Safety Assessment of Basic Yellow 87 as Used in Cosmetics

Status:	Draft Report for Panel Review
Release Date:	November 10, 2022
Panel Meeting Date:	December 5-6, 2022

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/ Writer, CIR.

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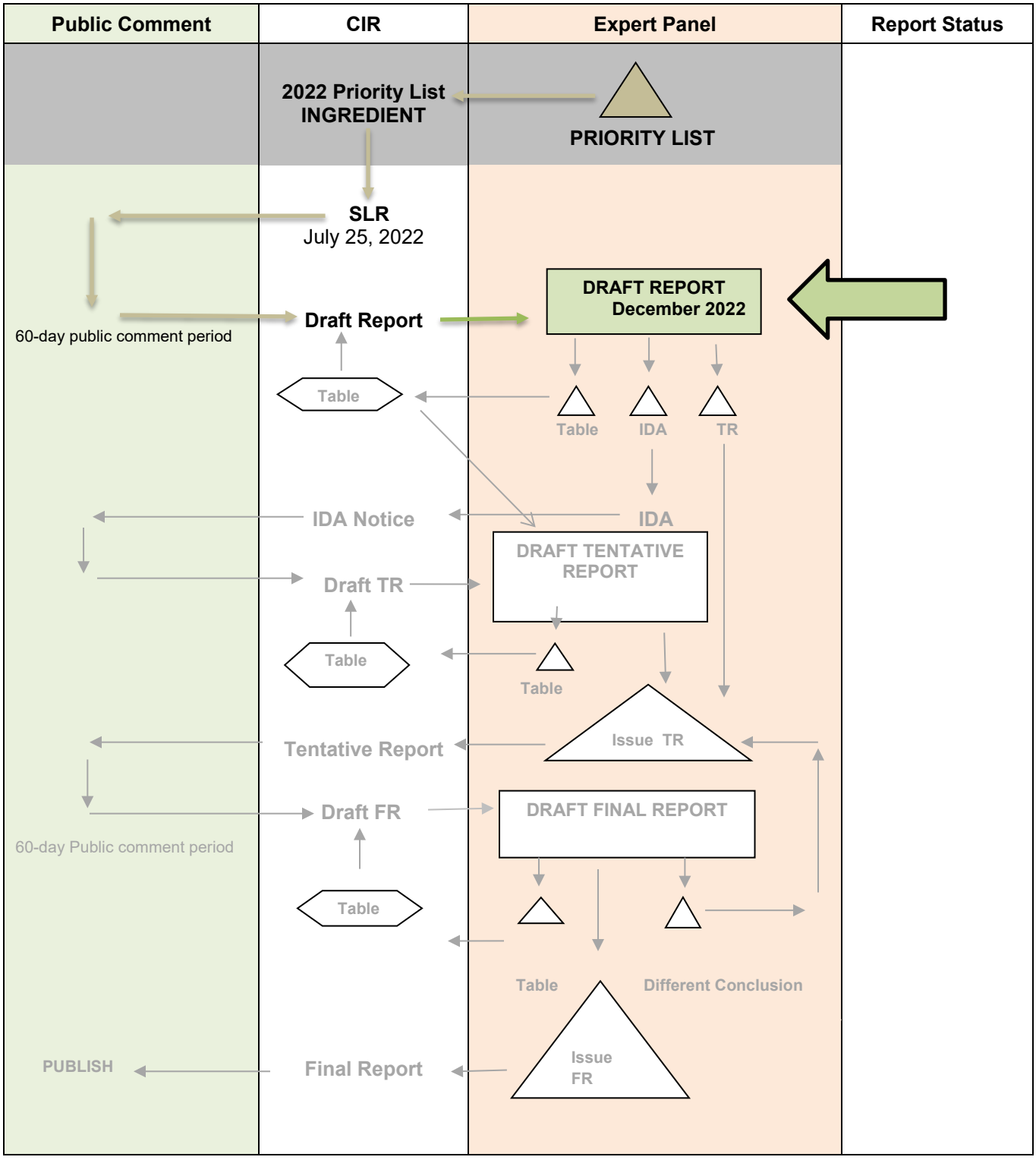
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Basic Yellow 87

MEETING December 2022



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, Senior Scientific Writer/Analyst, CIR
Monice M. Fiume, Senior Director, CIR
Date: November 10, 2022
Subject: Safety Assessment of Basic Yellow 87 as Used in Cosmetics

Enclosed is the Draft Report of the Safety Assessment of Basic Yellow 87 as Used in Cosmetics. (It is identified as *report_BasicYellow87_122022* in the pdf document.) The Scientific Literature Review (SLR) of this ingredient was issued by CIR on July 25, 2022. This ingredient is reported to function in cosmetics as a hair colorant.

According to 2022 VCRP survey data (*VCRP_BasicYellow87_122022*), Basic Yellow 87 is used in a total of 40 formulations. Of these reported uses, the majority (36) are in rinse-off hair coloring products. Four reported uses were in non-coloring hair products. One use in an aerosol hair color spray was also reported. The results of the concentration of use survey provided by the Council in 2022 (*data1_BasicYellow87_2022*) indicate that Basic Yellow 87 is used at up to 1% in hair dyes and colors and up to 0.02% in coloring shampoos.

At the September 2022 Panel meeting, a change to the current Use Table format was discussed. At that time, the Panel requested that both Use Table formats (i.e., the existing and the proposed format) be included in a Draft Report to provide a side-by-side comparison. That has been presented in this document to provide an example for a hair dye ingredient that has reported non-hair coloring uses. **CIR is asking that you compare the tables and provide your preference as to which format should be used in all future safety assessments.**

In addition to concentration of use survey data, the Council provided an oral short-term toxicity study, an in vitro dermal irritation study, a guinea pig maximization study, and an in vitro ocular irritation study (*data2_BasicYellow87_122022*). Comments provided by the Council on the SLR have been addressed (*PCPCcomments_BasicYellow87_122022* and *response-PCPCcomments_BasicYellow87_122022*).

Additional supporting documents for this report package include a flow chart (*flow_BasicYellow87_122022*), report history (*history_BasicYellow87_122022*), a search strategy (*search_BasicYellow87_122022*), and a data profile (*dataprofile_BasicYellow87_122022*).

If no further data are needed to reach a conclusion of safety, the Panel should formulate a Discussion and issue a Tentative Report. However, if additional data are required, the Panel should be prepared to identify those needs and issue an Insufficient Data Announcement.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: August 4, 2022

SUBJECT: Scientific Literature Review: Safety Assessment of Basic Yellow 87 as Used in Cosmetics (release date: July 25, 2022)

The Personal Care Products Council respectfully submits the following comments on the scientific literature review, Safety Assessment of Basic Yellow 87 as Used in Cosmetics.

Key Issues

Dermal Penetration, *In Vitro* – The actual results of the dermal penetration study in human skin using non-oxidative and oxidative formulations should be stated. Currently, the absorption values used by the SCCS are stated. These values are the means plus one standard deviation. The actual data from the study is presented in tables in the SCCS opinion. Please indicate why the SCCS "corrected" the absorption from the non-oxidative formulation (the formulation contained 0.9% Basic Yellow 87, while the SCCS was completing an MOS calculation for a 1% formulation).

Margin of Safety; Summary – The SCCS opinion is not completely clear about what 0.001 in their SED calculation represents. They are calculating the SED assuming a use concentration in hair dye of 1% and a retention factor of 0.1 (see SCCS notes of guidance for more information). It would be helpful if the CIR report noted that the calculation was for a product containing 1% Basic Yellow 87.

Additional Considerations

Abbreviations; Dermal Irritation and Sensitization - The "R" in DPRA stands for "reactivity" (rather than "reactive")

Chemical Properties; Table 1 – Although the SCCS opinion does not state the temperature at which the log P_{ow} was determined, it does state that the value was determined using OECD TG 107. The guideline indicates that the log P_{ow} should be determined at 20-25°C and that the temperature should be kept constant ±1 °C. At a minimum, the CIR report should state the OECD method.

Summary – To be consistent with the rest of the report, please change “MET-hemoglobin” to “methemoglobin”. In the paragraph on the developmental toxicity study, please correct “were observed were observed” (delete one “were observed”)

Table 5 – Please revise the following in the Results column of the second bacterial reverse mutation assay: “Test material did not induce point mutations by base pair changes or frameshifts observed” (delete “observed”). Please state the concentrations that were cytotoxic to Chinese hamster V79 cells. In the last study in this table, please correct “Test material did no induce” (change “no” to “not”).

Table 5, mouse lymphoma L5178Y cells – Because preparations from both rats and hamsters were used for metabolic activation in this study, more details should be provided in both the Procedure and Results column. Metabolic activation from only hamsters was used in the second study, and with this metabolic activation, Basic Yellow 87 was toxic to the cells even at the lowest concentration.

Table 5, human lymphocytes – It is misleading to state that “cytotoxicity was observed at 98.7 µg/ml”. This cytotoxicity was only observed in 1 of 2 cultures at 98.7 µg/ml. They did the assessment at this concentration from 200 cells of the second culture. There was no mention of cytotoxicity at the higher concentrations.

Table 6 – Please add Phototoxicity and Photosensitization to the title of this table.

Basic Yellow 87 - December 2022 – Christina Burnett	
Comment Submitter: Alexandra Kowcz, Personal Care Products Council **Date of Submission: August 4, 2022**	
Comment	**Response/Action**
Key Issue: Dermal Penetration, *In Vitro* – The actual results of the dermal penetration study in human skin using non-oxidative and oxidative formulations should be stated. Currently, the absorption values used by the SCCS are stated. These values are means plus one standard deviation. The actual data from the study is presented in tables in the SCCS opinion. Please indicate why the SCCS "corrected" the absorption from the non-oxidative formulation (the formulation contained 0.9% Basic Yellow 87, while the SCCS was completing an MOS calculation for a 1% formulation).	Corrected summarization of the data.
Key Issue: Margin of Safety; Summary – The SCCS opinion is not completely clear about what 0.001 in their SED calculation represents. They are calculating the SED assuming a use concentration in hair dye of 1% and a retention factor of 0.1 (see SCCS notes of guidance for more information). It would be helpful if the CIR report noted that the calculation was for a product containing 1% Basic Yellow 87.	Edit accepted.
Abbreviations; Dermal Irritation and Sensitization – The "R" in DPRA stands for "reactivity" (rather than "reactive").	Edit accepted.
Chemical Properties; Table 1 – Although the SCCS opinion does not state the temperature at which the log P_{ow} was determined, it dose state that the value was determined using OECD TG 107. The guideline indicates that the log P_{ow} should be determined at 20-25 ºC and that the temperature should be kept constant at $\pm$ 1 ºC. At a minimum, the CIR report should state the OECD method.	Test guideline temperature range added.
Summary – To be consistent with the rest of the report, please change "MET-hemoglobin" to "methemoglobin". In the paragraph on the developmental toxicity study, please correct "were observed were observed" (delete one "were observed").	Edits accepted.
Table 5 – Please revise the following in the Results column of the second bacterial reverse mutation assay: "Test material did not induce point mutations by base pair changes or frameshifts observed" (delete "observed"). Please state the concentrations that were cytotoxic to Chinese hamster V79 cells. In the last study in this table, please correct "Test material did no induce" (change "no" to "not").	Typographical errors corrected. Updated Chinese hamster V79 study to say "SCCS noted test material had a clear cytotoxic effect (no further details provided)." The opinion did not provide concentrations for cytotoxicity.
Table 5, mouse lymphoma L5178Y cells – Because preparations from both rats and hamsters were used for metabolic activation in this study, more details should be provided in both the Procedure and Results column. Metabolic activation from only hamsters was used in the second study, and with this metabolic activation, Basic Yellow 87 was toxic to the cells even at the lowest concentration.	Additional information on the metabolic activation utilized in the study and the outcome were added.
Table 5, human lymphocytes – It is misleading to state that "cytotoxicity was observed at 98.7 µg/ml". This cytotoxicity was only observed in 1 of 2 cultures at 98.7 µg/ml. They did the assessment at this concentration from 200 cells of the second culture. There was no mention of cytotoxicity at the higher concentrations.	Additional description was added.
Table 6 – Please add Phototoxicity and Photosensitization to the title of this table.	Edit accepted.

Basic Yellow 87 History

July 25, 2022– The Scientific Literature Review was issued for public comment.

August 8, 2022 – Unpublished data were received.

Basic Yellow 87 Data Profile* – December 2022 – Christina Burnett																														
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Basic Yellow 87	X		X	X	X	X	X	X			X			X	X	X			X	X		X	X		X	X			

* "X" indicates that new data were available in a category for the ingredient.

Basic Yellow 87

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Basic Yellow 87	68259-00-7	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Search Strategy for PubMed

(((68259-00-7[EC/RN Number]) OR (Pyridinium, 1- methyl-4-[(methylphenylhydrazono)methyl]-, methyl sulfate)) OR (269-503-2[EC/RN Number])) OR (Basic Yellow 87) - 31 returns, 0 relevant

LINKS

Search Engines

- Pubmed - http://www.ncbi.nlm.nih.gov/pubmed
- Connected Papers - https://www.connectedpapers.com/

Pertinent Websites

- wINCI - http://webdictionary.personalcarecouncil.org
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

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ABBREVIATIONS

ADME	absorption, distribution, metabolism, excretion
AUC	area under the curve
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
DPRA	direct peptide reactivity assay
ECHA	European Chemicals Agency
FDA	Food and Drug Administration
HPLC	high performance liquid chromatography
NOAEL	no-observable-adverse-effect-level
NOEL	no-observed-effect-level
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
SCCNFP	Scientific Committee on Cosmetic and Non-Food Products
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dose
TG	test guideline
US	United States
UV-Vis	ultraviolet-visible spectroscopy
VCRP	Voluntary Cosmetic Registration Program

INTRODUCTION

Basic Yellow 87 is reported to function as a hair colorant in cosmetic products, according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*).[1] This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Expert Panel for Cosmetic Ingredient Safety (Panel) typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/ supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some chemical and toxicological data on Basic Yellow 87 included in this safety assessment were obtained from robust summaries of data submitted to the European Chemicals Agency (ECHA) by companies as part of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) chemical registration process.[2] Additionally, data were obtained from opinions produced by the European Commission's Scientific Committee on Cosmetic and Non-Food Products (SCCNFP) and Scientific Committee on Consumer Safety (SCCS).[3,4] These data summaries are available on the ECHA and European Commission's database, respectively, and when deemed appropriate, information from the summaries has been included in this report.

CHEMISTRY

Definition and Structure

Basic Yellow 87 (CAS No. 68259-00-7) is a hair colorant that conforms to the structure in Figure 1.[1] It is reported to be used in semi-permanent and oxidative hair dye formulations, after mixing with an oxidative agent.[3]

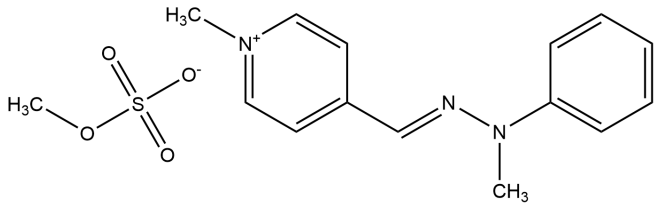

Figure 1. Basic Yellow 87

Chemical Properties

Available chemical properties of Basic Yellow 87 are provided in Table 1. Basic Yellow 87 is a yellow solid with the formula weight of 337.4 Da (methosulfate).[2,3] The log P_{ow} is -1.69 (20-25 ºC).

Method of Manufacture

No method of manufacturing data were found in the published literature, and unpublished methods were not submitted.

Composition/Impurities

The purity of Basic Yellow 87, as determined by high performance liquid chromatography (HPLC), was reported to be 61.1% - 92.6%.[3,4] Purity determined by ultraviolet-visible spectroscopy (UV-Vis) was reported to be 87.7% -92.9%.[4] Water content was reported to be $\leq$ 0.5%. Potential impurities and solvent residues may include $\leq$ 0.1% colored by-product and $\leq$ 0.1% isopropanol, respectively.[3] Salts of formulation or counter ions may include sodium chloride ($\leq$ 1.7%), methyl sulfate (up to 35.7%), and sulfate ($\leq$ 0.9%).[3,4] Heavy metal content was reported to be < 2 mg/kg (< 1 m/kg for mercury and cadmium, each).[4]

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics, and does not cover its use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush

delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2022 VCRP survey data, Basic Yellow 87 is used in a total of 40 formulations (Table 2).[5] Of these reported uses, the majority (36) are in rinse-off hair coloring products. Four reported uses were in non-coloring hair products. The results of the concentration of use survey provided by the Council in 2022 indicate that Basic Yellow 87 is used at up to 1% in hair dyes and colors and up to 0.02% in coloring shampoos.[6] [For comparison, Table 3 provides the frequency and concentration of use data by product category.]

Basic Yellow 87 is reported to be used in color sprays and could possibly be inhaled (concentration not reported).[5,6] In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.

Although products containing this ingredient may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of this ingredient (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

This ingredient is considered a coal tar hair dye for which regulations require caution statements and instructions regarding patch tests in order to be exempt from certain adulteration and color additive provisions of the US Federal Food, Drug, and Cosmetic Act. In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

> Caution - this product contains ingredients which may cause skin irritation on certain individuals and a preliminary test according to accompanying directions should be made. This product must not be used for dyeing the eyelashes or eyebrows; to do so may cause blindness.

Product labels shall also bear patch test instructions for determining whether the product causes skin irritation. However, whether or not patch testing prior to use is appropriate is not universally agreed upon. The Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 h after application of the test material and prior to the use of a hair dye formulation. Conversely, a report in Europe suggests that self-testing has severe limitations, and may even cause morbidity in consumers.[7,8] Hair dye products marketed and sold in the US, though, must follow the labeling requirements established by the Food, Drug, and Cosmetic Act.

In the European Union, Basic Yellow 87 is restricted to use in oxidative and non-oxidative hair dye products at a maximum concentration of 1.0%.[9] In 2003, the SCCNFP could not make a conclusion on the safety of Basic Yellow 87 due to methodological inadequacies in in vitro mammalian cell mutation tests.[3] However, in 2011, the SCCS concluded that Basic Yellow 87 "does not pose a risk to the health of the consumer when used in non-oxidative and oxidative hair dye formulations up to a concentration of 1.0% on-head."[4]

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

The percutaneous penetration/dermal absorption potential of a formulation containing 0.2% Basic Yellow 87 (88.6% - 92.6% pure) was studied using human female epidermis skin samples.[3] Using Franz diffusion cells, 90.2 - 109 mg/cm^2 (target dose 100 mg/cm^2) was applied to the skin surface for 30 min; the skin was then rinsed with warm water. The cells were then dismantled and a surface wipe, donor chamber rinse, filter paper support, tape strips, and the remaining skin samples were analyzed for test material content by HPLC (detection limit 2 ng/ml). The overall recovery of the applied dose was 98%. Permeation of the test material through the skin was detected in all but one of the cells treated with the formulation. The total percutaneous absorption of the test material (remaining in the skin + receptor phase) from the formulation was 0.082% of the applied dose, approximately equal to 0.16 µg/cm^2. The SCCNFP noted that the substance was not tested in the presence of an oxidizing agent.

In another study, rat (male HanBrl: WIST (SPF)) and human (female) split-thickness skin (200 µm each) was used to determine the percutaneous absorption of [^{14}C]Basic Yellow 87 (91.6% pure).[4] The study was performed in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 428. The skin samples were mounted in flow-through diffusion cells each consisting of a donor and receptor chamber (7 membranes/species). An area of 0.64 cm^2 was exposed to 179 µg/cm^2 of the test material. The penetration through the skin membranes was determined over a 24-h period under non-occluded conditions. The receptor fluid (physiological saline; 0.9% w/v) was delivered at a flow rate of about 3 ml/h during the test period, and the perfusate was collected in 1-h intervals for the first 6 h and then at 2-h intervals for

the remaining exposure period. Each skin membrane surface was rinsed 3 times with ethanol after 24 h. The skin membrane rinse fractions were combined according to the individual cells. The skin membranes were removed from the diffusion cell and stripped until the stratum corneum was removed from the skin membrane. Skin membranes remaining after stripping were digested in tissue solubilizer, the diffusion cells were washed with ethanol water (50/50 v/v), and the radioactivity was determined by liquid scintillation counting.

The total amount of the test material absorbed after 24 h, including that recovered from rat skin membrane and perfusates was 0.18% (standard deviation = 0.19%) of the applied dose. In the human skin membrane and perfusates 0.10% (standard deviation = 0.09%) was recovered. Of note, only 7 chambers were used per species, and in case of significant deviations, the conservative estimate of penetration was considered to be mean plus 2 standard deviations, i.e., 0.56% (1.0 $\mu g/cm^2$) in rats and 0.28% (0.50 $\mu g/cm^2$) in humans. It was concluded that Basic Yellow 87 penetrated at a low rate.[4,10]

In a similar percutaneous absorption study in human dermatomed skin (400 µm thickness), [^{14}C]Basic Yellow 87 (91.6% pure) was tested at 200 $\mu g/cm^2$ (nominal concentration) under both oxidative and non-oxidative conditions.[4] There were 9 membranes from 4 donors for each condition tested. After 30 min exposure, the membranes were each washed and and the skin was left unoccluded for the remainder of the 24-h experimental period. Under oxidative conditions, 0.18 $\pm$ 0.9% (equivalent to 0.31 $\pm$ 0.16 $\mu g/cm^2$) of Basic Yellow 87 was systemically available (mean value) from a formulation containing a final concentration of the dye at 0.975%. Under non-oxidative conditions, 0.17 $\pm$ 0.10% (equivalent to 0.33 $\pm$ 0.19 $\mu g/cm^2$) of Basic Yellow 87 was systemically available (mean value) from a formulation containing 0.9%. The SCCS considered these experiments well performed, and thus adjusted the penetration amount by using the mean plus one standard deviative; i.e., under oxidative conditions, 0.47 $\mu g/cm^2$ (0.28%) of Basic Yellow 87 was absorbed. Under non-oxidative conditions, 0.51 $\mu g/cm^2$ (0.27%) of Basic Yellow 87 was absorbed. According to the SCCS, the latter (non-oxidative) absorption value was corrected to 0.57 $\mu g/cm^2$ to allow for the calculation of the margin of safety with 1% Basic Yellow 87. The relevant cation absorbed under oxidative and non-oxidative conditions was 0.32 $\mu g/cm^2$ and 0.38 $\mu g/cm^2$, respectively.

Absorption, Distribution, Metabolism, Excretion (ADME)

Animal

Dermal

In an ADME study performed in accordance with OECD TG 417, 8 female Wistar rats (HanBrl:WIST (SPF) received 0.2 mg/cm^2 [^{14}C]Basic Yellow 87 (91.6% pure) dermally.[4] The concentration of radioactivity was determined in urine, feces, blood, plasma, and organs/tissues at different time points after administration. After 30 min, a skin wash and skin stripping were performed to remove any remaining test item and stratum corneum from the test site. The skin wash and skin strips were sampled to determine remaining amounts of the test material. Rats (4/timepoint) were killed at 24 h and at 96 h. Further methodology details were not provided. The results show that a very low fraction (0.3%) of the applied dose was absorbed from the skin into the systemic circulation. The concentrations of radioactivity for all blood sampling time points were below the limit of quantification. The amount of radioactivity determined in the stratum corneum was almost constant during the experimental period, accounting for 2.56% and 2.79% of the dose. It was concluded that Basic Yellow 87 was poorly absorbed.

Oral

In the same ADME study described above, 9 female rats received 10 mg/kg bw [^{14}C]Basic Yellow 87 (91.6% pure) via gavage.[4] Rats (3/timepoint) were killed at 24 h, at 48 h, and at 96 h. Further methodology details were not provided. Approximately 6% of the administered test material was absorbed from the gastrointestinal tract into systemic circulation. Oral absorption was fast, with a maximum concentration in blood and plasma reached 1 h after administration and accounting of 0.143 ppm and 0.283 ppm, respectively. A two-phase decrease of concentration was then observed, with an initial half-life of 7.5 and 5.6 h in blood and plasma, respectively, and a second half-life of 48 h (blood) and 45 h (plasma). Within 96 h after exposure, almost all of the test material was removed from the blood and plasma. The area under the curve (AUC) for 0 - 24 h was 1.72 µg·h/g for blood and 2.10 µg·h/g for plasma. The test material was rapidly excreted, predominately from feces (89% after 96 h). The test material was also excreted from urine (5.3% after 96 h). Approximately 0.1% of the dose was still remaining in tissue and carcass after 96 h. The highest residue levels were found at 24 h in the liver and kidneys, but at very low amounts. The metabolite pattern in urine revealed 1 major and 10 minor metabolite fractions. The major fractions represented more than 50% of the radioactivity in the urine or 2.5% of the dose, and was shown to contain a glucuronic acid conjugate of Basic Yellow 87 formed after hydroxylation of the phenyl moiety and its structural isomer. It was concluded that Basic Yellow 87 has low absorption after oral exposure.

In an oral bioavailability study, 15 female NMRI hybrid mice received 40 mg/kg bw Basic Yellow 87 (91.6% pure) in MilliQ water via a single gavage treatment.[4] The test material was radio-labelled and the study was performed in accordance with OECD TG 417. At 0.5, 1, 2, 4, and 24 h after treatment, 3 mice were killed, and the concentration of the test material was determined in the plasma and femur. No other tissues or endpoints were examined. Basic Yellow 87 was found to be rapidly absorbed in the gastrointestinal tract. The maximum concentration in plasma was observed 0.5 h after treatment and corresponded to 4.823 ppm equivalents/g. A two-phase decrease of the plasmatic concentration was observed with an initial

half-life of 1.2 h and a second half-life of 6 h. The AUC for 0 - 24 h for plasma was 14.25 µg·h/g. The maximum concentration in the femur was observed at 0.5 h and corresponded to 1.273 ppm equivalents/g. Depletion kinetics were similar to that observed in plasma, but with a slightly slower half initial half-life of 3.3 h and a terminal half-life of 13 h. The authors assumed that the radioactivity determined in the femur was predominately located in the bone marrow, and that it correlated to the unchanged test item or its metabolites.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Acute dermal and oral studies summarized here are described in Table 4. In an acute dermal study in rats, the LD_{50} for Basic Yellow 87 (87.7% pure) was greater than 2000 mg/kg bw.[3] In oral studies using rats, the LD_{50} in a limit study of Basic Yellow 87 (purity not reported) was between 500 and 1000 mg/kg bw in females and > 1500 mg/kg bw in males, when tested at up to 2000 mg/kg. [2,3] The LD_{50} was estimated to be 1000 mg/kg bw in another study where Basic Yellow 87 (purity not reported) was tested at 1000 mg/kg in male rats and at 500 mg/kg in female rats.[2]

Short-Term and Subchronic Toxicity Studies

Short-term and subchronic toxicity studies summarized here are described in Table 5. In a 2-wk gavage study, in which rats were dosed with up to 1000 mg/kg/d of a formulation containing 70% Basic Yellow 87, the no-observable-effect-level (NOEL) was 100 mg/kg/d.[11] All rats tested at 1000 mg/kg/d died or were killed before completion of study, and rats in the 300 mg/kg/d dose group exhibited higher absolute mean adrenal gland weights (males only), higher mean liver weights (both sexes), and epithelial cell hyperplasia and hyperkeratosis in the forestomach (males). In an oral study, rats that received up to 184 mg/kg bw Basic Yellow 87 (> 92% pure) in feed for 28-d study had decreased feed consumption, mean body weights, and body weight gains, slightly reduced total protein and globulin levels, and slightly increased albumin:globulin ratios at the highest doses tested. The no-observable-adverse-effect-level (NOAEL) for this study was 174 mg/kg bw/d and the NOEL was ~ 39 mg/kg bw/d. In a 13-wk dietary study, the NOAEL was 10 mg/kg bw/d in rats that received up to 245.2 mg/kg bw/d Basic Yellow 87 (> 92% pure).[3,4] Adverse effects included reduced feed and body weight gains (males), increase in methemoglobin levels (both sexes), and decreased white blood cell number (males) at the high dose and decreased total bilirubin levels (females) at mid and high doses.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

The teratogenic potential of Basic Yellow 87 (> 92% pure) was studied in mated female Wistar rats.[3] The study was performed in accordance with OECD TG 414. Groups of 22 females received 0, 20, 60, or 180 mg/kg bw of the test material in 4% carboxymethyl cellulose in twice-distilled water via gavage. The rats received the test material once daily from day 6 to day 17 of gestation. Body weights, feed consumption, mortality, and clinical signs of toxicity were recorded. On gestation day 21, all females were killed and maternal organs were examined. The uteri were weighed, and the fetuses were removed, weighed, and examine for sex and gross external abnormalities.

No maternal deaths were observed. No clinical signs were noted except for yellow feces and/or urine in the 60 and 180 mg/kg dose groups. Reduced feed consumption and weight gain were also observed at 60 and 180 mg/kg. No treatment-related changes were noted in the number of implantations, resorptions and fetuses, fetal weight, and external abnormalities, with the exception of 1 fetus with a cleft palate in the 20 mg/kg dose group, and 1 edematous fetus and a slight increase in fetal weight in the 180 mg/kg dose group. Some observed skeletal abnormalities were not considered related to the test material. Based on the results of this teratology study, the maternal and fetal NOAEL was determined to be 60 mg/kg bw/d.[3]

GENOTOXICITY STUDIES

In vitro and in vivo genotoxicity studies on Basic Yellow 87 summarized here are detailed in Table 6. Basic Yellow 87 was not mutagenic in Ames tests at up to 5000 µg/plate (87.7% and unreported purity), nor in a gene mutation test using Chinese hamster V79 cells, with and without metabolic activation, at up to 600 µg/ml (88.6% pure).[2-4] Basic Yellow 87 (91.6% pure; tested at up to 950 µg/ml) was mutagenic and/or clastogenic in a gene mutation test with mouse lymphoma cells, with and without metabolic activation; however, a chromosomal aberration test of Basic Yellow 87 (90.5% pure; tested at up to 288 µg/ml) was negative for clastogenic and/or aneugenic activity.[3,4] In vivo testing found that Basic Yellow 87 (88.6% pure) did not induce an increased frequency of polychromatic erythrocytes or increased mean number in normochromatic erythrocytes in a mammalian erythrocyte micronucleus test when mice were given a single oral dose by gavage at up to 125 mg/kg bw.[3] No increase in unscheduled DNA synthesis was observed in hepatocytes after rats were exposed to a single dose of up to 500 mg/kg Basic Yellow 87 (88.6% pure).

CARCINOGENICITY STUDIES

No carcinogenicity studies were found in the published literature, and unpublished data were not submitted.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Dermal irritation and sensitization studies on Basic Yellow 87 summarized here are detailed in Table 7. A mixture containing 0.24% Basic Yellow 87 (test concentration of 0.12% Basic Yellow 87 after dilution with another mixture) was predicted to be non-irritating in an EpiDerm™ skin model.[12] No dermal irritation was observed in rabbits tested with 0.5 g Basic Yellow 87 (87.7% pure) in 0.5 ml distilled water.[2,3] No skin reactions were observed in male and female Himalayan spotted guinea pigs that were treated for 15 d with up to 5% Basic Yellow 87 (> 92% pure).[3] Basic Yellow 87 was not peptide reactive in a direct peptide reactive assay (DPRA; purity tested not reported), and was not sensitizing in a guinea pig maximization test with 1% intradermal induction, a 50% topical induction, and a 50% challenge (tested at > 92% pure). A formulation with 70% Basic Yellow 87 produced sensitization in 10% of animals in a guinea pig maximization test with a 1% intradermal induction, a 25% topical induction, and a 25% challenge.[13]

Phototoxicity/Photosensitization Studies

Dermal phototoxicity/photosensitization studies on Basic Yellow 87 summarized here are also detailed in Table 7. Basic Yellow 87 was not phototoxic and did not induce photosensitization in Himalayan spotted albino guinea pigs at concentrations up to 50%.[3]

OCULAR IRRITATION STUDIES

In Vitro

In vitro and animal ocular irritation studies summarized here are detailed in Table 8. In an in vitro study using isolated chicken eyes, Basic Yellow 87 (99.2% pure) was irritating when tested neat and not irritating when tested at a 5% aqueous dilution.[2] A mixture containing 0.24% Basic Yellow 87 (test concentration of 0.12% Basic Yellow 87 after dilution with another mixture) was a mild irritant in a bovine corneal opacity and permeability assay.[14] In a rabbit ocular irritation study, Basic Yellow 87 (87.7% pure) was moderately irritating.[2,3]

MARGIN OF SAFETY

The SCCS calculated the margin of safety for a product containing 1% Basic Yellow 87 (non-oxidative conditions) to be 184.[4] This calculation is based on an adjusted NOAEL (10% bioavailability due to the low oral bioavailability as shown in an ADME study) of 0.676 mg/kg bw/d from a 13-wk oral rat study (as cation) and a systemic exposure dose (SED) of 0.0037 mg/kg bw (skin area surface of 580 cm^2 x absorption through skin of 0.38 (cation) $\mu g/cm^2$ x 0.001 (unit conversion)/typical human bw of 60 kg). The margin of safety under oxidative conditions was reported to be very similar.

HAIR DYE EPIDEMIOLOGY

Hair dyes may be broadly grouped into oxidative (permanent) and direct (temporary or semi-permanent) dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes consist of preformed colors. Basic Yellow 87 is reported to be used in semi-permanent and oxidative hair dye formulations. While the safety of individual hair dye ingredients is not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer. A detailed summary of the available hair dye epidemiology data is available at https://www.cir-safety.org/cir-findings.

SUMMARY

Basic Yellow 87 is reported to function as a hair colorant; specifically, it is used in semi-permanent and oxidative hair dye formulations, after mixing with an oxidative agent. According to 2022 VCRP survey data, Basic Yellow 87 is used in a total of 40 formulations. Of these reported uses, the majority (36) are in rinse-off hair coloring products. The results of the concentration of use survey provided by the Council in 2022 indicate that Basic Yellow 87 is used at up to 1% in hair dyes and colors and up to 0.02% in coloring shampoos.

In vitro percutaneous absorption studies in rat and human skin found that Basic Yellow 87 (88.6% - 92.6% pure) absorbed slowly. According to dermal and oral ADME studies in rats, Basic Yellow 87 (91.6% pure) does not readily absorb through the skin or the gastrointestinal tract. In the oral study, excretion mainly occurred in the feces. However, an oral bioavailability study in mice showed that Basic Yellow 87 (91.6% pure) rapidly absorbed in the gastrointestinal tract.

In an acute dermal study in rats, the LD_{50} for Basic Yellow 87 (87.7% pure) was greater than 2000 mg/kg bw. In oral studies using rats, the LD_{50} in a limit study of Basic Yellow 87 (purity not reported) was between 500 and 1000 mg/kg bw in females and > 1500 mg/kg bw in males when tested at up to 2000 mg/kg. The LD_{50} was estimated to be 1000 mg/kg bw in another study where Basic Yellow 87 (purity not reported) was tested at 1000 mg/kg in male rats and at 500 mg/kg in female rats.

A 2-wk gavage study, in which rats were dosed with up to 1000 mg/kg/d of a formulation containing 70% Basic Yellow 87, had a NOEL of 100 mg/kg/d. All rats tested at 1000 mg/kg/d died or were killed before completion of study and rats in the

300 mg/kg/d dose group exhibited higher absolute mean adrenal gland weights (males only), higher mean liver weights (both sexes), and epithelial cell hyperplasia and hyperkeratosis in the forestomach (males). In an oral study, rats that received up to 184 mg/kg bw Basic Yellow 87 (> 92% pure) in feed in a 28-d study had decreased fed consumption, mean body weights, and body weight gains and slightly reduced total protein and globulin levels and slightly increased albumin:globulin ratios at the highest doses tested. The NOAEL for this study was 174 mg/kg bw/d and the NOEL was ~ 39 mg/kg bw/d. In a 13-wk dietary study, the NOAEL was 10 mg/kg bw/d in rats that received up to 245 mg/kg bw/d Basic Yellow 87 (> 92% pure). Adverse effects included reduced feed and body weight gains (males), increase in methemoglobin levels (both sexes), and decreased white blood cell number (males) at high doses and decreased total bilirubin levels (females) at mid and high doses.

The maternal and fetal NOAEL for Basic Yellow 87 (> 92% pure) was determined to be 60 mg/kg bw/d in a teratogenic study in mated female rats. The dams received up to 180 mg/kg bw of the test material during days 6 - 17 of gestation. Reduced feed consumption and weight gain were observed in the mid and high dose groups and slight increase in fetal weight was observed in the high dose group.

Basic Yellow 87 was not mutagenic in Ames tests at up to 5000 µg/plate (87.7% and unreported purity), nor in a gene mutation test using Chinese hamster V79 cells, with and without metabolic activation, at up to 600 µg/ml (88.6% pure). Basic Yellow 87 (91.6% pure; tested at up to 950 µg/ml) was mutagenic and/or clastogenic in a gene mutation test with mouse lymphoma cells, with and without metabolic activation; however, a chromosomal aberration test of Basic Yellow 87 (90.5% pure; tested at up to 288 µg/ml) was negative for clastogenic and/or aneugenic activity. In vivo testing found that Basic Yellow 87 (88.6% pure) did not induce an increased frequency of polychromatic erythrocytes or increased mean number in normochromatic erythrocytes in a mammalian erythrocyte micronucleus test when mice were given a single oral dose by gavage at up to 125 mg/kg bw. No increase in unscheduled DNA synthesis was observed in hepatocytes after rats were exposed to a single dose of up to 500 mg/kg Basic Yellow 87 (88.6% pure).

A mixture containing 0.24% Basic Yellow 87 (test concentration of 0.12% Basic Yellow 87 after dilution with another mixture) was predicted to be non-irritating in an EpiDerm™ skin model. No dermal irritation was observed in rabbits tested with 0.5 g Basic Yellow 87 (87.7% pure) in 0.5 ml distilled water. No skin reactions were observed in male and female Himalayan spotted guinea pigs that were treated for 15 d with up to 5% Basic Yellow 87 (> 92% pure). Basic Yellow 87 was not peptide reactive in a DPRA (purity tested not reported), and was not sensitizing in a guinea pig maximization test with 1% intradermal induction, a 50% topical induction and a 50% challenge (tested at > 92% pure). A formulation with 70% Basic Yellow 87 produced sensitization in 10% of animals in a guinea pig maximization test with a 1% intradermal induction, a 25% topical induction, and a 25% challenge. Basic Yellow 87 was not phototoxic and did not induce photosensitization in Himalayan spotted albino guinea pigs at concentrations up to 50%.

In an in vitro study using isolated chicken eyes, Basic Yellow 87 (99.2% pure) was irritating when tested neat and not irritating when tested at a 5% aqueous dilution. A mixture containing 0.24% Basic Yellow 87 (test concentration of 0.12% Basic Yellow 87 after dilution with another mixture) was a mild irritant in a bovine corneal opacity and permeability assay. In a rabbit ocular irritation study, Basic Yellow 87 (87.7% pure) was moderately irritating.

A margin of safety for Basic Yellow 87 under non-oxidative conditions was calculated to be 184. This calculation was based on an adjusted NOAEL of 0.676 mg/kg bw/d from a 13-wk oral rat study and a SED of 0.0037 mg/kg bw. The margin of safety under oxidative conditions was reported to be very similar.

The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer.

No carcinogenicity studies on Basic Yellow 87 were found in the published literature, and unpublished data were not submitted.

DISCUSSION

To be developed.

CONCLUSION

To be determined.

TABLES

Table 1. Chemical properties for Basic Yellow 87

Property	Value	Reference
Physical Form	Yellow solid	2,3
Formula Weight (Da)	337.4 (methosulfate)	3
Density (tapped; g/ml @ 20 °C)	0.4	2
Vapor Pressure (mmHg @ 20 °C	$< 10 \times 10^{-10}$	2
@ 25 °C)	$< 4.1 \times 10^{-10}$	
Melting Point (°C)	140, not melting, decomposition at higher temperatures	3
	150-164, decomposition above 240	2
Water Solubility (g/l @ 20 °C)	40	3
	620	2
log $P_{o/w}$ (20-25 ºC)	-1.69	2,3

Table 2 Frequency (2022)[5] and concentration (2022)[6] of use according to duration and exposure.

	# of Uses	Max Conc of Use (%)
	Basic Yellow 87	
Totals*	**40**	**0.0007-1**
Duration of Use		
Leave-On	*3*	*NR*
Rinse Off	*37*	*0.0007-1*
Diluted for (Bath) Use	*NR*	*NR*
Exposure Type		
Eye Area	NR	NR
Incidental Ingestion	NR	NR
Incidental Inhalation-Spray	1	NR
Incidental Inhalation-Powder	NR	NR
Dermal Contact	NR	NR
Deodorant (underarm)	NR	NR
Hair - Non-Coloring	4	NR
Hair-Coloring	36	0.0007-1
Nail	NR	NR
Mucous Membrane	NR	NR
Baby Products	NR	NR

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
NR – not reported

Table 3. Frequency (2022)[5] and concentration (2022)[6] of use by product category for Basic Yellow 87.

