Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics

Status: Draft Tentative 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. Previous Panel members involved in this assessment: Daniel C. Liebler, Ph.D. and Ronald C. Shank, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., former Senior Scientific Analyst/Writer, and Regina Tucker, M.S., Scientific Analyst/Writer, CIR.

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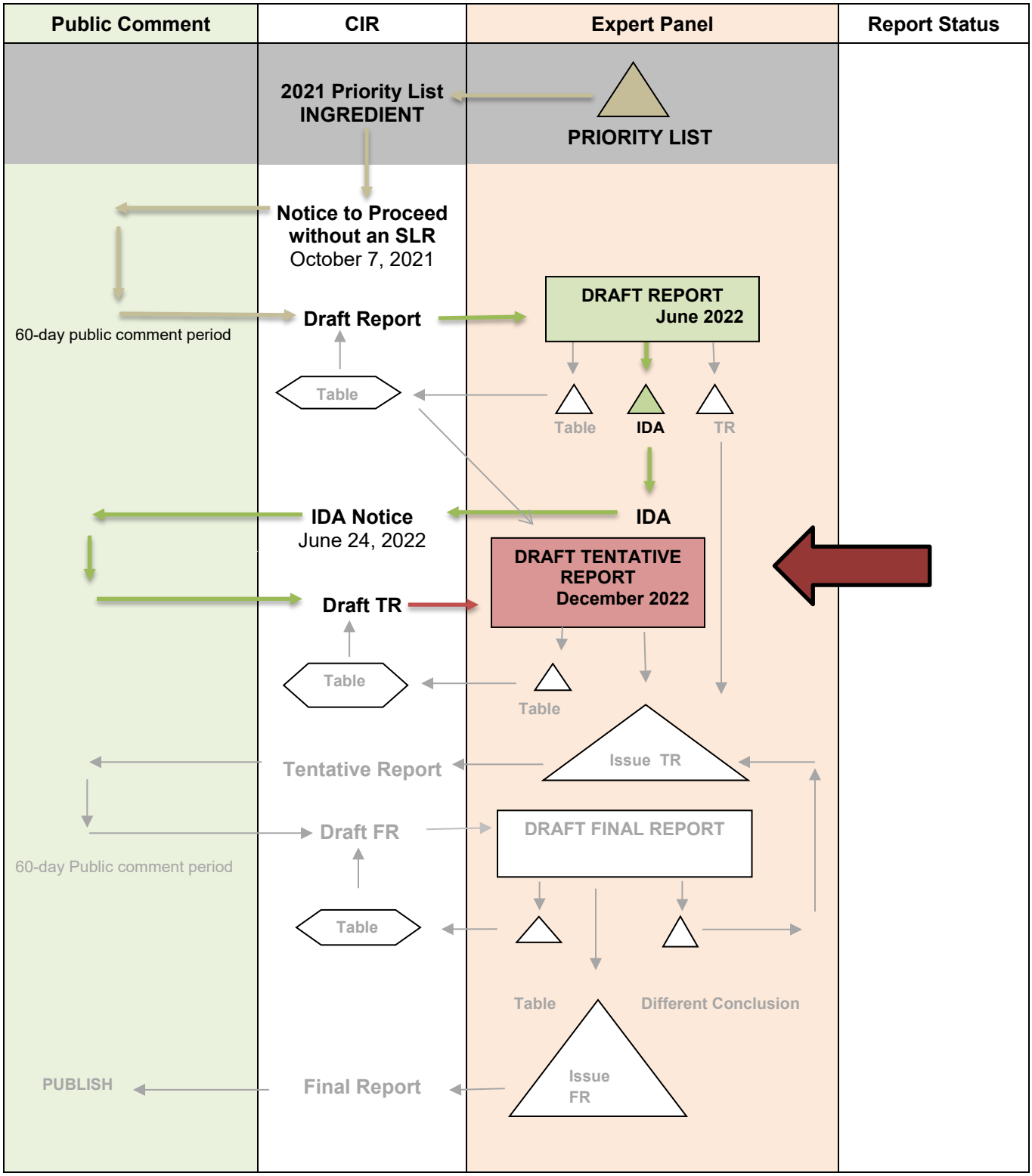
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Phytosteryl Glutamates

MEETING December 2022



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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Regina Tucker, M.S., Scientific Analyst/Writer, CIR
Date: November 10, 2022
Subject: Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics

Enclosed is the Draft Tentative Report on the Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics (*report_PhytosterylGlutamates_122022*). After reviewing the Draft Report at the June 2022 meeting, an Insufficient Data Announcement (IDA) on the 3 Phytosteryl Glutamate ingredients was issued by the Panel with the following data needs:

- Method of manufacturing
- Impurities data
- 28-day dermal toxicity
    - If positive, other toxicological endpoints, including developmental and reproductive toxicity, genotoxicity, and carcinogenicity data, may be needed
- Irritation and sensitization data for Phytosteryl/Octyldodecyl Lauroyl Glutamate at maximum concentration of use
- Ocular irritation data, if available

The following data were received, and have been incorporated into the current iteration of the report (as indicated by yellow highlighting):

- Unpublished data on Phytosteryl/Octyldodecyl Lauroyl Glutamate (*data1_PhytosterylGlutamates_122022*):
    - RCC. 1999. *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay with Phytosteryl/Octyldodecyl Lauroyl Glutamate (neat).
    - RCC. 1999. In vitro chromosome aberration assay in Chinese hamster V79 cells with Phytosteryl Octyldodecyl Lauroyl Glutamate (neat).
    - RCC. 1999. 28-Day oral toxicity (gavage) study in the Wistar rat (Phytosteryl/Octyldodecyl Lauroyl Glutamate neat).
    - Anonymous. 2015. Direct peptide reactivity assay data for L-glutamic acid, N-(1-oxododecyl), mixed (3. Beta., 24R)-ergost-5-en-3yl and 2-octyldodecyl and (3.beta.,22E)- stigmasta-5,220dien-3-yl and (3.beta.)-stigmast-5-en-3-yl esters (Phytosteryl Octyldodecyl Lauroyl Glutamate neat).
    - TKL Research. 2013. Human cumulative irritation patch test (facial essence with 1.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate)
- Unpublished data on Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate (*data2_PhytosterylGlutamates_122022*):
    - TKL Research. 2005. Human repeated insult patch test face cream containing 5% Phytosteryl/ Behenyl/Octyldodecyl Lauroyl Glutamate.
    - Institute for In Vitro Sciences, Inc. 2014. Tissue equivalent assay with EpiOcular™ cultures (1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate in a face cream).
    - North Cliff Consultants, Inc. 2014. Human cumulative irritation patch test (1% Phytosteryl/ Behenyl/Octyldodecyl Lauroyl Glutamate in a face cream).
- Additional unpublished data on Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate (*data3_PhytosterylGlutamates_122022*):
    - Anonymous. 2022. Summary information – Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate

Also included in this package for your review are the CIR report history (*history_PhtyosterylGlutamates_122022*), flow chart (*flow_PhytosterylGlutamates_122022*), literature search strategy (*search_PhytosterylGlutamates_122022*), ingredient data

profile (*dataprofile_PhytosterylGlutamates_122022*), transcripts from the June meeting (*transcripts_PhytosterylGlutamates_122022*), and 2022 FDA VCRP data (*VCRP_PhytosterylGlutamates_122022*).

A draft Abstract and Discussion have been included in this report version. The Panel should carefully consider these items, discuss the data (or lack thereof), and issue a Tentative Report with a safe, safe with qualifications, insufficient data, unsafe, or split conclusion, and identify any additional items for inclusion in the Discussion.

Phytosteryl Glutamates-History

October 2021

A Scientific Literature Review (SLR) Notice to Proceed was issued and the following data was requested:

- Chemistry information, including composition and structure, method of manufacture, and impurities data (including residual monomer content)
- Toxicokinetic data relevant to routes of exposure expected with cosmetic use
- Short-term, subchronic, and chronic dermal/oral toxicity data
- Developmental and reproductive toxicity data
- Genotoxicity data
- Carcinogenicity data
- Dermal irritation and sensitization data at maximum reported use concentrations
- Inhalation toxicity data; and
- Any other relevant safety information that may be available

The following unpublished data was received:

Summary data received for Phytosteryl/Octyldodecyl Lauroyl Glutamate:

- Repeated insult patch test mixture containing 5.999% Phytosteryl/Octyldodecyl Lauroyl Glutamate.
- Primary cutaneous tolerance: Cytotoxicity study performed on an EPISKIN® reconstructed human epidermis model (test mixture containing 1% Phytosteryl/Octyldodecyl Lauroyl Glutamate).

April 2022

The following unpublished data, all received from the Council, have been added to the draft report and are included for the Panel's review:

- Updated (2022) VCRP data
- Human skin irritation study on an epidermis model containing 1% Phytosteryl/Octyldodecyl Lauroyl Glutamate)
- Skin sensitization study (HRIPT) on a test mixture containing 5.999% Phytosteryl/Octyldodecyl Lauroyl Glutamate)

June 2022

The Panel issues an Insufficient Data Announcement, with the following data needs:

The additional data needed to determine safety for these cosmetic ingredients and address data insufficiencies include:

- Method of manufacturing
- Impurities
- Dermal toxicity (28-day dermal)
  If positive, other toxicological endpoints (e.g., developmental and reproductive toxicity, genotoxicity, carcinogenicity, etc.)
- Oral toxicity
- Sensitization and Irritation up 25% for Phytosteryl/Octyldodecyl Lauroyl Glutamate
- Ocular irritation

December 2022: Draft Tentative Report

The following unpublished data were received:

- Salmonella typhimurium and Escherichia coli reverse mutation assay
- In vitro chromosome aberration assay in Chinese hamster V79 cells with Phytosteryl Octyldodecyl Lauroyl Glutamate
- 28-Day oral toxicity (gavage) study in the Wistar rat (Phytosteryl/Octyldodecyl Lauroyl Glutamate).

- Direct peptide reactivity assay data for L-glutamic acid, N-(1-oxododecyl), mixed (3.Beta., 24R)-ergost-5-en-3yl and 2-octyldodecyl and (3.beta.,22E)- stigmasta-5,220dien-3-yl and (3.beta.)-stigmast-5-en-3-yl esters (Phytosteryl Octyldodecyl Lauroyl Glutamate).
- Human cumulative irritation patch test (facial essence with 1.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate)
- Human repeated insult patch test face cream containing 5% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate.
- Tissue equivalent assay with Epiocular™ cultures (1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate in a face cream).
- Human cumulative irritation patch test ((1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate in a face cream
- Summary information – Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate.

***Phytosteryl Glutamates* Ingredients Data Profile* –December 5-6, 2022 – Wilbur Johnson/Regina Tucker**

						Toxico-kinetics		Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	GRAS	Method of Mfg	Constituents	Impurities	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Silico	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Case Report	Other Clinical Reports
Phytosteryl/Octyldodecyl Lauroyl Glutamate	325								X							X	X			X					X					
Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate	25		X																			X					X			
Phytosteryl/Behenyl/Octyldodecyl/ Isostearyl Lauroyl Glutamate	1																													

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

Phytosteryl Glutamates

Ingredient	CAS #	InfoBase	SciFinder	PubMed		FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	ECE-TOC	Web
Phytosteryl/Octyldodecyl Lauroyl Glutamate	220465-88-3	Yes		0/0		No	No	No	No	No	No	No	No	No	No	No	No	Yes
Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate	No CAS No.	Yes		0/0		No	No	No	No	No	No	No	No	No	No	No	No	Yes
Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate	No CAS No.	Yes		0/0		No	No	No	No	No	No	No	No	No	No	No	No	Yes

Search Strategy

[*document search strategy used for SciFinder, PubMed, and Toxnet*]

[*identify total # of hits /# hits that were useful or examined for usefulness*]

LINKS

InfoBase (self-reminder that this info has been accessed; not a public website) - http://www.personalcarecouncil.org/science-safety/line-infobase
ScfFinder (usually a combined search for all ingredients in report; list # of this/# useful) - https://scifinder.cas.org/scifinder
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - http://www.ncbi.nlm.nih.gov/pubmed
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – https://toxnet.nlm.nih.gov/ (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)

FDA databases – http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (CFR); then,
list of all databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm; then,
https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus (Substances added to Food);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm (GRAS);
https://www.fda.gov/food/generally-recognized-safe-gras/gras-substances-scogs-database (SCOGS database);
http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives (indirect food additives list);
http://www.fda.gov/Drugs/InformationOnDrugs/default.htm (drug approvals and database);
http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf (OTC ingredient list);
http://www.accessdata.fda.gov/scripts/cder/iig/ (inactive ingredients approved for drugs)

EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - http://ec.europa.eu/growth/tools-databases/cosing/
ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.industrialchemicals.gov.au/chemical-information/search-assessments?assessmentcasnumber=39346-84-4

NTIS (National Technical Information Service) - http://www.ntis.gov/
NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/ (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - http://www.ecetoc.org/

JUNE 2022 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito's Team Meeting – June 16, 2022

Dr. Belsito - Yeah. Okie doke. And then the last one we have or is phytosteryl glutamates?

Dr. Rettie - So this is brand new.

Dr. Snyder - Yes.

Dr. Belsito - So the first question that I had is, have we reviewed any constituents that we can read across like fatty alcohols, moral glutamic acid phytosterols? I mean, I think we have no. Then there's some read across material we can bring in.

Monice Fiume (CIR) - So I believe I'm PDF page 10 in the intro. It does say some of the reports that have been reviewed previously. Are they useful?

Dr. Belsito - Yeah, I mean, that's what I mean. Can't we bring the way to in?

Monice Fiume (CIR) - OK.

Dr. Belsito - Because it's not been brought into this report.

Monice Fiume (CIR) - So Don, that's going to be up to the panel. Typically we refer to them and then provide the link that those reports are available unless those data are complete, read across and you want them brought into the report. Typically, we've been referring to them. We haven't brought the data in in a while, but whatever the panel prefers for use in the report is what we can do.

Dr. Belsito - OK. Well, I mean at this point, I thought it was insufficient for manufacturing and impurities. 28 day dermal and if absorbed, other endpoints such as DART and genotoxicity. Oral toxicity since it's used in lipsticks up to 20 percent, 25%. And sensitization or irritation up to 25%. And ocular irritation if available and I looks to me like I made a note that it was phytosteryl octadecyl glutamate, which was the one that was used and lipsticks. Is that correct?

At a very high concentration. 25% mucus membrane. Yeah. So I wanted sensitization and irritation, particularly on that one the fitsterlate-----. But I'm open to what other people might suggest.

Dr. Snyder - But we don't have any absorption, distribution, metabolism, excretion data at all.

Dr. Belsito - That's what I said, 28 dermal. So I said insufficient manufacturing impurities 28 day dermal if absorbed other endpoints such as DART and genotoxicity. Oral tox, because it's used in lipsticks up to 25%. And sensitization and irritation on phytosteryl octadecyl glutamate at 25%, and ocular irritation if available.

Dr. Snyder - Agree.

Dr. Rettie - Yeah, there's some tox testing, but it's only a 5% and the maximal concentration is much higher as you mentioned.

Dr. Belsito - Yeah. Right.

Dr. Klaassen - There's basically no data.

Dr. Belsito - I'm sorry I missed that. Right.

Dr. Klaassen - In essence this. This document basically contains no data.

Dr. Belsito - Right.

Dr. Klaassen - Get right down to it.

Dr. Liebler - Right, it's. Closest thing to an empty report we've ever gotten since I've been on the panel. So these just a couple of comments on some of the things that we talked about, the idea of using, prior reviewed ingredients like the phytosterols, for example. You know the phytosterols as read across. Like Laurel oil glutamate, for example, is read across. I'm on the RIFM panel we actually do something similar to this. Where we for systemic endpoints where it's going to be, you know, often oral administration and we're interested in repeat dose tox or repro and developmental. We have what we call a Tier 3 read across, which is if we have safety data for the ingredients of an Ester that would be hydrolyzed, for example, for the components of the Esther, and we have safety data on those, we can use a that sort of Tier 3 read across to clear the endpoint. We never use Tier 3 or practically never, I think for like skin sensitization for example. But we do for the systemic endpoints. We have no history of doing that kind of

read across with CIR, I mean honestly, CIR reviews have just not really developed the read across concept nearly as much as we have on the RIFM panel, so we don't have any way to just sort of jump in and start doing that. But that is I just want to mention that is something that could be considered how on the other hand, this is an empty report with practically no information and we should have information before we try and clear these by, you know, some kind of a Tier 3 read across. So I agree with an insufficient data analysis and seeing what we get out of these. You know the components are essentially innocuous, but you know we just have no data on the on the ingredients chemical properties, method of manufacture and purities et cetera.

Dr. Belsito - I mean, this is the first pass. We were virtually got nothing. So we've created a list and you know we can you know I guess, if I understand you correctly, my Monice? We're not going to bring in data from the other report. They'll just be links to those reports for us to look at. The data. Is that correct?

Monice Fiume (CIR) - So typically that's how we've done it. And Dan, actually, I'm trying to see which report it was in the past. We would create a table of reports that were done previously that might have relevant or like we wouldn't have called it a Tier 3 read across, but that type of use and we would have included the name of the ingredient in all of the data. Just a brief summary of what was seen. Once we started adding the link to the existing reports, we've sort of gotten away from including that table, but if that's what's helpful for this report and it's useful, we can bring it in in whatever format you would like to see it.

Dr. Klaassen - I think it would be helpful.

