69.0	31.3	87.4	5.0	41.9	0.0	103.8
8	67.8	23.0	116.8	8.8	50.8	0.0	244.3
9	71.9	36.9	108.3	6.4	36.8	0.0	125.8
10	112.8	43.6	133.4	13.8	58.4	0.0	486.3

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
1	145.9	3.53	104.9	2.70	1.98	69.90	43.26
2	145.6	4.00	104.3	2.69	1.70	72.90	43.24
3	144.7	4.01	103.4	2.70	1.82	67.29	40.46
4	146.8	3.41	107.0	2.59	1.57	71.39	43.23
5	146.8	3.93	104.9	2.78	1.61	72.62	42.72
6	146.3	4.16	104.6	2.83	1.85	75.22	43.85
7	148.1	3.28	106.5	2.75	1.90	72.60	44.19
8	146.4	3.84	104.5	2.69	1.82	71.51	41.70
9	146.8	3.80	105.1	2.71	1.73	74.81	53.43
10	148.1	3.70	106.4	2.79	1.81	74.70	44.53

Biochemistry
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MALES

Group 1 (0 mg/kg)

	GENERAL	
	GLOBULIN g/l	A/G RATIO
1	26.64	1.62
2	29.66	1.46
3	26.83	1.51
4	28.16	1.54
5	29.90	1.43
6	31.37	1.40
7	28.41	1.56
8	29.81	1.40
9	21.38	2.50
10	30.17	1.48

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MALES

Group 2 (100 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT µmol/l	BILI-T µmol/l	CHOLEST mmol/l	TRIGLY mmol/l	PHOS-LIP mmol/l
11	6.29	5.82	29.4	1.54	2.01	0.30	1.57
12	7.24	5.37	24.0	1.87	2.16	0.28	1.65
13	6.68	5.69	25.9	1.77	2.44	0.25	1.81
14	4.55	5.77	31.8	1.79	2.20	0.25	1.68
15	5.60	4.83	24.6	1.09	2.11	0.50	1.81
16	6.04	3.63	21.4	1.33	2.03	0.54	1.75
17	7.20	5.97	24.4	1.02	2.21	0.72	1.73
18	7.40	4.07	21.6	1.65	1.72	0.48	1.53
19	6.41	4.62	21.9	1.61	2.46	0.31	1.83
20	6.47	4.70	22.6	1.61	2.11	0.43	1.79

	GENERAL						
	ASAT U/l	ALAT U/l	LDH U/l	GLDH U/l	ALP U/l	GGT U/l	CK U/l
11	77.8	25.0	102.7	3.9	54.0	0.0	191.2
12	61.3	26.2	105.7	11.9	39.8	0.0	86.5
13	105.2	40.3	115.8	9.8	53.2	0.0	94.7
14	68.1	22.2	75.5	3.6	39.3	0.0	88.9
15	62.3	29.6	78.3	8.3	42.4	0.0	76.7
16	92.5	31.3	113.9	10.9	41.8	0.0	122.1
17	73.5	33.1	97.6	6.9	46.6	0.0	285.2
18	71.0	32.9	93.9	10.6	51.0	0.0	131.5
19	61.4	38.3	85.6	11.0	71.6	0.0	90.7
20	57.1	27.9	84.7	10.9	78.6	0.0	76.7

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
11	146.7	3.53	106.4	2.61	1.53	70.92	45.84
12	146.2	3.90	107.1	2.69	1.81	69.68	41.98
13	147.4	4.05	106.7	2.62	1.72	68.05	41.15
14	148.1	3.71	108.0	2.65	1.62	69.62	42.83
15	148.4	3.64	107.5	2.77	1.59	74.29	43.28
16	148.5	3.68	106.8	2.61	1.64	70.94	44.67
17	147.4	3.58	107.5	2.70	1.41	72.31	45.32
18	147.5	3.77	106.5	2.66	1.74	72.78	44.77
19	147.9	3.84	107.0	2.74	1.67	74.07	43.07
20	146.4	3.70	106.8	2.76	1.67	72.47	42.89

Biochemistry
After 13 Weeks
MALES

Group 2 (100 mg/kg)

	GENERAL	
	GLOBULIN g/l	A/G RATIO
11	25.08	1.83
12	27.70	1.52
13	26.90	1.53
14	26.79	1.60
15	31.01	1.40
16	26.27	1.70
17	26.99	1.68
18	28.01	1.60
19	31.00	1.39
20	29.58	1.45

Biochemistry
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MALES

Group 3 (300 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT µmol/l	BILI-T µmol/l	CHOLEST mmol/l	TRIGLY mmol/l	PHOS-LIP mmol/l
21	7.14	4.62	25.6	1.69	2.03	0.32	1.66
22	5.95	5.97	22.2	1.74	2.99	0.38	2.05
23	7.59	4.75	23.0	1.39	2.64	0.79	2.13
24	7.48	3.87	22.6	1.87	1.72	0.35	1.52
25	4.91	5.13	26.2	1.30	2.31	0.46	1.90
26	7.83	4.30	23.8	1.29	2.07	0.50	1.67
27	7.01	4.71	25.1	1.58	2.44	0.36	1.85
28	7.37	5.19	27.5	1.19	2.67	0.46	2.10
29	5.37	6.41	34.2	1.53	2.17	0.31	1.63
30	5.60	4.58	27.0	1.78	2.31	0.47	1.80

	GENERAL						
	ASAT U/l	ALAT U/l	LDH U/l	GLDH U/l	ALP U/l	GGT U/l	CK U/l
21	61.7	28.8	128.7	10.9	41.2	0.0	485.7
22	62.1	28.8	119.5	12.9	48.8	0.0	126.7
23	82.7	32.8	109.7	9.6	50.3	0.0	142.3
24	71.1	28.8	111.2	7.9	52.3	0.0	214.0
25	69.0	27.4	92.8	5.2	55.5	0.0	166.7
26	74.9	43.8	91.6	15.6	42.7	0.0	142.9
27	62.8	28.0	74.8	10.7	45.9	0.0	109.4
28	140.7	37.3	85.1	13.0	55.0	0.0	142.1
29	68.0	26.4	106.3	6.0	57.3	0.0	125.5
30	65.1	25.0	93.4	7.1	43.0	0.0	175.9

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
21	147.3	3.77	107.0	2.62	1.72	71.71	43.95
22	151.6	3.77	109.1	2.72	1.87	70.11	46.78
23	146.7	3.98	106.1	2.75	1.54	73.08	44.49
24	147.0	4.00	105.7	2.72	1.66	73.55	43.52
25	148.3	3.62	107.8	2.74	1.82	70.84	42.75
26	147.6	3.77	105.5	2.77	1.86	74.58	43.84
27	148.8	3.80	109.5	2.73	1.82	73.21	44.99
28	146.9	4.00	107.1	2.72	1.73	70.49	42.70
29	147.8	3.90	106.9	2.78	2.00	68.81	39.77
30	148.2	3.86	107.3	2.73	1.70	71.49	42.57

Biochemistry
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MALES

Group 3 (300 mg/kg)

	GENERAL	
	GLOBULIN g/l	A/G RATIO
21	27.76	1.58
22	23.33	2.01
23	28.59	1.56
24	30.03	1.45
25	28.09	1.52
26	30.74	1.43
27	28.22	1.59
28	27.79	1.54
29	29.04	1.37
30	28.92	1.47

Biochemistry
After 13 Weeks
MALES

Group 4 (1000 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT µmol/l	BILI-T µmol/l	CHOLEST mmol/l	TRIGLY mmol/l	PHOS-LIP mmol/l
31	7.03	4.63	24.2	2.04	2.80	0.70	1.98
32	6.52	4.79	26.8	1.68	1.83	0.39	1.66
33	7.27	5.02	22.5	1.48	2.16	0.36	1.71
34	6.55	4.80	28.9	1.48	2.32	0.39	1.95
35	5.97	6.12	28.3	1.76	2.14	0.35	1.67
36	6.40	5.20	26.6	1.52	2.58	1.08	2.11
37	5.54	4.82	27.1	1.59	1.89	0.39	1.56
38	5.96	5.40	28.6	1.73	2.48	0.34	1.81
39	8.21	4.99	26.3	1.97	2.33	0.79	1.82
40	7.68	5.35	25.3	1.65	2.69	0.55	2.00

	GENERAL						
	ASAT U/l	ALAT U/l	LDH U/l	GLDH U/l	ALP U/l	GGT U/l	CK U/l
31	64.5	29.1	82.6	10.4	54.5	0.0	89.9
32	78.9	33.2	103.6	13.8	62.5	0.0	149.0
33	69.1	28.6	116.7	12.3	37.5	0.0	259.9
34	71.7	20.9	83.5	8.4	56.8	0.0	247.8
35	60.0	29.1	110.0	6.2	37.6	0.0	147.5
36	65.5	41.4	72.7	13.7	45.0	0.0	92.8
37	63.4	21.6	92.9	6.1	44.7	0.0	214.5
38	91.7	29.6	88.2	8.9	55.6	0.0	110.1
39	101.8	44.2	109.2	13.7	57.0	0.0	105.5
40	65.2	27.3	106.9	6.6	43.7	0.0	181.5

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
31	146.0	4.15	107.2	2.74	1.70	71.33	43.78
32	147.8	3.80	108.0	2.79	1.95	71.07	43.40
33	146.7	3.79	106.4	2.77	2.00	69.06	40.64
34	146.5	3.70	106.8	2.73	1.85	74.15	42.24
35	148.0	3.58	106.5	2.71	1.76	72.50	43.72
36	147.8	3.83	107.2	2.77	1.84	74.06	45.55
37	148.7	3.79	107.4	2.72	1.85	72.63	42.92
38	148.7	3.80	107.9	2.69	1.64	71.56	43.28
39	147.8	4.11	106.0	2.80	1.49	75.69	46.99
40	148.6	3.73	108.4	2.69	1.39	70.88	43.13

Biochemistry
After 13 Weeks
MALES

Group 4 (1000 mg/kg)

	GENERAL	
	GLOBULIN g/l	A/G RATIO
31	27.55	1.59
32	27.67	1.57
33	28.42	1.43
34	31.91	1.32
35	28.78	1.52
36	28.51	1.60
37	29.71	1.44
38	28.28	1.53
39	28.70	1.64
40	27.75	1.55

Biochemistry
After 13 Weeks
FEMALES

Group 1 (0 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT µmol/l	BILI-T µmol/l	CHOLEST mmol/l	TRIGLY mmol/l	PHOS-LIP mmol/l
41	5.71	7.10	34.4	2.60	1.32	0.21	1.45
42	4.55	5.13	27.7	1.89	2.30	0.25	2.24
43	4.35	6.03	28.9	1.83	2.06	0.48	2.11
44	5.79	5.94	26.3	1.40	2.20	0.46	2.37
45	6.51	7.33	27.4	2.68	2.42	0.50	2.55
46	5.78	5.98	29.2	2.31	2.88	0.37	2.67
47	6.42	5.65	25.3	2.83	3.01	0.45	2.82
48	4.32	6.91	30.4	1.97	1.42	0.40	1.73
49	6.28	5.24	30.3	2.28	2.09	0.36	2.27
50	5.64	5.22	25.7	2.34	2.39	0.36	2.44

	GENERAL						
	ASAT U/l	ALAT U/l	LDH U/l	GLDH U/l	ALP U/l	GGT U/l	CK U/l
41	62.3	26.4	84.1	8.3	29.9	0.0	73.5
42	81.3	26.4	124.9	4.0	24.9	0.0	90.2
43	66.9	24.7	91.1	4.2	24.2	0.0	280.2
44	53.1	19.3	164.2	5.0	17.4	0.0	118.7
45	56.9	21.6	87.3	6.1	22.1	0.0	116.1
46	56.7	19.1	89.8	5.8	16.3	0.0	83.2
47	83.7	18.0	83.6	4.6	17.9	0.0	131.0
48	75.2	32.8	153.5	8.4	19.0	0.0	181.6
49	63.3	20.4	104.9	3.6	22.0	0.0	202.2
50	68.5	20.4	97.7	9.2	17.1	0.0	137.5

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
41	146.3	3.03	106.8	2.70	1.37	78.49	53.49
42	147.5	3.13	107.1	2.84	1.69	79.40	53.75
43	148.9	3.18	107.6	2.87	1.51	83.26	54.67
44	147.4	3.52	105.6	2.81	1.36	82.39	54.32
45	145.7	3.02	106.7	2.84	0.99	85.36	59.68
46	146.2	3.06	104.2	2.85	1.10	85.71	57.71
47	147.9	2.90	106.5	2.79	1.11	78.15	54.91
48	147.9	3.05	106.3	2.79	1.70	78.31	54.40
49	147.0	3.31	107.9	2.68	1.28	78.88	50.98
50	147.3	3.18	105.1	2.86	1.20	82.44	57.24

Biochemistry
After 13 Weeks
FEMALES

Group 1 (0 mg/kg)

	GENERAL	
	GLOBULIN g/l	A/G RATIO
41	25.00	2.14
42	25.65	2.10
43	28.59	1.91
44	28.07	1.94
45	25.68	2.32
46	28.00	2.06
47	23.24	2.36
48	23.91	2.28
49	27.90	1.83
50	25.20	2.27

Biochemistry
After 13 Weeks
FEMALES

Group 2 (100 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT µmol/l	BILI-T µmol/l	CHOLEST mmol/l	TRIGLY mmol/l	PHOS-LIP mmol/l
51	5.26	5.95	24.3	1.96	2.47	0.33	2.25
52	5.98	7.06	29.8	1.83	2.82	0.24	2.36
53	5.20	5.92	25.9	1.72	2.00	0.25	2.02
54	6.70	6.04	29.0	1.78	2.26	0.30	2.24
55	4.80	6.16	29.8	2.17	2.46	0.39	2.41
56	5.96	4.64	30.6	2.21	1.91	0.34	2.13
57	6.43	6.74	31.6	2.10	1.66	0.18	1.96
58	5.61	5.81	28.9	1.70	1.65	0.30	1.87
59	4.15	7.64	33.8	2.41	1.89	0.28	1.84
60	4.99	6.08	25.0	1.91	1.67	0.28	1.90

	GENERAL						
	ASAT U/l	ALAT U/l	LDH U/l	GLDH U/l	ALP U/l	GGT U/l	CK U/l
51	51.6	16.5	155.9	2.9	15.7	0.0	99.2
52	83.3	26.6	116.7	29.5	31.1	0.0	156.8
53	62.9	23.0	106.1	12.0	14.9	0.0	127.4
54	63.3	21.1	81.6	3.3	19.9	0.0	73.5
55	44.4	17.1	64.0	8.0	21.2	0.0	90.1
56	67.5	19.1	94.1	4.4	35.1	0.0	81.5
57	74.8	30.5	118.1	10.0	30.5	0.0	133.2
58	59.3	20.1	71.4	5.9	19.8	0.0	86.7
59	80.4	25.8	142.8	10.4	19.2	0.0	94.4
60	147.8	24.5	102.9	6.0	18.8	0.0	349.0

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
51	146.5	3.12	107.6	2.65	1.08	74.66	50.78
52	150.6	3.46	112.7	2.74	1.20	76.00	49.10
53	150.4	3.39	111.1	2.78	1.39	81.18	52.70
54	148.4	3.00	109.6	2.63	1.10	72.47	49.06
55	147.9	3.55	107.7	2.85	1.56	79.05	51.25
56	148.1	3.01	107.4	2.66	1.57	72.19	49.72
57	146.5	2.86	105.4	2.77	1.42	81.87	56.65
58	148.6	3.09	110.6	2.82	1.36	81.61	56.68
59	148.7	3.37	108.0	2.79	1.52	75.23	51.95
60	147.6	3.20	105.7	2.73	1.26	83.52	55.96

Biochemistry
After 13 Weeks
FEMALES

Group 2 (100 mg/kg)

	GENERAL	
	GLOBULIN g/l	A/G RATIO
51	23.88	2.13
52	26.90	1.83
53	28.48	1.85
54	23.41	2.10
55	27.80	1.84
56	22.47	2.21
57	25.22	2.25
58	24.93	2.27
59	23.28	2.23
60	27.56	2.03

Biochemistry
After 13 Weeks
FEMALES

Group 3 (300 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT µmol/l	BILI-T µmol/l	CHOLEST mmol/l	TRIGLY mmol/l	PHOS-LIP mmol/l
61	7.06	9.02	30.8	2.19	2.28	0.39	2.39
62	5.13	6.36	30.1	2.03	1.68	0.26	1.86
63	5.22	5.00	29.1	2.07	2.53	0.43	2.65
64	5.78	6.87	32.5	2.22	2.48	0.36	2.51
65	5.71	5.57	31.2	1.75	1.58	0.37	1.92
66	6.46	7.31	32.1	1.98	1.92	0.26	2.03
67	5.31	6.73	29.4	1.22	2.21	0.46	2.30
68	4.37	5.74	28.1	1.92	2.00	0.23	1.94
69	5.66	4.97	28.3	1.83	1.50	0.34	1.70
70	5.90	5.82	27.1	2.38	1.05	0.25	1.39

	GENERAL						
	ASAT U/l	ALAT U/l	LDH U/l	GLDH U/l	ALP U/l	GGT U/l	CK U/l
61	65.4	27.3	101.4	16.3	19.3	0.0	90.9
62	81.4	32.3	95.9	20.9	32.4	0.0	105.5
63	70.2	34.3	79.6	3.4	13.7	0.0	156.1
64	69.7	22.6	104.2	5.9	20.7	0.0	204.3
65	62.6	25.5	86.7	4.9	22.7	0.0	83.5
66	112.2	32.9	100.5	8.7	24.5	0.0	89.2
67	122.2	33.4	288.0	4.6	25.5	0.0	333.0
68	76.6	27.8	97.3	3.2	38.9	0.0	124.9
69	75.7	29.3	103.6	11.9	18.3	0.0	207.7
70	68.5	21.7	109.7	4.0	19.6	0.0	117.4

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
61	148.2	2.94	108.8	2.78	1.09	83.36	58.71
62	147.4	3.26	107.7	2.81	1.43	78.27	53.83
63	147.7	3.12	107.5	2.79	1.46	76.67	64.73
64	149.6	3.34	110.1	2.81	1.22	80.96	53.07
65	149.4	3.38	109.5	2.78	1.30	79.44	54.67
66	148.2	3.04	108.4	2.71	0.94	76.03	53.67
67	147.3	3.68	107.9	2.75	1.32	79.50	51.94
68	148.8	3.35	107.9	2.88	1.64	79.99	52.41
69	149.4	2.99	108.0	2.62	1.14	73.72	50.19
70	148.1	3.17	109.7	2.60	1.34	77.79	53.48

Biochemistry
After 13 Weeks
FEMALES

Group 3 (300 mg/kg)

	GENERAL	
	GLOBULIN g/l	A/G RATIO
61	24.65	2.38
62	24.44	2.20
63	11.94	5.42
64	27.89	1.90
65	24.77	2.21
66	22.36	2.40
67	27.56	1.88
68	27.58	1.90
69	23.53	2.13
70	24.31	2.20

Biochemistry
After 13 Weeks
FEMALES

Group 4 (1000 mg/kg)

	GENERAL						
	GLUCOSE mmol/l	UREA mmol/l	CREAT µmol/l	BILI-T µmol/l	CHOLEST mmol/l	TRIGLY mmol/l	PHOS-LIP mmol/l
71	7.24	6.36	32.7	1.93	2.58	0.42	2.50
72	7.24	7.75	35.3	1.97	2.00	0.36	2.09
73	5.33	6.27	29.5	1.92	2.21	0.34	2.21
74	5.56	5.63	30.2	1.75	1.43	0.23	1.52
75	5.80	6.56	31.4	2.34	2.35	0.26	2.29
76	5.67	6.45	26.5	1.95	3.05	0.23	2.71
77	6.44	5.55	26.4	1.86	2.70	0.38	2.63
78	6.07	5.86	26.4	2.31	2.03	0.32	2.02
79	6.10	5.80	24.4	2.24	1.48	0.25	1.68
80	5.26	6.33	31.5	2.41	2.16	0.36	2.17

	GENERAL						
	ASAT U/l	ALAT U/l	LDH U/l	GLDH U/l	ALP U/l	GGT U/l	CK U/l
71	68.3	19.2	79.4	5.9	17.4	0.0	89.5
72	61.9	23.0	106.3	4.1	23.9	0.0	99.4
73	61.8	20.4	71.1	5.1	25.1	0.0	93.5
74	107.3	26.4	95.0	5.0	19.4	0.0	114.8
75	67.8	20.2	127.5	3.9	22.7	0.0	303.3
76	52.8	20.4	124.5	6.7	19.3	0.0	95.4
77	112.2	24.6	183.6	17.1	22.6	0.0	483.4
78	62.7	16.2	109.3	3.9	17.6	0.0	266.9
79	55.4	19.5	88.3	4.6	21.0	0.0	73.0
80	58.9	21.4	70.3	4.0	19.7	0.0	73.5

	GENERAL						
	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	CALCIUM mmol/l	PHOSPHORUS mmol/l	PROTEIN g/l	ALBUMIN g/l
71	146.0	3.00	107.7	2.70	1.06	76.53	53.06
72	148.8	3.24	110.0	2.79	1.12	79.02	51.25
73	153.8	3.38	114.1	2.74	1.10	78.56	52.81
74	152.8	3.47	114.2	2.71	1.29	74.11	51.87
75	148.3	3.36	107.6	2.80	1.51	78.13	51.70
76	148.0	3.55	109.3	2.91	1.42	84.28	55.49
77	149.5	3.30	109.0	2.78	1.29	78.95	52.25
78	150.0	2.91	108.2	2.77	1.57	76.16	51.33
79	150.6	3.40	109.4	2.84	1.69	83.33	54.66
80	148.8	3.26	106.3	2.82	1.43	79.36	55.28

Biochemistry
After 13 Weeks
FEMALES

Group 4 (1000 mg/kg)

	GENERAL	
	GLOBULIN g/l	A/G RATIO
71	23.47	2.26
72	27.77	1.85
73	25.75	2.05
74	22.24	2.33
75	26.43	1.96
76	28.79	1.93
77	26.70	1.96
78	24.83	2.07
79	28.67	1.91
80	24.08	2.30

Urinalysis

Comments

a not enough 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 102	Female	Group 10	Reserve Removed
Animal 103	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	ERYTHROCYES
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	5.1	1.043	yellow	turbid	7.0	1	0.25
2	9.8	1.026	yellow	clear	6.0	0	0.25
3	13.7	1.019	yellow	clear	6.5	0	0.00
4	10.3	1.032	yellow	turbid	7.0	1	0.25
5	9.4	1.032	yellow	clear	6.5	1	0.25
6	4.3	1.046	yellow	turbid	8.0	1	0.75
7	10.1	1.027	yellow	clear	7.0	0	0.25
8	8.9	1.030	yellow	clear	7.0	1	0.25
9	14.4	1.018	light yel	clear	7.0	0	0.25
10	5.2	1.052	yellow	turbid	7.0	1	0.75

	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	0.5	0	0	0	0
3	0	0.5	0	0	0	0
4	0	0.5	0	0	0	100
5	0	0.5	0	0	0	25
6	0	1.5	0	0	0	100
7	0	0.5	0	0	0	25
8	0	0.5	0	0	0	25
9	0	0.0	0	0	0	25
10	0	1.5	0	0	0	100

Urinalysis
After 13 Weeks
MALES

Group 2 (100 mg/kg)

	GENERAL						
	VOLUME/18h ml	REL DENS rel. 1	COLOR	APPEARANCE	pH	NITRITE SCORE 0/1	PROTEIN g/l
11	14.4	1.016	light yel	clear	7.0	0	0.25
12	4.9	1.049	yellow	clear	7.0	0	0.75
13	5.4	1.046	yellow	clear	6.5	0	0.75
14	29.3	1.009	light yel	clear	7.0	0	0.00
15	11.6	1.020	yellow	clear	7.0	1	0.25
16	5.4	1.034	yellow	clear	7.0	1	0.25
17	6.6	1.030	yellow	clear	7.0	1	0.25
18	21.4	1.015	yellow	clear	8.0	0	0.00
19	6.2	1.039	yellow	clear	6.5	0	0.25
20	11.7	1.031	yellow	clear	7.0	1	0.25

	GENERAL					
	GLUCOSE mmol/l	KETONES mmol/l	UROBILI µmol/l	BILIRUBIN µmol/l	ERY per µl	LEU per µl
11	0	0.5	0	0	0	0
12	0	1.5	0	0	10	25
13	0	1.5	0	17	0	25
14	0	0.0	0	0	0	0
15	0	0.5	0	0	0	25
16	0	0.5	0	0	0	25
17	0	0.0	0	0	10	25
18	0	0.0	0	0	0	0
19	0	0.5	0	0	0	25
20	0	0.5	0	0	0	25

Urinalysis
After 13 Weeks
MALES

Group 3 (300 mg/kg)

	GENERAL						
	VOLUME/18h ml	REL DENS rel. 1	COLOR	APPEARANCE	pH	NITRITE SCORE 0/1	PROTEIN g/l
21	6.2	1.033	yellow	clear	6.5	0	0.25
22	6.0	1.039	yellow	clear	7.0	1	0.25
23	4.8	1.045	yellow	clear	6.0	0	0.75
24	9.2	1.022	yellow	turbid	7.0	1	0.25
25	7.7	1.018	yellow	turbid	7.0	1	0.25
26	4.7	1.044	yellow	turbid	6.5	1	0.75
27	13.3	1.020	light yel	clear	7.0	1	0.25
28	6.3	1.039	yellow	clear	6.0	0	0.25
29	4.7	1.053	yellow	clear	6.5	1	0.75
30	5.4	1.053	yellow	clear	6.0	0	0.25

	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	17	0	25
23	0	1.5	0	17	0	25
24	0	1.5	0	0	10	25
25	0	0.0	0	0	25	25
26	0	1.5	0	17	250	25
27	0	0.0	0	0	0	25
28	0	1.5	0	0	0	25
29	0	1.5	0	17	10	25
30	0	1.5	0	17	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	--- a	1.069 a	--- a	--- a	--- a	--- a	--- a
32	11.8	1.020	light yel	turbid	7.0	0	0.25
33	18.7	1.017	light yel	turbid	6.5	0	0.25
34	10.4	1.023	light yel	turbid	7.0	1	0.25
35	--- a	--- a	--- a	--- a	--- a	--- a	--- a
36	7.6	1.031	yellow	turbid	8.0	1	0.25
37	9.4	1.031	yellow	turbid	6.5	0	0.25
38	4.9	1.040	yellow	turbid	8.0	1	0.25
39	6.8	1.036	yellow	cloudy	6.5	0	0.25
40	5.6	1.038	yellow	cloudy	6.5	1	0.75

	GENERAL					
	GLUCOSE mmol/l	KETONES mmol/l	UROBILI µmol/l	BILIRUBIN µmol/l	ERY per µl	LEU per µl
31	--- a	--- a	--- a	--- a	--- a	--- a
32	0	0.5	0	0	0	25
33	0	0.0	0	0	10	25
34	0	0.5	0	0	0	25
35	--- a	--- a	--- a	--- a	--- a	--- a
36	0	0.5	0	0	0	25
37	0	1.5	0	0	0	25
38	0	0.5	0	0	0	100
39	0	0.0	0	17	0	25
40	0	0.5	0	0	0	25

a: See explanation on section cover page

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	4.1	1.050	yellow	clear	6.0	0	0.25
42	16.1	1.012	light yel	clear	6.0	0	0.00
43	35.8	1.006	light yel	clear	7.0	0	0.25
44	4.6	1.037	yellow	clear	6.5	0	0.25
45	7.7	1.025	yellow	clear	7.0	0	0.25
46	10.5	1.019	yellow	clear	6.5	0	0.25
47	18.0	1.011	light yel	clear	7.0	0	0.00
48	1.9	1.084	yellow	clear	5.0	0	0.75
49	4.2	1.039	yellow	clear	6.0	0	0.25
50	4.2	1.043	yellow	clear	6.0	0	0.25

	GENERAL					
	GLUCOSE mmol/l	KETONES mmol/l	UROBILI µmol/l	BILIRUBIN µmol/l	ERY per µl	LEU per µl
41	0	0.5	0	0	0	0
42	0	0.0	0	0	0	0
43	0	0.0	0	0	0	0
44	0	0.5	0	0	0	25
45	0	0.0	0	0	0	0
46	0	0.0	0	0	0	0
47	0	0.0	0	0	0	0
48	0	0.5	0	17	0	0
49	0	0.5	0	0	0	0
50	0	0.5	0	0	0	25

Urinalysis
After 13 Weeks
FEMALES

Group 2 (100 mg/kg)