Product Category	# of uses	Max conc of use	Likely Exposure Site
Hair Conditioner	1	NR	hair
Shampoo (non-coloring)	1	NR	hair
Other Hair Preparations	2	NR	hair
Hair Dyes and Colors (all types requiring caution statements and patch tests)	19	0.0007-1%	hair
Hair Rinses (coloring)	6	NR	hair
Hair Shampoos (coloring)	6	0.02%	hair
Hair Color Sprays (aerosol)	1	NR	hair
Other Hair Coloring Preparation	4	NR	hair
Totals	**40**	**0.0007-1%**	**hair**

Table 4. Acute toxicity studies on Basic Yellow 87

Animals	No./Group	Vehicle	Dose/Protocol	LD_{50}/Results	Reference
			DERMAL		
Crl:CD (SD)IGS BR rats	5 males and 5 females	none	2000 mg/kg (87.7% pure) in accordance with OECD TG 402; test sites (an area of approximately 10% of total surface area) were occluded for 24 h; test material moistened with water prior to application and removed with water and paper towel after treatment period; observed for signs of toxicity for 14 d	> 2000 mg/kg bw; no signs of dermal toxicity	[3]
			ORAL		
Crl:CD (SD)IGS BR rats	2 males and 2 females per group, except 5 males and 5 females in the high-dose group	water	500, 1000, 1500, or 2000 mg/kg (purity not reported) via single gavage dose (limit test)	LD_{50} between 500 and 1000 mg/kg in females and > 1500 mg/kg in males: all males survived the 500 and 1000 mg/kg doses and 1 male survived the 1500 mg/kg dose, no males survived the 2000 mg/kg dose; all females survived the 500 mg/kg dose but none survived the 1000-2000 mg/kg doses; enlarged heart observed in 1 male at 1500 mg/kg; dark-red lobes and dark areas on the lung of 1 male and 1 female at 500 mg/kg; no other visible lesions observed	[2,3]
Crl:CD (SD)IGS BR rats	5 males at 1000 mg/kg and 5 females at 500 mg/kg	water	Oral toxicity study in accordance with OECD TG 420	LD_{50} estimated as 1000 mg/kg; 2/5 males in the 1000 mg/kg died, no females died; prior to death, clinical signs in these animals included hypoactivity, ataxia, squinted eyes, liquid or mucoid feces, discolored feces, and/or discolored urine; distended stomach, ileum, duodenum, jejunum, colon, and bladder, and yellow fluid observed in 1 of the dead animals; remaining surviving males in the 1000 mg/kg and the 500 mg/kg females had similar clinical signs in addition to urine/fecal staining, crust around eyes and/or hair in the genital region; necropsy of surviving animals only showed a pale area in the liver of 1 female	[2]

Table 5. Repeated dose toxicity studies of Basic Yellow 87

Test Material Dose/Concentration	Animals/Group	Study Duration	Vehicle	Protocol	Results	Reference
ORAL						
0, 100, 300, or 1000 mg/kg/d of formulation containing 70% Basic Yellow 87	6 male and 6 female Sprague-Dawley rats per group	2-wk	water	Gavage study in accordance with OECD TG 407; animals received test material daily and were checked daily for mortality and clinical signs; feed consumption and body weight measured twice a week; hematology and blood chemistry investigation performed during week 2; all animals killed at study end and underwent necropsy; designated organs weighed and macroscopic lesions, liver, and kidneys submitted for microscopic examination	NOEL = 100 mg/kg/d; all rats in 1000 mg/kg/d dose group died or were killed prematurely after 7-15 d of treatment following numerous signs of poor clinical condition; necropsy of 1000 mg/kg/d dose group revealed all rats had dilatation/overdistension of the stomach; test material induced yellowish coloration of urine and feces in 100 and 300 mg/kg/d dose groups; ptyalism observed at all dose-levels in a dose-related manner; body weight gains and feed consumption of the 100 and 300 mg/kg/d dose groups was similar to controls, but it was markedly reduced in the 1000 mg/kg/d dose group; no significant findings in hematology for any dose group; higher urea nitrogen level and lower cholesterol level observed in males in 1000 mg/kg/d dose group; higher absolute mean adrenal gland weights observed in males in 300 mg/kg/d dose group and higher mean liver weights recorded in both sexes in the 300 mg/kg/d dose group; yellowish contents observed in urinary bladder of the males in the 300 mg/kg/d dose group; epithelial cell hyperplasia and hyperkeratosis in the forestomach observed in the 300 (males) and 1000 mg/kg/d (both sexes) dose groups	[11]
9, 38.8, or 174 mg/kg bw/d in males and 8.2, 40, or 184 mg/kg bw/d in females; purity > 92%	HanIbm: WIST rats; 5 males and 5 females per group, except 10 males and 10 females in controls and high dose groups	28-d study	feed	Study performed in accordance with OECD TG 407; controls received normal diet	NOAEL = 174 mg/kg bw/d and NOEL = ~ 39 mg/kg bw/d; yellow discoloration of feces noted in all high-dose rats, yellow urine discoloration observed in all animals that received test material; no toxicologically-significant effects on hematology, clinical biochemistry, or urinalysis observed; no abnormal findings in functional observational battery; feed intake, mean body weight, and body weight gain slightly lower in high-dose males; slightly reduced total protein and globulin level and slightly increased albumin:globulin ratios recorded in high-dose males	[3]
9.7, 48.5, or 245.2 mg/kg bw/d in males and 10.1, 48.9, or 245.0 mg/kg bw/d in females; purity > 92%	Wistar SPF-bred rats; 10 males and 10 females per group	13-wk study	feed	Study performed in accordance with OECD TG 408; control animals received normal diet	NOAEL = 10 mg/kg bw/d, corresponding to a dose of 6.76 mg/kg bw/d of the cation; no adverse effects observed in ophthalmologic or functional observational battery findings; colored feces observed in both sexes of the mid- and high-dose groups; high-dose females had increased urine pH, all tested females had decrease in uric acid levels; total bilirubin levels decreased in mid and high dose females; effects in only the high-dose group included: reduced feed and body weight gains (males), increase in methemoglobin levels (both sexes), decreased white blood cell number (males), increased platelet count (females), changes in creatinine levels, total protein amount, glucose levels, and changes in several organ/body weights and organ/brain ratios	[3,4]

Table 6. Genotoxicity studies on Basic Yellow 87

Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
IN VITRO					
33.3, 100, 333, 1000, 3300, or 5000 µg/plate following a dose range finding study of 6.67- 5000 µg/plate; purity = 87.7%	water	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2MvrA	Bacterial reverse mutation test in accordance with OECD TG 471; with and without S9 metabolic activation	Test material did not cause a positive increase in revertant frequencies, with or without metabolic activation	2,3
33.3, 100, 333, 1000, 2500, or 5000 µg/plate; purity not reported	twice-distilled water	*S. typhimurium* strains TA98 and TA100	Bacterial reverse mutation test in accordance with OECD TG 471; with and without metabolic activation	Test material did not induce point mutations by base pair changes or frameshifts	2
Test 1: 3, 10, 30 or 100 µg/ml without metabolic activation and 3.0, 30, 100, or 300 µg/ml with metabolic activation Test 2: 3, 10, 30, 50, 100, or 200 µg/ml without metabolic activation and 30, 50, 100, 300, 450, or 600 µg/ml with metabolic activation 88.6% pure	culture medium	Chinese hamster V79 cells	Mammalian cell gene mutation test at the HGPRT locus in accordance with OECD TG 476; with and without metabolic activation	Not mutagenic; no biologically-relevant statistically significant increase in mutant frequency observed in either test, with or without metabolic activation; SCCS noted test material had a clear cytotoxic effect (no further details provided)	3,4
Test 1: 118.8 - 950 µg/ml without metabolic activation and 59.4 - 712.5 µg/ml with metabolic activation Test 2: 200 - 600 µg/ml without metabolic activation and 30 - 120 µg/ml with metabolic activation 91.6% pure	deionized water	Mouse lymphoma L5178Y cells	Mammalian cell gene mutation test in accordance with OECD TG 476; with and without metabolic activation using rat and hamster S9-mix in Test 1 and only hamster S9-mix in Test 2	Mutagenic and/or clastogenic, with and without metabolic activation; concentration-dependent increase in mutant frequency observed, with genotoxic potency highest with metabolic activation; using hamster metabolic activation caused toxic effects at the lowest concentration in Test 2; ratio of small versus large colonies shifted towards small colonies; no further details	4
3.55 - 288 µg/ml; 90.5% pure	water	Human lymphocytes	Mammalian chromosomal aberration test in accordance with OECD TG 473; with and without metabolic activation	Negative for clastogenic and/or aneugenic activity, with and without metabolic activation; excessive cytotoxicity observed at 98.7 µg/ml in one culture, which required 200 cells to be scored from the duplicate culture – cytotoxicity was not described at higher concentrations	3
IN VIVO					
0, 12.5, 40, or 125 mg/kg bw; 88.6% pure	deionized water	Groups of 6 male and 6 female NMRI mice	Mammalian erythrocyte micronucleus test in accordance with OECD TG 474; single dose via gavage; groups of animals killed at 24, 48, or 72 h post-treatment; appropriate negative and positive controls used	Test material did not induce a statistically significant increase in the frequency of polychromatic erythrocytes; mean number of normochromatic erythrocytes not significantly increased after treatment as compared to controls	3
0, 250, or 500 mg/kg; 88.6% pure	not reported	Groups of 4 male Wistar HanIbm: WIST (SPF) rats	Unscheduled DNA synthesis test in accordance with OECD TG (draft) 486; single gavage dose; sampling times were 2 and 16 h post-treatment	Test material did not induce increased unscheduled DNA synthesis in hepatocytes	3

Table 7. Dermal irritation, dermal sensitization, and phototoxicity and photosensitization studies on Basic Yellow 87

Concentration/Dose	Test Population	Procedure	Results	Reference
		IRRITATION		
		IN VITRO		
Mixture containing 0.24% Basic Yellow 87; upon dilution; final test concentration of 0.12% Basic Yellow 87	reconstructed human epidermis	EpiDerm™ skin model; test material was diluted at a 1:1 ratio with another mixture prior to assessment; negative control was sterile calcium- magnesium free Dulbecco's phosphate buffered saline; the positive control was 5% sodium lauryl sulfate	Predicted to be non-irritating; mean viability = 104.3%	[12]
		ANIMAL		
0.5 g in 0.5 ml distilled water; 87.7% pure	2 male and 1 female New Zealand White rabbits	In accordance with OECD TG 404; semi-occlusive; test area = 6.25 cm^2; intact test sites for 4 h and then rinsed off; scoring of reactions at 0.5 to 1, 24, 48, and 72 h	Not irritating; primary dermal irritation index calculated to be 0.0; no evidence of corrosion; no evidence of treatment-related toxicity during treatment	[2,3]
0.5%, 1%, 3%, and 5% tested at 0.1 ml/ 7 cm^2; purity > 92%	Himalayan spotted guinea pigs; 4 males and 4 females	Study performed in accordance with OECD TG 402; 2 application sites were marked on the shaved backs of 6 treated animals; 2 animals were controls and treated with just vehicle (type not reported); a complete clock design was used so each concentration was tested 3 times on 3 different animals/sex; not occluded; treated skin flushed with water prior to each new application; skin shaved regularly and depilated on day 15 prior to final reading; skin reactions observed daily	No grading scores recorded on days 2 – 14 due to slight accumulation of test material on skin; no skin reaction was observed on final day after depilation	[3]
		SENSITIZATION		
		IN VITRO		
Not reported	lysine and cysteine peptides	DPRA; no further details provided	Not peptide reactive; no further details provided	[2]
		ANIMAL		
1% intradermal induction in physiological saline; 50% epidermal induction in twice-distilled water; challenge 50% in twice-distilled water; purity > 92%	15 female Himalayan spotted (GOHI, SPF-quality) guinea pigs	Guinea pig maximization test in accordance with OECD TG 406; 10 animals received test material, 5 were negative controls; intradermal induction (10 ml/site) included Freund's complete adjuvant followed 1 wk later with epidermal induction under occlusion, sites pre-treated with 10% sodium lauryl sulfate; 2 wk after induction, animals challenged with 50 % test material under occlusion	Not sensitizing; no reactions observed in the control or test groups during challenge	[2,3]
Formulation containing 70% Basic Yellow 87; 1% intradermal injection; 25% topical induction; challenge 25%; vehicle was sterile isotonic saline solution (0.9% sodium chloride)	Treatment group had 10 male and 10 female Dunkin-Hartley guinea pigs; control group had 5 males and 5 females	Guinea pig maximization test in accordance with OECD TG 406; intradermal induction (0.1 ml/site) included Freund's complete adjuvant followed 1 wk later with topical induction (0.5 ml) under occlusion for 48 h, sites pre-treated with 10% sodium lauryl sulfate in petrolatum; 2 wk after induction, animals challenged with 25% test material (0.5 ml) under occlusion for 24 h; animals killed at study end and cutaneous samples taken from challenge sites for histological examination	Skin coloration from test material prevented scoring for erythema, thus evaluation of skin sensitization performed by microscopic examination Histological examination revealed cutaneous reactions attributable to sensitization in 10% of animals treated with the test material.	[13]

Table 7. Dermal irritation, dermal sensitization, and phototoxicity and photosensitization studies on Basic Yellow 87

Concentration/Dose	Test Population	Procedure	Results	Reference
		PHOTOTOXICITY/PHOTOSENSITIZATION		
		ANIMAL		
0.025 ml/cm^2 dilution in concentrations of 10%, 15%, 25%, or 50% in water	15 female Himalayan spotted albino guinea pigs, 10 test and 5 control	Animals were treated with 2% dimethyl sulfoxide in ethanol to enhance skin penetration; test material applied topically and openly to 2 cm^2 areas on both flanks; 30 min after application, left flank exposed to 20 J/cm^2 UVA irradiation and right flank remained unexposed to light and served as reference; control animals exposed to UVA and vehicle; skin reactions evaluated at 24, 48, and 72 h after treatment	At 24 h, phototoxic reactions observed in 6 of the animals at 50% and 3 of the animals at 25%; positive reactions observed after 24 h in the non-radiated skin site of 1 of the animals at 50% and 2 of the animals at 25% were determined to be incidental and not related to the test material; no reactions observed at 48 or 72 h; no further details	[3]
0.1 ml /8 cm^2 of 50% in water	20 Himalayan spotted albino guinea pigs (sex not reported); additional 10 animals were controls	For induction, test material was applied epicutaneously to 8 cm^2 area in nuchal region that received 4 intradermal injections of Freund's complete adjuvant/ physiological saline; sites then exposed to 1.8 J/cm^2 UVB and 10 J/cm^2 UVA (5 total exposures in 2 wk); controls treated with only vehicle Challenge occurred 3 wk after beginning of induction on both flanks with test material at 10%, 15%, 25%, or 50% in water; test sites irradiated with 10 J/cm^2 UVA or left unirradiated; skin reactions evaluated at 24, 48, and 72 h post-challenge exposure	No reactions observed	[3]

Table 8. Ocular irritation studies on Basic Yellow 87

Concentration/Dose	Vehicle	Test Population	Procedure	Results	Reference
			IN VITRO		
99.2% pure Basic Yellow 87; 30 mg (neat) or 30 µl (5% aqueous dilution)	Not reported	Chicken eyes	Isolated chicken eye test; eyes exposed to single application for 10 s, followed by 20 ml saline rinse; corneal thickness, corneal opacity, and fluorescein retention measured; histopathology of corneas performed; negative control was saline and positive control was sodium hydroxide	Irritating when tested neat; not irritating at 5% dilution	[2]
Mixture containing 0.24% Basic Yellow 87; upon dilution; final concentration of 0.12% Basic Yellow 87	None	Bovine corneas	Bovine corneal opacity and permeability assay; test material was diluted at a 1:1 ratio with another mixture prior to assessment; negative control was sterile deionized water and the positive control was ethanol	Mild irritant; in vitro score = 3/3	[14]
			ANIMAL		
87.7% pure Basic Yellow 87; approximately 0.057 g/test eye	Neat	1 male and 2 female New Zealand White rabbits	Ocular irritation study in accordance with OECD TG 405; observations made 1, 24, 48, 72, and 96 h and 7, 14, and 21 d after instillation	Moderately irritating; no corneal effects observed; iritis (score of 1) observed 1 h after instillation in 1 animal, scores were 0 for other 2 animals and findings were reversible; redness observed in all animals from 1 to 72 h and in 1 animal for up to 21 d after instillation; chemosis and discharge noted in all animals up to 48 h after instillation	[2,3]

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Concentration of Use by FDA Product Category – Basic Yellow 87

Product Category	Maximum Concentration of Use
Hair dyes and colors	0.0007-1%
Hair shampoos (coloring)	0.02%

Information collected in 2021

Table prepared: January 10, 2022

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: August 8, 2022

SUBJECT: Studies on Basic Yellow 87

Anonymous. 1997. Two-week toxicity study by oral administration (gavage) in rats (test substances F 14529 contains 70% Basic Yellow 87).

Anonymous. 1997. Skin sensitization test in guinea pigs (maximization method of Magnussen, B. and Kligman, A.M.) (test material F14529 contains 70% Basic Yellow 87).

Anonymous. 2012. Skin irritation test using the EPIDERM™ skin model (test material 12AG30 contains 0.24% Basic Yellow 87; after mixing see results for 12AG30:AG32).

Anonymous. 2012. Bovine corneal opacity and permeability assay with optional histology (test material 12AG30 contains 0.24% Basic Yellow 87; after mixing see results for 12AG30:AG32).

[redacted]

STUDY TITLE

TWO-WEEK TOXICITY STUDY
BY ORAL ADMINISTRATION (GAVAGE) IN RATS

TEST SUBSTANCE

F 14529 contains 70% Basic Yellow 87

[redacted]

STUDY COMPLETION DATE

26 August 1997

[redacted]

CONTENTS

STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the principles of Good Laboratory Practice as described in:

. Council Directive 87/18/E.E.C. of 18 December 1986 on the harmonization of laws, regulations or administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (O.J. n° L 15 of 17.1.87),

. O.E.C.D. principles of Good Laboratory Practice, Decision Concerning Mutual Acceptance of Data in the Assessment of Chemicals, C(81)30(final) Annex 2. 12 May 1981,

. Décret N° 90-206 du 7 mars 1990 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 9 mars 1990), Ministère de l'Industrie et de l'Aménagement du Territoire.

This study was conducted in compliance with the following Animal Health regulation:

. Council Directive 86/609/E.E.C. of 24th November 1986 on the harmonization of laws, regulations or administrative provisions relating to the protection of animals used for experimental or other scientific purposes.

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

[redacted]

[redacted]

SCIENTISTS INVOLVED IN THE STUDY

[redacted]

Pharmacy/Toxicology

Macroscopic and
microscopic examinations

[redacted]

STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspections	Dates (day/month/year)		
	Inspections	Report to Study Director (*)	Report to Management (*)
Protocol	28 November 1996	28 November 1996	2 December 1996
Study	4 December 1996	16 December 1996	16 December 1996
Study	6 December 1996	9 December 1996	9 December 1996
Study	13 December 1996	16 December 1996	16 December 1996
Study	19 December 1996	23 December 1996	23 December 1996
Study	16 January 1997	4 February 1997	5 February 1997
Study	25 March 1997	28 March 1997	28 March 1997
Report	13 June 1997	16 June 1997	23 June 1997

[redacted]

(*) The dates mentioned correspond to the dates of signature of audit reports by Study Director and Management.

[redacted]

SUMMARY

The objective of this study was to evaluate the potential toxicity of the test substance F 14529 when administered daily by oral route (gavage) for two weeks in rats.
The test substance is a dye.

Methods

Four groups of six male and six female Sprague-Dawley rats received the test substance daily, by oral route (gavage), for two weeks, at 100, 300 and 1000 mg/kg/day. The test substance was administered as a suspension in water. An additional group received the vehicle alone (water) under the same conditions, and acted as a control group.
The animals were checked daily for mortality and clinical signs.
The food consumption and body weight of the animals were measured twice a week.
Haematology and blood biochemistry investigations were performed in all surviving animals during week 2.
At the end of the study, the animals were killed and a necropsy examination was performed. Designated organs were weighed and macroscopic lesions, liver and kidneys were submitted to a microscopic examination.

Results

Mortality
All males and females treated at 1000 mg/kg/day were found dead or were killed prematurely, after 7 to 15 days of treatment, following numerous signs of poor clinical condition.
Necropsy revealed in almost all these animals dilatation/overdistension of the stomach which may have contributed to their death via pressure on the diaphragm leading to respiratory failure.

Clinical signs
The test substance induced yellowish coloration of urine and faeces in almost all surviving animals treated with 100 or 300 mg/kg/day.
Ptyalism was observed at all dose-levels, in a dose-related manner.

Body weight and food consumption
The body weight gain and food consumption of the animals treated at 100 or 300 mg/kg/day were similar to that of the controls, whereas both weight gain and food consumption were markedly reduced at 1000 mg/kg/day.

Laboratory investigations

Haematology
No differences of toxicological importance were noted between treated and control animals.

Blood biochemistry
Higher urea nitrogen level and lower cholesterol level were observed in males treated with 1000 mg/kg/day.

Pathology

Organ weights (100 and 300 mg/kg/day groups)
Higher absolute mean adrenal gland weights were observed in males treated with 300 mg/kg/day and higher mean liver weights were recorded in both sexes at the same dose-level. A relationship to the treatment cannot be ruled out.

Macroscopic examination
Yellowish contents in the urinary bladder were seen in males treated with 300 mg/kg/day.

Microscopic examination
Epithelial cell hyperplasia and hyperkeratosis in the forestomach were observed in animals treated with 300 or 1000 mg/kg/day.

Conclusion

The test substance F 14529 was administered daily by oral route (gavage) over a period of two weeks to Sprague-Dawley rats, at 100, 300 or 1000 mg/kg/day.
The test substance did not produce any signs of toxicity at 100 mg/kg/day, and produced only minimal changes at 300 mg/kg/day (possible target organs: adrenals, liver and forestomach). The high dose-level (1000 mg/kg/day) was markedly toxic, leading to the death or sacrifice of all animals before the completion of the study.
Consequently, the No Observable Effect Level (NOEL) was established at 100 mg/kg/day.

1. INTRODUCTION

[redacted]

The rat was chosen because it is a rodent species generally accepted by regulatory authorities for this type of study. The Sprague-Dawley strain was selected due to the background data available from previous studies performed in our laboratory.
The oral route was used since it was expected to ensure an absorption of the test substance at least equal to the dermal route which is the route of exposure in humans.
The dose-levels were selected by the Sponsor.
The test substance is a dye.

The study is based on O.E.C.D. guideline No. 407, 27th July 1995.

2. MATERIALS AND METHODS

2.1. TEST AND CONTROL SUBSTANCES

2.1.1 Identification

2.1.1.1 Test substance

[redacted]

- protocol: F 14529 (or Jaune F 14529)
- labelling: F 14529

. batch number:
- protocol and labelling: MIP 2982

. description: yellow powder
. quantity and container : one glass flask with aluminium, containing 100 g
. date of receipt: 2 December 1996
. storage conditions: at room temperature, protected from light

At the finalisation of the study report, an analytical certificate was not available. Characterisation of the test substance, which appropriately defines the tested batch, was under the responsability of the Sponsor.

2.1.1.2 Vehicle

The vehicle was purified water, obtained by reverse osmosis from a milli-Ro 8 plus apparatus (Millipore S.A., 78057 Saint-Quentin en Yvelyne, France).

2.1.2 Preparation procedure

The test substance was administered as suspension in the vehicle.
The test substance was ground to a fine dust using a mortar and pestle, suspended in the vehicle in order to achieve the concentrations of 20, 60 and 200 mg/ml and then homogenized using a magnetic stirrer.
The test substance preparations were made daily. They were delivered to the animal room, protected from light, and maintained under continuous stirring during the dosing procedure.

2.2. TEST SYSTEM

2.2.1 Animals

A total of 52 Sprague-Dawley rats (26 males and 26 females) of the Crl CD® (SD) BR strain, *Caesarian Obtained, Barrier Sustained-Virus Antibody Free (COBS-VAF®)* were supplied by Charles River France (Saint Aubin-lès-Elbeuf, France) and received at C.I.T. on 28 November 1996.
On arrival, the animals were given a clinical examination to ensure that they were in good clinical condition.
A seven-day acclimatisation period to the conditions of the study preceded the beginning of the treatment period. The required number of animals (24 males and 24 females) was selected according to body weight and clinical examination and allocated, by sex, to the groups according to a computerized stratification procedure, so that the average body weight of each group was similar. The remaining animals were killed later.
Each animal was identified by an ear tattoo.
On the first day of treatment, the animals were approximately six weeks old and had a mean body weight of 187 g (179 g to 205 g) for the males and 169 g (155 g to 186 g) for the females.

2.2.2 Environmental conditions

[redacted] the animals were housed in a protected zone (one room during acclimatization, another room from the same zone, from day-1 onwards).
The environmental conditions in the animal rooms were set as follows:

. temperature	: 21± 2 °C,
. relative humidity	: 50 ± 20%,
. light/dark cycle	: 12h/12h (07:00 - 19:00),
. ventilation	: approximately 12 cycles/hour of filtered, non-recycled air.

The temperature and relative humidity were under continuous control and recording. The records were checked daily and retained. In addition to these daily checks, the housing conditions and corresponding instrumentation and equipment are verified and calibrated at regular intervals.
The animal room was disinfected before the arrival of the animals and cleaned regularly thereafter. Microbiological analyses of the air and the surfaces of the walls and floor of the animal room are performed regularly by an external laboratory.
[redacted]
The animals were housed in suspended wire-mesh cages (43.0 x 21.5 x 18.0 cm) and each cage contained two rats of the same sex and group. A metallic tray, containing autoclaved sawdust (SICSA, Alfortville, France), was placed under each cage.

Bacteriological analysis and detection of possible contaminants (pesticides, heavy metals) of the sawdust are performed periodically by external laboratories. The results of these analyses are archived at [redacted]
Bottles and sawdust were changed once a week.

In order to avoid bias in the study, the cages were placed in numerical order, vertically on the racks.

2.2.3 Food and water

The animals had free access to A04 C pelleted maintenance diet, batch No. 60918 (U.A.R., Villemoisson-sur-Orge, France) distributed weekly. The diet formula is presented in Appendix 1. Each batch of food was analysed by the supplier for composition and contaminant levels. The results of these analyses are archived at [redacted]
The animals had free access to bottles containing tap water, filtered using a 0.22 µm filter.

Bacteriological and chemical analyses of the diet and water and detection of possible contaminants (pesticides, heavy metals and nitrosamines) are performed regularly by external laboratories. The results of these analyses are archived at [redacted]

There were no other specific contaminants in the diet, water or sawdust at levels that may have interfered with or prejudiced the outcome of the study.

2.3. TREATMENT

2.3.1 Treatment groups

The dose-levels were specified by the Sponsor.

The groups, dose-levels and animal numbers are detailed in the following table:

Group	Animals per group	Dose-level (mg/kg/day)	Animal numbers
1	6 males 6 females	0	Q27891 to Q27896 Q27921 to Q27926
2	6 males 6 females	100	Q27897 to Q27902 Q27927 to Q27932
3	6 males 6 females	300	Q27903 to Q27908 Q27933 to Q27938
4	6 males 6 females	1000	Q27909 to Q27914 Q27939 to Q27944

2.3.2 Administration

The oral route was used since it was expected to ensure an absorption of the test substance at least equal to the dermal route which is the route of exposure in humans.
The test substance preparations were administered by gavage using a glass syringe fitted with a metal gavage tube.
Each animal was given the test substance once a day, at the same approximate daily time, seven days a week, over a period of two weeks (15 days, according to the necropsy date).
The quantity of test substance administered to the animals was adjusted according to the most recently recorded body weight.
Control animals received the vehicle alone, under the same conditions.
A constant dosage-volume of administration of 5 ml/kg was used.
The preparations were stirred continuously throughout the dosing procedure.

2.4. CLINICAL EXAMINATIONS

2.4.1 Morbidity and mortality

All animals were checked at least twice a day (including weekends and public holidays) for mortality and signs of morbidity.
Any animal showing signs of poor clinical condition, especially if death appeared imminent, was humanely killed (see § 2.6.1 Sacrifice).
Any animal found dead or killed due to poor clinical condition was subjected to a macroscopic examination. Whenever possible, a full spectrum of tissues was preserved and a microscopic examination performed (see § 2.6. Pathology).

2.4.2 Clinical signs

Clinical signs were observed for each animal at least once a day, at approximately the same time each day.

2.4.3 Body weight

Body weight was recorded for each animal once before the allocation of the animals into groups, on the first day of treatment, and then twice a week until the end of the study.

2.4.4 Food consumption

The quantity of food consumed by each animal was recorded twice a week over a three or four-day period throughout the study.
Food intake per animal and per day was calculated using the amount of food given and left in each food-hopper.
When one of the two animals from the same cage died, the number of days for which that animal was present in the cage was taken into consideration for the calculation of the food consumption.

2.5. LABORATORY INVESTIGATIONS

2.5.1 Blood collection

Blood samples were taken from the orbital sinus of the animals (before the daily treatment) under light ether anaesthesia and collected into tubes containing the appropriate anticoagulant (see below).
For blood samples, the animals were deprived of food for an overnight period of at least 14 hours.

2.5.2 Haematology

The following parameters were determined in all animals at the end of week 2 (day 14).

Parameter	Apparatus/Method	Unit
Blood collected on EDTA		
Erythrocytes (RBC)	Bayer Diagnostics H1 (1) Haematology Analyzer/laser	T/l
Haemoglobin (HB)	Bayer Diagnostics H1 Haematology Analyzer/Drabkin	g/dl
Mean Cell Volume (MCV)	Bayer Diagnostics H1 Haematology Analyzer/laser	fl
Packed Cell Volume (PCV)	Bayer Diagnostics H1 Haematology Analyzer/calculated	l/l
Mean Cell Haemoglobin Concentration (MCHC)	Bayer Diagnostics H1 Haematology Analyzer/calculated/ laser	g/dl
Mean Cell Haemoglobin (MCH)	Bayer Diagnostics H1 Haematology Analyzer/calculated	pg
Thrombocytes (PLAT)	Bayer Diagnostics H1 Haematology Analyzer/laser	G/l
Leucocytes (WBC)	Bayer Diagnostics H1 Haematology Analyzer/ peroxidase cytochemistry/laser morphometry	G/l
Differential white cell count with cell morphology	Bayer Diagnostics H1 Haematology Analyzer/ peroxidase cytochemistry/laser morphometry (a)	
. neutrophils (N)		% and G/l
. eosinophils (E)		% and G/l
. basophils (B)		% and G/l
. lymphocytes (L)		% and G/l
. monocytes (M)		% and G/l

(a) Blood smears were prepared for all sampled animals. If the samples were not accepted by the H1 Analyser, a microscopic control was determined after May Grünwald Giemsa staining (2)

(1) Bayer Diagnostics (92807 Puteaux, France)
(2) Merck Clévenot (77500 Chelles, France)

2.5.3 Blood biochemistry

The following parameters were determined in all animals at the end of week 2 (day 14).

Parameter	Apparatus/Method	Unit
Blood collected on lithium heparinate		
Sodium (Na^+)	Hitachi 717 Selective electrode (Boehringer) (1)	mmol/l
Potassium (K^+)	Hitachi 717 Selective electrode (Boehringer)	mmol/l
Chloride (Cl^-)	Hitachi 717 Selective electrode (Boehringer)	mmol/l
Glucose (GLUC)	Hitachi 717 GOD-PAP (Boehringer)	mmol/l
Urea (UREA)	Hitachi 717 Urease UV (Boehringer)	mmol/l
Creatinine (CREAT)	Hitachi 717 Jaffé without deproteinisation (Boehringer)	µmol/l
Total Bilirubin (TOT.BIL)	Hitachi 717 Jendrassik (Boehringer)	µmol/l
Total Proteins (PROT)	Hitachi 717 Biuret (Boehringer)	g/l
Albumin (ALB)	Hitachi 717 Bromocresol green (Boehringer)	g/l
Albumin/globulin ratio (A/G)	Hitachi 717 Calculated	1
Cholesterol (CHOL)	Hitachi 717 CHOD-PAP (Boehringer)	mmol/l
Alkaline phosphatase (ALP)	Hitachi 717 DGKC Standard/30 °C (Boehringer)	IU/l
Aspartate aminotransferase (ASAT)	Hitachi 717 IFCC Standard/30 °C (Boehringer)	IU/l
Alanine aminotransferase (ALAT)	Hitachi 717 IFCC Standard/30 °C (Boehringer)	IU/l

(1) Boehringer (38242 Meylan, France)

2.6. PATHOLOGY

2.6.1 Sacrifice

On completion of the treatment period, after at least 14 hours fasting, all surviving animals were asphyxiated using carbon dioxide and killed by exsanguination.
Throughout the study, any moribund animals were killed in the same way.

2.6.2 Organ weights

In all animals killed at the end of the treatment period, the body weight was recorded before sacrifice and the organs specified in the Tissue Procedures Table weighed wet as soon as possible after dissection.
Paired organs were weighed separately.
The ratio of organ weight to body weight (recorded immediately before sacrifice) was calculated for all animals killed on completion of the treatment period.

2.6.3 Macroscopic necropsy examination

A complete macroscopic necropsy examination was performed on all animals, including any that died during the study or were killed prematurely.
All macroscopic observations were recorded individually.

2.6.4 Preservation of tissues

For all animals including any that died during the study or were killed prematurely, the tissues specified in the Tissue Procedures Table were preserved in 10% buffered formalin (except for: (i) the eyes and Harderian glands which were fixed in Davidson's fixative, (ii) the testes and epididymides which were fixed in Bouin's fluid).

2.6.5 Microscopic examination

The tissues required for microscopic examination were embedded in paraffin wax, sectioned at approximately four microns in thickness and stained with hematoxylin-eosin.
Microscopic examination was performed on:

- all macroscopic lesions and tissues listed in the attached table for the animals of the control and high-dose groups (groups 1 and 4) killed at the end of the treatment period and in any animal that died or was killed prematurely,
- all macroscopic lesions of all the animals of the low and intermediate-dose groups (groups 2 and 3).

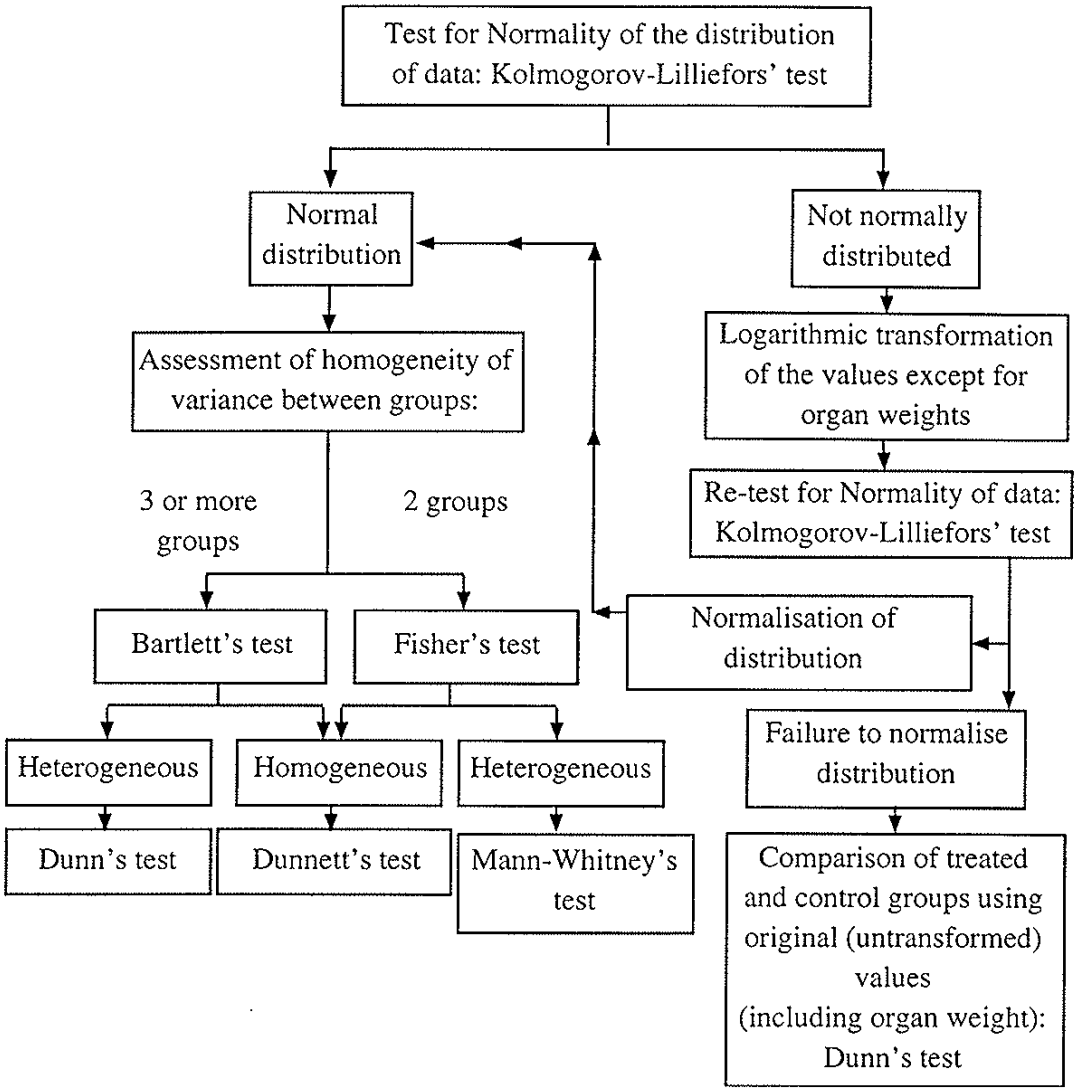
TISSUE PROCEDURES TABLE

Organs	Organ weights	Preservation of tissue	Microscopic examination
Macroscopic lesions		X	X
Adrenals	L+R	L+R	
Aorta		X	
Brain (including medulla/pons cerebellar and cerebral cortex)		X	
Caecum		X	
Colon		X	
Duodenum		X	
Epididymides		L+R	
Eyes with Harderian glands		L+R	
Femoral bone with articulation		X	
Heart	X	X	
Ileum		X	
Jejunum		X	
Kidneys	L+R	L+R	L+R
Liver	X	X	X
Lungs with bronchi		X	
Lymph nodes mandibular		X	
Lymph nodes mesenteric		X	
Mammary glands		X	
Oesophagus		X	
Ovaries	L+R	L+R	
Pancreas		X	
Pituitary gland		X	
Prostate		X	
Rectum		X	
Salivary glands (sublingual and submaxillary)		X	
Sciatic nerve		X	
Seminal vesicles		L+R	
Skeletal muscle		X	
Skin		X	
Spinal cord (cervical, thoracic and lumbar)		X	
Spleen	X	X	
Sternum with bone marrow		X	
Stomach with forestomach		X	
Testes	L+R	L+R	
Thymus	X	X	
Thyroids with parathyroids		L+R	
Tongue		X	
Trachea		X	
Urinary bladder		X	
Uterus (horns and cervix)		X	
Vagina		X	

X: action was undertaken L: left R: right

2.7. STATISTICAL ANALYSIS

The following sequence was used for the statistical analysis of body weight, food consumption, haematology, blood biochemistry and organ weight data:



2.8. ARCHIVES

The study documentation and materials, namely:
. protocol and amendments,
. raw data,
. correspondence,
. final report and possible amendments,
. tissues in preservative, blocks, histological slides,
. haematological slides,
. samples of the test and control substances,

[redacted]

2.9. CHRONOLOGY OF THE STUDY

The chronology of the study is summarized as follows:

Procedures	Dates	Study days
Protocol approved by:		
. Study Director	29 November 1996	
. Study Monitor	2 December 1996	
Arrival of the animals	28 November 1996	-7
. Preidentification and weighing	2 December 1996	-3
. Randomization and identification	3 December 1996	-2
First day of treatment	5 December 1996	1
Week 2		
. Haematology and blood biochemistry	18 December 1996	14
Week 3		
Day of necropsy	20 December 1996	16

2.10. PROTOCOL ADHERENCE

The study was performed in accordance with the original protocol No. [redacted]

3. RESULTS

3.1. CLINICAL EXAMINATIONS

3.1.1 Mortality (Appendix 2)

No mortality was observed during the study in the 0, 100 and 300 mg/kg/day groups.
All males and females treated at 1000 mg/kg/day were found dead or killed prematurely during the course of the treatment period, between days 7 and 16.
The mortality is summarized in the following table.

Incidence of unscheduled deaths

Day	7	8	9	10	11	12	13	14	15	16
. males	1FD+1KP		1FD					1KP		2FD
. females		1FD	2FD		1FD		1FD	1FD		
Cumulative	2/12	3/12	6/12		7/12		8/12	10/12		12/12

FD: Found Dead
KP: Killed Prematurely

Prior to death, these animals suffered from ptyalism (12/12), piloerection (11/12), round back (10/12), swollen abdomen (4/12), soft faeces (11/12) or yellowish-coloured urine, faeces or extremities (12/12). In addition, three males presented: dyspnea (1/3), locomotory difficulties (1/3), staggering gait (1/3), hypokinesia (3/3), cold to the touch (3/3) or eyes half closed (2/3). Furthermore, cessation of growth, or loss of weight were observed in these males and females respectively and this was correlated with a marked decrease in food consumption in both sexes.
Major necropsy findings observed in those animals found dead or killed prematurely included dilatation/overdistension (7/12) and yellow colour of the stomach (7/12), reduction in size of the spleen (6/12), enlarged adrenal glands (4/12) and in males reduced size of the prostate (5/6) and seminal vesicles (6/6).
Dilatation/overdistension of the stomach leading to pressure on the diaphragm and, consequently, to respiratory failure, may have been one of the factors contributing to death.

3.1.2 Clinical signs (Tables 1 and 2, Appendix 3)

During the study, clinical signs observed in surviving animals were as follows:
Yellow coloured urine and faeces was noted in animals treated with 100 mg/kg/day (5/6 males and 6/6 females) or 300 mg/kg/day (6/6 in both sexes). This was related to the dyeing properties of the test substance.
Ptyalism, at a dose-related incidence, (2/12 animals treated with 100 mg/kg/day and 12/12 animals treated with 300 or 1000 mg/kg/day) was also noted.

3.1.3 Body weight (Figures 1 and 2, Tables 3 and 4, Appendix 4)

When compared to the control values, no relevant difference in body weight gain was observed in animals treated with 100 or 300 mg/kg/day.

3.1.4 Food consumption (Tables 5 and 6, Appendix 5)

No difference in food consumption was noted between control animals and those treated with 100 mg/kg/day.

When compared to the control values, food consumption was slightly reduced in animals treated with 300 mg/kg/day (-5% and -8% in males and females respectively). Since the differences were slight, not statistically significant, and not correlated to a lower body weight gain, a relationship to the treatment was ruled out.

3.2. LABORATORY INVESTIGATIONS

N.B. The laboratory investigations were performed on day 14 of the treatment period in the three surviving males treated with 1000 mg/kg/day. No surviving females were present for sampling.

3.2.1 Haematology (Tables 7 and 8, Appendix 6)

No significant differences were observed between control animals those treated with 100 or 300 mg/kg/day.

A moderately higher mean neutrophil count was observed in males treated with 1000 mg/kg/day (4.47 G/l vs. 1.10 in the controls); this was the contribution of the two surviving animals. Considering the great variability of this parameter and the little number of animals involved, a relationship to the treatment was considered to be unlikely.

3.2.2 Blood biochemistry (Tables 9 and 10, Appendix 7)

No treatment-related changes were observed in animals given 100 and 300 mg/kg/day.

When compared with respective controls, the following differences were noted in the males given 1000 mg/kg/day:

. a higher mean urea level (x2.8). The individual values were above the upper limit of our historical background data (8.2 mmol/l).
. a lower mean cholesterol level (-54%). The individual values were below the lower limit of our historical background data (1.3 mmol/l).
. a higher ASAT activity (x2); this was the contribution of only one animal (Q27914 - ASAT 155 IU/l).

The higher urea level and the lower cholesterol level were considered to be treatment related. As the higher ASAT activity was the contribution of one animal and as there were no associated abnormalities in ALAT activity, this was considered to be without relationship to the treatment with the test substance.

The other differences noted in the two blood biochemical parameters (namely glucose level) were considered not to be of toxicological importance as they were minor and the individual values were within the range of our historical background data.

3.3. PATHOLOGY

3.3.1 Organ weights (Table 11, Appendix 8)

As organ weights of animals which died prematurely were not recorded, only the control and low and intermediate dose-groups are included.

Higher absolute mean adrenal gland weights were noted in males receiving 300 mg/kg/day (+19%).

Higher absolute mean liver weights were noted in males and females receiving 300 mg/kg/day (+18%, +15% respectively).

The above differences in organ weights may be treatment related but as no microscopic examination was performed, this could not be confirmed within the frame of this study.

3.3.2 Macroscopic necropsy examination (Table 12, Appendix 9)

Among the surviving animals, only yellow contents in the urinary bladder were seen in 5/6 males receiving 300 mg/kg/day. The above-mentioned finding was considered to be due to the physical properties of the test substance (dye).

All other macroscopic findings, not already mentioned under § 2.4.1 Mortality, were those which are commonly found in the untreated laboratory rat of this strain and age and their incidence (1/6 to 5/6 maximum) was considered to be of no toxicological importance.

3.3.3 Microscopic examination (Table 13, Appendix 9)

Epithelial cell hyperplasia and hyperkeratosis in the forestomach were seen in 5/5 males receiving 300 mg/kg/day and 4/5 females receiving 1000 mg/kg/day. This finding was considered to be treatment related.

All other microscopic findings were those which are commonly encountered in the untreated laboratory rat of this strain and age and their incidence (1/6 to 5/5 maximum) was considered to be of no toxicological importance.

4. CONCLUSION

The test substance F 14529 was administered daily by oral route (gavage) over a period of two weeks to Sprague-Dawley rats, at 100, 300 or 1000 mg/kg/day.
The test substance did not produce any signs of toxicity at 100 mg/kg/day, and produced only minimal changes at 300 mg/kg/day (possible target organs: adrenals, liver and forestomach). The high dose-level (1000 mg/kg/day) was markedly toxic, leading to the death or sacrifice of all animals before the completion of the study.
Consequently, the No Observable Effect Level (NOEL) was established at 100 mg/kg/day.