Dr. Liebler - Yeah. I think that this is a this is something that probably ought to be considered by the CIR panel, and by the time you've gone around with an insufficient data analysis and then you've got a, you know you've got whatever response back and then your face with the question of do we use data from reports on the component ingredients for these chemicals, you know, I'll be off the panel and you guys will be considering it. But I just wanted to point out that Tier 3 approach that the RIFM panel uses, it's easier to do that when you have a single chemical to single chemical read across situation think it's one of the you know, in the RIFM we review one target chemical and it might be an Ester. So we can do a tier three with the acid and the alcohol components of the Ester and it's very straightforward. With CIR, I think one of the barriers to read across has been we're trying to review a family of related chemicals and we may have some prior data on one component of a component of that family you know. And it it's just not something we've done. But on the other hand, you know, the principles and practice of doing it are there, we've even got one paper published in chemical research in toxicology on the grouping and which does talk about read across a little bit and then another manuscript that's heading for publication on doing this multi tiered read across for different endpoints. So I mean I think that the basic we're doing this will become better established in the literature and I think that CIR should look at incorporating that in some way in. As we go forward, it might not arrive in time for this report, but let's see what data we get.

Dr. Klaassen - Have we ever, in regard to this report, have we ever had a report on a number of these steroids that are in these molecules like beta, sitosterol and stigmasterol? I assume we probably have not.

Dr. Belsito - I think we've done that no? Didn't we do Phytosterol?

Monice Fiume (CIR) - We have the Phytosterol report.

Dr. Belsito - Yeah.

Dr. Rettie - Yeah.

Bart Heldreth (CIR) - Yes.

Dr. Klaassen - Just as a big Phytosterol report.

Dr. Belsito - Yes.

Monice Fiume (CIR) – I think it has individual ingredients in it let me bring it up.

Bart Heldreth (CIR) - Yeah, the Phytosterol report was very similar in the composition of which a steroid like molecules, where the where conscribed on there. So Sigma stereo and the beta stereo is all part of those mixtures in the phytosterols report.

Dr. Klaassen - I mean, the sittersterile. It's toxic to some people. And the reason why it is that some people don't have the transporter that transports it back into the gut.

Dr. Liebler - Ah.

Dr. Klaassen - So prevents it's absorption, but anyhow.

Dr. Liebler - Interesting.

Dr. Klaassen - That's just a little. And some people, there's a disease called sitosterol lomel. Because they can't they absorb more than the rest of us. You know, read across Dan. You know when we're talking about different fatty acids and lengths of fatty acids. Etcetera. Doesn't bother me. But steroids, for example, like these molecules, you know, there are so many endogenous chemicals and drugs that are derived from the steroid molecule that it scares me a little bit to do too much, read across on steroid type chemicals. Do you think I'm too conservative in that?

Dr. Liebler - No, I mean your comment in the context of the transporter for those of you who aren't aware, I mean Curt is probably the world's authority on toxicity mechanisms involving transporter biology and it's an interesting point well taken and I'm sure you could give us chapter and verse on other examples like this. Having said that about the transporters and asteroid steriles, I think, the whole idea read across is that in general concept it can seem real scary and dicey, but read across should always be framed as a specific structure to structure comparison in the context of a particular endpoint. Where the potential risks and benefits of doing the read across can be examined and justified or rejected. And so I would say read across should not be dismissed of preemptively, but if we're going to do it, it's really only valid as a chemical to chemical comparison. Where you got a chemical that you know has an appropriate data set for an endpoint, you've got a target chemical that doesn't have the data. The idea of reading across then would be only in the context of that specific endpoint and the other mitigating factors that could you know influence the interpretation need to be at least reasonably well understood. So like read across for repeat dose toxicity you know for you know compounds that might differ in chain length or you know with a double bond here or there that can be pretty easily assessed. Repro and developmental is usually a lot dicier for read across and then you know examples like these you know, with these, phytosterols, it depends on the endpoint. So I think your concerns well taking Curt, but I don't think it should necessarily be rejected out of hand. Until you're presented with a situation where you've got to a, you know, a specific structure, you need data for and you've got a candidate, then you can look at it and really compare the chemistry. What might be known about the pharmacology, et cetera, and then make a decision on whether you would accept the read across or not.

Dr. Belsito - Yeah. I mean to Dan's point that at on fragrance panel will oftentimes have three or four different reader cross materials for different endpoints. Simply in many cases, because there's not one single material that provides us for read across our all of those endpoints. And then typically we are reading across, there's a justification table at the end of the report that indicates what we're using to read across for and the rationale behind using that.

Dr. Klaassen - So at on as a as a medicinal chemist, does read across work great in pharmacology?

Dr. Liebler - I don't think it's ever used. There's no needs. There's no reason to do it.

Dr. Klaassen - Because it doesn't work in pharmacology.

Dr. Liebler - Well. It's not used because it's not needed or necessary or sufficient. I mean it's read across is used in the assessment of toxicity data for an endpoint.

Dr. Klaassen - For when you don't have data.

Dr. Liebler - Right.

Dr. Belsito - I mean, I think we can just try and look at it. You know what I mean. We have our data needs manufacturing and impurities. We're not going to be able to read across from. 28 day dermal. We may be, I don't know. Oral tox. We're going to have an issue cause of the high levels and lipsticks. And Curt already pointed out some concerns that he has with these steroid-like molecules and the phytosterols and we still have sensitization narration. So in ocular irritation, if it valuable. So there we're asking for literally every endpoint right now. If you can bring some set things in or just highlight the reports where you think we might want to bring information in, we can try and do it that way, but I mean this is insufficient and we have a list that I think everyone's agreed on correct?

Dr. Liebler - Yeah, I don't think we're going to be able to use the molecular weight as a get out of jail free card here. I mean that structure, the stratosphere, all Laurel glutamate on top of PDF 11. I mean, that's a that's roughly 750 molecular weight. It would be largely not absorbed it, but not completely, I think. It's always hard to predict, but. You know we it's not like a polymer where we can dismiss absorption completely.

Dr. Belsito - Right.

Dr. Liebler - So we just need to ask for the data and see what we get.

Dr. Belsito - OK. Any other comments? Allan. Paul, you've been very quiet.

Dr. Rettie - Well, it just seemed like we were going to ask for everything. So I was going to sit back and see what came in, but it was good to have this discussion because you're obviously looking to the next to the next iteration. The way you said, they're down about the molecular way. The other one, the octyldodecyl Laurel glutamate. We guess you're over 1000 with that one. That's probably one of the biggest, if not the biggest. So you're kind of a little and new man's land. Maybe some, you know, some dermal absorption for some and maybe less for others. Just based on the likelier weight. But it's a Gray area.

Dr. Liebler - Yep.

Dr. Belsito - Paul comments?

Dr. Snyder - I just felt that after your after you're opening insufficient data needs. I didn't think there was much to discuss at this point.

Dr. Belsito - OK.

Dr. Liebler - Right. Agree.

Cohen's Team Meeting – June 16, 2022

[Due to technical difficulties, transcripts were not available for the Cohen team meeting.

Full Panel – June 17, 2022

Dr. Bergfeld - OK. So moving on to the Phytosteryl glutamates Dr. Belsito.

Dr. Belsito - Yes, so. This includes three phytosterol glutamates. And they are mixed esters that each comprise laurel, would have tannic acid esterified with a mixture of phytosteryl *(inaudiable). Basically, this is the first time that we're looking at this, a document we did receive a significant amount of data but not overwhelming and I'm we were wondering to what extent we could bring in data from the fatty alcohols to Laurel, Lieutenaic acid, and the phytosterol reports that we previously had used or reviewed rather. But at this point we found this group to be insufficient for manufacturing impurities. 28-day dermal tox or other means of assessing absorption and depending upon absorption, other endpoints such as dark and genotoxicity may be needed. We need oral toxicity because it's used in lipsticks up to 25% sensitization and irritation, up to 25% for about a sterile opted vessel, glutamate. And we also need ocular irritation since it's used in high concentrations about the eye, *(inaudiable).

Dr. Bergfeld - And that's your motion.

Dr. Belsito - That's our motion.

Dr. Bergfeld - And you're going out for a what IDA?

Dr. Belsito - Yes.

Dr. Bergfeld - OK, Cohen?

Dr. Cohen - That's a second. We're perfectly harmonized.

Dr. Bergfeld - Very nice, good music, alright. I call the question, then all those opposed. Abstaining. Unanimously approved go out as an IDA with all those needs listed. Regina, do you need those listed again for you?

Regina Tucker (CIR) - No, I no, I have them. Thank you.

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ABBREVIATIONS

CFR	Code of Federal Regulations
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	US Consumer Product Safety Commission
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook*
FDA	Food and Drug Administration
HRIPT	human repeated insult patch test
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
NR	not reported
t_{50}	duration of exposure resulting in a 50% decrease in MTT conversion
US	United States
VCRP	Voluntary Cosmetic Registration Program
WHO	World Health Organization

DRAFT ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 3 phytosteryl glutamates as used in cosmetics. These ingredients are reported to function mainly as skin conditioning agents in cosmetics. The Panel reviewed the available data to determine the safety of these ingredients. The Panel concluded that… [to be determined].

INTRODUCTION

The safety of the following 3 phytosteryl glutamates as used in cosmetics is reviewed in this safety assessment.

Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate
Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate
Phytosteryl/Octyldodecyl Lauroyl Glutamate

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), all 3 phytosteryl glutamates are reported to function in cosmetics as skin conditioning agents, and Phytosteryl/Behenyl/Octyl-dodecyl/Isostearyl Lauroyl Glutamate is also reported to function as a hair conditioning agent (Table 1).[1] These ingredients are being reviewed together as they are all mixed esters of phytosterols, octyldodecanol (and other respective fatty alcohols), and lauroyl glutamic acid. The Expert Panel for Cosmetic Ingredient Safety (Panel) has previously reviewed the safety of several phytosterols and lauroyl glutamic acid. The phytosterols ingredient group were considered safe as used in the present practices of use and concentration (as described in that safety assessment).[2] Lauroyl glutamic acid was reviewed as part of the safety assessment of amino acid alkyl amides.[3] At the time of the assessment lauroyl glutamic acid was not in current use, but the Panel concluded it would be considered safe if used in product categories and at concentrations comparable to others in the group (as described in the safety assessment). The full reports on these ingredients can be accessed on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/ingredients).

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. The published data in this document were identified by conducting an exhaustive search of the world's literature. A list of the search engines and websites that are used, and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is available on the 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 may be provided by the cosmetics industry, as well as by other interested parties.

CHEMISTRY

Definition and Structure

Each of these ingredients comprise 2 core chemical structural residues, phytosterols and lauroyl glutamate. These ingredients also comprise certain fatty alkyl chains. The "/"in the names of these ingredients signifies mixtures. For example, Phytosteryl/Octyldodecyl Lauroyl Glutamate is a mixture of phytosteryl lauroyl glutamate and octyldodecyl lauroyl glutamate. The term "phytosteryl" also signifies a mixture, specifically of steroidal constituents derived from plants (i.e., "phyto"). The most common phytosterols (β-sitosterol, stigmasterol, avenasterol, campesterol, and campestanol) are illustrated in Figure 1, as is an example of connectivity with lauroyl glutamate.

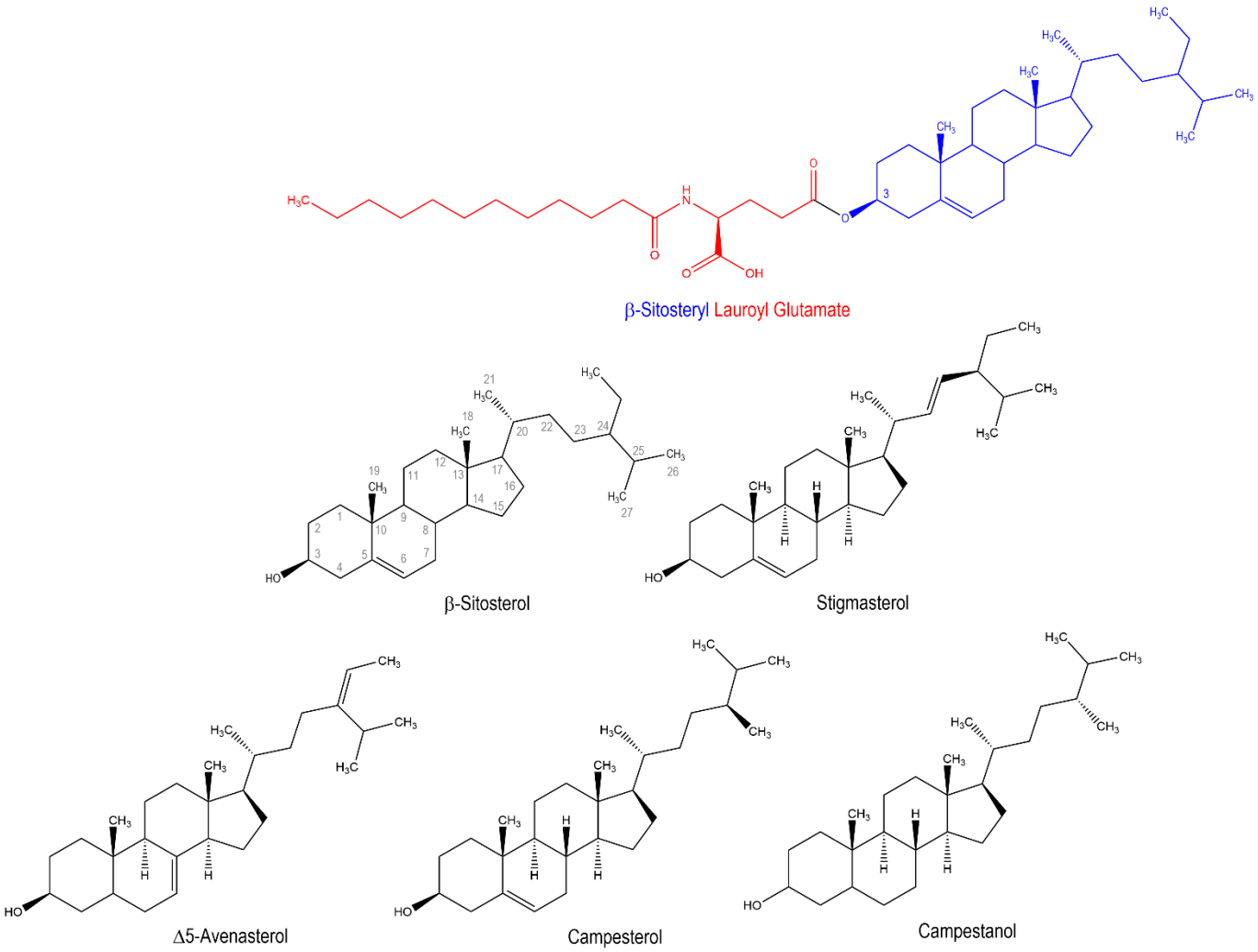

Figure 1. Phytosterols and phytosteryl connectivity

All such connectivities are the result of esterification via the 3-position alcohol functional group of one or more phytosterols. The connectivity of various fatty alkyl chains with lauroyl glutamate is similarly the result of esterification (e.g., octyldodecyl lauroyl glutamate (Figure 2)).

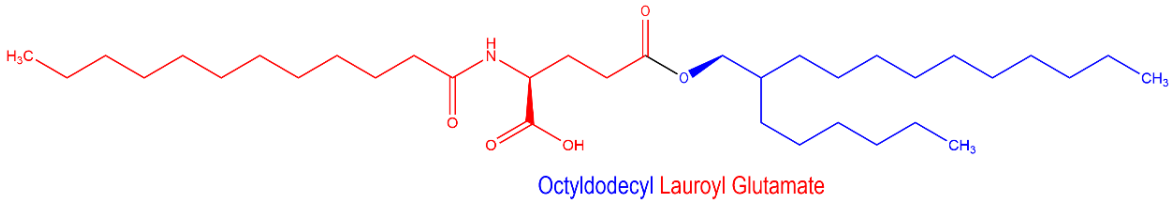

Figure 2. Octyldodecyl Lauroyl Glutamate

Accordingly, Phytosteryl/Octyldodecyl Lauroyl Glutamate is a mixture potentially comprising all of the above instances of esterified lauroyl glutamate. Likewise, Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate and Phytosteryl/Behenyl/ Octyldodecyl/Isostearyl Lauroyl Glutamate comprise similar such mixtures.

The definitions and reported functions of the phytosteryl glutamates included in this safety assessment are presented in Table 1.[1] The only ingredient with a CAS No. in this safety assessment is Phytosteryl/Octyldodecyl Lauroyl Glutamate (220465-88-33).

Chemical Properties

Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate

Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate is a white solid.[4] Results of gel permeation chromatography of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate in tetrahydrofuran are found in Table 2. The standard was polystyrene.

Method of Manufacture

Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate

A method of manufacture of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate involves the synthesis, cooling, washing, and drying of the ingredient.[4] This is followed by quality control and packing.

Impurities

Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate

In an arsenic and lead analysis of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate where the detection limit was 1 µg/g for arsenic and 2 µg/g for lead, neither arsenic nor lead was detected.[4] The loss on drying of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate (105°C, for 1 h) is 0.1%.

USE

Cosmetic

The safety of the cosmetic ingredients 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 these ingredients in cosmetics, and does not cover their 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 FDA VCRP data, Phytosteryl/Octyldodecyl Lauroyl Glutamate has the greatest frequency of use; it is reported to be used in 325 cosmetic products, 311 of which are leave-on products and over a third of which are in lipstick formulations (Table 3).[5] The results of the concentration of use survey conducted by the Council in 2021 indicate that Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate has the highest concentration of use; it is used at maximum use concentrations up to 25.6% in leave-on products (rouges).[6] The maximum concentration of use reported for Phytosteryl/Octyldodecyl Lauroyl Glutamate is very similar; it is reported to be used at up to 25% in rouges and in lipsticks.

Cosmetic products containing phytosteryl glutamates may incidentally come in contact with the eyes (e.g., Phytosteryl/Octyldodecyl Lauroyl Glutamate at concentrations up to 12% in eye shadow), and all 3 of these ingredients are used in products that are reported to be used in formulations that could be incidentally ingested and that come in contact with mucous membranes (e.g., Phytosteryl/Octyldodecyl Lauroyl Glutamate at concentrations up to 25% in lipstick). Use in baby products is also reported (e.g., Phytosteryl/Octyldodecyl Lauroyl Glutamate is used at up to 0.3% in baby lotions, oils, and creams).