	GENERAL						
	VOLUME/18h ml	REL DENS rel. 1	COLOR	APPEARANCE	pH	NITRITE SCORE 0/1	PROTEIN g/l
51	4.0	1.047	yellow	clear	6.0	0	0.25
52	21.6	1.011	light yel	clear	6.5	0	0.00
53	12.3	1.018	yellow	clear	6.0	0	0.25
54	8.7	1.030	yellow	clear	6.0	0	0.25
55	4.4	1.038	yellow	clear	6.0	0	0.75
56	10.2	1.016	yellow	clear	6.5	0	0.00
57	4.8	1.042	yellow	clear	6.0	0	0.75
58	5.8	1.026	yellow	clear	6.0	0	0.25
59	2.7	1.056	yellow	clear	5.0	1	0.75
60	24.6	1.008	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
51	0	0.5	0	0	0	0
52	0	0.0	0	0	0	0
53	0	0.0	0	0	0	0
54	0	0.0	0	0	0	0
55	0	0.5	0	0	0	0
56	0	0.0	0	0	0	0
57	0	0.5	0	0	0	25
58	0	0.0	0	0	0	0
59	0	0.5	0	17	0	0
60	0	0.0	0	0	0	0

Urinalysis
After 13 Weeks
FEMALES

Group 3 (300 mg/kg)

	GENERAL						
	VOLUME/18h ml	REL DENS rel. 1	COLOR	APPEARANCE	pH	NITRITE SCORE 0/1	PROTEIN g/l
61	2.7	1.051	yellow	clear	6.0	0	0.75
62	3.2	1.036	yellow	clear	6.5	0	0.25
63	14.9	1.015	yellow	clear	6.5	0	0.25
64	2.9	1.047	yellow	clear	5.0	1	0.75
65	3.0	1.050	yellow	clear	6.0	0	0.25
66	8.1	1.033	yellow	clear	5.0	0	0.25
67	5.8	1.027	yellow	clear	6.0	0	0.25
68	8.8	1.023	yellow	clear	6.0	0	0.25
69	13.6	1.015	yellow	clear	6.5	0	0.00
70	9.4	1.018	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
61	0	0.5	0	17	0	25
62	0	0.0	0	17	0	0
63	0	0.0	0	0	0	25
64	0	0.5	0	17	0	0
65	0	0.5	0	17	0	0
66	0	0.0	0	0	0	0
67	0	0.0	0	0	0	25
68	0	0.0	0	0	0	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	9.3	1.019	yellow	clear	6.0	0	0.25
72	5.6	1.035	yellow	clear	6.0	0	0.25
73	12.0	1.019	yellow	clear	6.0	0	0.25
74	9.2	1.020	yellow	clear	6.0	0	0.25
75	8.3	1.023	yellow	clear	7.0	1	0.25
76	3.1	1.060	yellow	clear	6.0	0	0.75
77	8.3	1.022	yellow	clear	6.5	0	0.25
78	2.7	1.051	yellow	clear	6.0	0	0.25
79	16.5	1.013	light yel	clear	6.0	0	0.00
80	2.3	1.051	yellow	clear	5.0	0	0.75

	GENERAL					
	GLUCOSE mmol/l	KETONES mmol/l	UROBILI µmol/l	BILIRUBIN µmol/l	ERY per µl	LEU per µl
71	0	0.0	0	0	0	0
72	0	0.0	0	0	0	0
73	0	0.0	0	0	0	0
74	0	0.0	0	0	0	0
75	0	0.0	0	0	0	25
76	0	0.5	0	17	0	0
77	0	0.0	0	0	0	0
78	0	0.5	0	0	0	25
79	0	0.0	0	0	0	0
80	0	0.5	0	17	0	0

ORGAN WEIGHTS (GRAM)

Comments

Exclusions

Not Reported

Animal 100	Male	Group 10	Reserve Removed
Animal 101	Male	Group 10	Reserve Removed
Animal 102	Female	Group 10	Reserve Removed
Animal 103	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	437.6	2.15	1.32	10.25	0.299	2.28	0.072
2	371.1	1.87	0.98	9.70	0.246	2.10	0.044
3	407.8	2.03	1.12	10.20	0.280	1.92	0.066
4	436.2	2.12	1.03	10.04	0.223	2.02	0.059
5	435.7	2.26	1.09	10.91	0.370	2.07	0.062
6	458.3	1.93	1.12	11.95	0.327	2.22	0.076
7	347.7	1.98	0.99	9.74	0.291	1.95	0.066
8	443.4	1.91	1.02	12.73	0.311	2.21	0.058
9	393.4	2.10	1.04	10.70	0.323	1.94	0.055
10	422.1	2.03	1.01	11.55	0.292	2.19	0.062

Animal	SPLEEN	TESTES	EPIDIDYMID
1	0.93	4.09	1.484
2	0.70	3.74	1.339
3	0.88	4.07	1.437
4	0.84	3.20	1.285
5	0.80	3.59	1.427
6	0.94	3.49	1.490
7	0.79	3.97	1.451
8	0.79	3.83	1.409
9	0.80	3.64	1.336
10	0.68	3.60	1.541

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
MALES

Group 2 (100 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
11	311.0	1.98	0.91	7.84	0.319	1.53	0.057
12	423.0	2.04	0.93	10.42	0.175	2.10	0.069
13	407.8	2.10	0.98	9.94	0.289	2.09	0.070
14	382.9	2.11	1.03	9.57	0.312	2.22	0.063
15	430.3	2.08	1.17	11.58	0.302	2.23	0.055
16	360.1	1.95	0.90	10.61	0.252	1.95	0.049
17	333.3	2.06	0.85	9.12	0.275	1.59	0.055
18	416.9	1.95	0.97	10.94	0.232	2.09	0.070
19	385.6	2.16	0.98	11.45	0.311	2.22	0.058
20	468.2	2.12	1.06	13.22	0.357	2.39	0.058

Animal	SPLEEN	TESTES	EPIDIDYMID
11	0.66	3.00	1.164
12	0.59	3.65	1.540
13	0.72	3.54	1.227
14	0.86	3.69	1.394
15	0.71	4.34	1.659
16	0.66	3.58	1.318
17	0.55	3.71	1.520
18	0.70	3.88	1.447
19	0.63	3.69	1.477
20	0.93	4.23	1.548

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
MALES

Group 3 (300 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
21	403.4	1.99	1.06	10.67	0.228	2.17	0.059
22	412.4	2.13	1.03	11.11	0.367	2.15	0.067
23	446.3	2.04	1.12	13.14	0.288	2.30	0.061
24	407.4	2.05	1.01	10.67	0.364	2.11	0.059
25	362.5	1.89	1.02	10.05	0.380	1.81	0.047
26	418.9	1.97	0.97	11.90	0.188	2.14	0.064
27	353.0	1.94	1.06	11.74	0.313	2.19	0.054
28	425.7	2.19	1.19	11.78	0.295	1.96	0.064
29	476.5	2.07	1.20	13.66	0.373	2.62	0.060
30	398.5	1.96	1.06	12.41	0.267	2.41	0.061

Animal	SPLEEN	TESTES	EPIDIDYMID
21	0.60	3.34	1.308
22	0.66	3.65	1.332
23	0.79	4.17	1.742
24	0.74	3.91	1.610
25	0.67	3.44	1.323
26	0.67	3.54	1.612
27	0.74	3.88	1.473
28	0.73	3.53	1.465
29	0.83	4.71	1.675
30	0.77	3.43	1.321

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
MALES

Group 4 (1000 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
31	441.7	2.09	1.18	11.38	0.372	2.19	0.070
32	376.1	1.95	1.05	8.80	0.275	1.90	0.059
33	484.6	2.36	1.16	12.22	0.358	2.50	0.080
34	465.4	2.13	1.08	11.99	0.296	2.08	0.066
35	371.0	1.97	1.08	10.04	0.268	1.94	0.056
36	374.0	2.00	0.84	10.46	0.333	1.74	0.055
37	464.3	2.14	1.13	12.76	0.250	2.21	0.065
38	384.1	2.08	0.98	10.24	0.319	2.08	0.072
39	392.8	2.14	0.94	10.35	0.204	1.90	0.078
40	376.6	2.04	0.93	9.98	0.275	1.75	0.058

Animal	SPLEEN	TESTES	EPIDIDYMID
31	0.94	3.97	1.616
32	0.79	3.50	1.465
33	0.86	4.13	1.513
34	0.79	4.25	1.599
35	0.80	3.69	1.450
36	0.58	3.89	1.566
37	0.83	4.09	1.702
38	0.70	4.04	1.519
39	0.62	3.89	1.579
40	0.58	3.93	1.667

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

Group 1 (0 mg/kg)

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
1	437.6	0.49	0.30	2.34	0.068	0.52	0.016
2	371.1	0.50	0.26	2.61	0.066	0.57	0.012
3	407.8	0.50	0.27	2.50	0.069	0.47	0.016
4	436.2	0.49	0.24	2.30	0.051	0.46	0.014
5	435.7	0.52	0.25	2.50	0.085	0.48	0.014
6	458.3	0.42	0.24	2.61	0.071	0.48	0.017
7	347.7	0.57	0.28	2.80	0.084	0.56	0.019
8	443.4	0.43	0.23	2.87	0.070	0.50	0.013
9	393.4	0.53	0.26	2.72	0.082	0.49	0.014
10	422.1	0.48	0.24	2.74	0.069	0.52	0.015

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYMID (%)
1	0.21	0.93	0.339
2	0.19	1.01	0.361
3	0.22	1.00	0.352
4	0.19	0.73	0.295
5	0.18	0.82	0.328
6	0.21	0.76	0.325
7	0.23	1.14	0.417
8	0.18	0.86	0.318
9	0.20	0.93	0.340
10	0.16	0.85	0.365

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

Group 2 (100 mg/kg)

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
11	311.0	0.64	0.29	2.52	0.103	0.49	0.018
12	423.0	0.48	0.22	2.46	0.041	0.50	0.016
13	407.8	0.51	0.24	2.44	0.071	0.51	0.017
14	382.9	0.55	0.27	2.50	0.081	0.58	0.016
15	430.3	0.48	0.27	2.69	0.070	0.52	0.013
16	360.1	0.54	0.25	2.95	0.070	0.54	0.014
17	333.3	0.62	0.26	2.74	0.083	0.48	0.017
18	416.9	0.47	0.23	2.62	0.056	0.50	0.017
19	385.6	0.56	0.25	2.97	0.081	0.58	0.015
20	468.2	0.45	0.23	2.82	0.076	0.51	0.012

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYMID (%)
11	0.21	0.96	0.374
12	0.14	0.86	0.364
13	0.18	0.87	0.301
14	0.22	0.96	0.364
15	0.17	1.01	0.386
16	0.18	0.99	0.366
17	0.17	1.11	0.456
18	0.17	0.93	0.347
19	0.16	0.96	0.383
20	0.20	0.90	0.331

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

Group 3 (300 mg/kg)

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
21	403.4	0.49	0.26	2.65	0.057	0.54	0.015
22	412.4	0.52	0.25	2.69	0.089	0.52	0.016
23	446.3	0.46	0.25	2.94	0.065	0.52	0.014
24	407.4	0.50	0.25	2.62	0.089	0.52	0.014
25	362.5	0.52	0.28	2.77	0.105	0.50	0.013
26	418.9	0.47	0.23	2.84	0.045	0.51	0.015
27	353.0	0.55	0.30	3.33	0.089	0.62	0.015
28	425.7	0.51	0.28	2.77	0.069	0.46	0.015
29	476.5	0.43	0.25	2.87	0.078	0.55	0.013
30	398.5	0.49	0.27	3.11	0.067	0.60	0.015

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYMID (%)
21	0.15	0.83	0.324
22	0.16	0.89	0.323
23	0.18	0.93	0.390
24	0.18	0.96	0.395
25	0.18	0.95	0.365
26	0.16	0.85	0.385
27	0.21	1.10	0.417
28	0.17	0.83	0.344
29	0.17	0.99	0.352
30	0.19	0.86	0.331

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

Group 4 (1000 mg/kg)

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
31	441.7	0.47	0.27	2.58	0.084	0.50	0.016
32	376.1	0.52	0.28	2.34	0.073	0.51	0.016
33	484.6	0.49	0.24	2.52	0.074	0.52	0.017
34	465.4	0.46	0.23	2.58	0.064	0.45	0.014
35	371.0	0.53	0.29	2.71	0.072	0.52	0.015
36	374.0	0.53	0.22	2.80	0.089	0.47	0.015
37	464.3	0.46	0.24	2.75	0.054	0.48	0.014
38	384.1	0.54	0.26	2.67	0.083	0.54	0.019
39	392.8	0.54	0.24	2.63	0.052	0.48	0.020
40	376.6	0.54	0.25	2.65	0.073	0.46	0.015

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYMID (%)
31	0.21	0.90	0.366
32	0.21	0.93	0.390
33	0.18	0.85	0.312
34	0.17	0.91	0.344
35	0.22	0.99	0.391
36	0.16	1.04	0.419
37	0.18	0.88	0.367
38	0.18	1.05	0.395
39	0.16	0.99	0.402
40	0.15	1.04	0.443

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

Group 1 (0 mg/kg)

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
1	2.15	61.40	476.74	13.907	106.05	3.349
2	1.87	52.41	518.72	13.155	112.30	2.353
3	2.03	55.17	502.46	13.793	94.58	3.251
4	2.12	48.58	473.58	10.519	95.28	2.783
5	2.26	48.23	482.74	16.372	91.59	2.743
6	1.93	58.03	619.17	16.943	115.03	3.938
7	1.98	50.00	491.92	14.697	98.48	3.333
8	1.91	53.40	666.49	16.283	115.71	3.037
9	2.10	49.52	509.52	15.381	92.38	2.619
10	2.03	49.75	568.97	14.384	107.88	3.054

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYMID (%)
1	43.26	190.23	69.023
2	37.43	200.00	71.604
3	43.35	200.49	70.788
4	39.62	150.94	60.613
5	35.40	158.85	63.142
6	48.70	180.83	77.202
7	39.90	200.51	73.283
8	41.36	200.52	73.770
9	38.10	173.33	63.619
10	33.50	177.34	75.911

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

Group 2 (100 mg/kg)

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
11	1.98	45.96	395.96	16.111	77.27	2.879
12	2.04	45.59	510.78	8.578	102.94	3.382
13	2.10	46.67	473.33	13.762	99.52	3.333
14	2.11	48.82	453.55	14.787	105.21	2.986
15	2.08	56.25	556.73	14.519	107.21	2.644
16	1.95	46.15	544.10	12.923	100.00	2.513
17	2.06	41.26	442.72	13.350	77.18	2.670
18	1.95	49.74	561.03	11.897	107.18	3.590
19	2.16	45.37	530.09	14.398	102.78	2.685
20	2.12	50.00	623.58	16.840	112.74	2.736

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYMID (%)
11	33.33	151.52	58.788
12	28.92	178.92	75.490
13	34.29	168.57	58.429
14	40.76	174.88	66.066
15	34.13	208.65	79.760
16	33.85	183.59	67.590
17	26.70	180.10	73.786
18	35.90	198.97	74.205
19	29.17	170.83	68.380
20	43.87	199.53	73.019

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

Group 3 (300 mg/kg)

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
21	1.99	53.27	536.18	11.457	109.05	2.965
22	2.13	48.36	521.60	17.230	100.94	3.146
23	2.04	54.90	644.12	14.118	112.75	2.990
24	2.05	49.27	520.49	17.756	102.93	2.878
25	1.89	53.97	531.75	20.106	95.77	2.487
26	1.97	49.24	604.06	9.543	108.63	3.249
27	1.94	54.64	605.15	16.134	112.89	2.784
28	2.19	54.34	537.90	13.470	89.50	2.922
29	2.07	57.97	659.90	18.019	126.57	2.899
30	1.96	54.08	633.16	13.622	122.96	3.112

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYMID (%)
21	30.15	167.84	65.729
22	30.99	171.36	62.535
23	38.73	204.41	85.392
24	36.10	190.73	78.537
25	35.45	182.01	70.000
26	34.01	179.70	81.827
27	38.14	200.00	75.928
28	33.33	161.19	66.895
29	40.10	227.54	80.918
30	39.29	175.00	67.398

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

Group 4 (1000 mg/kg)

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
31	2.09	56.46	544.50	17.799	104.78	3.349
32	1.95	53.85	451.28	14.103	97.44	3.026
33	2.36	49.15	517.80	15.169	105.93	3.390
34	2.13	50.70	562.91	13.897	97.65	3.099
35	1.97	54.82	509.64	13.604	98.48	2.843
36	2.00	42.00	523.00	16.650	87.00	2.750
37	2.14	52.80	596.26	11.682	103.27	3.037
38	2.08	47.12	492.31	15.337	100.00	3.462
39	2.14	43.93	483.64	9.533	88.79	3.645
40	2.04	45.59	489.22	13.480	85.78	2.843

Animal	SPLEEN (%)	TESTES (%)	EPIDIDYMID (%)
31	44.98	189.95	77.321
32	40.51	179.49	75.128
33	36.44	175.00	64.110
34	37.09	199.53	75.070
35	40.61	187.31	73.604
36	29.00	194.50	78.300
37	38.79	191.12	79.533
38	33.65	194.23	73.029
39	28.97	181.78	73.785
40	28.43	192.65	81.716

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
FEMALES

Group 1 (0 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
41	222.5	1.84	0.71	5.79	0.256	1.24	0.063
42	201.6	1.96	0.68	5.93	0.157	1.28	0.058
43	217.3	1.97	0.75	6.26	0.298	1.34	0.068
44	228.4	1.89	0.71	6.31	0.238	1.41	0.063
45	228.9	1.72	0.72	6.47	0.195	1.38	0.070
46	221.2	2.01	0.71	6.76	0.143	1.74	0.074
47	237.6	1.91	0.74	7.21	0.316	1.34	0.081
48	235.9	2.02	0.73	6.62	0.256	1.47	0.068
49	226.5	1.85	0.74	7.27	0.206	1.53	0.075
50	221.7	1.81	0.72	6.93	0.278	1.35	0.085

Animal	SPLEEN	OVARIES	UTERUS
41	0.49	0.094	0.89
42	0.53	0.094	0.71
43	0.53	0.097	0.66
44	0.57	0.099	1.56
45	0.51	0.089	1.02
46	0.38	0.049	0.73
47	0.57	0.079	0.95
48	0.53	0.089	0.80
49	0.62	0.103	0.82
50	0.54	0.060	0.92

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
FEMALES

Group 2 (100 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
51	244.7	2.01	0.75	7.42	0.399	1.45	0.064
52	245.2	1.99	0.85	7.33	0.346	1.62	0.092
53	229.5	1.97	0.72	6.84	0.188	1.36	0.072
54	246.3	1.89	0.75	7.46	0.291	1.50	0.078
55	255.6	1.91	0.67	6.87	0.217	1.37	0.067
56	237.9	1.87	0.66	7.58	0.304	1.57	0.066
57	225.1	1.86	0.76	6.86	0.274	1.48	0.077
58	268.2	1.98	0.74	7.80	0.276	1.47	0.076
59	229.9	1.80	0.69	6.23	0.310	1.21	0.072
60	228.1	1.91	0.69	7.31	0.213	1.46	0.083

Animal	SPLEEN	OVARIES	UTERUS
51	0.57	0.096	1.05
52	0.67	0.069	1.03
53	0.46	0.116	0.90
54	0.69	0.128	1.24
55	0.58	0.080	0.77
56	0.49	0.085	1.01
57	0.41	0.097	1.01
58	0.61	0.127	0.83
59	0.49	0.107	0.65
60	0.49	0.107	0.79

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
FEMALES

Group 3 (300 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
61	237.8	1.87	0.71	7.30	0.252	1.48	0.070
62	206.1	1.88	0.71	6.06	0.203	1.34	0.062
63	222.8	1.97	0.71	7.68	0.192	1.48	0.078
64	255.9	2.05	0.67	6.95	0.287	1.43	0.073
65	224.3	1.84	0.67	6.30	0.178	1.28	0.065
66	236.8	1.92	0.75	7.23	0.221	1.29	0.080
67	236.7	1.79	0.70	7.30	0.195	1.28	0.075
68	231.3	1.91	0.67	7.34	0.231	1.40	0.097
69	230.3	1.81	0.67	6.72	0.218	1.35	0.056
70	237.6	1.89	0.67	6.76	0.220	1.36	0.067

Animal	SPLEEN	OVARIES	UTERUS
61	0.56	0.093	1.00
62	0.44	0.096	1.19
63	0.61	0.074	1.00
64	0.75	0.093	1.35
65	0.52	0.109	0.91
66	0.52	0.079	0.88
67	0.52	0.103	0.67
68	0.58	0.133	0.70
69	0.46	0.098	1.11
70	0.52	0.110	0.80

ORGAN WEIGHTS (GRAM)
AFTER 13 WEEKS
FEMALES

Group 4 (1000 mg/kg)

Animal	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS
71	257.5	1.78	0.65	6.57	0.243	1.21	0.063
72	249.5	1.90	0.73	7.41	0.243	1.50	0.077
73	252.0	1.93	0.62	6.20	0.292	1.27	0.075
74	230.9	2.01	0.65	6.32	0.262	1.49	0.081
75	262.2	1.89	0.81	7.76	0.322	1.42	0.079
76	242.1	2.10	0.78	7.62	0.210	1.83	0.086
77	223.9	1.94	0.62	6.82	0.253	1.25	0.062
78	234.6	2.01	0.70	7.79	0.281	1.53	0.080
79	245.9	1.99	0.70	7.49	0.318	1.53	0.074
80	220.9	1.92	0.67	6.92	0.268	1.39	0.070

Animal	SPLEEN	OVARIES	UTERUS
71	0.52	0.087	0.88
72	0.49	0.118	0.95
73	0.47	0.100	1.31
74	0.70	0.103	1.17
75	0.65	0.105	1.06
76	0.54	0.077	0.99
77	0.42	0.072	0.73
78	0.59	0.142	0.98
79	0.48	0.127	1.37
80	0.58	0.095	1.10

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

Group 1 (0 mg/kg)

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
41	222.5	0.83	0.32	2.60	0.115	0.56	0.028
42	201.6	0.97	0.34	2.94	0.078	0.63	0.029
43	217.3	0.91	0.35	2.88	0.137	0.62	0.031
44	228.4	0.83	0.31	2.76	0.104	0.62	0.028
45	228.9	0.75	0.31	2.83	0.085	0.60	0.031
46	221.2	0.91	0.32	3.06	0.065	0.79	0.033
47	237.6	0.80	0.31	3.03	0.133	0.56	0.034
48	235.9	0.86	0.31	2.81	0.109	0.62	0.029
49	226.5	0.82	0.33	3.21	0.091	0.68	0.033
50	221.7	0.82	0.32	3.13	0.125	0.61	0.038

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
41	0.22	0.042	0.40
42	0.26	0.047	0.35
43	0.24	0.045	0.30
44	0.25	0.043	0.68
45	0.22	0.039	0.45
46	0.17	0.022	0.33
47	0.24	0.033	0.40
48	0.22	0.038	0.34
49	0.27	0.045	0.36
50	0.24	0.027	0.41

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

Group 2 (100 mg/kg)

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
51	244.7	0.82	0.31	3.03	0.163	0.59	0.026
52	245.2	0.81	0.35	2.99	0.141	0.66	0.038
53	229.5	0.86	0.31	2.98	0.082	0.59	0.031
54	246.3	0.77	0.30	3.03	0.118	0.61	0.032
55	255.6	0.75	0.26	2.69	0.085	0.54	0.026
56	237.9	0.79	0.28	3.19	0.128	0.66	0.028
57	225.1	0.83	0.34	3.05	0.122	0.66	0.034
58	268.2	0.74	0.28	2.91	0.103	0.55	0.028
59	229.9	0.78	0.30	2.71	0.135	0.53	0.031
60	228.1	0.84	0.30	3.20	0.093	0.64	0.036

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
51	0.23	0.039	0.43
52	0.27	0.028	0.42
53	0.20	0.051	0.39
54	0.28	0.052	0.50
55	0.23	0.031	0.30
56	0.21	0.036	0.42
57	0.18	0.043	0.45
58	0.23	0.047	0.31
59	0.21	0.047	0.28
60	0.21	0.047	0.35

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

Group 3 (300 mg/kg)

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
61	237.8	0.79	0.30	3.07	0.106	0.62	0.029
62	206.1	0.91	0.34	2.94	0.098	0.65	0.030
63	222.8	0.88	0.32	3.45	0.086	0.66	0.035
64	255.9	0.80	0.26	2.72	0.112	0.56	0.029
65	224.3	0.82	0.30	2.81	0.079	0.57	0.029
66	236.8	0.81	0.32	3.05	0.093	0.54	0.034
67	236.7	0.76	0.30	3.08	0.082	0.54	0.032
68	231.3	0.83	0.29	3.17	0.100	0.61	0.042
69	230.3	0.79	0.29	2.92	0.095	0.59	0.024
70	237.6	0.80	0.28	2.85	0.093	0.57	0.028

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
61	0.24	0.039	0.42
62	0.21	0.047	0.58
63	0.27	0.033	0.45
64	0.29	0.036	0.53
65	0.23	0.049	0.41
66	0.22	0.033	0.37
67	0.22	0.044	0.28
68	0.25	0.058	0.30
69	0.20	0.043	0.48
70	0.22	0.046	0.34

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

Group 4 (1000 mg/kg)

Animal	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
71	257.5	0.69	0.25	2.55	0.094	0.47	0.024
72	249.5	0.76	0.29	2.97	0.097	0.60	0.031
73	252.0	0.77	0.25	2.46	0.116	0.50	0.030
74	230.9	0.87	0.28	2.74	0.113	0.65	0.035
75	262.2	0.72	0.31	2.96	0.123	0.54	0.030
76	242.1	0.87	0.32	3.15	0.087	0.76	0.036
77	223.9	0.87	0.28	3.05	0.113	0.56	0.028
78	234.6	0.86	0.30	3.32	0.120	0.65	0.034
79	245.9	0.81	0.28	3.05	0.129	0.62	0.030
80	220.9	0.87	0.30	3.13	0.121	0.63	0.032

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
71	0.20	0.034	0.34
72	0.20	0.047	0.38
73	0.19	0.040	0.52
74	0.30	0.045	0.51
75	0.25	0.040	0.40
76	0.22	0.032	0.41
77	0.19	0.032	0.33
78	0.25	0.061	0.42
79	0.20	0.052	0.56
80	0.26	0.043	0.50

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

Group 1 (0 mg/kg)

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
41	1.84	38.59	314.67	13.913	67.39	3.424
42	1.96	34.69	302.55	8.010	65.31	2.959
43	1.97	38.07	317.77	15.127	68.02	3.452
44	1.89	37.57	333.86	12.593	74.60	3.333
45	1.72	41.86	376.16	11.337	80.23	4.070
46	2.01	35.32	336.32	7.114	86.57	3.682
47	1.91	38.74	377.49	16.545	70.16	4.241
48	2.02	36.14	327.72	12.673	72.77	3.366
49	1.85	40.00	392.97	11.135	82.70	4.054
50	1.81	39.78	382.87	15.359	74.59	4.696

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
41	26.63	5.109	48.37
42	27.04	4.796	36.22
43	26.90	4.924	33.50
44	30.16	5.238	82.54
45	29.65	5.174	59.30
46	18.91	2.438	36.32
47	29.84	4.136	49.74
48	26.24	4.406	39.60
49	33.51	5.568	44.32
50	29.83	3.315	50.83

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

Group 2 (100 mg/kg)

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
51	2.01	37.31	369.15	19.851	72.14	3.184
52	1.99	42.71	368.34	17.387	81.41	4.623
53	1.97	36.55	347.21	9.543	69.04	3.655
54	1.89	39.68	394.71	15.397	79.37	4.127
55	1.91	35.08	359.69	11.361	71.73	3.508
56	1.87	35.29	405.35	16.257	83.96	3.529
57	1.86	40.86	368.82	14.731	79.57	4.140
58	1.98	37.37	393.94	13.939	74.24	3.838
59	1.80	38.33	346.11	17.222	67.22	4.000
60	1.91	36.13	382.72	11.152	76.44	4.346

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
51	28.36	4.776	52.24
52	33.67	3.467	51.76
53	23.35	5.888	45.69
54	36.51	6.772	65.61
55	30.37	4.188	40.31
56	26.20	4.545	54.01
57	22.04	5.215	54.30
58	30.81	6.414	41.92
59	27.22	5.944	36.11
60	25.65	5.602	41.36

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

Group 3 (300 mg/kg)

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
61	1.87	37.97	390.37	13.476	79.14	3.743
62	1.88	37.77	322.34	10.798	71.28	3.298
63	1.97	36.04	389.85	9.746	75.13	3.959
64	2.05	32.68	339.02	14.000	69.76	3.561
65	1.84	36.41	342.39	9.674	69.57	3.533
66	1.92	39.06	376.56	11.510	67.19	4.167
67	1.79	39.11	407.82	10.894	71.51	4.190
68	1.91	35.08	384.29	12.094	73.30	5.079
69	1.81	37.02	371.27	12.044	74.59	3.094
70	1.89	35.45	357.67	11.640	71.96	3.545

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
61	29.95	4.973	53.48
62	23.40	5.106	63.30
63	30.96	3.756	50.76
64	36.59	4.537	65.85
65	28.26	5.924	49.46
66	27.08	4.115	45.83
67	29.05	5.754	37.43
68	30.37	6.963	36.65
69	25.41	5.414	61.33
70	27.51	5.820	42.33

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

Group 4 (1000 mg/kg)

Animal	BRAIN (GRAM)	HEART (%)	LIVER (%)	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)
71	1.78	36.52	369.10	13.652	67.98	3.539
72	1.90	38.42	390.00	12.789	78.95	4.053
73	1.93	32.12	321.24	15.130	65.80	3.886
74	2.01	32.34	314.43	13.035	74.13	4.030
75	1.89	42.86	410.58	17.037	75.13	4.180
76	2.10	37.14	362.86	10.000	87.14	4.095
77	1.94	31.96	351.55	13.041	64.43	3.196
78	2.01	34.83	387.56	13.980	76.12	3.980
79	1.99	35.18	376.38	15.980	76.88	3.719
80	1.92	34.90	360.42	13.958	72.40	3.646

Animal	SPLEEN (%)	OVARIES (%)	UTERUS (%)
71	29.21	4.888	49.44
72	25.79	6.211	50.00
73	24.35	5.181	67.88
74	34.83	5.124	58.21
75	34.39	5.556	56.08
76	25.71	3.667	47.14
77	21.65	3.711	37.63
78	29.35	7.065	48.76
79	24.12	6.382	68.84
80	30.21	4.948	57.29

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 102	Female	Group 10	Reserve Removed
Animal 103	Female	Group 10	Reserve Removed

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 1 (0 mg/kg)

Animal 1 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 2 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 3 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 4 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 5 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 6 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 7 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 8 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 9 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 1 (0 mg/kg)

Animal 10 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 2 (100 mg/kg)

Animal 11 PLANNED NECROPSY , 08-APR-2010

TESTES BOTH SIDES: REDUCED IN SIZE, D=20X10 MM.