5. REFERENCES

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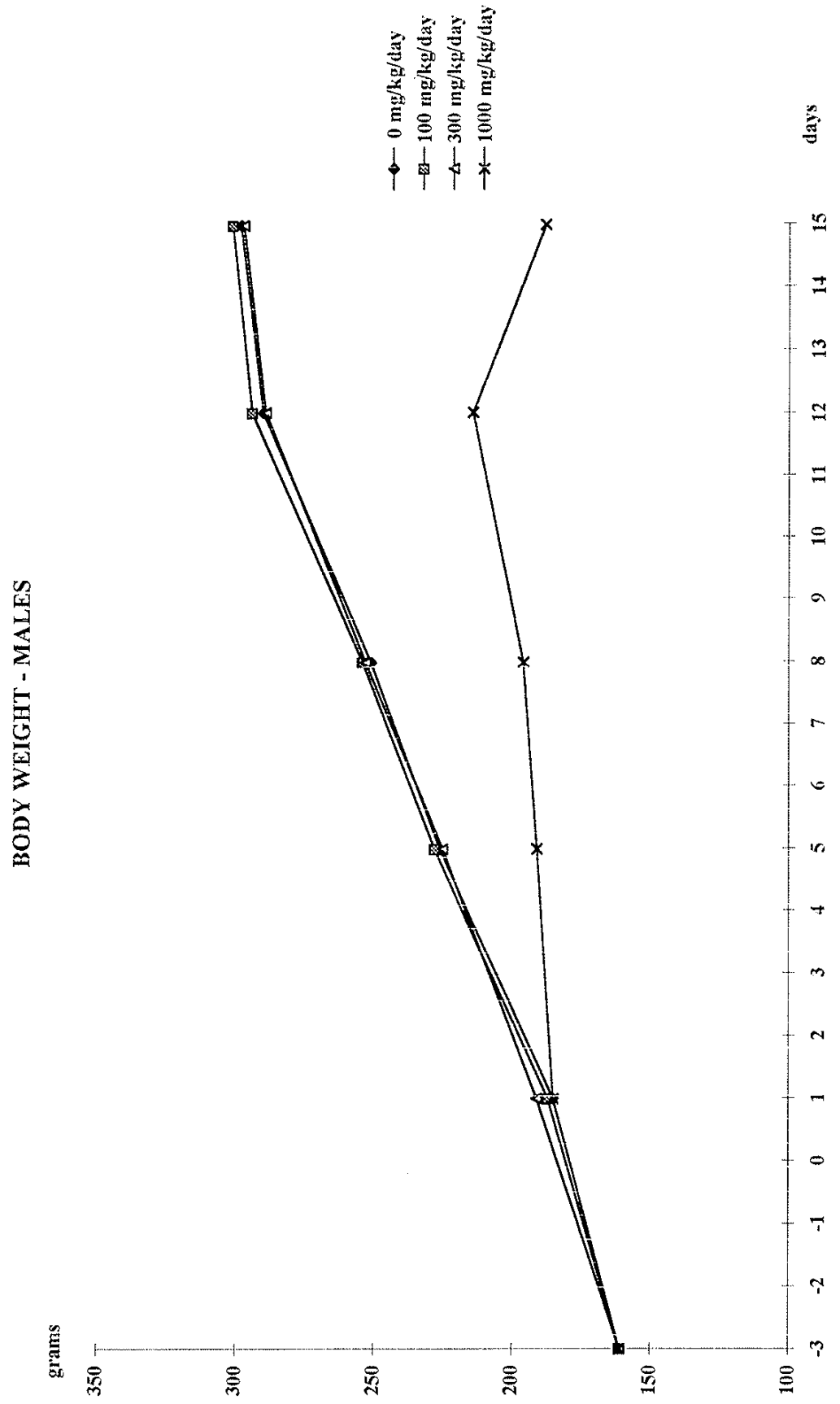
BODY WEIGHT - MALES
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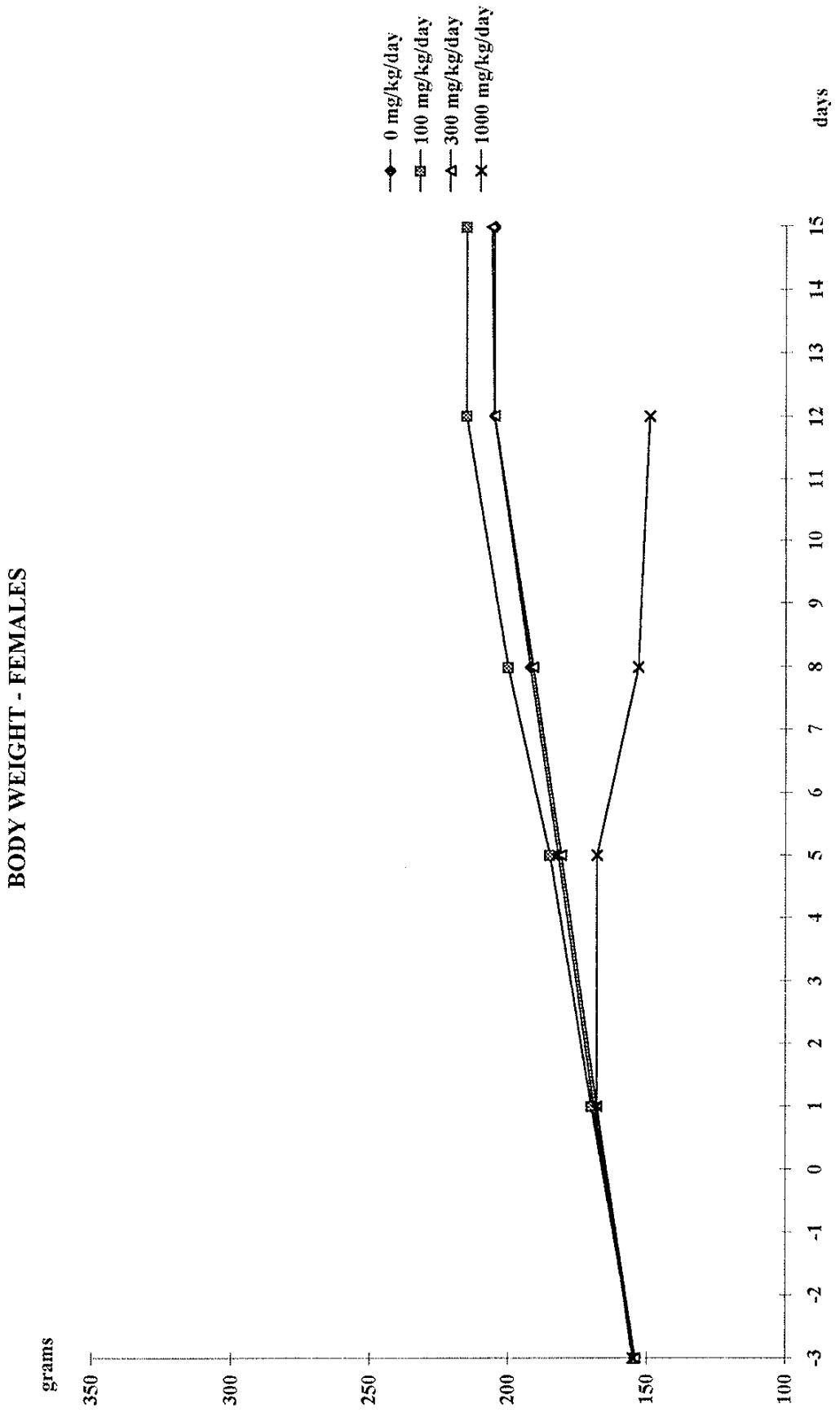
300
250
200
150
100
-3
-2
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
days
0 mg/kg/day
100 mg/kg/day
300 mg/kg/day
1000 mg/kg/day


BODY WEIGHT - FEMALES
grams
350
300
250
200
150
100
-3
-2
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
days
0 mg/kg/day
100 mg/kg/day
300 mg/kg/day
1000 mg/kg/day

CLINICAL SIGNS (SUMMARY TABLE)

Table: 1

Sex: male
Period: Day 1 to 16

Dose (mg/kg/d)	0		100		300		1000	
Observations	No	%	No	%	No	%	No	%
Cold to the touch	0	0	0	0	0	0	3	50
Piloerection	0	0	0	0	0	0	6	100
Round back	0	0	0	0	0	0	6	100
Startling	0	0	0	0	0	0	1	17
Hypokinesia	0	0	0	0	0	0	3	50
Bent head	0	0	0	0	0	0	3	50
Staggering gait	0	0	0	0	0	0	1	17
Locomotory difficulties	0	0	0	0	0	0	1	17
Dyspnea	0	0	0	0	0	0	1	17
Swollen abdomen	0	0	0	0	0	0	3	50
Ptyalism	0	0	2	33	6	100	6	100
Soft faeces	0	0	0	0	0	0	5	83
Eyes half-closed	0	0	0	0	0	0	2	33
Area of hair loss on back	0	0	0	0	0	0	1	17
Scattered hair	0	0	0	0	0	0	1	17
Yellow coloured fur	0	0	0	0	2	33	3	50
Yellow coloured extremities	0	0	0	0	6	100	6	100
Yellow coloured tail	0	0	0	0	0	0	1	17
Yellow coloured urine	0	0	6	100	6	100	6	100
Yellow coloured faeces	0	0	6	100	6	100	6	100
No clinical history	6	100	0	0	0	0	0	0

CLINICAL SIGNS (SUMMARY TABLE)

Table: 2

Sex: female
Period: Day 1 to 16

Dose (mg/kg/d)	0		100		300		1000	
Observations	No	%	No	%	No	%	No	%
Piloerection	0	0	0	0	0	0	5	83
Round back	0	0	0	0	0	0	4	67
Swollen abdomen	0	0	0	0	0	0	1	17
Ptyalism	0	0	0	0	6	100	6	100
Soft faeces	0	0	0	0	0	0	6	100
Yellow coloured fur	0	0	0	0	4	67	1	17
Yellow coloured extremities	0	0	0	0	6	100	6	100
Yellow coloured tail	0	0	0	0	0	0	1	17
Yellow coloured urine	0	0	6	100	6	100	6	100
Yellow coloured faeces	0	0	6	100	6	100	6	100
No clinical history	6	100	0	0	0	0	0	0

BODY WEIGHT
(mean values - g)

Table: 3

Sex: Male

Dose (mg/kg/d) Day		0	100	300	1000	
-3	M (1)	161	161	161	161	
	SD	3.6	4.0	6.6	4.8	
	n	6	6	6	6	
1	M (1)	185	187	191	185	
	SD	4.1	3.8	8.4	4.0	
	n	6	6	6	6	
5	M (1)	226	228	225	191	**
	SD	6.8	7.7	10.4	16.0	
	n	6	6	6	6	
8	M (3)	251	254	253	196	
	SD	6.9	8.1	12.8	21.8	
	n	6	6	6	4	
12	M (3)	290	294	289	214	
	SD	7.7	12.2	12.6	3.2	
	n	6	6	6	3	
15	M (1)	298	301	297	188	-
	SD	8.8	17.0	14.3	16.3	
	n	6	6	6	2	

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
\- Statistics excluded group

BODY WEIGHT
(mean values - g)

Table: 4

Sex: Female

Dose (mg/kg/d) Day		0	100	300	1000	
-3	M (1)	154	154	154	155	
	SD	6.0	6.6	5.6	6.0	
	n	6	6	6	6	
1	M (1)	169	170	168	168	
	SD	8.1	9.9	5.7	7.3	
	n	6	6	6	6	
5	M (1)	182	185	181	168	
	SD	8.6	13.0	8.3	7.4	
	n	6	6	6	6	
8	M (1)	192	200	191	153	**
	SD	8.2	14.2	9.2	7.5	
	n	6	6	6	6	
12	M (1)	205	215	205	149	-
	SD	8.7	17.6	10.0	18.4	
	n	6	6	6	2	
15	M (1)	205	215	206	-	-
	SD	9.0	17.9	12.0	-	
	n	6	6	6	0	

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
\- Statistics excluded group

FOOD CONSUMPTION
(mean values - g/animal/day)

Table: 5

Sex: Male

Dose (mg/kg/d) Day		0	100	300	1000	
1	M (3)	25.8	26.1	23.2	12.3	*
	SD	1.07	2.46	0.69	3.92	
	n	3	3	3	3	
5	M (3)	27.1	26.8	26.9	10.9	-
	SD	0.59	2.76	1.61	2.69	
	n	3	3	3	2	
8	M (3)	28.0	28.5	26.5	12.6	-
	SD	1.19	3.09	0.85	0.35	
	n	3	3	3	2	
12	M (3)	22.3	22.8	21.4	7.0	-
	SD	1.12	2.23	1.38	-	
	n	3	3	3	1	

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

FOOD CONSUMPTION
(mean values - g/animal/day)

Table: 6

Sex: Female

Dose (mg/kg/d) Day		0	100	300	1000	
1	M (3)	18.0	19.0	15.7	9.3	*
	SD	0.56	2.32	0.67	0.76	
	n	3	3	3	3	
5	M (3)	18.1	19.4	17.2	6.9	
	SD	1.17	2.35	0.35	1.31	
	n	3	3	3	3	
8	M (3)	20.2	19.4	17.9	8.1	-
	SD	2.05	2.76	0.46	0.85	
	n	3	3	3	2	
12	M (3)	15.2	16.7	15.3	-	-
	SD	0.49	2.10	0.72	-	
	n	3	3	3	0	

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
\- Statistics excluded group

HAEMATOLOGY
(mean values)

Table: 7

Sex: Male
Time: Week 2

Dose (mg/kg/d)		0	100	300	1000
WBC	M (3)	12.69	10.47	14.77	10.96 -
G/l	SD	2.827	2.875	1.861	2.171
	n	4	5	6	2
RBC	M (3)	7.40	7.35	7.27	8.28 -
T/l	SD	0.295	0.101	0.432	0.375
	n	4	5	6	2
HB	M (3)	14.9	15.0	14.7	15.0 -
g/dl	SD	0.40	0.40	0.51	0.50
	n	4	5	6	2
PCV	M (3)	0.45	0.46	0.45	0.45 -
l/l	SD	0.015	0.010	0.011	0.000
	n	4	5	6	2
MCV	M (3)	60.9	62.4	61.9	54.7 -
fl	SD	0.62	0.96	2.55	2.05
	n	4	5	6	2
MCH	M (3)	20.2	20.5	20.3	18.1 -
pg	SD	0.38	0.43	0.74	0.14
	n	4	5	6	2
MCHC	M (3)	33.1	32.8	32.9	33.1 -
g/dl	SD	0.39	0.47	0.31	0.99
	n	4	5	6	2
PLAT	M (3)	1272	1272	1284	1288 -
G/l	SD	99.2	215.7	144.2	258.1
	n	4	5	6	2

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
\- Statistics excluded group

HAEMATOLOGY
(mean values)

Table: 7 (continued)

Sex: Male
Time: Week 2

Dose (mg/kg/d)		0	100	300	1000
N	M (3)	9.0	12.5	11.4	40.5 -
%	SD	2.07	3.97	2.01	3.04
	n	4	5	6	2
E	M (3)	0.8	0.8	0.7	1.0 -
%	SD(K)	0.14	0.20	0.12	0.42
	n	4	5	6	2
B	M (3)	0.3	0.2	0.3	0.1 -
%	SD	0.15	0.08	0.06	0.00
	n	4	5	6	2
L	M (3)	88.7	85.1	85.9	55.5 -
%	SD	2.47	4.25	2.05	4.81
	n	4	5	6	2
M	M (3)	1.3	1.4	1.9	2.9 -
%	SD	0.47	0.24	0.51	1.27
	n	4	5	6	2
N	M (3)	1.10	1.33	1.68	4.47 -
G/l	SD	0.116	0.576	0.354	1.216
	n	4	5	6	2
E	M (3)	0.10	0.09	0.11	0.12 -
G/l	SD	0.010	0.018	0.014	0.078
	n	4	5	6	2
B	M (3)	0.04	0.02	0.04	0.02 -
G/l	SD	0.028	0.017	0.014	0.007
	n	4	5	6	2
L	M (3)	11.30	8.88	12.67	6.03 -
G/l	SD	2.743	2.451	1.547	0.679
	n	4	5	6	2
M	M (3)	0.16	0.15	0.28	0.33 -
G/l	SD	0.058	0.044	0.110	0.198
	n	4	5	6	2

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
\- Statistics excluded group

HAEMATOLOGY
(mean values)

Table: 8

Sex: Female
Time: Week 2

Dose (mg/kg/d)		0	100	300	1000	
WBC	M (1)	9.66	9.40	12.76	-	-
G/l	SD	2.870	1.207	1.603	-	
	n	5	5	5	0	
RBC	M (3)	7.33	7.43	7.50	-	-
T/l	SD	0.118	0.565	0.173	-	
	n (B)	5	5	5	0	
HB	M (1)	15.0	14.5	15.1	-	-
g/dl	SD	0.51	0.99	0.37	-	
	n	5	5	5	0	
PCV	M (1)	0.43	0.43	0.44	-	-
l/l	SD	0.006	0.031	0.013	-	
	n	5	5	5	0	
MCV	M (1)	59.1	57.2 *	58.5	-	-
fl	SD	0.65	1.72	0.86	-	
	n	5	5	5	0	
MCH	M (1)	20.4	19.5 *	20.1	-	-
pg	SD	0.47	0.57	0.51	-	
	n	5	5	5	0	
MCHC	M (1)	34.6	34.2	34.4	-	-
g/dl	SD	0.83	0.20	0.40	-	
	n	5	5	5	0	
PLAT	M (1)	1306	1324	1229	-	-
G/l	SD	137.8	71.3	156.1	-	
	n	5	5	5	0	

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
\- Statistics excluded group

HAEMATOLOGY
(mean values)

Table: 8 (continued)

Sex: Female
Time: Week 2

Dose (mg/kg/d)		0	100	300	1000	
N	M (1)	12.1	15.0	13.1	-	-
%	SD	4.12	3.70	4.56	-	
	n	5	5	5	0	
E	M (1)	1.1	1.3	1.3	-	-
%	SD	0.40	0.53	0.21	-	
	n	5	5	5	0	
B	M (1)	0.2	0.2	0.3	-	-
%	SD	0.10	0.08	0.09	-	
	n	5	5	5	0	
L	M (1)	85.0	81.5	83.5	-	-
%	SD	4.55	3.82	4.43	-	
	n	5	5	5	0	
M	M (1)	1.6	1.9	1.9	-	-
%	SD	0.55	0.45	0.47	-	
	n	5	5	5	0	
N	M (1)	1.10	1.43	1.64	-	-
G/l	SD	0.317	0.480	0.498	-	
	n	5	5	5	0	
E	M (1)	0.10	0.13	0.16 *	-	-
G/l	SD	0.018	0.052	0.016	-	
	n	5	5	5	0	
B	M (1)	0.02	0.02	0.04	-	-
G/l	SD	0.013	0.008	0.015	-	
	n	5	5	5	0	
L	M (1)	8.28	7.64	10.70	-	-
G/l	SD	2.745	0.829	1.805	-	
	n	5	5	5	0	
M	M (1)	0.16	0.19	0.24	-	-
G/l	SD	0.062	0.045	0.051	-	
	n	5	5	5	0	

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

BLOOD BIOCHEMISTRY
(mean values)

Table: 9

Sex: Male
Time: Week 2

Dose (mg/kg/d)		0	100	300	1000
Na+	M (3)	145.2	145.9	144.7	141.5
mmol/l	SD	1.01	0.51	1.35	1.37
	n	6	6	6	3
K+	M (3)	3.52	3.74	3.40	3.85
mmol/l	SD	0.747	0.567	0.119	1.092
	n	6	6	6	3
Cl-	M (3)	103.0	104.2	104.6 *	98.4
mmol/l	SD	0.45	1.35	0.87	4.54
	n	6	6	6	3
GLUC	M (3)	4.70	4.66	6.06 *	5.63
mmol/l	SD	0.359	0.532	0.781	0.900
	n	6	6	6	3
UREA	M (3)	3.7	4.0	4.1	10.3 **
mmol/l	SD	0.23	0.67	0.44	0.70
	n	6	6	6	3
CREAT	M (3)	42	40	39	40
µmol/l	SD	2.4	5.4	1.7	1.2
	n	6	6	6	3

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

BLOOD BIOCHEMISTRY
(mean values)

Table: 9 (continued)

Sex: Male
Time: Week 2

Dose (mg/kg/d)		0	100	300	1000	
PROT	M (3)	67	66	67	60	*
g/l	SD	1.9	1.5	2.6	1.5	
	n	6	6	6	3	
ALB	M (3)	34	33	35	33	
g/l	SD	1.0	1.0	0.8	3.6	
	n	6	6	6	3	
A/G	M (3)	1.00	1.03	1.07	1.22	
1	SD	0.060	0.058	0.077	0.219	
	n	6	6	6	3	
TOT.BIL.	M	1	1	1	2	
µmol/l	SD(K)	0.0	0.0	0.0	0.0	
	n	6	6	6	3	
CHOL	M (3)	2.2	2.0	1.7	1.0	**
mmol/l	SD	0.19	0.45	0.33	0.21	
	n	6	6	6	3	
TRIG	M (3)	0.81	1.10	0.92	0.74	
mmol/l	SD	0.245	0.476	0.429	0.137	
	n	6	6	6	3	
ALP	M (3)	452	469	529	220	
IU/l	SD	105.9	102.5	104.7	84.6	
	n	6	6	6	3	
ASAT	M (3)	54	53	71	104	*
IU/l	SD	13.2	9.2	11.1	43.9	
	n	6	6	6	3	
ALAT	M (3)	30	31	18	19	
IU/l	SD	14.1	8.6	3.6	5.7	
	n	6	6	6	3	

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

BLOOD BIOCHEMISTRY
(mean values)

Table: 10

Sex: Female
Time: Week 2

Dose (mg/kg/d)		0	100	300	1000
Na+ mmol/l	M (1)	143.8	142.9	141.4 *	- -
	SD	1.72	1.56	1.61	-
	n	6	6	6	0
K+ mmol/l	M (1)	3.16	3.23	3.60 *	- -
	SD	0.346	0.289	0.241	-
	n	6	6	6	0
Cl- mmol/l	M (1)	105.1	103.9	104.0	- -
	SD	2.04	1.38	1.26	-
	n	6	6	6	0
GLUC mmol/l	M (1)	5.48	6.13	6.65	- -
	SD	0.828	1.196	0.798	-
	n	6	6	6	0
UREA mmol/l	M (1)	6.1	5.4	4.7	- -
	SD	1.59	0.49	0.78	-
	n	6	6	6	0
CREAT µmol/l	M (1)	49	43	43	- -
	SD	5.6	1.9	5.0	-
	n	6	6	6	0

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

BLOOD BIOCHEMISTRY
(mean values)

Table: 10 (continued)

Sex: Female
Time: Week 2

Dose (mg/kg/d)		0	100	300	1000	
PROT	M (1)	71	71	70	-	-
g/l	SD	2.8	3.0	0.8	-	
	n	6	6	6	0	
ALB	M (1)	37	37	35	-	-
g/l	SD	1.8	1.6	1.5	-	
	n	6	6	6	0	
A/G	M (1)	1.06	1.08	1.02	-	-
1	SD	0.085	0.050	0.098	-	
	n	6	6	6	0	
TOT.BIL.	M	1	1	1	-	-
µmol/l	SD(K)	0.5	0.4	0.4	-	
	n	6	6	6	0	
CHOL	M (1)	1.6	2.1	1.5	-	-
mmol/l	SD	0.34	0.44	0.28	-	
	n	6	6	6	0	
TRIG	M (1)	0.38	0.67 *	0.58	-	-
mmol/l	SD	0.147	0.144	0.245	-	
	n	6	6	6	0	
ALP	M (1)	326	283	272	-	-
IU/l	SD	32.6	52.7	41.8	-	
	n	6	6	6	0	
ASAT	M (1)	62	47 *	62	-	-
IU/l	SD	9.0	6.9	8.1	-	
	n	6	6	6	0	
ALAT	M (1)	14	14	15	-	-
IU/l	SD	7.6	3.1	5.2	-	
	n	6	6	6	0	

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
\- Statistics excluded group

Table: 11

SUMMARY TABLE OF BODY/ORGAN WEIGHTS AND STATISTICS
STATUS AT NECROPSY: K0
SEX: MALE

ORGAN	DOSE GROUP: / NO.ANIMALS:	1	2	3	4
		6	6	6	6
FINAL BODY WEIGHT	n:	6	6	6	
MEAN WEIGHT (G)	:	270.6	270.1	265.4	
STD. DEVIATION	:	8.87	13.86	12.76	
LIVER	n:	6	6	6	
MEAN WEIGHT (G)	:	8.87	9.43	10.20*	
STD. DEVIATION	:	0.739	0.750	0.988	
MEAN % BODY	:	3.28	3.50	3.85	
STD. DEVIATION	:	0.246	0.412	0.331	
KIDNEYS	n:	6	6	6	
MEAN WEIGHT (G)	:	2.42	2.45	2.30	
STD. DEVIATION	:	0.172	0.163	0.118	
MEAN % BODY	:	0.894	0.909	0.867	
STD. DEVIATION	:	0.056	0.047	0.044	
TESTES	n:	6	6	6	
MEAN WEIGHT (G)	:	2.75	3.05	3.03	
STD. DEVIATION	:	0.792	0.256	0.128	
MEAN % BODY	:	1.01	1.13	1.14	
STD. DEVIATION	:	0.280	0.093	0.057	
THYMUS	n:	6	6	6	
MEAN WEIGHT (G)	:	0.598	0.602	0.568	
STD. DEVIATION	:	0.083	0.128	0.082	
MEAN % BODY	:	0.221	0.224	0.213	
STD. DEVIATION	:	0.026	0.054	0.022	
ADRENAL GLANDS	n:	6	6	6	
MEAN WEIGHT (G)	:	0.052	0.057	0.062#	
STD. DEVIATION	:	0.002	0.006	0.011	
MEAN % BODY	:	0.019	0.021	0.023	
STD. DEVIATION	:	0.001	0.003	0.003	

Table: 11 (continued)

```
--------------------------------------------------------------------------
SUMMARY TABLE OF BODY/ORGAN WEIGHTS AND STATISTICS
STATUS AT NECROPSY:  K0
SEX:  MALE
--------------------------------------------------------------------------

            DOSE GROUP:     1         2         3         4
ORGAN       NO.ANIMALS:     6         6         6         6
--------------------------------------------------------------------------
SPLEEN                n:    6         6         6
      MEAN WEIGHT (G) :   0.617     0.659     0.571
      STD. DEVIATION  :   0.066     0.028     0.099
      MEAN % BODY     :   0.228     0.244     0.215
      STD. DEVIATION  :   0.029     0.015     0.033
..........................................................................
HEART                 n:    6         6         6
      MEAN WEIGHT (G) :    1.12      1.16      1.15
      STD. DEVIATION  :   0.085     0.125     0.093
      MEAN % BODY     :   0.414     0.429     0.433
      STD. DEVIATION  :   0.022     0.037     0.040
..........................................................................


---------
#/##):DUNN'S TEST AT 5% (#) OR 1% (##) LEVEL
*/**):DUNNETT'S TEST BASED ON POOLED VARIANCES AT 5% (*) OR 1% (**) LEVEL
Assigned control group(s) : 1,
```

Table: 11 (continued)

SUMMARY TABLE OF BODY/ORGAN WEIGHTS AND STATISTICS
STATUS AT NECROPSY: K0
SEX: FEMALE

ORGAN	DOSE GROUP: / NO.ANIMALS:	1 6	2 6	3 6	4 6
FINAL BODY WEIGHT	n:	6	6	6	
MEAN WEIGHT (G)	:	183.0	190.3	182.2	
STD. DEVIATION	:	8.78	17.90	9.59	
LIVER	n:	6	6	6	
MEAN WEIGHT (G)	:	5.56	6.11	6.56#	
STD. DEVIATION	:	0.177	0.708	0.526	
MEAN % BODY	:	3.04	3.21	3.60	
STD. DEVIATION	:	0.083	0.116	0.222	
KIDNEYS	n:	6	6	6	
MEAN WEIGHT (G)	:	1.61	1.71	1.65	
STD. DEVIATION	:	0.109	0.194	0.064	
MEAN % BODY	:	0.879	0.899	0.907	
STD. DEVIATION	:	0.060	0.057	0.076	
THYMUS	n:	6	6	6	
MEAN WEIGHT (G)	:	0.442	0.381	0.459	
STD. DEVIATION	:	0.079	0.080	0.077	
MEAN % BODY	:	0.241	0.200	0.252	
STD. DEVIATION	:	0.036	0.036	0.042	
ADRENAL GLANDS	n:	6	6	6	
MEAN WEIGHT (G)	:	0.062	0.066	0.071*	
STD. DEVIATION	:	0.005	0.005	0.002	
MEAN % BODY	:	0.034	0.035	0.039	
STD. DEVIATION	:	0.004	0.003	0.002	
SPLEEN	n:	6	6	6	
MEAN WEIGHT (G)	:	0.451	0.413	0.422	
STD. DEVIATION	:	0.068	0.045	0.065	
MEAN % BODY	:	0.246	0.218	0.232	
STD. DEVIATION	:	0.028	0.022	0.033	

Table: 11 (continued)

```
------------------------------------------------------------------------
SUMMARY TABLE OF BODY/ORGAN WEIGHTS AND STATISTICS
STATUS AT NECROPSY:  K0
SEX:  FEMALE
------------------------------------------------------------------------

            DOSE GROUP:     1         2         3         4
ORGAN       NO.ANIMALS:     6         6         6         6
------------------------------------------------------------------------
HEART                  n:    6         6         6
      MEAN WEIGHT (G) :   0.849     0.861     0.824
      STD. DEVIATION  :   0.134     0.086     0.090
      MEAN % BODY     :   0.463     0.453     0.452
      STD. DEVIATION  :   0.065     0.034     0.036
........................................................................
OVARIES                n:    6         6         6
      MEAN WEIGHT (G) :   0.113     0.110     0.121
      STD. DEVIATION  :   0.015     0.009     0.021
      MEAN % BODY     :   0.062     0.058     0.066
      STD. DEVIATION  :   0.008     0.006     0.010
........................................................................


---------
*/**):DUNNETT'S TEST BASED ON POOLED VARIANCES AT 5% (*) OR 1% (**) LEVEL
#/##):DUNN'S TEST AT 5% (#) OR 1% (##) LEVEL
Assigned control group(s) : 1,

END OF REPORT SECTION

                                                15034 / PDS PATHDATA SYSTEM TM
```

Table: 12

NUMBER OF ANIMALS WITH NECROPSY FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +

DOSE GROUP:	1		2		3		4	
SEX:	M	F	M	F	M	F	M	F
ORGAN/FINDING NO.ANIMALS:	6	6	6	6	6	6	6	6
LIVER :								
- FOCI YELLOWISH								1
KIDNEYS :								
- DILATED PELVIS		2			1	1	1	
ILEUM :								
- DILATATION							1	
- YELLOWISH COLOR					3	1	3	1
- YELLOWISH CONTENTS					5		2	1
JEJUNUM :								
- DILATATION							1	
- YELLOWISH COLOR					1		3	1
- YELLOWISH CONTENTS					1		1	1
DUODENUM :								
- DILATATION							2	2
- YELLOWISH COLOR								1
- YELLOWISH CONTENTS							2	2
CAECUM :								
- DILATATION							1	1
- YELLOWISH COLOR					6	4	3	1
- YELLOWISH CONTENTS							2	2
COLON :								
- YELLOWISH COLOR					1		2	
- YELLOWISH CONTENTS								1
RECTUM :								
- YELLOWISH COLOR							1	
- YELLOWISH CONTENTS								1

Table: 12 (continued)

NUMBER OF ANIMALS WITH NECROPSY FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +

DOSE GROUP:	1		2		3		4	
SEX:	M	F	M	F	M	F	M	F
ORGAN/FINDING NO.ANIMALS:	6	6	6	6	6	6	6	6
STOMACH :								
- BLACKISH DEPOSIT								1
- DILATATION							2	5
- FOCI BROWNISH/BLACKISH								1
- PURPLISH CONTENTS								1
- YELLOWISH COLOR							3	4
- YELLOWISH CONTENTS							2	3
FORESTOMACH :								
- DILATATION								1
- FOCI GREYISH/WHITISH								2
- THINNED								1
- YELLOWISH COLOR					5		1	4
SEMINAL VESICLES :								
- REDUCED IN SIZE							6	
PROSTATE :								
- REDUCED IN SIZE							5	
TESTES :								
- CRYPTORCHIDISM							1	
- REDUCED IN SIZE	1							
- SOFT	1							
THYMUS :								
- REDUCED IN SIZE							1	2
ADRENAL GLANDS :								
- ENLARGED							1	3
- REDUCED IN SIZE							1	
SPLEEN :								
- REDUCED IN SIZE							5	1
URINARY BLADDER :								
- YELLOWISH CONTENTS					5			

Table: 12 (continued)

NUMBER OF ANIMALS WITH NECROPSY FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +

DOSE GROUP:	1		2		3		4	
SEX:	M	F	M	F	M	F	M	F
ORGAN/FINDING NO.ANIMALS:	6	6	6	6	6	6	6	6
UTERUS :								
- REDUCED IN SIZE								1
- SEROUS CONTENTS		1		1		2		
SKIN :								
- ALOPECIA							1	
URINARY CONTENTS :								
EXTREMITIES/HAIR :								
- YELLOWISH COLOR					6	6	6	6

15034 / PDS PATHDATA SYSTEM TM

Table: 13

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +
MG/KG/DAY: 0, 100, 300, 1000

	DOSE GROUP:	1		2		3		4	
	SEX:	M	F	M	F	M	F	M	F
ORGAN/FINDING	NO.ANIMALS:	6	6	6	6	6	6	6	6
LIVER	NO. EXAM.:	6	6					6	6
- AREA COAG.HEPAT.NEC.									1
- MONONUCLEAR CEL.AGG.		4	5						
- FIBROPLASIA PERIPOR.		1							
- PELIOSIS		1							1
- TENSION LIPIDOSIS		1	1						
- FIBROSIS, INTERLOB.		1							
KIDNEYS	NO. EXAM.:	6	6			1	1	6	6
- DILATED PELVIS		2				1	1		
- TUBULAR BASOPHILIA			1						
- TUBULAR DILATATION			1						
- PERITUBULAR FIBROSIS			1						
JEJUNUM	NO. EXAM.:					1		4	2
- MUCOSAL HAEMORRHAGE								1	
CAECUM	NO. EXAM.:					6	4	4	3
- MUCOSAL HAEMORRHAGE								1	1
COLON	NO. EXAM.:					1		2	1
- CAP.HAEMORRHAGE									1
STOMACH	NO. EXAM.:					2		4	6
- EROSION						2			2
FORESTOMACH	NO. EXAM.:					5		1	5
- EPITHEL.CEL.HYPERPL.						5			4
- HYPERKERATOSIS						5			4
- ULCERATION									2
- INFL.CEL.INF.,MUCOSA									1
SEMINAL VESICLES	NO. EXAM.:							6	
- HYPOSECRETION								4	
- EPITHELIUM ATROPHY								3	

Table: 13 (continued)

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +
MG/KG/DAY: 0, 100, 300, 1000

DOSE GROUP:	1		2		3		4	
SEX:	M	F	M	F	M	F	M	F
ORGAN/FINDING NO.ANIMALS:	6	6	6	6	6	6	6	6
PROSTATE NO. EXAM.:							5	
- HYPOSECRETION							2	
- INTERS.MONO.CEL.AGG.							1	
TESTES NO. EXAM.:	1						1	
- ATROPHY OF SEM.TUBU.	1							
THYMUS NO. EXAM.:							1	2
- CAPILLARY HAEMORRH.							1	2
- INVOLUTION							1	2
ADRENAL GLANDS NO. EXAM.:							2	3
- CORT.CEL.HYPERTROPHY								2
SPLEEN NO. EXAM.:							5	1
- LYMPHOID DEPLETION							5	1
URINARY BLADDER NO. EXAM.:					5			
- EOSINOPH.MATERIAL					2			
UTERUS NO. EXAM.:		1		1		2		1
- DILATED LUMEN		1		1		2		
- ENDOMETRITIS								1
SKIN NO. EXAM.:							1	
- ACANTHOSIS							1	

APPENDICES

1. Diet formula

Ref: A04

COMPLETE DIET

RAT AND MOUSE MAINTENANCE DIET

Appearance: 15 mm diameter pellets or powder

Conditioning: 25 kg double paper bag with aluminium on the outside

Daily portion: Rat 18-25 g, Mouse 5-10 g, water *ad libitum*.

FORMULA %

Cereals and cereal biproducts	88
Vegetable protein (soya bean meal, yeast)	7
Animal protein (fish)	2
Vitamin and mineral mixture	3

AVERAGE ANALYSIS %

Calorific value (KCal/kg)	2900
Moisture	12
Proteins	17
Lipids	3
Carbohydrates (N.F.E.)	58.7
Fibre	4
Minerals (ash)	5

AMINO ACID VALUES
(calculated in mg/kg)

Arginine	9800
Cystine	2300
Lysine	8500
Methionine	3200
Tryptophan	1900
Glycine	8100

FATTY ACID VALUES
(calculated in mg/kg)

Palmitic acid	2600
Palmitoleic acid	Traces
Stearic acid	500
Oleic acid	8000
Linoleic acid	14500
Linolenic acid	Traces

MINERALS (calculated in mg/kg)

	Nat val.	CMV val.	Total
P	5900	0	5900
Ca	3300	5000	8300
K	6700	0	6700
Na	300	1600	1900
Mg	1900	100	2000
Mn	50	40	90
Fe	90	150	240
Cu	15	15	30
Zn	40	45	85
Co	T	1.5	1.5
I	0.3	0	0.3

VITAMINS (calculated per kg)

	Nat val.	CMV val.	Total
Vitamin A	Traces	7500 IU	7500 IU
Vitamin D3	Traces	1500 IU	1500 IU
Vitamin B1	6 mg	1 mg	7 mg
Vitamin B2	2 mg	4.5 mg	6.5 mg
Vitamin B3	10 mg	6.5 mg	16.5 mg
Vitamin B6	1.3 mg	1.3 mg	2.6 mg
Vitamin B12	0.01 mg	0.01 mg	0.02 mg
Vitamin E	15 mg	15 mg	30 mg
Vitamin K3	0.25 mg	2.25 mg	2.5 mg
Vitamin PP	60 mg	15 mg	75 mg
Folic acid	0.5 mg	0 mg	0.5 mg
Biotin	0.04 mg	0 mg	0.04 mg
Choline	1200 mg	400 mg	1600 mg

Available under quality "Control Ref.: A04 C "

U.A.R., 7 rue Galliéni, 91360 Villemoisson - Tel: 69.04.03.57 - Fax : 69.04.81.97
(Ref. Doc. UAR: 1992)

2. Mortality

-: dead animal
/: missing value
DK: Decision of Killing

INDIVIDUAL FATE

Dose: 0 mg/kg/day
Sex: male

Animal No.	Week of death	Day of death	Date	Reason for sacrifice or death
Q27891	3	16	20/12/96	Final sacrifice
Q27892	3	16	20/12/96	Final sacrifice
Q27893	3	16	20/12/96	Final sacrifice
Q27894	3	16	20/12/96	Final sacrifice
Q27895	3	16	20/12/96	Final sacrifice
Q27896	3	16	20/12/96	Final sacrifice

INDIVIDUAL FATE

Dose: 100 mg/kg/day
Sex: male

Animal No.	Week of death	Day of death	Date	Reason for sacrifice or death
Q27897	3	16	20/12/96	Final sacrifice
Q27898	3	16	20/12/96	Final sacrifice
Q27899	3	16	20/12/96	Final sacrifice
Q27900	3	16	20/12/96	Final sacrifice
Q27901	3	16	20/12/96	Final sacrifice
Q27902	3	16	20/12/96	Final sacrifice

INDIVIDUAL FATE

Dose: 300 mg/kg/day
Sex: male

Animal No.	Week of death	Day of death	Date	Reason for sacrifice or death
Q27903	3	16	20/12/96	Final sacrifice
Q27904	3	16	20/12/96	Final sacrifice
Q27905	3	16	20/12/96	Final sacrifice
Q27906	3	16	20/12/96	Final sacrifice
Q27907	3	16	20/12/96	Final sacrifice
Q27908	3	16	20/12/96	Final sacrifice

INDIVIDUAL FATE

Dose: 1000 mg/kg/day
Sex: male

Animal No.	Week of death	Day of death	Date	Reason for sacrifice or death
Q27909	2	9	13/12/96	Found dead
Q27910	2	14	18/12/96	Killed prematurely-DK
Q27911	1	7	11/12/96	Killed prematurely-DK
Q27912	1	7	11/12/96	Found dead
Q27913	3	16	20/12/96	Found dead
Q27914	3	16	20/12/96	Found dead

INDIVIDUAL FATE

Dose: 0 mg/kg/day
Sex: female

Animal No.	Week of death	Day of death	Date	Reason for sacrifice or death
Q27921	3	16	20/12/96	Final sacrifice
Q27922	3	16	20/12/96	Final sacrifice
Q27923	3	16	20/12/96	Final sacrifice
Q27924	3	16	20/12/96	Final sacrifice
Q27925	3	16	20/12/96	Final sacrifice
Q27926	3	16	20/12/96	Final sacrifice

INDIVIDUAL FATE

Dose: 100 mg/kg/day
Sex: female

Animal No.	Week of death	Day of death	Date	Reason for sacrifice or death
Q27927	3	16	20/12/96	Final sacrifice
Q27928	3	16	20/12/96	Final sacrifice
Q27929	3	16	20/12/96	Final sacrifice
Q27930	3	16	20/12/96	Final sacrifice
Q27931	3	16	20/12/96	Final sacrifice
Q27932	3	16	20/12/96	Final sacrifice

INDIVIDUAL FATE

Dose: 300 mg/kg/day
Sex: female

Animal No.	Week of death	Day of death	Date	Reason for sacrifice or death
Q27933	3	16	20/12/96	Final sacrifice
Q27934	3	16	20/12/96	Final sacrifice
Q27935	3	16	20/12/96	Final sacrifice
Q27936	3	16	20/12/96	Final sacrifice
Q27937	3	16	20/12/96	Final sacrifice
Q27938	3	16	20/12/96	Final sacrifice

INDIVIDUAL FATE

Dose: 1000 mg/kg/day
Sex: female

Animal No.	Week of death	Day of death	Date	Reason for sacrifice or death
Q27939	2	13	17/12/96	Found dead
Q27940	2	8	12/12/96	Found dead
Q27941	2	9	13/12/96	Found dead
Q27942	2	9	13/12/96	Found dead
Q27943	2	14	18/12/96	Found dead
Q27944	2	11	15/12/96	Found dead

3. Individual clinical signs

CLINICAL HISTORY - INDIVIDUAL FINDINGS

Dose: 0 mg/kg/day
Sex: male

Animal No.	Day of death	Clinical history
Q27891	16	Final sacrifice No clinical history
Q27892	16	Final sacrifice No clinical history
Q27893	16	Final sacrifice No clinical history
Q27894	16	Final sacrifice No clinical history
Q27895	16	Final sacrifice No clinical history
Q27896	16	Final sacrifice No clinical history

CLINICAL HISTORY - INDIVIDUAL FINDINGS

Dose: 100 mg/kg/day
Sex: male

Animal No.	Day of death	Clinical history	
Q27897	16	Final sacrifice	
		Yellow coloured urine	From day 4
		Ptyalism	From day 5
		Yellow coloured faeces	From day 5
Q27898	16	Final sacrifice	
		Yellow coloured urine	From day 4
		Ptyalism	Day 5 to 6
		Yellow coloured faeces	From day 5
Q27899	16	Final sacrifice	
		Yellow coloured urine	From day 4
		Yellow coloured faeces	From day 5
Q27900	16	Final sacrifice	
		Yellow coloured urine	From day 4
		Yellow coloured faeces	From day 5
Q27901	16	Final sacrifice	
		Yellow coloured urine	From day 4
		Yellow coloured faeces	From day 5
Q27902	16	Final sacrifice	
		Yellow coloured urine	From day 4
		Yellow coloured faeces	From day 5

CLINICAL HISTORY - INDIVIDUAL FINDINGS

Dose: 300 mg/kg/day
Sex: male

Animal No.	Day of death	Clinical history	
Q27903	16	Final sacrifice	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 5
		Yellow coloured fur	From day 9
		Yellow coloured extremities	From day 15
Q27904	16	Final sacrifice	
		Ptyalism	From day 2
		Yellow coloured urine	From day 3
		Yellow coloured faeces	From day 5
		Yellow coloured extremities	From day 15
Q27905	16	Final sacrifice	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 5
		Yellow coloured extremities	From day 15
Q27906	16	Final sacrifice	
		Ptyalism	From day 2
		Yellow coloured urine	From day 3
		Yellow coloured faeces	From day 5
		Yellow coloured extremities	From day 15
Q27907	16	Final sacrifice	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 5
		Yellow coloured extremities	From day 15
Q27908	16	Final sacrifice	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 5
		Yellow coloured extremities	From day 15
		Yellow coloured fur	From day 15

CLINICAL HISTORY - INDIVIDUAL FINDINGS

Dose: 1000 mg/kg/day
Sex: male

Animal No.	Day of death	Clinical history	
Q27909	09	Found dead	
		Ptyalism	From day 2
		Yellow coloured urine	From day 3
		Yellow coloured faeces	From day 4
		Piloerection	From day 5
		Round back	From day 5
		Scattered hair	From day 5
		Soft faeces	From day 6
		Yellow coloured extremities	From day 7
Q27910	14	Killed prematurely-DK	
		Yellow coloured urine	From day 3
		Yellow coloured faeces	From day 4
		Piloerection	From day 5
		Ptyalism	From day 5
		Round back	From day 5
		Yellow coloured tail	From day 5
		Soft faeces	From day 6
		Yellow coloured extremities	From day 7
		Yellow coloured fur	From day 8
		Swollen abdomen	From day 13
		Cold to the touch	Day 14
		Dyspnea	Day 14
		Eyes half-closed	Day 14
		Hypokinesia	Day 14
		Staggering gait	Day 14
Q27911	07	Killed prematurely-DK	
		Ptyalism	From day 3
		Yellow coloured urine	From day 3
		Yellow coloured faeces	From day 4
		Piloerection	From day 5
		Round back	From day 5
		Yellow coloured extremities	From day 5
		Cold to the touch	From day 6
		Hypokinesia	From day 6
		Soft faeces	From day 6
		Bent head	From day 7
		Locomotory difficulties	From day 7
		Startling	From day 7

CLINICAL HISTORY - INDIVIDUAL FINDINGS

Dose: 1000 mg/kg/day
Sex: male

Animal No.	Day of death	Clinical history	
Q27912	07	Found dead	
		Ptyalism	From day 3
		Yellow coloured urine	From day 3
		Yellow coloured faeces	From day 4
		Area of hair loss on back	From day 5
		Piloerection	From day 5
		Round back	From day 5
		Soft faeces	From day 5
		Yellow coloured extremities	From day 5
		Cold to the touch	From day 6
		Eyes half-closed	From day 6
		Hypokinesia	From day 6
Q27913	16	Found dead	
		Ptyalism	From day 2
		Yellow coloured urine	From day 3
		Yellow coloured faeces	From day 4
		Yellow coloured extremities	From day 6
		Yellow coloured fur	From day 6
		Swollen abdomen	From day 13
		Bent head	From day 15
		Piloerection	From day 15
		Round back	From day 15
Q27914	16	Found dead	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 4
		Soft faeces	From day 6
		Yellow coloured extremities	From day 6
		Yellow coloured fur	From day 6
		Piloerection	From day 13
		Round back	From day 13
		Swollen abdomen	From day 13
		Bent head	From day 15

CLINICAL HISTORY - INDIVIDUAL FINDINGS

Dose: 0 mg/kg/day
Sex: female

Animal No.	Day of death	Clinical history
Q27921	16	Final sacrifice No clinical history
Q27922	16	Final sacrifice No clinical history
Q27923	16	Final sacrifice No clinical history
Q27924	16	Final sacrifice No clinical history
Q27925	16	Final sacrifice No clinical history
Q27926	16	Final sacrifice No clinical history