Some of these ingredients are used in cosmetic products that could possibly be inhaled; for example, Phytosteryl/Octyldodecyl Lauroyl Glutamate is reported to be used in hairsprays (concentration not reported), aerosol deodorant (up to 0.1%), and in face powders at concentrations up to 5%. 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. There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable. However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

Although products containing some of these ingredients 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 these ingredients (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.

The phytosteryl glutamates reviewed in this safety assessment are not restricted from use in any way under the rules governing cosmetic products in the European Union.[7]

TOXICOKINETIC STUDIES

Data on toxicokinetic effects of phytosteryl glutamate ingredients reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Data on the acute toxicity of the phytosteryl glutamate ingredients reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

Short-Term Toxicity Studies

Oral

Phytosteryl/Octyldodecyl Lauroyl Glutamate

In a short-term oral toxicity study, a daily dose of Phytosteryl/Octydodecyl Lauroyl Glutamate was administered by gavage to SPF-bred Wistar rats of both sexes at dose levels of 50, 200, or 1000 mg/kg for 28 d.[8] During the experiment, clinical signs, outside cage observation, food consumption, and body weights were recorded. Functional observational battery, locomotor activity, and grip strength were performed during week 4. After the dosing period, blood samples were drawn for hematology and blood chemistry profile. Histological examinations were performed on organs and tissues. No test substance-related clinical signs were noted, along with no changes in functional observational battery, grip strength, locomotor activity, food consumption, and body weight. Changes in hematology or clinical chemistry parameters were also not reported. There were no reported experimental effects on organ weights; macroscopic and microscopic examination found no changes in experimental animals.

Subchronic and Chronic Toxicity Studies

Data on the subchronic and chronic toxicity of the phytosteryl glutamates reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Data on the developmental and reproductive toxicity of phytosteryl glutamates reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

GENOTOXICITY STUDIES

In Vitro

Phytosteryl Octyldodecyl Lauroyl Glutamate

The mutagenicity of Phytosteryl/Octyldodecyl Lauroyl Glutamate was examined in a reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 and *Escherichia coli* strain WP2 uvr A.[9] Doses of 33, 100, 333, 1000, 2500, and 5000 µg/plate were tested. The assay was performed in two independent experiments with and without liver microsomal activation. The test was performed in triplicate. The vehicle and positive controls produced appropriate responses. The test article did not cause a positive increase in the number of revertants per plate in any of the tester strains either in the presence or absence of metabolic activation. Under the conditions of the assay, Phytosteryl/Octyldodecyl Lauroyl Glutamate was not mutagenic.

An in vitro assay was performed to assess the potential of Phytosteryl/Octyldodecyl Lauroyl Glutamate in 0.5% acetone to induce structural chromosome aberrations in Chinese hamsters V79 cells in two parallel cell cultures.[10] In both independent experiments, no biologically relevant increase in the number of cells carrying structural chromosomal aberrations were observed after treatment with the test substance ranging in concentration from 2.5 µg/ml to 2500 µg/ml, with and without S9 liver activation. One instance of increase in 1250 µg/ml concentration in the absence of S9 was noted, however this was not reproducible and was not considered biologically relevant. Thus, the test substance was considered to be non-clastogenic.

CARCINOGENICITY STUDIES

Data on the carcinogenicity of phytosteryl glutamates reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Dermal irritation and sensitization studies are described in Table 4, and summarized below.

According to summary data, an in vitro cell viability assay was performed, using EpiSkin™ reconstituted human epidermis. The test substance, a product containing 1% Phytosteryl/Octyldodecyl Lauroyl Glutamate, was predicted to be non-

irritating.[11] In a 24-h patch test in which an occlusive patch containing 15 µl of a Phytosteryl/Behenyl/Octyldodecyl Lauroyl cream (concentration not stated) was applied to 31 subjects, the test article was determined to be a non-irritant.[4] In a human cumulative irritation patch test with 25 subjects that took place over 7 d, a face cream containing 1% Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate was determined to be non-irritating.[12] A 7-d semi-occlusive cumulative irritation patch study with a face cream containing 1.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate was performed with 38 subjects; no evidence of irritation was observed.[13]

In a direct peptide reactivity assay (DPRA), Phytosteryl/Octydodecyl Lauroyl Glutamate was prepared as a 100 mM stock solution and tested for cysteine and lysine depletion.[14] Both peptides showed minimal reactivity. In a human repeated insult patch test (HRIPT), a face cream containing 5% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate (102 subjects, tested neat, occlusive patch) was not a sensitizer.[15] In another HRIPT, Phytosteryl/Octyldodecyl Lauroyl Glutamate (219 subjects; tested neat, occlusive patch) was not an irritant or a sensitizer.[16]

OCULAR IRRITATION STUDIES

Phytosteryl/Behenyl/Octydodecyl Lauroyl Glutamate

A tissue equivalent assay, measuring the conversion of 3-[4,5,-dimethylthiazol-2-y1]-2,5-diphenyltetrazolium bromide (MTT) by EpiOcular™ cultures, was performed to test the potential ocular irritancy of a face cream containing 1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate.[17] Approximately 100 µl of each test article was administered undiluted and then incubated. A face cream containing 1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate did not reduce MTT in the absence of viable tissue; the t_{50} (duration of exposure resulting in a 50% decrease in MTT conversion) was > 24 h.

SUMMARY

The safety of 3 phytosteryl glutamates as used in cosmetics is reviewed in this safety assessment. According to the *Dictionary*, Phytosteryl/Octyldodecyl Lauroyl Glutamate and Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate are reported to function in cosmetics as skin conditioning agents and Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate is reported to function as a hair conditioning agent and skin conditioning agent.

According to 2022 FDA VCRP data, Phytosteryl/Octyldodecyl Lauroyl Glutamate has the greatest frequency of use; it is reported to be used in 325 cosmetic products, (311 leave-on products and 14 rinse-off products). The results of a concentration of use survey conducted by the Council in 2021 indicate Phytosteryl/Octyldodecyl Lauroyl Glutamate has the highest concentration of use; it is used at maximum use concentrations up to 25.6% in leave-on products. The maximum concentration of use reported for Phytosteryl/Octyldodecyl Lauroyl Glutamate is very similar; it is reported to be used at up to 25% in rouges and in lipsticks.

The short-term oral toxicity of Phytosteryl/Octydodecyl Lauroyl Glutamate was administered by gavage to SPF-bred Wistar rats of both sexes at dose levels of 50, 200, or 1000 mg/kg for 28 d. No experimental substance-related clinical signs were noted, along with no changes in functional observational battery, grip strength, locomotor activity, food consumption, and body weight were noted. Changes in hematology or clinical chemistry parameters, organ weights, or macroscopic and microscopic findings were also not observed.

Phytosteryl/Octyldodecyl Lauroyl Glutamate was not mutagenic in a reverse mutation assay, with or without metabolic activation, when tested at up to 5000 µg/plate. It was also not genotoxic, with or without metabolic activation, in a chromosomal aberration when tested at up to 2500 µg/plate in Chinese hamster V79 cells.

An in vitro cell viability assay was performed, using EpiSkin™ reconstituted human epidermis. The test substance, a product containing 1% Phytosteryl Octyldodecyl Lauroyl Glutamate, was predicted to be non-irritating. In a 24-h patch test in which an occlusive patch containing 15 µl of a Phytosteryl/Behenyl/Octyldodecyl Lauroyl cream (concentration not stated) was applied to 31 subjects, the test article was determined to be a non-irritant. In a human cumulative irritation patch test with 25 subjects that took place over 7 d, a face cream containing 1% Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate was determined to be non-irritating. A 7-d semi-occlusive cumulative irritation patch study with a face cream containing 1.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate was performed with 38 subjects; no evidence of irritation was observed.

In a DPRA, Phytosteryl/Octyldodecyl Lauroyl Glutamate was prepared in a 100 mM stock solution and tested for cysteine and lysine depletion. Both peptides showed minimal reactivity. An HRIPT with a face cream containing 5% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate (102 subjects, tested neat, occlusive patch) was not a sensitizer. In another HRIPT, Phytosteryl/Octyldodecyl Lauroyl Glutamate (5.999%, 219 subjects; tested neat, occlusive patch) was not an irritant or sensitizer.

A tissue equivalent assay measuring the conversion of MTT by EpiOcular™ cultures to test the potential ocular irritancy of a test article containing 1% Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate in a face cream was performed. Approximately 100µl of each test article was administered undiluted. A time range finding assay was performed with exposure times ranging from 1-16, and a definitive assay whereby three to five exposure times were treated in duplicate. In both cases the test articles did not reduce MTT in the absence of viable tissue.

DISCUSSION

[Note: This Discussion is in draft form, and changes will be made following the Panel meeting.]

This assessment reviews the safety of 3 Phytosteryl Glutamates. The Panel reviewed the available data and concluded [TBD].

The Panel discussed the issue of incidental inhalation exposure resulting from these ingredients (for example, Phytosteryl/Octyldodecyl Lauroyl Glutamate is reported to be used in hairsprays (concentration not reported), aerosol deodorant (up to 0.1 %), and in face powders (at concentrations up to 5%). Inhalation toxicity data were not available. However, the Panel noted that in aerosol products, the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which these ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

To be determined.

TABLES

Table 1. Definitions and reported functions of the ingredients in this safety assessment[1]

Ingredient/CAS No.	Definition	Function(s)
Phytosteryl/Octyldodecyl Lauroyl Glutamate 220465-88-3	Phytosteryl/Octyldodecyl Lauroyl Glutamate is the mixed ester of phytosterol and octyldodecanol with lauroyl glutamic acid.	Skin-Conditioning Agents – Occlusive
Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate	Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate is the mixed ester of phytosterol, behenyl alcohol, and octyldodecanol with lauroyl glutamic acid.	Skin-Conditioning Agents – Occlusive
Phytosteryl/Behenyl/Octyldodecyl/ Isostearyl Lauroyl Glutamate	Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate is the mixed ester of phytosterols, behenyl alcohol, octyldodecanol and isostearyl alcohol with lauroyl glutamic acid.	Hair Conditioning Agents; Skin-Conditioning Agents – Emollient

Table 2. Gel permeation chromatography of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate [4]

Peak No.	No. Avg. Molecular Weight	Weight average molecular weight	Size average molecular weight	Molecular weight at the highest peak	Degree of dispersion	Area%
1	1,344	1,372	1,402	1,389	1.021	74.2
2	746	757	768	765	1.015	13.8
3	383	396	409	388	1.034	12.0

Table 3. Frequency (2022)[5] and concentration (2021)[3] of use according to duration and exposure

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Phytosteryl/Octyldodecyl Lauroyl Glutamate		Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate		Phytosteryl/Behenyl/Octyldodecyl/ Isostearyl Lauroyl Glutamate	
Totals*	**325**	**0.005-25**	**25**	**NR**	**1**	**0.00028-25.6**
Duration of Use						
Leave-On	*311*	*0.01-25*	*25*	*NR*	*1*	*0.03-25.6*
Rinse-Off	*14*	*0.005-2*	*NR*	*NR*	*NR*	*0.00028-1*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	26	0.1-12	4	NR	NR	1-8.6
Incidental Ingestion	133	1-25	1	NR	1	0.1-7
Incidental Inhalation-Spray	1; 94[a]; 40[b]	0.1-2 [a]	4 [a];4 [b]	NR	NR	0.2 [a]
Incidental Inhalation-Powder	40[b]	5; 0.01-8[c]	4 [b]	NR	NR	1; 0.03-5[c]
Dermal Contact	178	0.005-25	24	NR	NR	0.00028-25.6
Deodorant (underarm)	NR	not spray: 0.1 spray: 0.1	NR	NR	NR	NR
Hair – Non-Coloring	13	0.1-2	NR	NR	NR	0.2
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	1	NR	NR	NR	NR	NR
Mucous Membrane	133	0.005-25	1	NR	1	0.1-7
Baby Products	NR	0.3	NR	NR	NR	NR

NR = Not Reported
* 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.
[a] It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays
[b] Not specified these products are sprays or powders, but it is possible the use can be as a spray or powder, therefore the information is captured in both categories
[c] It is possible that these products may be powders, but it is not specified whether the reported uses are powders

Table 4. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION					
In Vitro					
Mixture containing 1 % Phytosteryl/Octyldodecyl Lauroyl Glutamate	150 mg ± 5 mg, applied in duplicate	2 different lots of reconstructed human epidermis (EpiSkin™)	Epidermis was prepared; a negative and positive control were tested in triplicate. At the end of incubation an MTT test was performed. Samples were plated, biopsied, and the epidermis was separated from the collagen and transferred to tubes. Cell viability was then determined. Acceptability and expression of results followed	Mean viability greater than 50% is interpreted as being potentially non-irritant; in two samples the mean viability resulted in 81.1% and 72.4%, thus this mixture is considered potentially non-irritant.	11
Human					
Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate cream (concentration not specified)	15 µl, applied as is.	31 subjects	24-h occlusive patch test. The test sample was applied to the backs of subjects and fixed with plaster. Reactions were scored after 30 min, and at 24 and 48 h after patch removal.	One subject had a 0.5 score after 30 min that resolved to 0 at 24 and 48 h after patch removal. Another subject had a score of 0.5 only at 48 h after patch removal. All other subjects had scores of 0 at all time points. This cream is considered a non-irritant on human skin.	4
Face cream containing 1% Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate	0.2 ml, applied neat	25 subjects	Human cumulative irritation patch test. On study day 1, a semi-occlusive patch containing 0.2.ml of the test sample was applied to the backs of subjects for 23 (+/- 1) h. On study days 2-6, patches were removed and graded 30 min following patch removal using a 60-W daylight blue bulb. Patches were then reapplied to the same area on the subjects. On study day 7, patches were removed and graded.	On the last day of the study, 5 subjects exhibited elevated irritation grades (≥ 2). All elevated grades were resolved. Two adverse events occurred during the course of the study, but were related to study procedures (i.e., tape irritation), not the test study material. Based on the cumulative irritation index, no unexpected skin conditions were observed and the test material elicited skin responses similar to the negative irritant control.	12
Facial essence containing 1.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate	0.2 ml, applied neat	38 subjects	A 7-d semi-occlusive cumulative irritation patch study was performed. Distilled water served as the negative control and 0.75% SLS served as a positive control. Prior to the first application, sites were wiped with 70% isopropyl alcohol. Two-tenths (0.2) ml of the test sample was applied with a 2cm x 2cm pad to the back and upper arm for 23 (± 1) h and then removed. After patch removal sites were evaluated, and the responses recorded. This was repeated daily for 7 d.	Under the conditions employed in the study, the subjects showed no evidence of irritation.	13
SENSITIZATION					
In Chemico					
Phytosteryl/Octyldodecyl Lauroyl Glutamate	acetonitrile		A DPRA measuring reactivity (percent depletion) of cysteine and lysine peptides was conducted on Phytosteryl/Octydodecyl Lauroyl Glutamate dissolved in acetonitrile. The positive control was in the appropriate range for both peptides cysteine 60.8%<mean<100%; Lysine: 40.2<mean<69.4%)	The percent peptide depletion value of cysteine was 1.8% and 0.1% for lysine. Depletion less than 14.9 is considered to have no, or minimal reactivity, and is predicted to be negative for dermal sensitization. The control had the expected results and was predicted to have minimal reactivity.	14
Human					
Face cream containing 5% Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate	0.2 ml applied as neat	102 subjects	HRIPT evaluating sensitization potential. During induction product was placed on an occlusive patch (2 cm x 2 cm) no longer than 15 min prior to patch application. The induction phase consisted of nine 24-h applications made over 3 wk. After a 10–15-d non-treatment period, challenge patches were applied for 24 h to previously untreated sites. Reactions were scored at 48 h and 72 h after patch removal.	During induction, no reactions were reported, and none were observed for any of the subjects at challenge. Under the conditions employed in this study, there was no evidence of sensitization to the product.	15

Table 4. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Mixture containing 5.999% Phytosteryl/ Octyldodecyl Lauroyl Glutamate	0.2ml applied as supplied.	219 subjects	HRIPT evaluating sensitization potential. During induction, product was placed on an occlusive patch (2 cm x 2 cm), which was applied to the infrascapular area of the back (either to right or left of midline), or to the upper arm. Induction phase consisted of nine 24-h applications made over 4 consecutive weeks. After a 10-15 d non-treatment period, challenge patches were applied for 24 h to previously untreated sites. Reactions were scored at 48 h and 72 h after patch removal.	During induction, no reactions were reported, and none were observed for any of the subjects at challenge. Under the conditions employed in this study, there was no evidence of sensitization to the product.	[16]

Abbreviations: HRIPT – human repeated insult patch test; SIOPT – single insult occlusive patch test; SLS – sodium lauryl sulfate

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Memorandum

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

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

DATE: August 16, 2022

SUBJECT: Phytosteryl/Octyldodecyl Lauroyl Glutamate

RCC. 1999. *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay with Phytosteryl/Octyldodecyl Lauroyl Glutamate (neat).

RCC. 1999. *In vitro* chromosome aberration assay in Chinese hamster V79 cells with Phytosteryl Octyldodecyl Lauroyl Glutamate (neat).

RCC. 1999. 28-Day oral toxicity (gavage) study in the Wistar rat (Phytosteryl/Octyldodecyl Lauroyl Glutamate neat).

Anonymous. 2015. Direct peptide reactivity assay data for L-glutamic acid, N-(1-oxododecyl), mixed (3.Beta., 24R)-ergost-5-en-3yl and 2-octyldodecyl and (3.beta.,22E)-stigmasta-5,220dien-3-yl and (3.beta.)-stigmast-5-en-3-yl esters (Phytosteryl Octyldodecyl Lauroyl Glutamate neat).