Animal 12 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 13 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 14 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 15 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 16 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 17 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 18 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 19 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 2 (100 mg/kg)

Animal 20 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 3 (300 mg/kg)

Animal 21 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 22 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 23 PLANNED NECROPSY , 08-APR-2010

PANCREAS DISCOLORATION, REDDISH.

Animal 24 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 25 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 26 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 27 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 28 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 29 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 3 (300 mg/kg)

Animal 30 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 4 (1000 mg/kg)

Animal 31	PLANNED NECROPSY , 08-APR-2010	
THYMUS	FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.	
Animal 32	PLANNED NECROPSY , 08-APR-2010	
NO FINDINGS NOTED		
Animal 33	PLANNED NECROPSY , 08-APR-2010	
NO FINDINGS NOTED		
Animal 34	PLANNED NECROPSY , 08-APR-2010	
NO FINDINGS NOTED		
Animal 35	PLANNED NECROPSY , 08-APR-2010	
NO FINDINGS NOTED		
Animal 36	PLANNED NECROPSY , 08-APR-2010	
KIDNEYS	RIGHT SIDE: PELVIC DILATION.	
Animal 37	PLANNED NECROPSY , 08-APR-2010	
NO FINDINGS NOTED		
Animal 38	PLANNED NECROPSY , 08-APR-2010	
NO FINDINGS NOTED		
Animal 39	PLANNED NECROPSY , 08-APR-2010	
NO FINDINGS NOTED		

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
MALES

Group 4 (1000 mg/kg)

Animal 40 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

Group 1 (0 mg/kg)

Animal 41 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 42 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 43 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 44 PLANNED NECROPSY , 08-APR-2010

THYMUS FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.

Animal 45 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 46 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 47 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 48 PLANNED NECROPSY , 08-APR-2010

MANDIBULAR L.NODE FOCUS/FOCI, ISOLATED, D=1 MM, DARK RED.

Animal 49 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

Group 1 (0 mg/kg)

Animal 50 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

Group 2 (100 mg/kg)

Animal 51 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 52 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 53 PLANNED NECROPSY , 08-APR-2010

MANDIBULAR L.NODE FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.

Animal 54 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 55 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 56 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 57 PLANNED NECROPSY , 08-APR-2010

THYMUS FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.

Animal 58 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 59 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

Group 2 (100 mg/kg)

Animal 60 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

Group 3 (300 mg/kg)

Animal 61 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 62 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 63 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 64 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 65 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 66 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 67 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 68 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 69 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

Group 3 (300 mg/kg)

Animal 70 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

Group 4 (1000 mg/kg)

Animal 71 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 72 PLANNED NECROPSY , 08-APR-2010

EYES RIGHT SIDE: DESTROYED DURING BLOOD COLLECTION.

Animal 73 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 74 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 75 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 76 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 77 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 78 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

Animal 79 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
AFTER 13 WEEKS
ALL NECROPSIES
FEMALES

Group 4 (1000 mg/kg)

Animal 80 PLANNED NECROPSY , 08-APR-2010

NO FINDINGS NOTED

REPORT (PART II OF II)

Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083)

13-Week 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 GmbH**
Feldbergstrasse 78
64293 Darmstadt / Germany

Study Identification: Harlan Laboratories Study **C68821**

Version: Final

Study Completion Date: 14-Dec-2010

APPENDIX I - CHEMICAL ANALYSIS OF FEED

LUFA-ITL GmbH

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www.agrolab.de

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LUFA - ITL Dr.-Hell-Str. 6, 24107 Kiel

PROVIMI KLIBA AG
RINAUSTRASSE
4303 KAISERAUGST / SCHWEIZ
SCHWEIZ

Date 02.09.2009
Customer no. 1209835
Page 1 of 2

TEST REPORT

Order no. 633558

Sample No.	**808512**
Order No.	**GLP Schadstoffuntersuchung**
Sample Arrival	**19.08.2009**
Sample code	**TEKLAD GLOBAL REDENT 2014 PELL. 10 MM ECKIG** **Alleinfuttermittel für Mäuse und Ratten** **Rezeptur 3253 - GLP-Batch 58/09** **Fabr.-Code: 0908014 (pelletiert) - Hergestellt: 17.08.09 - MHD: 17.02.10**
Sample packing	**2 x plastic bag**

	Unit	Result	limits acc. GV-SOLAS A-08-2001		Substance	Method
Trace-Elements/Heavy-Metals						
Copper	mg/kg	**10,7**			OM	VDLUFA VII 2.2.2.6
Selenium	mg/kg	**0,22**			OM	VDLUFA VII 2.2.2.5; ICPMS
Cadmium	mg/kg	**0,07**	0,4		OM	VDLUFA VII 2.2.2.5; ICPMS
Lead	mg/kg	**0,21**	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	**0,32**	1		OM	VDLUFA VII 2.2.2.5; ICPMS
Mycotoxins						
Aflatoxine B1	µg/kg	**<1,00**	10		OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine B2	µg/kg	**<1,00**	5		OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine G1	µg/kg	**<1,00**	5		OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine G2	µg/kg	**<1,00**	5		OM	HPLC-VDLUFA Bd. III, 16.1.4
Sum Aflatoxines	µg/kg	**n.d.**			OM	calculated
PCB						
PCB 28	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
PCB 52	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
PCB 101	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
PCB 118	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
PCB 138	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
PCB 153	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
PCB 180	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
sum PCB	mg/kg	**n.d.**	0,05		OM	calculated
Organochlorous-Pesticides GC-Multiresidueanalysis						
Dieldrin	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
HCH-gamma (Lindan)	mg/kg	**<0,005**	0,1		OM	acc. to §64 LFGB L00.00-34
Heptachlor	mg/kg	**<0,00500**			OM	acc. to §64 LFGB L00.00-34
Heptachlorepoxide-cis	mg/kg	**<0,00500**			OM	acc. to §64 LFGB L00.00-34
Heptachlorepoxide-trans	mg/kg	**<0,00500**			OM	acc. to §64 LFGB L00.00-34
o,p-DDD	mg/kg	**<0,00500**			OM	acc. to §64 LFGB L00.00-34

LUFA-ITL GmbH

AGROLAB Laborgruppe
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Date 02.09.2009
Customer no. 1209835
Page 2 of 2

Order no. 633558 Sample No. 808512

	Unit	Result	limits acc. GV-SOLAS A-08-2001		Substance	Method	
o,p-DDE	mg/kg	**<0,00500**			OM	acc. to §64 LFGB L00.00-34	
o,p-DDT	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34	
p,p-DDD	mg/kg	**<0,00500**			OM	acc. to §64 LFGB L00.00-34	
p,p-DDE	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34	
p,p-DDT	mg/kg	**<0,00500**			OM	acc. to §64 LFGB L00.00-34	
Sum DDTs	mg/kg	**n.d.**	0,05		OM	calculated	
Sum Heptachlor	mg/kg	**n.d.**	0,01		OM	calculated	
Organo-Phosphorous Pesticides GC-Multiresidueanalysis							
Malathion	mg/kg	**<0,010**	1		OM	acc. to §64 LFGB L00.00-34	
nitrosamines							
N-Nitrosodibutylamin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
N-Nitrosodiethylamin	µg/kg	**<5,00**	10		OM	GC-Inhousemethod	
N-Nitrosodiisopropylamin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
N-Nitrosodimethylamin	µg/kg	**<5,00**	10		OM	GC-Inhousemethod	
N-Nitrosodipropylamin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
N-Nitrosomethylethylamin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
N-Nitrosomorpholin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
N-Nitrosopiperidin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
N-Nitrosopyrrolidin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
Sum Nitrosamines	µg/kg	**n.d.**			OM	calculated	
Estrogenes							
dienestrol	µg/kg	**<10,0**			OM	no object	v)
diethyl stilbestrol	µg/kg	**<1,00**			OM	no object	v)
hexestrol	µg/kg	**<2,00**			OM	no object	v)
Sum Estrogenes	µg/kg	**n.d.**			OM	calculated	v)

Explanation: "<", n.d.: not detected, below limit of detection .
The actual limit of detection can be different to the standard value for a particular analysis due to matrix effects or insufficient sample volume.
Remark: OM=original matter, DM=dry matter
v) Remitted to an accredited laboratory

LUFA - ITL Dr. E. Hansen, Tel. 0431/1228-130
feeding stuffs

LUFA-ITL Kiel Dr.-Hell-Straße 6

External laboratory

TIERGESUNDHEITSDIENST, SENATOR-GERAUER STR 23, 85586 POING
Parameter
Sum Estrogenes
Zentrale Analytik - Organische Henkel KGaA, Henkelstrasse 67 ¿ Gebäude Z43, 40589 Düsseldorf
Parameter
Sum Nitrosamines

The analytical results are valid for the delivered sample material only. The testing period is the time between the receipt of the sample and the reporting date. Validation of results is not possible for samples of unknown origin .

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LUFA - ITL Dr.-Hell-Str. 6, 24107 Kiel

PROVIMI KLIBA AG
RINAUSTRASSE
4303 KAISERAUGST / SCHWEIZ
SCHWEIZ

Date 09.12.2009
Customer no. 1209835

TEST REPORT

Order no. 665888

Sample No. **53076**
Order No. **GLP Schadstoffuntersuchung**
Sample Arrival **30.11.2009**
Sample code **TEKLAD GLOBAL RODENT 2914 C - Pel. 10 mm eckig**
Alleinfuttermittel für Mäuse und Ratten
Rezeptur 3255 - GLP-Batch 82/09
Fabr.-Code: 0911023 - Hergestellt: 26.11.09 - MHD: 26.08.10
Sample packing **plastic bag**

	Unit	Result	limits acc. GV-SOLAS A-08-2001		Substance	Method
Trace-Elements/Heavy-Metals						
Copper	mg/kg	**12,2**			OM	VDLUFA VII 2.2.2.6
Selenium	mg/kg	**<0,10**			OM	VDLUFA VII 2.2.2.5; ICPMS
Cadmium	mg/kg	**0,07**	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	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine B2	µg/kg	**<1,00**	5		OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine G1	µg/kg	**<1,00**	5		OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine G2	µg/kg	**<1,00**	5		OM	HPLC-VDLUFA Bd. III, 16.1.4
Sum Aflatoxines	µg/kg	**n.d.**			OM	calculated
PCB						
PCB 28	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
PCB 52	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
PCB 101	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
PCB 118	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
PCB 138	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
PCB 153	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
PCB 180	mg/kg	**<0,0020**			OM	acc. to §64 LFGB L00.00-34
sum PCB	mg/kg	**n.d.**	0,05		OM	calculated
Organochlorous-Pesticides GC-Multiresidueanalysis						
Dieldrin	mg/kg	**<0,002**			OM	acc. to §64 LFGB L00.00-34
HCH-gamma (Lindan)	mg/kg	**<0,002**	0,1		OM	acc. to §64 LFGB L00.00-34
Heptachlor	mg/kg	**<0,00200**			OM	acc. to §64 LFGB L00.00-34
Heptachlorepoxide-cis	mg/kg	**<0,00200**			OM	acc. to §64 LFGB L00.00-34
Heptachlorepoxide-trans	mg/kg	**<0,00200**			OM	acc. to §64 LFGB L00.00-34
o,p-DDD	mg/kg	**<0,00200**			OM	acc. to §64 LFGB L00.00-34

LUFA-ITL GmbH

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eMail: zentrale@lufa-itl.de

Date 09.12.2009
Customer no. 1209835

Order no. 665888 Sample No. 53076

	Unit	Result	limits acc. GV-SOLAS A-08-2001		Substance	Method	
o,p-DDE	mg/kg	**<0,00200**			OM	acc. to §64 LFGB L00.00-34	
o,p-DDT	mg/kg	**<0,002**			OM	acc. to §64 LFGB L00.00-34	
p,p-DDD	mg/kg	**<0,00200**			OM	acc. to §64 LFGB L00.00-34	
p,p-DDE	mg/kg	**<0,00200**			OM	acc. to §64 LFGB L00.00-34	
p,p-DDT	mg/kg	**<0,00200**			OM	acc. to §64 LFGB L00.00-34	
Sum DDTs	mg/kg	**n.d.**	0,05		OM	calculated	
Sum Heptachlor	mg/kg	**n.d.**	0,01		OM	calculated	
Organo-Phosphorous Pesticides GC-Multiresidueanalysis							
Malathion	mg/kg	**<0,010**	1		OM	acc. to §64 LFGB L00.00-34	
nitrosamines							
N-Nitrosodibutylamin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
N-Nitrosodiethylamin	µg/kg	**<5,00**	10		OM	GC-Inhousemethod	
N-Nitrosodiisopropylamin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
N-Nitrosodimethylamin	µg/kg	**<5,00**	10		OM	GC-Inhousemethod	
N-Nitrosodipropylamin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
N-Nitrosomethylethylamin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
N-Nitrosomorpholin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
N-Nitrosopiperidin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
N-Nitrosopyrrolidin	µg/kg	**<5,00**			OM	GC-Inhousemethod	
Sum Nitrosamines	µg/kg	**n.d.**			OM	calculated	
Estrogenes							
dienestrol	µg/kg	**<10,0**			OM	no object	**v)**
diethyl stilbestrol	µg/kg	**<1,00**			OM	no object	**v)**
hexestrol	µg/kg	**<2,00**			OM	no object	**v)**
Sum Estrogenes	µg/kg	**n.d.**			OM	calculated	**v)**

Explanation: "<", n.d.: not detected, below limit of detection .
The actual limit of detection can be different to the standard value for a particular analysis due to matrix effects or insufficient sample volume.
Remark: OM=original matter, DM=dry matter
v) Remitted to an accredited laboratory

LUFA - ITL Dr. Wehage, Tel. 0431/1228-220

This electronically transmitted report was checked and released. It's in accordance with the requirements of DIN EN ISO/IEC 17025:2005 for simplified reports and valid without signature.

Copies

PROVIMI KLIBA AG

External laboratory

TIERGESUNDHEITSDIENST, SENATOR-GERAUER STR 23, 85586 POING
Parameter
Sum Estrogenes
Zentrale Analytik - Organische Henkel KGaA, Henkelstrasse 67 ¿ Gebäude Z43, 40589 Düsseldorf
Parameter
Sum Nitrosamines

LUFA-ITL GmbH

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Date 09.12.2009
Customer no. 1209835
Page 3 of 3

Order no. 665888 Sample No. 53076

The analytical results are valid for the delivered sample material only. The testing period is the time between the receipt of the sample and the reporting date. Validation of results is not possible for samples of unknown origin .

APPENDIX II - DRINKING WATER ANALYSIS

harlan™

REVISED
BACTERIOLOGICAL ASSAY OF DRINKING WATER, ITINGEN

Official Laboratory	Liestal, July 28, 2009
Basel-Landschaft	Ref.no. 200076925
Sampling point:	59.99.N Net water Harlan Laboratories Ltd. Itingen, Room No. 10
Sampled on:	July 13, 2009
Sample:	H_2O, Harlan Laboratories Ltd.
Time of sampling	07.25
Water temperature (°C)	16.6

BACTERIOLOGICAL TEST:

Aerobic mesophilic bacteria / ml	0
E.coli / 100 ml	0
Enterococci / 100 ml	0
Costridium perfringens / 100mL	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)

Official Laboratory
The Official Chemist
(b) (6)

(signed Dr. P. Wenk)

harlan™

CHEMICAL WATER ANALYSIS, ITINGEN

Official Laboratory Basel-Landschaft	Liestal, July 28, 2009 Ref. no. 200076926
Sampling point:	59.99.N, Net water Halan Ltd., Itingen, Room no. 10
Sampled on:	July 13, 2009
Time of sampling	07.25
Water temperature (°C)	---

CHEMICAL TEST:

Appearance			clear, colourless
Odor			not remarkable
Taste			not remarkable
UV-absorption at 254 nm/100 cm			1.19
pH value			7.25
Oxygen demand	($KMnO_4$ cons.)	mg/l	2.2
Turbidity	FNU		0.02
Chloride	Cl^-	mg/l	24.0
Nitrate	NO_3^-	mg/l	17.0
Sulphate	SO_4^{--}	mg/l	115.0
Nitrite	NO_2^-	mg/l	<0.005
Total hardness		fr.H°	39.8
Alkaline hardness		fr.H°	27.1
Non carbonate hardness		fr.H°	12.7
Calcium	Ca^{++}	mg/l	139.0
Magnesium	Mg^{++}	mg/l	12.2

ASSESSMENT:

At the time of sampling, the tested chemical parameters met the requirements for drinking water according to "Artikel 3 der Verordnung über Trink-, Quell-, und Mineralwasser (SR 817.022.102)

Official Laboratory
The Official Chemist

(b) (6)

(signed Dr. P. Wen

KANTONALES LABORATORIUM BASEL-LANDSCHAFT

CONTAMINANT ASSAY OF DRINKING WATER, ITINGEN

Harlan Laboratories Study.: C58021

Date of Sampling: July 13, 2009

Sample: H_2O, Harlan Laboratories Ltd., Itingen, Room No. 10

PARAMETER	ASSAY LEVEL µg/l	LIMIT * µg/l
Lindane	< 0.05	0.1
Heptachlor	< 0.05	0.1
Malathion	< 0.05	0.1
DDT, total	< 0.05	0.1
Dieldrin	< 0.05	0.1
Cadmium	< 0.5	5
Arsenic	< 3	50
Lead	< 3	50
Mercury	< 1	1
Selenium	< 3	10
Copper	< 4	1500
PCBs (28, 52, 101, 138, 153, 180)	< 0.05	0.1
Nitrosamines, total (DMN, DEN, NPIP, NMORPH)	< 0.05	-----

< 0.05 = less than 0.05 microgram per liter

* Schweizer Lebensmittelbuch

Issued by for Dr. D. Flade

(b) (6)

September 08, 2009

harlan™

BACTERIOLOGICAL ASSAY OF DRINKING WATER, ITINGEN

Official Laboratory	Liestal, February 02, 2010
Basel-Landschaft	Ref.no. 200082807
Sampling point:	59.99.N Net water Harlan Laboratories Ltd. Itingen, Room No. 10
Sampled on:	January 27, 2010
Sample:	H_2O, Harlan Laboratories Ltd.
Time of sampling	7.35
Water temperature (°C)	9.6

BACTERIOLOGICAL TEST:

Aerobic mesophilic bacteria / ml	14
E.coli / 100 ml	0
Enterococci / 100 ml	0
Costridium perfringens / 100mL	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)

Official Laboratory
The Official Chemist
(b) (6)

(signed Dr. P. Wenk)

CHEMICAL WATER ANALYSIS, ITINGEN

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Official Laboratory
Basel-Landschaft

Liestal, February 17, 2010
Ref. no. 200082808

Sampling point: 35.991.N, Net water
Harlan Laboratories Ltd., Füllinsdorf, Bldg.2

Sampled on: January 27, 2010

Time of sampling 07.45

Water temperature (°C) 9.6°C

CHEMICAL TEST:

Appearance			clear, colourless
Odor			not remarkable
Taste			not remarkable
UV-absorption at 254 nm/100 cm			1.50
Conductivity		µS/cm	616
Oxygen demand	($KMnO_4$ cons.)	mg/l	1.9
Turbidity	FNU		0.59
Chloride	Cl^-	mg/l	15.4
Nitrate	NO_3^-	mg/l	13.2
Sulphate	SO_4^{--}	mg/l	65.0
Nitrite	NO_2^-	mg/l	<0.005
Total hardness		fr.H°	35.3
Alkaline hardness		fr.H°	27.7
Non carbonate hardness		fr.H°	7.6
Calcium	Ca^{++}	mg/l	127.5
Magnesium	Mg^{++}	mg/l	8.4
Fluoride		mg/l	0.11
Dry residue		mg/l	440
Phosphate as P		mg/l	0.007
pH-value			7.15

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)

Official Laboratory
The Official Chemist

(b) (6)

(signed Dr. P. Wenk)

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CONTAMINANT ASSAY OF DRINKING WATER, ITINGEN

Harlan Laboratories Study: C82130

Date of Sampling: January 27, 2010

Sample: H_2O, Harlan Laboratories Ltd., Itingen, Room No. 10

PARAMETER	ASSAY LEVEL µg/l	LIMIT * µg/l
Lindane	< 0.05	0.1
Heptachlor	< 0.05	0.1
Malathion	< 0.05	0.1
DDT, total	< 0.05	0.1
Dieldrin	< 0.05	0.1
Cadmium	< 0.5	5
Arsenic	< 3	50
Lead	< 3	50
Mercury	< 1	1
Selenium	< 3	10
Copper	< 4	1500
PCBs (28, 52, 101, 138, 153, 180)	< 0.05	0.1
Nitrosamines, total (DMN, DEN, NPIP, NMORPH)	< 0.05	-----

< 0.05 = less than 0.05 microgram per liter

* Schweizer Lebensmittelbuch

Issued by Dr. D. Burger

(b) (6)

March 03, 2010

APPENDIX III - FORMULATION ANALYSIS

Determination of Maltogenic Amylase in Dose Formulations

CONTENTS

LIST OF TABLES

1 MATERIALS AND METHODS

1.1 Test Item

Information about the test item EL 2009 083 (test product, dose and mode of administration), used within the in-life phase, are given in the main study report. The test item was used as analytical reference item.

1.2 Study Samples and Storage

Detailed information concerning the in-life phase is provided in the main study report.

Application formulations were prepared at the test site and were transferred to the person responsible for dose formulation analysis (Dr. C. Bachmann, Füllinsdorf / Switzerland). Samples were stored at 2-8 °C after arrival until measurement. For benchtop stability (4h, RT) samples were stored at RT and then stored at 2-8 °C until analysis. For detailed information, see table below:

Dose Formulation Samples	Preparation	Shipment	Arrival	Analysis
10 (Homogeneity)	14-Dec-2009	14-Dec-2009	14-Dec-2009	14-Dec-2009
3 (4h, RT Stability)	14-Dec-2009	14-Dec-2009	14-Dec-2009	14-Dec-2009
3 (7-day, 2 - 8 °C Stability)	14-Dec-2009	14-Dec-2009	14-Dec-2009	21-Dec-2009
10 (Homogeneity)	07-Jan-2010	07-Jan-2010	07-Jan-2010	07-Jan-2010
10 (Homogeneity)	11-Feb-2010	11-Feb-2010	11-Feb-2010	11-Feb-2010
10 (Homogeneity)	01-Apr-2010	01-Apr-2010	01-Apr-2010	06-Apr-2010

1.3 Reagents and Materials

All further reagents (including supplier, grade) and materials used in this study are listed in the raw data.

Reagent	Catalog No	Batch	Supplier
D-Glucose Kit	10716251035	11115200	R-Biopharm / Germany

1.4 Analytical Method

1.4.1 Principle of the Assay

Maltogenic α-amylase catalyses the hydrolysis of (1-4) α-D-glucosidic linkages in maltotriose polysaccharide. A successive remove of α-maltose residues from the non-reducing end of the chains of maltose occurs and glucose is released. Glucose is converted to glucose-6-phosphate by hexokinase in the presence of adenine triphosphate (ATP). Glucose-6-phosphate is then converted to 6-phosphogluconate and NADPH by glucose-6-phosphate dehydrogenase. The released NADPH is measured spectrophotometrically at a wavelength 340 nm. The formation of NADPH is proportional to the glucose formed.

1.4.2 Preparation of Standards and Study Samples

A stock solution of test item (100 mg/mL in water, Batch: AA09087A3) was diluted with dilution solvent to standards of 2500, 5000 and 10000 ng/mL. Quality control samples (QCs) with concentrations of 4000 and/or 8000 ng/mL were prepared by dilution of the highest standard (10000 ng/mL).

Dose formulation samples were diluted with dilution solvent to fit the range of the standard curve.

1.4.3 Procedure

For the determination of the content of maltogenic α-amylase (test item) a spectrophotometric assay was applied.

1. 100 µL substrate (maltotriose: 20 mg/mL in 100 mM citric acid buffer, pH 5.0) was added to 100 µL of standard, quality control or sample and incubated at 37 °C for 30 minutes. (For Blank: 100 µL of dilution solvent was used instead of sample)

2. The reaction was stopped by adding 100 µL of stop solution (120 mM sodium hydroxide).

3. 1 mL of solution 1 from D-Glucose kit (lyophilized powder diluted with 45 mL deionized water) and 2 mL deionized water was added. Solution was mixed and incubated for 2 minutes.

4. Absorption A_{340} (A1) was measured. (Deionized water was used as reference).

5. Suspension 2 (20 µL) was added and incubation solution was mixed.

6. Samples were mixed and incubated for 15 minutes at 37 °C.

7. Absorption A_{340} (A2) was measured.

1.4.4 Evaluation

For calculation the value obtained from measurement A1 was subtracted from that of measurement A2. A linear fitting of the optical density (A2-A1) against the nominal concentration was performed using Microsoft Excel. Blank was subtracted.

2 RESULTS

2.1 In-Study Validation

(See Table 1)

The analytical range of this assay included up to 3 standard concentrations of 2500, 5000 and 10000 ng/mL. The acceptance criteria were fully met as accuracies of 93.9% to 106.8% were obtained for at least 75% of the standard samples and the correlation coefficient was ≥0.985 (total 5 runs).

(See Table 2)

QC samples (QCs) at concentrations of 4000 and/or 8000 ng test item/mL were used in each run (total 5 runs). The acceptance criteria were fully met as accuracies of 93.1% to 115.8% were obtained for at least 67% of the QCs.

2.2 Dose Formulation

2.2.1 Stability

(See Table 3)

After storage at room temperature for 4 hours accuracies of 102.3 to 103.6% were observed for dose formulations.

In dose formulations prepared on 14-Dec-2009 and stored for 7 days at 2-8 °C accuracies of 85.3 to 99.3% were found.

2.2.2 Dose Formulation Samples

(See Table 4)

All results obtained fulfilled the requirements of acceptance criteria. Accuracies of 84.0 to 120.1% (Group 2), 85.2 to 107.8% (Group 3) and 82.8 to 121.2% (Group 4) were observed.

No analyte could be detected in formulation of Group 1 (vehicle).