CLINICAL HISTORY - INDIVIDUAL FINDINGS

Dose: 100 mg/kg/day
Sex: female

Animal No.	Day of death	Clinical history	
Q27927	16	Final sacrifice	
		Yellow coloured urine	From day 4
		Yellow coloured faeces	From day 5
Q27928	16	Final sacrifice	
		Yellow coloured urine	From day 4
		Yellow coloured faeces	From day 5
Q27929	16	Final sacrifice	
		Yellow coloured urine	From day 4
		Yellow coloured faeces	From day 5
Q27930	16	Final sacrifice	
		Yellow coloured urine	From day 4
		Yellow coloured faeces	From day 5
Q27931	16	Final sacrifice	
		Yellow coloured urine	From day 4
		Yellow coloured faeces	From day 5
Q27932	16	Final sacrifice	
		Yellow coloured urine	From day 4
		Yellow coloured faeces	From day 5

CLINICAL HISTORY - INDIVIDUAL FINDINGS

Dose: 300 mg/kg/day
Sex: female

Animal No.	Day of death	Clinical history	
Q27933	16	Final sacrifice	
		Ptyalism	From day 2
		Yellow coloured urine	From day 3
		Yellow coloured faeces	From day 5
		Yellow coloured fur	From day 12
		Yellow coloured extremities	From day 15
Q27934	16	Final sacrifice	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 5
		Yellow coloured extremities	From day 15
Q27935	16	Final sacrifice	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 5
		Yellow coloured extremities	From day 15
		Yellow coloured fur	From day 15
Q27936	16	Final sacrifice	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 5
		Yellow coloured fur	From day 12
		Yellow coloured extremities	From day 15
Q27937	16	Final sacrifice	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 5
		Yellow coloured extremities	From day 15
		Yellow coloured fur	From day 15
Q27938	16	Final sacrifice	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 5
		Yellow coloured extremities	From day 15

CLINICAL HISTORY - INDIVIDUAL FINDINGS

Dose: 1000 mg/kg/day
Sex: female

Animal No.	Day of death	Clinical history	
Q27939	13	Found dead	
		Ptyalism	From day 2
		Yellow coloured extremities	From day 2
		Yellow coloured urine	From day 3
		Yellow coloured faeces	From day 4
		Piloerection	From day 6
		Soft faeces	From day 7
		Yellow coloured tail	From day 9
Q27940	08	Found dead	
		Ptyalism	From day 2
		Yellow coloured extremities	From day 2
		Yellow coloured urine	From day 3
		Yellow coloured faeces	From day 4
		Piloerection	From day 6
		Soft faeces	From day 7
Q27941	09	Found dead	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 4
		Piloerection	From day 5
		Round back	From day 5
		Soft faeces	From day 5
		Yellow coloured extremities	From day 7
Q27942	09	Found dead	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 4
		Piloerection	From day 5
		Round back	From day 5
		Soft faeces	From day 6
		Yellow coloured extremities	From day 7
Q27943	14	Found dead	
		Ptyalism	From day 2
		Yellow coloured urine	From day 3
		Yellow coloured faeces	From day 4
		Piloerection	From day 5
		Round back	From day 5
		Yellow coloured extremities	From day 5
		Soft faeces	From day 6
		Swollen abdomen	From day 13

CLINICAL HISTORY - INDIVIDUAL FINDINGS

Dose: 1000 mg/kg/day
Sex: female

Animal No.	Day of death	Clinical history	
Q27944	11	Found dead	
		Yellow coloured urine	From day 3
		Ptyalism	From day 4
		Yellow coloured faeces	From day 4
		Round back	From day 5
		Yellow coloured extremities	From day 5
		Soft faeces	From day 6
		Yellow coloured fur	From day 8

4. Individual body weight values

-: dead animal
/: missing value

BODY WEIGHT
(g)

Dose: 0 mg/kg/day
Sex: Male

Day Animal No.	-3	1	5	8	12	15
Q27891	157	179	216	242	279	290
Q27892	158	183	223	249	290	300
Q27893	161	185	231	255	294	301
Q27894	161	184	221	245	284	285
Q27895	165	190	229	253	290	306
Q27896	166	189	234	261	301	307
Mean	161	185	226	251	290	298
SD	3.6	4.1	6.8	6.9	7.7	8.8

Dose: 100 mg/kg/day
Sex: Male

Day Animal No.	-3	1	5	8	12	15
Q27897	155	183	216	247	283	284
Q27898	158	184	223	248	285	292
Q27899	160	186	226	253	298	306
Q27900	161	190	229	249	282	282
Q27901	164	186	233	264	310	320
Q27902	166	193	238	265	305	319
Mean	161	187	228	254	294	301
SD	4.0	3.8	7.7	8.1	12.2	17.0

Dose: 300 mg/kg/day
Sex: Male

Day Animal No.	-3	1	5	8	12	15
Q27903	152	181	219	244	286	300
Q27904	158	187	215	239	276	282
Q27905	159	187	218	245	281	283
Q27906	162	196	230	263	292	301
Q27907	163	190	223	257	285	296
Q27908	172	205	243	272	312	321
Mean	161	191	225	253	289	297
SD	6.6	8.4	10.4	12.8	12.6	14.3

BODY WEIGHT
(g)

Dose: 1000 mg/kg/day
Sex: Male

Day Animal No.	-3	1	5	8	12	15
Q27909	155	180	186	164	-	-
Q27910	158	187	201	202	212	-
Q27911	160	181	176	-	-	-
Q27912	162	185	169	-	-	-
Q27913	163	184	202	208	213	176
Q27914	169	191	209	211	218	199
Mean	161	185	191	196	214	188
SD	4.8	4.0	16.0	21.8	3.2	16.3

BODY WEIGHT
(g)

Dose: 0 mg/kg/day
Sex: Female

Day Animal No.	-3	1	5	8	12	15
Q27921	147	160	171	181	191	190
Q27922	150	164	177	186	199	204
Q27923	151	167	179	192	205	205
Q27924	155	167	179	192	208	205
Q27925	156	173	188	201	209	211
Q27926	164	183	195	202	216	217
Mean	154	169	182	192	205	205
SD	6.0	8.1	8.6	8.2	8.7	9.0

Dose: 100 mg/kg/day
Sex: Female

Day Animal No.	-3	1	5	8	12	15
Q27927	146	155	166	181	193	195
Q27928	150	170	180	192	206	208
Q27929	151	169	188	208	225	228
Q27930	155	172	182	194	203	198
Q27931	156	170	191	202	219	220
Q27932	165	186	205	222	242	241
Mean	154	170	185	200	215	215
SD	6.6	9.9	13.0	14.2	17.6	17.9

Dose: 300 mg/kg/day
Sex: Female

Day Animal No.	-3	1	5	8	12	15
Q27933	148	164	175	190	208	209
Q27934	150	160	168	181	191	197
Q27935	151	165	179	179	195	187
Q27936	153	170	184	195	204	214
Q27937	158	170	188	199	212	212
Q27938	163	176	190	201	217	219
Mean	154	168	181	191	205	206
SD	5.6	5.7	8.3	9.2	10.0	12.0

BODY WEIGHT
(g)

Dose: 1000 mg/kg/day
Sex: Female

Day Animal No.	-3	1	5	8	12	15
Q27939	149	163	166	153	136	-
Q27940	149	161	168	155	-	-
Q27941	153	163	156	140	-	-
Q27942	153	166	170	159	-	-
Q27943	161	177	179	160	162	-
Q27944	163	177	168	148	-	-
Mean	155	168	168	153	149	-
SD	6.0	7.3	7.4	7.5	18.4	-

5. Individual food consumption values

-: dead animal
/: missing value
1: day 1 to day 4
5: day 5 to day 7
8: day 8 to day 11
12: day 12 to day 14

FOOD CONSUMPTION
(g/animal/day)

Dose: 0 mg/kg/day
Sex: Male

Day Animal No.	1	5	8	12
- Q27891	24.6	26.4	26.6	21.5
Q27892	24.6	26.4	26.6	21.5
- Q27893	26.7	27.3	28.5	21.9
Q27894	26.7	27.3	28.5	21.9
- Q27895	26.0	27.5	28.8	23.6
Q27896	26.0	27.5	28.8	23.6
Mean	25.8	27.1	28.0	22.3
SD	1.07	0.59	1.19	1.12

Dose: 100 mg/kg/day
Sex: Male

Day Animal No.	1	5	8	12
- Q27897	23.5	23.9	25.7	20.3
Q27898	23.5	23.9	25.7	20.3
- Q27899	26.3	27.0	27.9	23.7
Q27900	26.3	27.0	27.9	23.7
- Q27901	28.4	29.4	31.8	24.5
Q27902	28.4	29.4	31.8	24.5
Mean	26.1	26.8	28.5	22.8
SD	2.46	2.76	3.09	2.23

Dose: 300 mg/kg/day
Sex: Male

Day Animal No.	1	5	8	12
- Q27903	22.8	25.1	25.7	21.1
Q27904	22.8	25.1	25.7	21.1
- Q27905	22.8	27.6	26.5	20.2
Q27906	22.8	27.6	26.5	20.2
- Q27907	24.0	28.1	27.4	22.9
Q27908	24.0	28.1	27.4	22.9
Mean	23.2	26.9	26.5	21.4
SD	0.69	1.61	0.85	1.38

FOOD CONSUMPTION
(g/animal/day)

Dose: 1000 mg/kg/day
Sex: Male

Day Animal No.	1	5	8	12
- Q27909	13.9	9.0	12.8	-
Q27910	13.9	9.0	12.8	-
- Q27911	7.8	-	-	-
Q27912	7.8	-	-	-
- Q27913	15.1	12.8	12.3	7.0
Q27914	15.1	12.8	12.3	7.0
Mean	12.3	10.9	12.6	7.0
SD	3.92	2.69	0.35	-

FOOD CONSUMPTION
(g/animal/day)

Dose: 0 mg/kg/day
Sex: Female

Day Animal No.	1	5	8	12
- Q27921	18.6	16.8	22.6	15.4
Q27922	18.6	16.8	22.6	15.4
- Q27923	17.5	19.0	19.1	15.5
Q27924	17.5	19.0	19.1	15.5
- Q27925	17.9	18.6	19.0	14.6
Q27926	17.9	18.6	19.0	14.6
Mean	18.0	18.1	20.2	15.2
SD	0.56	1.17	2.05	0.49

Dose: 100 mg/kg/day
Sex: Female

Day Animal No.	1	5	8	12
- Q27927	17.1	17.1	16.8	14.9
Q27928	17.1	17.1	16.8	14.9
- Q27929	18.4	19.3	19.1	16.2
Q27930	18.4	19.3	19.1	16.2
- Q27931	21.6	21.8	22.3	19.0
Q27932	21.6	21.8	22.3	19.0
Mean	19.0	19.4	19.4	16.7
SD	2.32	2.35	2.76	2.10

Dose: 300 mg/kg/day
Sex: Female

Day Animal No.	1	5	8	12
- Q27933	15.4	17.0	17.6	14.8
Q27934	15.4	17.0	17.6	14.8
- Q27935	15.3	17.0	17.6	14.9
Q27936	15.3	17.0	17.6	14.9
- Q27937	16.5	17.6	18.4	16.1
Q27938	16.5	17.6	18.4	16.1
Mean	15.7	17.2	17.9	15.3
SD	0.67	0.35	0.46	0.72

FOOD CONSUMPTION
(g/animal/day)

Dose: 1000 mg/kg/day
Sex: Female

Day Animal No.	1	5	8	12
- Q27939	10.1	7.3	7.5	-
Q27940	10.1	7.3	-	-
- Q27941	9.1	5.4	-	-
Q27942	9.1	5.4	-	-
- Q27943	8.6	7.9	8.7	-
Q27944	8.6	7.9	8.7	-
Mean	9.3	6.9	8.1	-
SD	0.76	1.31	0.85	-

6. Individual values of haematological parameters

KEY TO ABBREVIATIONS USED FOR HAEMATOLOGY

WBC	: leucocytes	N	: neutrophils
RBC	: erythrocytes	E	: eosinophils
HB	: haemoglobin	B	: basophils
PCV	: packed cell volume	L	: lymphocytes + L.U.C. (Large Unstained Cells)
MCV	: mean cell volume	M	: monocytes
MCH	: mean cell haemoglobin		
MCHC	: mean cell haemoglobin concentration		
PLAT	: thrombocytes		

EXPLANATION FOR MISSING VALUE

-	: dead animal	cg	: coagulated sample
ns	: not sampled	i	: insufficient sample
h	: haemolysed sample	np	: not performed
nd	: not detectable	a	: aberrant value
u	: unreadable slide	l	: lactescent
nc	: not coagulable	t	: technical problem
m	: missing value		

HAEMATOLOGY

Time: Week 2
Dose: 0 mg/kg/day
Sex: Male

Animal No.	WBC G/l	RBC T/l	HB g/dl	PCV l/l	MCV fl	MCH pg	MCHC g/dl	PLAT G/l
Q27891	cg	cg	cg	cg	cg	cg	cg	cg
Q27892	12.44	7.16	14.7	0.44	61.0	20.5	33.6	1239
Q27893	14.06	7.57	14.9	0.46	60.1	19.7	32.7	1270
Q27894	15.39	7.72	15.5	0.47	61.0	20.1	32.9	1171
Q27895	8.85	7.13	14.6	0.44	61.6	20.5	33.2	1407
Q27896	cg	cg	cg	cg	cg	cg	cg	cg
Mean	12.69	7.40	14.9	0.45	60.9	20.2	33.1	1272
SD	2.827	0.295	0.40	0.015	0.62	0.38	0.39	99.2

Dose: 100 mg/kg/day
Sex: Male

Animal No.	WBC G/l	RBC T/l	HB g/dl	PCV l/l	MCV fl	MCH pg	MCHC g/dl	PLAT G/l
Q27897	cg	cg	cg	cg	cg	cg	cg	cg
Q27898	7.18	7.38	15.2	0.47	63.3	20.6	32.5	1554
Q27899	8.85	7.17	14.6	0.45	62.5	20.4	32.6	1046
Q27900	14.86	7.42	15.6	0.47	62.7	21.1	33.6	1079
Q27901	11.04	7.40	14.7	0.45	60.8	19.9	32.7	1275
Q27902	10.40	7.36	15.1	0.46	62.9	20.5	32.5	1407
Mean	10.47	7.35	15.0	0.46	62.4	20.5	32.8	1272
SD	2.875	0.101	0.40	0.010	0.96	0.43	0.47	215.7

Dose: 300 mg/kg/day
Sex: Male

Animal No.	WBC G/l	RBC T/l	HB g/dl	PCV l/l	MCV fl	MCH pg	MCHC g/dl	PLAT G/l
Q27903	14.24	6.98	14.8	0.45	64.5	21.2	32.9	1258
Q27904	16.48	7.85	15.6	0.47	59.9	19.9	33.3	1500
Q27905	13.39	7.19	14.4	0.44	60.5	20.1	33.1	1053
Q27906	17.68	6.63	14.1	0.44	65.7	21.3	32.4	1283
Q27907	13.55	7.51	14.8	0.45	60.2	19.7	32.8	1339
Q27908	13.27	7.47	14.7	0.45	60.3	19.7	32.7	1269
Mean	14.77	7.27	14.7	0.45	61.9	20.3	32.9	1284
SD	1.861	0.432	0.51	0.011	2.55	0.74	0.31	144.2

HAEMATOLOGY

Time: Week 2
Dose: 1000 mg/kg/day
Sex: Male

Animal No.	WBC G/l	RBC T/l	HB g/dl	PCV l/l	MCV fl	MCH pg	MCHC g/dl	PLAT G/l
Q27909	-	-	-	-	-	-	-	-
Q27910	9.42	8.01	14.6	0.45	56.1	18.2	32.4	1470
Q27911	-	-	-	-	-	-	-	-
Q27912	-	-	-	-	-	-	-	-
Q27913	12.49	8.54	15.3	0.45	53.2	18.0	33.8	1105
Q27914	cg	cg	cg	cg	cg	cg	cg	cg
Mean	10.96	8.28	15.0	0.45	54.7	18.1	33.1	1288
SD	2.171	0.375	0.50	0.000	2.05	0.14	0.99	258.1

HAEMATOLOGY

Time: Week 2
Dose: 0 mg/kg/day
Sex: Male

Animal No.	N %	E %	B %	L %	M %	N G/l	E G/l	B G/l	L G/l	M G/l
Q27891	cg	cg	cg	cg	cg	cg	cg	cg	cg	cg
Q27892	8.0	0.8	0.2	90.1	0.9	0.99	0.09	0.02	11.21	0.11
Q27893	9.0	0.7	0.4	88.3	1.7	1.26	0.10	0.05	12.41	0.24
Q27894	7.0	0.7	0.4	91.0	0.8	1.08	0.11	0.07	14.01	0.13
Q27895	11.8	1.0	0.1	85.4	1.6	1.05	0.09	0.01	7.56	0.14
Q27896	cg	cg	cg	cg	cg	cg	cg	cg	cg	cg
Mean	9.0	0.8	0.3	88.7	1.3	1.10	0.10	0.04	11.30	0.16
SD	2.07	0.14	0.15	2.47	0.47	0.116	0.010	0.028	2.743	0.058

Dose: 100 mg/kg/day
Sex: Male

Animal No.	N %	E %	B %	L %	M %	N G/l	E G/l	B G/l	L G/l	M G/l
Q27897	cg	cg	cg	cg	cg	cg	cg	cg	cg	cg
Q27898	9.8	0.9	0.2	87.9	1.3	0.70	0.07	0.01	6.31	0.09
Q27899	8.5	0.9	0.2	89.3	1.2	0.75	0.08	0.02	7.90	0.11
Q27900	11.0	0.5	0.3	86.9	1.3	1.64	0.07	0.05	12.92	0.19
Q27901	17.9	1.0	0.3	79.4	1.4	1.98	0.11	0.03	8.78	0.16
Q27902	15.4	0.9	0.1	81.8	1.8	1.60	0.10	0.01	8.51	0.18
Mean	12.5	0.8	0.2	85.1	1.4	1.33	0.09	0.02	8.88	0.15
SD	3.97	0.20	0.08	4.25	0.24	0.576	0.018	0.017	2.451	0.044

Dose: 300 mg/kg/day
Sex: Male

Animal No.	N %	E %	B %	L %	M %	N G/l	E G/l	B G/l	L G/l	M G/l
Q27903	10.4	0.8	0.2	87.0	1.6	1.48	0.11	0.02	12.39	0.23
Q27904	12.1	0.7	0.3	84.4	2.5	2.00	0.11	0.05	13.91	0.42
Q27905	10.7	0.8	0.2	87.1	1.3	1.43	0.10	0.03	11.65	0.18
Q27906	11.0	0.6	0.3	85.8	2.4	1.94	0.11	0.05	15.16	0.42
Q27907	14.9	0.9	0.2	82.6	1.4	2.02	0.12	0.02	11.19	0.19
Q27908	9.0	0.6	0.3	88.2	1.9	1.19	0.08	0.04	11.71	0.25
Mean	11.4	0.7	0.3	85.9	1.9	1.68	0.11	0.04	12.67	0.28
SD	2.01	0.12	0.06	2.05	0.51	0.354	0.014	0.014	1.547	0.110

HAEMATOLOGY

Time: Week 2
Dose: 1000 mg/kg/day
Sex: Male

Animal No.	N %	E %	B %	L %	M %	N G/l	E G/l	B G/l	L G/l	M G/l
Q27909	-	-	-	-	-	-	-	-	-	-
Q27910	38.3	0.7	0.1	58.9	2.0	3.61	0.06	0.01	5.55	0.19
Q27911	-	-	-	-	-	-	-	-	-	-
Q27912	-	-	-	-	-	-	-	-	-	-
Q27913	42.6	1.3	0.1	52.1	3.8	5.33	0.17	0.02	6.51	0.47
Q27914	cg	cg	cg	cg	cg	cg	cg	cg	cg	cg
Mean	40.5	1.0	0.1	55.5	2.9	4.47	0.12	0.02	6.03	0.33
SD	3.04	0.42	0.00	4.81	1.27	1.216	0.078	0.007	0.679	0.198

HAEMATOLOGY

Time: Week 2
Dose: 0 mg/kg/day
Sex: Female

Animal No.	WBC G/l	RBC T/l	HB g/dl	PCV l/l	MCV fl	MCH pg	MCHC g/dl	PLAT G/l
Q27921	7.96	7.46	15.7	0.44	59.0	21.1	35.7	1291
Q27922	5.76	7.36	14.9	0.43	58.1	20.3	34.9	1243
Q27923	10.13	7.21	14.3	0.43	59.2	19.8	33.5	1547
Q27924	11.39	7.41	15.1	0.44	59.8	20.4	34.1	1245
Q27925	cg	cg	cg	cg	cg	cg	cg	cg
Q27926	13.07	7.20	14.8	0.43	59.5	20.6	34.6	1206
Mean	9.66	7.33	15.0	0.43	59.1	20.4	34.6	1306
SD	2.870	0.118	0.51	0.006	0.65	0.47	0.83	137.8

Dose: 100 mg/kg/day
Sex: Female

Animal No.	WBC G/l	RBC T/l	HB g/dl	PCV l/l	MCV fl	MCH pg	MCHC g/dl	PLAT G/l
Q27927	cg	cg	cg	cg	cg	cg	cg	cg
Q27928	7.85	7.61	14.2	0.42	54.6	18.6	34.1	1303
Q27929	11.12	8.25	16.0	0.47	56.5	19.4	34.3	1260
Q27930	8.99	6.73	13.3	0.39	58.0	19.8	34.1	1284
Q27931	9.16	7.15	14.3	0.41	58.0	19.9	34.4	1328
Q27932	9.89	7.39	14.8	0.44	59.0	20.0	33.9	1443
Mean	9.40	7.43	14.5	0.43	57.2	19.5	34.2	1324
SD	1.207	0.565	0.99	0.031	1.72	0.57	0.20	71.3

Dose: 300 mg/kg/day
Sex: Female

Animal No.	WBC G/l	RBC T/l	HB g/dl	PCV l/l	MCV fl	MCH pg	MCHC g/dl	PLAT G/l
Q27933	15.43	7.43	15.1	0.43	58.4	20.3	34.8	965
Q27934	12.87	7.78	15.1	0.45	57.6	19.4	33.8	1212
Q27935	cg	cg	cg	cg	cg	cg	cg	cg
Q27936	12.43	7.49	15.5	0.45	59.5	20.6	34.7	1294
Q27937	11.39	7.31	14.5	0.42	57.7	19.8	34.3	1350
Q27938	11.69	7.47	15.3	0.44	59.2	20.5	34.6	1322
Mean	12.76	7.50	15.1	0.44	58.5	20.1	34.4	1229
SD	1.603	0.173	0.37	0.013	0.86	0.51	0.40	156.1

HAEMATOLOGY

Time: Week 2
Dose: 1000 mg/kg/day
Sex: Female

Animal No.	WBC G/l	RBC T/l	HB g/dl	PCV l/l	MCV fl	MCH pg	MCHC g/dl	PLAT G/l
Q27939	-	-	-	-	-	-	-	-
Q27940	-	-	-	-	-	-	-	-
Q27941	-	-	-	-	-	-	-	-
Q27942	-	-	-	-	-	-	-	-
Q27943	-	-	-	-	-	-	-	-
Q27944	-	-	-	-	-	-	-	-
Mean	-	-	-	-	-	-	-	-
SD	-	-	-	-	-	-	-	-

HAEMATOLOGY

Time: Week 2
Dose: 0 mg/kg/day
Sex: Female

Animal No.	N %	E %	B %	L %	M %	N G/l	E G/l	B G/l	L G/l	M G/l
Q27921	17.2	1.2	0.3	78.9	2.5	1.37	0.10	0.02	6.28	0.20
Q27922	13.8	1.7	0.1	83.4	1.0	0.79	0.10	0.01	4.81	0.06
Q27923	13.1	0.7	0.1	84.5	1.6	1.32	0.07	0.01	8.56	0.16
Q27924	6.3	0.8	0.2	91.2	1.4	0.72	0.10	0.03	10.38	0.16
Q27925	cg	cg	cg	cg	cg	cg	cg	cg	cg	cg
Q27926	10.0	0.9	0.3	87.1	1.7	1.30	0.12	0.04	11.38	0.22
Mean	12.1	1.1	0.2	85.0	1.6	1.10	0.10	0.02	8.28	0.16
SD	4.12	0.40	0.10	4.55	0.55	0.317	0.018	0.013	2.745	0.062

Dose: 100 mg/kg/day
Sex: Female

Animal No.	N %	E %	B %	L %	M %	N G/l	E G/l	B G/l	L G/l	M G/l
Q27927	cg	cg	cg	cg	cg	cg	cg	cg	cg	cg
Q27928	15.9	1.6	0.1	80.9	1.3	1.25	0.13	0.01	6.36	0.11
Q27929	18.9	1.2	0.3	77.8	1.8	2.10	0.14	0.03	8.65	0.20
Q27930	9.1	1.1	0.1	87.6	2.2	0.82	0.10	0.01	7.87	0.20
Q27931	14.4	0.7	0.2	82.1	2.5	1.32	0.07	0.02	7.52	0.23
Q27932	16.9	2.1	0.2	78.9	1.9	1.67	0.21	0.02	7.80	0.19
Mean	15.0	1.3	0.2	81.5	1.9	1.43	0.13	0.02	7.64	0.19
SD	3.70	0.53	0.08	3.82	0.45	0.480	0.052	0.008	0.829	0.045

Dose: 300 mg/kg/day
Sex: Female

Animal No.	N %	E %	B %	L %	M %	N G/l	E G/l	B G/l	L G/l	M G/l
Q27933	8.5	1.1	0.4	88.4	1.5	1.32	0.17	0.06	13.65	0.24
Q27934	9.6	1.0	0.2	87.0	2.2	1.24	0.13	0.03	11.20	0.28
Q27935	cg	cg	cg	cg	cg	cg	cg	cg	cg	cg
Q27936	20.0	1.3	0.2	77.2	1.4	2.48	0.16	0.03	9.59	0.17
Q27937	14.5	1.5	0.2	82.0	1.8	1.65	0.17	0.02	9.34	0.20
Q27938	12.7	1.4	0.3	83.1	2.5	1.49	0.16	0.04	9.70	0.29
Mean	13.1	1.3	0.3	83.5	1.9	1.64	0.16	0.04	10.70	0.24
SD	4.56	0.21	0.09	4.43	0.47	0.498	0.016	0.015	1.805	0.051

HAEMATOLOGY

Time: Week 2
Dose: 1000 mg/kg/day
Sex: Female

Animal No.	N %	E %	B %	L %	M %	N G/l	E G/l	B G/l	L G/l	M G/l
Q27939	-	-	-	-	-	-	-	-	-	-
Q27940	-	-	-	-	-	-	-	-	-	-
Q27941	-	-	-	-	-	-	-	-	-	-
Q27942	-	-	-	-	-	-	-	-	-	-
Q27943	-	-	-	-	-	-	-	-	-	-
Q27944	-	-	-	-	-	-	-	-	-	-
Mean	-	-	-	-	-	-	-	-	-	-
SD	-	-	-	-	-	-	-	-	-	-

7. Individual values of blood biochemical parameters

KEY TO ABBREVIATIONS USED FOR BLOOD BIOCHEMISTRY

Na^+	: sodium	ALB	: albumin
K^+	: potassium	A/G	: albumin/globulin ratio
Cl^-	: chloride	CHOL	: cholesterol
GLUC	: glucose	TRIG	: triglycerides
UREA	: urea	ALP	: alkaline phosphatase
CREAT	: creatinine	ASAT	: aspartate aminotransferase
TOT.BIL	: total bilirubin	ALAT	: alanine aminotransferase
PROT	: total proteins		

EXPLANATION FOR MISSING VALUE

-	: dead animal	cg	: coagulated sample
ns	: not sampled	i	: insufficient sample
h	: haemolysed sample	np	: not performed
nd	: not detectable	a	: aberrant value
l	: lactescent	t	: technical problem
m	: missing value		

BLOOD BIOCHEMISTRY

Time: Week 2
Dose: 0 mg/kg/day
Sex: Male

Animal No.	Na+ mmol/l	K+ mmol/l	Cl- mmol/l	GLUC mmol/l	UREA mmol/l	CREAT µmol/l
Q27891	144.1	3.60	102.6	4.37	3.7	40
Q27892	144.2	3.31	102.6	4.66	4.0	45
Q27893	145.9	3.09	103.3	4.35	3.3	39
Q27894	144.9	3.30	103.7	4.82	3.6	43
Q27895	145.3	2.84	103.2	5.33	3.7	41
Q27896	146.7	4.95	102.7	4.69	3.7	44
Mean	145.2	3.52	103.0	4.70	3.7	42
SD	1.01	0.747	0.45	0.359	0.23	2.4

Dose: 100 mg/kg/day
Sex: Male

Animal No.	Na+ mmol/l	K+ mmol/l	Cl- mmol/l	GLUC mmol/l	UREA mmol/l	CREAT µmol/l
Q27897	145.2	4.29	104.5	4.19	5.0	50
Q27898	146.7	3.16	103.6	4.16	2.9	36
Q27899	146.0	3.47	104.6	4.68	4.0	43
Q27900	145.6	4.57	103.6	5.60	3.9	37
Q27901	145.6	3.30	102.4	4.84	4.0	37
Q27902	146.0	3.62	106.4	4.50	3.9	39
Mean	145.9	3.74	104.2	4.66	4.0	40
SD	0.51	0.567	1.35	0.532	0.67	5.4

Dose: 300 mg/kg/day
Sex: Male

Animal No.	Na+ mmol/l	K+ mmol/l	Cl- mmol/l	GLUC mmol/l	UREA mmol/l	CREAT µmol/l
Q27903	146.2	3.34	105.8	5.54	4.4	38
Q27904	145.8	3.29	103.9	6.47	4.5	41
Q27905	143.3	3.27	104.5	6.98	3.9	37
Q27906	143.6	3.47	105.5	6.81	3.6	40
Q27907	143.5	3.56	103.7	5.39	4.4	38
Q27908	145.7	3.49	104.1	5.19	3.5	41
Mean	144.7	3.40	104.6	6.06	4.1	39
SD	1.35	0.119	0.87	0.781	0.44	1.7

BLOOD BIOCHEMISTRY

Time: Week 2
Dose: 1000 mg/kg/day
Sex: Male

Animal No.	Na+ mmol/l	K+ mmol/l	Cl- mmol/l	GLUC mmol/l	UREA mmol/l	CREAT µmol/l
Q27909	-	-	-	-	-	-
Q27910	141.7	3.27	103.2	5.65	10.8	39
Q27911	-	-	-	-	-	-
Q27912	-	-	-	-	-	-
Q27913	140.0	3.17	94.2	6.52	10.6	39
Q27914	142.7	5.11	97.7	4.72	9.5	41
Mean	141.5	3.85	98.4	5.63	10.3	40
SD	1.37	1.092	4.54	0.900	0.70	1.2

BLOOD BIOCHEMISTRY

Time: Week 2
Dose: 0 mg/kg/day
Sex: Male

Animal No.	PROT g/l	ALB g/l	A/G l	TOT.BIL. µmol/l	CHOL mmol/l	TRIG mmol/l	ALP IU/l	ASAT IU/l	ALAT IU/l
Q27891	66	32	0.94	1	2.0	0.97	425	64	28
Q27892	67	34	1.03	1	2.1	0.59	381	67	18
Q27893	70	34	0.94	1	2.4	0.75	422	50	31
Q27894	67	35	1.09	1	2.3	0.82	565	51	26
Q27895	65	33	1.03	1	2.2	0.53	325	61	20
Q27896	69	34	0.97	1	1.9	1.19	595	31	57
Mean	67	34	1.00	1	2.2	0.81	452	54	30
SD	1.9	1.0	0.060	0.0	0.19	0.245	105.9	13.2	14.1

Dose: 100 mg/kg/day
Sex: Male

Animal No.	PROT g/l	ALB g/l	A/G l	TOT.BIL. µmol/l	CHOL mmol/l	TRIG mmol/l	ALP IU/l	ASAT IU/l	ALAT IU/l
Q27897	68	34	1.00	1	2.9	1.64	549	43	43
Q27898	66	34	1.06	1	2.1	0.90	512	59	28
Q27899	66	32	0.94	1	1.8	1.75	360	40	37
Q27900	64	32	1.00	1	1.7	0.97	405	62	23
Q27901	64	33	1.06	1	1.8	0.74	611	55	32
Q27902	65	34	1.10	1	1.8	0.62	379	59	20
Mean	66	33	1.03	1	2.0	1.10	469	53	31
SD	1.5	1.0	0.058	0.0	0.45	0.476	102.5	9.2	8.6

Dose: 300 mg/kg/day
Sex: Male

Animal No.	PROT g/l	ALB g/l	A/G l	TOT.BIL. µmol/l	CHOL mmol/l	TRIG mmol/l	ALP IU/l	ASAT IU/l	ALAT IU/l
Q27903	63	34	1.17	1	1.7	0.53	628	73	18
Q27904	68	36	1.13	1	1.3	1.52	548	90	20
Q27905	69	34	0.97	1	2.1	1.00	358	58	22
Q27906	68	35	1.06	1	2.0	0.73	520	66	15
Q27907	70	35	1.00	1	1.5	0.44	641	73	12
Q27908	65	34	1.10	1	1.4	1.29	476	64	19
Mean	67	35	1.07	1	1.7	0.92	529	71	18
SD	2.6	0.8	0.077	0.0	0.33	0.429	104.7	11.1	3.6

BLOOD BIOCHEMISTRY

Time: Week 2
Dose: 1000 mg/kg/day
Sex: Male

Animal No.	PROT g/l	ALB g/l	A/G l	TOT.BIL. µmol/l	CHOL mmol/l	TRIG mmol/l	ALP IU/l	ASAT IU/l	ALAT IU/l
Q27909	-	-	-	-	-	-	-	-	-
Q27910	62	36	1.38	2	0.9	0.62	302	79	24
Q27911	-	-	-	-	-	-	-	-	-
Q27912	-	-	-	-	-	-	-	-	-
Q27913	60	34	1.31	2	0.8	0.72	224	79	21
Q27914	59	29	0.97	2	1.2	0.89	133	155	13
Mean	60	33	1.22	2	1.0	0.74	220	104	19
SD	1.5	3.6	0.219	0.0	0.21	0.137	84.6	43.9	5.7

BLOOD BIOCHEMISTRY

Time: Week 2
Dose: 0 mg/kg/day
Sex: Female

Animal No.	Na+ mmol/l	K+ mmol/l	Cl- mmol/l	GLUC mmol/l	UREA mmol/l	CREAT µmol/l
Q27921	142.7	3.24	103.9	5.07	4.4	45
Q27922	143.8	3.57	105.0	5.07	4.8	41
Q27923	145.8	2.67	107.1	6.27	5.3	47
Q27924	145.5	3.06	108.0	4.79	8.7	57
Q27925	143.9	2.93	103.1	4.91	6.8	52
Q27926	141.2	3.51	103.4	6.77	6.5	50
Mean	143.8	3.16	105.1	5.48	6.1	49
SD	1.72	0.346	2.04	0.828	1.59	5.6

Dose: 100 mg/kg/day
Sex: Female

Animal No.	Na+ mmol/l	K+ mmol/l	Cl- mmol/l	GLUC mmol/l	UREA mmol/l	CREAT µmol/l
Q27927	144.0	3.25	103.5	5.04	5.7	43
Q27928	144.1	3.03	105.3	5.97	5.5	44
Q27929	144.6	2.97	102.9	6.78	4.6	40
Q27930	141.3	3.46	104.7	5.46	5.1	41
Q27931	142.2	3.68	105.2	5.32	6.0	45
Q27932	141.0	3.00	101.9	8.23	5.4	42
Mean	142.9	3.23	103.9	6.13	5.4	43
SD	1.56	0.289	1.38	1.196	0.49	1.9

Dose: 300 mg/kg/day
Sex: Female

Animal No.	Na+ mmol/l	K+ mmol/l	Cl- mmol/l	GLUC mmol/l	UREA mmol/l	CREAT µmol/l
Q27933	140.3	3.77	104.0	5.73	5.3	40
Q27934	142.6	3.54	105.1	5.82	4.0	39
Q27935	143.9	3.45	105.0	6.67	4.6	48
Q27936	139.8	4.01	103.5	6.99	3.6	47
Q27937	140.2	3.45	101.7	7.88	5.7	48
Q27938	141.6	3.39	104.4	6.78	4.7	37
Mean	141.4	3.60	104.0	6.65	4.7	43
SD	1.61	0.241	1.26	0.798	0.78	5.0

BLOOD BIOCHEMISTRY

Time: Week 2
Dose: 1000 mg/kg/day
Sex: Female

Animal No.	Na+ mmol/l	K+ mmol/l	Cl- mmol/l	GLUC mmol/l	UREA mmol/l	CREAT µmol/l
Q27939	-	-	-	-	-	-
Q27940	-	-	-	-	-	-
Q27941	-	-	-	-	-	-
Q27942	-	-	-	-	-	-
Q27943	-	-	-	-	-	-
Q27944	-	-	-	-	-	-
Mean	-	-	-	-	-	-
SD	-	-	-	-	-	-

BLOOD BIOCHEMISTRY

Time: Week 2
Dose: 0 mg/kg/day
Sex: Female

Animal No.	PROT g/l	ALB g/l	A/G 1	TOT.BIL. µmol/l	CHOL mmol/l	TRIG mmol/l	ALP IU/l	ASAT IU/l	ALAT IU/l
Q27921	67	35	1.09	1	1.0	0.26	368	72	10
Q27922	72	39	1.18	1	1.5	0.37	304	66	27
Q27923	71	35	0.97	1	1.6	0.24	360	64	9
Q27924	69	35	1.03	2	1.5	0.36	299	54	18
Q27925	75	37	0.97	1	1.8	0.40	292	69	7
Q27926	72	38	1.12	2	2.0	0.65	330	49	10
Mean	71	37	1.06	1	1.6	0.38	326	62	14
SD	2.8	1.8	0.085	0.5	0.34	0.147	32.6	9.0	7.6

Dose: 100 mg/kg/day
Sex: Female

Animal No.	PROT g/l	ALB g/l	A/G 1	TOT.BIL. µmol/l	CHOL mmol/l	TRIG mmol/l	ALP IU/l	ASAT IU/l	ALAT IU/l
Q27927	71	37	1.09	2	2.1	0.76	211	50	18
Q27928	71	38	1.15	1	1.9	0.51	284	40	12
Q27929	76	39	1.05	1	1.9	0.80	264	55	10
Q27930	69	36	1.09	1	1.7	0.56	252	53	12
Q27931	67	35	1.09	1	1.8	0.56	333	48	17
Q27932	70	35	1.00	1	2.9	0.84	353	38	13
Mean	71	37	1.08	1	2.1	0.67	283	47	14
SD	3.0	1.6	0.050	0.4	0.44	0.144	52.7	6.9	3.1

Dose: 300 mg/kg/day
Sex: Female

Animal No.	PROT g/l	ALB g/l	A/G 1	TOT.BIL. µmol/l	CHOL mmol/l	TRIG mmol/l	ALP IU/l	ASAT IU/l	ALAT IU/l
Q27933	70	36	1.06	1	1.4	0.50	305	56	23
Q27934	69	37	1.16	1	1.8	0.59	224	65	18
Q27935	70	35	1.00	1	1.2	0.38	274	52	11
Q27936	71	33	0.87	1	1.6	0.64	259	71	15
Q27937	69	34	0.97	1	1.2	0.36	333	70	9
Q27938	70	36	1.06	2	1.8	1.03	234	56	12
Mean	70	35	1.02	1	1.5	0.58	272	62	15
SD	0.8	1.5	0.098	0.4	0.28	0.245	41.8	8.1	5.2

BLOOD BIOCHEMISTRY

Time: Week 2
Dose: 1000 mg/kg/day
Sex: Female

Animal No.	PROT g/l	ALB g/l	A/G l	TOT.BIL. µmol/l	CHOL mmol/l	TRIG mmol/l	ALP IU/l	ASAT IU/l	ALAT IU/l
Q27939	-	-	-	-	-	-	-	-	-
Q27940	-	-	-	-	-	-	-	-	-
Q27941	-	-	-	-	-	-	-	-	-
Q27942	-	-	-	-	-	-	-	-	-
Q27943	-	-	-	-	-	-	-	-	-
Q27944	-	-	-	-	-	-	-	-	-
Mean	-	-	-	-	-	-	-	-	-
SD	-	-	-	-	-	-	-	-	-

8. Individual organ weights

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

Explanation of symbols:

- = Tissue/organ not weighed
* = Tissue/organ weighed after fixation

15034 / PDS PATHDATA SYSTEM TM

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 1, 0 mg/kg/day
SEX : MALE

ANIMAL NUMBER	:	Q27891	Q27892	Q27893	Q27894
DAYS ON TEST	:	16	16	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0	K0	K0
STATUS AT NECROPSY	:	K0	K0	K0	K0
PROSECTOR	:	VR	FC	FC	FC
FINAL BODY WEIGHT	G:	263.000	274.700	279.400	256.800
LIVER	G:	8.335	8.199	8.934	8.746
	% BODY :	3.169	2.985	3.198	3.406
	% BRAIN:				
KIDNEYS	G:	2.141	2.293	2.606	2.443
	RIGHT:	1.046	1.169	1.317	1.212
	LEFT :	1.095	1.124	1.289	1.231
	% BODY :	0.814	0.835	0.933	0.951
	% BRAIN:				
TESTES	G:	3.058	3.165	3.166	1.148
	RIGHT:	1.549	1.584	1.550	0.598
	LEFT :	1.509	1.581	1.616	0.550
	% BODY :	1.163	1.152	1.133	0.447
	% BRAIN:				
THYMUS	G:	0.464	0.660	0.702	0.570
	% BODY :	0.176	0.240	0.251	0.222
	% BRAIN:				
ADRENAL GLANDS	G:	0.054	0.052	0.054	0.051
	RIGHT:	0.025	0.026	0.025	0.023
	LEFT :	0.029	0.026	0.029	0.028
	% BODY :	0.021	0.019	0.019	0.020
	% BRAIN:				

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 1, 0 mg/kg/day
SEX : MALE

ANIMAL NUMBER	:	Q27891	Q27892	Q27893	Q27894
SPLEEN	G:	0.536	0.651	0.606	0.711
	% BODY :	0.204	0.237	0.217	0.277
	% BRAIN:				
HEART	G:	1.098	1.184	1.226	0.983
	% BODY :	0.417	0.431	0.439	0.383
	% BRAIN:				

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 1, 0 mg/kg/day
SEX : MALE

ANIMAL NUMBER	:	Q27895	Q27896
DAYS ON TEST	:	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0
STATUS AT NECROPSY	:	K0	K0
PROSECTOR	:	FC	VR
FINAL BODY WEIGHT	G:	271.900	277.600
LIVER	G:	8.726	10.269
	% BODY :	3.209	3.699
	% BRAIN:		
KIDNEYS	G:	2.520	2.518
	RIGHT:	1.297	1.247
	LEFT :	1.223	1.271
	% BODY :	0.927	0.907
	% BRAIN:		
TESTES	G:	3.102	2.836
	RIGHT:	1.487	1.418
	LEFT :	1.615	1.418
	% BODY :	1.141	1.022
	% BRAIN:		
THYMUS	G:	0.578	0.615
	% BODY :	0.213	0.222
	% BRAIN:		
ADRENAL GLANDS	G:	0.049	0.053
	RIGHT:	0.023	0.025
	LEFT :	0.026	0.028
	% BODY :	0.018	0.019
	% BRAIN:		

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 1, 0 mg/kg/day
SEX : MALE

ANIMAL NUMBER		Q27895	Q27896
SPLEEN	G:	0.645	0.551
	% BODY :	0.237	0.198
	% BRAIN:		
HEART	G:	1.150	1.090
	% BODY :	0.423	0.393
	% BRAIN:		

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 1, 0 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER	:	Q27921	Q27922	Q27923	Q27924
DAYS ON TEST	:	16	16	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0	K0	K0
STATUS AT NECROPSY	:	K0	K0	K0	K0
PROSECTOR	:	ML	FC	VR	CLF
FINAL BODY WEIGHT	G:	168.900	182.900	183.200	180.200
LIVER	G:	5.295	5.502	5.438	5.633
	% BODY :	3.135	3.008	2.968	3.126
	% BRAIN:				
KIDNEYS	G:	1.569	1.735	1.613	1.415
	RIGHT:	0.786	0.847	0.803	0.745
	LEFT :	0.783	0.888	0.810	0.670
	% BODY :	0.929	0.949	0.880	0.785
	% BRAIN:				
THYMUS	G:	0.362	0.505	0.455	0.327
	% BODY :	0.214	0.276	0.248	0.181
	% BRAIN:				
ADRENAL GLANDS	G:	0.061	0.060	0.058	0.072
	RIGHT:	0.027	0.029	0.028	0.034
	LEFT :	0.034	0.031	0.030	0.038
	% BODY :	0.036	0.033	0.032	0.040
	% BRAIN:				
SPLEEN	G:	0.339	0.444	0.454	0.431
	% BODY :	0.201	0.243	0.248	0.239
	% BRAIN:				
HEART	G:	0.648	0.879	0.858	0.863
	% BODY :	0.384	0.481	0.468	0.479
	% BRAIN:				