TKL Research. 2013. Human cumulative irritation patch test (facial essence with 1.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate).

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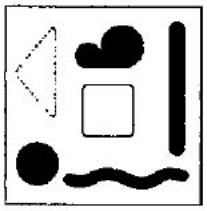
[redacted]



6/6 [redacted]

ok

RCC - CCR PROJECT [redacted]

Project ID of the Contracting Institute: RCC [redacted]

SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI REVERSE MUTATION ASSAY

WITH

Phytosteryl Octyldodecyl lauroyl glutamate

[redacted]

REPORT

Study Completion Date:

May 14, 1999

RCC

COPY OF GLP CERTIFICATE

HESSISCHES MINISTERIUM
FÜR UMWELT, ENERGIE,
JUGEND, FAMILIE UND
GESUNDHEIT

GLP-Bescheinigung

Bescheinigung

Hiermit wird bestätigt, daß die Prüfeinrichtung
RCC Cytotest Cell Research GmbH
in 64380 Roßdorf
In den Leppsteinswiesen 19
(Ort, Anschrift)
der RCC/CCR Holding Verwaltungs GmbH
(Firma)
am 25./26. Februar 1998
(Datum)

von der für die Überwachung zuständigen Behörden über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist.

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden:

Prüfungen zur Bestimmung der toxikologischen Eigenschaften
Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)

Certificate

It is hereby certified that the test facility
RCC Cytotest Cell Research GmbH
in 64380 Roßdorf
In den Leppsteinswiesen 19
(location, address)
of RCC/CCR Holding Verwaltungs GmbH
(company name)
on 25./26. Februar 1998
(date)

was inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice:

Toxicity studies

Mutagenicity studies

Im Auftrag

Dr. Hecker

(Dr. Hecker) Wiesbaden, den 30. März 1998

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PREFACE

General

Sponsor: [redacted]

Study Monitor:

Testing Facility: **R C C**
CYTOTEST CELL RESEARCH GMBH
In den Leppsteinswiesen 19
D-64380 Roßdorf

RCC-CCR Project No.: [redacted]

Contracting Institute: R C C LTD.
REGISTRATION AND CONSULTING DIVISION
CH-4452 Itingen

RCC Project No.: [redacted]

Test Article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

RCC-CCR Test Article No.: [redacted]

Title: Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay with [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

Project Staff

Study Director: Dr. Hans-Eric Wollny

Management: Markus Arenz

Quality Assurance Unit: Frauke Hermann

Schedule

Date of Protocol and German Translation:	January 25, 1999
Start of Experiments:	February 09, 1999
End of Experiments:	February 22, 1999
Date of Draft:	March 03, 1999
Date of 2nd Draft	April 19, 1999
Date of Final Report:	May 14, 1999

Project Staff Signatures

Study Director — Dr. Hans-Eric Wollny

Wollny

Date: May 14, 1999

Management — Markus Arenz

ppa G. Völkel

Date: May 14, 1999

Quality Assurance

The study was performed in compliance with:

„Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, „Anhang 1" (Annexe 1) dated July 25, 1994 („BGBl. I 1994", pp. 1703), last revision: May 14, 1997

"The OECD Principles of Good Laboratory Practice", Paris 1981

Guidelines

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

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

EEC Directive 92/69, L 383 A, Annexe V, B 13, B 14 dated December 29, 1992

Archiving

RCC Cytotest Cell Research, D-64380 Roßdorf will archive the following data for 15 years:

Raw data, protocol, and a copy of the report.

The following sample will be archived for at least 2 years following the date on which the report is audited by the Quality Assurance Unit and also at least until the next inspection of RCC Cytotest Cell Research by the GLP-authority:

A sample of the test article

If there are no other instructions by the sponsor the raw data and the above mentioned material will be discarded at the end of the archiving period.

Deviations to Protocol

Test Article:

Present:

Aggregate State
at Room Temperature: liquid

New:

Aggregate State
at Room Temperature: **oily liquid**

Reason for the deviation: requested by the sponsor

S9 (Preparation by R C C - C C R)

Present:

The protein concentration in the S9 preparation **usually is between 20 and 45** mg/ml.

New:

The protein concentration in the S9 preparation was **50.4** mg/ml (lot no. 181298).

Reason for the deviation: updating

These deviations had no detrimental impact on the outcome of the study.

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STATEMENT OF COMPLIANCE

Project Number: [redacted]

Test Article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

Study Director: Dr. Hans-Eric Wollny

Title: Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay with [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

This study performed in the testing facility of RCC Cytotest Cell Research was conducted in compliance with Good Laboratory Practice Regulations.

„Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, „Anhang 1" (Annexe 1) dated July 25, 1994 („BGBl. I 1994", pp. 1703), last revision: May 14, 1997

"The OECD Principles of Good Laboratory Practice", Paris 1981

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

Study Director

RCC - CCR
Dr. Hans-Eric Wollny

Wollny
.....

Date: May 21, 1999

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QUALITY ASSURANCE UNIT

R C C Cytotest Cell Research GmbH
In den Leppsteinswiesen 19,
D-64380 Roßdorf

Statement

Project Number: [redacted]

Test Article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

Study Director: Dr. Hans-Eric Wollny

Title: Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay with [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

This report was audited by the Quality Assurance Unit and the conduct of this study was inspected on the following dates.

Phases and Dates of QAU Inspections/ Audits			Dates of Reports to the Study Director and to Management	
Protocol Audit:	January	26, 1999	January	26, 1999
Process Inspection:	February	19, 1999	February	19, 1999
Draft Audit:	March	04, 1999	March	04, 1999

Head of Quality Assurance Unit — Frauke Hermann

F. Hermann

Date: May 19, 1999

SUMMARY OF RESULTS

This study was performed to investigate the potential of [redacted] (Phytosteryl Octyldodecyl lauroyl glutamate) to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 100, and the Escherichia coli strain WP2 uvrA.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test article was tested at the following concentrations:

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

The plates incubated with the test article showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments.

No relevant toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation in experiment I.

Toxic effects were observed at the following concentrations in experiment II:

Strain	Without S9 mix	With S9 mix
TA 1535	1000 - 5000 µg/plate	/
TA 1537	33 - 5000 µg/plate	1000 µg/plate
TA 98	5000 µg/plate	/
TA 100	1000 µg/plate	/
WP2 uvrA	/	/

/ no relevant toxic effects observed

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

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

Conclusion

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

Therefore, [redacted] (Phytosteryl Octyldodecyl lauroyl glutamate) is considered to be non-mutagenic in this Salmonella typhimurium and Escherichia coli reverse mutation assay.

OBJECTIVE

Aims of the Study

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

Reasons for the Study

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

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

The Salmonella typhimurium histidine (his) and the E. coli tryptophan (trp) reversion system measures $his^- \rightarrow his^+$ and $trp^- \rightarrow trp^+$ reversions, respectively. The S. typhimurium and Escherichia coli strains are constructed to differentiate between base pair (TA 1535, TA 100, WP2 uvrA) and frameshift (TA 1537, TA 98) mutations.

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

To establish a dose response effect 6 dose levels with adequately spaced intervals were tested. The maximum dose level was 5000 µg/plate.

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

MATERIALS AND METHODS

Test Article

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

Name:	[redacted] Phytosteryl Octyldodecyl lauroyl glutamate
Batch No.:	808010
Aggregate State at Room Temperature:	oily liquid
Colour:	pale yellow
Analysis:	HPLC
Purity:	100 %
Stability in solvent:	7 days in water
Storage:	room temperature
Expiration Date:	March 31, 2000

On the day of the experiment, the test article [redacted] Phytosteryl Octyldodecyl lauroyl glutamate was dissolved in acetone (purity ≥ 99 %; MERCK, D-64293 Darmstadt). The solvent was chosen because of its solubility properties and its relative non-toxicity to the bacteria.

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

Controls

Negative Controls

Concurrent untreated and solvent controls were performed.

Positive Control Substances

Without metabolic activation

Strains:	TA 1535, TA 100
Name:	sodium azide, NaN_3
Supplier:	SERVA, D-69042 Heidelberg
Catalogue No.:	30175
Purity:	at least 99 %
Dissolved in:	water deionised
Concentration:	10 µg/plate

Strains:	TA 1537, TA 98
Name:	4-nitro-o-phenylene-diamine, 4-NOPD
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	N 9504
Purity:	> 99.9 %
Dissolved in:	DMSO (MERCK, D-64293 Darmstadt; purity > 99 %)
Concentration:	10 µg/plate in TA 98, 50 µg/plate in TA 1537

Strains:	WP2 uvrA
Name:	methyl methane sulfonate, MMS
Supplier:	Merck-Schuchardt, D-85662 Hohenbrunn
Catalogue No.:	820775
Purity:	> 99.0 %
Dissolved in:	water deionised
Concentration:	5 µl/plate

With metabolic activation

Strains:	TA 1535, TA 1537, TA 98, TA 100, WP2 uvrA
Name:	2-aminoanthracene, 2-AA
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	A 1381
Purity:	97.5 %
Dissolved in:	DMSO (MERCK, D-64293 Darmstadt; purity > 99 %)
Concentration:	2.5 µg/plate in strains TA 1535, TA 1537, TA 98, and TA 100 10 µg/plate in strain WP2 uvrA

The stability of the positive control substances in solution was unknown but a mutagenic response in the expected range means a biological stability demonstration. The dilutions of the stock solutions were prepared on the day of the experiment and used immediately.

Test System

Characterisation of the Salmonella typhimurium Strains and E. coli Strain

The histidine dependent strains are derived from S. typhimurium strain LT2 through a mutation in the histidine locus. Additionally due to the "deep rough" (rfa-minus) mutation they possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. A further mutation causes a reduction in the activity of an excision repair system. The latter alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named "uvrB-minus". In the strains TA 98 and TA 100 the R-factor plasmid pKM 101 carries the ampicillin resistance marker.

Strain WP2 (5) and its derivatives all carry the same defect in one of the genes for tryptophan biosynthesis. Tryptophan-independent (Trp^+) mutants (revertants) can arise either by a base change at the site of the original alteration or by a base change elsewhere in the chromosome so that the original defect is suppressed. This second possibility can occur in several different ways so that the system seems capable of detecting all types of mutagen which substitute one base for another. Additionally, the uvrA derivative is deficient in the DNA repair process (excision repair damage). Such a repair-deficient strain may be more readily mutated by agents.

When summarised the mutations of the TA strains and the E. coli strain, used in this study can be described as follows:

Salmonella typhimurium

TA1537:	his C 3076; rfa^-; $uvrB^-$:	frame shift mutations
TA 98:	his D 3052; rfa^-; $uvrB^-$;R-factor:	" " "
TA1535:	his G 46; rfa^-; $uvrB^-$:	base-pair substitutions
TA 100:	his G 46; rfa^-; $uvrB^-$;R-factor:	" "

Escherichia coli

WP2 uvrA:	trp; $uvrA^-$:	base-pair substitutions and others

Regular checking of the properties of the strains regarding the membrane permeability and ampicillin resistance as well as spontaneous mutation rates is performed in RCC Cytotest Cell Research according to Ames et al. (1). In this way it was ensured that the experimental conditions set down by Ames were fulfilled.

The bacterial strains TA 1535, TA 98, and TA 100 were obtained from Dr. B.N. Ames (University of California, 94720 Berkeley, U.S.A.). The bacterial strain TA 1537 was obtained from BASF (D-67063 Ludwigshafen). The bacterial strain WP2 uvrA was obtained from Dr. Heinz Träger, Knoll AG, D-67008 Ludwigshafen.

Storage

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

Precultures

From the thawed ampoules of the strains 0.5 ml suspension was transferred into 250 ml Erlenmeyer flasks containing 20 ml nutrient medium. A solution of 20 µl ampicillin (25 µg/ml) was added to the strains TA 98 and TA 100. This nutrient medium contains per litre:

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

The bacterial cultures were incubated in a shaking water bath for 8 hours at 37° C.

Selective Agar

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

Overlay Agar

The overlay agar contains per litre:

for Salmonella strains:	for Escherichia coli:
6.0 g MERCK Agar Agar*	6.0 g MERCK Agar Agar*
6.0 g NaCl*	6.0 g NaCl*
10.5 mg L-Histidine×HCl×H_2O*	2.5 mg Tryptophan*
12.2 mg Biotin*	

* (MERCK, D-64293 Darmstadt)

Sterilisations were performed at 121° C in an autoclave.

Mammalian Microsomal Fraction S9 Mix

S9 (Preparation by R C C - C C R)

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

The S9 liver microsomal fraction was obtained from the livers of 8 - 12 weeks old male rats, strain Wistar HanIbm (BRL, CH-4414 Füllinsdorf; weight approx. 220 - 320 g) which received daily applications of 80 mg/kg b.w. Phenobarbital i.p. dissolved in aqua deionised (Desitin; D-22335 Hamburg) and β-Naphthoflavone orally dissolved in corn oil (Aldrich, D-89555 Steinheim) on three subsequent days. The livers were prepared 24 hours after the last treatment.

After decapitation of the anaesthetised animals the livers of the animals were removed, washed in 150 mM KCl and homogenised. The homogenate was diluted 1+3 in KCl and centrifuged cold at 9,000 g for 10 minutes at 4° C. A stock of the supernatant containing the microsomes was frozen in ampoules and stored at -80° C. Small numbers of the ampoules are kept at - 20° C for up to one week before use. The protein content was determined using an analysis kit of Bio-Rad Laboratories, D-80939 München (Bio-Rad protein assay, Catalogue No. 5000006).

The protein concentration in the S9 preparation was 50.4 mg/ml (lot no. 181298).

S9 Mix

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 co-factor solution. The amount of S9 supernatant was 15% v/v in the cultures. The concentrated co-factor solution yields the following concentrations in the S9 mix:

8 mM $MgCl_2$
33 mM KCl
5 mM Glucose-6-phosphate
5 mM NADP

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

During the experiment the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames et al.(2).

Pre-Experiment for Toxicity

To evaluate the toxicity of the test article a pre-experiment was performed with strains TA 98 and TA 100. Eight concentrations were tested for toxicity and mutation induction with three plates each. The experimental conditions in this pre-experiment were the same as described below for the experiment I (plate incorporation test).

Toxicity of the test article results in a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.

Dose Selection

Based upon the results of this pre-experiment the concentrations applied in the main experiments were chosen. The concentration range covered two logarithmic decades. Two independent experiments were performed.

According to the dose selection criteria the test article was tested at the following concentrations:

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

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

Evaluable plates (>0 colonies) at five concentrations or more.

Experimental Performance

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

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

- 100 µl Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
- 500 µl S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
- 100 µl Bacteria suspension (cf. test system, pre-culture of the strains),
- 2000µl Overlay agar

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

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

Data Recording

The colonies were counted using the AUTOCOUNT (Artek Systems Corporation, BIOSYS GmbH, D-61184 Karben). The counter was connected to an IBM AT compatible PC with printer to print out the individual values and the means from the plates for each concentration together with standard deviations and enhancement factors as compared to the spontaneous reversion rates (see tables of results).

Acceptability of the Assay

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

- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of our historical data
- the positive control substances should produce a significant increase in mutant colony frequencies

Evaluation of Results

A test article is considered positive if either a dose related and reproducible increase in the number of revertants or a biologically relevant and reproducible increase for at least one test concentration is induced.

A test article producing neither a reproducible and dose related increase in the number of revertants, nor a biologically relevant and reproducibly positive response at any one of the test points is considered non-mutagenic in this system.

A mutagenic response is described as follows:

A test article is considered mutagenic if in the strains TA 98, TA 100, and WP2 uvrA the number of reversions will be at least twice as high and in the strains TA 1535 and TA 1537 at least three times higher as compared to the spontaneous reversion rate (3,4).

Also, a dose-dependent and reproducible increase in the number of revertants is regarded as an indication of possibly existing mutagenic potential of the test article regardless whether the highest dose induced the above described enhancement factors or not.

Biometry

No statistical evaluation of the data is required.

DISCUSSION OF RESULTS

The test article [redacted] (Phytosteryl Octyldodecyl lauroyl glutamate) was assessed for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA 1535, TA 1537, TA 98 and TA 100 and the Escherichia coli strain WP2 uvrA.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration and the controls were tested in triplicate. The test article was tested at the following concentrations:

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

The plates incubated with the test article showed normal background growth up to 5000 µg/plate with and without S9 mix in both experiments.

No relevant toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation in experiment I.

Toxic effects were observed at the following concentrations in experiment II:

Strain	Without S9 mix	With S9 mix
TA 1535	1000 - 5000 µg/plate	/
TA 1537	33 - 5000 µg/plate	1000 µg/plate
TA 98	5000 µg/plate	/
TA 100	1000 µg/plate	/
WP2 uvrA	/	/

/ no relevant toxic effects observed

The toxic effects were more pronounced in the second experiment since the pre-incubation test design is more sensitive towards both, the mutagenic and toxic potential of a test item.

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

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

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

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ANNEXE: TABLES OF RESULTS

Pre-Experiment for Toxicity

8 concentrations were tested for toxicity and mutation induction with each 3 plates.

The results are given in the following table:

Table 1:

Substance	Concentration per plate [µg]	Revertants per plate TA 98		TA 100	
		–	+	–	+*
Negative control	–	19	27	105	133
Solvent control	–	24	31	97	157
4-NOPD	10.0	332	/	/	/
Sodium azide	10.0	/	/	529	/
2-aminoanthracene	2.5	/	271	/	747
Test article					
	3	29	36	90	110
	10	28	26	90	133
	33	30	31	89	101
	100	33	32	120	110
	333	27	32	105	116
	1000	37	25	115	88
	2500	43	27	89	102
	5000	30	37	93	111

The plates with the test article showed normal background growth up to 5000.0 µg/plate in strain TA 98 and TA 100.