Table 1 Accuracy of Standards

Analysis	R^2	Std 1 10000 ng/mL	Std 2 5000 ng/mL	Std 3 2500 ng/mL
14-Dec-2009	0.9995	100.3	99.4	105.4
14-Dec-2009		99.9	99.4	96.2
21-Dec-2009	0.9852	106.8	97.6	104.6
21-Dec-2009		93.9	97.9	101.4
07-Jan-2010	0.9973	102.7	n.a.	97.3
07-Jan-2010		97.3	n.a.	102.7
11-Feb-2010	0.9993	99.7	103.4	99.9
11-Feb-2010		100.1	97.9	98.4
06-Apr-2010	0.9985	100.7	102.5	94.3
06-Apr-2010		98.9	99.8	102.5

n.a. = not available

Table 2 Accuracy of QCs

Analysis	QC 1 8000 ng/mL	QC 2 4000 ng/mL
14-Dec-2009	108.0%	n.a.
14-Dec-2009	104.0%	n.a.
21-Dec-2009	105.4%	n.a.
21-Dec-2009	101.9%	n.a.
07-Jan-2010	113.3%	94.6%
07-Jan-2010	115.8%	93.1%
11-Feb-2010	97.6%	95.5%
11-Feb-2010	102.2%	95.5%
06-Apr-2010	103.7%	93.1%
06-Apr-2010	102.5%	104.2%

n.a. = not available

Table 3 Stability of Dose Formulations

Benchtop Stability RT 4 hours				
Arrival	**Analysis**	**Group**	**Sample ID**	**Accuracy [%]**
14-Dec-2009	14-Dec-2009	2	2D	102.8
14-Dec-2009	14-Dec-2009	3	3D	103.6
14-Dec-2009	14-Dec-2009	4	4D	102.3

7-Day Stability 2-8°C				
Arrival	**Analysis**	**Group**	**Sample ID**	**Accuracy [%]**
14-Dec-2009	21-Dec-2009	2	2E	99.3
14-Dec-2009	21-Dec-2009	3	3E	85.3
14-Dec-2009	21-Dec-2009	4	4E	89.6

Table 4 Individual Results

Arrival	Analysis	Group	Sample ID	Accuracy [%]	Homogeneity CV [%]
14-Dec-2009	14-Dec-2009	1	1	n.a.	n.a.
14-Dec-2009	14-Dec-2009	2	2A	105.3	2.87
14-Dec-2009	14-Dec-2009	2	2B	109.6	
14-Dec-2009	14-Dec-2009	2	2C	103.7	
14-Dec-2009	14-Dec-2009	3	3A	107.8	2.68
14-Dec-2009	14-Dec-2009	3	3B	102.6	
14-Dec-2009	14-Dec-2009	3	3C	103.4	
14-Dec-2009	14-Dec-2009	4	4A	107.5	5.9
14-Dec-2009	14-Dec-2009	4	4B	105.6	
14-Dec-2009	14-Dec-2009	4	4C	117.7	

n.a. not applicable (vehicle, no analyte detected)

Table 4 continued

Arrival	Analysis	Group	Sample ID	Accuracy [%]	Homogeneity CV [%]
07-Jan-2010	07-Jan-2010	1	1	n.a.	n.a.
07-Jan-2010	07-Jan-2010	2	2A	86.3	6.25
07-Jan-2010	07-Jan-2010	2	2B	84	
07-Jan-2010	07-Jan-2010	2	2C	94.5	
07-Jan-2010	07-Jan-2010	3	3A	99.2	4.98
07-Jan-2010	07-Jan-2010	3	3B	94.5	
07-Jan-2010	07-Jan-2010	3	3C	104.4	
07-Jan-2010	07-Jan-2010	4	4A	84.8	4.10
07-Jan-2010	07-Jan-2010	4	4B	90.8	
07-Jan-2010	07-Jan-2010	4	4C	84.5	
11-Feb-2010	11-Feb-2010	1	1	n.a.	n.a.
11-Feb-2010	11-Feb-2010	2	2A	89.2	2.43
11-Feb-2010	11-Feb-2010	2	2B	86.3	
11-Feb-2010	11-Feb-2010	2	2C	90.5	
11-Feb-2010	11-Feb-2010	3	3A	85.2	2.08
11-Feb-2010	11-Feb-2010	3	3B	88	
11-Feb-2010	11-Feb-2010	3	3C	88.6	
11-Feb-2010	11-Feb-2010	4	4A	82.8	1.87
11-Feb-2010	11-Feb-2010	4	4B	83.5	
11-Feb-2010	11-Feb-2010	4	4C	85.8	
01-Apr-2010	06-Apr-2010	1	1	n.a.	n.a.
01-Apr-2010	06-Apr-2010	2	2A	110.8	6.42
01-Apr-2010	06-Apr-2010	2	2B	105.9	
01-Apr-2010	06-Apr-2010	2	2C	120.1	
01-Apr-2010	06-Apr-2010	3	3A	99.7	1.60
01-Apr-2010	06-Apr-2010	3	3B	96.9	
01-Apr-2010	06-Apr-2010	3	3C	97.1	
01-Apr-2010	06-Apr-2010	4	4A	112.8	9.24
01-Apr-2010	06-Apr-2010	4	4B	100.7	
01-Apr-2010	06-Apr-2010	4	4C	121.2	

n.a. not applicable (vehicle, no analyte detected)

APPENDIX IV - CLINICAL LABORATORY INVESTIGATIONS

CLINICAL LABORATORY INVESTIGATIONS

Blood and Urine Sampling: After 13 Weeks: 08-Apr-2010

Blood samples were drawn from the retro-orbital plexus 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.

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
Mean corpuscular hemoglobin	MCH	fmol	Advia 120
Mean corpuscular hemoglobin concentration	MCHC	mmol/L	Advia 120

[1] ADVIA 120 hematology system (Bayer)

Parameter	Abbreviation	Unit		Instrumentation
Hemoglobin concentration distribution width	HDW	mmol/L		Advia 120
		relative	absolute	
Reticulocyte count	RETI	rel.1	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
Leukocyte count, total	WBC	G/L		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
Platelet count	PLATELETS	G/L		Advia 120

Hemoglobin Derivatives

Parameter	Abbre-viation	Unit	Method	Instrumentation
Methemoglobin	MET-HB	rel. 1	Spectrometry, results given as ratio of total hemoglobin	OSM3[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] Hemoximeter OSM3

Coagulation

Parameter	Abbre-viation	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	Abbre-viation	Unit	Method	Instrumentation
Glucose		mmol/L	Hexokinase/G6P-DH	Hitachi 917[4]
Urea		mmol/L	Urease/GLDH	Hitachi 917
Creatinine	CREAT	µmol/L	Enzymatic colorimetric test	Hitachi 917
Bilirubin, total	BILI-T	µmol/L	Reaction with 2,5-Dichloro-phenyl-diazonium salt	Hitachi 917
Cholesterol, total	CHOLEST	mmol/L	Enzymatic, CHOD/PAP	Hitachi 917
Triglycerides	TRIGLY	mmol/L	Glycerol-Kinase GPO/PAP method	Hitachi 917
Phospholipids	PHOS-LIP	mmol/L	Phospholipase-Cholinoxidase-Peroxidase-reaction	Hitachi 917
Aspartate aminotransferase EC 2.6.1.1[5]	ASAT	U/L 37 ºC	MDH/NADH coupled reaction	Hitachi 917
Alanine aminotransferase EC 2.6.1.2	ALAT	U/L 37 ºC	LDH/NADH coupled reaction	Hitachi 917
Lactate dehydrogenase EC 1.1.1.27	LDH	U/L 37 ºC	NADH/LDH coupled reaction using pyruvate as substrate	Hitachi 917
Glutamate dehydrogenase EC 1.4.1.3	GLDH	U/L 37 ºC	Standard method, optimized (DGKC)	Hitachi 917
Alkaline phosphatase EC 3.1.3.1	ALP	U/L 37 ºC	p-Nitrophenyl-phosphate as substrate	Hitachi 917
Gamma-glutamyl transferase EC 2.3.2.2	GGT	U/L 37 ºC	Substrate: L-gamma-glutamyl-3-carboxy-4-nitroanilide	Hitachi 917
Creatine kinase EC 2.7.3.2	CK	U/L 37 ºC	HK/ATP and G6P-DH/NADPH coupled reaction method	Hitachi 917

[4] Hitachi 917 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	Abbre-viation	Unit	Method	Instrumentation
Sodium		mmol/L	Ion selective electrode	Hitachi 917
Potassium		mmol/L	Ion selective electrode	Hitachi 917
Chloride		mmol/L	Ion selective electrode	Hitachi 917
Calcium		mmol/L	o-Cresolphthalein complexone method	Hitachi 917
Phosphorus		mmol/L	Phosphomolybdate reaction	Hitachi 917
Protein, total	PROTEIN	g/L	Biuret reaction	Hitachi 917
Albumin		g/L	Bromocresol green method	Hitachi 917
Globulin		g/L	Calculated value (total protein minus albumin)	
Albumin / Globulin Ratio	A/G RATIO		Calculated value (albumin / globulin)	

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 Miditron (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	Miditron[8]
Nitrite		score	0 (negative), 1 (positive)	Miditron
Protein		g/L	0, 0.25, 0.75, 1.50, 5.00	Miditron
Glucose		mmol/L	0, 3, 6, 17, 56	Miditron
Ketones		mmol/L	0, 0.5, 1.5, 5.0, 15.0	Miditron
Urobilinogen	UROBILI	µmol/L	0, 17, 68, 135, 203	Miditron
Bilirubin		µmol /L	0, 17, 50, 100	Miditron
Erythrocytes	ERY	per µL	0, 10, 25, 50, 150, 250	Miditron
Leukocytes	LEU	per µL	0, 25, 100, 500	Miditron

[6] Mettler balance
[7] Clinical Refractometer SU-202, Kernco
[8] Miditron semi-automated urine chemistry analyzer and reagent test strips, Roche Diagnostics

HISTORICAL DATA - HEMATOLOGY

STRAIN: RAT / HanRcc:WIST (MALES) AGE: FROM 19 TO 40 WEEKS
DATA COLLECTION PERIOD: 29-JAN-02 TO 12-JUN-07

PARAMETER	UNIT	N	MEAN	STAND.DEV	95% TOLERANCE LIMITS	
ERYTHROCYTES (RBC)	T/l	1598	8.88	0.46	7.98	9.78
HEMOGLOBIN (HB)	mmol/l	1598	10.0	0.4	9.2	10.8
HEMATOCRIT (HCT)	rel. 1	1598	0.45	0.02	0.41	0.49
MEAN CORPUSCULAR VOLUME (MCV)	fl	1598	50.7	2.4	45.9	55.4
RED CELL VOL. DISTR. WIDTH (RDW)	rel. 1	1544	0.151	0.041	0.117	0.272
MEAN CORPUSCULAR HEMOGLOBIN (MCH)	fmol	1598	1.13	0.06	1.03	1.26
MEAN CORPUSCULAR HEMOGLOBIN CONC.	mmol/l	1598	22.23	1.05	20.45	24.57
HEMOGLOBIN CONC. DISTR. WIDTH	mmol/l	1544	1.79	0.20	1.40	2.14
RETICULOCYTE COUNT.....						
RETICULOCYTE (REL)	rel. 1	1598	0.020	0.004	0.013	0.029
RETICULOCYTE (ABS)	G/l	1598	176	34	119	250
MATURITY INDEX (L-RETI)	rel. 1	1544	0.597	0.123	0.378	0.798
MATURITY INDEX (M-RETI)	rel. 1	1544	0.309	0.056	0.187	0.392
MATURITY INDEX (H-RETI)	rel. 1	1544	0.094	0.088	0.011	0.315
LEUKOCYTES, TOTAL (WBC)	G/l	1598	6.22	1.49	3.74	9.53
DIFF.WBC COUNT (REL).....						
NEUTROPHILS (NEUT)	rel. 1	761	0.209	0.055	0.119	0.339
EOSINOPHILS (EOS)	rel. 1	1598	0.019	0.007	0.010	0.035
BASOPHILS (BASO)	rel. 1	1598	0.004	0.003	0.001	0.013
LYMPHOCYTES (LYMPH)	rel. 1	1598	0.743	0.059	0.611	0.842
MONOCYTES (MONO)	rel. 1	1598	0.022	0.007	0.011	0.038
LARGE UNSTAINED CELLS (LUC)	rel. 1	1598	0.008	0.005	0.002	0.021
DIFF.WBC COUNT (ABS).....						
NEUTROPHILS (NEUT)	G/l	761	1.31	0.45	0.66	2.29
EOSINOPHILS (EOS)	G/l	1598	0.12	0.05	0.05	0.22
BASOPHILS (BASO)	G/l	1598	0.03	0.02	0.00	0.08
LYMPHOCYTES (LYMPH)	G/l	1598	4.64	1.23	2.59	7.39
MONOCYTES (MONO)	G/l	1598	0.14	0.05	0.06	0.26
LARGE UNSTAINED CELLS (LUC)	G/l	1598	0.05	0.04	0.01	0.13
THROMBOCYTES (PLATELETS)	G/l	1598	912	120	708	1168
METHEMOGLOBIN (MET-HB)	rel. 1	634	0.009	0.002	0.005	0.012
HEINZ BODIES	rel. 1	230	0.000	0.000	0.000	0.000
COAGULATION.....						
PROTHROMBIN TIME (PT)	rel. 1	1603	0.81	0.07	0.70	0.97
PARTIAL THROMBOPLASTIN TIME (PTT)	sec	1602	21.2	4.0	14.4	29.9

HISTORICAL DATA - HEMATOLOGY

STRAIN: RAT / HanRcc:WIST (FEMALES) AGE: FROM 19 TO 40 WEEKS
DATA COLLECTION PERIOD: 29-JAN-02 TO 12-JUN-07

PARAMETER	UNIT	N	MEAN	STAND.DEV	95% TOLERANCE LIMITS	
ERYTHROCYTES (RBC)	T/l	1645	8.05	0.41	7.25	8.86
HEMOGLOBIN (HB)	mmol/l	1645	9.6	0.4	8.8	10.4
HEMATOCRIT (HCT)	rel. 1	1645	0.43	0.02	0.39	0.47
MEAN CORPUSCULAR VOLUME (MCV)	fl	1645	53.6	2.4	49.1	58.5
RED CELL VOL. DISTR. WIDTH (RDW)	rel. 1	1589	0.133	0.036	0.105	0.249
MEAN CORPUSCULAR HEMOGLOBIN (MCH)	fmol	1645	1.20	0.05	1.10	1.30
MEAN CORPUSCULAR HEMOGLOBIN CONC.	mmol/l	1645	22.33	0.95	20.64	24.32
HEMOGLOBIN CONC. DISTR. WIDTH	mmol/l	1589	1.46	0.15	1.17	1.76
RETICULOCYTE COUNT.....						
RETICULOCYTE (REL)	rel. 1	1645	0.023	0.006	0.014	0.035
RETICULOCYTE (ABS)	G/l	1645	186	43	114	274
MATURITY INDEX (L-RETI)	rel. 1	1589	0.563	0.124	0.334	0.783
MATURITY INDEX (M-RETI)	rel. 1	1589	0.323	0.056	0.200	0.420
MATURITY INDEX (H-RETI)	rel. 1	1589	0.114	0.111	0.009	0.383
LEUKOCYTES, TOTAL (WBC)	G/l	1645	3.64	1.08	1.91	6.14
DIFF.WBC COUNT (REL).....						
NEUTROPHILS (NEUT)	rel. 1	793	0.197	0.065	0.099	0.343
EOSINOPHILS (EOS)	rel. 1	1645	0.021	0.010	0.009	0.045
BASOPHILS (BASO)	rel. 1	1645	0.004	0.003	0.001	0.012
LYMPHOCYTES (LYMPH)	rel. 1	1645	0.754	0.070	0.598	0.860
MONOCYTES (MONO)	rel. 1	1645	0.020	0.007	0.010	0.038
LARGE UNSTAINED CELLS (LUC)	rel. 1	1645	0.008	0.004	0.002	0.018
DIFF.WBC COUNT (ABS).....						
NEUTROPHILS (NEUT)	G/l	793	0.69	0.25	0.34	1.31
EOSINOPHILS (EOS)	G/l	1645	0.07	0.03	0.03	0.15
BASOPHILS (BASO)	G/l	1645	0.01	0.01	0.00	0.04
LYMPHOCYTES (LYMPH)	G/l	1645	2.77	0.93	1.30	4.86
MONOCYTES (MONO)	G/l	1645	0.07	0.03	0.03	0.15
LARGE UNSTAINED CELLS (LUC)	G/l	1645	0.03	0.02	0.01	0.08
THROMBOCYTES (PLATELETS)	G/l	1645	963	133	723	1235
METHEMOGLOBIN (MET-HB)	rel. 1	644	0.009	0.002	0.005	0.012
HEINZ BODIES	rel. 1	295	0.000	0.000	0.000	0.000
COAGULATION.....						
PROTHROMBIN TIME (PT)	rel. 1	1643	0.83	0.07	0.70	0.98
PARTIAL THROMBOPLASTIN TIME (PTT)	sec	1633	24.1	7.0	13.5	38.2

HISTORICAL DATA - CLINICAL BIOCHEMISTRY

STRAIN: RAT / HanRcc:WIST (MALES) AGE: FROM 19 TO 40 WEEKS
DATA COLLECTION PERIOD: 29-JAN-02 TO 12-JUN-07

PARAMETER	UNIT	N	MEAN	STAND.DEV	95% TOLERANCE LIMITS	
GLUCOSE	mmol/l	1613	5.49	1.07	3.83	8.14
UREA	mmol/l	1612	5.36	0.84	3.99	7.19
CREATININE	µmol/l	1611	27.8	3.3	21.9	35.0
BILIRUBIN, TOTAL	µmol/l	1581	1.69	0.35	1.06	2.42
CHOLESTEROL, TOTAL	mmol/l	1612	1.83	0.39	1.15	2.70
TRIGLYCERIDES	mmol/l	1612	0.46	0.24	0.20	1.08
PHOSPHOLIPIDS	mmol/l	1557	1.56	0.25	1.09	2.07
ASPARTATE AMINOTRANSFERASE (ASAT)	U/l	1613	77.4	13.4	59.7	108.2
ALANINE AMINOTRANSFERASE (ALAT)	U/l	1613	33.8	7.1	22.9	48.9
LACTATE DEHYDROGENASE (LDH)	U/l	1410	200.6	159.2	82.8	547.5
GLUTAMATE-DEHYDROGENASE (GLDH)	U/l	1241	8.4	5.2	4.1	17.6
ALKALINE PHOSPHATASE (ALP)	U/l	1611	58.5	13.3	37.3	87.1
GAMMA-GLUTAMYLTRANSFERASE (GGT)	U/l	1575	0.0	0.1	0.0	0.0
CREATINE KINASE (CK)	U/l	1492	170.8	125.8	80.1	479.8
SODIUM	mmol/l	1612	143.7	3.2	138.5	149.2
POTASSIUM	mmol/l	1612	3.74	0.31	3.22	4.47
CHLORIDE	mmol/l	1612	104.3	2.5	99.9	109.2
CALCIUM	mmol/l	1613	2.76	0.11	2.55	2.97
PHOSPHORUS	mmol/l	1612	1.77	0.20	1.37	2.17
PROTEIN, TOTAL	g/l	1612	67.56	2.85	62.10	73.54
ALBUMIN	g/l	1410	42.20	2.03	38.69	46.22
GLOBULIN	g/l	1410	25.37	2.31	21.15	29.84
A/G RATIO	-	1410	1.68	0.21	1.39	2.06

HISTORICAL DATA - CLINICAL BIOCHEMISTRY

STRAIN: RAT / HanRcc:WIST (FEMALES) AGE: FROM 19 TO 40 WEEKS
DATA COLLECTION PERIOD: 29-JAN-02 TO 12-JUN-07

PARAMETER	UNIT	N	MEAN	STAND.DEV	95% TOLERANCE LIMITS	
GLUCOSE	mmol/l	1642	5.21	0.96	3.67	7.41
UREA	mmol/l	1642	6.54	1.02	4.72	8.70
CREATININE	µmol/l	1641	32.3	4.1	25.0	41.7
BILIRUBIN, TOTAL	µmol/l	1597	2.13	0.57	1.20	3.46
CHOLESTEROL, TOTAL	mmol/l	1640	1.62	0.46	0.85	2.61
TRIGLYCERIDES	mmol/l	1641	0.33	0.11	0.18	0.57
PHOSPHOLIPIDS	mmol/l	1586	1.72	0.39	1.05	2.59
ASPARTATE AMINOTRANSFERASE (ASAT)	U/l	1642	82.7	32.4	56.9	159.8
ALANINE AMINOTRANSFERASE (ALAT)	U/l	1642	32.1	20.2	17.7	77.9
LACTATE DEHYDROGENASE (LDH)	U/l	1428	195.1	107.2	79.3	456.2
GLUTAMATE-DEHYDROGENASE (GLDH)	U/l	1247	15.8	34.9	3.4	81.9
ALKALINE PHOSPHATASE (ALP)	U/l	1639	21.6	6.4	12.1	36.4
GAMMA-GLUTAMYLTRANSFERASE (GGT)	U/l	1593	0.0	0.2	0.0	0.0
CREATINE KINASE (CK)	U/l	1521	153.3	105.1	70.7	414.1
SODIUM	mmol/l	1642	142.7	2.6	137.8	147.8
POTASSIUM	mmol/l	1642	3.27	0.32	2.73	3.90
CHLORIDE	mmol/l	1642	105.1	2.4	100.6	110.3
CALCIUM	mmol/l	1642	2.76	0.12	2.53	2.99
PHOSPHORUS	mmol/l	1642	1.39	0.25	0.90	1.86
PROTEIN, TOTAL	g/l	1642	71.43	4.10	63.62	79.36
ALBUMIN	g/l	1434	49.20	3.39	42.49	55.58
GLOBULIN	g/l	1434	22.33	2.30	18.23	27.08
A/G RATIO	-	1434	2.23	0.39	1.78	2.81

HISTORICAL DATA - URINALYSIS

STRAIN: RAT / HanRcc:WIST (MALES) AGE: FROM 19 TO 40 WEEKS
DATA COLLECTION PERIOD: 29-JAN-02 TO 12-JUN-07

PARAMETER	UNIT	N	MEAN	STAND.DEV	95% TOLERANCE LIMITS	
VOLUME/18h	ml	1551	8.3	4.6	2.2	22.1
RELATIVE DENSITY	rel. 1	699	1.045	0.023	1.014	1.108
pH	-	1600	6.5	0.5	6.0	7.0
PROTEIN	g/l	1600	0.35	0.25	0.00	0.75
GLUCOSE	mmol/l	1600	0	0	0	0
KETONES	mmol/l	1600	0.6	0.6	0.0	1.5
UROBILINOGEN	µmol/l	1600	0	1	0	0
BILIRUBIN	µmol/l	1600	2	5	0	17
ERYTHROCYES	per µl	1600	11	19	0	25
LEUCOCYTES	per µl	1600	28	41	0	100

HISTORICAL DATA - URINALYSIS

STRAIN: RAT / HanRcc:WIST (FEMALES) AGE: FROM 19 TO 40 WEEKS
DATA COLLECTION PERIOD: 29-JAN-02 TO 12-JUN-07

PARAMETER	UNIT	N	MEAN	STAND.DEV	95% TOLERANCE LIMITS	
VOLUME/18h	ml	1581	7.1	5.5	1.5	22.5
RELATIVE DENSITY	rel. 1	711	1.040	0.025	1.010	1.104
pH	-	1630	6.0	0.5	5.0	7.0
PROTEIN	g/l	1630	0.22	0.20	0.00	0.75
GLUCOSE	mmol/l	1630	0	0	0	0
KETONES	mmol/l	1630	0.2	0.3	0.0	1.5
UROBILINOGEN	µmol/l	1630	0	3	0	0
BILIRUBIN	µmol/l	1630	2	5	0	17
ERYTHROCYES	per µl	1630	3	12	0	10
LEUCOCYTES	per µl	1630	6	21	0	25

APPENDIX V - HISTOPATHOLOGY

Histopathology Examination

CONTENTS

LIST OF TABLES

Summary Tables

Individual Tables

[1] Animal organ finding table

1 MATERIALS AND METHODS

1.1 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 2 100	Group 3 300	Group 4 1000
Males	01 - 10	11 - 20	21 - 30	31 - 40
Females	41 - 50	51 - 60	61 - 70	71 - 80

* Control animals were treated with the vehicle only

1.2 Necropsy and Histopathology

Necropsies and histological preparation of the tissues were performed at Harlan Laboratories Ltd., Itingen / Switzerland.
All animals were weighed, anesthetized by intraperitoneal injection of pentobarbitone, sacrificed by exsanguinations, and necropsied. Descriptions of all macroscopic abnormalities were recorded. Samples of the following tissues and organs were collected from all animals at necropsy and, unless otherwise indicated, fixed in neutral phosphate buffered 4% formaldehyde solution:

Tissues / Organs	Weight	Collect	Examine
Adrenal glands	X	X	X
Aorta		X	X
Bone (sternum, femur including joint)		X	X
Bone marrow (femur)		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 Bouin's solution)	X	X	X
Esophagus		X	X
Eyes w/optic nerve (fixed in Davidson's solution)		X	X

Tissues / Organs	Weight	Collect	Examine
Harderian gland (fixed in Davidson's solution)		X	X
Heart including auricles	X	X	X
Ileum, with Peyer's patches		X	X
Jejunum with Peyer's patches		X	X
Kidneys	X	X	X
Larynx		X	X
Lacrimal gland, exorbital		X	X
Liver	X	X	X
Lungs, filled w/formalin at necropsy		X	X
Lymph nodes – mesenteric and mandibular		X	X
Mammary gland area		X	X
Nasal cavity		X	X
Ovaries	X	X	X
Pancreas		X	X
Pharynx		X	X
Pituitary gland		X	X
Prostate gland incl. coagulating glands		X	X
Rectum		X	X
Salivary glands - mandibular, sublingual		X	X
Sciatic nerve		X	X
Seminal vesicles		X	X
Skeletal muscle		X	X
Skin		X	X
Spinal cord - cervical, midthoracic, lumbar		X	X
Spleen	X	X	X
Stomach		X	X
Testes (fixed in Bouin's solution)	X	X	X
Thymus	X	X	X
Thyroid (incl. parathyroid gland, if possible)		X	X
Tongue		X	X
Trachea		X	X
Urinary bladder, filled w/formalin at necropsy		X	X

Tissues / Organs	Weight	Collect	Examine
Uterus with vagina	X	X	X
All gross lesions		X	X

Slides of all organs and tissues (see list above) from all animals of the control and high dose group (group 1 and 4, respectively), and all gross lesions from all animals of all groups were processed, embedded in paraffin, cut at a nominal thickness of 2 - 4 micrometers, stained with hematoxylin & eosin (H&E) and examined by light microscope by the study pathologist.

1.3 Data Compilation

The animal data and necropsy findings were transferred electronically via transfer file into the PathData System. Due to a limited space for the heading data in the PathData System, the test item name "Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083)" was abbreviated to "Maltogenic amylase".

The microscopic findings were recorded during histopathologic examination by the pathologist and directly entered into the PathData System. The slides were evaluated during May 2010.

Histologic changes were described, wherever possible, according to distribution, severity and morphologic character. Severity scores were assigned as given under "Explanation of Codes and Symbols".

All microscopic findings are listed in the "Table of Individual Microscopic Findings", along with an explanation of the codes and symbols used. Computer-generated incidence tables derived from these data are also presented as well as the complete narrative of the macroscopic and microscopic findings.

1.4 Peer Review

The following sections were reviewed by Dr. H. Iwata (Department of Pathology, Harlan Laboratories Ltd., Itingen / Switzerland): all organs and tissues of animals no. 1, 40, 41 and 80.

The assessment of the study pathologist and reviewing pathologist compared favorably.

2 RESULTS

2.1 Mortality

All animals survived their scheduled study period.

2.2 Macroscopic Findings

There were no gross lesions that could be attributed to treatment with the test item. All gross lesions recorded were considered to be within the range of normal background alterations.

2.3 Microscopic Findings

All findings recorded were within the range of normal background lesions which may be recorded in animals of this strain and age.

3 DISCUSSION AND CONCLUSION

All animals survived their scheduled study period. There were no gross lesions that could be attributed to treatment with the test item.

Under the conditions of this study, the test item Maltogenic amylase from *Bacillus stearothermophilus* in *Bacillus subtilis* (EL 2009083) produced no morphological evidence of toxicological properties in the organs and tissues examined.