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 1, 0 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER		Q27921	Q27922	Q27923	Q27924
OVARIES	G:	0.097	0.121	0.104	0.128
	RIGHT:	0.053	0.061	0.053	0.063
	LEFT :	0.044	0.060	0.051	0.065
	% BODY :	0.057	0.066	0.057	0.071
	% BRAIN:				

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 1, 0 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER	:	Q27925	Q27926
DAYS ON TEST	:	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0
STATUS AT NECROPSY	:	K0	K0
PROSECTOR	:	CLF	ML
FINAL BODY WEIGHT	G:	186.900	195.800
LIVER	G:	5.730	5.744
	% BODY :	3.066	2.934
	% BRAIN:		
KIDNEYS	G:	1.669	1.636
	RIGHT:	0.851	0.829
	LEFT :	0.818	0.807
	% BODY :	0.893	0.836
	% BRAIN:		
THYMUS	G:	0.491	0.511
	% BODY :	0.263	0.261
	% BRAIN:		
ADRENAL GLANDS	G:	0.064	0.058
	RIGHT:	0.031	0.030
	LEFT :	0.033	0.028
	% BODY :	0.034	0.030
	% BRAIN:		
SPLEEN	G:	0.541	0.496
	% BODY :	0.289	0.253
	% BRAIN:		
HEART	G:	1.058	0.786
	% BODY :	0.566	0.401
	% BRAIN:		

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 1, 0 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER		Q27925	Q27926
OVARIES	G:	0.130	0.099
	RIGHT:	0.062	0.054
	LEFT :	0.068	0.045
	% BODY :	0.070	0.051
	% BRAIN:		

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 2, 100 mg/kg/day
SEX : MALE

ANIMAL NUMBER	:	Q27897	Q27898	Q27899	Q27900
DAYS ON TEST	:	16	16	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0	K0	K0
STATUS AT NECROPSY	:	K0	K0	K0	K0
PROSECTOR	:	VR	VR	FC	VR
FINAL BODY WEIGHT	G:	254.600	264.000	270.300	258.400
LIVER	G:	10.393	8.881	8.526	10.265
	% BODY :	4.082	3.364	3.154	3.973
	% BRAIN:				
KIDNEYS	G:	2.396	2.247	2.318	2.495
	RIGHT:	1.209	1.134	1.180	1.249
	LEFT :	1.187	1.113	1.138	1.246
	% BODY :	0.941	0.851	0.858	0.966
	% BRAIN:				
TESTES	G:	3.090	3.344	2.858	2.646
	RIGHT:	1.535	1.665	1.437	1.318
	LEFT :	1.555	1.679	1.421	1.328
	% BODY :	1.214	1.267	1.057	1.024
	% BRAIN:				
THYMUS	G:	0.506	0.600	0.526	0.855
	% BODY :	0.199	0.227	0.195	0.331
	% BRAIN:				
ADRENAL GLANDS	G:	0.053	0.056	0.060	0.061
	RIGHT:	0.025	0.027	0.030	0.029
	LEFT :	0.028	0.029	0.030	0.032
	% BODY :	0.021	0.021	0.022	0.024
	% BRAIN:				

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 2, 100 mg/kg/day
SEX : MALE

ANIMAL NUMBER		Q27897	Q27898	Q27899	Q27900
SPLEEN	G:	0.662	0.625	0.657	0.675
	% BODY :	0.260	0.237	0.243	0.261
	% BRAIN:				
HEART	G:	1.096	1.115	1.327	0.977
	% BODY :	0.430	0.422	0.491	0.378
	% BRAIN:				

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 2, 100 mg/kg/day
SEX : MALE

ANIMAL NUMBER	:	Q27901	Q27902
DAYS ON TEST	:	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0
STATUS AT NECROPSY	:	K0	K0
PROSECTOR	:	FC	VR
FINAL BODY WEIGHT	G:	287.500	285.700
LIVER	G:	9.277	9.224
	% BODY :	3.227	3.229
	% BRAIN:		
KIDNEYS	G:	2.600	2.668
	RIGHT:	1.320	1.340
	LEFT :	1.280	1.328
	% BODY :	0.904	0.934
	% BRAIN:		
TESTES	G:	3.172	3.209
	RIGHT:	1.595	1.559
	LEFT :	1.577	1.650
	% BODY :	1.103	1.123
	% BRAIN:		
THYMUS	G:	0.550	0.575
	% BODY :	0.191	0.201
	% BRAIN:		
ADRENAL GLANDS	G:	0.065	0.047
	RIGHT:	0.032	0.025
	LEFT :	0.033	0.022
	% BODY :	0.023	0.016
	% BRAIN:		

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 2, 100 mg/kg/day
SEX : MALE

ANIMAL NUMBER		Q27901	Q27902
SPLEEN	G:	0.632	0.700
	% BODY :	0.220	0.245
	% BRAIN:		
HEART	G:	1.259	1.181
	% BODY :	0.438	0.413
	% BRAIN:		

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 2, 100 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER	:	Q27927	Q27928	Q27929	Q27930
DAYS ON TEST	:	16	16	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0	K0	K0
STATUS AT NECROPSY	:	K0	K0	K0	K0
PROSECTOR	:	CLF	ML	ML	CLF
FINAL BODY WEIGHT	G:	170.600	184.400	201.700	173.700
LIVER	G:	5.512	5.854	6.689	5.515
	% BODY :	3.231	3.175	3.316	3.175
	% BRAIN:				
KIDNEYS	G:	1.667	1.502	1.869	1.490
	RIGHT:	0.847	0.779	0.909	0.783
	LEFT :	0.820	0.723	0.960	0.707
	% BODY :	0.977	0.815	0.927	0.858
	% BRAIN:				
THYMUS	G:	0.350	0.257	0.436	0.349
	% BODY :	0.205	0.139	0.216	0.201
	% BRAIN:				
ADRENAL GLANDS	G:	0.067	0.071	0.064	0.056
	RIGHT:	0.031	0.036	0.031	0.027
	LEFT :	0.036	0.035	0.033	0.029
	% BODY :	0.039	0.039	0.032	0.032
	% BRAIN:				
SPLEEN	G:	0.393	0.457	0.464	0.355
	% BODY :	0.230	0.248	0.230	0.204
	% BRAIN:				
HEART	G:	0.810	0.747	0.986	0.842
	% BODY :	0.475	0.405	0.489	0.485
	% BRAIN:				

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 2, 100 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER		Q27927	Q27928	Q27929	Q27930
OVARIES	G:	0.106	0.099	0.114	0.108
	RIGHT:	0.049	0.049	0.057	0.066
	LEFT :	0.057	0.050	0.057	0.042
	% BODY :	0.062	0.054	0.057	0.062
	% BRAIN:				

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 2, 100 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER	:	Q27931	Q27932
DAYS ON TEST	:	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0
STATUS AT NECROPSY	:	K0	K0
PROSECTOR	:	CLF	ML
FINAL BODY WEIGHT	G:	193.700	217.900
LIVER	G:	5.839	7.259
	% BODY :	3.014	3.331
	% BRAIN:		
KIDNEYS	G:	1.774	1.967
	RIGHT:	0.874	0.937
	LEFT :	0.900	1.030
	% BODY :	0.916	0.903
	% BRAIN:		
THYMUS	G:	0.483	0.413
	% BODY :	0.249	0.190
	% BRAIN:		
ADRENAL GLANDS	G:	0.065	0.070
	RIGHT:	0.029	0.033
	LEFT :	0.036	0.037
	% BODY :	0.034	0.032
	% BRAIN:		
SPLEEN	G:	0.375	0.434
	% BODY :	0.194	0.199
	% BRAIN:		
HEART	G:	0.847	0.933
	% BODY :	0.437	0.428
	% BRAIN:		

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 2, 100 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER		Q27931	Q27932
OVARIES	G:	0.127	0.108
	RIGHT:	0.059	0.052
	LEFT :	0.068	0.056
	% BODY :	0.066	0.050
	% BRAIN:		

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 3, 300 mg/kg/day
SEX : MALE

ANIMAL NUMBER	:	Q27903	Q27904	Q27905	Q27906
DAYS ON TEST	:	16	16	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0	K0	K0
STATUS AT NECROPSY	:	K0	K0	K0	K0
PROSECTOR	:	VR	FC	VR	VR
FINAL BODY WEIGHT	G:	268.400	249.000	254.300	270.700
LIVER	G:	10.495	9.716	9.337	12.034
	% BODY :	3.910	3.902	3.672	4.446
	% BRAIN:				
KIDNEYS	G:	2.380	2.213	2.146	2.465
	RIGHT:	1.179	1.168	1.126	1.257
	LEFT :	1.201	1.045	1.020	1.208
	% BODY :	0.887	0.889	0.844	0.911
	% BRAIN:				
TESTES	G:	3.070	2.923	2.888	3.019
	RIGHT:	1.519	1.454	1.441	1.557
	LEFT :	1.551	1.469	1.447	1.462
	% BODY :	1.144	1.174	1.136	1.115
	% BRAIN:				
THYMUS	G:	0.647	0.446	0.527	0.579
	% BODY :	0.241	0.179	0.207	0.214
	% BRAIN:				
ADRENAL GLANDS	G:	0.054	0.055	0.053	0.066
	RIGHT:	0.027	0.026	0.025	0.032
	LEFT :	0.027	0.029	0.028	0.034
	% BODY :	0.020	0.022	0.021	0.024
	% BRAIN:				

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 3, 300 mg/kg/day
SEX : MALE

ANIMAL NUMBER		Q27903	Q27904	Q27905	Q27906
SPLEEN	G:	0.603	0.546	0.438	0.730
	% BODY :	0.225	0.219	0.172	0.270
	% BRAIN:				
HEART	G:	1.094	1.263	1.073	1.161
	% BODY :	0.408	0.507	0.422	0.429
	% BRAIN:				

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 3, 300 mg/kg/day
SEX : MALE

ANIMAL NUMBER	:	Q27907	Q27908
DAYS ON TEST	:	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0
STATUS AT NECROPSY	:	K0	K0
PROSECTOR	:	FC	FC
FINAL BODY WEIGHT	G:	264.800	285.000
LIVER	G:	9.532	10.110
	% BODY :	3.600	3.547
	% BRAIN:		
KIDNEYS	G:	2.338	2.245
	RIGHT:	1.226	1.145
	LEFT :	1.112	1.100
	% BODY :	0.883	0.788
	% BRAIN:		
TESTES	G:	3.250	3.011
	RIGHT:	1.619	1.507
	LEFT :	1.631	1.504
	% BODY :	1.227	1.056
	% BRAIN:		
THYMUS	G:	0.539	0.667
	% BODY :	0.204	0.234
	% BRAIN:		
ADRENAL GLANDS	G:	0.060	0.083
	RIGHT:	0.029	0.040
	LEFT :	0.031	0.043
	% BODY :	0.023	0.029
	% BRAIN:		

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 3, 300 mg/kg/day
SEX : MALE

ANIMAL NUMBER		Q27907	Q27908
SPLEEN	G:	0.512	0.595
	% BODY :	0.193	0.209
	% BRAIN:		
HEART	G:	1.041	1.246
	% BODY :	0.393	0.437
	% BRAIN:		

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 3, 300 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER	:	Q27933	Q27934	Q27935	Q27936
DAYS ON TEST	:	16	16	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0	K0	K0
STATUS AT NECROPSY	:	K0	K0	K0	K0
PROSECTOR	:	CLF	ML	ML	CLF
FINAL BODY WEIGHT	G:	186.100	173.700	168.700	184.700
LIVER	G:	6.751	5.695	6.229	7.198
	% BODY :	3.628	3.279	3.692	3.897
	% BRAIN:				
KIDNEYS	G:	1.608	1.649	1.711	1.644
	RIGHT:	0.820	0.842	0.870	0.846
	LEFT :	0.788	0.807	0.841	0.798
	% BODY :	0.864	0.949	1.014	0.890
	% BRAIN:				
THYMUS	G:	0.517	0.494	0.362	0.557
	% BODY :	0.278	0.284	0.215	0.302
	% BRAIN:				
ADRENAL GLANDS	G:	0.074	0.070	0.067	0.071
	RIGHT:	0.035	0.032	0.030	0.035
	LEFT :	0.039	0.038	0.037	0.036
	% BODY :	0.040	0.040	0.040	0.038
	% BRAIN:				
SPLEEN	G:	0.488	0.447	0.327	0.490
	% BODY :	0.262	0.257	0.194	0.265
	% BRAIN:				
HEART	G:	0.882	0.742	0.692	0.913
	% BODY :	0.474	0.427	0.410	0.494
	% BRAIN:				

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 3, 300 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER	:	Q27933	Q27934	Q27935	Q27936
OVARIES	G:	0.121	0.099	0.099	0.140
	RIGHT:	0.066	0.052	0.046	0.068
	LEFT :	0.055	0.047	0.053	0.072
	% BODY :	0.065	0.057	0.059	0.076
	% BRAIN:				

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 3, 300 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER	:	Q27937	Q27938
DAYS ON TEST	:	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0
STATUS AT NECROPSY	:	K0	K0
PROSECTOR	:	CLF	ML
FINAL BODY WEIGHT	G:	184.300	195.600
LIVER	G:	6.825	6.671
	% BODY :	3.703	3.411
	% BRAIN:		
KIDNEYS	G:	1.720	1.550
	RIGHT:	0.875	0.776
	LEFT :	0.845	0.774
	% BODY :	0.933	0.792
	% BRAIN:		
THYMUS	G:	0.440	0.384
	% BODY :	0.239	0.196
	% BRAIN:		
ADRENAL GLANDS	G:	0.071	0.070
	RIGHT:	0.036	0.031
	LEFT :	0.035	0.039
	% BODY :	0.039	0.036
	% BRAIN:		
SPLEEN	G:	0.378	0.404
	% BODY :	0.205	0.207
	% BRAIN:		
HEART	G:	0.892	0.825
	% BODY :	0.484	0.422
	% BRAIN:		

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 3, 300 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER	:	Q27937	Q27938
OVARIES	G:	0.150	0.114
	RIGHT:	0.071	0.062
	LEFT :	0.079	0.052
	% BODY :	0.081	0.058
	% BRAIN:		

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 4, 1000 mg/kg/day
SEX : MALE

ANIMAL NUMBER	Q27909	Q27910	Q27911	Q27912
DAYS ON TEST	9	14	7	7
DATE OF NECROPSY	13-DEC-96	18-DEC-96	11-DEC-96	11-DEC-96
DEFINED SACR.GROUP	K0	K0	K0	K0
STATUS AT NECROPSY	+1	+2	+2	+1
PROSECTOR	CL	TH	SAM	SAM

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 4, 1000 mg/kg/day
SEX : MALE

ANIMAL NUMBER	:	Q27913	Q27914
DAYS ON TEST	:	16	16
DATE OF NECROPSY	:	20-DEC-96	20-DEC-96
DEFINED SACR.GROUP	:	K0	K0
STATUS AT NECROPSY	:	+1	+1
PROSECTOR	:	PP	PP

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 4, 1000 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER	Q27939	Q27940	Q27941	Q27942
DAYS ON TEST	13	8	9	9
DATE OF NECROPSY	17-DEC-96	12-DEC-96	13-DEC-96	13-DEC-96
DEFINED SACR.GROUP	K0	K0	K0	K0
STATUS AT NECROPSY	+1	+1	+1	+1
PROSECTOR	ML	SP	CL	CL

TABLE OF INDIVIDUAL BODY/ORGAN WEIGHTS

DOSE GROUP : 4, 1000 mg/kg/day
SEX : FEMALE

ANIMAL NUMBER	:	Q27943	Q27944
DAYS ON TEST	:	14	11
DATE OF NECROPSY	:	18-DEC-96	15-DEC-96
DEFINED SACR.GROUP	:	K0	K0
STATUS AT NECROPSY	:	+1	+1
PROSECTOR	:		CLF

9. Individual macroscopic and microscopic examinations

EXPLANATION OF CODES AND SYMBOLS

CODES AND SYMBOLS USED AT ANIMAL LEVEL

M	= MALE ANIMAL
F	= FEMALE ANIMAL
K0	= TERMINAL SACRIFICE GROUP
+	= INTERCURRENT DEATH/KILLED PREMATURELY
+1	= FOUND DEAD
+2	= KILLED MORIBUND

CODES AND SYMBOLS USED AT ORGAN LEVEL:

G	= GROSS OBSERVATION CHECKED OFF HISTOLOGICALLY
!	= GROSS OBSERVAT.NOT CHECKED OFF HISTOLOGICALLY
'	= HISTOLOGIC EXAMINATION NOT REQUIRED
+	= ORGAN EXAMINED, FINDINGS PRESENT
-	= ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

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
(	= FINDING UNILATERAL IN PAIRED ORGANS
*	= COMMENT IN TEXT OF INDIVIDUAL ANIMAL DATA

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day

ANIMAL NUMBER :	Q2 7891 MK0	Q2 7892 MK0	Q2 7893 MK0	Q2 7894 MK0	Q2 7895 MK0	Q2 7896 MK0
GENERAL OBSERVATION	'	'	'	'	'	'
LIVER	+	+	+	+	+	-
- MONONUCLEAR CEL.AGG.	1.	.	1.	1.	1.	.
- FIBROPLASIA PERIPOR.	.	2.	.	.	.	.
- PELIOSIS	.	.	2.	.	.	.
- TENSION LIPIDOSIS	.	.	.	1.	.	.
- FIBROSIS, INTERLOB.	.	.	.	.	2.	.
KIDNEYS	-	-	-	-	+G	+G
- DILATED PELVIS	.	.	.	.	3*	3*
ILEUM	'	'	'	'	'	'
JEJUNUM	'	'	'	'	'	'
DUODENUM	'	'	'	'	'	'
CAECUM	'	'	'	'	'	'
COLON	'	'	'	'	'	'
RECTUM	'	'	'	'	'	'
STOMACH	'	'	'	'	'	'
FORESTOMACH	'	'	'	'	'	'
SEMINAL VESICLES	'	'	'	'	'	'
PROSTATE	'	'	'	'	'	'
TESTES	'	'	'	+G	'	'
- ATROPHY OF SEM.TUBU.				3*		
THYMUS	'	'	'	'	'	'
ADRENAL GLANDS	'	'	'	'	'	'

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day

ANIMAL NUMBER :	Q2 7891 MK0	Q2 7892 MK0	Q2 7893 MK0	Q2 7894 MK0	Q2 7895 MK0	Q2 7896 MK0
SPLEEN	'	'	'	'	'	'
URINARY BLADDER	'	'	'	'	'	'
SKIN	'	'	'	'	'	'
LUNGS WITH BRONCHI	'	'	'	'	'	'
EPIDIDYMIDES	'	'	'	'	'	'
THYROID GLANDS	'	'	'	'	'	'
PARATHYROID GLANDS	'	'	'	'	'	'
SCIATIC NERVE	'	'	'	'	'	'
SKELETAL MUSCLE	'	'	'	'	'	'
SPINAL CORD	'	'	'	'	'	'
AORTA	'	'	'	'	'	'
BRAIN	'	'	'	'	'	'
EYES	'	'	'	'	'	'
HARDERIAN GLANDS	'	'	'	'	'	'
FEMORAL BONE(+ART.)	'	'	'	'	'	'
STERNUM (+ B.M.)	'	'	'	'	'	'
HEART	'	'	'	'	'	'
MESENT. LYMPH NODES	'	'	'	'	'	'
MANDIBUL. LYMPH NODE	'	'	'	'	'	'
MAMMARY GLAND(S)	'	'	'	'	'	'

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day

ANIMAL NUMBER :	Q2 7891 MK0	Q2 7892 MK0	Q2 7893 MK0	Q2 7894 MK0	Q2 7895 MK0	Q2 7896 MK0
OESOPHAGUS	,	,	,	,	,	,
PANCREAS	,	,	,	,	,	,
PITUITARY GLAND	,	,	,	,	,	,
SUBMAXIL./SUBLINGUAL	,	,	,	,	,	,
TRACHEA	,	,	,	,	,	,
TONGUE	,	,	,	,	,	,
URINARY CONTENTS	,	,	,	,	,	,
OTHER GEN. COMMENTS	,	,	,	,	,	,
EXTREMITIES/HAIR	,	,	,	,	,	,

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day

ANIMAL NUMBER :	Q2 7921 FK0	Q2 7922 FK0	Q2 7923 FK0	Q2 7924 FK0	Q2 7925 FK0	Q2 7926 FK0
GENERAL OBSERVATION	,	,	,	,	,	,
LIVER	+	+	+	+	+	+
- MONONUCLEAR CEL.AGG.	.	1.	1.	1.	1.	1.
- TENSION LIPIDOSIS	1.	.	.	.	.	.
KIDNEYS	-	-	+	-	-	-
- TUBULAR BASOPHILIA	.	.	1.	.	.	.
- TUBULAR DILATATION	.	.	1.	.	.	.
- PERITUBULAR FIBROSIS	.	.	(1.	.	.	.
ILEUM	,	,	,	,	,	,
JEJUNUM	,	,	,	,	,	,
DUODENUM	,	,	,	,	,	,
CAECUM	,	,	,	,	,	,
COLON	,	,	,	,	,	,
RECTUM	,	,	,	,	,	,
STOMACH	,	,	,	,	,	,
FORESTOMACH	,	,	,	,	,	,
THYMUS	,	,	,	,	,	,
ADRENAL GLANDS	,	,	,	,	,	,
SPLEEN	,	,	,	,	,	,
URINARY BLADDER	,	,	,	,	,	,
UTERUS	,	,	,	+G	,	,
- DILATED LUMEN				3*		
SKIN	,	,	,	,	,	,

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day

ANIMAL NUMBER :	Q2 7921 FK0	Q2 7922 FK0	Q2 7923 FK0	Q2 7924 FK0	Q2 7925 FK0	Q2 7926 FK0
LUNGS WITH BRONCHI	,	,	,	,	,	,
THYROID GLANDS	,	,	,	,	,	,
PARATHYROID GLANDS	,	,	,	,	,	,
SCIATIC NERVE	,	,	,	,	,	,
SKELETAL MUSCLE	,	,	,	,	,	,
SPINAL CORD	,	,	,	,	,	,
AORTA	,	,	,	,	,	,
BRAIN	,	,	,	,	,	,
EYES	,	,	,	,	,	,
HARDERIAN GLANDS	,	,	,	,	,	,
FEMORAL BONE(+ART.)	,	,	,	,	,	,
STERNUM (+ B.M.)	,	,	,	,	,	,
HEART	,	,	,	,	,	,
MESENT. LYMPH NODES	,	,	,	,	,	,
MANDIBUL. LYMPH NODE	,	,	,	,	,	,
MAMMARY GLAND(S)	,	,	,	,	,	,
OESOPHAGUS	,	,	,	,	,	,
OVARIES	,	,	,	,	,	,
PANCREAS	,	,	,	,	,	,
PITUITARY GLAND	,	,	,	,	,	,

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day

ANIMAL NUMBER :	Q2 7921 FK0	Q2 7922 FK0	Q2 7923 FK0	Q2 7924 FK0	Q2 7925 FK0	Q2 7926 FK0
SUBMAXIL./SUBLINGUAL	,	,	,	,	,	,
TRACHEA	,	,	,	,	,	,
TONGUE	,	,	,	,	,	,
URINARY CONTENTS	,	,	,	,	,	,
VAGINA	,	,	,	,	,	,
OTHER GEN. COMMENTS	,	,	,	,	,	,
EXTREMITIES/HAIR	,	,	,	,	,	,

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day

ANIMAL NUMBER :	Q2 7897 MK0	Q2 7898 MK0	Q2 7899 MK0	Q2 7900 MK0	Q2 7901 MK0	Q2 7902 MK0
GENERAL OBSERVATION	,	,	,	,	,	,
LIVER	,	,	,	,	,	,
KIDNEYS	,	,	,	,	,	,
ILEUM	,	,	,	,	,	,
JEJUNUM	,	,	,	,	,	,
DUODENUM	,	,	,	,	,	,
CAECUM	,	,	,	,	,	,
COLON	,	,	,	,	,	,
RECTUM	,	,	,	,	,	,
STOMACH	,	,	,	,	,	,
FORESTOMACH	,	,	,	,	,	,
SEMINAL VESICLES	,	,	,	,	,	,
PROSTATE	,	,	,	,	,	,
TESTES	,	,	,	,	,	,
THYMUS	,	,	,	,	,	,
ADRENAL GLANDS	,	,	,	,	,	,
SPLEEN	,	,	,	,	,	,
URINARY BLADDER	,	,	,	,	,	,
SKIN	,	,	,	,	,	,
LUNGS WITH BRONCHI	,	,	,	,	,	,

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day

ANIMAL NUMBER :	Q2 7897 MK0	Q2 7898 MK0	Q2 7899 MK0	Q2 7900 MK0	Q2 7901 MK0	Q2 7902 MK0
EPIDIDYMIDES	,	,	,	,	,	,
THYROID GLANDS	,	,	,	,	,	,
PARATHYROID GLANDS	,	,	,	,	,	,
SCIATIC NERVE	,	,	,	,	,	,
SKELETAL MUSCLE	,	,	,	,	,	,
SPINAL CORD	,	,	,	,	,	,
AORTA	,	,	,	,	,	,
BRAIN	,	,	,	,	,	,
EYES	,	,	,	,	,	,
HARDERIAN GLANDS	,	,	,	,	,	,
FEMORAL BONE(+ART.)	,	,	,	,	,	,
STERNUM (+ B.M.)	,	,	,	,	,	,
HEART	,	,	,	,	,	,
MESENT. LYMPH NODES	,	,	,	,	,	,
MANDIBUL. LYMPH NODE	,	,	,	,	,	,
MAMMARY GLAND(S)	,	,	,	,	,	,
OESOPHAGUS	,	,	,	,	,	,
PANCREAS	,	,	,	,	,	,
PITUITARY GLAND	,	,	,	,	,	,
SUBMAXIL./SUBLINGUAL	,	,	,	,	,	,

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day

ANIMAL NUMBER :	Q2 7897 MK0	Q2 7898 MK0	Q2 7899 MK0	Q2 7900 MK0	Q2 7901 MK0	Q2 7902 MK0
TRACHEA	,	,	,	,	,	,
TONGUE	,	,	,	,	,	,
URINARY CONTENTS	,	,	,	,	,	,
OTHER GEN. COMMENTS	,	,	,	,	,	,
EXTREMITIES/HAIR	,	,	,	,	,	,

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day

ANIMAL NUMBER :	Q2 7927 FK0	Q2 7928 FK0	Q2 7929 FK0	Q2 7930 FK0	Q2 7931 FK0	Q2 7932 FK0
GENERAL OBSERVATION	,	,	,	,	,	,
LIVER	,	,	,	,	,	,
KIDNEYS	,	,	,	,	,	,
ILEUM	,	,	,	,	,	,
JEJUNUM	,	,	,	,	,	,
DUODENUM	,	,	,	,	,	,
CAECUM	,	,	,	,	,	,
COLON	,	,	,	,	,	,
RECTUM	,	,	,	,	,	,
STOMACH	,	,	,	,	,	,
FORESTOMACH	,	,	,	,	,	,
THYMUS	,	,	,	,	,	,
ADRENAL GLANDS	,	,	,	,	,	,
SPLEEN	,	,	,	,	,	,
URINARY BLADDER	,	,	,	,	,	,
UTERUS	+G	,	,	,	,	,
- DILATED LUMEN	3*					
SKIN	,	,	,	,	,	,
LUNGS WITH BRONCHI	,	,	,	,	,	,
THYROID GLANDS	,	,	,	,	,	,

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day

ANIMAL NUMBER :	Q2 7927 FK0	Q2 7928 FK0	Q2 7929 FK0	Q2 7930 FK0	Q2 7931 FK0	Q2 7932 FK0
PARATHYROID GLANDS	,	,	,	,	,	,
SCIATIC NERVE	,	,	,	,	,	,
SKELETAL MUSCLE	,	,	,	,	,	,
SPINAL CORD	,	,	,	,	,	,
AORTA	,	,	,	,	,	,
BRAIN	,	,	,	,	,	,
EYES	,	,	,	,	,	,
HARDERIAN GLANDS	,	,	,	,	,	,
FEMORAL BONE(+ART.)	,	,	,	,	,	,
STERNUM (+ B.M.)	,	,	,	,	,	,
HEART	,	,	,	,	,	,
MESENT. LYMPH NODES	,	,	,	,	,	,
MANDIBUL. LYMPH NODE	,	,	,	,	,	,
MAMMARY GLAND(S)	,	,	,	,	,	,
OESOPHAGUS	,	,	,	,	,	,
OVARIES	,	,	,	,	,	,
PANCREAS	,	,	,	,	,	,
PITUITARY GLAND	,	,	,	,	,	,
SUBMAXIL./SUBLINGUAL	,	,	,	,	,	,
TRACHEA	,	,	,	,	,	,

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day

ANIMAL NUMBER :	Q2 7927 FK0	Q2 7928 FK0	Q2 7929 FK0	Q2 7930 FK0	Q2 7931 FK0	Q2 7932 FK0
TONGUE	,	,	,	,	,	,
URINARY CONTENTS	,	,	,	,	,	,
VAGINA	,	,	,	,	,	,
OTHER GEN. COMMENTS	,	,	,	,	,	,
EXTREMITIES/HAIR	,	,	,	,	,	,

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day

ANIMAL NUMBER :	Q2 7903 MK0	Q2 7904 MK0	Q2 7905 MK0	Q2 7906 MK0	Q2 7907 MK0	Q2 7908 MK0
GENERAL OBSERVATION	'	'	'	'	'	'
LIVER	'	'	'	'	'	'
KIDNEYS	'	+G	'	'	'	'
- DILATED PELVIS		3*				
ILEUM	'	-G	-G	-G	-G	-G
JEJUNUM	'	-G	'	'	'	'
DUODENUM	'	'	'	'	'	'
CAECUM	-G	-G	-G	-G	-G	-G
COLON	'	'	-G	'	'	'
RECTUM	'	'	'	'	'	'
STOMACH	+	+	'	'	'	'
- EROSION	2.	2.				
FORESTOMACH	+G	+G	+G	+G	'	+G
- EPITHEL.CEL.HYPERPL.	2*	2*	2*	2*		3*
- HYPERKERATOSIS	1.	1.	2.	2.		3.
SEMINAL VESICLES	'	'	'	'	'	'
PROSTATE	'	'	'	'	'	'
TESTES	'	'	'	'	'	'
THYMUS	'	'	'	'	'	'
ADRENAL GLANDS	'	'	'	'	'	'
SPLEEN	'	'	'	'	'	'

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day

ANIMAL NUMBER :	Q2 7903 MK0	Q2 7904 MK0	Q2 7905 MK0	Q2 7906 MK0	Q2 7907 MK0	Q2 7908 MK0
URINARY BLADDER	+G	-G	'	-G	-G	+G
- EOSINOPH.MATERIAL	2*	.		.	.	3*
SKIN	'	'	'	'	'	'
LUNGS WITH BRONCHI	'	'	'	'	'	'
EPIDIDYMIDES	'	'	'	'	'	'
THYROID GLANDS	'	'	'	'	'	'
PARATHYROID GLANDS	'	'	'	'	'	'
SCIATIC NERVE	'	'	'	'	'	'
SKELETAL MUSCLE	'	'	'	'	'	'
SPINAL CORD	'	'	'	'	'	'
AORTA	'	'	'	'	'	'
BRAIN	'	'	'	'	'	'
EYES	'	'	'	'	'	'
HARDERIAN GLANDS	'	'	'	'	'	'
FEMORAL BONE(+ART.)	'	'	'	'	'	'
STERNUM (+ B.M.)	'	'	'	'	'	'
HEART	'	'	'	'	'	'
MESENT. LYMPH NODES	'	'	'	'	'	'
MANDIBUL. LYMPH NODE	'	'	'	'	'	'
MAMMARY GLAND(S)	'	'	'	'	'	'

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day

ANIMAL NUMBER :	Q2 7903 MK0	Q2 7904 MK0	Q2 7905 MK0	Q2 7906 MK0	Q2 7907 MK0	Q2 7908 MK0
OESOPHAGUS	'	'	'	'	'	'
PANCREAS	'	'	'	'	'	'
PITUITARY GLAND	'	'	'	'	'	'
SUBMAXIL./SUBLINGUAL	'	'	'	'	'	'
TRACHEA	'	'	'	'	'	'
TONGUE	'	'	'	'	'	'
URINARY CONTENTS	'	'	'	'	'	'
OTHER GEN. COMMENTS	'	'	'	'	'	'
EXTREMITIES/HAIR	'!	'!	'!	'!	'!	'!

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day

ANIMAL NUMBER :	Q2 7933 FK0	Q2 7934 FK0	Q2 7935 FK0	Q2 7936 FK0	Q2 7937 FK0	Q2 7938 FK0
GENERAL OBSERVATION	'	'	'	'	'	'
LIVER	'	'	'	'	'	'
KIDNEYS	'	'	+G	'	'	'
- DILATED PELVIS			2*			
ILEUM	-G	'	'	'	'	'
JEJUNUM	'	'	'	'	'	'
DUODENUM	'	'	'	'	'	'
CAECUM	-G	'	-G	-G	-G	'
COLON	'	'	'	'	'	'
RECTUM	'	'	'	'	'	'
STOMACH	'	'	'	'	'	'
FORESTOMACH	'	'	'	'	'	'
THYMUS	'	'	'	'	'	'
ADRENAL GLANDS	'	'	'	'	'	'
SPLEEN	'	'	'	'	'	'
URINARY BLADDER	'	'	'	'	'	'
UTERUS	'	+G	'	+G	'	'
- DILATED LUMEN		2*		2*		
SKIN	'	'	'	'	'	'
LUNGS WITH BRONCHI	'	'	'	'	'	'
THYROID GLANDS	'	'	'	'	'	'

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day

ANIMAL NUMBER :	Q2 7933 FK0	Q2 7934 FK0	Q2 7935 FK0	Q2 7936 FK0	Q2 7937 FK0	Q2 7938 FK0
PARATHYROID GLANDS	,	,	,	,	,	,
SCIATIC NERVE	,	,	,	,	,	,
SKELETAL MUSCLE	,	,	,	,	,	,
SPINAL CORD	,	,	,	,	,	,
AORTA	,	,	,	,	,	,
BRAIN	,	,	,	,	,	,
EYES	,	,	,	,	,	,
HARDERIAN GLANDS	,	,	,	,	,	,
FEMORAL BONE(+ART.)	,	,	,	,	,	,
STERNUM (+ B.M.)	,	,	,	,	,	,
HEART	,	,	,	,	,	,
MESENT. LYMPH NODES	,	,	,	,	,	,
MANDIBUL. LYMPH NODE	,	,	,	,	,	,
MAMMARY GLAND(S)	,	,	,	,	,	,
OESOPHAGUS	,	,	,	,	,	,
OVARIES	,	,	,	,	,	,
PANCREAS	,	,	,	,	,	,
PITUITARY GLAND	,	,	,	,	,	,
SUBMAXIL./SUBLINGUAL	,	,	,	,	,	,
TRACHEA	,	,	,	,	,	,

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day

ANIMAL NUMBER :	Q2 7933 FK0	Q2 7934 FK0	Q2 7935 FK0	Q2 7936 FK0	Q2 7937 FK0	Q2 7938 FK0
TONGUE	'	'	'	'	'	'
URINARY CONTENTS	'	'	'	'	'	'
VAGINA	'	'	'	'	'	'
OTHER GEN. COMMENTS	'	'	'	'	'	'
EXTREMITIES/HAIR	'!	'!	'!	'!	'!	'!

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day

ANIMAL NUMBER :	Q2 7909 M+1	Q2 7910 M+2	Q2 7911 M+2	Q2 7912 M+1	Q2 7913 M+1	Q2 7914 M+1
GENERAL OBSERVATION	'	'	'	'	'	'
LIVER	-	-	-	-	-	-
KIDNEYS	-	-	-	-G	-	-
ILEUM	-G	-G	-G	-G	'	'
JEJUNUM	+G	-G	-G	-G	'	'
- MUCOSAL HAEMORRHAGE	1*	.	.	.		
DUODENUM	-G	-G	'	'	'	'
CAECUM	+G	-G	-G	-G	'	'
- MUCOSAL HAEMORRHAGE	3*	.	.	.		
COLON	'	-G	'	-G	'	'
RECTUM	'	'	'	-G	'	'
STOMACH	-G	-G	-G	-G	'	'
FORESTOMACH	-G	'	'	'	'	'
SEMINAL VESICLES	-G	-G	+G	+G	+G	+G
- HYPOSECRETION	.	.	2.	3.	3.	3*
- EPITHELIUM ATROPHY	.	.	1*	3*	3*	.
PROSTATE	'	-G	+G	+G	-G	-G
- HYPOSECRETION		.	2*	2*	.	.
- INTERS.MONO.CEL.AGG.		.	1.	.	.	.
TESTES	'	'	-G	'	'	'
THYMUS	'	'	'	+G	'	'
- CAPILLARY HAEMORRH.				3.		
- INVOLUTION				3*		
ADRENAL GLANDS	'	-G	'	-G	'	'

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day

ANIMAL NUMBER :	Q2 7909 M+1	Q2 7910 M+2	Q2 7911 M+2	Q2 7912 M+1	Q2 7913 M+1	Q2 7914 M+1
SPLEEN	+G	'	+G	+G	+G	+G
- LYMPHOID DEPLETION	2*		2*	3*	3*	2*
URINARY BLADDER	'	'	'	'	'	'
SKIN	'	'	'	+G	'	'
- ACANTHOSIS				1*		
LUNGS WITH BRONCHI	'	'	'	'	'	'
EPIDIDYMIDES	'	'	'	'	'	'
THYROID GLANDS	'	'	'	'	'	'
PARATHYROID GLANDS	'	'	'	'	'	'
SCIATIC NERVE	'	'	'	'	'	'
SKELETAL MUSCLE	'	'	'	'	'	'
SPINAL CORD	'	'	'	'	'	'
AORTA	'	'	'	'	'	'
BRAIN	'	'	'	'	'	'
EYES	'	'	'	'	'	'
HARDERIAN GLANDS	'	'	'	'	'	'
FEMORAL BONE(+ART.)	'	'	'	'	'	'
STERNUM (+ B.M.)	'	'	'	'	'	'
HEART	'	'	'	'	'	'
MESENT. LYMPH NODES	'	'	'	'	'	'
MANDIBUL. LYMPH NODE	'	'	'	'	'	'

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day

ANIMAL NUMBER :	Q2 7909 M+1	Q2 7910 M+2	Q2 7911 M+2	Q2 7912 M+1	Q2 7913 M+1	Q2 7914 M+1
MAMMARY GLAND(S)	'	'	'	'	'	'
OESOPHAGUS	'	'	'	'	'	'
PANCREAS	'	'	'	'	'	'
PITUITARY GLAND	'	'	'	'	'	'
SUBMAXIL./SUBLINGUAL	'	'	'	'	'	'
TRACHEA	'	'	'	'	'	'
TONGUE	'	'	'	'	'	'
URINARY CONTENTS	'	'	'	'	'	'
OTHER GEN. COMMENTS	'!	'	'	'	'!	'!
EXTREMITIES/HAIR	'!	'!	'!	'!	'!	'!

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day

ANIMAL NUMBER :	Q2 7939 F+1	Q2 7940 F+1	Q2 7941 F+1	Q2 7942 F+1	Q2 7943 F+1	Q2 7944 F+1
GENERAL OBSERVATION	'	'	'	'	'	'
LIVER	-	+	-	-	+G	-
- AREA COAG.HEPAT.NEC.	.	.	.	.	3*	.
- PELIOSIS	.	1.	.	.	.	.
KIDNEYS	-	-	-	-	-	-
ILEUM	'	-G	'	'	'	-G
JEJUNUM	'	-G	'	'	'	-G
DUODENUM	-G	-G	'	'	-G	-G
CAECUM	'	+G	-G	'	'	-G
- MUCOSAL HAEMORRHAGE		2.	.			.
COLON	'	'	'	'	'	+G
- CAP.HAEMORRHAGE						2.
RECTUM	'	'	'	'	'	-G
STOMACH	-G	-G	-G	+G	+G	-G
- EROSION	.	.	.	1*	1*	.
FORESTOMACH	+G	-G	'	+G	+G	+G
- EPITHEL.CEL.HYPERPL.	2.	.		3.	3.	2.
- HYPERKERATOSIS	3.	.		3*	3*	2*
- ULCERATION	3*	.		3.	.	.
- INFL.CEL.INF.,MUCOSA	.	.		.	.	3.
THYMUS	+G	'	'	'	+G	'
- CAPILLARY HAEMORRH.	3.				3.	
- INVOLUTION	2*				2*	
ADRENAL GLANDS	+G	'	+G	'	-G	'
- CORT.CEL.HYPERTROPHY	2*		2*		.	

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day

ANIMAL NUMBER :	Q2 7939 F+1	Q2 7940 F+1	Q2 7941 F+1	Q2 7942 F+1	Q2 7943 F+1	Q2 7944 F+1
SPLEEN	'	'	'	'	+G	'
- LYMPHOID DEPLETION					2*	
URINARY BLADDER	'	'	'	'	'	'
UTERUS	+G	'	'	'	'	'
- ENDOMETRITIS	2.					
SKIN	'	'	'	'	'	'
LUNGS WITH BRONCHI	'	'	'	'	'	'
THYROID GLANDS	'	'	'	'	'	'
PARATHYROID GLANDS	'	'	'	'	'	'
SCIATIC NERVE	'	'	'	'	'	'
SKELETAL MUSCLE	'	'	'	'	'	'
SPINAL CORD	'	'	'	'	'	'
AORTA	'	'	'	'	'	'
BRAIN	'	'	'	'	'	'
EYES	'	'	'	'	'	'
HARDERIAN GLANDS	'	'	'	'	'	'
FEMORAL BONE(+ART.)	'	'	'	'	'	'
STERNUM (+ B.M.)	'	'	'	'	'	'
HEART	'	'	'	'	'	'
MESENT. LYMPH NODES	'	'	'	'	'	'
MANDIBUL. LYMPH NODE	'	'	'	'	'	'

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day

ANIMAL NUMBER :	Q2 7939 F+1	Q2 7940 F+1	Q2 7941 F+1	Q2 7942 F+1	Q2 7943 F+1	Q2 7944 F+1
MAMMARY GLAND(S)	'	'	'	'	'	'
OESOPHAGUS	'	'	'	'	'	'
OVARIES	'	'	'	'	'	'
PANCREAS	'	'	'	'	'	'
PITUITARY GLAND	'	'	'	'	'	'
SUBMAXIL./SUBLINGUAL	'	'	'	'	'	'
TRACHEA	'	'	'	'	'	'
TONGUE	'	'	'	'	'	'
URINARY CONTENTS	'	'	'	'	'	'
VAGINA	'	'	'	'	'	'
OTHER GEN. COMMENTS	'	'	'!	'!	'	'
EXTREMITIES/HAIR	'!	'!	'!	'!	'!	'!

ANIMAL HEADING DATA
DOSE GROUP : 1, 0 mg/kg/day

ANIMAL NUMBER	SEX M/F	DEFINED AND FINAL STATE OF NECROPSY		TEST DAYS	FIRST AND LAST DAY UNDER TEST		DATE OF NECROPSY
Q27891	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27892	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27893	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27894	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27895	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27896	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27921	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27922	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27923	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27924	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27925	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27926	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day MALE

* STATE AT NECROPSY: KO
DAYS ON TEST : 16 * ANIMAL NO. : Q27891

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
-MONONUCLEAR CELL AGGREGATION, GRADE 1
KIDNEYS:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27892

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* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
-FIBROPLASIA PERIPORTAL, GRADE 2
KIDNEYS:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27893

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* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
-MONONUCLEAR CELL AGGREGATION, GRADE 1
-PELIOSIS, GRADE 2
KIDNEYS:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP     :  1, 0 mg/kg/day                                          MALE
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* STATE AT NECROPSY: K0
  DAYS ON TEST      :    16                        * ANIMAL NO. :    Q27894
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* NECROPSY FINDINGS

  TESTES:
    01: SOFT, REDUCED IN SIZE.
  ALL OTHER ORGANS WITHOUT NECROPSY OBSERVATIONS


* MICROSCOPIC FINDINGS

  LIVER:
   -MONONUCLEAR CELL AGGREGATION, GRADE 1
   -TENSION LIPIDOSIS, GRADE 1
  KIDNEYS:
    ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED
  TESTES:
   -ATROPHY OF SEMINIFEROUS TUBULES, GRADE 3
    CORRELATING WITH NECROPSY FINDING(S).


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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP     :  1, 0 mg/kg/day                                          MALE
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* STATE AT NECROPSY: K0
  DAYS ON TEST      :    16                        * ANIMAL NO. :    Q27895
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* NECROPSY FINDINGS

  KIDNEYS:
    01: RIGHT: DILATED PELVIS.
  ALL OTHER ORGANS WITHOUT NECROPSY OBSERVATIONS


* MICROSCOPIC FINDINGS

  LIVER:
   -MONONUCLEAR CELL AGGREGATION, GRADE 1
   -FIBROSIS, INTERLOBULAR, GRADE 2
  KIDNEYS:
   -DILATED PELVIS, GRADE 3
    CORRELATING WITH NECROPSY FINDING(S).