According to the dose selection criteria, the test article was tested at the following concentrations:

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

* – = without S9 mix; + = with S9 mix, / = not performed

Experiment I: Plate Incorporation Test

Test article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

S9 mix from : Rat liver (Batch R 181298)

Test strain: TA 1535

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	13	11	16	13	2.5	
Solvent Control	13	21	12	15	4.9	1.0
Positive Control#	550	629	533	571	51.2	37.2
33	17	21	12	17	4.5	1.1
100	17	12	22	17	5.0	1.1
333	14	13	13	13	0.6	0.9
1000	13	13	13	13	0.0	0.8
2500	16	10	12	13	3.1	0.8
5000	12	10	7	10	2.5	0.6

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	7	10	12	10	2.5	
Solvent Control	12	13	13	13	0.6	1.0
Positive Control##	189	149	168	169	20.0	13.3
33	9	8	17	11	4.9	0.9
100	8	9	10	9	1.0	0.7
333	12	13	7	11	3.2	0.8
1000	14	7	11	11	3.5	0.8
2500	10	11	16	12	3.2	1.0
5000	16	12	17	15	2.6	1.2

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

sodium azide 10 µg/plate
2-aminoanthracene 2.5 µg/plate

Experiment I: Plate Incorporation Test

Test article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

S9 mix from : Rat liver (Batch R 181298)

Test strain: TA 1537

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	22	11	15	16	5.6	
Solvent Control	11	9	9	10	1.2	1.0
Positive Control#	144	112	102	119	21.9	12.3
33	12	13	9	11	2.1	1.2
100	16	17	14	16	1.5	1.6
333	12	13	8	11	2.6	1.1
1000	8	15	10	11	3.6	1.1
2500	10	17	16	14	3.8	1.5
5000	14	12	9	12	2.5	1.2

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	11	13	10	11	1.5	
Solvent Control	11	20	16	16	4.5	1.0
Positive Control##	120	120	122	121	1.2	7.7
33	10	13	11	11	1.5	0.7
100	6	8	8	7	1.2	0.5
333	13	6	11	10	3.6	0.6
1000	16	8	11	12	4.0	0.7
2500	15	9	11	12	3.1	0.7
5000	12	12	9	11	1.7	0.7

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

4-nitro-o-phenylene-diamine 50 µg/plate
2-aminoanthracene 2.5 µg/plate

Experiment I: Plate Incorporation Test

Test article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

S9 mix from : Rat liver (Batch R 181298)

Test strain: TA 98

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	22	18	18	19	2.3	
Solvent Control	28	18	27	24	5.5	1.0
Positive Control#	246	390	359	332	75.8	13.6
33	23	34	34	30	6.4	1.2
100	36	37	25	33	6.7	1.3
333	31	25	25	27	3.5	1.1
1000	32	33	46	37	7.8	1.5
2500	43	37	49	43	6.0	1.8
5000	31	36	22	30	7.1	1.2

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	23	27	32	27	4.5	
Solvent Control	22	37	35	31	8.1	1.0
Positive Control##	243	294	277	271	26.0	8.7
33	35	29	28	31	3.8	1.0
100	29	40	28	32	6.7	1.0
333	37	23	35	32	7.6	1.0
1000	27	23	24	25	2.1	0.8
2500	23	28	31	27	4.0	0.9
5000	38	34	38	37	2.3	1.2

$$\text{* enhancement factor} = \frac{\Sigma_{\text{revertants / concentr. test article}}}{\Sigma_{\text{revertants / solvent control}}}$$

4-nitro-o-phenylene-diamine 10 µg/plate
2-aminoanthracene 2.5 µg/plate

Experiment I: Plate Incorporation Test

Test article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

S9 mix from : Rat liver (Batch R 181298)

Test strain: TA 100

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	82	87	146	105	35.6	
Solvent Control	113	83	96	97	15.0	1.0
Positive Control#	424	645	518	529	110.9	5.4
33	84	95	89	89	5.5	0.9
100	138	129	93	120	23.8	1.2
333	96	138	81	105	29.5	1.1
1000	97	124	125	115	15.9	1.2
2500	80	92	94	89	7.6	0.9
5000	101	90	89	93	6.7	1.0

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	111	135	152	133	20.6	
Solvent Control	161	158	151	157	5.1	1.0
Positive Control##	749	747	746	747	1.5	4.8
33	115	106	83	101	16.5	0.6
100	111	105	113	110	4.2	0.7
333	81	93	175	116	51.2	0.7
1000	72	78	115	88	23.3	0.6
2500	70	98	137	102	33.7	0.6
5000	107	86	139	111	26.7	0.7

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

sodium azide 10 µg/plate
2-aminoanthracene 2.5 µg/plate

Experiment I: Plate Incorporation Test

Test article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

S9 mix from : Rat liver (Batch R 181298)

Test strain: WP2 uvrA

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	30	38	40	36	5.3	
Solvent Control	42	39	26	36	8.5	1.0
Positive Control#	654	545	567	589	57.6	16.5
33	23	37	30	30	7.0	0.8
100	35	38	48	40	6.8	1.1
333	30	22	18	23	6.1	0.7
1000	33	37	30	33	3.5	0.9
2500	33	38	39	37	3.2	1.0
5000	30	35	40	35	5.0	1.0

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	44	39	38	40	3.2	
Solvent Control	39	46	30	38	8.0	1.0
Positive Control##	171	170	187	176	9.5	4.6
33	40	40	34	38	3.5	1.0
100	39	36	33	36	3.0	0.9
333	46	41	42	43	2.6	1.1
1000	36	49	40	42	6.7	1.1
2500	32	43	42	39	6.1	1.0
5000	39	49	49	46	5.8	1.2

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

methyl methane sulfonate 5 µl/plate
2-aminoanthracene 10 µg/plate

Experiment II: Pre-Incubation Test

Test article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

S9 mix from : Rat liver (Batch R 181298)

Test strain: TA 1535

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	13	15	15	14	1.2	
Solvent Control	12	13	13	13	0.6	1.0
Positive Control#	488	513	464	488	24.5	38.6
33	9	9	8	9	0.6	0.7
100	18	16	16	17	1.2	1.3
333	8	12	12	11	2.3	0.8
1000	3	6	3	4	1.7	0.3
2500	2	7	5	5	2.5	0.4
5000	5	6	5	5	0.6	0.4

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	10	19	15	15	4.5	
Solvent Control	12	12	15	13	1.7	1.0
Positive Control##	281	266	272	273	7.5	21.0
33	16	16	14	15	1.2	1.2
100	9	9	13	10	2.3	0.8
333	14	12	12	13	1.2	1.0
1000	14	14	14	14	0.0	1.1
2500	14	10	9	11	2.6	0.8
5000	16	17	16	16	0.6	1.3

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# sodium azide 10 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment II: Pre-Incubation Test

Test article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

S9 mix from : Rat liver (Batch R 181298)

Test strain: TA 1537

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	11	12	15	13	2.1	
Solvent Control	14	14	16	15	1.2	1.0
Positive Control#	125	98	128	117	16.5	8.0
33	6	7	6	6	0.6	0.4
100	9	8	8	8	0.6	0.6
333	3	4	7	5	2.1	0.3
1000	8	8	5	7	1.7	0.5
2500	5	6	6	6	0.6	0.4
5000	7	6	7	7	0.6	0.5

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	11	16	10	12	3.2	
Solvent Control	13	19	13	15	3.5	1.0
Positive Control##	164	175	181	173	8.6	11.6
33	12	11	7	10	2.6	0.7
100	12	8	8	9	2.3	0.6
333	11	16	21	16	5.0	1.1
1000	9	4	7	7	2.5	0.4
2500	12	7	8	9	2.6	0.6
5000	9	14	14	12	2.9	0.8

$$\text{* enhancement factor} = \frac{\Sigma_{\text{revertants / concentr. test article}}}{\Sigma_{\text{revertants / solvent control}}}$$

4-nitro-o-phenylene-diamine 50 µg/plate
2-aminoanthracene 2.5 µg/plate

Experiment II: Pre-Incubation Test

Test article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

S9 mix from : Rat liver (Batch R 181298)

Test strain: TA 98

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	16	16	17	16	0.6	
Solvent Control	17	17	17	17	0.0	1.0
Positive Control#	299	290	240	276	31.8	16.3
33	19	14	6	13	6.6	0.8
100	15	35	21	24	10.3	1.4
333	6	11	11	9	2.9	0.5
1000	12	13	13	13	0.6	0.7
2500	9	9	8	9	0.6	0.5
5000	5	8	8	7	1.7	0.4

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	20	17	19	19	1.5	
Solvent Control	21	16	15	17	3.2	1.0
Positive Control##	274	292	168	245	67.0	14.1
33	9	17	14	13	4.0	0.8
100	17	15	20	17	2.5	1.0
333	17	18	26	20	4.9	1.2
1000	16	13	20	16	3.5	0.9
2500	22	15	15	17	4.0	1.0
5000	15	29	18	21	7.4	1.2

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

4-nitro-o-phenylene-diamine 10 µg/plate
2-aminoanthracene 2.5 µg/plate

Experiment II: Pre-Incubation Test

Test article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

S9 mix from : Rat liver (Batch R 181298)

Test strain: TA 100

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	85	82	98	88	8.5	
Solvent Control	102	104	102	103	1.2	1.0
Positive Control#	1088	1073	1038	1066	25.7	10.4
33	53	115	103	90	32.9	0.9
100	57	56	60	58	2.1	0.6
333	51	52	37	47	8.4	0.5
1000	49	44	44	46	2.9	0.4
2500	51	67	42	53	12.7	0.5
5000	51	45	56	51	5.5	0.5

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	78	86	84	83	4.2	
Solvent Control	78	78	77	78	0.6	1.0
Positive Control##	670	581	599	617	47.1	7.9
33	71	62	61	65	5.5	0.8
100	58	83	66	69	12.8	0.9
333	91	85	94	90	4.6	1.2
1000	70	77	75	74	3.6	1.0
2500	73	70	66	70	3.5	0.9
5000	73	61	84	73	11.5	0.9

$$\text{* enhancement factor} = \frac{\Sigma_{\text{revertants / concentr. test article}}}{\Sigma_{\text{revertants / solvent control}}}$$

sodium azide 10 µg/plate
2-aminoanthracene 2.5 µg/plate

Experiment II: Pre-Incubation Test

Test article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

S9 mix from : Rat liver (Batch R 181298)

Test strain: WP2 uvrA

without S9 mix

Concentration µg/plate	Plate 1	Plate 2	Plate 3	Revertants / plate mean	s.d.	factor*
Negative Control	31	32	31	31	0.6	
Solvent Control	31	28	28	29	1.7	1.0
Positive Control#	250	251	259	253	4.9	8.7
33	35	27	28	30	4.4	1.0
100	31	32	32	32	0.6	1.1
333	31	17	29	26	7.6	0.9
1000	22	32	23	26	5.5	0.9
2500	24	28	41	31	8.9	1.1
5000	35	47	36	39	6.7	1.4

with S9 mix

Concentration µg/plate	Plate 1	Plate 2	Plate 3	Revertants / plate mean	s.d.	factor*
Negative Control	42	30	55	42	12.5	
Solvent Control	38	31	31	33	4.0	1.0
Positive Control##	198	190	197	195	4.4	5.9
33	35	27	38	33	5.7	1.0
100	31	28	38	32	5.1	1.0
333	42	34	31	36	5.7	1.1
1000	37	29	25	30	6.1	0.9
2500	26	22	22	23	2.3	0.7
5000	32	49	24	35	12.8	1.1

$$\text{* enhancement factor} = \frac{\Sigma\,\text{revertants / concentr. test article}}{\Sigma\,\text{revertants / solvent control}}$$

methyl methane sulfonate 5 µl/plate
2-aminoanthracene 10 µg/plate

Summary of Results

Test article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

S9 mix from: Rat liver (Batch R 181298)

without S9 mix

Concentration µg/plate	Revertants/plate mean from three plates									
	TA 1535		TA 1537		TA 98		TA 100		WP2 uvrA	
	I	II	I	II	I	II	I	II	I	II
Negative control	13	14	16	13	19	16	105	88	36	31
Solvent control	15	13	10	15	24	17	97	103	36	29
Positive control#	571	488	119	117	332	276	529	1066	589	253
33	17	9	11	6	30	13	89	90	30	30
100	17	17	16	8	33	24	120	58	40	32
333	13	11	11	5	27	9	105	47	23	26
1000	13	4	11	7	37	13	115	46	33	26
2500	13	5	14	6	43	9	89	53	37	31
5000	10	5	12	7	30	7	93	51	35	39

with S9 Mix

Concentration µg/plate	Revertants/plate mean from three plates									
	TA 1535		TA 1537		TA 98		TA 100		WP2 uvrA	
	I	II	I	II	I	II	I	II	I	II
Negative control	10	15	11	12	27	19	133	83	40	42
Solvent control	13	13	16	15	31	17	157	78	38	33
Positive control##	169	273	121	173	271	245	747	617	176	195
33	11	15	11	10	31	13	101	65	38	33
100	9	10	7	9	32	17	110	69	36	32
333	11	13	10	16	32	20	116	90	43	36
1000	11	14	12	7	25	16	88	74	42	30
2500	12	11	12	9	27	17	102	70	39	23
5000	15	16	11	12	37	21	111	73	46	35

Sodium azide (10.0 µg/plate) strains TA 1535 and TA 100
4-nitro-o-phenylene-diamine strains TA 1537 (50 µg/plate) and TA 98 (10.0 µg/plate)
Methyl methane sulfonate (5 µl/plate) strain WP2 uvrA

2-aminoanthracene (2.5 µg/plate) strains TA 1535, TA 1537, TA 98, and TA 100
2-aminoanthracene (10.0 µg/plate) strain WP2 uvrA

HISTORICAL CONTROL DATA

Negative Control (without metabolic activation)				
1535	1537	98	100	WP2 uvrA
9 - 29	5 - 28	15 - 57	77 - 189	28-63

Solvent Control (without metabolic activation)				
1535	1537	98	100	WP2 uvrA
9 - 25	4 - 28	14 - 58	77 - 225	26-65

Positive Control (without metabolic activation)				
1535	1537	98	100	WP2 uvrA
68 - 814	42 - 191	86 - 450	460 - 910	156 - 1076

These values represent our historical control data since 1995.

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[redacted]

PROJECT ID OF CONTRACTING INSTITUTE

RCC PROJECT [redacted]

RCC-CCR PROJECT [redacted]

IN VITRO CHROMOSOME ABERRATION ASSAY IN CHINESE HAMSTER V79 CELLS WITH

[redacted]

Phytosteryl Octyldodecyl lauroyl glutamate

REPORT

Study Completion Date:

September 15, 1999

RCC

COPY OF GLP CERTIFICATE

HESSISCHES MINISTERIUM
FÜR UMWELT, ENERGIE,
JUGEND, FAMILIE UND
GESUNDHEIT

GLP-Bescheinigung

Bescheinigung

Hiermit wird bestätigt, daß die Prüfeinrichtung
RCC Cytotest Cell Research GmbH
in 64380 Roßdorf
in den Leppsteinswiesen 19
(Ort, Anschrift)
der RCC/CCR Holding Verwaltungs GmbH
(Firma)
am 25./26. Februar 1998
(Datum)

von der für die Überwachung zuständigen Behörden über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist.

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden:

Prüfungen zur Bestimmung der toxikologischen Eigenschaften
Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)

Certificate

It is hereby certified that the test facility
RCC Cytotest Cell Research GmbH
in 64380 Roßdorf
in den Leppsteinswiesen 19
(location, address)
of RCC/CCR Holding Verwaltungs GmbH
(company name)
on 25./26. Februar 1998
(date)

was inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice:

Toxicity studies

Mutagenicity studies

Im Auftrag

Dr. Hecker

(Dr. Hecker) Wiesbaden, den 30. März 1998

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PREFACE

General

Sponsor:	[redacted]
Study Monitor:	
Testing Facility:	RCC CYTOTEST CELL RESEARCH GMBH In den Leppsteinswiesen 19 D-64380 Roßdorf
RCC-CCR Project No.:	629302
Contracting Institute:	RCC Ltd. Registration & Consulting Division CH-4452 Itingen
RCC Project No.:	[redacted]
Test Article:	[redacted] Phytosteryl Octyldodecyl lauroyl glutamate
RCC-CCR Test Article No.:	[redacted]
Title:	*In vitro* Chromosome Aberration Assay in Chinese Hamster V79 Cells with ELDEW PS-203

Project Staff

Study Director:	Dr. Andreas Czich
Management:	Markus Arenz
Head of Quality Assurance Unit:	Frauke Hermann

Schedule

Date of Protocol and German Translation:	January	26, 1999
Start of Experiments:	February	15, 1999
End of Experiments:	July	26, 1999
Date of Draft:	August	09, 1999
Date of Final Report:	September	15, 1999

Project Staff Signatures

Study Director — Dr. Andreas Czich

Date: September 15, 1999

Management — Markus Arenz

Date: September 15, 1999

Good Laboratory Practice

The study was performed in compliance with:

„Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, „Anhang 1" (Annexe 1), dated July 25, 1994 („BGBl. I 1994", pp. 1703), last revision dated May 14, 1997.

„The OECD Principles of Good Laboratory Practice", Paris, 1981.

Guidelines

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

Ninth Addendum to the OECD Guidelines for Testing of Chemicals, February 1998, adopted July 21, 1997, Guideline Nr.473 „*In vitro* Mammalian Cytogenetic Test".

EEC Directive 92/69, L 383 A, Annexe V, B 10, dated December 29, 1992.

Archiving

RCC Cytotest Cell Research GmbH, D-64380 Roßdorf will archive the following data for 15 years:

Raw data, protocol, and a copy of the report.

A sample of the test article will be archived for at least 2 years following the date on which the report is audited by the Quality Assurance Unit and also at least until the next inspection of RCC Cytotest Cell Research by the GLP-authority.