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SUMMARY TABLES Harlan Laboratories C68821

TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
HEART WITH AURICLES :	10	—	—	10
— Mononuclear foci				
GRADE 1 :	4	—	—	4
GRADE 2 :	1	—	—	2
TOTAL AFFECTED :	5	—	—	6
MEAN SEVERITY :	1.2	—	—	1.3
— Myocardial necrosis				
GRADE 1 :	4	—	—	3
TOTAL AFFECTED :	4	—	—	3
MEAN SEVERITY :	1.0	—	—	1.0
— Myocardial fibrosis				
GRADE 1 :	2	—	—	3
TOTAL AFFECTED :	2	—	—	3
MEAN SEVERITY :	1.0	—	—	1.0
TRACHEA :	10	—	—	10
— Glandular dilation				
GRADE 1 :	1	—	—	1
GRADE 2 :	1	—	—	1
TOTAL AFFECTED :	2	—	—	2
MEAN SEVERITY :	1.5	—	—	1.5
— Mononuclear infiltr.				
GRADE 1 :	5	—	—	4
GRADE 2 :	—	—	—	2
TOTAL AFFECTED :	5	—	—	6
MEAN SEVERITY :	1.0	—	—	1.3

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SUMMARY TABLES Harlan Laboratories C68821

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
LIVER :	10	—	—	10
— Fatty change				
GRADE 1 :	5	—	—	7
TOTAL AFFECTED :	5	—	—	7
MEAN SEVERITY :	1.0	—	—	1.0
— Inflammatory foci				
GRADE 1 :	9	—	—	7
GRADE 2 :	1	—	—	3
TOTAL AFFECTED :	10	—	—	10
MEAN SEVERITY :	1.1	—	—	1.3
— Glycogen increased				
GRADE 1 :	2	—	—	5
TOTAL AFFECTED :	2	—	—	5
MEAN SEVERITY :	1.0	—	—	1.0
— Hematopoietic foci				
GRADE 1 :	1	—	—	2
TOTAL AFFECTED :	1	—	—	2
MEAN SEVERITY :	1.0	—	—	1.0
— Peribiliary inflamm.				
GRADE 1 :	—	—	—	2
GRADE 2 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	2
MEAN SEVERITY :	2.0	—	—	1.0
— Focal necrosis				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0

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SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
SPLEEN :	10	—	—	10
— Extramed.hemopoiesis				
GRADE 1 :	8	—	—	8
GRADE 2 :	2	—	—	2
TOTAL AFFECTED :	10	—	—	10
MEAN SEVERITY :	1.2	—	—	1.2
— Hemosiderin deposits				
GRADE 1 :	6	—	—	7
GRADE 2 :	4	—	—	3
TOTAL AFFECTED :	10	—	—	10
MEAN SEVERITY :	1.4	—	—	1.3
MESENT. LYMPH NODE :	10	—	—	10
— Lymphoid hyperplasia				
GRADE 1 :	3	—	—	3
GRADE 2 :	2	—	—	1
TOTAL AFFECTED :	5	—	—	4
MEAN SEVERITY :	1.4	—	—	1.3
KIDNEYS :	10	—	—	10
— Hyaline droplets				
GRADE 1 :	4	—	—	4
TOTAL AFFECTED :	4	—	—	4
MEAN SEVERITY :	1.0	—	—	1.0
— Tubular basophilia				
GRADE 1 :	4	—	—	4
TOTAL AFFECTED :	4	—	—	4
MEAN SEVERITY :	1.0	—	—	1.0

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SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
KIDNEYS CONT'D.	10	—	—	10
— Mononuclear infiltr.				
GRADE 1 :	7	—	—	3
TOTAL AFFECTED :	7	—	—	3
MEAN SEVERITY :	1.0	—	—	1.0
— Hyaline casts				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
— Pelvic dilation				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0
— Papillary mineraliz.				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
URINARY BLADDER :	10	—	—	10
— Perivasc. infiltrat.				
GRADE 1 :	3	—	—	1
GRADE 2 :	—	—	—	1
TOTAL AFFECTED :	3	—	—	2
MEAN SEVERITY :	1.0	—	—	1.5

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SUMMARY TABLES Harlan Laboratories C68821

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TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
STOMACH :	10	—	—	10
— Glandular dilation				
GRADE 1 :	2	—	—	—
TOTAL AFFECTED :	2	—	—	—
MEAN SEVERITY :	1.0	—	—	—
— Epith. vacuolation				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
— Incr. infl. infiltr.				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0
PEYERS PATCHES JEJ. :	10	—	—	10
— Lymphoid hyperplasia				
GRADE 1 :	6	—	—	3
TOTAL AFFECTED :	6	—	—	3
MEAN SEVERITY :	1.0	—	—	1.0
PEYERS PATCHES ILEUM :	10	—	—	10
— Lymphoid hyperplasia				
GRADE 1 :	9	—	—	8
GRADE 2 :	1	—	—	—
TOTAL AFFECTED :	10	—	—	8
MEAN SEVERITY :	1.1	—	—	1.0

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SUMMARY TABLES Harlan Laboratories C68821

TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
LUNGS :	10	—	—	10
— Alveolar macrophages				
GRADE 1 :	6	—	—	7
GRADE 2 :	1	—	—	—
TOTAL AFFECTED :	7	—	—	7
MEAN SEVERITY :	1.1	—	—	1.0
— Alveolar hemorrhages				
GRADE 1 :	5	—	—	3
TOTAL AFFECTED :	5	—	—	3
MEAN SEVERITY :	1.0	—	—	1.0
— Alveolitis				
GRADE 2 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	2.0
— Vascular mineraliz.				
GRADE 1 :	6	—	—	8
TOTAL AFFECTED :	6	—	—	8
MEAN SEVERITY :	1.0	—	—	1.0
— Perivascular infilt.				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0
— Osseous metaplasia				
GRADE 1 :	1	—	—	3
TOTAL AFFECTED :	1	—	—	3
MEAN SEVERITY :	1.0	—	—	1.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
LUNGS CONT'D.	10	—	—	10
— Mucoid secretion				
GRADE 1 :	1	—	—	1
TOTAL AFFECTED :	1	—	—	1
MEAN SEVERITY :	1.0	—	—	1.0
THYMUS :	9	—	—	10
— Atrophy/involution				
GRADE 1 :	9	—	—	8
TOTAL AFFECTED :	9	—	—	8
MEAN SEVERITY :	1.0	—	—	1.0
EPIDIDYMIDES :	10	—	—	10
— Mononuclear infiltr.				
GRADE 1 :	3	—	—	3
TOTAL AFFECTED :	3	—	—	3
MEAN SEVERITY :	1.0	—	—	1.0
— Vasc./Perivasculitis				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
— Epith. vacuolation				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
PANCREAS :	10	—	1	10
— Mononuclear infiltr.				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0
MANDIB.LYMPH NODE :	10	—	—	10
— Lymphoid hyperplasia				
GRADE 1 :	4	—	—	3
TOTAL AFFECTED :	4	—	—	3
MEAN SEVERITY :	1.0	—	—	1.0
ADRENAL CORTICES :	10	—	—	10
— Vacuolation, Z.fasc.				
GRADE 1 :	1	—	—	1
TOTAL AFFECTED :	1	—	—	1
MEAN SEVERITY :	1.0	—	—	1.0
SCIATIC NERVE :	10	—	—	10
— Single fiber degen.				
GRADE 1 :	2	—	—	2
TOTAL AFFECTED :	2	—	—	2
MEAN SEVERITY :	1.0	—	—	1.0
SKIN/SUBCUTIS :	10	—	—	10
— Inflammatory infilt.				
GRADE 1 :	—	—	—	2
TOTAL AFFECTED :	—	—	—	2
MEAN SEVERITY :	—	—	—	1.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
SKIN/SUBCUTIS CONT'D.	10	—	—	10
— Epiderm. hyperplasia				
GRADE 1 :	1	—	—	1
TOTAL AFFECTED :	1	—	—	1
MEAN SEVERITY :	1.0	—	—	1.0
— Hyperkeratosis				
GRADE 1 :	1	—	—	2
TOTAL AFFECTED :	1	—	—	2
MEAN SEVERITY :	1.0	—	—	1.0
— Ulceration				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
EYES :	10	—	—	10
— Retinal rosette(s)				
GRADE 1 :	2	—	—	2
TOTAL AFFECTED :	2	—	—	2
MEAN SEVERITY :	1.0	—	—	1.0
— Retro—orb.hemorrhage				
GRADE 1 :	5	—	—	4
GRADE 2 :	4	—	—	5
TOTAL AFFECTED :	9	—	—	9
MEAN SEVERITY :	1.4	—	—	1.6
— Peri—orb.inflammat.				
GRADE 1 :	4	—	—	5
TOTAL AFFECTED :	4	—	—	5
MEAN SEVERITY :	1.0	—	—	1.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
HARDERIAN GLANDS :	10	—	—	10
— Porphyrin deposits				
GRADE 1 :	8	—	—	4
TOTAL AFFECTED :	8	—	—	4
MEAN SEVERITY :	1.0	—	—	1.0
— Mononuclear infiltr.				
GRADE 1 :	2	—	—	—
GRADE 2 :	—	—	—	1
TOTAL AFFECTED :	2	—	—	1
MEAN SEVERITY :	1.0	—	—	2.0
SKELETAL MUSCLE :	10	—	—	10
— Mononuclear foci				
GRADE 1 :	6	—	—	6
TOTAL AFFECTED :	6	—	—	6
MEAN SEVERITY :	1.0	—	—	1.0
— Myofiber degenerat.				
GRADE 1 :	5	—	—	6
TOTAL AFFECTED :	5	—	—	6
MEAN SEVERITY :	1.0	—	—	1.0
PROSTATE GLAND :	10	—	—	10
— Mononuclear infiltr.				
GRADE 1 :	2	—	—	—
TOTAL AFFECTED :	2	—	—	—
MEAN SEVERITY :	1.0	—	—	—

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
JOINT :	10	—	—	10
— Chronic inflammation				
GRADE 1 :	—	—	—	1
GRADE 2 :	1	—	—	1
TOTAL AFFECTED :	1	—	—	2
MEAN SEVERITY :	2.0	—	—	1.5
— Synovialitis				
GRADE 1 :	1	—	—	3
GRADE 2 :	1	—	—	—
TOTAL AFFECTED :	2	—	—	3
MEAN SEVERITY :	1.5	—	—	1.0
BONE MARROW, FEMUR :	10	—	—	10
— Fatty replacement				
GRADE 1 :	7	—	—	5
GRADE 2 :	3	—	—	5
TOTAL AFFECTED :	10	—	—	10
MEAN SEVERITY :	1.3	—	—	1.5
EXORBITAL LACR.GLDS. :	10	—	—	10
— Harderian alteration				
GRADE 1 :	4	—	—	2
TOTAL AFFECTED :	4	—	—	2
MEAN SEVERITY :	1.0	—	—	1.0
— Mononuclear infiltr.				
GRADE 1 :	3	—	—	2
TOTAL AFFECTED :	3	—	—	2
MEAN SEVERITY :	1.0	—	—	1.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
EXORBITAL LACR.GLDS. :	10	—	—	10
— Degeneration acinar				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
LARYNX :	10	—	—	10
— Inflamm. infiltrates				
GRADE 1 :	4	—	—	4
GRADE 2 :	3	—	—	3
GRADE 3 :	1	—	—	—
TOTAL AFFECTED :	8	—	—	7
MEAN SEVERITY :	1.6	—	—	1.4
— Luminal hemorrhages				
GRADE 1 :	1	—	—	1
GRADE 2 :	—	—	—	1
TOTAL AFFECTED :	1	—	—	2
MEAN SEVERITY :	1.0	—	—	1.5
— Mucoid secretion				
GRADE 2 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	2.0
— Luminal foreign body				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
NASAL CAVITY,LEV.I :	10	—	—	10
— Luminal hemorrhages				
GRADE 1 :	2	—	—	—
TOTAL AFFECTED :	2	—	—	—
MEAN SEVERITY :	1.0	—	—	—
— Goblet cell hyperpl.				
GRADE 1 :	1	—	—	1
TOTAL AFFECTED :	1	—	—	1
MEAN SEVERITY :	1.0	—	—	1.0
NASAL CAVITY,LEV.III :	10	—	—	10
— Hyaline inclusions				
GRADE 1 :	3	—	—	5
TOTAL AFFECTED :	3	—	—	5
MEAN SEVERITY :	1.0	—	—	1.0
— Luminal hemorrhages				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
— Goblet cell hyperpl.				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
NASAL CAVITY,LEV.IV :	10	—	—	10
— Hyaline inclusions				
GRADE 1 :	1	—	—	3
TOTAL AFFECTED :	1	—	—	3
MEAN SEVERITY :	1.0	—	—	1.0
PHARYNX :	10	—	—	10
— Mononuclear infiltr.				
GRADE 1 :	2	—	—	1
TOTAL AFFECTED :	2	—	—	1
MEAN SEVERITY :	1.0	—	—	1.0
— Mucoid secretion				
GRADE 1 :	3	—	—	1
TOTAL AFFECTED :	3	—	—	1
MEAN SEVERITY :	1.0	—	—	1.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
HEART WITH AURICLES :	10	—	—	10
— Mononuclear foci				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
— Myocardial necrosis				
GRADE 1 :	—	—	—	2
TOTAL AFFECTED :	—	—	—	2
MEAN SEVERITY :	—	—	—	1.0
TRACHEA :	10	—	—	10
— Glandular dilation				
GRADE 1 :	1	—	—	5
TOTAL AFFECTED :	1	—	—	5
MEAN SEVERITY :	1.0	—	—	1.0
— Mononuclear infiltr.				
GRADE 1 :	4	—	—	8
TOTAL AFFECTED :	4	—	—	8
MEAN SEVERITY :	1.0	—	—	1.0
LIVER :	10	—	—	10
— Fatty change				
GRADE 1 :	2	—	—	5
TOTAL AFFECTED :	2	—	—	5
MEAN SEVERITY :	1.0	—	—	1.0
— Inflammatory foci				
GRADE 1 :	10	—	—	9
TOTAL AFFECTED :	10	—	—	9
MEAN SEVERITY :	1.0	—	—	1.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
LIVER CONT'D.	10	—	—	10
— Glycogen increased				
GRADE 1 :	—	—	—	3
TOTAL AFFECTED :	—	—	—	3
MEAN SEVERITY :	—	—	—	1.0
— Hematopoietic foci				
GRADE 1 :	1	—	—	3
TOTAL AFFECTED :	1	—	—	3
MEAN SEVERITY :	1.0	—	—	1.0
— Hepatocell. pigment				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
SPLEEN :	10	—	—	10
— Extramed.hemopoiesis				
GRADE 1 :	6	—	—	7
GRADE 2 :	—	—	—	2
TOTAL AFFECTED :	6	—	—	9
MEAN SEVERITY :	1.0	—	—	1.2
— Hemosiderin deposits				
GRADE 2 :	10	—	—	9
GRADE 3 :	—	—	—	1
TOTAL AFFECTED :	10	—	—	10
MEAN SEVERITY :	2.0	—	—	2.1

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
MESENT. LYMPH NODE :	10	—	—	10
— Lymphoid hyperplasia				
GRADE 1 :	3	—	—	6
GRADE 2 :	1	—	—	—
TOTAL AFFECTED :	4	—	—	6
MEAN SEVERITY :	1.3	—	—	1.0
KIDNEYS :	10	—	—	10
— Corticomed. mineral.				
GRADE 1 :	1	—	—	1
TOTAL AFFECTED :	1	—	—	1
MEAN SEVERITY :	1.0	—	—	1.0
— Mononuclear infiltr.				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
— Papillary mineraliz.				
GRADE 1 :	2	—	—	—
TOTAL AFFECTED :	2	—	—	—
MEAN SEVERITY :	1.0	—	—	—
URINARY BLADDER :	10	—	—	10
— Perivasc. infiltrat.				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
STOMACH :	10	—	—	10
— Glandular dilation				
GRADE 1 :	2	—	—	2
TOTAL AFFECTED :	2	—	—	2
MEAN SEVERITY :	1.0	—	—	1.0
— Focal inflammation				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0
PEYERS PATCHES JEJ. :	8	—	—	10
— Lymphoid hyperplasia				
GRADE 1 :	1	—	—	2
TOTAL AFFECTED :	1	—	—	2
MEAN SEVERITY :	1.0	—	—	1.0
— Mineralization				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0
PEYERS PATCHES ILEUM :	10	—	—	10
— Lymphoid hyperplasia				
GRADE 1 :	3	—	—	7
TOTAL AFFECTED :	3	—	—	7
MEAN SEVERITY :	1.0	—	—	1.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
LUNGS :	10	—	—	10
— Alveolar macrophages				
GRADE 1 :	7	—	—	7
TOTAL AFFECTED :	7	—	—	7
MEAN SEVERITY :	1.0	—	—	1.0
— Alveolar hemorrhages				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0
— Alveolitis				
GRADE 2 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	2.0
— Vascular mineraliz.				
GRADE 1 :	3	—	—	4
TOTAL AFFECTED :	3	—	—	4
MEAN SEVERITY :	1.0	—	—	1.0
— Mononuclear foci				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
— Mucoid secretion				
GRADE 1 :	3	—	—	1
TOTAL AFFECTED :	3	—	—	1
MEAN SEVERITY :	1.0	—	—	1.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
LUNGS CONT'D.	10	—	—	10
— Bronch. hyperplasia				
GRADE 2 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	2.0
THYMUS :	10	1	—	10
— Atrophy/involution				
GRADE 1 :	10	1	—	9
TOTAL AFFECTED :	10	1	—	9
MEAN SEVERITY :	1.0	1.0	—	1.0
— Hemorrhages				
GRADE 1 :	—	—	—	2
GRADE 2 :	1	1	—	—
TOTAL AFFECTED :	1	1	—	2
MEAN SEVERITY :	2.0	2.0	—	1.0
PANCREAS :	10	—	—	10
— Acinar cell atrophy				
GRADE 2 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	2.0	—	—	—
MANDIB.LYMPH NODE :	10	1	—	10
— Lymphoid hyperplasia				
GRADE 1 :	1	1	—	1
TOTAL AFFECTED :	1	1	—	1
MEAN SEVERITY :	1.0	1.0	—	1.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
SCIATIC NERVE :	10	—	—	10
— Single fiber degen.				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
SKIN/SUBCUTIS :	10	—	—	10
— Inflammatory infilt.				
GRADE 1 :	2	—	—	2
TOTAL AFFECTED :	2	—	—	2
MEAN SEVERITY :	1.0	—	—	1.0
EYES :	10	—	—	10
— Retro—orb.hemorrhage				
GRADE 1 :	2	—	—	4
GRADE 2 :	5	—	—	5
GRADE 3 :	2	—	—	1
TOTAL AFFECTED :	9	—	—	10
MEAN SEVERITY :	2.0	—	—	1.7
— Peri—orb.inflammat.				
GRADE 1 :	2	—	—	5
TOTAL AFFECTED :	2	—	—	5
MEAN SEVERITY :	1.0	—	—	1.0
HARDERIAN GLANDS :	10	—	—	10
— Porphyrin deposits				
GRADE 1 :	6	—	—	3
TOTAL AFFECTED :	6	—	—	3
MEAN SEVERITY :	1.0	—	—	1.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
HARDERIAN GLANDS :	10	—	—	10
— Mononuclear infiltr.				
GRADE 1 :	1	—	—	2
TOTAL AFFECTED :	1	—	—	2
MEAN SEVERITY :	1.0	—	—	1.0
SKELETAL MUSCLE :	10	—	—	10
— Mononuclear foci				
GRADE 1 :	6	—	—	4
TOTAL AFFECTED :	6	—	—	4
MEAN SEVERITY :	1.0	—	—	1.0
— Myofiber degenerat.				
GRADE 1 :	2	—	—	4
TOTAL AFFECTED :	2	—	—	4
MEAN SEVERITY :	1.0	—	—	1.0
TONGUE :	10	—	—	10
— Mononuclear infiltr.				
GRADE 1 :	2	—	—	—
TOTAL AFFECTED :	2	—	—	—
MEAN SEVERITY :	1.0	—	—	—
VAGINA :	10	—	—	10
— Cystic mucosal deg.				
GRADE 1 :	1	—	—	—
GRADE 2 :	1	—	—	1
TOTAL AFFECTED :	2	—	—	1
MEAN SEVERITY :	1.5	—	—	2.0

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
VAGINA CONT'D.	10	—	—	10
— Mucification				
GRADE 2 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	2.0	—	—	—
JOINT :	10	—	—	10
— Synovialitis				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0
BONE MARROW, FEMUR :	10	—	—	10
— Fatty replacement				
GRADE 1 :	8	—	—	8
GRADE 2 :	2	—	—	2
TOTAL AFFECTED :	10	—	—	10
MEAN SEVERITY :	1.2	—	—	1.2
EXORBITAL LACR.GLDS. :	10	—	—	10
— Harderian alteration				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0
LARYNX :	10	—	—	10
— Inflamm. infiltrates				
GRADE 1 :	4	—	—	3
GRADE 2 :	1	—	—	2
TOTAL AFFECTED :	5	—	—	5
MEAN SEVERITY :	1.2	—	—	1.4

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
LARYNX CONT'D.	10	—	—	10
— Luminal hemorrhages				
GRADE 1 :	2	—	—	1
TOTAL AFFECTED :	2	—	—	1
MEAN SEVERITY :	1.0	—	—	1.0
— Mucoid secretion				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—
NASAL CAVITY,LEV.I :	10	—	—	10
— Goblet cell hyperpl.				
GRADE 1 :	—	—	—	2
TOTAL AFFECTED :	—	—	—	2
MEAN SEVERITY :	—	—	—	1.0
— Inflamm. infiltrates				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0
NASAL CAVITY,LEV.II :	10	—	—	10
— Inflamm. infiltrates				
GRADE 1 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	—
MEAN SEVERITY :	1.0	—	—	—

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SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
NASAL CAVITY,LEV.III :	10	—	—	10
— Hyaline inclusions				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0
— Goblet cell hyperpl.				
GRADE 1 :	1	—	—	1
TOTAL AFFECTED :	1	—	—	1
MEAN SEVERITY :	1.0	—	—	1.0
NASAL CAVITY,LEV.IV :	10	—	—	10
— Hyaline inclusions				
GRADE 1 :	—	—	—	2
TOTAL AFFECTED :	—	—	—	2
MEAN SEVERITY :	—	—	—	1.0
PHARYNX :	10	—	—	10
— Mucoid secretion				
GRADE 1 :	—	—	—	1
TOTAL AFFECTED :	—	—	—	1
MEAN SEVERITY :	—	—	—	1.0
— Luminal hemorrhages				
GRADE 1 :	—	—	—	1
GRADE 2 :	1	—	—	—
TOTAL AFFECTED :	1	—	—	1
MEAN SEVERITY :	2.0	—	—	1.0

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
HEART WITH AURICLES :	10	—	—	10
— Mononuclear foci :	5	—	—	6
— Myocardial necrosis :	4	—	—	3
— Myocardial fibrosis :	2	—	—	3
TRACHEA :	10	—	—	10
— Glandular dilation :	2	—	—	2
— Mononuclear infiltr.:	5	—	—	6
LIVER :	10	—	—	10
— Fatty change :	5	—	—	7
— Inflammatory foci :	10	—	—	10
— Glycogen increased :	2	—	—	5
— Hematopoietic foci :	1	—	—	2
— Peribiliary inflamm.:	1	—	—	2
— Focal necrosis :	—	—	—	1
SPLEEN :	10	—	—	10
— Extramed.hemopoiesis:	10	—	—	10
— Hemosiderin deposits:	10	—	—	10
MESENT. LYMPH NODE :	10	—	—	10
— Lymphoid hyperplasia:	5	—	—	4
KIDNEYS :	10	—	—	10
— Hyaline droplets :	4	—	—	4
— Tubular basophilia :	4	—	—	4
— Mononuclear infiltr.:	7	—	—	3
— Hyaline casts :	1	—	—	—
— Pelvic dilation :	—	—	—	1
— Papillary mineraliz.:	1	—	—	—
URINARY BLADDER :	10	—	—	10
— Perivasc. infiltrat.:	3	—	—	2

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
STOMACH :	10	—	—	10
— Glandular dilation :	2	—	—	—
— Epith. vacuolation :	1	—	—	—
— Incr. infl. infiltr.:	—	—	—	1
PEYERS PATCHES JEJ. :	10	—	—	10
— Lymphoid hyperplasia:	6	—	—	3
PEYERS PATCHES ILEUM :	10	—	—	10
— Lymphoid hyperplasia:	10	—	—	8
LUNGS :	10	—	—	10
— Alveolar macrophages:	7	—	—	7
— Alveolar hemorrhages:	5	—	—	3
— Alveolitis :	—	—	—	1
— Vascular mineraliz. :	6	—	—	8
— Perivascular infilt.:	—	—	—	1
— Osseous metaplasia :	1	—	—	3
— Mucoid secretion :	1	—	—	1
THYMUS :	9	—	—	10
— Cyst (s) :	6	—	—	4
— Atrophy/involution :	9	—	—	8
— No histol. correlate:	—	—	—	1
TESTES :	10	1	—	10
— No histol. correlate:	—	1	—	—
— Fibrotic nodule :	—	—	—	1
EPIDIDYMIDES :	10	—	—	10
— Mononuclear infiltr.:	3	—	—	3
— Vasc./Perivasculitis:	1	—	—	—
— Epith. vacuolation :	—	—	—	1
PANCREAS :	10	—	1	10
— Mononuclear infiltr.:	—	—	—	1
— Congestion :	—	—	1	—

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
MANDIB.LYMPH NODE :	10	—	—	10
— Lymphoid hyperplasia:	4	—	—	3
THYROID GLAND :	10	—	—	10
— Ductal remnants :	2	—	—	2
— Thymic ectopia :	—	—	—	1
ADRENAL CORTICES :	10	—	—	10
— Vacuolation, Z.fasc.:	1	—	—	1
— Extra—adrenal tissue:	—	—	—	1
SCIATIC NERVE :	10	—	—	10
— Single fiber degen. :	2	—	—	2
SKIN/SUBCUTIS :	10	—	—	10
— Inflammatory infilt.:	—	—	—	2
— Epiderm. hyperplasia:	1	—	—	1
— Hyperkeratosis :	1	—	—	2
— Ulceration :	1	—	—	—
EYES :	10	—	—	10
— Retinal rosette(s) :	2	—	—	2
— Retro—orb.hemorrhage:	9	—	—	9
— Peri—orb.inflammat. :	4	—	—	5
— Hyaloid remnants :	1	—	—	1
HARDERIAN GLANDS :	10	—	—	10
— Porphyrin deposits :	8	—	—	4
— Mononuclear infiltr.:	2	—	—	1
SKELETAL MUSCLE :	10	—	—	10
— Mononuclear foci :	6	—	—	6
— Myofiber degenerat. :	5	—	—	6
PROSTATE GLAND :	10	—	—	10
— Mononuclear infiltr.:	2	—	—	—

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				MALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
JOINT :	10	—	—	10
— Chronic inflammation:	1	—	—	2
— Synovialitis :	2	—	—	3
BONE MARROW, FEMUR :	10	—	—	10
— Fatty replacement :	10	—	—	10
EXORBITAL LACR.GLDS. :	10	—	—	10
— Harderian alteration:	4	—	—	2
— Mononuclear infiltr.:	3	—	—	2
— Degeneration acinar :	1	—	—	—
LARYNX :	10	—	—	10
— Inflamm. infiltrates:	8	—	—	7
— Luminal hemorrhages :	1	—	—	2
— Mucoid secretion :	—	—	—	1
— Luminal foreign body:	—	—	—	1
NASAL CAVITY,LEV.I :	10	—	—	10
— Luminal hemorrhages :	2	—	—	—
— Goblet cell hyperpl.:	1	—	—	1
NASAL CAVITY,LEV.III :	10	—	—	10
— Hyaline inclusions :	3	—	—	5
— Luminal hemorrhages :	1	—	—	—
— Goblet cell hyperpl.:	1	—	—	—
NASAL CAVITY,LEV.IV :	10	—	—	10
— Hyaline inclusions :	1	—	—	3
PHARYNX :	10	—	—	10
— Mononuclear infiltr.:	2	—	—	1
— Mucoid secretion :	3	—	—	1
PITUITARY GLAND :	10	—	—	10
— Cystic cleft :	—	—	—	2

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
HEART WITH AURICLES :	10	—	—	10
— Mononuclear foci :	1	—	—	—
— Myocardial necrosis :	—	—	—	2
TRACHEA :	10	—	—	10
— Glandular dilation :	1	—	—	5
— Mononuclear infiltr.:	4	—	—	8
LIVER :	10	—	—	10
— Fatty change :	2	—	—	5
— Inflammatory foci :	10	—	—	9
— Glycogen increased :	—	—	—	3
— Hematopoietic foci :	1	—	—	3
— Hepatocell. pigment :	1	—	—	—
SPLEEN :	10	—	—	10
— Extramed.hemopoiesis:	6	—	—	9
— Hemosiderin deposits:	10	—	—	10
MESENT. LYMPH NODE :	10	—	—	10
— Lymphoid hyperplasia:	4	—	—	6
KIDNEYS :	10	—	—	10
— Corticomed. mineral.:	1	—	—	1
— Mononuclear infiltr.:	1	—	—	—
— Papillary mineraliz.:	2	—	—	—
URINARY BLADDER :	10	—	—	10
— Perivasc. infiltrat.:	1	—	—	—
STOMACH :	10	—	—	10
— Glandular dilation :	2	—	—	2
— Focal inflammation :	—	—	—	1
PEYERS PATCHES JEJ. :	8	—	—	10
— Lymphoid hyperplasia:	1	—	—	2
— Mineralization :	—	—	—	1

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
PEYERS PATCHES ILEUM :	10	—	—	10
— Lymphoid hyperplasia:	3	—	—	7
LUNGS :	10	—	—	10
— Alveolar macrophages:	7	—	—	7
— Alveolar hemorrhages:	—	—	—	1
— Alveolitis :	—	—	—	1
— Vascular mineraliz. :	3	—	—	4
— Mononuclear foci :	1	—	—	—
— Mucoid secretion :	3	—	—	1
— Bronch. hyperplasia :	—	—	—	1
THYMUS :	10	1	—	10
— Cyst (s) :	10	1	—	7
— Atrophy/involution :	10	1	—	9
— Hemorrhages :	1	1	—	2
PANCREAS :	10	—	—	10
— Acinar cell atrophy :	1	—	—	—
MANDIB.LYMPH NODE :	10	1	—	10
— Lymphoid hyperplasia:	1	1	—	1
— No histol. correlate:	1	1	—	—
THYROID GLAND :	10	—	—	10
— Ductal remnants :	1	—	—	1
— Thymic ectopia :	1	—	—	2
ADRENAL CORTICES :	10	—	—	10
— Extra—adrenal tissue:	1	—	—	2
SCIATIC NERVE :	10	—	—	10
— Single fiber degen. :	1	—	—	—
SKIN/SUBCUTIS :	10	—	—	10
— Inflammatory infilt.:	2	—	—	2

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
EYES :	10	—	—	10
— Retro—orb.hemorrhage:	9	—	—	10
— Peri—orb.inflammat. :	2	—	—	5
— Hyaloid remnants :	1	—	—	—
HARDERIAN GLANDS :	10	—	—	10
— Porphyrin deposits :	6	—	—	3
— Mononuclear infiltr.:	1	—	—	2
SKELETAL MUSCLE :	10	—	—	10
— Mononuclear foci :	6	—	—	4
— Myofiber degenerat. :	2	—	—	4
TONGUE :	10	—	—	10
— Mononuclear infiltr.:	2	—	—	—
UTERUS :	10	—	—	10
— Distended lumina :	—	—	—	2
VAGINA :	10	—	—	10
— Cycle:Proestrus :	1	—	—	4
— Cycle:Estrus :	2	—	—	4
— Cycle:Metestrus :	6	—	—	2
— Cycle:Diestrus :	1	—	—	—
— Cystic mucosal deg. :	2	—	—	1
— Mucification :	1	—	—	—
JOINT :	10	—	—	10
— Synovialitis :	—	—	—	1
BONE MARROW, FEMUR :	10	—	—	10
— Fatty replacement :	10	—	—	10
EXORBITAL LACR.GLDS. :	10	—	—	10
— Harderian alteration:	—	—	—	1

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

SEX :				FEMALE
DOSE GROUP:	01	02	03	04
NO.ANIMALS:	10	10	10	10
LARYNX :	10	—	—	10
— Inflamm. infiltrates:	5	—	—	5
— Luminal hemorrhages :	2	—	—	1
— Mucoid secretion :	1	—	—	—
NASAL CAVITY,LEV.I :	10	—	—	10
— Goblet cell hyperpl.:	—	—	—	2
— Inflamm. infiltrates:	—	—	—	1
NASAL CAVITY,LEV.II :	10	—	—	10
— Inflamm. infiltrates:	1	—	—	—
NASAL CAVITY,LEV.III :	10	—	—	10
— Hyaline inclusions :	—	—	—	1
— Goblet cell hyperpl.:	1	—	—	1
NASAL CAVITY,LEV.IV :	10	—	—	10
— Hyaline inclusions :	—	—	—	2
PHARYNX :	10	—	—	10
— Mucoid secretion :	—	—	—	1
— Luminal hemorrhages :	1	—	—	1
PITUITARY GLAND :	10	—	—	10
— Cyst :	1	—	—	—
— Cystic cleft :	1	—	—	—

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CORRELATION TABLE: NECROPSY — MICROSCOPY DOSE GROUP 01, FEMALE

NECROPSY OBSERVATION	CORRESPONDING MICROSCOPIC FINDING
	ANIMAL NO: 44
THYMUS	
— 01: FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.	— Hemorrhages, focal/multifocal, grade 2.
	ANIMAL NO: 48
MANDIBULAR LYMPH NODE	
— 01: FOCUS/FOCI, ISOLATED, D=1 MM, DARK RED.	— No histological correlate, bilateral.