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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27896

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* NECROPSY FINDINGS

KIDNEYS:
01: RIGHT: DILATED PELVIS.
ALL OTHER ORGANS WITHOUT NECROPSY OBSERVATIONS

* MICROSCOPIC FINDINGS

LIVER:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED
KIDNEYS:
-DILATED PELVIS, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27921

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
-TENSION LIPIDOSIS, GRADE 1
KIDNEYS:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day FEMALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27922

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* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
-MONONUCLEAR CELL AGGREGATION, GRADE 1
KIDNEYS:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day FEMALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27923

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* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
-MONONUCLEAR CELL AGGREGATION, GRADE 1
KIDNEYS:
-TUBULAR BASOPHILIA, GRADE 1
-TUBULAR DILATATION, GRADE 1
-PERITUBULAR FIBROSIS, UNILATERAL, GRADE 1

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27924

* NECROPSY FINDINGS

UTERUS (HORNS AND CERVIX):
01: BOTH HORNS: SEROUS CONTENTS.
ALL OTHER ORGANS WITHOUT NECROPSY OBSERVATIONS

* MICROSCOPIC FINDINGS

LIVER:
-MONONUCLEAR CELL AGGREGATION, GRADE 1
KIDNEYS:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED
UTERUS (HORNS AND CERVIX):
-DILATED LUMEN, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day FEMALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27925

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* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
-MONONUCLEAR CELL AGGREGATION, GRADE 1
KIDNEYS:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, 0 mg/kg/day FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27926

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:
-MONONUCLEAR CELL AGGREGATION, GRADE 1
KIDNEYS:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

ANIMAL HEADING DATA
DOSE GROUP : 2, 100 mg/kg/day

ANIMAL NUMBER	SEX M/F	DEFINED AND FINAL STATE OF NECROPSY		TEST DAYS	FIRST AND LAST DAY UNDER TEST		DATE OF NECROPSY
Q27897	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27898	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27899	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27900	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27901	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27902	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27927	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27928	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27929	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27930	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27931	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27932	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27897

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27898

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* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27899

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27900

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* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27901

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27902

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27927

* NECROPSY FINDINGS

UTERUS (HORNS AND CERVIX):
01: RIGHT HORN: SEROUS CONTENTS.

* MICROSCOPIC FINDINGS

UTERUS (HORNS AND CERVIX):
-DILATED LUMEN, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day FEMALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27928

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* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27929

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27930

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day FEMALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27931

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* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 2, 100 mg/kg/day FEMALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27932

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* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

ANIMAL HEADING DATA
DOSE GROUP : 3, 300 mg/kg/day

ANIMAL NUMBER	SEX M/F	DEFINED AND FINAL STATE OF NECROPSY		TEST DAYS	FIRST AND LAST DAY UNDER TEST		DATE OF NECROPSY
Q27903	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27904	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27905	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27906	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27907	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27908	M	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27933	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27934	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27935	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27936	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27937	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27938	F	K0	K0	16	05-DEC-96	20-DEC-96	20-DEC-96

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27903

* NECROPSY FINDINGS

CAECUM:
01: MUCOSA: YELLOWISH COLOR.
FORESTOMACH:
01: MUCOSA: YELLOWISH COLOR.
URINARY BLADDER:
01: YELLOWISH CONTENTS, LIQUID.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

CAECUM:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
STOMACH:
-EROSION, GRADE 2
FORESTOMACH:
-EPITHELIAL CELL HYPERPLASIA, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
-HYPERKERATOSIS, GRADE 1
URINARY BLADDER:
-EOSINOPHILIC MATERIAL IN LUMEN, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
PROBABLY PROTEINACEOUS

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27904

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* NECROPSY FINDINGS

KIDNEYS:
01: LEFT: DILATED PELVIS.
ILEUM:
01: YELLOWISH CONTENTS, LIQUID.
02: MUCOSA: YELLOWISH COLOR.
JEJUNUM:
01: YELLOWISH CONTENTS, LIQUID.
02: MUCOSA: YELLOWISH COLOR.
CAECUM:
01: MUCOSA: YELLOWISH COLOR.
FORESTOMACH:
01: MUCOSA: YELLOWISH COLOR.
URINARY BLADDER:
01: YELLOWISH CONTENTS, LIQUID.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

KIDNEYS:
-DILATED PELVIS, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).
ILEUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
JEJUNUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
CAECUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
STOMACH:
-EROSION, GRADE 2
FORESTOMACH:
-EPITHELIAL CELL HYPERPLASIA, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
-HYPERKERATOSIS, GRADE 1
URINARY BLADDER:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day MALE

CONT./FF. ANIMAL NO. : Q27904

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27905

* NECROPSY FINDINGS

ILEUM:
01: YELLOWISH CONTENTS, LIQUID.
CAECUM:
01: MUCOSA: YELLOWISH COLOR.
COLON:
01: MUCOSA: YELLOWISH COLOR.
FORESTOMACH:
01: MUCOSA: YELLOWISH COLOR.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

ILEUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
CAECUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
COLON:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
FORESTOMACH:
-EPITHELIAL CELL HYPERPLASIA, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
-HYPERKERATOSIS, GRADE 2
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27906

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* NECROPSY FINDINGS

ILEUM:
01: YELLOWISH CONTENTS, THICK.
CAECUM:
01: MUCOSA: YELLOWISH COLOR.
FORESTOMACH:
01: MUCOSA: YELLOWISH COLOR.
URINARY BLADDER:
01: YELLOWISH CONTENTS, LIQUID.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

ILEUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
CAECUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
FORESTOMACH:
-EPITHELIAL CELL HYPERPLASIA, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
-HYPERKERATOSIS, GRADE 2
URINARY BLADDER:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27907

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* NECROPSY FINDINGS

ILEUM:
01: MUCOSA: YELLOWISH COLOR.
02: YELLOWISH CONTENTS, LIQUID.
CAECUM:
01: MUCOSA: YELLOWISH COLOR.
URINARY BLADDER:
01: YELLOWISH CONTENTS, LIQUID.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

ILEUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
CAECUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
URINARY BLADDER:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
NO MICROSCOPIC FINDINGS NOTED.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27908

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* NECROPSY FINDINGS

ILEUM:
01: YELLOWISH CONTENTS, LIQUID.
02: MUCOSA: YELLOWISH COLOR.
CAECUM:
01: MUCOSA: YELLOWISH COLOR.
FORESTOMACH:
01: MUCOSA: YELLOWISH COLOR.
URINARY BLADDER:
01: YELLOWISH CONTENTS, LIQUID.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

ILEUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
CAECUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
FORESTOMACH:
-EPITHELIAL CELL HYPERPLASIA, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).
-HYPERKERATOSIS, GRADE 3
URINARY BLADDER:
-EOSINOPHILIC MATERIAL IN LUMEN, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).
PROBABLY PROTEINACEOUS
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27933

* NECROPSY FINDINGS

ILEUM:
01: MUCOSA: YELLOWISH COLOR.
CAECUM:
01: MUCOSA: YELLOWISH COLOR.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

ILEUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
CAECUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
NO MICROSCOPIC FINDINGS NOTED.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day FEMALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27934

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* NECROPSY FINDINGS

UTERUS (HORNS AND CERVIX):
01: BOTH HORNS: SEROUS CONTENTS.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

UTERUS (HORNS AND CERVIX):
-DILATED LUMEN, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day FEMALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27935

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* NECROPSY FINDINGS

KIDNEYS:
01: RIGHT: DILATED PELVIS.
CAECUM:
01: MUCOSA: YELLOWISH COLOR.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

KIDNEYS:
-DILATED PELVIS, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
CAECUM:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP     :  3, 300 mg/kg/day                                      FEMALE
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* STATE AT NECROPSY: K0
  DAYS ON TEST      :    16                        * ANIMAL NO. :    Q27936
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* NECROPSY FINDINGS

  CAECUM:
    01: MUCOSA: YELLOWISH COLOR.
  UTERUS (HORNS AND CERVIX):
    01: BOTH HORNS: SEROUS CONTENTS.
  EXTREMITIES/HAIR:
    01: YELLOWISH COLOR.


* MICROSCOPIC FINDINGS

  CAECUM:
    ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED
    NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
  UTERUS (HORNS AND CERVIX):
   -DILATED LUMEN, GRADE 2
    CORRELATING WITH NECROPSY FINDING(S).


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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day FEMALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27937

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* NECROPSY FINDINGS

CAECUM:
01: MUCOSA: YELLOWISH COLOR.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

CAECUM:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 3, 300 mg/kg/day FEMALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : Q27938

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* NECROPSY FINDINGS

EXTREMITIES/HAIR:
01: YELLOWISH COLOR.
ALL OTHER ORGANS WITHOUT NECROPSY OBSERVATIONS

* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

ANIMAL HEADING DATA
DOSE GROUP : 4, 1000 mg/kg/day

ANIMAL NUMBER	SEX M/F	DEFINED AND FINAL STATE OF NECROPSY	TEST DAYS	FIRST AND LAST DAY UNDER TEST		DATE OF NECROPSY
Q27909	M	K0 +1	9	05-DEC-96	13-DEC-96	13-DEC-96
Q27910	M	K0 +2	14	05-DEC-96	18-DEC-96	18-DEC-96
Q27911	M	K0 +2	7	05-DEC-96	11-DEC-96	11-DEC-96
Q27912	M	K0 +1	7	05-DEC-96	11-DEC-96	11-DEC-96
Q27913	M	K0 +1	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27914	M	K0 +1	16	05-DEC-96	20-DEC-96	20-DEC-96
Q27939	F	K0 +1	13	05-DEC-96	17-DEC-96	17-DEC-96
Q27940	F	K0 +1	8	05-DEC-96	12-DEC-96	12-DEC-96
Q27941	F	K0 +1	9	05-DEC-96	13-DEC-96	13-DEC-96
Q27942	F	K0 +1	9	05-DEC-96	13-DEC-96	13-DEC-96
Q27943	F	K0 +1	14	05-DEC-96	18-DEC-96	18-DEC-96
Q27944	F	K0 +1	11	05-DEC-96	15-DEC-96	15-DEC-96

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day MALE

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* STATE AT NECROPSY: K0/+1
DAYS ON TEST : 9 * ANIMAL NO. : Q27909

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* NECROPSY FINDINGS

ILEUM:
01: DILATATION, YELLOWISH CONTENTS, LIQUID.
JEJUNUM:
01: DILATATION, YELLOWISH CONTENTS, LIQUID.
DUODENUM:
01: DILATATION, YELLOWISH CONTENTS, LIQUID.
CAECUM:
01: DILATATION, YELLOWISH CONTENTS, LIQUID.
STOMACH:
01: DILATATION, MARKED.
02: YELLOWISH CONTENTS, THICK.
03: MUCOSA: YELLOWISH COLOR.
FORESTOMACH:
01: MUCOSA: YELLOWISH COLOR.
SEMINAL VESICLES:
01: REDUCED IN SIZE.
SPLEEN:
01: REDUCED IN SIZE.
OTHER GENERAL COMMENTS:
01: YELLOWISH ORGANS.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

ILEUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
JEJUNUM:
-MUCOSAL HAEMORRHAGE, GRADE 1
CORRELATING WITH NECROPSY FINDING(S).
DUODENUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
CAECUM:
-MUCOSAL HAEMORRHAGE, GRADE 3
EARLY AUTOLYSIS
CORRELATING WITH NECROPSY FINDING(S).

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP     :  4, 1000 mg/kg/day                                       MALE
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                                          CONT./FF. ANIMAL NO. :    Q27909
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  STOMACH:
    NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
  FORESTOMACH:
    NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
  SEMINAL VESICLES:
    NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
  SPLEEN:
   -LYMPHOID DEPLETION, GRADE 2
    CORRELATING WITH NECROPSY FINDING(S).
  ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.


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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day MALE

* STATE AT NECROPSY: K0/+2
DAYS ON TEST : 14 * ANIMAL NO. : Q27910

* NECROPSY FINDINGS

ILEUM:
01: MUCOSA: YELLOWISH COLOR.
JEJUNUM:
01: MUCOSA: YELLOWISH COLOR.
DUODENUM:
01: DILATATION, YELLOWISH CONTENTS, THICK.
CAECUM:
01: MUCOSA: YELLOWISH COLOR.
COLON:
01: MUCOSA: YELLOWISH COLOR.
STOMACH:
01: DILATATION, MARKED.
02: YELLOWISH CONTENTS, THICK.
SEMINAL VESICLES:
01: REDUCED IN SIZE.
PROSTATE:
01: REDUCED IN SIZE.
ADRENAL GLANDS:
01: ENLARGED.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.
ALL OTHER ORGANS WITHOUT NECROPSY OBSERVATIONS

* MICROSCOPIC FINDINGS

ILEUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
JEJUNUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
DUODENUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
CAECUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
COLON:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day MALE

CONT./FF. ANIMAL NO. : Q27910

STOMACH:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
SEMINAL VESICLES:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
PROSTATE:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
ADRENAL GLANDS:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
NO MICROSCOPIC FINDINGS NOTED.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day MALE

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* STATE AT NECROPSY: K0/+2
DAYS ON TEST : 7 * ANIMAL NO. : Q27911

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* NECROPSY FINDINGS

ILEUM:
01: MUCOSA: YELLOWISH COLOR.
02: YELLOWISH CONTENTS, LIQUID.
JEJUNUM:
01: MUCOSA: YELLOWISH COLOR.
CAECUM:
01: MUCOSA: YELLOWISH COLOR.
02: YELLOWISH CONTENTS, LIQUID.
STOMACH:
01: MUCOSA: YELLOWISH COLOR.
SEMINAL VESICLES:
01: REDUCED IN SIZE.
PROSTATE:
01: REDUCED IN SIZE.
TESTES:
01: CRYPTORCHIDISM.
SPLEEN:
01: REDUCED IN SIZE.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.
ALL OTHER ORGANS WITHOUT NECROPSY OBSERVATIONS

* MICROSCOPIC FINDINGS

ILEUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
JEJUNUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
CAECUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
STOMACH:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
SEMINAL VESICLES:
-HYPOSECRETION, GRADE 2
-EPITHELIUM ATROPHY, GRADE 1
CORRELATING WITH NECROPSY FINDING(S).

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day MALE

CONT./FF. ANIMAL NO. : Q27911

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PROSTATE:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
-HYPOSECRETION, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
-INTERSTITIAL MONONUCLEAR CELL AGGREGATION, GRADE 1
TESTES:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
SPLEEN:
-LYMPHOID DEPLETION, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day MALE

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* STATE AT NECROPSY: K0/+1
DAYS ON TEST : 7 * ANIMAL NO. : Q27912

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* NECROPSY FINDINGS

KIDNEYS:
01: RIGHT: DILATED PELVIS.
ILEUM:
01: MUCOSA: YELLOWISH COLOR.
JEJUNUM:
01: MUCOSA: YELLOWISH COLOR.
CAECUM:
01: MUCOSA: YELLOWISH COLOR.
COLON:
01: MUCOSA: YELLOWISH COLOR.
RECTUM:
01: MUCOSA: YELLOWISH COLOR.
STOMACH:
01: MUCOSA: YELLOWISH COLOR.
SEMINAL VESICLES:
01: REDUCED IN SIZE.
PROSTATE:
01: REDUCED IN SIZE.
THYMUS:
01: REDUCED IN SIZE.
ADRENAL GLANDS:
01: RIGHT: REDUCED IN SIZE.
SPLEEN:
01: REDUCED IN SIZE.
SKIN:
01: DORSAL REGION: ALOPECIA, APPROX 1.5 CM LONG, APPROX 1.5 CM WIDE, (A).
EXTREMITIES/HAIR:
01: YELLOWISH COLOR

* MICROSCOPIC FINDINGS

KIDNEYS:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
ILEUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day MALE

CONT./FF. ANIMAL NO. : Q27912

JEJUNUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
CAECUM:
EARLY AUTOLYSIS
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
COLON:
EARLY AUTOLYSIS
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
RECTUM:
EARLY AUTOLYSIS
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
STOMACH:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
SEMINAL VESICLES:
-HYPOSECRETION, GRADE 3
-EPITHELIUM ATROPHY, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).
PROSTATE:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
-HYPOSECRETION, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
THYMUS:
-CAPILLARY HAEMORRHAGE, GRADE 3
-INVOLUTION, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).
ADRENAL GLANDS:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
SPLEEN:
-LYMPHOID DEPLETION, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).
SKIN:
-ACANTHOSIS, GRADE 1
CORRELATING WITH NECROPSY FINDING(S).
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day MALE

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* STATE AT NECROPSY: K0/+1
DAYS ON TEST : 16 * ANIMAL NO. : Q27913

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* NECROPSY FINDINGS

SEMINAL VESICLES:
01: REDUCED IN SIZE.
PROSTATE:
01: REDUCED IN SIZE.
SPLEEN:
01: REDUCED IN SIZE.
OTHER GENERAL COMMENTS:
01: YELLOWISH ORGANS.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

SEMINAL VESICLES:
-HYPOSECRETION, GRADE 3
-EPITHELIUM ATROPHY, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).
PROSTATE:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
SPLEEN:
-LYMPHOID DEPLETION, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day MALE

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* STATE AT NECROPSY: K0/+1
DAYS ON TEST : 16 * ANIMAL NO. : Q27914

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* NECROPSY FINDINGS

SEMINAL VESICLES:
01: REDUCED IN SIZE.
PROSTATE:
01: REDUCED IN SIZE.
SPLEEN:
01: REDUCED IN SIZE.
OTHER GENERAL COMMENTS:
01: YELLOWISH ORGANS.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

SEMINAL VESICLES:
-HYPOSECRETION, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).
PROSTATE:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
SPLEEN:
-LYMPHOID DEPLETION, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP     :  4, 1000 mg/kg/day                                      FEMALE
--------------------------------------------------------------------------------

* STATE AT NECROPSY: K0/+1
  DAYS ON TEST      :    13                        * ANIMAL NO. :    Q27939
................................................................................

* NECROPSY FINDINGS

  DUODENUM:
    01: DILATATION.
  STOMACH:
    01: MUCOSA: YELLOWISH COLOR.
  FORESTOMACH:
    01: MUCOSA: YELLOWISH COLOR.
    02: MUCOSA: FOCI GREYISH/WHITISH, SEVERAL, UP TO 0.2 CM IN
        DIAMETER, DEPRESSED.
    03: MUCOSA: THINNED.
  THYMUS:
    01: REDUCED IN SIZE.
  ADRENAL GLANDS:
    01: ENLARGED.
  UTERUS (HORNS AND CERVIX):
    01: REDUCED IN SIZE.
  EXTREMITIES/HAIR:
    01: YELLOWISH COLOR.
  ALL OTHER ORGANS WITHOUT NECROPSY OBSERVATIONS


* MICROSCOPIC FINDINGS

  DUODENUM:
    NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
  STOMACH:
    NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
    EARLY AUTOLYSIS
  FORESTOMACH:
   -EPITHELIAL CELL HYPERPLASIA, GRADE 2
   -HYPERKERATOSIS, GRADE 3
   -ULCERATION, GRADE 3
    CORRELATING WITH NECROPSY FINDING(S).
  THYMUS:
   -CAPILLARY HAEMORRHAGE, GRADE 3
   -INVOLUTION, GRADE 2
    CORRELATING WITH NECROPSY FINDING(S).
```

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day FEMALE

CONT./FF. ANIMAL NO. : Q27939

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ADRENAL GLANDS:
-CORTICAL CELL HYPERTROPHY, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
UTERUS (HORNS AND CERVIX):
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
-ENDOMETRITIS, GRADE 2
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP     :  4, 1000 mg/kg/day                                      FEMALE
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* STATE AT NECROPSY: K0/+1
  DAYS ON TEST      :     8                        * ANIMAL NO. :    Q27940
................................................................................

* NECROPSY FINDINGS

  ILEUM:
    01: MUCOSA: YELLOWISH COLOR.
  JEJUNUM:
    01: MUCOSA: YELLOWISH COLOR.
  DUODENUM:
    01: MUCOSA: YELLOWISH COLOR.
  CAECUM:
    01: MUCOSA: YELLOWISH COLOR.
  STOMACH:
    01: DILATATION.
    02: MUCOSA: BLACKISH DEPOSIT.
    03: MUCOSA: YELLOWISH COLOR.
  FORESTOMACH:
    01: DILATATION.
    02: MUCOSA: YELLOWISH COLOR.
  EXTREMITIES/HAIR:
    01: YELLOWISH COLOR.
  ALL OTHER ORGANS WITHOUT NECROPSY OBSERVATIONS


* MICROSCOPIC FINDINGS

  LIVER:
   -PELIOSIS, GRADE 1
  ILEUM:
    NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
  JEJUNUM:
    NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
  DUODENUM:
    NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
  CAECUM:
    NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
    EARLY AUTOLYSIS
   -MUCOSAL HAEMORRHAGE, GRADE 2
  FORESTOMACH:
    NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
```

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day FEMALE

CONT./FF. ANIMAL NO. : Q27940

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day FEMALE

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* STATE AT NECROPSY: K0/+1
DAYS ON TEST : 9 * ANIMAL NO. : Q27941

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* NECROPSY FINDINGS

CAECUM:
01: DILATATION, YELLOWISH CONTENTS, LIQUID.
STOMACH:
01: DILATATION, MARKED.
02: PURPLISH CONTENTS, THICK.
ADRENAL GLANDS:
01: ENLARGED.
OTHER GENERAL COMMENTS:
02: YELLOWISH ORGANS.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

CAECUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
AUTOLYSIS
STOMACH:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
ADRENAL GLANDS:
-CORTICAL CELL HYPERTROPHY, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day FEMALE

* STATE AT NECROPSY: K0/+1
DAYS ON TEST : 9 * ANIMAL NO. : Q27942

* NECROPSY FINDINGS

STOMACH:
01: MUCOSA: YELLOWISH COLOR.
02: MUCOSA: FOCI BROWNISH/BLACKISH, MANY, UP TO 0.3 CM IN DIAMETER.
03: YELLOWISH CONTENTS, THICK.
04: DILATATION, MARKED.
FORESTOMACH:
01: YELLOWISH COLOR.
OTHER GENERAL COMMENTS:
01: YELLOWISH ORGANS.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

STOMACH:
-EROSION, GRADE 1
CORRELATING WITH NECROPSY FINDING(S).
FORESTOMACH:
-EPITHELIAL CELL HYPERPLASIA, GRADE 3
-HYPERKERATOSIS, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).
-ULCERATION, GRADE 3
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day FEMALE

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* STATE AT NECROPSY: K0/+1
DAYS ON TEST : 14 * ANIMAL NO. : Q27943

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* NECROPSY FINDINGS

LIVER:
01: LEFT LATERAL LOBE: DORSAL FACE, FOCI YELLOWISH, SEVERAL, UP TO 0.2 CM IN DIAMETER.
DUODENUM:
01: DILATATION, YELLOWISH CONTENTS, THICK.
STOMACH:
01: DILATATION, MARKED.
02: YELLOWISH CONTENTS, THICK.
FORESTOMACH:
01: MUCOSA: FOCI GREYISH/WHITISH, SEVERAL, UP TO 0.2 CM IN DIAMETER, RAISED.
THYMUS:
01: REDUCED IN SIZE, (A).
ADRENAL GLANDS:
01: ENLARGED.
SPLEEN:
01: REDUCED IN SIZE.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.

* MICROSCOPIC FINDINGS

LIVER:
-AREA OF COAGULATIVE HEPATOCELLULAR NECROSIS, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).
DUODENUM:
EARLY AUTOLYSIS
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
STOMACH:
-EROSION, GRADE 1
CORRELATING WITH NECROPSY FINDING(S).
FORESTOMACH:
-EPITHELIAL CELL HYPERPLASIA, GRADE 3
-HYPERKERATOSIS, GRADE 3
CORRELATING WITH NECROPSY FINDING(S).

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day FEMALE

CONT./FF. ANIMAL NO. : Q27943

THYMUS:
-CAPILLARY HAEMORRHAGE, GRADE 3
-INVOLUTION, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
ADRENAL GLANDS:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
SPLEEN:
-LYMPHOID DEPLETION, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day FEMALE

* STATE AT NECROPSY: K0/+1
DAYS ON TEST : 11 * ANIMAL NO. : Q27944

* NECROPSY FINDINGS

ILEUM:
01: YELLOWISH CONTENTS, LIQUID.
JEJUNUM:
01: YELLOWISH CONTENTS, LIQUID.
DUODENUM:
01: YELLOWISH CONTENTS, LIQUID.
CAECUM:
01: YELLOWISH CONTENTS, LIQUID.
COLON:
01: YELLOWISH CONTENTS, LIQUID.
RECTUM:
01: YELLOWISH CONTENTS, LIQUID.
STOMACH:
01: DILATATION, MARKED.
02: YELLOWISH CONTENTS, THICK.
03: MUCOSA: YELLOWISH COLOR.
FORESTOMACH:
01: MUCOSA: YELLOWISH COLOR.
EXTREMITIES/HAIR:
01: YELLOWISH COLOR.
ALL OTHER ORGANS WITHOUT NECROPSY OBSERVATIONS

* MICROSCOPIC FINDINGS

ILEUM:
EARLY AUTOLYSIS
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
JEJUNUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
DUODENUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
CAECUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
EARLY AUTOLYSIS

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, 1000 mg/kg/day FEMALE

CONT./FF. ANIMAL NO. : Q27944

COLON:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
-CAPILLARY HAEMORRHAGE, GRADE 2
RECTUM:
NO MICROSCOPIC LESION(S) CORRELATING TO NECROPSY FINDING(S)
FORESTOMACH:
-EPITHELIAL CELL HYPERPLASIA, GRADE 2
-HYPERKERATOSIS, GRADE 2
CORRELATING WITH NECROPSY FINDING(S).
-INFLAMMATORY CELL INFILTRATION, MUCOSA, GRADE 3
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

[redacted]

STUDY TITLE

SKIN SENSITIZATION TEST IN GUINEA-PIGS (Maximization method of Magnusson, B. and Kligman, A.M.)

TEST SUBSTANCES

F15812, F15813 and F14529

F14529 contains 70% Basic Yellow 87

[redacted]

STUDY COMPLETION DATE

7 April 1997

[redacted]

CONTENTS

[redacted]

STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the following Principles of Good Laboratory Practice Regulations:

. O.E.C.D. principles of Good Laboratory Practice, Decision Concerning Mutual Acceptance of Data in the Assessment of Chemicals, C(81)30(final) Annex 2. May 12, 1981.

. Décret N° 90-206 du 7 mars 1990 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 9 mars 1990), Ministère de l'Industrie et de l'Aménagement du Territoire.

This study was also performed in compliance with the following Animal Health Regulation:

. Council Directive 86/609/E.E.C. of 24th November 1986 on the harmonization of laws, regulations or administrative provisions relating to the protection of animals used for experimental or other scientific purposes.

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

[redacted]

AUTHENTICATION OF PATHOLOGICAL DATA

Macroscopic and
microscopic examinations

[redacted]

OTHER SCIENTISTS INVOLVED IN THIS STUDY

[redacted]

[redacted]

STATEMENT OF QUALITY ASSURANCE UNIT

1. Specific study inspections

Type of inspections	Dates		
	Inspections	Report to Study Director (*)	Report to Management (*)
Protocol	10 Oct. 96	10 Oct. 96	10 Oct. 96
Report	4 March 97	11 March 97	11 March 97

2. Routine inspections performed on other studies of the same type according to a frequency defined in Q.A.U. procedures

Inspected phase	Dates		
	Inspections	Report to Study Director (*)	Report to Management (*)
Test substance/preparation	6 Sept. 96	9 Sept. 96	9 Sept. 96
Treatment/test substance	2 Aug. 96	5 Aug. 96	5 Aug. 96
Animals/housing	2 Sept. 96	6 Sept. 96	6 Sept. 96

[redacted]

(*) The dates mentioned correspond to the dates of signature of audit reports by Study Director and Management.

[redacted]

SUMMARY

[redacted] the potential of the test substances F15812 (batch No. MIP 3100-12), F15813 (batch No. MIP 2890-110) and F14529 (batch No. MIP 2982) to induce delayed contact hypersensitivity was evaluated in guinea-pigs according to the maximization method of Magnusson and Kligman and to O.E.C.D. (No. 406, 17th July 1992) and E.C. (92/69/E.E.C., B_6, 31st July 1992) guidelines. The study was conducted in compliance with the Principles of Good Laboratory Practice Regulations.

Methods

Seventy guinea-pigs were allocated to four groups: a control group 1 (five males and five females) and three treated groups (ten males and ten females each).

On day 1, intradermal injections of Freund's complete adjuvant mixed with each test substance (treated groups) or the vehicles (control group) were performed in the dorsal region between the shoulders.

On day 7, the same region received a topical application of sodium lauryl sulfate in vaseline (10% w/w) in order to induce local irritation.

On day 8, this same test site was treated by topical application of each test substance (treated groups) or the vehicles (control group) and was covered by an occlusive dressing for 48 hours.

After a rest period of 12 days, all animals of the treated groups were challenged by a topical application of each test substance to the right flank and was covered by an occlusive dressing for 24 hours. The left flank served as control and received the vehicle only.

All animals of the control group were treated, in the same experimental conditions, with the three test substances and the vehicles.

Test substances and vehicles were maintained under an occlusive dressing for 24 hours. Skin reactions were evaluated approximately 24 and 48 hours later.

Test substances concentrations were as follows:

Induction
Group 2
. intradermal injections (day 1): F15812 at 1% (w/w) in sterile isotonic saline solution (0.9% NaCl)
. topical application (day 8): F15812 at 25% (w/w) in sterile isotonic saline solution (0.9% NaCl).

Group 3
. intradermal injections (day 1): F15813 at 1% (w/w) in paraffin oil
. topical application (day 8): F15813 at 10% (w/w) in paraffin oil.

Group 4
. intradermal injections (day 1): F14529 at 1% (w/w) in sterile isotonic saline solution (0.9% NaCl)
. topical application (day 8): F14529 at 25% (w/w) in sterile isotonic saline solution (0.9% NaCl).

Challenge
Group 1
. posterior left flank: vehicle (0.9% NaCl or paraffin oil)
. anterior left flank: F15813 at 10% (w/w) in paraffin oil
. anterior right flank: F15812 at 25% (w/w) in sterile isotonic saline solution (0.9% NaCl)
. posterior right flank: F14529 at 25% (w/w) in sterile isotonic saline solution (0.9% NaCl).

Group 2
. F15812 at 25% (w/w) in sterile isotonic saline solution (0.9% NaCl).

Group 3
. F15813 at 10% (w/w) in paraffin oil.

Group 4
. F14529 at 25% (w/w) in sterile isotonic saline solution (0.9% NaCl).

At the end of the study, animals were killed and cutaneous samples were taken from the challenge application sites from all the animals.

Histological examination was performed on all samples from animals which were challenged with the test substances.

The sensitivity of the guinea-pigs in C.I.T. experimental conditions was checked with a positive sensitizer: 2,4-DINITRO CHLOROBENZENE. During induction period, the test substance was applied at 0.1% (w/w) (day 1) and 1% (w/w) (day 8) concentrations. At cutaneous challenge application, a 0.5% (w/w) concentration was tested on the right flank.

The interpretation of results was carried out according to the classification criteria laid down in Council Directive 93/21/E.E.C. (27th April 1993) adapting to technical progress for the eighteenth time Council Directive 67/548/E.E.C.

Results

No clinical signs and no deaths related to treatment were noted during the study.

A. Control group 1
No cutaneous reactions were observed at the site which received the vehicle.
A colouration of the skin was noted at all application sites which received the three test substances, as follows:

- Test substance F15812
A red colouration of the skin, which could mask a well-defined (1/10 animals at the 24-hour reading, 2/10 animals at the 48-hour reading) or moderate (9/10 animals at the 24-hour reading, 8/10 animals at the 48-hour reading) erythema was observed.

- Test substance F15813
A purple colouration of the skin, which could mask a very slight (3/10 animals at the 24-hour reading, 5/10 animals at the 48-hour reading), well-defined (5/10 animals at the 24-hour reading, 3/10 animals at the 48-hour reading) or moderate (1/10 animals) erythema was noted; a well-defined erythema was also observed in one animal at both readings.

- Test substance F14529
A yellow colouration of the skin, which could mask a well-defined erythema was noted in all animals, 24 and 48 hours after the removal of the pads of the cutaneous challenge application.

B. Treated group 2: test substance F15812
A red colouration of the skin, which could mask a well-defined (14/19 animals at the 24-hour reading, 15/19 animals at the 48-hour reading) or moderate (5/19 animals at the 24-hour reading, 4/19 animals at the 48-hour reading) erythema was observed. Dryness of the skin was also noted at the 48-hour reading, but no oedema was recorded.

C. Treated group 3: test substance F15813
A very slight, well-defined, moderate or severe erythema was observed in 4/20, 7/20, 8/20 and 1/20 animals, respectively, at the 24 and 48-hour readings. A slight oedema was noted in 1/20 animals at the 24-hour reading and in 7/20 animals at the 48-hour reading; a severe oedema was noted in 1/20 animals at the 24-hour reading and in 3/20 animals at the 48-hour reading. Dryness of the skin and crusts were also noted at the 48-hour reading in 10/20 and 2/20 animals, respectively, and a slight purple colouration of the skin was recorded in almost all animals at both readings.

D. Treated group 4: test substance F14529
A yellow colouration of the skin, which could mask a very slight (10/20 animals) or well-defined (10/20 animals) erythema was observed at the 24 and 48-hour readings. No oedema was noted.

The histological examination performed on skin samples of all animals revealed the presence of a sensitization reaction in 100% animals treated with the test substance F15812, in 95% animals treated with the test substance F15813 and in 10% animals treated with the test substance F14529.

Conclusion

Skin colouration did not allow scoring of erythema. Therefore, evaluation of skin sensitization was only possible by microscopic examination of the test sites. Cutaneous reactions attributable to a sensitization potential were observed in 100% animals treated with the test substance F15812 (batch No. MIP 3100-12), in 95% animals treated with the test substance F15813 (batch No. MIP 2890-110) and in 10% animals treated with the test substance F14529 (batch No. MIP 2982).

1. INTRODUCTION

The objective of this study, performed according to the maximization method of Magnusson and Kligman (1), was to evaluate the potential of the test substances F15812, F15813 and F14529 to induce delayed contact hypersensitivity in guinea-pigs.

The results of the study are of value in predicting the contact sensitization potential of the test material in Man.

The study was conducted in compliance with:
. O.E.C.D. guideline No. 406, 17th July 1992.
. E.C. Directive No. 92/69/E.E.C., B_6, 31st July 1992.

2. MATERIALS AND METHODS

2.1. TEST AND CONTROL SUBSTANCES

2.1.1 Test substances

The test substances F15812, F15813 and F14529 used in the study was supplied by [redacted]

2.1.1.1 First test substance

Documentation supplied by the Sponsor identified the test substance as follows:
. name:
  - protocol and labelling: F15812
. batch number:
  - protocol and labelling: MIP 3100-12
. description: dark purple powder
. container: one glass flask
. date of receipt: 30 September 1996
. storage conditions: at room temperature.

At the finalization of the study report, an analytical certificate was not available.
Characterization of the test substance, which appropriately define the tested batch, is under responsibility of the Sponsor.

(1) Magnusson, B.; Kligman, A.M.: The identification of contact allergens by animal assay. The guinea-pig maximization test. J. Invest. Derm. 52: 268-276 (1969).

2.1.1.2 Second test substance

Documentation supplied by the Sponsor identified the test substance as follows:
. name:
  - protocol and labelling: F15813
. batch number:
  - protocol and labelling: MIP 2890-110
. description: blackish powder
. container: one glass flask
. date of receipt: 27 September 1996
. storage conditions: at room temperature.

At the finalization of the study report, an analytical certificate was not available.
Characterization of the test substance, which appropriately define the tested batch, is under responsibility of the Sponsor.

2.1.1.3 Third test substance

Documentation supplied by the Sponsor identified the test substance as follows:
. name:
  - protocol and labelling: F14529
. batch number:
  - protocol and labelling: MIP 2982
. description: yellow powder
. container: one glass flask
. date of receipt: 30 September 1996
. storage conditions: at room temperature.

At the finalization of the study report, an analytical certificate was not available.
Characterization of the test substance, which appropriately define the tested batch, is under responsibility of the Sponsor.

2.1.2 Vehicles

The choice of the vehicles was based on tests to check visually the homogeneity of the preparation (for topical and intradermal injections) and its free passage through a needle (for intradermal injections). The highest concentration which satisfied these criteria was called the maximal practicable concentration.
The vehicle used were sterile isotonic saline solution (0.9% NaCl), batch No. LD 5002 (Laboratoire Fresenius, 92316 Sèvres, France) and paraffin oil, batch No. 8101 (Coopérative Pharmaceutique Française, 77000 Melun, France).

2.1.3 Preparation

Each test substance was prepared at appropriate concentrations (see § 2.3.3).
The test substance of group 3 (F15813) was finely pulverised before being incorporated in the vehicle.
All preparations were made freshly on the morning of administration and any unused material was discarded that same day.

2.1.4 Other substances

The other substances used were Freund's complete adjuvant, batch Nos. 084H8800 and 026H8900 (Sigma, 38297 Saint-Quentin-Fallavier, France); sodium lauryl sulfate, batch No. 115H0759 (Sigma, 38297 Saint-Quentin-Fallavier, France) and vaseline, batch No. 0036 (Coopérative Pharmaceutique Française, 77000 Melun, France).

2.2. TEST SYSTEM

2.2.1 Animals

Species and strain: Dunkin-Hartley guinea-pigs.
Reason for this choice: species recommended by the international regulations for sensitization studies. The strain used has been shown to produce a satisfactory sensitization response using known positive sensitizers.
Breeder: Centre d'Elevage Lebeau, 78950 Gambais, France.
Number: 70 animals (35 males and 35 nulliparous and non-pregnant females).
Allocation of the animals to the groups: on day -1, the animals were weighed and randomly allocated to four groups: a control group 1 consisting of ten animals (five males and five females) and three treated groups consisting of 20 animals (ten males and ten females each).
Weight: on day 1, the animals were approximately three months old and had a mean body weight ± standard deviation of 311 ± 16 g for the males and 342 ± 14 g for the females.
Acclimatization: at least five days before the beginning of the study.
Identification of the animals: ear-tattoo.

2.2.2 Environmental conditions

During the acclimatization period and throughout the study, the conditions in the animal room were set as follows:
. temperature: 21 ± 2°C
. relative humidity: 30 to 70%
. light/dark cycle: 12 h/12 h
. ventilation: about 12 cycles/hour of filtered, non-recycled air.
The temperature and relative humidity were recorded continuously and records retained.
The housing conditions (temperature, relative humidity and ventilation) were checked monthly.
During the acclimatization period and throughout the study, the animals were housed individually in polycarbonate cages (48 cm x 27 cm x 20 cm) equipped with a polypropylene bottle.
Dust-free sawdust was provided as litter (SICSA, 92142 Alfortville, France).

Bacteriological analysis of the sawdust and detection of possible contaminants (pesticides, heavy metals) are performed periodically.

2.2.3 Food and water

During the study, the animals had free access to "106 pelleted diet" (U.A.R., 91360 Villemoisson-sur-Orge, France).
Each batch of food was analysed (composition and contaminants) by the supplier.
The diet formula is presented in appendix 1.

Drinking water filtered by a F.G. Millipore membrane (0.22 micron) was provided *ad libitum*.
Bacteriological and chemical analysis of the water and diet and detection of possible contaminants (pesticides, heavy metals and nitrosamines) are performed periodically.
Results are archived at C.I.T.

It was verified that no contaminants in the diet or water at levels likely to influence the outcome of the study were present.

2.3. TREATMENT

2.3.1 Preliminary test

A preliminary test was conducted in order to determine the concentrations to be tested in the main study.

By intradermal route:
. 24 hours before treatment, the dorsal region of the animals was clipped,
. each test substance was prepared in an appropriate vehicle,
. intradermal administrations of the test substance (0.1 ml) at different concentrations were performed in the dorsal region between the shoulders,
. cutaneous reactions were evaluated approximately 24, 48 hours and six days after injection.

By cutaneous route:
. 24 hours before treatment, both flank regions of the animals were clipped,
. each test substance was prepared in an appropriate vehicle,
. each test substance (0.5 ml for each concentration) was prepared on a dry gauze pad of approximately 4 cm^2 which was held in place by an occlusive dressing for 24 hours,
. cutaneous reactions were evaluated approximately 24 and 48 hours after removal of the dressings.

Criteria for selection of concentrations

The following criteria were used:
. the concentrations should be well-tolerated systemically and locally,
. intradermal injections should cause moderate irritant effect (no necrosis or ulceration of the skin),
. topical application for the induction should cause at most weak or moderate skin reactions or be the maximal practicable concentration,
. topical application for the challenge should be the highest concentration which does not cause irritant effect.

2.3.2 Main study

2.3.2.1 Preparation of the animals

For all animals and before each treatment, the application sites were:
. clipped on days -1 and 7 (scapular area 4 cm x 2 cm),
. clipped and shaved on day 21 (each flank 2 cm x 2 cm),
. shaved again on day 23 before the 24-hour reading,
. clipped again on day 25 (each flank 2 cm x 2 cm).

2.3.3 Induction phase by intradermal and cutaneous routes

2.3.3.1 Intradermal route

On day 1, six injections were made deep into the dermis of a clipped area (4 cm x 2 cm) in the dorsal region between the shoulders, using a needle (diameter: 0.50 x 16 mm, Térumo: C.M.L., 77140 Nemours, France) mounted on a 1 ml glass syringe (0.01 ml graduations, Record: Carrieri, 75005 Paris, France).

Three injections of 0.1 ml were made into each side of this shoulder region, as follows:

	Injection sites*	
Control group 1		
	Anterior	1: FCA diluted at 50% (v/v) with 0.9% NaCl or paraffin oil
	Middle	2: 0.9% NaCl or paraffin oil
	Posterior	3: mixture of 50/50 (w/v) of 1 and 2
Treated group 2		
F15812	Anterior	1: FCA diluted at 50% (v/v) with 0.9% NaCl
	Middle	2: test substance at 1% (w/w) in NaCl 0.9%
	Posterior	3: mixture of 50/50 (w/v) of 1 and 2
Treated group 3		
F15813	Anterior	1: FCA diluted at 50% (v/v) with 0.9% NaCl
	Middle	2: test substance at 1% (w/w) in paraffin oil
	Posterior	3: mixture of 50/50 (w/v) of 1 and 2
Treated group 4		
F14529	Anterior	1: FCA diluted at 50% (v/v) with 0.9% NaCl
	Middle	2: test substance at 1% (w/w) in NaCl
	Posterior	3: mixture of 50/50 (w/v) of 1 and 2

* : three pairs of sites
FCA: Freund's complete adjuvant

2.3.3.2 Cutaneous route

On day 7, the scapular area was clipped. As the test substance was not strongly irritant during the preliminary tests, the animals were treated with 0.5 ml of sodium lauryl sulfate (10% w/w) in vaseline in order to induce local irritation.

On day 8, a topical application to the region of the intradermal injections (4 cm x 2 cm) was performed.

Control group 1
. application of 0.5 ml of the vehicle (0.9% NaCl or paraffin oil).

Treated groups 2, 3 and 4
. application of 0.5 ml of each test substance at the chosen concentration.

Each test substance and vehicle were prepared on a dry gauze pad (Coopérative Pharmaceutique Française, 77000 Melun, France), which was then applied to the dorsal region between the shoulders and held in place for 48 hours by means of an adhesive hypoallergenic dressing (Laboratoires de Pansements et d'Hygiène, 21300 Chenove, France) and an adhesive anallergenic waterproof plaster (Laboratoire des Professions Médicales, 92240 Malakoff, France).

On removal of the dressing, if present, any residual test substance was removed by means of a moistened gauze pad.