Microscopic slides will be archived for at least 12 years.

If there are no other instructions by the sponsor the raw data and the above mentioned material will be discarded at the end of the archiving period.

Deviations from the Protocol

Test Article

Aggregate state at
Room Temperature: **oily** liquid

Positive Control Substances

Without metabolic activation

Name:	EMS; Ethylmethane sulfonate
Supplier:	Merck-Schuchardt, D-85662 Hohenbrunn
Catalogue no.:	820774 (purity: > 98 %)
Dissolved in:	nutrient medium
Final Concentration:	600 µg/ml = 4.8 mM **(continuous exposure)**
	1000 µg/ml = 8.0 mM (4 h exposure)

S9 (Preparation by RCC Cytotest Cell Research)

The protein concentration in the S9 preparation is usually between 20 and 45 mg/ml. The protein concentration was **50.4 mg/ml** (Lot. No.: 181298) in the pre-test and 35.3 mg/ml (Lot. No.: 180399) in both main experiments.

Reason for the deviations (printed in bold letters): updating.

These deviations had no detrimental impact on the outcome of the study.

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STATEMENT OF COMPLIANCE

Project Number: [redacted]

Test Article: [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

Study Director: Dr. Andreas Czich

Title: *In vitro* Chromosome Aberration Assay in Chinese Hamster V79 Cells with [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

This study performed in the testing facility of RCC Cytotest Cell Research was conducted in compliance with Good Laboratory Practice Regulations.

„Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, „Anhang 1" (Annexe 1), dated July 25, 1994 („BGBl. I 1994", pp. 1703), last revision dated May 14, 1997.

„The OECD Principles of Good Laboratory Practice", Paris, 1981.

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

Study Director

RCC Cytotest Cell Research GmbH
Dr. Andreas Czich

A. Czich

Date: September 16, 1999

STATEMENT OF QUALITY ASSURANCE UNIT

Project Number:

Test Item: Phytosteryl Octyldodecyl lauroyl glutamate

Study Director: Dr. Andreas Czich

Title: *In vitro* Chromosome Aberration Assay in Chinese Hamster V79 Cells with Phytosteryl Octyldodecyl lauroyl glutamate

The facility of RCC Cytotest Cell Research GmbH and the routine procedures used during the type of study were inspected periodically and the results were reported to the Study Director and the Management.

The protocol and this report were audited by the Quality Assurance Unit and the conduct of the study was inspected process-based on the following dates:

Phases and Dates of QAU Inspections/ Audits		Dates of Reports to the Study Director and to Management
Protocol Audit:	January 28, 1999	January 28, 1999
Process Inspection:	April 21, 1999	April 21, 1999
Draft Audit:	August 10, 1999	August 10, 1999

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

Head of Quality Assurance Unit Frauke Hermann

F. Hermann

Date: September 16, 1999

SUMMARY OF RESULTS

The test article Phytosteryl Octyldodecyl lauroyl glutamate [redacted], dissolved in acetone, was assessed for its potential to induce structural chromosome aberrations in V79 cells of the Chinese hamster *in vitro* in two independent experiments. Following study design was performed:

	without S9-mix			with S9-mix	
	exp. I	exp. II		exp. I	exp. II
Exposure period	4 h	18 h	28 h	4 h	4 h
Recovery	14 h	-	-	14 h	24 h
Preparation interval	18 h	18 h	28 h	18 h	28 h

In each experimental group two parallel cultures were set up. Per culture 100 metaphase plates were scored for structural chromosome aberrations.

The highest applied concentration in the range finding pre-test on toxicity (2500 µg/ml) was chosen with regard to the solubility properties of the test article in accordance to the OECD Guideline 473.

Dose selection of the cytogenetic experiments was performed considering the toxicity data of the pre-test (page 23). The chosen treatment concentrations and the rational are described on page 16 and in table 2 (page 16). The evaluated experimental points and the results are summarised in table 1 (page 10/11).

In both independent experiments, no biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment with the test article. A single not reproduced increases after treatment with an intermediate concentration (1250 µg/ml) in experiment I in the absence of S9 mix must be regarded as being biologically irrelevant.

In addition, no increase in the frequencies of polyploid metaphases was found after treatment with the test article as compared to the frequencies of the controls.

Appropriate mutagens were used as positive controls. They induced statistically significant increases ($p < 0.05$) in cells with structural chromosome aberrations (see page 31).

Conclusion

In conclusion, it can be stated that in the study described and under the experimental conditions reported, the test article did not induce structural chromosome aberrations as determined by the chromosome aberration test in V79 cells (Chinese hamster cell line) *in vitro*.

Therefore, Phytosteryl Octyldodecyl lauroyl glutamate [redacted] **is considered to be non-clastogenic** in this chromosome aberration test.

Table 1: Summary of results of the chromosomal aberration study with [redacted]

Exp.	Preparation interval	Concentration of [redacted] in µg/ml	Polyploid cells in %	Mitotic index in % of control	Aberrant cells in % incl. gaps	excl. gaps*	exchanges
		Exposure period 4 h without S9 mix					
I	18 h	negative control	5.1	100.0	2.5	1.5	0.0
		solvent control[1]	3.2	100.0	2.5	1.5	0.0
		positive control[3]	4.0	76.0	26.0	26.0[S]	16.0
		78.1	3.0	107.0	0.0	0.0	0.0
		156.3	3.1	100.0	1.0	0.5	0.0
		625.0	5.0	97.6	4.0	4.0	4.0
		1250.0	6.8	122.6	5.5	5.5[S]	3.5
		2500.0	6.0	62.4	0.5	0.5	0.0
		Exposure period 18 h without S9 mix					
II	18 h	negative control	4.1	100.0	2.0	0.5	0.0
		solvent control[1]	3.8	100.0	2.0	2.0	0.5
		positive control[2]	3.2	57.3	22.5	20.5[S]	9.5
		78.1	4.4	93.0	3.0	2.0	0.5
		156.3	3.8	108.4	2.5	1.5	0.0
		312.5	3.6	94.6	2.0	0.5	0.5
		1250.0	2.6	111.5	2.0	0.5	0.0
		2500.0	4.6	99.1	1.0	0.5	0.0
		Exposure period 28 h without S9 mix					
II	28 h	solvent control[1]	5.5	100.0	3.5	2.0	1.0
		1250.0	2.8	99.0	3.0	1.0	0.0
		2500.0	3.0	101.0	2.5	1.5	0.0

* inclusive cells carrying exchanges
P precipitation occurred
S aberration frequency statistically significant higher than corresponding solvent control values
1 acetone 0.5 %
2 EMS 600 µg/ml
3 EMS 1000 µg/ml

Table 1 cont.: Summary of results of the chromosomal aberration study with [redacted] Phytosteryl Octyldodecyl lauroyl glutamate

Exp.	Preparation interval	Concentration of [redacted] Phytosteryl Octyldodecyl lauroyl glutamate in µg/ml	Polyploid cells in %	Mitotic index in % of control	Aberrant cells in % incl. gaps	excl. gaps*	exchanges
Exposure period 4 h with S9 mix							
I	18 h	negative control	3.4	100.0	1.5	1.0	0.0
		solvent control[1]	3.1	100.0	1.5	1.0	0.5
		positive control[2]	2.8	65.8	14.0	13.5[S]	7.0
		9.4	2.8	110.7	3.5	3.5	1.0
		18.8	2.7	95.4	3.0	2.5	1.5
		37.5	3.1	68.3	2.0	1.5	0.0
		75.0	2.6	85.8	1.5	0.0	0.0
		300.0[P]	1.5	93.6	2.0	1.0	0.0
II	28 h	negative control	5.0	100.0	2.0	1.0	0.0
		solvent control[1]	6.0	100.0	1.0	1.0	0.0
		positive control[2]	5.4	95.4	10.0	9.5[S]	3.0
		78.1	6.5	114.5	1.0	0.5	0.5
		156.3	3.7	101.2	2.5	2.0	0.0
		312.5	5.3	93.8	1.5	0.5	0.0
		2500.0	4.6	86.4	0.0	0.0	0.0

* inclusive cells carrying exchanges
P precipitation occurred
S aberration frequency statistically significant higher than corresponding solvent control values
1 acetone 0.5 %
2 CPA 0.71 µg/ml

INTRODUCTION

According to national and international acts chemicals have to be tested before introduction to the market for a possible hazard to humans and environment. Genotoxicity studies provide important information for the assessment of the mutagenic potential of these substances (1, 2). The *in vitro* Chromosome Aberration Assay performed in this study is an essential part of genotoxicity test batteries of substances.

This *in vitro* test is an assay for the detection of structural chromosomal aberrations. Such aberrations are frequently lethal to the damaged cells (3, 4). However, cytogenetic damage in somatic cells is an indicator of a potential to induce more subtle chromosomal damage that may be compatible with cell division. Similar damage induced in germ cells may lead to heritable cytogenetic abnormalities. Heritable cytogenetic abnormalities are known to have deleterious effects in man, e.g. induction of neoplastic events or birth defects. Also, chromosome abnormalities in somatic cells may become one of the reasons, why a transformed cell population developes possibly leading to neoplastic processes.

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

For treatment, cell cultures should be in exponential growth to guarantee that there are cells in all stages of the cell cycle (i.e. an asynchronous population). Since the normal cell cycle time is 12 h (see page 14) and the guidelines require fixation times of about 1.5-fold of the normal cell cycle, a fixation time of around 18 hours is appropriate. Because there may be chemicals which induce a very extensive mitotic delay at clastogenic concentrations or may be clastogenic only when cells have passed through more than one cell cycle after the beginning of treatment an additional later sampling time (28 h) should be included (5). Due to the limited capacity for metabolic activation of potential mutagens an exogenous metabolic activation system is necessary to include (6).

The frequency of polyploid/endoreduplicated cells should also be noted so that the level in control cultures can be monitored and their induction by the test article can be recorded especially when a late sampling time is used.

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

Aims of the Study

Phytosteryl Octyldodecyl lauroyl glutamate

This *in vitro* assay was performed to assess the potential of [redacted] to induce structural chromosome aberrations. Evaluation of cytogenetic damage induced in V79 cells (cell line from the lung of the Chinese Hamster) in the absence and the presence of metabolic activation was performed in two independent experiments at one preparation interval (18 h) in experiment I and at two preparation intervals (18 h and 28 h) in experiment II.

MATERIALS AND METHODS

Test Article

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

Name:	[redacted] Phytosteryl Octyldodecyl lauroyl glutamate
Batch No.:	808010
Aggregate state at Room Temperature:	oily liquid
Molecular Weight:	958
Colour:	pale yellow
Analysis:	HPLC
Purity:	100 %
Stability in solvent:	7 days in water
Storage:	room temperature
Expiration Date:	March 31, 2000

On the day of the experiment (immediately before treatment), the test article was dissolved in acetone (E. MERCK, D-64293 Darmstadt; purity 99.5 %). The final concentration of acetone in the culture medium was 0.5 % (v/v). The solvent was chosen to its solubility properties and its relative nontoxicity to the cell cultures.

Controls

Negative Controls

Concurrent negative controls (culture medium) and solvent controls (acetone) were performed.

Positive Control Substances

Without metabolic activation

Name:	EMS; Ethylmethane sulfonate
Supplier:	Merck-Schuchardt, D-85662 Hohenbrunn
Catalogue no.:	820774 (purity: > 98 %)
Dissolved in:	nutrient medium
Final Concentration:	600 µg/ml = 4.8 mM (continuous exposure) 1000 µg/ml = 8.0 mM (4 h exposure)

Solution prepared on day of experiment.

The stability of the positive control substance in solution was proven by the mutagenic response in the expected range.

With metabolic activation

Name:	CPA; Cyclophosphamide
Supplier:	Aldrich Chemie, D-89555 Steinheim
Catalogue no.:	21.870-7 (purity 98 %)
Dissolved in:	nutrient medium
Final Concentration:	0.71 µg/ml = 2.5 µM

The stability of CPA at room temperature is good. At 25° C only 3.5 % of its potency is lost after 24 hours (7).

Test System

Reasons for the Choice of the Cell Line V79

The V79 cell line has been used successfully for many years in *in vitro* experiments. Especially the high proliferation rate (doubling time of clone V79/T5 in stock cultures: 12 h) and a reasonable plating efficiency of untreated cells (as a rule more than 70 %) both necessary for the appropriate performance of the study, recommend the use of this cell line. The cells have a stable karyotype with a modal chromosome number of 22.

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

Cell Cultures

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

Thawed stock cultures were propagated at 37° C in 80 cm^2 plastic flasks (GREINER, D-72632 Frickenhausen). About 5 x 10^5 cells per flask were seeded into 15 ml of MEM (Minimal Essential Medium; SEROMED; D-12247 Berlin) supplemented with 10 % fetal calf serum (FCS; Boehringer Mannheim, D-68261 Mannheim). The cells were subcultured twice weekly. The cell cultures were incubated at 37° C in a humidified atmosphere with 4.5 % carbon dioxide (95.5 % air).

Mammalian Microsomal Fraction S9 Mix

S9 (Preparation by RCC Cytotest Cell Research)

The S9 liver microsomal fraction was obtained from the livers of 8 - 12 weeks old male rats, strain Wistar Hanlbm (BRL, CH-4414 Füllinsdorf; weight approx. 220 - 320 g) which received daily applications of 80 mg/kg b.w. phenobarbital i.p. dissolved in deionised water (Desitin; D-22335 Hamburg) and β-naphthoflavone orally dissolved in corn oil (Aldrich, D-89555 Steinheim) on three subsequent days. The livers were prepared 24 hours after the last treatment.

After decapitation of the anaesthetised animals, the livers of the animals were removed, washed in 150 mM KCl and homogenized. The homogenate, diluted 1+3 with KCl was centrifuged at 9000 g for 10 minutes (4° C). Aliquotes of the supernatant containing the microsomal fraction were frozen and stored in ampoules at -80° C. Small numbers of the ampoules were kept at -20° C for up to one week. The protein content was determined using the analysis kit of Bio-Rad Laboratories, D-80939 München: Bio-Rad protein assay, Catalogue No. 5000006.

The protein concentration in the S9 preparation is usually between 20 and 45 mg/ml. The protein concentration was 50.4 mg/ml (Lot. No.: 181298) in the pre-test and 35.3 mg/ml (Lot. No.: 180399) in both main experiments.

S9 Mix

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/ml in the cultures. Cofactors were added to the S9 mix to reach the following concentrations:

8 mM $MgCl_2$
33 mM KCl
5 mM glucose-6-phosphate
4 mM NADP

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

During the experiment the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames et al. (1).

Pre-Test on Toxicity

A pre-test on cell growth inhibition with 4 h and 24 h treatment was performed in order to determine the toxicity of the test article (8). Cytotoxicity was determined using concentrations separated by no more than a factor of 2 - 3.33. The general experimental conditions in this pre-test were the same as described below for the cytogenetic main experiment. The following method was used:

In a quantitative assessment, exponentially growing cell cultures (seeding 15600 cells/slide, with regard to the culture time 72 h) were treated with the test article for simulating the conditions of the main experiment. A qualitative evaluation of cell number and cell morphology was made 4 h after start of treatment. 24 h after start of treatment the cells were stained and in 10 coordinate defined fields of the slides (2 slides per treatment group) the cells were counted. The cell number of the treatment groups is given as % cells in relation to the control.

Dose Selection

Phytosteryl Octyldodecyl lauroyl glutamate

The highest concentration used in the pre-test was chosen with regard to the current OECD Guideline for *in vitro* mammalian cytogenetic tests. With respect to the solubility of [redacted] in acetone (highest soluble concentration 500 mg/ml), 2500 µg/ml of [redacted] were applied as top concentration for treatment of the cultures in the pre-test. Test article concentrations between 19.5 and 2500 µg/ml (with and without S9-mix) were chosen for the evaluation of cytotoxicity. Since the test article was floating on the culture medium, the observation of insolubility in culture medium was difficult. However, taking all results into account it can be stated, that at concentrations of 156,6 µg/ml and above, the test article was not completely dissolved in culture medium.

In the absence of S9 mix, no clear dose related toxicity was observed. The experiment was repeated two times due to missing toxicity. In the reported experiment, 2500 µg/ml was chosen as top treatment concentration. The applied concentrations in the cytogenetic experiments are presented in table 2.

Phytosteryl Octyldodecyl lauroyl glutamate

Table 2: Doses applied in the Chromosome aberration assay with [redacted]

Preparation interval	Exposure period	Exp.	Concentrations in µg/ml					
			without S9 mix					
18 h	4 h	I*	2.5	5.0	10.0	20.0	30.0	40.0
18 h	4 h	I*	20.0	40.0	80.0	160.0	320.0	640.0
18 h	4 h	I	**78.1**	**156.3**	312.5	**625.0**	**1250.0**	**2500.0**
18 h	18 h	II	**78.1**	**156.3**	**312.5**	625.0	**1250.0**	**2500.0**
28 h	28 h	II			312.5	625.0	**1250.0**	**2500.0**
			with S9 mix					
18 h	4 h	I	**9.4**	**18.8**	**37.5**	**75.0**	150.0	**300.0**
28 h	4 h	II	**78.1**	**156.3**	**312.5**	625.0	1250.0	**2500.0**

* was not evaluated for cytogenetic damage due to missing toxicity
evaluated experimental points were printed in bold letters

In all experimental parts, at least the lowest evaluated concentration did not exhibit a test item film on the top of the culture medium. Due to the difficulty to determine exactly the insoluble concentration, an extended concentration range was used for the evaluation of cytogenetic damage.

Experimental Performance

Schedule

	without S9-mix			with S9-mix	
	exp. I	exp. II		exp. I	exp. II
Exposure period	4 h	18 h	28 h	4 h	4 h
Recovery	14 h	-	-	14 h	24 h
Preparation interval	18 h	18 h	28 h	18 h	28 h

Seeding of the Cultures

Exponentially growing stock cultures more than 50 % confluent were treated with trypsin at 37 °C for approximately 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium and a single cell suspension was prepared. The trypsin concentration was 0.2 % in Ca-Mg-free salt solution (Trypsin: Difco Laboratories, Detroit, USA).