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CORRELATION TABLE: NECROPSY — MICROSCOPY DOSE GROUP 02, MALE

NECROPSY OBSERVATION	CORRESPONDING MICROSCOPIC FINDING
	ANIMAL NO: 11
TESTES	
— 01: BOTH SIDES: REDUCED IN SIZE, D=20X10 MM.	— No histological correlate, bilateral.

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CORRELATION TABLE: NECROPSY — MICROSCOPY DOSE GROUP 02, FEMALE

NECROPSY OBSERVATION	CORRESPONDING MICROSCOPIC FINDING
	ANIMAL NO: 53
MANDIBULAR LYMPH NODE	
— 01: FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.	— No histological correlate, bilateral.
	ANIMAL NO: 57
THYMUS	
— 01: FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.	— Hemorrhages, focal/multifocal, grade 2.

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CORRELATION TABLE: NECROPSY — MICROSCOPY DOSE GROUP 03, MALE

NECROPSY OBSERVATION	CORRESPONDING MICROSCOPIC FINDING
	ANIMAL NO: 23
PANCREAS	
— 01: DISCOLORATION, REDDISH.	— Congestion.

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CORRELATION TABLE: NECROPSY — MICROSCOPY DOSE GROUP 04, MALE

NECROPSY OBSERVATION	CORRESPONDING MICROSCOPIC FINDING
	ANIMAL NO: 31
THYMUS	
— 01: FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.	— No histological correlate.
	ANIMAL NO: 36
KIDNEYS	
— 01: RIGHT SIDE: PELVIC DILATION.	— Pelvic dilation, unilateral, grade 1.

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CORRELATION TABLE: NECROPSY — MICROSCOPY DOSE GROUP 04, FEMALE

NECROPSY OBSERVATION	CORRESPONDING MICROSCOPIC FINDING
	ANIMAL NO: 72
EYES	
— 01: RIGHT SIDE: DESTROYED DURING BLOOD COLLECTION.	— Retro—orbital hemorrhage, unilateral, grade 3.

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EXPLANATION OF CODES AND SYMBOLS

CODES AND SYMBOLS USED AT ANIMAL LEVEL:

M = Male animal
F = Female animal
K0 = Terminal sacrifice group

CODES AND SYMBOLS USED AT ORGAN LEVEL:

G = Gross observation checked off histologically
0 = Tissue not present for histologic examination
' = Histologic examination not required
+ = Organ examined, findings present
— = Organ examined, no pathologic findings noted (AOFT only)
(= Only one of paired organs examined/present

CODES AND SYMBOLS USED AT FINDING LEVEL:

GRADE 1 = Minimal / very few / very small
GRADE 2 = Slight / few / small
GRADE 3 = Moderate / moderate number / moderate size
P = Finding present, severity not scored
(= Finding unilateral in paired organs

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, 0 mg/kg

ANIMAL NUMBER :	1	2	3	4	5	6	7	8	9	10
	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0
HEART WITH AURICLES	—	+	+	—	+	—	—	+	+	+
— Mononuclear foci	.	1.	1.	.	1.	.	.	2.	.	1.
— Myocardial necrosis	.	.	1.	.	.	.	.	1.	1.	1.
— Myocardial fibrosis	.	.	.	.	.	.	.	1.	1.	.
AORTA	—	—	—	—	—	—	—	—	—	—
ESOPHAGUS	—	—	—	—	—	—	—	—	—	—
TRACHEA	—	+	—	—	+	—	—	+	+	+
— Glandular dilation	.	1.	.	.	2.	.	.	.	.	.
— Mononuclear infiltr.	.	1.	.	.	1.	.	.	1.	1.	1.
LIVER	+	+	+	+	+	+	+	+	+	+
— Fatty change	1.	.	.	1.	.	1.	.	.	1.	1.
— Inflammatory foci	1.	1.	2.	1.	1.	1.	1.	1.	1.	1.
— Glycogen increased	.	.	.	.	.	.	1.	1.	.	.
— Hematopoietic foci	.	.	1.	.	.	.	.	.	.	.
— Peribiliary inflamm.	.	.	.	.	2.	.	.	.	.	.
SPLEEN	+	+	+	+	+	+	+	+	+	+
— Extramed.hemopoiesis	1.	1.	1.	2.	2.	1.	1.	1.	1.	1.
— Hemosiderin deposits	1.	2.	1.	2.	1.	2.	1.	1.	1.	2.
MESENT. LYMPH NODE	+	—	+	+	+	—	+	—	—	—
— Lymphoid hyperplasia	2.	.	1.	1.	1.	.	2.	.	.	.
KIDNEYS	+	+	+	+	+	+	+	+	+	+
— Hyaline droplets	.	1.	.	1.	.	(1.	.	1.	.	.
— Tubular basophilia	(1.	.	.	.	(1.	(1.	.	.	(1.	.
— Mononuclear infiltr.	(1.	.	(1.	(1.	(1.	.	(1.	(1.	.	1.
— Hyaline casts	.	.	.	.	.	(1.	.	.	.	.
— Papillary mineraliz.	.	.	.	.	(1.	.	.	.	.	.
URINARY BLADDER	+	+	—	—	+	—	—	—	—	—
— Perivasc. infiltrat.	1.	1.	.	.	1.	.	.	.	.	.

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, 0 mg/kg

ANIMAL NUMBER :	1	2	3	4	5	6	7	8	9	10
	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0
STOMACH	+	—	—	—	—	+	—	—	—	+
— Glandular dilation	.	.	.	.	.	1.	.	.	.	1.
— Epith. vacuolation	1.	.	.	.	.	.	.	.	.	.
DUODENUM	—	—	—	—	—	—	—	—	—	—
JEJUNUM	—	—	—	—	—	—	—	—	—	—
PEYERS PATCHES JEJ.	+	+	—	+	+	—	—	—	+	+
— Lymphoid hyperplasia	1.	1.	.	1.	1.	.	.	.	1.	1.
ILEUM	—	—	—	—	—	—	—	—	—	—
PEYERS PATCHES ILEUM	+	+	+	+	+	+	+	+	+	+
— Lymphoid hyperplasia	1.	1.	1.	1.	1.	1.	2.	1.	1.	1.
CECUM	—	—	—	—	—	—	—	—	—	—
COLON	—	—	—	—	—	—	—	—	—	—
RECTUM	—	—	—	—	—	—	—	—	—	—
LUNGS	+	+	+	+	—	+	+	+	+	+
— Alveolar macrophages	1.	1.	1.	1.	.	2.	1.	.	1.	.
— Alveolar hemorrhages	1.	1.	1.	.	.	1.	.	.	.	1.
— Vascular mineraliz.	1.	1.	.	.	.	.	1.	1.	1.	1.
— Osseous metaplasia	.	1.	.	.	.	.	.	.	.	.
— Mucoid secretion	.	.	.	.	.	.	.	.	1.	.
THYMUS	+	+	+	+	+	+	0	+	+	+
— Cyst (s)	.	P.	.	P.	P.	P.	.	.	P.	P.
— Atrophy/involution	1.	1.	1.	1.	1.	1.	.	1.	1.	1.
TESTES	—	—	—	—	—	—	—	—	—	—
EPIDIDYMIDES	+	—	—	+	—	+	—	—	+	—
— Mononuclear infiltr.	1.	.	.	.	.	(1.	.	.	(1.	.
— Vasc./Perivasculitis	.	.	.	(1.	.	.	.	.	.	.

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, 0 mg/kg

ANIMAL NUMBER :	1	2	3	4	5	6	7	8	9	10
	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0
MANDIBULAR GLANDS	—	—	—	—	—	—	—	—	—	—
SUBLINGUAL GLANDS	—	—	(—	—	—	—	(—	(—	—	—
PANCREAS	—	—	—	—	—	—	—	—	—	—
MANDIB.LYMPH NODE	—	+	—	+	—	+	—	—	—	+
— Lymphoid hyperplasia	.	1.	.	1.	.	1.	.	.	.	1.
THYROID GLAND	—	—	+	—	—	—	—	+	—	—
— Ductal remnants	.	.	P.	.	.	.	.	(P.	.	.
PARATHYROID GLANDS	(—	(—	(—	—	(—	—	—	(—	(—	(—
ADRENAL CORTICES	+	—	—	—	—	—	—	—	—	—
— Vacuolation, Z.fasc.	1.	.	.	.	.	.	.	.	.	.
ADRENAL MEDULLAS	—	—	—	—	—	—	—	—	—	—
SCIATIC NERVE	—	—	+	—	—	+	—	—	—	—
— Single fiber degen.	.	.	1.	.	.	1.	.	.	.	.
SKIN/SUBCUTIS	—	—	+	—	+	—	—	—	—	—
— Epiderm. hyperplasia	.	.	1.	.	.	.	.	.	.	.
— Hyperkeratosis	.	.	1.	.	.	.	.	.	.	.
— Ulceration	.	.	.	.	1.	.	.	.	.	.
MAMMARY GLAND AREA	—	0	—	—	—	—	—	—	—	—
EYES	+	+	+	+	—	+	+	+	+	+
— Retinal rosette(s)	.	(1.	.	.	.	.	.	.	(1.	.
— Retro—orb.hemorrhage	(1.	(2.	(1.	(1.	.	(2.	(2.	(2.	(1.	(1.
— Peri—orb.inflammat.	.	.	.	(1.	.	(1.	.	(1.	.	(1.
— Hyaloid remnants	.	.	.	.	.	.	(P.	.	.	.
OPTIC NERVES	(—	—	—	(—	—	—	(—	(—	—	—

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, 0 mg/kg

ANIMAL NUMBER :	1	2	3	4	5	6	7	8	9	10
	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0
HARDERIAN GLANDS	+	+	+	+	+	+	—	+	+	+
— Porphyrin deposits	(1.	(1.	1.	.	1.	(1.	.	1.	(1.	1.
— Mononuclear infiltr.	.	(1.	.	(1.	.	.	.	.	.	.
CEREBRUM	—	—	—	—	—	—	—	—	—	—
CEREBELLUM	—	—	—	—	—	—	—	—	—	—
MEDULLA OBLONGATA	—	—	—	—	—	—	—	—	—	—
PONS	—	—	—	—	—	—	—	—	—	—
SKELETAL MUSCLE	+	+	+	+	+	+	+	—	—	+
— Mononuclear foci	1.	1.	.	1.	.	1.	1.	.	.	1.
— Myofiber degenerat.	1.	.	1.	1.	1.	.	.	.	.	1.
TONGUE	—	—	—	—	—	—	—	—	—	—
PROSTATE GLAND	—	—	—	—	+	—	+	—	—	—
— Mononuclear infiltr.	.	.	.	.	1.	.	1.	.	.	.
COAGULATING GLANDS	—	—	(—	—	—	—	—	—	(—	—
SEMINAL VESICLES	—	—	—	—	—	—	—	—	—	—
STERNUM	—	—	—	—	—	—	—	—	—	—
FEMUR	—	—	—	—	—	—	—	—	—	—
JOINT	—	—	—	—	—	+	+	+	—	—
— Chronic inflammation	.	.	.	.	.	2.	.	.	.	.
— Synovialitis	.	.	.	.	.	.	2.	1.	.	.
BONE MARROW, FEMUR	+	+	+	+	+	+	+	+	+	+
— Fatty replacement	1.	1.	1.	2.	1.	1.	1.	2.	1.	2.

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, 0 mg/kg

ANIMAL NUMBER :	1	2	3	4	5	6	7	8	9	10
	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0
EXORBITAL LACR.GLDS.	+	+	+	+	—	—	—	—	+	—
— Harderian alteration	.	(1.	(1.	(1.	.	.	.	.	1.	.
— Mononuclear infiltr.	.	(1.	(1.	.	.	.	.	.	1.	.
— Degeneration acinar	(1.	.	.	.	.	.	.	.	.	.
LARYNX	+	+	+	+	—	+	+	+	+	+
— Inflamm. infiltrates	2.	1.	1.	1.	.	3.	2.	.	1.	2.
— Luminal hemorrhages	.	.	.	.	.	.	.	1.	.	.
NASAL CAVITY,LEV.I	—	—	—	+	—	—	+	—	—	+
— Luminal hemorrhages	.	.	.	1.	.	.	1.	.	.	.
— Goblet cell hyperpl.	.	.	.	.	.	.	.	.	.	1.
NASAL CAVITY,LEV.II	—	—	—	—	—	—	—	—	—	—
NASAL CAVITY,LEV.III	+	—	—	—	—	+	+	—	—	+
— Hyaline inclusions	1.	.	.	.	.	1.	.	.	.	1.
— Luminal hemorrhages	.	.	.	.	.	.	1.	.	.	.
— Goblet cell hyperpl.	.	.	.	.	.	.	.	.	.	1.
NASAL CAVITY,LEV.IV	—	—	—	—	+	—	—	—	—	—
— Hyaline inclusions	.	.	.	.	1.	.	.	.	.	.
PHARYNX	+	—	+	+	—	+	—	—	—	+
— Mononuclear infiltr.	.	.	.	1.	.	1.	.	.	.	.
— Mucoid secretion	1.	.	1.	.	.	.	.	.	.	1.
PITUITARY GLAND	—	—	—	—	—	—	—	—	—	—
SPINAL CORD, CERVIC.	—	—	—	—	—	—	—	—	—	—
SPINAL CORD, THORAC.	—	—	—	—	—	—	—	—	—	—
SPINAL CORD, LUMBAR	—	—	—	—	—	—	—	—	—	—

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, 0 mg/kg

ANIMAL NUMBER :	41 FK0	42 FK0	43 FK0	44 FK0	45 FK0	46 FK0	47 FK0	48 FK0	49 FK0	50 FK0
HEART WITH AURICLES	—	—	—	—	—	—	—	+	—	—
— Mononuclear foci	.	.	.	.	.	.	.	1.	.	.
AORTA	—	—	—	—	—	—	—	—	—	—
ESOPHAGUS	—	—	—	—	—	—	—	—	—	—
TRACHEA	+	—	+	+	—	—	+	—	+	—
— Glandular dilation	.	.	1.	.	.	.	.	.	.	.
— Mononuclear infiltr.	1.	.	.	1.	.	.	1.	.	1.	.
LIVER	+	+	+	+	+	+	+	+	+	+
— Fatty change	1.	.	.	.	.	.	.	1.	.	.
— Inflammatory foci	1.	1.	1.	1.	1.	1.	1.	1.	1.	1.
— Hematopoietic foci	.	.	.	.	.	.	.	.	1.	.
— Hepatocell. pigment	.	.	.	.	.	.	.	1.	.	.
SPLEEN	+	+	+	+	+	+	+	+	+	+
— Extramed.hemopoiesis	.	.	.	1.	1.	1.	.	1.	1.	1.
— Hemosiderin deposits	2.	2.	2.	2.	2.	2.	2.	2.	2.	2.
MESENT. LYMPH NODE	—	—	—	+	+	+	—	—	—	+
— Lymphoid hyperplasia	.	.	.	1.	1.	2.	.	.	.	1.
KIDNEYS	—	+	—	+	—	—	+	+	—	—
— Corticomed. mineral.	.	.	.	1.	.	.	.	.	.	.
— Mononuclear infiltr.	.	(1.	.	.	.	.	.	.	.	.
— Papillary mineraliz.	.	.	.	.	.	.	(1.	1.	.	.
URINARY BLADDER	—	—	—	—	—	—	—	—	—	+
— Perivasc. infiltrat.	.	.	.	.	.	.	.	.	.	1.
STOMACH	+	—	—	—	—	—	—	+	—	—
— Glandular dilation	1.	.	.	.	.	.	.	1.	.	.
DUODENUM	—	—	—	—	—	—	—	—	—	—
JEJUNUM	—	—	—	—	—	—	—	—	—	—

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, 0 mg/kg

ANIMAL NUMBER :	41 FK0	42 FK0	43 FK0	44 FK0	45 FK0	46 FK0	47 FK0	48 FK0	49 FK0	50 FK0
PEYERS PATCHES JEJ.	—	—	0	—	—	—	0	—	+	—
— Lymphoid hyperplasia	.	.	.	.	.	.	.	.	1.	.
ILEUM	—	—	—	—	—	—	—	—	—	—
PEYERS PATCHES ILEUM	+	+	—	—	—	—	—	—	—	+
— Lymphoid hyperplasia	1.	1.	.	.	.	.	.	.	.	1.
CECUM	—	—	—	—	—	—	—	—	—	—
COLON	—	—	—	—	—	—	—	—	—	—
RECTUM	—	—	—	—	—	—	—	—	—	—
LUNGS	—	+	+	+	+	+	—	+	+	+
— Alveolar macrophages	.	1.	1.	.	1.	1.	.	1.	1.	1.
— Vascular mineraliz.	.	.	1.	.	.	1.	.	.	1.	.
— Mononuclear foci	.	.	.	.	.	.	.	.	.	1.
— Mucoid secretion	.	.	1.	1.	.	.	.	.	1.	.
THYMUS	+	+	+	+G	+	+	+	+	+	+
— Cyst (s)	P.	P.	P.	P.	P.	P.	P.	P.	P.	P.
— Atrophy/involution	1.	1.	1.	1.	1.	1.	1.	1.	1.	1.
— Hemorrhages	.	.	.	2.	.	.	.	.	.	.
OVARIES	—	—	—	—	—	—	—	—	—	—
MANDIBULAR GLANDS	—	—	—	—	—	—	—	—	—	—
SUBLINGUAL GLANDS	—	—	—	—	—	—	—	—	—	—
PANCREAS	—	—	—	—	—	—	—	+	—	—
— Acinar cell atrophy	.	.	.	.	.	.	.	2.	.	.
MANDIB.LYMPH NODE	—	—	—	—	—	—	—	+G	+	—
— Lymphoid hyperplasia	.	.	.	.	.	.	.	.	1.	.
— No histol. correlate	.	.	.	.	.	.	.	P.	.	.

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, 0 mg/kg

ANIMAL NUMBER :	41 FK0	42 FK0	43 FK0	44 FK0	45 FK0	46 FK0	47 FK0	48 FK0	49 FK0	50 FK0
THYROID GLAND	—	—	—	+	—	—	+	—	—	—
— Ductal remnants	.	.	.	.	.	.	(P.	.	.	.
— Thymic ectopia	.	.	.	(P.	.	.	.	.	.	.
PARATHYROID GLANDS	(—	—	(—	(—	—	0	(—	—	(—	(—
ADRENAL CORTICES	—	—	—	—	—	—	—	—	+	—
— Extra—adrenal tissue	.	.	.	.	.	.	.	.	(P.	.
ADRENAL MEDULLAS	—	—	—	—	—	—	—	—	—	—
SCIATIC NERVE	—	—	—	—	—	+	—	—	—	—
— Single fiber degen.	.	.	.	.	.	1.	.	.	.	.
SKIN/SUBCUTIS	—	+	—	—	—	—	+	—	—	—
— Inflammatory infilt.	.	1.	.	.	.	.	1.	.	.	.
MAMMARY GLAND AREA	—	—	—	—	—	—	—	—	—	—
EYES	+	+	+	—	+	+	+	+	+	+
— Retro—orb.hemorrhage	(2.	(3.	(2.	.	(1.	(1.	(2.	(3.	(2.	(2.
— Peri—orb.inflammat.	.	.	.	.	.	.	.	(1.	.	(1.
— Hyaloid remnants	.	.	.	.	.	.	(P.	.	.	.
OPTIC NERVES	—	(—	—	—	—	—	(—	—	—	(—
HARDERIAN GLANDS	—	+	—	+	+	+	—	+	+	—
— Porphyrin deposits	.	1.	.	1.	1.	(1.	.	1.	1.	.
— Mononuclear infiltr.	.	.	.	.	(1.	.	.	.	.	.
CEREBRUM	—	—	—	—	—	—	—	—	—	—
CEREBELLUM	—	—	—	—	—	—	—	—	—	—
PONS	—	—	—	—	—	—	—	—	—	—
SKELETAL MUSCLE	—	+	+	+	+	—	—	+	—	+
— Mononuclear foci	.	1.	1.	1.	1.	.	.	1.	.	1.
— Myofiber degenerat.	.	1.	.	.	1.	.	.	.	.	.

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, 0 mg/kg

ANIMAL NUMBER :	41 FK0	42 FK0	43 FK0	44 FK0	45 FK0	46 FK0	47 FK0	48 FK0	49 FK0	50 FK0
TONGUE	—	—	—	+	—	—	—	+	—	—
— Mononuclear infiltr.	.	.	.	1.	.	.	.	1.	.	.
UTERUS	—	—	—	—	—	—	—	—	—	—
VAGINA	+	+	+	+	+	+	+	+	+	+
— Cycle:Proestrus	.	.	.	P.	.	.	.	.	.	.
— Cycle:Estrus	.	.	.	.	P.	.	.	.	P.	.
— Cycle:Metestrus	P.	P.	P.	.	.	P.	P.	.	.	P.
— Cycle:Diestrus	.	.	.	.	.	.	.	P.	.	.
— Cystic mucosal deg.	.	.	.	.	.	.	.	1.	.	2.
— Mucification	.	.	.	.	.	2.	.	.	.	.
STERNUM	—	—	—	—	—	—	—	—	—	—
FEMUR	—	—	—	—	—	—	—	—	—	—
JOINT	—	—	—	—	—	—	—	—	—	—
BONE MARROW, FEMUR	+	+	+	+	+	+	+	+	+	+
— Fatty replacement	1.	1.	1.	1.	2.	1.	2.	1.	1.	1.
EXORBITAL LACR.GLDS.	—	—	—	—	—	—	—	—	—	—
LARYNX	+	—	+	+	—	+	+	+	+	—
— Inflamm. infiltrates	1.	.	1.	1.	.	.	1.	2.	.	.
— Luminal hemorrhages	.	.	.	.	.	.	1.	.	1.	.
— Mucoid secretion	.	.	.	.	.	1.	.	.	.	.
NASAL CAVITY,LEV.I	—	—	—	—	—	—	—	—	—	—
NASAL CAVITY,LEV.II	—	+	—	—	—	—	—	—	—	—
— Inflamm. infiltrates	.	1.	.	.	.	.	.	.	.	.
NASAL CAVITY,LEV.III	—	—	—	—	—	—	—	—	—	+
— Goblet cell hyperpl.	.	.	.	.	.	.	.	.	.	1.
NASAL CAVITY,LEV.IV	—	—	—	—	—	—	—	—	—	—

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, 0 mg/kg

ANIMAL NUMBER :	41 FK0	42 FK0	43 FK0	44 FK0	45 FK0	46 FK0	47 FK0	48 FK0	49 FK0	50 FK0
PHARYNX	+	—	—	—	—	—	—	—	—	—
— Luminal hemorrhages	2.	.	.	.	.	.	.	.	.	.
PITUITARY GLAND	—	—	+	+	—	—	—	—	—	—
— Cyst	.	.	P.	.	.	.	.	.	.	.
— Cystic cleft	.	.	.	P.	.	.	.	.	.	.
SPINAL CORD, CERVIC.	—	—	—	—	—	—	—	—	—	—
SPINAL CORD, THORAC.	—	—	—	—	—	—	—	—	—	—
SPINAL CORD, LUMBAR	—	—	—	—	—	—	—	—	—	—

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 02, 100 mg/kg

ANIMAL NUMBER :	11	12	13	14	15	16	17	18	19	20
	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0
TESTES	+G	'	'	'	'	'	'	'	'	'
— No histol. correlate	P.									

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 02, 100 mg/kg

ANIMAL NUMBER :	51	52	53	54	55	56	57	58	59	60
	FK0	FK0	FK0	FK0	FK0	FK0	FK0	FK0	FK0	FK0
THYMUS	'	'	'	'	'	'	+G	'	'	'
— Cyst (s)							P.			
— Atrophy/involution							1.			
— Hemorrhages							2.			
MANDIB.LYMPH NODE	'	'	+G	'	'	'	'	'	'	'
— Lymphoid hyperplasia			1.							
— No histol. correlate			P.							

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 03, 300 mg/kg

ANIMAL NUMBER :	21	22	23	24	25	26	27	28	29	30
	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0
PANCREAS	'	'	+G	'	'	'	'	'	'	'
— Congestion			P.							