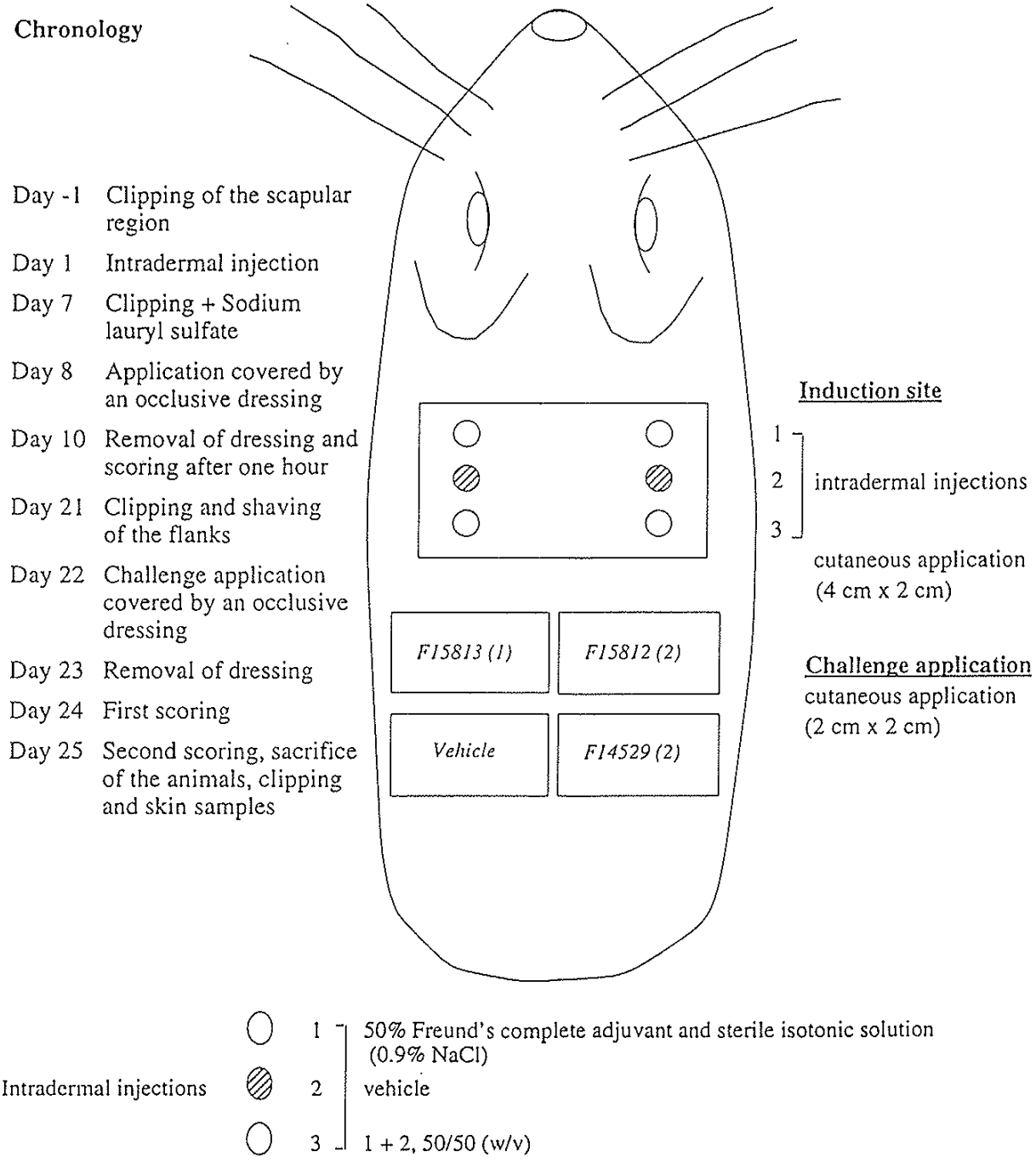
Cutaneous reactions were recorded one hour after removal of the occlusive dressing.

2.3.3.3 Challenge phase

On day 22, the animals from treated groups 2, 3 and 4 received an application of 0.5 ml of each test substance at the chosen concentration on the posterior right flank, and 0.5 ml of the vehicle corresponding on the posterior left flank. The animals of control group received, under the same experimental conditions, at the chosen concentration the test substance F15813 on the anterior left flank, the test substance F15812 on the anterior right flank, the test substance F14529 on the posterior right flank and the vehicle (0.9% NaCl for males and paraffin oil for females) on the posterior left flank. This application was performed using a 1 ml plastic syringe (0.01 ml graduations, Térumo: C.M.L., 77140 Nemours, France). Each test substance and vehicle were prepared on a dry gauze pad (Coopérative Pharmaceutique Française, 77000 Melun, France), then applied to a 4 cm^2 (2 cm x 2 cm) clipped area of the skin. The gauze pad was held in contact with the skin for 24 hours by means of an occlusive, hypoallergenic dressing (Laboratoires de Pansements et d'Hygiène, 21300 Chenove, France) and an adhesive anallergenic waterproof plaster (Laboratoire des Professions Médicales, 92240 Malakoff, France).
On removal of the dressing, if present, any residual test substance was removed by means of a moistened gauze pad.

2.4. SUMMARY DIAGRAMS

Figure 1: control group



(1) at a concentration of 10% (w/w)

(2) at a concentration of 25% (w/w)

Figure 2: treated group 2

Chronology

Day -1 Clipping of the scapular region

Day 1 Intradermal injection

Day 7 Clipping + Sodium lauryl sulfate

Day 8 Application covered by an occlusive dressing

Day 10 Removal of dressing and scoring after one hour

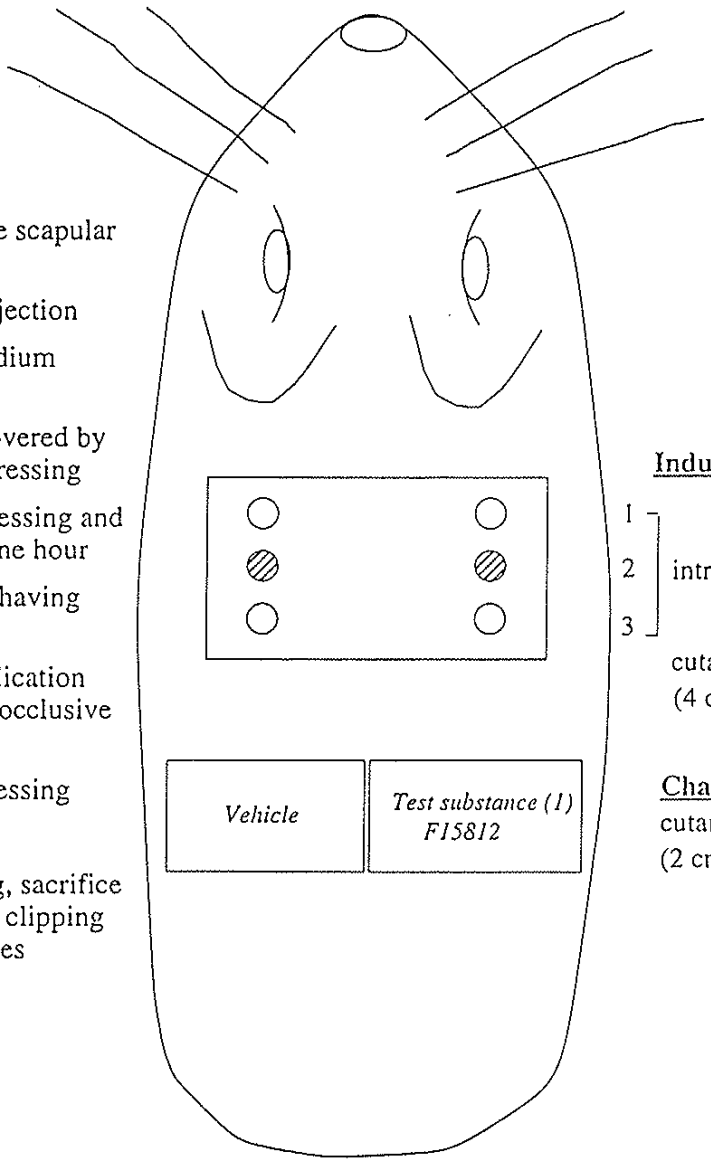
Day 21 Clipping and shaving of the flanks

Day 22 Challenge application covered by an occlusive dressing

Day 23 Removal of dressing

Day 24 First scoring

Day 25 Second scoring, sacrifice of the animals, clipping and skin samples



Intradermal injections

1 — 50% Freund's complete adjuvant and sterile isotonic solution (0.9% NaCl)

2 — test substance at the chosen concentration

3 — 1 + 2, 50/50 (w/v)

(1) at a concentration of 25% (w/w)

Figure 3: treated group 3

Chronology

Day -1 Clipping of the scapular region

Day 1 Intradermal injection

Day 7 Clipping + Sodium lauryl sulfate

Day 8 Application covered by an occlusive dressing

Day 10 Removal of dressing and scoring after one hour

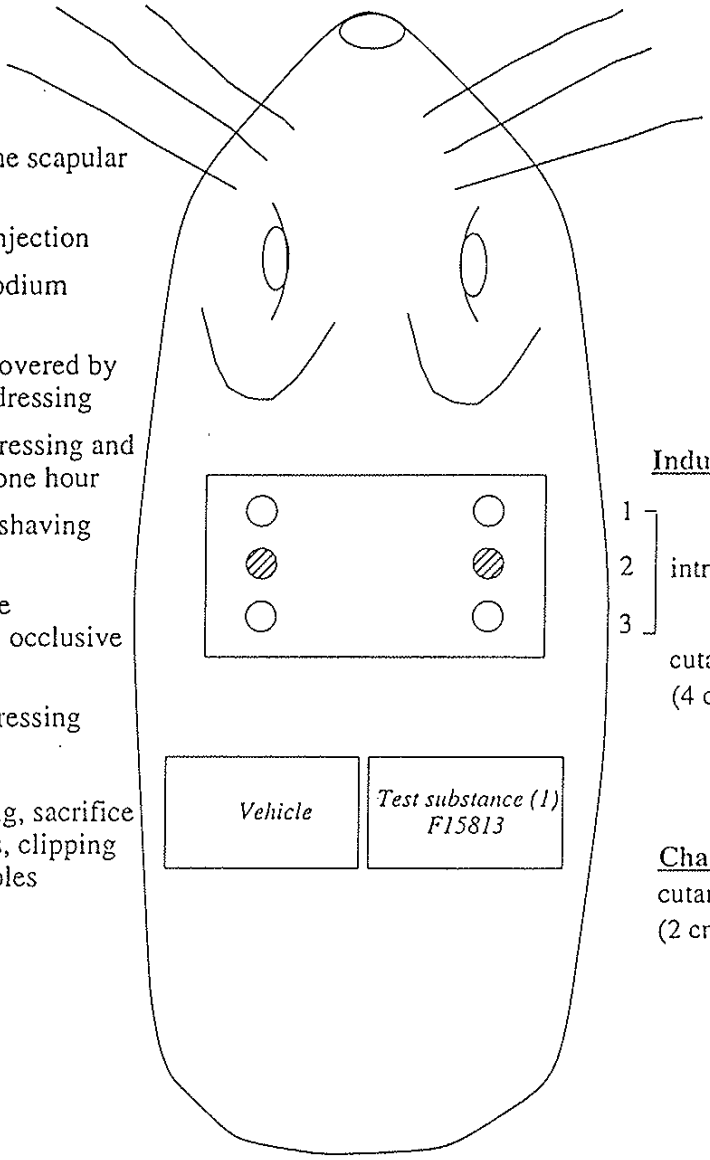
Day 21 Clipping and shaving of the flanks

Day 22 First challenge covered by an occlusive dressing

Day 23 Removal of dressing

Day 24 First scoring

Day 25 Second scoring, sacrifice of the animals, clipping and skin samples



Intradermal injections

○ 1 50% Freund's complete adjuvant and sterile isotonic solution (0.9% NaCl)

▨ 2 test substance at the chosen concentration

○ 3 1 + 2, 50/50 (w/v)

(1) at a concentration of 10% (w/w)

Figure 4: treated group 4

Chronology

Day -1 Clipping of the scapular region

Day 1 Intradermal injection

Day 7 Clipping + Sodium lauryl sulfate

Day 8 Application covered by an occlusive dressing

Day 10 Removal of dressing and scoring after one hour

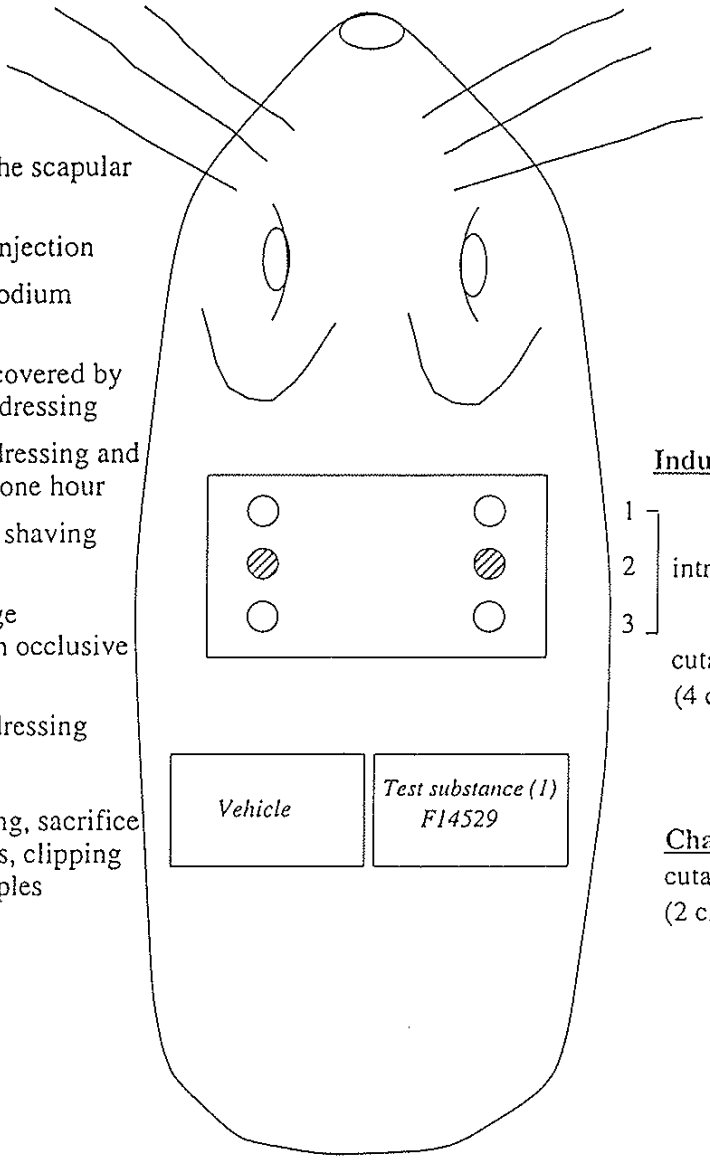
Day 21 Clipping and shaving of the flanks

Day 22 First challenge covered by an occlusive dressing

Day 23 Removal of dressing

Day 24 First scoring

Day 25 Second scoring, sacrifice of the animals, clipping and skin samples



Intradermal injections

1 — 50% Freund's complete adjuvant and sterile isotonic solution (0.9% NaCl)

2 — test substance at the chosen concentration

3 — 1 + 2, 50/50 (w/v)

(1) at a concentration of 25% (w/w)

2.5. SCORING OF CUTANEOUS REACTIONS

Twenty-four and 48 hours after the challenge application, both flanks of the treated and control animals were observed in order to evaluate cutaneous reactions, according to the following scale:

Erythema and eschar formation

. No erythema	0
. Very slight erythema (barely perceptible)	1
. Well-defined erythema	2
. Moderate to severe erythema	3
. Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema formation

. No oedema	0
. Very slight oedema (barely perceptible)	1
. Slight oedema (visible swelling with well-defined edges)	2
. Moderate oedema (visible swelling raised more than 1 millimetre)	3
. Severe oedema (visible swelling raised more than 1 millimetre and extending beyond the area of exposure)	4

Any other lesions were noted.

2.6. CLINICAL EXAMINATIONS

The animals were observed twice a day during the study in order to check for clinical signs and mortality.

2.7. BODY WEIGHT

The animals were weighed individually on the day of allocation into the groups, on the first day of the study (day 1), on days 8 and 15 and on the last day of the study (day 25).

2.8. PATHOLOGY

2.8.1 Necropsy

Macroscopic examination of the main organs was performed on the animal found dead during the study.

At the end of the study, all the surviving animals were killed by CO_2 inhalation in excess. No necropsy was performed.

2.8.2 Cutaneous samples

At the end of the study, skin samples were taken from the posterior left and right flanks of all the surviving animals. The samples were preserved in 10% buffered formalin.

2.8.3 Microscopic examination

Tissues taken for microscopic examination were embedded in paraplast, sectioned at approximately 4 microns in thickness and stained with hemalum-eosin.
After agreement with the Study Monitor, histological examination was performed on all cutaneous samples from animals which were challenged with the test substances.

2.9. DETERMINATION OF THE ALLERGENICITY LEVEL

The treated animals show a positive reaction if macroscopic cutaneous reactions are clearly visible (erythema and/or oedema ≥ 2) and if the treated animals have a greater intensity or duration of response than the maximum reaction seen in control animals, or, if macroscopic reactions are confirmed at microscopic examination as being due to the sensitization process. Sensitization reactions are characterized at microscopic examination by basal spongiosis, reactional acanthosis of the epidermis and infiltration of mononucleated cells into the dermis (1).

Determination of the allergenicity level
The allergenicity level of the test substance is calculated by comparing the number of animals showing positive reactions with the number of surviving treated animals at the end of the study.

% of animals showing a reaction	Allergenicity level	Classification
0 - 8	I	very weak
9 - 28	II	weak
29 - 64	III	moderate
65 - 80	IV	strong
81 - 100	V	very strong

According to the Commission Directive 93/21/E.E.C., when the reactions are positive in at least 30% of the treated animals, the test substance has sensitization properties and the sentence "R 43: May cause sensitization by skin contact" must be applied.

(1) Duprat, P. ; Delsaut. L. ; Gradiski, D. ; Lepage, M. : Investigations histo-pathologiques et cytologiques lors de la mise en évidence, chez le cobaye, d'une allergie cutanée de type retardé. Revue Méd. Vét. 127: 7, 1083-1101 (1976).

[redacted]

2.10. CHRONOLOGY OF THE STUDY

The chronology of the study is summarized as follows:

Procedure	Date	Day
Arrival of the animals	17 October 1996	-5
Weighing and allocation of the animals into groups	21 October 1996	-1
Weighing, induction by intradermal injection	22 October 1996	1
Laurylsulfate application	28 October 1996	7
Weighing, induction by cutaneous route	29 October 1996	8
Removal of occlusive dressings and scoring of local reactions after one hour	31 October 1996	10
Weighing	5 November 1996	15
Challenge cutaneous application	12 November 1996	22
Removal of occlusive dressings	13 November 1996	23
Scoring of cutaneous reactions after		
. 24 hours	14 November 1996	24
. 48 hours	15 November 1996	25
Weighing, sacrifice of the animals and skin samples	15 November 1996	25

2.11. ARCHIVES

The study documentation and materials, namely:
. protocol and possible amendments,
. raw data,
. correspondence,
. final report and possible amendments,
. histological specimens:
  - tissues in preservative
  - blocks and slides

[redacted]

3. RESULTS

3.1. PRELIMINARY STUDY

3.1.1 Administration by intradermal route

Test substance: F15812

The maximal concentration which could pass through a needle was 1% (w/w). One test was performed in order to determine if this concentration was well-tolerated.

Animal number	Concentration of the test substance % (w/w)	Scoring after treatment 24 hours	48 hours	6 days
male 02	1	Cr*	Cr*	A/Cr*
female 02	1	Cr*	Cr*	A/Cr*

Cr* : red colouration which could mask an eventual irritation
A : crusts

Concentration chosen for the main study was 1% (w/w).

Test substance: F15813

The maximal concentration which could pass through a needle was 1% (w/w). One test was performed in order to determine if this concentration was well-tolerated.

Animal number	Concentration of the test substance % (w/w)	Scoring after treatment 24 hours	48 hours	6 days
male 03	1	Cv*	Cv*	Cv*
female 03	1	Cv*	Cv*	Cv*

Cr* : purple colouration which could mask an eventual irritation

Concentration chosen for the main study was 1% (w/w).

Test substance: F14529
Several tests were performed in order to determine the concentration to be used in the main study.

Animal number	Concentration of the test substance % (w/w)	Scoring after treatment 24 hours	48 hours	6 days
male 01	10	N/Cj*	N/Cj*	N/Cj*
	5	N/Cj*	N/Cj*	N/Cj*
	1	I/Cj*	I/Cj*	I/Cj*
female 01	10	-	-	-
	5	-	-	-
	1	-	-	-

Cj* : yellow colouration
I : irritation
N : necrosis
- : dead animal

Concentration chosen for the main study was 1% (w/w).

3.1.2 Application by cutaneous route

Test substance: F15812
The maximal practicable concentration was 25% (w/w). Several tests were performed in order to determine the concentrations to be used in the main study.

Animal number	Concentration of the test substance % (w/w)		Scoring after removal of the dressing (1) 24 hours E	O	48 hours E	O
First assay						
male 02	25	RF	C3r	0	C3r	0
		LF	C2r	0	C2r	0
female 02	25	RF	C3r	0	C3r	0
		LF	C2r	0	C2r	0
Second assay						
male 04	10	RF	C3r	0	C3r	0
	5	LF	C2r	0	C2r	0
female 04	10	RF	C2r	0	C2r	0
	5	LF	C1r	0	C1r	0

E : erythema
O : oedema
RF : right flank
LF : left flank
(1) : Residual test substance was wiped off with a moistened gauze pad
C1r : red colouration which could mask a very slight erythema
C2r : red colouration which could mask a well-defined erythema
C3r : red colouration which could mask a moderate to severe erythema

Shaving of the test site was performed at the 24-hour reading in order to try to avoid masking effect by the test substance colouration.

Concentration chosen for the topical application of the induction phase (day 8) and for the challenge application was 25% (w/w).

Test substance: F15813
The maximal practicable concentration was 10% (w/w). Several tests were performed in order to determine the concentrations to be used in the main study.

Animal number	Concentration of the test substance % (w/w)		Scoring after removal of the dressing (1) 24 hours E	O	48 hours E	O
First assay						
male 03	10	RF	C1v	0	C1v	0
	10	LF	C2v	0	C1v	0
female 03	10	RF	C1v	0	C1v	0
	10	LF	C2v	0	C1v	0
Second assay						
male 05	10	RF	C1v	0	C1v	0
	5	LF	C1v	0	C1v	0
female 05	10	RF	C1v	0	C1v	0
	5	LF	C1v	0	C1v	0

E : erythema
O : oedema
RF : right flank
LF : left flank
(1) : Residual test substance was wiped off with a moistened gauze pad
C1v : purple colouration which could mask a very slight erythema
C2v : purple colouration which could mask a well-defined erythema

Shaving of the test site was performed at the 24-hour reading in order to try to avoid masking effect by the test substance colouration.

Concentration chosen for the topical application of the induction phase (day 8) and for the challenge application was 10% (w/w).

Test substance: F14529

The maximal practicable concentration was 25% (w/w). Several tests were performed in order to determine the concentrations to be used in the main study.

Animal number	Concentration of the test substance % (w/w)		Scoring after removal of the dressing (1) 24 hours E	O	48 hours E	O
First assay						
male 01	25	RF	C2j	0	C1j	0
	25	LF	C2j	0	C1j	0
female 01	25	RF	-	-	-	-
	25	LF	-	-	-	-
Second assay						
male 06	10	RF	C1j	0	C1j	0
	5	LF	C2j	0	C1j	0
female 06	10	RF	C3j	0	C3j	0
	5	LF	C2j	0	C1j	0

E : erythema
O : oedema
RF : right flank
LF : left flank
(1) : Residual test substance was wiped off with a moistened gauze pad
C1j : yellow colouration which could mask a very slight erythema
C2j : yellow colouration which could mask a well-defined erythema
C3j : yellow colouration which could mask a moderate to severe erythema
- : dead animal

Shaving of the test site was performed the day at the 24-hour reading in order to try to avoid masking effect by the test substance colouration.

3.2. MAIN STUDY

3.2.1 Clinical examinations

No clinical signs were observed during the study, but the female No. 113 (group 2, treated with F15812) was found dead on day 21. No clinical signs were noted prior to death. As such spontaneous mortality is sometimes observed in this species, and as no macroscopic changes were observed at necropsy, this death was not attributed to treatment.

The body weight gain of the treated animals was normal when compared to that of the control animals (figures 5 and 6, appendix 2).

3.2.2 Scoring of cutaneous reactions

3.2.2.1 End of the induction period

On day 10, after topical application of the induction period, signs of irritation were observed at the test site (dorsal region between shoulders) in the control group; red, purple and yellow colouration of the skin were observed at the test site (dorsal region between shoulders) in the treated groups 2, 3 and 4, respectively.

3.2.2.2 Challenge application

Residual test substance was removed by means of a gauze pad moistened with water.
Scoring of the skin reactions was as follows:

Sex	Animal number	Control group							
		24 hours							
		Erythema				Oedema			
		FAG	FPG	FAD	FPD	FAG	FPG	FAD	FPD
Male	81	C1v	0	C3r	C2j	0	0	0	0
	82	C1v	0	C2r	C2j	0	0	0	0
	83	2/Cv	0	C3r	C2j	0	0	0	0
	84	C2v	0	C3r	C2j	0	0	0	0
	85	C2v	0	C3r	C2j	0	0	0	0
Female	106	C2v	0	C3r	C2j	0	0	0	0
	107	C2v	0	C3r	C2j	0	0	0	0
	108	C1v	0	C3r	C2j	0	0	0	0
	109	C2v	0	C3r	C2j	0	0	0	0
	110	C3v	0	C3r	C2j	0	0	0	0
		48 hours							
		Erythema				Oedema			
		FAG	FPG	FAD	FPD	FAG	FPG	FAD	FPD
Male	81	C1v	0	C3r	C2j	0	0	0	0
	82	C1v	0	C2r	C2j	0	0	0	0
	83	2/Cv	0	C3r	C2j	0	0	0	0
	84	C1v	0	C3r	C2j	0	0	0	0
	85	C2v	0	C3r	C2j	0	0	0	0
Female	106	C1v	0	C2r	C2j	0	0	0	0
	107	C2v	0	C3r	C2j	0	0	0	0
	108	C1v	0	C3r	C2j	0	0	0	0
	109	C2v	0	C3r	C2j	0	0	0	0
	110	C3v	0	C3r	C2j	0	0	0	0

FAG : left anterior flank (F15813 at a concentration of 10%)
FPG : left posterior flank (0.9% NaCl for males and paraffin oil for females)
FAD : right anterior flank (F15812 at a concentration of 25%)
FPD : right posterior flank (F14529 at a concentration of 25%)
Cv : purple colouration of the skin
C1v : purple colouration of the skin which could mask a very slight erythema
C2v : purple colouration of the skin which could mask a well-defined erythema
C3v : purple colouration of the skin which could mask a moderate to severe erythema
C2r : red colouration of the skin which could mask a well-defined erythema
C3r : red colouration of the skin which could mask a moderate to severe erythema
C2j : yellow colouration of the skin which could mask a well-defined erythema

Sex	Animal number	Treated group 2							
		24 hours				48 hours			
		Erythema		Oedema		Erythema		Oedema	
		FG	FD	FG	FD	FG	FD	FG	FD
Male	86	0	C3r	0	0	0	C3r/S	0	0
	87	0	C3r	0	0	0	C3r/S	0	0
	88	0	C2r	0	0	0	C2r	0	0
	89	0	C2r	0	0	0	C2r	0	0
	90	0	C2r	0	0	0	C2r	0	0
	91	0	C2r	0	0	0	C2r	0	0
	92	0	C2r	0	0	0	C2r	0	0
	93	0	C2r	0	0	0	C2r	0	0
	94	0	C2r	0	0	0	C2r/S	0	0
	95	0	C2r	0	0	0	C2r/S	0	0
Female	111	0	C2r	0	0	0	C2r	0	0
	112	0	C2r	0	0	0	C2r	0	0
	113	-	-	-	-	-	-	-	-
	114	0	C3r	0	0	0	C2r/S	0	0
	115	0	C3r	0	0	0	C3r/S	0	0
	116	0	C2r	0	0	0	C2r	0	0
	117	0	C3r	0	0	0	C3r/S	0	0
	118	0	C2r	0	0	0	C2r/S	0	0
	119	0	C2r	0	0	0	C2r	0	0
	120	0	C2r	0	0	0	C2r	0	0

FG : left flank (0.9% NaCl)
FD : right flank (F15812 at a concentration of 25%)
S : dryness of the skin
C2r : red colouration of the skin which could mask a well-defined erythema
C3r : red colouration of the skin which could mask a moderate to severe erythema
- : dead animal

Sex	Animal number	Treated group 3							
		24 hours				48 hours			
		Erythema		Oedema		Erythema		Oedema	
		FG	FD	FG	FD	FG	FD	FG	FD
Male	96	0	1/Cv	0	0	0	1/Cv	0	0
	97	0	2/Cv	0	0	0	2/Cv/S	0	0
	98	0	1/Cv	0	0	0	2/Cv/S	0	0
	99	0	1/Cv	0	0	0	1/Cv	0	0
	100	0	3/Cv	0	0	0	3/Cv/S	0	2
	101	0	2/Cv	0	0	0	2/Cv/S	0	0
	102	0	3/Cv	0	0	0	3/Cv/S	0	2
	103	0	2/Cv	0	0	0	2/Cv/S	0	0
	104	0	2/Cv	0	0	0	1/Cv	0	0
	105	0	2/Cv	0	0	0	2/Cv	0	0
Female	121	0	3/Cv	0	0	0	3/Cv/S	0	4
	122	0	2/Cv	0	0	0	2/Cv	0	2
	123	0	3/Cv	0	0	0	3/Cv	0	2
	124	0	3/Cv	0	0	0	3/Cv	0	2
	125	0	3/Cv	0	2	0	3/Cv/A	0	4
	126	0	3/Cv	0	0	0	3/Cv	0	2
	127	0	1/Cv	0	0	0	1/Cv	0	0
	128	0	3/Cv	0	0	0	3/Cv/S	0	2
	129	0	2/Cv	0	0	0	2/Cv/S	0	0
	130	0	4	0	4	0	4/A/S	0	4

FG : left flank (paraffin oil)
FD : right flank (F15813 at a concentration of 10%)
S : dryness of the skin
A : crusts
Cv : purple colouration of the skin

Sex	Animal number	Treated group 4							
		24 hours				48 hours			
		Erythema		Oedema		Erythema		Oedema	
		FG	FD	FG	FD	FG	FD	FG	FD
Male	131	0	C1j	0	0	0	C1j	0	0
	132	0	C1j	0	0	0	C1j	0	0
	133	0	C2j	0	0	0	C2j	0	0
	134	0	C1j	0	0	0	C1j	0	0
	135	0	C1j	0	0	0	C1j	0	0
	136	0	C2j	0	0	0	C2j	0	0
	137	0	C1j	0	0	0	C1j	0	0
	138	0	C2j	0	0	0	C2j	0	0
	139	0	C2j	0	0	0	C2j	0	0
	140	0	C1j	0	0	0	C1j	0	0
Female	141	0	C2j	0	0	0	C2j	0	0
	142	0	C1j	0	0	0	C1j	0	0
	143	0	C1j	0	0	0	C1j	0	0
	144	0	C1j	0	0	0	C1j	0	0
	145	0	C2j	0	0	0	C2j	0	0
	146	0	C1j	0	0	0	C1j	0	0
	147	0	C2j	0	0	0	C2j	0	0
	148	0	C2j	0	0	0	C2j	0	0
	149	0	C2j	0	0	0	C2j	0	0
	150	0	C2j	0	0	0	C2j	0	0

FG : left flank (0.9% NaCl)
FD : right flank (F14529 at a concentration of 25%)
C1j : yellow colouration of the skin which could mask a very slight erythema
C2j : yellow colouration of the skin which could mask a well-defined erythema

Control group 1
No cutaneous reactions were observed at the site which received the vehicle.
A colouration of the skin was noted at all application sites which received the three test substances.

Test substance F15812
A red colouration of the skin, which could mask a well-defined (grade 2 - 1/10 animals at the 24-hour reading, 2/10 animals at the 48-hour reading) or moderate (grade 3 - 9/10 animals at the 24-hour reading, 8/10 animals at the 48-hour reading) erythema was observed.

Test substance F15813
A purple colouration of the skin, which could mask a very slight (grade 1 - 3/10 animals at the 24-hour reading, 5/10 animals at the 48-hour reading), well-defined (grade 2 - 5/20 animals at the 24-hour reading, 3/10 animals at the 48-hour reading) or moderate (grade 3 - 1/10 animals) erythema was noted; a well-defined erythema (grade 2) was also observed in one animal at both readings.

Test substance F14529
A yellow colouration of the skin, which could mask a well-defined erythema (grade 2) was noted in all animals, 24 and 48 hours after the removal of the pads of the cutaneous challenge application.

Treated group 2: test substance F15812
A red colouration of the skin, which could mask a well-defined (grade 2 - 14/19 animals at the 24-hour reading, 15/19 animals at the 48-hour reading) or moderate (grade 3 - 5/19 animals at the 24-hour reading, 4/19 animals at the 48-hour reading) erythema was observed. Dryness of the skin was also noted at the 48-hour reading, but no oedema was recorded.

Treated group 3: test substance F15813
A very slight (grade 1), well-defined (grade 2), moderate (grade 3) or severe erythema (grade 4) was observed in 4/20, 7/20, 8/20 and 1/20 animals, respectively, at the 24 and 48-hour readings. A slight oedema (grade 2) was noted in 1/20 animals at the 24-hour reading and in 7/20 animals at the 48-hour reading; a severe oedema (grade 4) was noted in 1/20 animals at the 24-hour reading and in 3/20 animals at the 48-hour reading. Dryness of the skin and crusts were also noted at the 48-hour reading in 10/20 and 2/20 animals, respectively, and a slight purple colouration of the skin was recorded in almost all animals at both readings.

Treated group 4: test substance F14529
A yellow colouration of the skin, which could mask a very slight (grade 1 - 10/20 animals) or well-defined (grade 2 - 10/20 animals) erythema was observed at the 24 and 48-hour readings. No oedema was noted.

3.2.3 Pathology (appendix 4)

3.2.3.1 Necropsy
Macroscopic examination of the main organs of the animal (No. 113) found dead during the study revealed no abnormalities.

[redacted]

3.2.3.2 Microscopic examination

Control group:

Animals challenged with F15812:

Minimal or slight (1/5 males and 1/5 females) acanthosis was observed on the challenged skin of 4/5 males and all the females. This was sometimes associated with minimal or slight hyperkeratosis and with minimal exocytosis and oedema of the epidermis for 1/5 females and with minimal inflammatory cell infiltration of the upper dermis in another female. Moderate chronic cellulitis of the lower dermis was noted for 1/5 females (No. 107).

Taking into account the nature (chronic) of the lesion, this was considered to be of spontaneous nature.

Animals challenged with F15813:

Minimal or slight (2/5 males and 3/5 females) acanthosis was observed on the challenged skin of 4/5 males and all the females. This was sometimes associated with minimal hyperkeratosis and with minimal exocytosis and oedema of the epidermis and minimal inflammatory cell infiltration of the upper dermis for 1/5 females. Ectasia of the blood vessels in the dermis was noted for 2/5 females together with extravasation of red blood cells in the dermis for one of those two females.

Animals challenged with F14529:

Minimal or slight (1/5 females) acanthosis was observed on the challenged skin of 3/5 males and 3/5 females. This was sometimes associated with minimal hyperkeratosis and with minimal exocytosis and oedema of the epidermis in 1/5 females. Minimal inflammatory cell infiltration of the upper dermis was noted in 1/5 males without any other associated histopathological finding.

The histopathological findings noted on the skin challlenged with F15812, F15813 and F14529 were considered to be due at least in part to the irritative effect of shaving and possibly to a minimal irritative effect of the test substances.

Treated groups:

Taking into consideration that the one experimental difference between treated and control animals was the induction by the test subtances, the histopathological results of the treated animals were compared with those of the controls. The treated animals bearing histopathological findings of higher incidence and/or severity than the most affected controls were considered to be sensitized by the test substance.

Induction with F15812:

The histopathological findings found in each animal given F15812 were compared with control female No. 107.

All the treated animals showed higher severity of acanthosis (moderate or marked), except for female No. 111 (slight) together with minimal or slight hyperkeratosis and associated with minimal, slight or moderate inflammatory cell infiltration of the upper dermis for 9/10 males and all the females, exocytosis of inflammatory cells in the epidermis and/or oedema of the epidermis in 9/10 males and 7/9 females. In addition, extravasation of red blood cells and/or ectasia of the blood vessels were noted in the upper dermis of some animals.

Taking into account the higher incidence and/or severity of hyperkeratosis, acanthosis, inflammatory cell infiltration of the upper dermis in the treated animals and their association with exocytosis and/or oedema of the epidermis in almost all the males and females, each of the treated males or females was considered to be sensitized by the test substance F15812.

Induction by F15813:
The histopathological findings found in each animal given F15813 were compared with control female No. 110.
Consequently, male No. 104 showing minimal hyperkeratosis and slight acanthosis was considered to be negative for hypersensitization.
Although acanthosis and hyperkeratosis were similar to those of control female No. 110, due to the presence of slight inflammatory cell infiltration in the upper dermis of the challenged skin, female No. 127 was considered to be sensitized with the test substance.

All the other males and females were considered to be sensitized with the test substance F15813 as they showed moderate or marked acanthosis together with minimal, slight, moderate or marked hyperkeratosis and minimal, slight, moderate or marked inflammatory cell infiltration of the upper dermis. Hyperkeratosis contained sometimes nuclear debris in some animals; this was considered to be due to exocytosis of inflammatory cells through the epidermis. Minimal or slight exocytosis of inflammatory cells and oedema of the epidermis were also found in 6/9 males and 9/10 females, sometimes together with formation of spongiotic bullae containing granulocytes. Ectasia of the blood vessels of the dermis and/or extravasation of red blood cells were noted in some animals.

Induction with F14529:
The histopathological findings found in each animal given F14529 were compared with control female No. 110.
Consequently male Nos. 131, 136, 137, 138 and 140 were considered to be negative for sensitization. In addition, as minimal inflammatory cell infiltration of the upper dermis can be found in controls (male No. 85), the treated males Nos. 134, 135 and 139 were also considered to be negative for hypersensitization. On the contrary, the two remaining males Nos. 132 and 133 which showed slight acanthosis and slight hyperkeratosis together with minimal inflammatory cell infiltration of the upper dermis, were considered to be sensitized.

Taking into account that minimal inflammatory cell infiltration can be observed in some controls (male No. 85) the histopathological findings noted for female No. 146 were considered to be negative for hypersensitization as minimal hyperkeratosis, slight acanthosis, minimal exocytosis of inflammatory cells in the epidermis were similar to those findings found in control female No. 110. In addition, minimal extravasation of red blood cells can be found occasionally in control animals.
As all the other females showed histopathological findings of lower incidence and/or severity than those found in control female No. 110, all the females given F14529 were considered to be negative for hypersensitization.

Due to the low severity of the histopathological findings observed in the males given F14529, the sensitization potential of the test substance F14529 must be considered to be very weak.

4. CONCLUSION

Skin colouration did not allow scoring of erythema. Therefore, evaluation of skin sensitization was only possible by microscopic examination of the test sites. Cutaneous reactions attributable to a sensitization potential were observed in 100% animals treated with the test substance F15812 (batch No. MIP 3100-12), in 95% animals treated with the test substance F15813 (batch No. MIP 2890-110) and in 10% animals treated with the test substance F14529 (batch No. MIP 2982).

Figure 5: Male body weight (g)

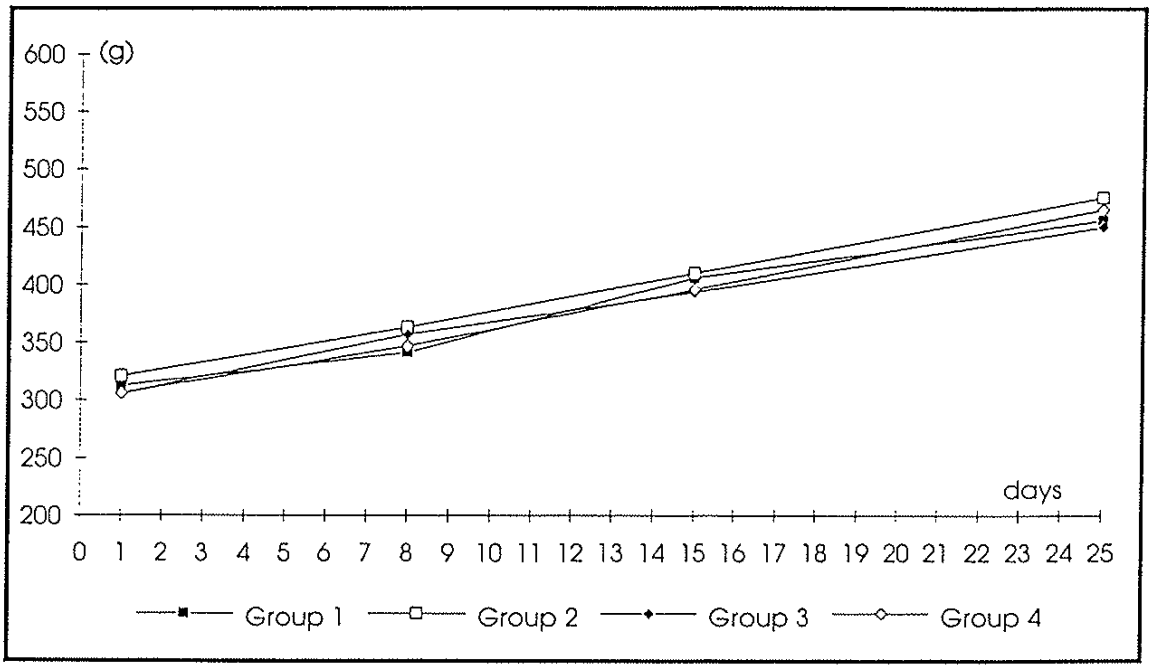
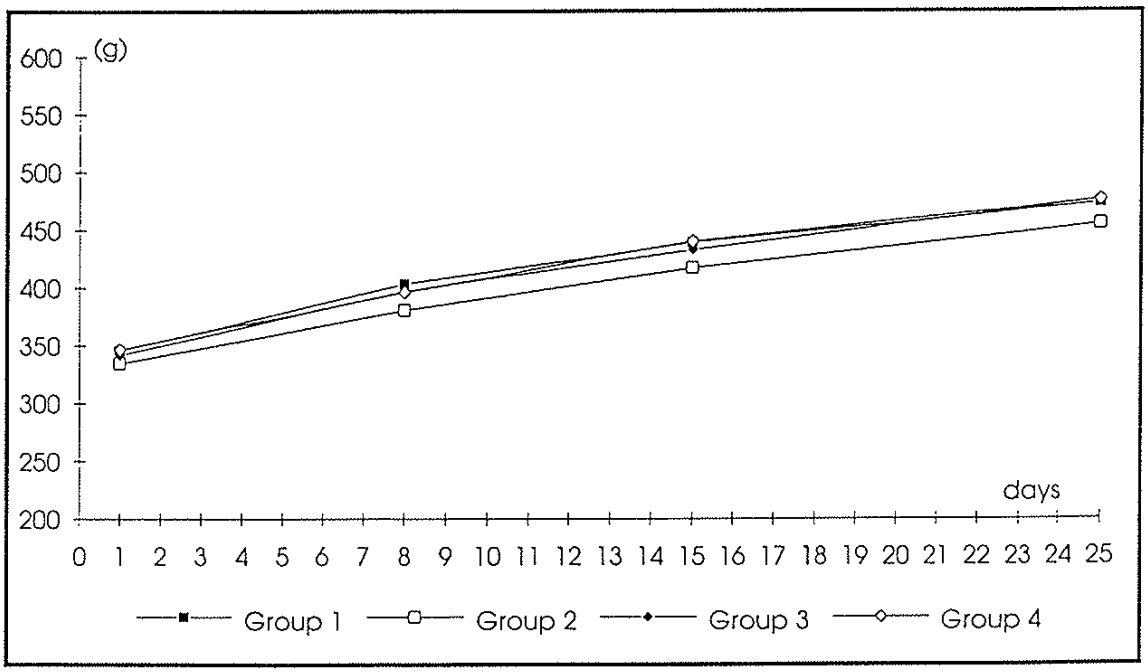


Figure 6: Female body weight (g)



APPENDICES

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1. Diet formula

Ref: 106

COMPLETE DIET

GUINEA-PIG MAINTENANCE DIET

Appearance: 4.5 mm diameter granules

Conditioning: bags of 25 kgs

Daily portion: Guinea-pigs 35-50 g, water *ad libitum*.

FORMULA %

Cereals	42
Grain biproducts and legumes	46
Vegetable protein (soya bean meal, yeast)	9
Vitamin and mineral mixture	3

AVERAGE ANALYSIS %

Calorific value (KCal/kg)	2600
Moisture	10
Proteins	17
Lipids	3
Carbohydrates (N.F.E.)	49
Fibre	13
Minerals (ash)	8

AMINO ACID VALUES
(calculated in mg/kg)

Arginine	8500
Cystine	2500
Lysine	7200
Methionine	2100
Tryptophan	2000
Glycine	6000

FATTY ACID VALUES
(calculated in mg/kg)

Palmitic acid	3600
Palmitoleic acid	0
Stearic acid	700
Oleic acid	5900
Linoleic acid	11200
Linolenic acid	3000

MINERALS (calculated in mg/kg)

	Nat. val.	CMV val.	Total
P	7400	1400	8800
Ca	5400	5600	11000
K	12000	0	12000
Na	1300	1950	3250
Mg	3270	130	3400
Mn	60	40	100
Fe	170	150	320
Cu	10	15	25
Zn	40	45	85
Co	0.1	1.5	1.6
I	0	0	0
Cl	0	0	0

VITAMINS (calculated per kg)

	Nat. val.	CMV val.	Total
Vitamin A	3500 IU	7500 IU	11000 IU
Vitamin D3	30 IU	2000 IU	2030 IU
Vitamin B1	6 mg	6.4 mg	12.4 mg
Vitamin B2	5 mg	6.4 mg	11.4 mg
Vitamin B3	22 mg	26 mg	48 mg
Vitamin B6	0.7 mg	2.7 mg	3.4 mg
Vitamin B12	0.003 mg	0.012 mg	0.015 mg
Vitamin C	0 mg	400 mg	400 mg
Vitamin E	15 mg	60 mg	75 mg
Vitamin K3	5 mg	12.6 mg	17.6 mg
Vitamin PP	97 mg	14.5 mg	111.5 mg
Folic acid	2.2 mg	1.3 mg	3.5 mg
P.A.B. acid	0 mg	2.5 mg	2.5 mg
Biotin	0.02 mg	0.06 mg	0.08 mg
Choline	1010 mg	60 mg	1070 mg
Meso-Inositol	0 mg	62.5 mg	62.5 mg

This food is supplemented with stabilized coated vitamin C, avoiding the need of other food substances (greenery, ascorbic acid) if used within 4 months of date of manufacture.