The Ca-Mg-free salt solution was composed as follows (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose	1000 mg
$NaHC0_3$	350 mg

Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/l EDTA (Ethylene diamine tetraacetic acid).

The cells were seeded into Quadriperm dishes (Heraeus, D-63450 Hanau) which contained microscopic slides (at least 2 chambers per dish and test group). In each chamber 1×10^4 - 6×10^4 cells were seeded with regard to preparation time. The medium was MEM + 10 % FCS (complete medium).

Treatment

Exposure period 4 hours

The culture medium of exponentially growing cell cultures was replaced with serum-free medium (for treatment with S9 mix) or complete medium (for treatment without S9 mix) with 10 % FCS (v/v), containing the test article. For the treatment with metabolic activation 50 µl/ml S9 mix per ml culture medium were added. Concurrent negative, solvent and positive controls were performed. After 4 h the cultures were washed twice with "Saline G" and then the cells were cultured in complete medium for the remaining culture time.

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

NaCl	8000 mg
KCl	400 mg
Glucose	1100 mg
$Na_2HP0_4x7H_20$	290 mg
KH_2P0_4	150 mg

pH was adjusted to 7.2

Exposure period 18 and 28 hours

The culture medium of exponentially growing cell cultures was replaced with complete medium (10 % FCS) containing different concentrations of the test article without S9 mix. The medium was not changed until preparation of the cells. Concurrent negative, solvent and positive controls were performed.

All cultures were incubated at 37° C in a humidified atmosphere with 4.5 % CO_2 (95.5 % air).

Preparation of the Cultures

16 h and 26 h, respectively after the start of the treatment colcemid was added (0.2 µg/ml culture medium) to the cultures. 2 h later, the cells on the slides were treated in the chambers with hypotonic solution (0.4 % KCl) for 20 min at 37° C. After incubation in the hypotonic solution the cells were fixed with 3 + 1 methanol + glacial acetic acid. Per experiment both slides per group were prepared. After preparation the cells were stained with Giemsa (E. Merck, D-64293 Darmstadt).

Additionally, two cultures per treatment group, not treated with Colcemid, were set up in parallel. These cultures were stained in order to determine microscopically the cell number within 10 defined fields per slide. The toxicity of the substance is given as reduction of % cells as compared to the solvent control.

Analysis of Metaphase Cells

Evaluation of the cultures was performed (according to standard protocol of the "Arbeitsgruppe der Industrie, Cytogenetik" (9)) using NIKON microscopes with 100x oil immersion objectives. Breaks, fragments, deletions, exchanges and chromosome disintegrations were recorded as structural chromosome aberrations. Gaps were recorded as well but not included in the calculation of the aberration rates. 100 well spread metaphases per culture were scored for cytogenetic damage on coded slides. Only metaphases with characteristic chromosome numbers of 22 ± 1 were included in the analysis. To describe a cytotoxic effect the mitotic index (% cells in mitosis) was determined. In addition, the number of polyploid cells was determined (% polyploid metaphases; in the case of this aneuploid cell line polyploid means a near tetraploid karyotype).

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Data Recording

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

Acceptability of the Assay

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

a) The number of structural aberrations found in the negative and/or solvent controls falls within the range of our historical laboratory control data: 0.00 % - 4.00 %.

b) The positive control substances should produce significant increases in the number of cells with structural chromosome aberrations, which are within the range of the laboratories historical control data:

test group final concentration	range	test group final concentration	range
without S9 mix		**with S9 mix**	
EMS 600 µg/ml	9.0 - 39.0	CPA 0.47 - 0.93 µg/ml	7.5 - 49.5

Evaluation of Results

A test article is classified as non-mutagenic if:
- the number of induced structural chromosome aberrations in all evaluated dose groups are in the range of our historical control data (0.0 - 4.0 % aberrant cells exclusive gaps).
- no significant increase of the number of structural chromosome aberrations are observed.

A test article is classified as mutagenic if:
- the number of induced structural chromosome aberrations are not in the range of our historical control data (0.0 - 4.0 % aberrant cells exclusive gaps).
- either a concentration-related or a significant increase of the number of structural chromosome aberrations are observed.

Statistical significance was confirmed by means of the Fischer's exact test (10) ($p < 0.05$). However, both biological and statistical significance should be considered together. If the a.m. criteria for the test article are not clearly met, the classification with regard to the historical data and the biological relevance is discussed and/or a confirmatory experiment is performed.

RESULTS AND DISCUSSION

The test article Phytosteryl Octyldodecyl lauroyl glutamate [redacted], dissolved in acetone, was assessed for its potential to induce structural chromosome aberrations in V79 cells of the Chinese hamster *in vitro* in the **absence** and **presence** of metabolic activation by S9 mix. Two independent experiments were performed. In experiment I, the exposure period was 4 h with and without metabolic activation. In experiment II the exposure period was 4 h with S9 mix and 18 h and 28 h without S9 mix. The chromosomes were prepared 18 h (exp. I and II) and 28 h (exp. II) after start of treatment with the test article. In each experimental group two parallel cultures were set up. Per culture 100 metaphase plates were scored for structural chromosome aberrations.

The highest concentration used in the range finding pre-test was chosen with regard to the current OECD Guideline for *in vitro* mammalian cytogenetic tests. With respect to the solubility of Phytosteryl Octyldodecyl lauroyl glutamate [redacted] in Acetone (highest soluble concentration 500 mg/ml), 2500 µg/ml of Phytosteryl Octyldodecyl lauroyl glutamate [redacted] were applied as top concentration for treatment of the cultures in the pre-test. Test article concentrations between 19.5 and 2500 µg/ml (with and without S9-mix) were chosen for the evaluation of cytotoxicity.

Since the test article was floating on the culture medium, the observation of insolubility in culture medium was difficult. However, taking all observations into account it can be stated, that at concentrations of 156,6 µg/ml and above, the test article was not completely dissolved in culture medium.

In this pre-test on toxicity cell numbers 24 h after start of treatment were scored as indicator for cytotoxicity. In the **absence** of S9 mix, no clear dose related toxicity could be observed (see dose selection). Therefore, 2500 µg/ml was chosen as top treatment concentration. In the **presence** of S9 mix, the cell number was reduced to 13 % of the controls after treatment with 312.5 µg/ml (see table 4, page 23). However, this toxicity was not reproduced in experiment I with 300 µg/ml (see table 5, page 24). Therefore, 2500 µg/ml was chosen as top treatment concentration in experiment II.

No influence of the test article on the pH value or osmolarity was observed (solvent control: 367 mOsm, pH 7.3 versus 345 mOsm and pH 7.3 at 2500 µg/ml).

Table 3: Evaluated experimental points after treatment with Phytosteryl Octyldodecyl lauroyl [redacted]

Exp.	Preparation interval	Exposure period	concentrations in µg/ml				
			without S9 mix				
I	18 h	4 h	78.1	156.3	625.0	1250.0	2500.0
II	18 h	18 h	78.1	156.3	312.5	1250.0	2500.0
II	28 h	28 h				1250.0	2500.0
			with S9 mix				
I	18 h	4 h	9.4	18.8	37.5	75.0	300.0
II	28 h	4 h		78.1	156.3	312.5	2500.0

In this study, neither reduced mitotic indices nor reduced cell numbers < 50 % of control could be observed up to the highest evaluated concentrations. However, in the **absence** of S9 mix in experiment I after treatment with 2500 µg/ml a slightly reduced mitotic index was observed (see table 6, page 25). In the **presence** of S9 mix slightly reduced cell numbers were observed at the border of insolubility in culture medium in experiment I after treatment with 75 µg/ml (see table 5, page 24).

In both experiments, in the **absence** and **presence** of S9 mix, no biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. In the **absence** and in the **presence** of S9 mix, the aberration rates of the cells after treatment with the test article (exp. I: 0.0 % - 4.0 %; exp. II: 0.0 % - 2.0 %) were near to the range of the solvent control values (exp. I: 1.0 % - 1.5 %; exp. II: 1.0 % - 2.0 %) and within the range of our historical control data (0.0 % - 4.0 %), except in experiment I in the **absence** of S9 mix after treatment with 1250 µg/ml. At this experimental point, a single significant increase in experiment I after 4 h exposure was observed. This increase (5.5 % aberrant cells exclusive gaps) slightly beyond our historical control data range (0.0 % - 4.0 %) was caused by a low solvent control value at a concentration exhibiting test article insolubility. Since this single finding was observed at an intermediate concentration without dose dependency, and was not reproduced in experiment II, neither at interval 28 h nor at interval 18 h it has to be regarded as being biologically irrelevant.

Tables 6 and 9 show the occurrence of polyploid metaphases. In both experiments, no biologically relevant increase in the rate of polyploid metaphases was found after treatment with the test article as compared to the rates of the solvent controls.

In both experiments, EMS (600 and 1000 µg/ml, respectively) and CPA (0.71 µg/ml) were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

In conclusion, it can be stated that in the study described and under the experimental conditions reported, the test article [redacted] did not induce structural chromosome aberrations in V79 cells (Chinese hamster cell line).

Phytosteryl Octyldodecyl lauroyl glutamate

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ANNEXE: TABLES OF RESULTS

Pre-Test on Toxicity

In the pre-test the toxicity of the test article was examined using the determination of the cell number. Cell numbers of two cultures (10 coordinate defined fields per culture) were determined for each experimental group.

Phytosteryl Octyldodecyl lauroyl glutamate

Table 4: Cytotoxicity of [redacted] to cultures of Chinese hamster cell line V79.

without S9 mix, 4 h exposure			without S9 mix, 24 h exposure		
Concentration µg/ml	number of cells	% of solvent control	Concentration µg/ml	number of cells	% of solvent control
solvent control	428	100	solvent control	200	100
19.5	418	98	19.5	219	110
39.1	105	24	39.1	128	64
78.1	124	29	78.1	236	118
156.3	238	56	156.3	380	190
312.5	403	94	312.5	332	166
625.0	202	47	625.0	243	121
1250.0	191	45	1250.0	230	115
2500.0	251	59	2500.0	145	73

with S9 mix, 4 h exposure		
Concentration µg/ml	number of cells	% of solvent control
solvent control	200	100
19.5	240	120
39.1	113	57
78.1	72	36
156.3	148	74
312.5	27	13
625.0	45	22
1250.0	187	93
2500.0	17	8

Experiment I and II: Determination of Toxicity

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

Table 5: Number of cells in % of solvent control

without metabolic activation (S9-mix)					
Experiment I: 4 h exposure			Experiment II: continuous exposure		
Preparation interval	Concentration in µg/ml	Cells in % of solvent control	Preparation interval	Concentration in µg/ml	Cells in % of solvent control
18 h	**78.1**	110	18 h	**78.1**	105
"	**156.3**	122	"	**156.3**	90
"	312.5	125	"	**312.5**	108
"	**625.0**	64	"	625.0	76
"	**1250.0**	94	"	**1250.0**	105
"	**2500.0**	88	"	**2500.0**	131
			28 h	312.5	132
			"	625.0	119
			"	**1250.0**	120
			"	**2500.0**	94

with metabolic activation (S9-mix)					
Experiment I: 4 h exposure			Experiment II: 4 h exposure		
Preparation interval	Concentration in µg/ml	Cells in % of solvent control	Preparation interval	Concentration in µg/ml	Cells in % of solvent control
18 h	**9.4**	132	28 h	**78.1**	126
"	**18.8**	119	"	**156.3**	101
"	**37.5**	109	"	**312.5**	111
"	**75.0**	106	"	625.0	109
"	150.0	113	"	1250.0	167
"	**300.0**	139	"	**2500.0**	121

experimental groups evaluated for cytogenetic damage were printed in bold letters

Experiment I

Table 6: Number of polyploid cells and mitotic index; exposure period 4 h; preparation interval 18 h

Treatment group	conc. per ml	S9 mix	preparation interval	polyploid cells* culture 1	culture 2	total	%	mitotic index** absolute 1	absolute 2	mean	%***
Neg. control		-	18 h	39	12	51	5.1	11.7	14.5	13.1	100.0
Solv. control#	0.5 %	-	18 h	12	20	32	3.2	14.4	14.3	14.4	100.0
Pos. control##	1000.0 µg	-	18 h	18	22	40	4.0	11.9	8.0	10.0	76.0
Test article	78.1 µg	-	18 h	18	12	30	3.0	16.6	14.1	15.4	107.0
"	156.3 µg	-	18 h	20	11	31	3.1	14.7	14.0	14.4	100.0
"	625.0 µg	-	18 h	26	24	50	5.0	14.0	14.0	14.0	97.6
"	1250.0 µg	-	18 h	38	30	68	6.8	16.8	18.4	17.6	122.6
"	2500.0 µg	-	18 h	38	22	60	6.0	8.7	9.2	9.0	62.4
Neg. control		+	18 h	14	20	34	3.4	13.4	12.9	13.2	100.0
Solv. control#	0.5 %	+	18 h	18	13	31	3.1	14.4	13.7	14.1	100.0
Pos. control###	0.71 µg	+	18 h	15	13	28	2.8	9.1	8.2	8.7	65.8
Test article	9.4 µg	+	18 h	14	14	28	2.8	15.1	16.0	15.6	110.7
"	18.8 µg	+	18 h	11	16	27	2.7	13.9	12.9	13.4	95.4
"	37.5 µg	+	18 h	14	17	31	3.1	9.5	9.7	9.6	68.3
"	75.0 µg	+	18 h	14	12	26	2.6	12.5	11.6	12.1	85.8
"	300.0 µg	+	18 h	9	6	15	1.5	13.8	12.5	13.2	93.6

* The number of polyploid cells was determined of each test group in a sample of 500 cells per culture

** The mitotic index was determined in a sample of 1000 cells per culture of each test group

*** For the positive control groups, the relative values of the mitotic index are related to the negative controls; for the test article treatment groups the values are related to the solvent controls

\# acetone

EMS

CPA

Table 7: Structural chromosome aberrations Experiment I; exposure period 4 h; preparation interval 18 h; without S9 mix

slide no.	cells scored	% aberrant cells incl. gaps	excl. gaps*	with exchanges	aberrations: gaps g	ig	chromatid type b	f	d	ex	chromosome type ib	if	id	cx	other ma	cd
					without S9 mix											
Negative control																
1	100				2	0	0	1	0	0	0	0	0	0	0	0
2	100				0	0	2	0	0	0	0	0	0	0	0	0
1 + 2	200	2.5	1.5	0.0	2	0	2	1	0	0	0	0	0	0	0	0
Solvent control: acetone 0.5 %																
1	100				2	0	0	0	0	0	0	2	0	0	0	0
2	100				0	0	1	1	0	0	0	0	0	0	0	0
1 + 2	200	2.5	1.5	0.0	2	0	1	1	0	0	0	2	0	0	0	0
Positive control: EMS 1000.0 µg / ml																
1	100				3	0	6	5	0	17	8	1	0	0	1	0
2	100				1	0	10	3	0	24	4	2	0	1	1	0
1 + 2	200	26.0	26.0	16.0	4	0	16	8	0	41	12	3	0	1	2	0
Test article: 78.1 µg / ml																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0	0	0	0
Test article: 156.3 µg /ml																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	0	1	0	0	0	0	0	0	0	0
1 + 2	200	1.0	0.5	0.0	1	0	0	1	0	0	0	0	0	0	0	0
Test article: 625.0 µg / ml																
1	100				0	0	0	0	0	8	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	4.0	4.0	4.0	0	0	0	0	0	8	0	0	0	0	0	0
Test article: 1250.0 µg / ml																
1	100				0	0	2	0	0	7	0	0	0	0	0	0
2	100				0	0	1	0	0	0	0	0	0	0	1	0
1 + 2	200	5.5	5.5	3.5	0	0	3	0	0	7	0	0	0	0	1	0
Test article: 2500.0 µg / ml																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	0.5	0.5	0.0	0	0	1	0	0	0	0	0	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 8: Structural chromosome aberrations Experiment I; exposure period 4 h; preparation interval 18 h; with S9 mix

slide no.	cells scored	% aberrant cells incl. gaps	excl. gaps*	with exchanges	aberrations: gaps g	ig	chromatid type b	f	d	ex	chromosome type ib	if	id	cx	other ma	cd
					with S9 mix											
Negative control																
1	100				0	0	0	1	0	0	1	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	1.5	1.0	0.0	1	0	0	1	0	0	1	0	0	0	0	0
Solvent control: acetone 0.5 %																
1	100				0	0	0	1	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	1	0	0	0	0	0	0
1 + 2	200	1.5	1.0	0.5	1	0	0	1	0	1	0	0	0	0	0	0
Positive control: CPA 0.71 µg /ml																
1	100				1	0	4	1	0	11	0	1	0	0	0	0
2	100				0	0	8	5	0	7	2	2	0	0	3	0
1 + 2	200	14.0	13.5	7.0	1	0	12	6	0	18	2	3	0	0	3	0
Test article: 9.4 µg / ml																
1	100				0	0	1	0	0	0	0	0	0	0	1	0
2	100				0	0	2	0	0	2	1	0	0	0	0	0
1 + 2	200	3.5	3.5	1.0	0	0	3	0	0	2	1	0	0	0	1	0
Test article: 18.8 µg /ml																
1	100				1	0	0	0	0	3	0	1	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	1	0
1 + 2	200	3.0	2.5	1.5	1	0	0	0	0	3	0	1	0	0	1	0
Test article: 37.5 µg / ml																
1	100				1	0	0	0	0	0	0	0	0	0	2	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	2.0	1.5	0.0	1	0	1	0	0	0	0	0	0	0	2	0
Test article: 75.0 µg / ml																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				2	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	1.5	0.0	0.0	3	0	0	0	0	0	0	0	0	0	0	0
Test article: 300.0 µg / ml																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	1	0	0	0	0	2	0	0	0	0
1 + 2	200	2.0	1.0	0.0	2	0	1	0	0	0	0	2	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Experiment II