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 mg/kg

ANIMAL NUMBER :	31 MK0	32 MK0	33 MK0	34 MK0	35 MK0	36 MK0	37 MK0	38 MK0	39 MK0	40 MK0
HEART WITH AURICLES	+	+	+	—	+	—	—	+	—	+
— Mononuclear foci	1.	2.	2.	.	1.	.	.	1.	.	1.
— Myocardial necrosis	.	1.	1.	.	1.	.	.	.	.	.
— Myocardial fibrosis	1.	1.	.	.	.	.	.	.	.	1.
AORTA	—	—	—	—	—	—	—	—	—	—
ESOPHAGUS	—	—	—	—	—	—	—	—	—	—
TRACHEA	+	+	+	+	+	—	+	—	+	+
— Glandular dilation	2.	1.	.	.	.	.	.	.	.	.
— Mononuclear infiltr.	.	.	2.	1.	1.	.	1.	.	2.	1.
LIVER	+	+	+	+	+	+	+	+	+	+
— Fatty change	1.	1.	1.	1.	.	1.	.	1.	1.	.
— Inflammatory foci	1.	2.	1.	1.	1.	2.	1.	1.	1.	2.
— Glycogen increased	.	.	.	.	.	1.	1.	1.	1.	1.
— Hematopoietic foci	.	.	1.	.	.	.	1.	.	.	.
— Peribiliary inflamm.	.	1.	.	.	.	.	.	.	1.	.
— Focal necrosis	.	.	.	.	.	.	1.	.	.	.
SPLEEN	+	+	+	+	+	+	+	+	+	+
— Extramed.hemopoiesis	1.	1.	2.	2.	1.	1.	1.	1.	1.	1.
— Hemosiderin deposits	1.	1.	1.	2.	1.	1.	2.	1.	1.	2.
MESENT. LYMPH NODE	—	+	—	—	+	—	—	+	+	—
— Lymphoid hyperplasia	.	1.	.	.	1.	.	.	2.	1.	.
KIDNEYS	+	+	+	—	+	+G	+	+	+	—
— Hyaline droplets	.	.	1.	.	1.	.	1.	1.	.	.
— Tubular basophilia	(1.	.	.	.	(1.	.	(1.	.	(1.	.
— Mononuclear infiltr.	.	(1.	.	.	.	1.	.	(1.	.	.
— Pelvic dilation	.	.	.	.	.	(1.	.	.	.	.
URINARY BLADDER	—	—	+	—	—	—	+	—	—	—
— Perivasc. infiltrat.	.	.	1.	.	.	.	2.	.	.	.
STOMACH	—	—	—	—	—	—	—	+	—	—
— Incr. infl. infiltr.	.	.	.	.	.	.	.	1.	.	.

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SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 mg/kg

ANIMAL NUMBER :	31	32	33	34	35	36	37	38	39	40
	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0
DUODENUM	—	—	—	—	—	—	—	—	—	—
JEJUNUM	—	—	—	—	—	—	—	—	—	—
PEYERS PATCHES JEJ.	—	—	+	—	—	—	+	—	+	—
— Lymphoid hyperplasia	.	.	1.	.	.	.	1.	.	1.	.
ILEUM	—	—	—	—	—	—	—	—	—	—
PEYERS PATCHES ILEUM	—	+	+	+	+	+	+	—	+	+
— Lymphoid hyperplasia	.	1.	1.	1.	1.	1.	1.	.	1.	1.
CECUM	—	—	—	—	—	—	—	—	—	—
COLON	—	—	—	—	—	—	—	—	—	—
RECTUM	—	—	—	—	—	—	—	—	—	—
LUNGS	+	+	+	+	+	+	+	+	+	+
— Alveolar macrophages	1.	1.	.	1.	1.	1.	1.	.	1.	.
— Alveolar hemorrhages	.	.	1.	.	.	.	1.	1.	.	.
— Alveolitis	.	.	.	.	2.	.	.	.	.	.
— Vascular mineraliz.	.	1.	1.	1.	1.	1.	.	1.	1.	1.
— Perivascular infilt.	.	.	.	.	1.	.	.	.	.	.
— Osseous metaplasia	1.	1.	1.	.	.	.	.	.	.	.
— Mucoid secretion	.	.	.	.	.	.	.	1.	.	.
THYMUS	+G	—	+	+	+	—	+	+	+	+
— Cyst (s)	.	.	P.	.	.	.	P.	.	P.	P.
— Atrophy/involution	1.	.	1.	1.	1.	.	1.	1.	1.	1.
— No histol. correlate	P.	.	.	.	.	.	.	.	.	.
TESTES	—	—	—	—	—	—	—	+	—	—
— Fibrotic nodule	.	.	.	.	.	.	.	P.	.	.
EPIDIDYMIDES	+	—	—	—	+	+	—	—	+	—
— Mononuclear infiltr.	(1.	.	.	.	1.	.	.	.	(1.	.
— Epith. vacuolation	.	.	.	.	.	(1.	.	.	.	.

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SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 mg/kg

ANIMAL NUMBER :	31 MK0	32 MK0	33 MK0	34 MK0	35 MK0	36 MK0	37 MK0	38 MK0	39 MK0	40 MK0
MANDIBULAR GLANDS	—	—	—	—	—	—	—	—	—	—
SUBLINGUAL GLANDS	—	—	—	—	—	—	—	—	—	(—
PANCREAS	—	—	—	—	—	—	—	—	+	—
— Mononuclear infiltr.	.	.	.	.	.	.	.	.	1.	.
MANDIB.LYMPH NODE	+	—	—	+	—	—	—	—	+	—
— Lymphoid hyperplasia	1.	.	.	1.	.	.	.	.	1.	.
THYROID GLAND	+	—	—	—	—	+	—	—	—	+
— Ductal remnants	(P.	.	.	.	.	.	.	.	.	(P.
— Thymic ectopia	.	.	.	.	.	(P.	.	.	.	.
PARATHYROID GLANDS	(—	(—	(—	(—	(—	—	—	—	—	—
ADRENAL CORTICES	—	+	—	+	—	—	—	—	—	—
— Vacuolation, Z.fasc.	.	.	.	1.	.	.	.	.	.	.
— Extra—adrenal tissue	.	(P.	.	.	.	.	.	.	.	.
ADRENAL MEDULLAS	—	—	—	—	—	—	—	—	—	—
SCIATIC NERVE	—	—	+	—	—	—	—	—	—	+
— Single fiber degen.	.	.	1.	.	.	.	.	.	.	1.
SKIN/SUBCUTIS	—	—	—	—	+	+	—	—	—	—
— Inflammatory infilt.	.	.	.	.	1.	1.	.	.	.	.
— Epiderm. hyperplasia	.	.	.	.	1.	.	.	.	.	.
— Hyperkeratosis	.	.	.	.	1.	1.	.	.	.	.
MAMMARY GLAND AREA	—	—	—	—	—	0	0	0	—	—
EYES	+	+	+	+	+	+	+	+	+	+
— Retinal rosette(s)	.	.	.	.	.	.	.	.	(1.	(1.
— Retro—orb.hemorrhage	.	(2.	(2.	(1.	(2.	(1.	(2.	(1.	(2.	(1.
— Peri—orb.inflammat.	.	.	.	.	.	(1.	(1.	(1.	(1.	(1.
— Hyaloid remnants	(P.	.	.	.	.	.	.	.	.	.
OPTIC NERVES	(—	—	—	—	—	—	—	—	(—	—

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SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 mg/kg

ANIMAL NUMBER :	31 MK0	32 MK0	33 MK0	34 MK0	35 MK0	36 MK0	37 MK0	38 MK0	39 MK0	40 MK0
HARDERIAN GLANDS	+	—	+	—	+	—	—	+	+	—
— Porphyrin deposits	1.	.	1.	.	.	.	.	(1.	1.	.
— Mononuclear infiltr.	.	.	.	.	(2.	.	.	.	.	.
CEREBRUM	—	—	—	—	—	—	—	—	—	—
CEREBELLUM	—	—	—	—	—	—	—	—	—	—
MEDULLA OBLONGATA	—	—	—	—	—	—	—	—	—	—
PONS	—	—	—	—	—	—	—	—	—	—
SKELETAL MUSCLE	+	+	—	+	+	+	—	+	—	+
— Mononuclear foci	1.	1.	.	1.	.	1.	.	1.	.	1.
— Myofiber degenerat.	1.	1.	.	1.	1.	1.	.	1.	.	.
TONGUE	—	—	—	—	—	—	—	—	—	—
PROSTATE GLAND	—	—	—	—	—	—	—	—	—	—
COAGULATING GLANDS	—	—	—	—	—	(—	—	—	(—	—
SEMINAL VESICLES	—	—	—	—	—	—	—	—	—	—
STERNUM	—	—	—	—	—	—	—	—	—	—
FEMUR	—	—	—	—	—	—	—	—	—	—
JOINT	—	—	—	—	+	+	+	—	+	—
— Chronic inflammation	.	.	.	.	.	.	2.	.	1.	.
— Synovialitis	.	.	.	.	1.	1.	.	.	1.	.
BONE MARROW, FEMUR	+	+	+	+	+	+	+	+	+	+
— Fatty replacement	1.	1.	1.	1.	2.	2.	1.	2.	2.	2.
EXORBITAL LACR.GLDS.	—	—	—	+	+	—	+	—	+	—
— Harderian alteration	.	.	.	.	(1.	.	(1.	.	.	.
— Mononuclear infiltr.	.	.	.	(1.	.	.	.	.	(1.	.

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 mg/kg

ANIMAL NUMBER :	31 MK0	32 MK0	33 MK0	34 MK0	35 MK0	36 MK0	37 MK0	38 MK0	39 MK0	40 MK0
LARYNX	—	—	+	+	—	+	+	+	+	+
— Inflamm. infiltrates	.	.	2.	1.	.	2.	1.	1.	1.	2.
— Luminal hemorrhages	.	.	1.	2.	.	.	.	.	.	.
— Mucoid secretion	.	.	.	.	.	.	2.	.	.	.
— Luminal foreign body	.	.	.	.	.	.	.	1.	.	.
NASAL CAVITY,LEV.I	+	—	—	—	—	—	—	—	—	—
— Goblet cell hyperpl.	1.	.	.	.	.	.	.	.	.	.
NASAL CAVITY,LEV.II	—	—	—	—	—	—	—	—	—	—
NASAL CAVITY,LEV.III	—	+	—	+	+	+	—	+	—	—
— Hyaline inclusions	.	1.	.	1.	1.	1.	.	1.	.	.
NASAL CAVITY,LEV.IV	—	+	—	—	—	+	—	+	—	—
— Hyaline inclusions	.	1.	.	.	.	1.	.	1.	.	.
PHARYNX	—	—	+	—	—	—	—	—	—	+
— Mononuclear infiltr.	.	.	.	.	.	.	.	.	.	1.
— Mucoid secretion	.	.	1.	.	.	.	.	.	.	.
PITUITARY GLAND	—	—	+	—	—	—	+	—	—	—
— Cystic cleft	.	.	P.	.	.	.	P.	.	.	.
SPINAL CORD, CERVIC.	—	—	—	—	—	—	—	—	—	—
SPINAL CORD, THORAC.	—	—	—	—	—	—	—	—	—	—
SPINAL CORD, LUMBAR	—	—	—	—	—	—	—	—	—	—

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 mg/kg

ANIMAL NUMBER :	71 FK0	72 FK0	73 FK0	74 FK0	75 FK0	76 FK0	77 FK0	78 FK0	79 FK0	80 FK0
HEART WITH AURICLES	—	—	—	—	—	—	—	+	—	+
— Myocardial necrosis	.	.	.	.	.	.	.	1.	.	1.
AORTA	—	—	—	—	—	—	—	—	—	—
ESOPHAGUS	—	—	—	—	—	—	—	—	—	—
TRACHEA	+	+	+	+	—	+	—	+	+	+
— Glandular dilation	.	.	1.	.	.	1.	.	1.	1.	1.
— Mononuclear infiltr.	1.	1.	1.	1.	.	1.	.	1.	1.	1.
LIVER	+	+	+	+	+	+	+	+	+	+
— Fatty change	.	1.	.	1.	.	1.	.	1.	.	1.
— Inflammatory foci	1.	.	1.	1.	1.	1.	1.	1.	1.	1.
— Glycogen increased	.	.	.	.	.	.	.	1.	1.	1.
— Hematopoietic foci	.	.	1.	.	.	1.	.	.	1.	.
SPLEEN	+	+	+	+	+	+	+	+	+	+
— Extramed.hemopoiesis	1.	1.	2.	1.	2.	1.	1.	1.	.	1.
— Hemosiderin deposits	2.	3.	2.	2.	2.	2.	2.	2.	2.	2.
MESENT. LYMPH NODE	+	+	—	—	+	+	—	—	+	+
— Lymphoid hyperplasia	1.	1.	.	.	1.	1.	.	.	1.	1.
KIDNEYS	—	+	—	—	—	—	—	—	—	—
— Corticomed. mineral.	.	1.	.	.	.	.	.	.	.	.
URINARY BLADDER	—	—	—	—	—	—	—	—	—	—
STOMACH	—	—	—	—	+	+	—	—	—	+
— Glandular dilation	.	.	.	.	1.	.	.	.	.	1.
— Focal inflammation	.	.	.	.	.	1.	.	.	.	.
DUODENUM	—	—	—	—	—	—	—	—	—	—
JEJUNUM	—	—	—	—	—	—	—	—	—	—

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 mg/kg

ANIMAL NUMBER :	71 FK0	72 FK0	73 FK0	74 FK0	75 FK0	76 FK0	77 FK0	78 FK0	79 FK0	80 FK0
PEYERS PATCHES JEJ.	—	—	+	—	—	—	+	—	+	—
— Lymphoid hyperplasia	.	.	1.	.	.	.	1.	.	.	.
— Mineralization	.	.	.	.	.	.	.	.	1.	.
ILEUM	—	—	—	—	—	—	—	—	—	—
PEYERS PATCHES ILEUM	+	+	—	+	+	—	+	+	+	—
— Lymphoid hyperplasia	1.	1.	.	1.	1.	.	1.	1.	1.	.
CECUM	—	—	—	—	—	—	—	—	—	—
COLON	—	—	—	—	—	—	—	—	—	—
RECTUM	—	—	—	—	—	—	—	—	—	—
LUNGS	+	+	—	+	+	+	—	+	+	+
— Alveolar macrophages	1.	1.	.	1.	1.	1.	.	.	1.	1.
— Alveolar hemorrhages	.	.	.	.	.	.	.	.	.	1.
— Alveolitis	.	.	.	.	.	.	.	.	.	2.
— Vascular mineraliz.	.	1.	.	1.	1.	.	.	1.	.	.
— Mucoid secretion	.	.	.	.	.	.	.	.	.	1.
— Bronch. hyperplasia	.	.	.	.	.	.	.	2.	.	.
THYMUS	+	+	+	+	+	+	+	+	+	+
— Cyst (s)	P.	P.	.	P.	P.	.	.	P.	P.	P.
— Atrophy/involution	1.	1.	1.	1.	1.	.	1.	1.	1.	1.
— Hemorrhages	1.	.	.	.	.	1.	.	.	.	.
OVARIES	—	—	—	—	—	—	—	—	—	—
MANDIBULAR GLANDS	—	—	—	—	—	—	—	—	—	—
SUBLINGUAL GLANDS	—	—	—	—	—	—	—	—	—	—
PANCREAS	—	—	—	—	—	—	—	—	—	—
MANDIB.LYMPH NODE	—	—	—	—	—	—	—	—	—	+
— Lymphoid hyperplasia	.	.	.	.	.	.	.	.	.	1.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 mg/kg

ANIMAL NUMBER :	71 FK0	72 FK0	73 FK0	74 FK0	75 FK0	76 FK0	77 FK0	78 FK0	79 FK0	80 FK0
THYROID GLAND	—	—	+	—	—	+	—	+	—	—
— Ductal remnants	.	.	(P.	.	.	.	.	.	.	.
— Thymic ectopia	.	.	.	.	.	(P.	.	(P.	.	.
PARATHYROID GLANDS	—	—	(—	0	—	(—	—	—	—	—
ADRENAL CORTICES	—	—	—	—	—	—	+	—	+	—
— Extra—adrenal tissue	.	.	.	.	.	.	(P.	.	(P.	.
ADRENAL MEDULLAS	—	—	—	—	—	—	0	—	—	—
SCIATIC NERVE	—	—	—	—	—	—	—	—	—	—
SKIN/SUBCUTIS	—	—	—	—	+	—	—	—	+	—
— Inflammatory infilt.	.	.	.	.	1.	.	.	.	1.	.
MAMMARY GLAND AREA	—	—	—	—	—	—	—	—	—	—
EYES	+	+G	+	+	+	+	+	+	+	+
— Retro—orb.hemorrhage	(1.	(3.	(2.	(2.	(2.	(1.	(1.	(1.	(2.	(2.
— Peri—orb.inflammat.	.	.	(1.	.	.	.	(1.	(1.	(1.	(1.
OPTIC NERVES	—	—	—	—	—	—	—	—	—	—
HARDERIAN GLANDS	+	+	—	—	—	—	+	—	+	—
— Porphyrin deposits	(1.	1.	.	.	.	.	.	.	(1.	.
— Mononuclear infiltr.	.	(1.	.	.	.	.	(1.	.	.	.
CEREBRUM	—	—	—	—	—	—	—	—	—	—
CEREBELLUM	—	—	—	—	—	—	—	—	—	—
MEDULLA OBLONGATA	—	—	—	—	—	—	—	—	—	—
PONS	—	—	—	—	—	—	—	—	—	—
SKELETAL MUSCLE	+	+	+	+	+	—	+	+	+	—
— Mononuclear foci	1.	1.	.	1.	1.	.	.	.	.	.
— Myofiber degenerat.	.	.	1.	.	.	.	1.	1.	1.	.

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 mg/kg

ANIMAL NUMBER :	71	72	73	74	75	76	77	78	79	80
	FK0	FK0	FK0	FK0	FK0	FK0	FK0	FK0	FK0	FK0
TONGUE	—	—	—	—	—	—	—	—	—	—
UTERUS	—	—	+	—	—	—	—	—	+	—
— Distended lumina	.	.	P.	.	.	.	.	.	P.	.
VAGINA	+	+	+	+	+	+	+	+	+	+
— Cycle:Proestrus	.	.	.	P.	P.	.	.	.	P.	P.
— Cycle:Estrus	P.	P.	P.	.	.	.	P.	.	.	.
— Cycle:Metestrus	.	.	.	.	.	P.	.	P.	.	.
— Cystic mucosal deg.	.	.	.	.	.	.	.	.	.	2.
STERNUM	—	—	—	—	—	—	—	—	—	—
FEMUR	—	—	—	—	—	—	—	—	—	—
JOINT	—	—	—	—	+	—	—	—	—	—
— Synovialitis	.	.	.	.	1.	.	.	.	.	.
BONE MARROW, FEMUR	+	+	+	+	+	+	+	+	+	+
— Fatty replacement	1.	1.	1.	1.	1.	1.	1.	2.	2.	1.
EXORBITAL LACR.GLDS.	—	—	—	—	—	—	+	—	—	—
— Harderian alteration	.	.	.	.	.	.	1.	.	.	.
LARYNX	—	—	+	+	+	+	+	—	—	+
— Inflamm. infiltrates	.	.	1.	1.	1.	.	2.	.	.	2.
— Luminal hemorrhages	.	.	.	.	.	1.	.	.	.	.
NASAL CAVITY,LEV.I	—	+	—	—	+	+	—	—	—	—
— Goblet cell hyperpl.	.	.	.	.	1.	1.	.	.	.	.
— Inflamm. infiltrates	.	1.	.	.	.	.	.	.	.	.
NASAL CAVITY,LEV.II	—	—	—	—	—	—	—	—	—	—
NASAL CAVITY,LEV.III	—	—	—	+	—	—	—	—	—	—
— Hyaline inclusions	.	.	.	1.	.	.	.	.	.	.
— Goblet cell hyperpl.	.	.	.	1.	.	.	.	.	.	.

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 mg/kg

ANIMAL NUMBER :	71 FK0	72 FK0	73 FK0	74 FK0	75 FK0	76 FK0	77 FK0	78 FK0	79 FK0	80 FK0
NASAL CAVITY,LEV.IV	+	—	—	+	—	—	—	—	—	—
— Hyaline inclusions	1.	.	.	1.	.	.	.	.	.	.
PHARYNX	—	—	—	—	+	—	+	—	—	—
— Mucoid secretion	.	.	.	.	1.	.	.	.	.	.
— Luminal hemorrhages	.	.	.	.	.	.	1.	.	.	.
PITUITARY GLAND	—	—	—	—	—	—	—	—	—	—
SPINAL CORD, CERVIC.	—	—	—	—	—	—	—	—	—	—
SPINAL CORD, THORAC.	—	—	—	—	—	—	—	—	—	—
SPINAL CORD, LUMBAR	—	—	—	—	—	—	—	—	—	—

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ANIMAL HEADING DATA
DOSE GROUP : 01, 0 mg/kg

ANIMAL NUMBER	SEX M/F	DEFINED AND FINAL STATE OF NECROPSY		TEST DAYS	FIRST AND LAST DAY UNDER TEST		DATE OF NECROPSY
1	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
2	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
3	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
4	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
5	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
6	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
7	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
8	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
9	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
10	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
41	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
42	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
43	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
44	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
45	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
46	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
47	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
48	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
49	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
50	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10

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SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 1

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 1
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 2
KIDNEYS:
—Tubular basophilia, unilateral, grade 1
—Mononuclear cell infiltrates, unilateral, grade 1
URINARY BLADDER:
—Perivascular mononuclear cell infiltrates, grade 1
STOMACH:
—Vacuolation, squamous epithelium, limiting ridge, grade 1
PEYER'S PATCHES (JEJUNUM):
—Lymphoid hyperplasia, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Alveolar hemorrhages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
THYMUS:
—Atrophy/involution, grade 1
EPIDIDYMIDES:
—Mononuclear cell infiltrates, bilateral, grade 1

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 1

PARATHYROID GLANDS:
Only one of paired organs examined/present
ADRENAL CORTICES:
—Vacuolation, Zona fasciculata, bilateral, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 1
OPTIC NERVES:
Only one of paired organs examined/present
HARDERIAN GLANDS:
—Porphyrin deposits, unilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
—Single myofiber degeneration, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
EXORBITAL LACRIMAL GLANDS:
—Degeneration acinar cells, focal, unilateral, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 2
NASAL CAVITY, LEVEL III:
—Hyaline inclusions, nasal septum, grade 1
PHARYNX:
—Mucoid secretion, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 2

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Mononuclear cell foci, grade 1
TRACHEA:
—Glandular dilation, grade 1
—Mononuclear cell infiltrates, grade 1
LIVER:
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
KIDNEYS:
—Hyaline droplets, bilateral, grade 1
URINARY BLADDER:
—Perivascular mononuclear cell infiltrates, grade 1
PEYER'S PATCHES (JEJUNUM):
—Lymphoid hyperplasia, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Alveolar hemorrhages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
—Osseous metaplasia, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
MANDIBULAR LYMPH NODE:
—Lymphoid hyperplasia, bilateral, grade 1

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 2

PARATHYROID GLANDS:
Only one of paired organs examined/present
MAMMARY GLAND AREA:
Tissue not present for histologic examination
EYES:
—Retinal rosette(s), unilateral, grade 1
—Retro—orbital hemorrhage, unilateral, grade 2
HARDERIAN GLANDS:
—Porphyrin deposits, unilateral, grade 1
—Mononuclear cell infiltrates, unilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
EXORBITAL LACRIMAL GLANDS:
—Harderian alteration, unilateral, grade 1
—Mononuclear cell infiltrates, unilateral, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 3

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 3

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Mononuclear cell foci, grade 1
—Myocardial necrosis, grade 1
LIVER:
—Inflammatory cell foci, grade 2
—Hematopoietic foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 1
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
KIDNEYS:
—Mononuclear cell infiltrates, unilateral, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Alveolar hemorrhages, focal/multifocal, grade 1
THYMUS:
—Atrophy/involution, grade 1
SUBLINGUAL GLANDS:
Only one of paired organs examined/present
THYROID GLAND (BOTH LOBES):
—Ductal remnants, bilateral
PARATHYROID GLANDS:
Only one of paired organs examined/present
SCIATIC NERVE:
—Single nerve fiber degeneration, grade 1
SKIN/SUBCUTIS:
—Epidermal hyperplasia, grade 1
—Hyperkeratosis, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 3

HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1
SKELETAL MUSCLE:
—Single myofiber degeneration, grade 1
COAGULATING GLANDS (ANTERIOR PROSTATE):
Only one of paired organs examined/present
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
EXORBITAL LACRIMAL GLANDS:
—Harderian alteration, unilateral, grade 1
—Mononuclear cell infiltrates, unilateral, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1
PHARYNX:
—Mucoid secretion, with plant particles, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 4

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 2
—Hemosiderin deposits, grade 2

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 4

MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
KIDNEYS:
—Hyaline droplets, bilateral, grade 1
—Mononuclear cell infiltrates, unilateral, grade 1
PEYER'S PATCHES (JEJUNUM):
—Lymphoid hyperplasia, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
EPIDIDYMIDES:
—Vasculitis/perivasculitis, unilateral, grade 1
MANDIBULAR LYMPH NODE:
—Lymphoid hyperplasia, bilateral, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 1
—Peri—orbital inflammation, unilateral, grade 1
OPTIC NERVES:
Only one of paired organs examined/present
HARDERIAN GLANDS:
—Mononuclear cell infiltrates, unilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
—Single myofiber degeneration, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 2
EXORBITAL LACRIMAL GLANDS:
—Harderian alteration, unilateral, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1
NASAL CAVITY, LEVEL I:
—Luminal hemorrhages, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 4

PHARYNX:
—Mononuclear cell infiltrates, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 5

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Mononuclear cell foci, grade 1
TRACHEA:
—Glandular dilation, grade 2
—Mononuclear cell infiltrates, grade 1
LIVER:
—Inflammatory cell foci, grade 1
—Peribiliary inflammation, chronic, grade 2
SPLEEN:
—Extramedullary hemopoiesis, grade 2
—Hemosiderin deposits, grade 1
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
KIDNEYS:
—Tubular basophilia, unilateral, grade 1
—Mononuclear cell infiltrates, unilateral, grade 1
—Papillary mineralization, focal/multifocal, unilateral, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 5

URINARY BLADDER:
—Perivascular mononuclear cell infiltrates, grade 1
PEYER'S PATCHES (JEJUNUM):
—Lymphoid hyperplasia, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
PARATHYROID GLANDS:
Only one of paired organs examined/present
SKIN/SUBCUTIS:
—Ulceration, focal, grade 1
HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1
SKELETAL MUSCLE:
—Single myofiber degeneration, grade 1
PROSTATE GLAND:
—Mononuclear cell infiltrates, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
NASAL CAVITY, LEVEL IV:
—Hyaline inclusions, nasal septum, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 6

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
KIDNEYS:
—Hyaline droplets, unilateral, grade 1
—Tubular basophilia, unilateral, grade 1
—Hyaline casts, unilateral, grade 1
STOMACH:
—Mucosal gland dilation, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 2
—Alveolar hemorrhages, focal/multifocal, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
EPIDIDYMIDES:
—Mononuclear cell infiltrates, unilateral, grade 1
MANDIBULAR LYMPH NODE:
—Lymphoid hyperplasia, bilateral, grade 1
SCIATIC NERVE:
—Single nerve fiber degeneration, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
—Peri—orbital inflammation, unilateral, grade 1

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 6

HARDERIAN GLANDS:
—Porphyrin deposits, unilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
JOINT:
—Chronic inflammation, focal, Ligamentum patellae, grade 2
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 3
NASAL CAVITY, LEVEL III:
—Hyaline inclusions, nasal septum, grade 1
PHARYNX:
—Mononuclear cell infiltrates, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 7

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
—Inflammatory cell foci, grade 1
—Increased hepatocytic glycogen deposits, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 7

MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 2
KIDNEYS:
—Mononuclear cell infiltrates, unilateral, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 2
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
THYMUS:
Tissue not present for histologic examination
SUBLINGUAL GLANDS:
Only one of paired organs examined/present
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
—Hyaloir arterial remnants, unilateral
OPTIC NERVES:
Only one of paired organs examined/present
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
PROSTATE GLAND:
—Mononuclear cell infiltrates, perivascular, grade 1
JOINT:
—Synovialitis, chronic, focal/multifocal, grade 2
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 2
NASAL CAVITY, LEVEL I:
—Luminal hemorrhages, grade 1
NASAL CAVITY, LEVEL III:
—Luminal hemorrhages, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 8

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Mononuclear cell foci, grade 2
—Myocardial necrosis, grade 1
—Myocardial fibrosis, grade 1
TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Inflammatory cell foci, grade 1
—Increased hepatocytic glycogen deposits, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 1
KIDNEYS:
—Hyaline droplets, bilateral, grade 1
—Mononuclear cell infiltrates, unilateral, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Vascular mineralization, grade 1
THYMUS:
—Atrophy/involution, grade 1
SUBLINGUAL GLANDS:
Only one of paired organs examined/present
THYROID GLAND (BOTH LOBES):
—Ductal remnants, unilateral
PARATHYROID GLANDS:
Only one of paired organs examined/present

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 8

EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
—Peri—orbital inflammation, unilateral, grade 1
OPTIC NERVES:
Only one of paired organs examined/present
HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1
JOINT:
—Synovialitis, chronic, focal/multifocal, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 2
LARYNX:
—Luminal hemorrhages, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 9