U.A.R., 7 rue Galliéni, 91360 Villemoisson - Tel: 69.04.03.57 - Fax : 69.04.81.97
(Ref. Doc. UAR: 1992)

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2. Individual body weight values

INDIVIDUAL BODY WEIGHT VALUES
(g)

Groups	Sex	Animals	Days							
			-1	1	(1)	8	(1)	15	(1)	25
4	Male	131	324	318	46	364	41	405	15	420
		132	318	319	53	372	40	412	73	485
		133	285	295	36	331	45	376	69	445
		134	286	289	35	324	46	370	77	447
		135	326	336	10	346	70	416	117	533
		136	301	305	15	320	60	380	65	445
		137	297	287	35	322	56	378	57	435
		138	298	306	40	346	25	371	34	405
		139	292	305	71	376	40	416	105	521
		140	300	299	66	365	69	434	79	513
		M	303	306	41	347	49	396	69	465
		SD	15	15	20	22	14	23	30	45
	Female	141	352	356	56	412	32	444	47	491
		142	356	360	67	427	55	482	47	529
		143	353	357	48	405	47	452	20	472
		144	354	365	50	415	41	456	39	495
		145	306	312	48	360	30	390	21	411
		146	338	345	53	398	40	438	32	470
		147	370	369	44	413	75	488	62	550
		148	331	339	51	390	56	446	31	477
		149	345	342	37	379	39	418	27	445
		150	333	327	29	356	27	383	29	412
		M	344	347	48	396	44	440	36	475
		SD	18	18	10	24	15	35	13	45

(1) = Body weight gain
M = Mean
SD = Standard Deviation

[redacted]

3. Positive control to check the sensitivity of Dunkin-Hartley guinea-pigs

Purpose: check the sensitivity of Dunkin-Hartley Guinea-pigs (Centre d'Elevage Lebeau) to a positive control test article

Method	:	Magnusson and Kligman	
Test substance	:	2,4-DINITRO CHLOROBENZENE	
C.I.T. Study - Date	:	(CIT/Study No. 14420 TSG) - June 1996	
Number of animals	:	ten females	
Induction	:	0.1% 1%	intradermal route day 1 cutaneous route day 8
Challenge application:		0.5% paraffin oil	right flank left flank

Conclusion

Under our experimental conditions and according to the Magnusson and Kligman method, the test substance 2,4-DINITRO CHLOROBENZENE at a concentration of 0.5% (w/w) induced positive skin sensitization reactions in 50% of the guinea-pigs.

INDIVIDUAL REACTIONS: CHALLENGE PHASE
MACROSCOPIC FINDINGS

Group	Sex	Animals	24-hour				48-hour				72-hour				Conclusion	
			Erythema		Oedema		Erythema		Oedema		Erythema		Oedema			
			LF	RF	LF	RF	LF	RF	LF	RF	LF	RF	LF	RF		
Treated	Female	96	0	0/S	0	0	0	1/S	0	0	0	1/S	0	0	-	-
		97	0	1/S/A	0	0	0	1/S/A	0	0	0	1/S/A	0	0	-	+
		98	0	1/S/A	0	0	0	2/S/A	0	0	0	1/S/A	0	0	-	+
		99	0	0/S	0	0	0	0/S	0	0	0	0/S	0	0	-	-
		100	0	1/S	0	2	0	1/S/A	0	0	0	1/S/A	0	0	-	+
		101	0	1	0	2	0	1/S	0	0	0	0/S	0	0	-	+
		102	0	1/S	0	0	0	1/S	0	0	0	0/S	0	0	-	-
		103	0	1	0	0	0	0/S	0	0	0	0/S	0	0	-	-
		104	0	2	0	0	0	1/S	0	0	0	0/S	0	0	-	+
		105	0	1/S	0	0	0	1/S	0	0	0	0/S	0	0	-	-

- : negative
+ : hypersensitizing reactions
S : dryness of the skin
A : crusts
LF: left flank
RF: right flank

4. Individual macroscopic and microscopic examinations

[redacted]

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:

!	= GROSS OBSERVAT.NOT CHECKED OFF HISTOLOGICALLY
'	= HISTOLOGIC EXAMINATION NOT REQUIRED
+	= ORGAN EXAMINED, FINDINGS PRESENT
-	= ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

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
GRADE 4	= MARKED / MANY / LARGE
*	= COMMENT IN TEXT OF INDIVIDUAL ANIMAL DATA

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 1, CONTROL

ANIMAL NUMBER :	81 MK0	82 MK0	83 MK0	84 MK0	85 MK0	106 FK0	107 FK0	108 FK0	109 FK0	110 FK0
GENERAL OBSERVATION	'!	'!	'!	'!	'!	'!	'!	'!	'!	'!
SKIN F15812	-	+	+	+	+	+	+	+	+	+
- HYPERKERATOSIS	.	.	1.	.	1.	1.	2.	1.	.	.
- ACANTHOSIS	.	1.	1.	1.	2.	1.	2.	1.	1.	1.
- EXOCYTOSIS,EPIDERMIS	.	.	.	.	.	1.	.	.	.	.
- OEDEMA,EPIDERMIS	.	.	.	.	.	1.	.	.	.	.
- INFL.CEL.INF.,UP.DE.	.	.	.	.	.	.	1.	.	.	.
- CHRONIC CELLULITIS	.	.	.	.	.	.	3.	.	.	.
SKIN F15813	+	+	+	-	+	+	+	+	+	+
- HYPERKERATOSIS	.	1.	.	.	1.	1.	1.	1.	1.	1.
- ACANTHOSIS	1.	2.	1.	.	2.	1.	2.	2.	1.	2.
- EXOCYTOSIS,EPIDERMIS	.	.	.	.	.	.	.	.	.	1.
- OEDEMA,EPIDERMIS	.	.	.	.	.	.	.	.	.	1.
- INFL.CEL.INF.,UP.DE.	.	.	.	.	.	.	.	.	.	1.
- VASCULA.ECTAS.,DERM.	.	.	.	.	.	.	1.	1.	.	.
- RED BLO.CEL.EXTR.,D.	.	.	.	.	.	.	1.	.	.	.
SKIN F14529	+	+	+	-	+	-	+	-	+	+
- HYPERKERATOSIS	1.	.	1.	.	.	.	.	.	1.	1.
- ACANTHOSIS	1.	1.	1.	.	.	.	1.	.	1.	2.
- EXOCYTOSIS,EPIDERMIS	.	.	.	.	.	.	.	.	.	1.
- OEDEMA,EPIDERMIS	.	.	.	.	.	.	.	.	.	1.
- INFL.CEL.INF.,UP.DE.	.	.	.	.	1.	.	.	.	.	.

[redacted]

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529

ANIMAL NUMBER :	131 MK0	132 MK0	133 MK0	134 MK0	135 MK0	136 MK0	137 MK0	138 MK0	139 MK0	140 MK0
GENERAL OBSERVATION	'!	'!	'!	'!	'!	'!	'!	'!	'!	'!
SKIN F15812	'	'	'	'	'	'	'	'	'	'
SKIN F15813	'	'	'	'	'	'	'	'	'	'
SKIN F14529	-	+	+	+	+	+	+	-	+	+
- HYPERKERATOSIS	.	2.	2.	1.	1.	.	1.	.	1.	1.
- ACANTHOSIS	.	2.	2.	1.	2.	1.	2.	.	2.	2.
- EXOCYTOSIS,EPIDERMIS	.	.	.	.	.	.	.	.	1.	.
- OEDEMA,EPIDERMIS	.	.	.	.	.	.	.	.	1.	1.
- INFL.CEL.INF.,UP.DE.	.	1.	1.	1.	1.	.	.	.	1.	.

[redacted]

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529

ANIMAL NUMBER :	141 FK0	142 FK0	143 FK0	144 FK0	145 FK0	146 FK0	147 FK0	148 FK0	149 FK0	150 FK0
GENERAL OBSERVATION	'!	'!	'!	'!	'!	'!	'!	'!	'!	'!
SKIN F15812	'	'	'	'	'	'	'	'	'	'
SKIN F15813	'	'	'	'	'	'	'	'	'	'
SKIN F14529	+	+	+	+	+	+	+	+	+	+
- HYPERKERATOSIS	.	1.	.	.	1.	1.	1.	1.	1.	1.
- ACANTHOSIS	1.	1.	1.	1.	1.	2.	2.	1.	1.	2.
- EXOCYTOSIS,EPIDERMIS	.	1.	.	1.	.	1.	.	.	.	.
- OEDEMA,EPIDERMIS	.	1.	.	1*	.	.	.	.	.	.
- INFL.CEL.INF.,UP.DE.	.	.	.	.	.	1.	.	.	.	.
- VASCULA.ECTAS.,DERM.	.	.	.	.	.	.	1.	1.	.	.
- RED BLO.CEL.EXTR.,D.	.	.	.	.	.	1.	.	.	.	.

[redacted]

ANIMAL HEADING DATA
DOSE GROUP : 1, CONTROL

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
81	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
82	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
83	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
84	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
85	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
106	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
107	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
108	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
109	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
110	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, CONTROL MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 81

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F15812:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED
SKIN F15813:
-ACANTHOSIS, FOCAL, GRADE 1
SKIN F14529:
-HYPERKERATOSIS, FOCAL, GRADE 1
-ACANTHOSIS, FOCAL, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, CONTROL MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 82

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F15812:
-ACANTHOSIS, MULTIFOCAL, GRADE 1
SKIN F15813:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, DIFFUSE, GRADE 2
SKIN F14529:
-ACANTHOSIS, FOCAL, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, CONTROL MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 83

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F15812:
-HYPERKERATOSIS, FOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 1
SKIN F15813:
-ACANTHOSIS, MULTIFOCAL, GRADE 1
SKIN F14529:
-HYPERKERATOSIS, FOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, CONTROL MALE

---

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 84

.....

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F15812:
-ACANTHOSIS, MULTIFOCAL, GRADE 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, CONTROL MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 85

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F15812:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, DIFFUSE, GRADE 2
SKIN F15813:
-HYPERKERATOSIS, DIFFUSE, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 2
SKIN F14529:
-INFLAMMATORY CELL INFILTRATION, UPPER DERMIS, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, CONTROL FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 106

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F15812:
-HYPERKERATOSIS, FOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 1
-EXOCYTOSIS, EPIDERMIS, FOCAL, GRADE 1
-OEDEMA, EPIDERMIS, FOCAL, GRADE 1
SKIN F15813:
-HYPERKERATOSIS, FOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 1
SKIN F14529:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 1, CONTROL FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 107

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F15812:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 2
-ACANTHOSIS, MULTIFOCAL, GRADE 2
-INFLAMMATORY CELL INFILTRATION, UPPER DERMIS, GRADE 1
-CHRONIC CELLULITIS, GRADE 3
SKIN F15813:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, DIFFUSE, GRADE 2
-VASCULAR ECTASIA, UPPER DERMIS, GRADE 1
-RED BLOOD CELL EXTRAVASATION, UPPER DERMIS, GRADE 1
SKIN F14529:
-ACANTHOSIS, FOCAL, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, CONTROL FEMALE

---

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 108

.....

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F15812:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 1
SKIN F15813:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 2
-VASCULAR ECTASIA, UPPER DERMIS, FOCAL, GRADE 1
SKIN F14529:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, CONTROL FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 109

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F15812:
-ACANTHOSIS, MULTIFOCAL, GRADE 1
SKIN F15813:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 1
SKIN F14529:
-HYPERKERATOSIS, FOCAL, GRADE 1
-ACANTHOSIS, FOCAL, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 1, CONTROL FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 110

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F15812:
-ACANTHOSIS, MULTIFOCAL, GRADE 1
SKIN F15813:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, DIFFUSE, GRADE 2
-EXOCYTOSIS, EPIDERMIS, FOCAL, GRADE 1
-OEDEMA, EPIDERMIS, FOCAL, GRADE 1
-INFLAMMATORY CELL INFILTRATION, UPPER DERMIS, GRADE 1
SKIN F14529:
-HYPERKERATOSIS, FOCAL, GRADE 1
-ACANTHOSIS, FOCAL, GRADE 2
-EXOCYTOSIS, EPIDERMIS, FOCAL, GRADE 1
-OEDEMA, EPIDERMIS, GRADE 1

[redacted]

ANIMAL HEADING DATA
DOSE GROUP : 4, F14529

ANIMAL NUMBER	SEX M/F	DEFINED AND FINAL STATE OF NECROPSY		TEST DAYS	FIRST AND LAST DAY UNDER TEST		DATE OF NECROPSY
131	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
132	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
133	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
134	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
135	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
136	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
137	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
138	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
139	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
140	M	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
141	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
142	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
143	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
144	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
145	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
146	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
147	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
148	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
149	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96
150	F	K0	K0	25	22-OCT-96	15-NOV-96	15-NOV-96

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 131

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 MALE

---

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 132

.....

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 2
-ACANTHOSIS, MULTIFOCAL, GRADE 2
-INFLAMMATORY CELL INFILTRATION, UPPER DERMIS, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 133

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 2
-ACANTHOSIS, DIFFUSE, GRADE 2
-INFLAMMATORY CELL INFILTRATION, UPPER DERMIS, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 134

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* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 1
-INFLAMMATORY CELL INFILTRATION, UPPER DERMIS, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 135

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, DIFFUSE, GRADE 2
-INFLAMMATORY CELL INFILTRATION, UPPER DERMIS, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 136

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-ACANTHOSIS, FOCAL, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 137

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* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, FOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 2

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 MALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 138

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
ORGAN EXAMINED, NO PATHOLOGIC FINDINGS NOTED

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 139

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* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 2
-EXOCYTOSIS, EPIDERMIS, MULTIFOCAL, GRADE 1
-OEDEMA, EPIDERMIS, MULTIFOCAL, GRADE 1
-INFLAMMATORY CELL INFILTRATION, UPPER DERMIS, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 MALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 140

.....

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 2
-OEDEMA, EPIDERMIS, FOCAL, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 141

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-ACANTHOSIS, FOCAL, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 142

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, FOCAL, GRADE 1
-ACANTHOSIS, FOCAL, GRADE 1
-EXOCYTOSIS, EPIDERMIS, FOCAL, GRADE 1
-OEDEMA, EPIDERMIS, FOCAL, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 143

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-ACANTHOSIS, FOCAL, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 4, F14529 FEMALE

---

* STATE AT NECROPSY: K0

DAYS ON TEST : 25 * ANIMAL NO. : 144

.....

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-ACANTHOSIS, MULTIFOCAL, GRADE 1
-EXOCYTOSIS, EPIDERMIS, BIFOCAL, GRADE 1
-OEDEMA, EPIDERMIS, GRADE 1
BIFOCAL

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 FEMALE

---

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 145

.....

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 146

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, FOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 2
-EXOCYTOSIS, EPIDERMIS, FOCAL, GRADE 1
-INFLAMMATORY CELL INFILTRATION, UPPER DERMIS, GRADE 1
-RED BLOOD CELL EXTRAVASATION, DERMIS, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 147

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 2
-VASCULAR ECTASIA, DERMIS, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 148

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, FOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 1
-VASCULAR ECTASIA, DERMIS, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 149

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, MULTIFOCAL, GRADE 1
-ACANTHOSIS, MULTIFOCAL, GRADE 1

[redacted]

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 4, F14529 FEMALE

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* STATE AT NECROPSY: K0
DAYS ON TEST : 25 * ANIMAL NO. : 150

.....

* NECROPSY FINDINGS

GENERAL OBSERVATION:
01: SEE CUTANEOUS REACTIONS.
NO OTHER NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SKIN F14529:
-HYPERKERATOSIS, DIFFUSE, GRADE 1
-ACANTHOSIS, DIFFUSE, GRADE 2

FINAL REPORT

Study Title

SKIN IRRITATION TEST (SIT)
USING THE EPIDERM™ SKIN MODEL

conducted on a mixture that contains 0.24% Basic Yellow 87(12AG30) (as described in the study protocol, the mixture was diluted at a 1:1 ratio with another mixture before being assessed, therefore the final concentration of Basic Yellow 87 tested in the assay was 0.12%.) (in the results the tested mixture is 12AG30:AG32)

Test Articles

[redacted]

[redacted]

Study Completion Date

25 September 2012

[redacted]

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[redacted]

SIGNATURE PAGE

SKIN IRRITATION TEST (SIT)
USING THE EPIDERM™ SKIN MODEL

Initiation Date:	5 July 2012
Completion Date:	25 September 2012
Sponsor:	[redacted]
Sponsor's Representative:	
Testing Facility:	
Archive Location:	
Study Director:	[redacted]
Laboratory Manager:	
Laboratory Supervisor:	

[redacted]

TEST ARTICLE RECEIPT

[redacted] Article Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions*
12AG30	[redacted]	clear red semi-viscous liquid	20 June 2012	room temperature
12AG31		clear yellow semi-viscous liquid	20 June 2012	room temperature
12AG32		clear colorless non-viscous liquid	20 June 2012	room temperature

* - Protected from exposure to light

SKIN IRRITATION TEST (SIT) USING THE EPIDERM™ SKIN MODEL

[redacted]

INTRODUCTION

The purpose of this study was to evaluate the skin irritation potential of the test articles, supplied by [redacted] in the context of identification and classification of skin irritation hazard according to the UN GHS and EU classification system (Category 2/R38 or No label). The skin irritation potential was evaluated based upon measuring the relative conversion of MTT (3-[4,5 - dimethylthiazol-2-yl] - 2,5 - diphenyltetrazolium bromide)[1] in the test article-treated tissues after exposure to each test article for a 60-minute exposure period, followed by a 42-hour post-exposure expression period. Skin irritation potential of the test articles was predicted if the relative viability was less than or equal to 50%. The protocol was based upon the EpiDerm™ SOP, Version 7.0 (Revised March 2009), Protocol for: In vitro EpiDerm™ skin irritation test (EPI-200-SIT), for use with MatTek Corporation's reconstructed human epidermal model EpiDerm (EPI-200)[2]. The protocol met the requirements of the OECD guideline, "In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method" (TG **439**)[3].

Each test article was tested in at least one valid definitive assay to determine the identification and classification of skin irritation hazard according to the UN GHS and EU classification system (Category 2/R38 or No label). The laboratory phase of the study was conducted from 10 July 2012 to 13 July 2012 at [redacted]

[redacted]

[redacted]

MATERIALS AND METHODS

Receipt of the EpiDerm™ Skin Model

Upon receipt of the EpiDerm™ Skin Kit (MatTek Corporation), the solutions were stored as indicated by the manufacturer. The EpiDerm™ tissues were stored at 2-8°C until use. On the day prior to testing, EpiDerm™ Maintenance Medium was set to room temperature prior to use. Nine-tenths mL of Maintenance Medium were aliquotted into the appropriate wells of 6-well plates. Each 6-well plate was labeled with the test article, positive control, or negative control. Each EpiDerm™ tissue was inspected for air bubbles between the agarose gel and Millicell® insert prior to opening the sealed package. Tissue inserts with air bubbles covering greater than 50% of the Millicell® area were not used. The 24-well shipping containers were removed from the plastic bag and their surfaces were disinfected with 70% ethanol. The EpiDerm™ tissues were transferred aseptically into the 6-well plates. The EpiDerm™ tissues were then incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air (standard culture conditions) for 60±5 minutes. After 60 minutes, the EpiDerm™ tissues were transferred to appropriate wells containing 0.9 mL of fresh warmed (to 37°C) Maintenance Medium. The plates were returned to the incubator for 18±3 hours to acclimate the tissues.

Test Article Preparation

As instructed by the Sponsor, the test articles, [redacted] and [redacted] were each diluted with the test article [redacted] at a ratio of 1:1 (v/v). Each test article dilution was prepared on the day of use by pipeting 500 µL of the test article into a prelabeled conical tube. Five hundred microliters of the test article, [redacted] were then added, and then mixed to form a homogenous solution. The dilutions were dosed within 3 minutes of preparation. For the remainder of this report, each test article dilution is referred to as the test article.

Assessment of Test Article/Nylon Mesh Compatibility

Prior to performing the assay, the compatibility of each the test article with the nylon mesh was evaluated. Nylon meshes (MatTek Corporation) were placed on a slide and 30 µL of each test article were applied. A negative control, 30 µL of sterile, Calcium and Magnesium Free Dulbecco's Phosphate Buffered Saline (CMF-DPBS) (Quality Biological, Inc.), was tested concurrently. The slides holding the treated meshes were placed into a petri dish and incubated at standard culture conditions for 60±1 minutes. Using a microscope, each mesh was checked after 60 minutes of exposure to assess any interaction between the test articles and the mesh.

The test articles were not observed to interact with the nylon mesh, and therefore a nylon mesh was used to aid in the spreading of the test article after dosing the EpiDerm™ tissues.

Assessment of Direct Test Article Reduction of MTT

Each test article was added to a 1.0 mg/mL MTT (Sigma) solution in warm Dulbecco's Modified Eagle's Medium (DMEM) containing 2 mM L-glutamine (MTT Addition Medium) to assess its ability to directly reduce MTT. Approximately 30 µL of each test article were added to 1 mL of the MTT solution and the mixtures were incubated in the dark at standard culture conditions for at least one hour. A negative control, 30 µL of sterile, Calcium and Magnesium

Free Dulbecco's Phosphate Buffered Saline (CMF-DPBS) (Quality Biological, Inc.), was tested concurrently. If the MTT solution color turned blue/purple, the test article was presumed to have reduced the MTT. Water insoluble test materials may show direct reduction (darkening) only at the interface between the test article and the medium.

In cases where the test article was shown to reduce MTT, only those test articles that remained bound to the tissue after rinsing, resulting in a false MTT reduction signal, could present a problem. To evaluate whether residual test article was binding to the tissue and leading to a false MTT reduction signal, a functional check (using freeze-killed control tissue) was performed as described in the section labeled "Killed Controls (KC)".

The test articles, [redacted] was observed to reduce MTT directly in the absence of viable cells. A killed control experiment was performed concurrently in the screening assay to determine the extent of the direct MTT reduction (if any) by the test article. The test article, [redacted], was not observed to directly reduce MTT in the absence of viable cells. However due to the color of the test article (clear red) and possible interference with the MTT signal, a killed control experiment was performed for this test article as well.

pH Determination

The pH of each test article was measured using pH paper (EMD Chemicals Inc.). Initially, each test article was added to pH paper with a 0-14 pH range in 1.0 pH unit increments to approximate a narrow pH range. Next, each test article was added to pH paper with a narrower range of 0-6 or 5-10 pH units with 0.5 pH unit increments, to obtain a more accurate pH value. A pH value was not able to be obtained for the test article, [redacted] since the test article discolored the pH paper. The pH value obtained from the narrower range pH paper for the test article, [redacted] is presented in Table 1.

Controls

The definitive assay included a negative control and a positive control. The negative control was 30 µL of sterile, CMF-DPBS (Quality Biological, Inc.) and the positive control was 30 µL of 5% Sodium Lauryl Sulfate (SLS). Both the positive and negative controls were tested in triplicate, and at the same exposure time as the test articles (60±1 minutes).

Skin Irritation Test (SIT) Definitive Assay

The test articles were tested in one valid definitive trial. After the overnight incubation for 18±3 hours, the 6-well plates containing the EpiDerm™ tissues were removed from the incubator and placed at room temperature for at least 5 minutes prior to dosing.

The EpiDerm™ tissues were treated in triplicate with each test article for 60±1 minutes. Thirty microliters of each test article were applied to each of three tissues at 1 minute intervals per tissue. A nylon mesh was placed gently over the dose to spread the test article. If necessary, the mesh was gently pressed down to assure even spreading. The EpiDerm™ tissues were tested in triplicate with the positive or negative control for 60±1 minutes. Thirty microliters of each control were applied to each of three tissues at 1 minute intervals per tissue. Immediately after control administration onto the tissue, a nylon mesh was placed gently over the dose to spread the

negative and positive controls. The plates with dosed tissues were kept in the laminar flow hood until the last tissue was dosed. After the last tissue was dosed, all of plates were transferred to the incubator for 35±1 minutes at standard culture conditions. After 35 minutes, all of the plates were removed from the incubator, placed into the laminar flow hood and kept at room temperature until the exposure period was completed for the first dosed tissue.

After 60±1 minutes of test or control article exposure, the tissues were rinsed with sterile, CMF-DPBS by filling and emptying the tissue insert 15 times. A stream of CMF-DPBS was directed onto the tissue surface. For the test and control articles where a mesh was used, the mesh was carefully removed with forceps (if necessary) after the 5th rinse. After the removal of the mesh, the rinsing procedure of the tissue continued for 10 times. After the 15th rinse, each of the 3 inserts per treatment group (test substance, positive and negative control) was completely submerged, gently swirled, and rinse media dumped in a beaker containing approximately 150 mL of CMF-DPBS and specifically assigned for each treatment group; this procedure was repeated three times for each insert of each treatment group. Finally, the tissues were rinsed once more on the inside and outside of the tissue insert with sterile CMF-DPBS from the wash bottle, and the excess CMF-DPBS was decanted. The bottoms of the tissue inserts were blotted on sterile paper towels and the inserts were transferred to new 6-well plates containing 0.9 mL of fresh warmed (to 37°C) Maintenance Medium. The tissue surface was carefully blotted with sterile cotton-tipped applicators to remove any excess moisture, and the tissue surface was visually observed for residual test article using a dissecting scope. The tissues were then placed into the incubator at standard culture conditions for a post-treatment expression incubation of 42±2 hours. After an initial 24±1 hours of incubation, the 6-well plates were removed from the incubator and the tissues were transferred into new 6-well plates pre-filled with 0.9 mL fresh Maintenance Medium warmed to approximately 37°C. The tissues were placed back into the incubator at standard culture conditions for an additional 18±1 hours for the remainder of the 42±2 hour post-treatment expression incubation.

MTT Preparation

A 10X stock of MTT prepared in PBS (filtered at time of batch preparation) was thawed and diluted in warm MTT Addition Medium to produce a 1.0 mg/mL solution no more than two hours before use. Three hundred microliters of the MTT solution were added to each designated well of a pre-labeled 24-well plate.

After the total 42±1 hours post-exposure expression incubation, the 6-well plates were removed from the incubator. Each tissue was blotted on a sterile paper towel and transferred to an appropriate well containing 0.3 mL of MTT solution. The 24-well MTT plates were incubated at standard culture conditions for 3±0.1 hours.

After the 3±0.1 hour incubation, the EpiDerm™ tissues were submerged, gently swirled, and rinse media decanted in a beaker containing approximately 150 mL of CMF-DPBS three times. The tissue was then blotted on absorbent paper, cleared of excess liquid, and transferred to a prelabelled 24-well plate containing 2.0 mL of isopropanol in each designated well. The plate was covered with parafilm and shaken for at least 2 hours at room temperature to extract the MTT. At the end of the extraction period, the insert was gently agitated up and down in its extractant well. The tissues were pierced with forceps to allow the extract to flow back into the well from which the insert was removed, and the Millicell® inserts were discarded. The extract solution was mixed (homogenized by pipetting up and down three times) and two x 200 µL

aliquots were transferred to the appropriate wells of a 96-well plate. Two hundred µL of isopropanol were added to the wells designated as blanks. The absorbance at 570 nm (OD_{570}) of each well was measured with a Molecular Devices Vmax plate reader with the AUTOMIX function selected.

Killed Controls (KC)

To evaluate whether residual test article was binding to the tissue and leading to a false MTT reduction signal, a functional check (using freeze-killed control tissue) was performed. Freeze-killed tissues were received from MatTek Corporation, and were stored in the freezer until use.

[redacted]

For each test article a single killed tissue was treated with the test article in the normal fashion for 1 hour. The rinsing, MTT exposure, and solvent extraction procedures were performed exactly as described for the viable tissues. Single killed-control tissues were treated with the negative control for 1 hour. A small amount of MTT reduction is expected from the residual NADH and associated enzymes within the killed tissue. This background reduction of MTT will be compared to the MTT reduction observed in the test article-treated killed-control tissues using calculations described in the Presentation of Data.

Presentation of Data

The mean OD_{570} value of the blank wells was calculated. Individual blank-corrected OD_{570} values for each of the duplicate aliquots of each test article or control tissue was determined by subtracting the mean OD_{570} value of the blank wells from their individual OD_{570} values. All calculations were performed using an Excel spreadsheet.

$$\text{Corrected Individual } OD_{570} = \text{Individual } OD_{570} - \text{mean Blank } OD_{570}$$

Mean corrected OD_{570} values were calculated for each individual test article and control tissue from the duplicate aliquots. The group mean of the corrected OD_{570} values for the negative controls were calculated.

The following calculations were performed to determine whether test article residues on the tissues could directly reduce MTT in the killed control tissues. The raw OD_{550} value for the negative control killed control was subtracted from the raw OD_{550} values for each of the test article-treated killed controls, to determine the net OD_{550} values of the test article-treated killed controls.

$$\text{Net } OD_{550} \text{ for each test article KC} = \text{Raw } OD_{550} \text{ test article KC} - \text{Raw } OD_{550} \text{ negative control KC}$$

The net OD_{550} values represent the amount of reduced MTT due to direct reduction by test article residues. For the test articles [redacted] and [redacted] there was little or no direct MTT reduction in the test article-treated killed control compared to the negative control-treated killed controls. Therefore, the MTT reduction observed in the test article-treated viable tissue was ascribed to the viable cells, and no further calculations to address direct MTT reduction were required.

The following % of Control viability calculations were made for each individual tissue:

$$\% \text{ Viability} = \frac{\text{Mean Corrected } OD_{570} \text{ of Aliquots of Individual Test Article or Control Tissue}}{\text{Corrected Group Mean } OD_{570} \text{ of Negative Control}} \times 100$$

The individual % of Control viability values were tabulated for each individual tissue. Mean (and standard deviation) viability values were calculated for the test article and control treatment groups. Finally, the mean viability values were plotted on a bar graph (with 1 standard deviation error bar) for the test article and positive control.

Evaluation of Test Results

The following Prediction Model was endorsed by the European Centre for the Validation of Alternative Methods (ECVAM) Scientific Advisory Committee (ESAC) for the prediction of skin irritation. A test article was predicted to be an irritant (EU Classification R38) when the mean relative viability of the three treated tissues is less than or equal to 50% of the mean viability of the negative control.

In Vitro Result	*In Vivo* Prediction
mean tissue viability $\leq$ 50%	Irritant (I); R38
mean tissue viability > 50%	Non-irritant (NI)

Criteria for a Valid Test

The assay was accepted when the following criteria were met: 1) the positive control (5% SLS) resulted in a mean tissue viability $\leq$ 20%, 2) the mean OD_{570} value of the negative control tissues was $\geq$ 1.000 and < 2.500, and 3) the standard deviations of the positive and negative control calculated from individual percent tissue viabilities of the three identically treated replicates were < 18%.

[redacted]

RESULTS AND DISCUSSION

The test articles, [redacted] and [redacted] were tested using the EpiDerm™ Skin Model for the Skin Irritation Test (SIT). Table 1 summarizes the results of the Skin Irritation Test (SIT) for the test articles and the positive control. The raw and analyzed data for the test articles and the negative and positive controls are included in Appendix B. The mean OD_{570} of the negative control, CMF-DPBS, was 2.167. The mean viability of the positive control, 5% SLS, was 6.3%. The standard deviation calculated from individual percent tissue viabilities of the 3 identically treated replicates was < 18 for the positive control and negative control. Since the acceptance criteria were met, the assay was considered valid.

The test article, [redacted] was not observed to directly reduce MTT in the absence of viable cells, however due to the color of the test article (clear red) and possible interference with the MTT endpoint, the Study Director elected to perform a killed control experiment. The test article, [redacted], was determined to directly reduce MTT. Therefore, a killed-control experiment was performed for this test article as well. The results of the killed control experiment showed that there was little or no direct MTT reduction in the test article-treated killed controls (for both test articles) compared to the negative control-treated killed controls and the MTT reduction in the test article-treated viable tissue was ascribed to the viable cells.

Notes: the following observations have been made upon the evaluation of tissue surface upon removal of the test article:

- All EpiDerm™ tissues treated with test article [redacted] stained orange at edges and blisters were noted on tissues #2 and #3.
- The killed control tissue treated with test article [redacted] stained orange throughout and blisters or bubbles were noted on the surface of the tissue.
- All EpiDerm™ tissues treated with test article [redacted] were darkly colored around the edges and blisters were noted on tissues #2 and #3.
- The killed control tissue treated with [redacted] stained dark grey/blue throughout and blisters or bubbles were noted on the surface of the tissue.

Based upon the results of this assay, the test articles were predicted to be non-skin irritating, and thus would not require an R38 label.

SIT Results Using the EpiDerm™ Skin Model

Table 1

[redacted] Article Number	Sponsor's Designation	Mean Viability (%)	Skin Irritation Prediction	pH
12AG30/12AG32	[redacted]	104.3	Non-Irritant	DpH
12AG31/12AG32		106.4	Non-Irritant	5.5
Positive Control	5% SLS	6.3	Irritant	NA

NA – Not Applicable
DpH – Discolored pH paper, a pH value was not able to be determined since the test article discolored the pH paper.

FINAL REPORT

Study Title

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY WITH OPTIONAL HISTOLOGY

Test Articles

[redacted]

conducted on a mixture that contains 0.24% Basic Yellow 87(12AG30) (as described in the study protocol, the mixture was diluted at a 1:1 ratio with another mixture before being assessed, therefore the final concentration of Basic Yellow 87 tested in the assay was 0.12%.) (in the results the tested mixture is 12AG30:AG32)

Authors

[redacted]

Study Completion Date

8 August 2012

Performing Laboratory

[redacted]

TABLE OF CONTENTS

[redacted]

SIGNATURE PAGE

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY WITH OPTIONAL HISTOLOGY

Initiation Date: 8 July 2012

Completion Date: 8 August 2012

Sponsor: [redacted]

Sponsor's Representative:

Testing Facility:

Archive Location:

Study Director: [redacted]

Laboratory Manager:

Laboratory Supervisor:

[redacted]

TEST ARTICLE RECEIPT

[redacted] Article Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions*
12AG30	[redacted]	clear red semi-viscous liquid	20 June 2012	room temperature
12AG31		clear yellow semi-viscous liquid	20 June 2012	room temperature
12AG32		clear colorless non-viscous liquid	20 June 2012	room temperature

* - Protected from exposure to light

[redacted]

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY WITH OPTIONAL HISTOLOGY

[redacted]

INTRODUCTION

The Bovine Corneal Opacity and Permeability Assay (BCOP) was used to assess the potential ocular irritancy of the test articles to isolated bovine corneas. Bovine corneas, obtained as a by-product from freshly slaughtered animals, were mounted in special holders and exposed to the test articles. An *in vitro* score was determined for the test article based on the induction of opacity and permeability (to fluorescein) in the isolated bovine corneas.

The purpose of this study was to evaluate the potential ocular irritancy of the test articles, supplied by [redacted] as measured by changes in opacity and permeability (to fluorescein) in isolated bovine corneas. The laboratory phase of this study was conducted on 17 July 2012 at [redacted] Four corneas were treated with each test article. Based on changes in corneal opacity and permeability (relative to the control corneas), an *in vitro* score was determined.

[redacted]

MATERIALS AND METHODS

Bovine Eyes

Bovine eyes were obtained from a local abattoir as a by-product from freshly slaughtered animals (J.W. TREUTH & SONS, Inc., Baltimore, MD). The eyes were excised and then placed in Hanks' Balanced Salt Solution, containing Penicillin/Streptomycin (HBSS), and transported to the laboratory on ice packs. Immediately upon receipt of the eyes into the laboratory, preparation of the corneas was initiated.

Preparation of Corneas

The eyes were grossly examined for damage and those exhibiting defects were discarded. The tissue surrounding the eyeball was carefully pulled away and the cornea was excised such that a 2 to 3 mm rim of sclera was present around the cornea. The isolated corneas were then stored in a petri dish containing HBSS until they were mounted in a corneal holder. The corneas were mounted in the holders with the endothelial side against the O-ring of the posterior chamber. The anterior chamber was then positioned on top of the cornea and the screws were tightened. Starting with the posterior chamber, the two chambers were then filled with Minimum Essential Medium (EMEM) without phenol red, containing 1% fetal bovine serum and 2 mM L-glutamine (Complete MEM). Each corneal holder was uniquely identified with a number written in permanent marker, on both the anterior and posterior chambers. The corneal holders were incubated at 32 ± 1°C for a minimum of 1 hour.

Controls

The positive control used in this study was ethanol (Pharmco). The negative control used in this study was sterile, deionized water (Quality Biological).

Test Article Preparation

As instructed by the Sponsor, the test articles, [redacted] and [redacted], were each mixed 1:1 (v/v) with the test article, [redacted] and dosed onto the corneas within 3 minutes of the test article mixture preparation

Test Article pH Determination

A determination of the pH of the test article mixtures was attempted using pH paper (EMD Chemicals Inc.). The test articles were added to 0-14 pH paper with 1.0 pH unit increments. Since the test articles discolored the pH paper, pH values could not be determined.

Bovine Corneal Opacity and Permeability Assay

After a minimum of 1 hour of incubation, the corneas were removed from the incubator. The medium was removed from both chambers and replaced with fresh Complete MEM. The initial opacity was determined for each cornea using an Electro Design OP-KIT opacitometer. Any cornea with an initial opacity greater than 7 was not used in the assay. The treatment of each cornea was identified with the test article number written in permanent marker on colored tape, affixed to each holder. The medium was then removed from the anterior chamber and replaced with the test article, positive control, or negative control.

[redacted]

Method for Testing Liquid or Surfactant Materials

The test articles, [redacted] and [redacted], were each tested as a 1:1 (v/v) mixture with test article, [redacted]. An aliquot of 750 µL of the test article mixtures, positive control, or negative control was introduced into the anterior chamber while slightly rotating the holder to ensure uniform distribution over the cornea. Due to their viscous nature, the test article mixtures were administered directly onto the exposed cornea using a positive displacement pipet. Each treated cornea was completely covered with the test article. Four corneas were incubated in the presence of each test article at 32 ± 1°C for 10 minutes. Three corneas were incubated in the presence of the positive control at 32 ± 1°C for 10 minutes. Three corneas were incubated in the presence of the negative control at 32 ± 1°C for 10 minutes. After the 10-minute exposure time, the control or test article treatments were removed. The epithelial side of the corneas was washed at least three times with Complete MEM (containing phenol red) to ensure total removal of the control or test articles. The corneas were then given a final rinse with Complete MEM (without phenol red). For the corneas directly exposed to the test article mixtures (without anterior chamber window), the test article mixture was removed from the treated corneas by rinsing the exposed epithelium of the corneas (special care was taken not to spray the corneas directly) with Complete MEM (with phenol red). The chamber windows were returned to the chambers when most or all of the test article mixture had been removed. The rinsing process continued in the same manner as the positive and negative control corneas. The anterior chambers were refilled with fresh Complete MEM (without phenol red) and an opacity measurement was performed. The corneas were returned to the incubator for approximately 2 hours after which a final measure of opacity was obtained.

After the final opacity measurement was performed, the medium was removed from both chambers of the holder. The posterior chamber was filled with fresh Complete MEM and 1 mL of a 4 mg/mL fluorescein solution was added to the anterior chamber. The corneas were then incubated in a horizontal position (anterior side up) for approximately 90 minutes at 32 ± 1°C. At the end of the 90-minute incubation period, the medium was removed from the posterior chamber and placed into tubes numbered corresponding to chamber number. Aliquots of 360 µL from the numbered tubes were placed into their designated wells on a 96-well plate. The optical density at 490 nm (OD_{490}) was determined using a Molecular Devices *V*max kinetic microplate reader. If the OD_{490} value of a control or test article sample was 1.500 or above, a 1:5 dilution of the sample was prepared in Complete MEM (to bring the OD_{490} value within the linear range of the platereader). A 360 µL sample of each 1:5 dilution was transferred to its specified well on the 96-well plate. The plate was read again and the final reading was saved to a designated print file.

Fixation of Corneas

After the medium was removed for the permeability determination, each cornea was carefully separated from its corneal holder and transferred to an individual prelabeled tissue cassette containing a biopsy sponge. The endothelial surface of each cornea was placed on the sponge to protect it. The cassettes were placed in 10% neutral buffered formalin to fix the corneal tissue for at least 24 hours. The fixed corneas will be stored up to one year.

Histological Evaluation

As instructed by the Sponsor, a histological evaluation was not performed.

[redacted]

Presentation of Data

Opacity Measurement: The change in opacity for each cornea (including the negative control corneas) was calculated by subtracting the initial opacity reading from the final opacity reading. These values were then corrected by subtracting from each the average change in opacity observed for the negative control corneas. The mean opacity value of each treatment group was calculated by averaging the corrected opacity values of each cornea for that treatment condition.

Permeability Measurement: The mean OD_{490} value for the blank wells was calculated. The mean blank OD_{490} value was then subtracted from the raw OD_{490} value of each well (corrected OD_{490}). Any dilutions that were made to bring the OD_{490} readings into the linear range of the platereader (OD_{490} should be less than 1.500), had each diluted OD_{490} reading multiplied by the dilution factor. The final corrected OD_{490} values of the test articles and the positive control was then calculated by subtracting the average corrected OD_{490} value of the negative control corneas from the corrected OD_{490} value of each treated cornea:

Final Corrected OD_{490} = (raw OD_{490} – mean blank OD_{490}) – average corrected negative control OD_{490}

The mean OD_{490} value of each treatment group was calculated by averaging the final corrected OD_{490} values of the treated corneas for that treatment condition.

The following formula was used to determine the *in vitro* score:

In Vitro Score = Mean Opacity Value + (15 x Mean OD_{490} Value)

Criteria for Determination of a Valid Test

The BCOP assay was accepted when the positive control (ethanol) caused an *in vitro* score that fell within two standard deviations of the historical mean.

[redacted]

RESULTS AND DISCUSSION

Bovine Corneal Opacity and Permeability Assay

Table 1 summarizes the opacity, permeability, and *in vitro* score for each test article mixture. Table 2 summarizes the opacity, permeability and *in vitro* score for the positive control. Since the results of the positive control fell within two standard deviations of the historical mean (within a range of 39.2 to 63.9), the assay was considered valid. The opacity and permeability data for the individual corneas may be found in Appendix B.

The following classification system was established by Sina et al.[1] based on studies with a wide range of test materials. While this classification system provides a good initial guide to interpretation of these *in vitro* data, these specific ranges may not be applicable to all classes of materials.

In Vitro Score:

from 0 to 25	= mild irritant
from 25.1 to 55	= moderate irritant
from 55.1 and above	= severe irritant

Table 1
BCOP Results of the Test Articles

Assay Date	[redacted] Article Number	Sponsor's Designation	Conc.	Exposure Time	Opacity Value	OD_{490} Value	*In Vitro* Score	pH
17 July 2012	12AG30:AG32	[redacted]	1:1 (v/v)	10 minutes	3.3	0.004	3.3	DpH
	12AG31:AG32		1:1 (v/v)	10 minutes	3.5	0.001	3.5	DpH

Table 2
BCOP Results of the Positive Control

Assay Date	Positive Control	Exposure Time	Mean Opacity Value	Mean OD_{490} Value	*In Vitro* Score
17 July 2012	Ethanol	10 minutes	40.3	0.994	55.2

[1]Sina, J.F., Galer, D.M., Sussman, R.G., Gautheron, P.D., Sargent, E.V., Leong, B., Shah, P.V., Curren, R.D., and Miller, K. (1995) A collaborative evaluation of seven alternatives to the Draize eye irritation test using pharmaceutical intermediates. **Fundamental and Applied Toxicology** 26:20-31.

[redacted]

2022 FDA VCRP Raw Data

BASIC YELLOW 87	Hair Conditioner	1
BASIC YELLOW 87	Shampoos (non-coloring)	1
BASIC YELLOW 87	Other Hair Preparations	2
BASIC YELLOW 87	Hair Dyes and Colors (all types requiring caution statements and patch tests)	19
BASIC YELLOW 87	Hair Rinses (coloring)	6
BASIC YELLOW 87	Hair Shampoos (coloring)	6
BASIC YELLOW 87	Hair Color Sprays (aerosol)	1
BASIC YELLOW 87	Other Hair Coloring Preparation	4