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Myocardial necrosis, grade 1
—Myocardial fibrosis, grade 1
TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 9

SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 1
KIDNEYS:
—Tubular basophilia, unilateral, grade 1
PEYER'S PATCHES (JEJUNUM):
—Lymphoid hyperplasia, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
—Mucoid secretion, with inflammatory cells, bronchiolar, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
EPIDIDYMIDES:
—Mononuclear cell infiltrates, unilateral, grade 1
PARATHYROID GLANDS:
Only one of paired organs examined/present
EYES:
—Retinal rosette(s), unilateral, grade 1
—Retro—orbital hemorrhage, unilateral, grade 1
HARDERIAN GLANDS:
—Porphyrin deposits, unilateral, grade 1
COAGULATING GLANDS (ANTERIOR PROSTATE):
Only one of paired organs examined/present
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
EXORBITAL LACRIMAL GLANDS:
—Harderian alteration, bilateral, grade 1
—Mononuclear cell infiltrates, bilateral, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 9

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 10

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Mononuclear cell foci, grade 1
—Myocardial necrosis, grade 1
TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
KIDNEYS:
—Mononuclear cell infiltrates, bilateral, grade 1
STOMACH:
—Mucosal gland dilation, grade 1
PEYER'S PATCHES (JEJUNUM):
—Lymphoid hyperplasia, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg MALE

CONT./FF. ANIMAL NO. : 10

LUNGS:
—Alveolar hemorrhages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
MANDIBULAR LYMPH NODE:
—Lymphoid hyperplasia, bilateral, grade 1
PARATHYROID GLANDS:
Only one of paired organs examined/present
EYES:
—Retro—orbital hemorrhage, unilateral, grade 1
—Peri—orbital inflammation, unilateral, grade 1
HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
—Single myofiber degeneration, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 2
LARYNX:
—Inflammatory cell infiltrates, grade 2
NASAL CAVITY, LEVEL I:
—Goblet cell hyperplasia, grade 1
NASAL CAVITY, LEVEL III:
—Hyaline inclusions, nasal septum, grade 1
—Goblet cell hyperplasia, grade 1
PHARYNX:
—Mucoid secretion, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 41

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
SPLEEN:
—Hemosiderin deposits, grade 2
STOMACH:
—Mucosal gland dilation, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
PARATHYROID GLANDS:
Only one of paired organs examined/present
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
VAGINA:
—Metestrus phase of the estrus cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1
PHARYNX:
—Luminal hemorrhages, grade 2
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 42

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
—Inflammatory cell foci, grade 1
SPLEEN:
—Hemosiderin deposits, grade 2
KIDNEYS:
—Mononuclear cell infiltrates, unilateral, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
SKIN/SUBCUTIS:
—Inflammatory cell infiltrates, dermal, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 3
OPTIC NERVES:
Only one of paired organs examined/present
HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
—Single myofiber degeneration, grade 1
VAGINA:
—Metestrus phase of the estrus cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 42

NASAL CAVITY, LEVEL II:
—Inflammatory cell infiltrates, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 43

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

TRACHEA:
—Glandular dilation, grade 1
LIVER:
—Inflammatory cell foci, grade 1
SPLEEN:
—Hemosiderin deposits, grade 2
PEYER'S PATCHES (JEJUNUM):
Tissue not present for histologic examination
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
—Mucoid secretion, with inflammatory cells, bronchiolar, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
PARATHYROID GLANDS:
Only one of paired organs examined/present

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 43

EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
VAGINA:
—Metestrus phase of the estrus cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1
PITUITARY GLAND:
—Cyst
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 44

* NECROPSY FINDINGS

THYMUS:
01: FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.
NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 44

MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
KIDNEYS:
—Corticomedullary mineralization, focal/multifocal, bilateral, grade 1
LUNGS:
—Mucoid secretion, with inflammatory cells, bronchiolar, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
—Hemorrhages, focal/multifocal, grade 2
This finding corresponds to necropsy observation no: 01.
THYROID GLAND (BOTH LOBES):
—Thymic ectopia, unilateral
PARATHYROID GLANDS:
Only one of paired organs examined/present
HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
TONGUE:
—Mononuclear cell infiltrates, grade 1
VAGINA:
—Proestrus phase of the estrous cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1
PITUITARY GLAND:
—Cystic cleft
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 45

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 1
HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1
—Mononuclear cell infiltrates, unilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
—Single myofiber degeneration, grade 1
VAGINA:
—Estrus phase of the estrous cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 2
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 46

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 2
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
PARATHYROID GLANDS:
Tissue not present for histologic examination
SCIATIC NERVE:
—Single nerve fiber degeneration, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 1
HARDERIAN GLANDS:
—Porphyrin deposits, unilateral, grade 1
VAGINA:
—Metestrus phase of the estrus cycle
—Mucification, grade 2
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Mucoid secretion, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 46

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 47

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Inflammatory cell foci, grade 1
SPLEEN:
—Hemosiderin deposits, grade 2
KIDNEYS:
—Papillary mineralization, focal/multifocal, unilateral, grade 1
PEYER'S PATCHES (JEJUNUM):
Tissue not present for histologic examination
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
THYROID GLAND (BOTH LOBES):
—Ductal remnants, unilateral
PARATHYROID GLANDS:
Only one of paired organs examined/present
SKIN/SUBCUTIS:
—Inflammatory cell infiltrates, dermal, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 47

EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
—Hyaloir arterial remnants, unilateral
OPTIC NERVES:
Only one of paired organs examined/present
VAGINA:
—Metestrus phase of the estrus cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 2
LARYNX:
—Inflammatory cell infiltrates, grade 1
—Luminal hemorrhages, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 48

* NECROPSY FINDINGS

MANDIBULAR LYMPH NODE:
01: FOCUS/FOCI, ISOLATED, D=1 MM, DARK RED.
NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Mononuclear cell foci, grade 1
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
—Hepatocellular intracytoplasmic hemosiderin—like pigment, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 48

SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
KIDNEYS:
—Papillary mineralization, focal/multifocal, bilateral, grade 1
STOMACH:
—Mucosal gland dilation, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
PANCREAS:
—Acinar cell atrophy, focal, grade 2
MANDIBULAR LYMPH NODE:
—No histological correlate, bilateral
This finding corresponds to necropsy observation no: 01.
EYES:
—Retro—orbital hemorrhage, unilateral, grade 3
—Peri—orbital inflammation, unilateral, grade 1
HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
TONGUE:
—Mononuclear cell infiltrates, grade 1
VAGINA:
—Diestrus phase of the estrous cycle
—Cystic mucosal degeneration, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 2
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 49

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Inflammatory cell foci, grade 1
—Hematopoietic foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
PEYER'S PATCHES (JEJUNUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
—Mucoid secretion, with inflammatory cells, bronchiolar, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
MANDIBULAR LYMPH NODE:
—Lymphoid hyperplasia, bilateral, grade 1
PARATHYROID GLANDS:
Only one of paired organs examined/present
ADRENAL CORTICES:
—Extra—adrenal tissue, unilateral
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 49

VAGINA:
—Estrus phase of the estrous cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Luminal hemorrhages, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 50

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
URINARY BLADDER:
—Perivascular mononuclear cell infiltrates, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Mononuclear cell focus/i, grade 1

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 01, 0 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 50

THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
PARATHYROID GLANDS:
Only one of paired organs examined/present
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
—Peri—orbital inflammation, unilateral, grade 1
OPTIC NERVES:
Only one of paired organs examined/present
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
VAGINA:
—Metestrus phase of the estrus cycle
—Cystic mucosal degeneration, grade 2
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
NASAL CAVITY, LEVEL III:
—Goblet cell hyperplasia, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

ANIMAL HEADING DATA
DOSE GROUP : 02, 100 mg/kg

ANIMAL NUMBER	SEX M/F	DEFINED AND FINAL STATE OF NECROPSY		TEST DAYS	FIRST AND LAST DAY UNDER TEST		DATE OF NECROPSY
11	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
12	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
13	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
14	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
15	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
16	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
17	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
18	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
19	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
20	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
51	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
52	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
53	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
54	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
55	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
56	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
57	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
58	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
59	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
60	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 100 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 11

* NECROPSY FINDINGS

TESTES:
01: BOTH SIDES: REDUCED IN SIZE, D=20X10 MM.
NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

TESTES:
—No histological correlate, bilateral
This finding corresponds to necropsy observation no: 01.

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* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 12

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 100 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 13

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 14

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 100 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 15

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 16

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 100 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 17

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 18

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 100 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 19

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 20

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 100 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 51

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 52

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 100 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 53

* NECROPSY FINDINGS

MANDIBULAR LYMPH NODE:
01: FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.
NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

MANDIBULAR LYMPH NODE:
—Lymphoid hyperplasia, bilateral, grade 1
—No histological correlate, bilateral
This finding corresponds to necropsy observation no: 01.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 54

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 100 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 55

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 56

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 100 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 57

* NECROPSY FINDINGS

THYMUS:
01: FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.
NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
—Hemorrhages, focal/multifocal, grade 2
This finding corresponds to necropsy observation no: 01.

---

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 58

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 100 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 59

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 60

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

ANIMAL HEADING DATA
DOSE GROUP : 03, 300 mg/kg

ANIMAL NUMBER	SEX M/F	DEFINED STATE OF NECROPSY	FINAL STATE OF NECROPSY	TEST DAYS	FIRST DAY UNDER TEST	LAST DAY UNDER TEST	DATE OF NECROPSY
21	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
22	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
23	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
24	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
25	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
26	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
27	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
28	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
29	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
30	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
61	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
62	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
63	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
64	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
65	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
66	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
67	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
68	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
69	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
70	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
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SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 03, 300 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 21

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 22

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 03, 300 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 23

* NECROPSY FINDINGS

PANCREAS:
01: DISCOLORATION, REDDISH.
NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

PANCREAS:
—Congestion
This finding corresponds to necropsy observation no: 01.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 24

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 03, 300 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 25

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 26

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 03, 300 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 27

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 28

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 03, 300 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 29

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 30

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 03, 300 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 61

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 62

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 03, 300 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 63

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 64

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 03, 300 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 65

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 66

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 03, 300 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 67

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 68

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 03, 300 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 69

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 70

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

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TEST ARTICLE : Maltogenic amylase *PATHOL. NO.:* 71087 TAT
TEST SYSTEM : RAT, 13—Week, Oral (Gavage) *DATE* : 13—DEC—10
SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

ANIMAL HEADING DATA
DOSE GROUP : 04, 1000 mg/kg

ANIMAL NUMBER	SEX M/F	DEFINED STATE OF NECROPSY	FINAL STATE OF NECROPSY	TEST DAYS	FIRST DAY UNDER TEST	LAST DAY UNDER TEST	DATE OF NECROPSY
31	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
32	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
33	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
34	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
35	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
36	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
37	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
38	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
39	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
40	M	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
71	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
72	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
73	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
74	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
75	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
76	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
77	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
78	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
79	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10
80	F	K0	K0	91	07—JAN—10	07—APR—10	08—APR—10

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 31

* NECROPSY FINDINGS

THYMUS:
01: FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.
NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Mononuclear cell foci, grade 1
—Myocardial fibrosis, grade 1
TRACHEA:
—Glandular dilation, grade 2
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 1
KIDNEYS:
—Tubular basophilia, unilateral, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Osseous metaplasia, grade 1
THYMUS:
—Atrophy/involution, grade 1
—No histological correlate
This finding corresponds to necropsy observation no: 01.
EPIDIDYMIDES:
—Mononuclear cell infiltrates, unilateral, grade 1
MANDIBULAR LYMPH NODE:
—Lymphoid hyperplasia, bilateral, grade 1
THYROID GLAND (BOTH LOBES):
—Ductal remnants, unilateral

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 31

PARATHYROID GLANDS:
Only one of paired organs examined/present
EYES:
—Hyaloir arterial remnants, unilateral
OPTIC NERVES:
Only one of paired organs examined/present
HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
—Single myofiber degeneration, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
NASAL CAVITY, LEVEL I:
—Goblet cell hyperplasia, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 32

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Mononuclear cell foci, grade 2
—Myocardial necrosis, grade 1
—Myocardial fibrosis, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 32

TRACHEA:
—Glandular dilation, grade 1
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 2
—Peribiliary inflammation, chronic, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 1
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
KIDNEYS:
—Mononuclear cell infiltrates, unilateral, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
—Osseous metaplasia, grade 1
PARATHYROID GLANDS:
Only one of paired organs examined/present
ADRENAL CORTICES:
—Extra—adrenal tissue, unilateral
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
—Single myofiber degeneration, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
NASAL CAVITY, LEVEL III:
—Hyaline inclusions, nasal septum, grade 1
NASAL CAVITY, LEVEL IV:
—Hyaline inclusions, nasal septum, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 33

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Mononuclear cell foci, grade 2
—Myocardial necrosis, grade 1
TRACHEA:
—Mononuclear cell infiltrates, grade 2
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
—Hematopoietic foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 2
—Hemosiderin deposits, grade 1
KIDNEYS:
—Hyaline droplets, bilateral, grade 1
URINARY BLADDER:
—Perivascular mononuclear cell infiltrates, grade 1
PEYER'S PATCHES (JEJUNUM):
—Lymphoid hyperplasia, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar hemorrhages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
—Osseous metaplasia, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 33

PARATHYROID GLANDS:
Only one of paired organs examined/present
SCIATIC NERVE:
—Single nerve fiber degeneration, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 2
—Luminal hemorrhages, grade 1
PHARYNX:
—Mucoid secretion, grade 1
PITUITARY GLAND:
—Cystic cleft
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 34

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 34

* MICROSCOPIC FINDINGS

TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 2
—Hemosiderin deposits, grade 2
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
THYMUS:
—Atrophy/involution, grade 1
MANDIBULAR LYMPH NODE:
—Lymphoid hyperplasia, bilateral, grade 1
PARATHYROID GLANDS:
Only one of paired organs examined/present
ADRENAL CORTICES:
—Vacuolation, Zona fasciculata, bilateral, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
—Single myofiber degeneration, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
EXORBITAL LACRIMAL GLANDS:
—Mononuclear cell infiltrates, unilateral, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1
—Luminal hemorrhages, grade 2

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 34

NASAL CAVITY, LEVEL III:
—Hyaline inclusions, nasal septum, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 35

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Mononuclear cell foci, grade 1
—Myocardial necrosis, grade 1
TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 1
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
KIDNEYS:
—Hyaline droplets, bilateral, grade 1
—Tubular basophilia, unilateral, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 35

LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Alveolitis, chronic, multifocal, with alv.wall hyperplasia, grade 2
—Vascular mineralization, grade 1
—Perivascular cell infiltration, grade 1
THYMUS:
—Atrophy/involution, grade 1
EPIDIDYMIDES:
—Mononuclear cell infiltrates, bilateral, grade 1
PARATHYROID GLANDS:
Only one of paired organs examined/present
SKIN/SUBCUTIS:
—Inflammatory cell infiltrates, dermal, with hemorrhage, grade 1
—Epidermal hyperplasia, focal, grade 1
—Hyperkeratosis, focal, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
HARDERIAN GLANDS:
—Mononuclear cell infiltrates, unilateral, grade 2
SKELETAL MUSCLE:
—Single myofiber degeneration, grade 1
JOINT:
—Synovialitis, chronic, focal/multifocal, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 2
EXORBITAL LACRIMAL GLANDS:
—Harderian alteration, unilateral, grade 1
NASAL CAVITY, LEVEL III:
—Hyaline inclusions, nasal septum, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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SPONSOR : AB Enzymes GmbH *PathData©System V6.2b5*

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 36

* NECROPSY FINDINGS

KIDNEYS:
01: RIGHT SIDE: PELVIC DILATION.
NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 2
—Increased hepatocytic glycogen deposits, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 1
KIDNEYS:
—Mononuclear cell infiltrates, bilateral, grade 1
—Pelvic dilation, unilateral, grade 1
This finding corresponds to necropsy observation no: 01.
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
EPIDIDYMIDES:
—Epithelial vacuolation, unilateral, grade 1
THYROID GLAND (BOTH LOBES):
—Thymic ectopia, unilateral
SKIN/SUBCUTIS:
—Inflammatory cell infiltrates, dermal, grade 1
—Hyperkeratosis, grade 1
MAMMARY GLAND AREA:
Tissue not present for histologic examination

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 36

EYES:
—Retro—orbital hemorrhage, unilateral, grade 1
—Peri—orbital inflammation, unilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
—Single myofiber degeneration, grade 1
COAGULATING GLANDS (ANTERIOR PROSTATE):
Only one of paired organs examined/present
JOINT:
—Synovialitis, chronic, focal/multifocal, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 2
LARYNX:
—Inflammatory cell infiltrates, grade 2
NASAL CAVITY, LEVEL III:
—Hyaline inclusions, nasal septum, grade 1
NASAL CAVITY, LEVEL IV:
—Hyaline inclusions, nasal septum, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 37

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 37

* MICROSCOPIC FINDINGS

TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Inflammatory cell foci, grade 1
—Increased hepatocytic glycogen deposits, grade 1
—Hematopoietic foci, grade 1
—Focal hepatocellular necrosis, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
KIDNEYS:
—Hyaline droplets, bilateral, grade 1
—Tubular basophilia, unilateral, grade 1
URINARY BLADDER:
—Perivascular mononuclear cell infiltrates, grade 2
PEYER'S PATCHES (JEJUNUM):
—Lymphoid hyperplasia, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Alveolar hemorrhages, focal/multifocal, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
MAMMARY GLAND AREA:
Tissue not present for histologic examination
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
—Peri—orbital inflammation, unilateral, grade 1
JOINT:
—Chronic inflammation, focal, Ligamentum patellae, grade 2
BONE MARROW (FEMUR):
—Fatty replacement, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 37

EXORBITAL LACRIMAL GLANDS:
—Harderian alteration, unilateral, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1
—Mucoid secretion, grade 2
PITUITARY GLAND:
—Cystic cleft
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 38

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Mononuclear cell foci, grade 1
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
—Increased hepatocytic glycogen deposits, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 1
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 2
KIDNEYS:
—Hyaline droplets, bilateral, grade 1
—Mononuclear cell infiltrates, unilateral, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 38

STOMACH:
—Increased inflammatory cell infiltrates, limiting ridge, grade 1
LUNGS:
—Alveolar hemorrhages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
—Mucoid secretion, with inflammatory cells, bronchiolar, grade 1
THYMUS:
—Atrophy/involution, grade 1
TESTES:
—Fibrotic nodule, Tunica albuginea, bilateral
MAMMARY GLAND AREA:
Tissue not present for histologic examination
EYES:
—Retro—orbital hemorrhage, unilateral, grade 1
—Peri—orbital inflammation, unilateral, grade 1
HARDERIAN GLANDS:
—Porphyrin deposits, unilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
—Single myofiber degeneration, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 2
LARYNX:
—Inflammatory cell infiltrates, grade 1
—Luminal foreign body, grade 1
NASAL CAVITY, LEVEL III:
—Hyaline inclusions, nasal septum, grade 1
NASAL CAVITY, LEVEL IV:
—Hyaline inclusions, nasal septum, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 39

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

TRACHEA:
—Mononuclear cell infiltrates, grade 2
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
—Increased hepatocytic glycogen deposits, grade 1
—Peribiliary inflammation, chronic, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 1
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
KIDNEYS:
—Tubular basophilia, unilateral, grade 1
PEYER'S PATCHES (JEJUNUM):
—Lymphoid hyperplasia, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
EPIDIDYMIDES:
—Mononuclear cell infiltrates, unilateral, grade 1
PANCREAS:
—Mononuclear cell infiltrates, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 39

MANDIBULAR LYMPH NODE:
—Lymphoid hyperplasia, bilateral, grade 1
EYES:
—Retinal rosette(s), unilateral, grade 1
—Retro—orbital hemorrhage, unilateral, grade 2
—Peri—orbital inflammation, unilateral, grade 1
OPTIC NERVES:
Only one of paired organs examined/present
HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1
COAGULATING GLANDS (ANTERIOR PROSTATE):
Only one of paired organs examined/present
JOINT:
—Chronic inflammation, grade 1
—Synovialitis, chronic, focal/multifocal, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 2
EXORBITAL LACRIMAL GLANDS:
—Mononuclear cell infiltrates, unilateral, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 40

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 40

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Mononuclear cell foci, grade 1
—Myocardial fibrosis, grade 1
TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Inflammatory cell foci, grade 2
—Increased hepatocytic glycogen deposits, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Vascular mineralization, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
SUBLINGUAL GLANDS:
Only one of paired organs examined/present
THYROID GLAND (BOTH LOBES):
—Ductal remnants, unilateral
SCIATIC NERVE:
—Single nerve fiber degeneration, grade 1
EYES:
—Retinal rosette(s), unilateral, grade 1
—Retro—orbital hemorrhage, unilateral, grade 1
—Peri—orbital inflammation, unilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 2
LARYNX:
—Inflammatory cell infiltrates, grade 2

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg MALE

CONT./FF. ANIMAL NO. : 40

PHARYNX:
—Mononuclear cell infiltrates, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 71

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
—Hemorrhages, focal/multifocal, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 1
HARDERIAN GLANDS:
—Porphyrin deposits, unilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
VAGINA:
—Estrus phase of the estrous cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 71

NASAL CAVITY, LEVEL IV:
—Hyaline inclusions, nasal septum, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 72

* NECROPSY FINDINGS

EYES:
01: RIGHT SIDE: DESTROYED DURING BLOOD COLLECTION.
NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Fatty change, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 3
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
KIDNEYS:
—Corticomedullary mineralization, focal/multifocal, bilateral, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Vascular mineralization, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 72

THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 3
This finding corresponds to necropsy observation no: 01.
HARDERIAN GLANDS:
—Porphyrin deposits, bilateral, grade 1
—Mononuclear cell infiltrates, unilateral, grade 1
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
VAGINA:
—Estrus phase of the estrous cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
NASAL CAVITY, LEVEL I:
—Inflammatory cell infiltrates, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 73

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 73

* MICROSCOPIC FINDINGS

TRACHEA:
—Glandular dilation, grade 1
—Mononuclear cell infiltrates, grade 1
LIVER:
—Inflammatory cell foci, grade 1
—Hematopoietic foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 2
—Hemosiderin deposits, grade 2
PEYER'S PATCHES (JEJUNUM):
—Lymphoid hyperplasia, grade 1
THYMUS:
—Atrophy/involution, grade 1
THYROID GLAND (BOTH LOBES):
—Ductal remnants, unilateral
PARATHYROID GLANDS:
Only one of paired organs examined/present
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
—Peri—orbital inflammation, unilateral, grade 1
SKELETAL MUSCLE:
—Single myofiber degeneration, grade 1
UTERUS:
—Distended lumina of uterine horns, cyclic change
VAGINA:
—Estrus phase of the estrous cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 74

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

TRACHEA:
—Mononuclear cell infiltrates, grade 1
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Vascular mineralization, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
PARATHYROID GLANDS:
Tissue not present for histologic examination
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
VAGINA:
—Proestrus phase of the estrous cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 74

NASAL CAVITY, LEVEL III:
—Hyaline inclusions, nasal septum, grade 1
—Goblet cell hyperplasia, grade 1
NASAL CAVITY, LEVEL IV:
—Hyaline inclusions, nasal septum, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 75

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 2
—Hemosiderin deposits, grade 2
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
STOMACH:
—Mucosal gland dilation, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Vascular mineralization, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 75

THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
SKIN/SUBCUTIS:
—Inflammatory cell infiltrates, dermal, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
SKELETAL MUSCLE:
—Mononuclear cell focus/i, grade 1
VAGINA:
—Proestrus phase of the estrous cycle
JOINT:
—Synovialitis, chronic, focal/multifocal, grade 1
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 1
NASAL CAVITY, LEVEL I:
—Goblet cell hyperplasia, grade 1
PHARYNX:
—Mucoid secretion, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 76

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 76

* MICROSCOPIC FINDINGS

TRACHEA:
—Glandular dilation, grade 1
—Mononuclear cell infiltrates, grade 1
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
—Hematopoietic foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
STOMACH:
—Focal inflammation, chronic, forestomach, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
THYMUS:
—Hemorrhages, focal/multifocal, grade 1
THYROID GLAND (BOTH LOBES):
—Thymic ectopia, unilateral
PARATHYROID GLANDS:
Only one of paired organs examined/present
EYES:
—Retro—orbital hemorrhage, unilateral, grade 1
VAGINA:
—Metestrus phase of the estrus cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Luminal hemorrhages, grade 1
NASAL CAVITY, LEVEL I:
—Goblet cell hyperplasia, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 77

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
—Inflammatory cell foci, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
PEYER'S PATCHES (JEJUNUM):
—Lymphoid hyperplasia, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
THYMUS:
—Atrophy/involution, grade 1
ADRENAL CORTICES:
—Extra—adrenal tissue, unilateral
ADRENAL MEDULLAS:
Tissue not present for histologic examination
EYES:
—Retro—orbital hemorrhage, unilateral, grade 1
—Peri—orbital inflammation, unilateral, grade 1
HARDERIAN GLANDS:
—Mononuclear cell infiltrates, unilateral, grade 1
SKELETAL MUSCLE:
—Single myofiber degeneration, grade 1
VAGINA:
—Estrus phase of the estrous cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
EXORBITAL LACRIMAL GLANDS:
—Harderian alteration, bilateral, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 77

LARYNX:
—Inflammatory cell infiltrates, grade 2
PHARYNX:
—Luminal hemorrhages, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 78

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Myocardial necrosis, grade 1
TRACHEA:
—Glandular dilation, grade 1
—Mononuclear cell infiltrates, grade 1
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
—Increased hepatocytic glycogen deposits, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Vascular mineralization, grade 1
—Bronchiolar epithelial hyperplasia, focal, grade 2

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 78

THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
THYROID GLAND (BOTH LOBES):
—Thymic ectopia, unilateral
EYES:
—Retro—orbital hemorrhage, unilateral, grade 1
—Peri—orbital inflammation, unilateral, grade 1
SKELETAL MUSCLE:
—Single myofiber degeneration, grade 1
VAGINA:
—Metestrus phase of the estrus cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 2
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 79

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

TRACHEA:
—Glandular dilation, grade 1
—Mononuclear cell infiltrates, grade 1
LIVER:
—Inflammatory cell foci, grade 1
—Increased hepatocytic glycogen deposits, grade 1
—Hematopoietic foci, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 79

SPLEEN:
—Hemosiderin deposits, grade 2
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
PEYER'S PATCHES (JEJUNUM):
—Mineralization, focal/multifocal, grade 1
PEYER'S PATCHES (ILEUM):
—Lymphoid hyperplasia, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
ADRENAL CORTICES:
—Extra—adrenal tissue, unilateral
SKIN/SUBCUTIS:
—Inflammatory cell infiltrates, dermal, grade 1
EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
—Peri—orbital inflammation, unilateral, grade 1
HARDERIAN GLANDS:
—Porphyrin deposits, unilateral, grade 1
SKELETAL MUSCLE:
—Single myofiber degeneration, grade 1
UTERUS:
—Distended lumina of uterine horns, cyclic change
VAGINA:
—Proestrus phase of the estrous cycle
BONE MARROW (FEMUR):
—Fatty replacement, grade 2
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 91 * ANIMAL NO. : 80

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

HEART WITH AURICLES:
—Myocardial necrosis, grade 1
TRACHEA:
—Glandular dilation, grade 1
—Mononuclear cell infiltrates, grade 1
LIVER:
—Fatty change, grade 1
—Inflammatory cell foci, grade 1
—Increased hepatocytic glycogen deposits, grade 1
SPLEEN:
—Extramedullary hemopoiesis, grade 1
—Hemosiderin deposits, grade 2
MESENTERIC LYMPH NODE:
—Lymphoid hyperplasia, grade 1
STOMACH:
—Mucosal gland dilation, grade 1
LUNGS:
—Alveolar macrophages, focal/multifocal, grade 1
—Alveolar hemorrhages, focal/multifocal, grade 1
—Alveolitis, chronic, multifocal, with alv.wall hyperplasia, grade 2
—Mucoid secretion, with inflammatory cells, bronchiolar, grade 1
THYMUS:
—Cyst (s)
—Atrophy/involution, grade 1
MANDIBULAR LYMPH NODE:
—Lymphoid hyperplasia, bilateral, grade 1

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 1000 mg/kg FEMALE

CONT./FF. ANIMAL NO. : 80

EYES:
—Retro—orbital hemorrhage, unilateral, grade 2
—Peri—orbital inflammation, unilateral, grade 1
VAGINA:
—Proestrus phase of the estrous cycle
—Cystic mucosal degeneration, grade 2
BONE MARROW (FEMUR):
—Fatty replacement, grade 1
LARYNX:
—Inflammatory cell infiltrates, grade 2
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.