Table 9: Number of polyploid cells and mitotic index;
preparation interval 18 and 28 h without S9 mix: exposure period 18 and 28 h;
preparation interval 28 h with S9 mix: exposure period 4 h

Treatment group	conc. per ml	S9 mix	preparation interval	polyploid cells* culture 1	culture 2	total	%	mitotic index** absolute 1	absolute 2	mean	%***
Neg. control		-	18 h	26	15	41	4.1	16.3	21.2	18.8	100.0
Solv. control#	0.5 %	-	18 h	18	20	38	3.8	20.8	21.9	21.4	100.0
Pos. control##	600.0 µg	-	18 h	10	22	32	3.2	12.5	9.0	10.8	57.3
Test article	78.1 µg	-	18 h	26	18	44	4.4	19.0	20.7	19.9	93.0
"	156.3 µg	-	18 h	22	16	38	3.8	24.1	22.2	23.2	108.4
"	312.5 µg	-	18 h	14	22	36	3.6	17.7	22.7	20.2	94.6
"	1250.0 µg	-	18 h	13	13	26	2.6	23.0	24.6	23.8	111.5
"	2500.0 µg	-	18 h	30	16	46	4.6	18.3	24.0	21.2	99.1
Solv. control#	0.5 %	-	28 h	28	27	55	5.5	20.1	18.2	19.2	100.0
Test article	1250.0 µg	-	28 h	16	12	28	2.8	19.4	18.5	19.0	99.0
"	2500.0 µg	-	28 h	12	18	30	3.0	20.4	18.3	19.4	101.0
Neg. control		+	28 h	28	22	50	5.0	17.8	12.5	15.2	100.0
Solv. control#	0.5 %	+	28 h	26	34	60	6.0	18.6	13.8	16.2	100.0
Pos. control###	0.71 µg	+	28 h	20	34	54	5.4	14.9	14.0	14.5	95.4
Test article	78.1 µg	+	28 h	35	30	65	6.5	19.3	17.8	18.6	114.5
"	156.3 µg	+	28 h	11	26	37	3.7	15.7	17.1	16.4	101.2
"	312.5 µg	+	28 h	25	28	53	5.3	13.7	16.7	15.2	93.8
"	2500.0 µg	+	28 h	22	24	46	4.6	14.9	13.1	14.0	86.4

* The number of polyploid cells was determined of each test group in a sample of 500 cells per culture

** The mitotic index was determined in a sample of 1000 cells per culture of each test group

*** For the positive control groups, the relative values of the mitotic index are related to the negative controls; for the test article treatment groups the values are related to the solvent controls

\# acetone

EMS

CPA

Table 10: Structural chromosome aberrations Experiment II; exposure period 18 h; preparation interval 18 h; without S9 mix

slide no.	cells scored	% aberrant cells incl. gaps	excl. gaps*	with exchanges	aberrations gaps g	ig	chromatid type b	f	d	ex	chromosome type ib	if	id	cx	other ma	cd
					without S9 mix											
Negative control																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				2	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	2.0	0.5	0.0	3	0	1	0	0	0	0	0	0	0	0	0
Solvent control: acetone 0.5 %																
1	100				0	0	0	1	0	1	0	1	0	0	2	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	2.0	2.0	0.5	0	0	0	1	0	1	0	1	0	0	2	0
Positive control: EMS 600.0 µg / ml																
1	100				3	0	13	3	0	8	9	0	0	0	2	0
2	100				5	1	1	4	0	12	2	0	0	0	1	0
1 + 2	200	22.5	20.5	9.5	8	1	14	7	0	20	11	0	0	0	3	0
Test article: 78.1 µg / ml																
1	100				0	0	0	0	0	1	0	0	0	0	1	0
2	100				2	0	0	1	0	0	0	1	0	0	0	0
1 + 2	200	3.0	2.0	0.5	2	0	0	1	0	1	0	1	0	0	1	0
Test article: 156.3 µg /ml																
1	100				0	0	1	1	0	0	0	1	0	0	0	0
2	100				2	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	2.5	1.5	0.0	2	0	1	1	0	0	0	1	0	0	0	0
Test article: 312.5 µg / ml																
1	100				3	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	2	0	0	0	0	0	0
1 + 2	200	2.0	0.5	0.5	3	0	0	0	0	2	0	0	0	0	0	0
Test article: 1250.0 µg / ml																
1	100				2	0	0	0	0	0	0	1	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	2.0	0.5	0.0	3	0	0	0	0	0	0	1	0	0	0	0
Test article: 2500.0 µg / ml																
1	100				1	0	0	1	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	1.0	0.5	0.0	1	0	0	1	0	0	0	0	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 11: Structural chromosome aberrations Experiment II; preparation interval 28 h; exposure period 28 h without S9 mix; exposure period 4 h with S9 mix

slide no.	cells scored	% aberrant cells incl. gaps	excl. gaps*	with exchanges	aberrations: gaps g	ig	chromatid type b	f	d	ex	chromosome type ib	if	id	cx	other ma	cd
					without S9 mix											
Solvent control: acetone 0.5 %																
1	100				2	0	1	0	0	0	0	0	0	0	0	0
2	100				2	0	0	1	0	1	1	0	0	2	0	0
1 + 2	200	3.5	2.0	1.0	4	0	1	1	0	1	1	0	0	2	0	0
Test article: 1250.0 µg / ml																
1	100				2	0	0	0	0	0	0	0	0	0	0	0
2	100				2	0	0	1	0	0	0	1	0	0	0	0
1 + 2	200	3.0	1.0	0.0	4	0	0	1	0	0	0	1	0	0	0	0
Test article: 2500.0 µg / ml																
1	100				1	0	1	0	0	0	0	0	0	0	0	0
2	100				1	0	1	1	0	0	0	2	0	0	0	0
1 + 2	200	2.5	1.5	0.0	2	0	2	1	0	0	0	2	0	0	0	0
					with S9 mix											
Negative control:																
1	100				1	1	0	1	0	0	0	1	1	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	2.0	1.0	0.0	1	1	0	1	0	0	0	1	1	0	0	0
Solvent control: acetone 0.5 %																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	1	2	0	0	0	0	0	0	0	0
1 + 2	200	1.0	1.0	0.0	0	0	1	2	0	0	0	0	0	0	0	0
Positive control: CPA 0.71 µg /ml																
1	100				0	0	2	2	0	3	1	2	0	0	1	0
2	100				2	0	1	2	0	3	0	5	0	1	0	0
1 + 2	200	10.0	9.5	3.0	2	0	3	4	0	6	1	7	0	1	1	0
Test article: 78.1 µg / ml																
1	100				1	0	0	0	0	0	0	0	0	1	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	1.0	0.5	0.5	1	0	0	0	0	0	0	0	0	1	0	0
Test article: 156.3 µg / ml																
1	100				0	0	2	0	0	0	1	2	0	0	0	0
2	100				1	0	0	0	0	0	0	1	0	0	0	0
1 + 2	200	2.5	2.0	0.0	1	0	2	0	0	0	1	3	0	0	0	0
Test article: 312.5 µg / ml																
1	100				1	0	1	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	1.5	0.5	0.0	2	0	1	0	0	0	0	0	0	0	0	0
Test article: 2500.0 µg / ml																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

BIOMETRY

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

Experiment I

Solvent control versus		preparation interval	exposure period	S9 mix	p-value
Test group	78.1 µg/ml	18 h	4 h	-	n.c.
"	156.3 µg/ml	18 h	4 h	-	n.c.
"	625.0 µg/ml	18 h	4 h	-	0.07
"	1250.0 µg/ml	18 h	4 h	-	0.016[S]
"	2500.0 µg/ml	18 h	4 h	-	n.c.
"	9.4 µg/ml	18 h	4 h	+	0.053
"	18.8 µg/ml	18 h	4 h	+	0.143
"	37.5 µg/ml	18 h	4 h	+	0.343
"	75.0 µg/ml	18 h	4 h	+	n.c.
"	300.0 µg/ml	28 h	4 h	+	n.c.
Negative control versus Positive control					
EMS	1000 µg/ml	18 h	4 h	-	< 0.001[S]
CPA	0.71 µg/ml	18 h	4 h	+	< 0.001[S]

Experiment II

Solvent control versus		preparation interval	exposure period	S9 mix	p-value
Test group	78.1 µg/ml	18 h	18 h	-	n.c.
"	156.3 µg/ml	18 h	18 h	-	n.c.
"	312.5 µg/ml	18 h	18 h	-	n.c.
"	1250.0 µg/ml	18 h	18 h	-	n.c.
"	2500.0 µg/ml	18 h	18 h	-	n.c.
"	1250.0 µg/ml	28 h	28 h	-	n.c.
"	2500.0 µg/ml	28 h	28 h	-	n.c.
"	78.1 µg/ml	28 h	4 h	+	n.c.
"	156.3 µg/ml	28 h	4 h	+	0.225
"	312.5 µg/ml	28 h	4 h	+	n.c.
"	2500.0 µg/ml	28 h	4 h	+	n.c.
Negative control versus Positive control					
EMS	600 µg/ml	18 h	18 h	-	< 0.001[S]
CPA	0.71 µg/ml	28 h	4 h	+	< 0.001[S]

n.c. = not calculated as the aberration rate is equal or lower than the control rate
[S] aberration rate is statistically significant higher than the control rate

HISTORICAL LABORATORY CONTROL DATA

Chinese hamster V79 cell cultures from 1993 to 1996

without S9 mix										
		aberrant cells								
		inclusive gaps			exclusive gaps			with exchanges		
test group final concentration	cells scored	range	mean	calculated normal range*	range	mean	calculated. normal range*	range	mean	calculated normal range*
negative control										
acetone 1 % (v/v)	1200	0.0-5.5	2.3	0.4-4.2	0.0-4.0	1.4	0.0-2.9	0.0-1.0	0.2	0.0-0.6
culture medium DMEM/F12	64700	0.0-6.0	2.1	0.8-3.4	0.0-4.0	1.1	0.2-2.0	0.0-1.0	0.1	0.0-0.3
DMSO 1 % (v/v)	42400	0.0-8.5	2.8	1.3-4.3	0.0-4.0	1.3	0.4-2.2	0.0-1.5	0.1	0.0-0.4
ETOH 1 % (v/v)	3800	1.0-5.5	3.1	1.6-4.6	0.0-3.5	1.4	0.4-2.4	0.0-1.0	0.1	0.0-0.4
deionised water 10 % (v/v)	14000	0.0-6.5	2.3	0.8-3.8	0.0-4.0	1.2	0.2-2.2	0.0-0.5	0.1	0.0-0.3
Total	**126100**	**0.0-8.5**	**2.1-3.1**	**0.4-4.6**	0.0-4.0	**1.1-1.4**	**0.0-2.9**	**0.0-1.5**	**0.0-0.2**	**0.0-0.6**
positive control										
EMS 600 µg/ml	47500	9.0-39.5	22.0	16.3-27.7	9.0-39.0	18.9	13.4-24.4	1.0-25.0	11.3	7.6-15.2

* mean ± standard deviation

[redacted]

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[redacted]

Chinese hamster V79 cell cultures from 1993 to 1996

with S9 mix										
		aberrant cells								
		inclusive gaps			exclusive gaps			with exchanges		
test group final concentration	cells scored	range	mean	calculated normal range*	range	mean	calculated. normal range*	range	mean	calculated normal range*
negative control										
acetone 1 % (v/v)	1200	1.5-3.5	2.2	1.4-3.0	0.5-3.5	1.4	0.3-2.5	0.0-0.5	0.2	0.0-0.5
culture medium DMEM/F12	64900	0.0-7.5	2.5	1.6-3.4	0.0-4.0	1.2	0.9-1.5	0.0-2.0	0.2	0.0-0.5
DMSO 1 % (v/v)	43000	0.0-8.5	2.4	1.0-3.8	0.0-4.0	1.4	0.5-2.3	0.0-2.0	0.2	0.0-0.5
ETOH 1 % (v/v)	4000	0.0-5.5	2.7	1.2-4.2	0.0-4.0	1.5	0.4-2.6	0.0-1.0	0.2	0.0-0.5
deionised water 10 % (v/v)	14200	0.0-6.0	2.2	0.8-3.6	0.0-4.0	1.2	0.2-2.2	0.0-1.0	0.2	0.0-0.5
Total	127300	**0.0-8.5**	**2.2-2.7**	**0.8-4.2**	**0.0-4.0**	**1.2-1.5**	**0.2-2.6**	**0.0-1.5**	**0.1-0.2**	**0.0-0.6**
positive control										
CPA 0.47 - 0.93 µg/ml	48700	8.5-52.0	17.1	11.1-23.1	7.5-49.5	15.3	9.5-21.1	1.0-25.5	7.3	4.1-10.5

* mean ± standard deviation

[redacted]

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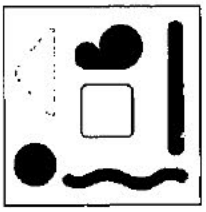
[redacted]



RCC PROJECT [redacted]

Phytosteryl octyldodecyl lauroyl glutamate

[redacted]

28-DAY ORAL TOXICITY (GAVAGE) STUDY IN THE WISTAR RAT

REPORT

Author: W.H. Braun, H. Luetkemeier, K. Biedermann, Dr. K. Heider

Sponsor: [redacted]

Study Completion Date: July 23, 1999

RCC

[redacted]

RCC PROJECT [redacted]

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RCC PROJECT

1 PREFACE

1.1 GENERAL

Title	Phytosteryl octyldodecyl lauroyl glutamate: 28-Day Oral Toxicity (Gavage) Study in the Wistar Rat
Sponsor	
Study Monitor	
Test Facilities	a) RCC Ltd Toxicology Division CH – 4452 Itingen / Switzerland b) RCC Ltd Toxicology Division CH – 4414 Füllinsdorf / Switzerland c) RCC Ltd Environmental Chemistry & Pharmanalytics Division CH – 4452 Itingen / Switzerland d) EPS, Experimental Pathology Services AG CH – 4132 Muttenz / Switzerland
RCC Project Number	
Test Article	Phytosteryl octyldodecyl lauroyl glutamate
Test System	Rat

1.2 PROJECT STAFF

Study Director	W.H. Braun (a)
Deputy Study Director	Dr. E. Rosner (a)
Technical Coordinator	D. Frei (a)
Laboratory Coordinator	P. Althaus-Ravenstijn (a)
Clinical Laboratory Investigations	H. Luetkemeier (b)
Necropsy/Histotechnique	Dr. K. Weber (a)

1.2.1 PRINCIPAL INVESTIGATORS

Analytical Chemistry	K. Biedermann (c)
Study Pathologist	Dr. K. Heider (d)

1.3 SCHEDULE

Experimental Start	16 Feb 1999
Experimental Completion	23 Mar 1999
Delivery of Animals	16 Feb 1999
Acclimatization/Pretest	16 – 22 Feb 1999
Administration/Treatment	23 Feb – 22 Mar 1999
Termination (Necropsy)	23 Mar 1999

1.4 ARCHIVING

RCC Ltd (CH-4452 Itingen / Switzerland) will archive the following data for at least 10 years:

Protocol, report, amendments, raw data, specimens of laboratory investigations, specimens and slides of pathology and histopathology and the test article reference sample. No data will be discarded without the Sponsor's consent.

1.5 TEST GUIDELINES

The study procedures described in this report conform with the following guidelines:

Directive 96 / 54 / EC, B. 7. "Repeated dose (28 days) toxicity (oral)", September 30, 1996.

"Repeated Dose Oral Toxicity - Rodent: 28-day or 14-day Study", OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects, Number 407, July 27,1995.

1.6 ACCREDITATION

The test facility "RCC Ltd" is accredited according to EN 45001 under accreditation number STS 085 by the Swiss Accreditation Service. As specified by the Guidelines of the Swiss Accreditation Service, the test results described in this report relate only to the items tested and information on the error of measurement (such as confidence intervals, if relevant) is available upon request. This report shall not be reproduced, except in full, without the written approval of the test facility.

[redacted]

RCC PROJECT [redacted]

1.7 SIGNATURES

STUDY DIRECTOR: W.H. Braun

date: 23 July 1999

MANAGEMENT: (for) T. R. Allen

date: 23-Jul-1999

The signatures of K. Biedermann (Analytical Chemistry) and Dr. K. Heider (Study Pathologist) are included in the relevant attached reports.

[redacted]

RCC PROJECT [redacted]

1.8 QUALITY ASSURANCE STATEMENT

RCC Ltd
Toxicology Division
CH-4452 Itingen / Switzerland

PROJECT NUMBER [redacted]

TEST ARTICLE [redacted] Phytosteryl octyldodecyl lauroyl glutamate

STUDY DIRECTOR W.H. Braun

TITLE [redacted] Phytosteryl octyldodecyl lauroyl glutamate: 28-Day Oral Toxicity (Gavage) Study in the Wistar Rat

The conduct of this study was subjected to periodic inspections and the report audited by the RCC Quality Assurance Unit. The dates are given below.

Dates of QA Inspections/Audits	Types of QA Inspections/Audits	Dates of Reports to the Study Director and to Management
10 Feb 1999	Protocol audit	10 Feb 1999
23 Feb 1999	Study-based inspection	23 Feb 1999
12 Mar 1999	Study-based inspection	12 Mar 1999
23 Mar 1999	Study-based inspection	23 Mar 1999
21 Apr 1999	Process-based inspection	21 Apr 1999
14 May 1999	Process-based inspection	14 May 1999
16/17/18 June 1999	Report audit	18 June 1999

Manager, Quality Assurance Unit: Dr. G. Menne

date 23-JUL-1999

[redacted]

RCC PROJECT [redacted]

GOOD LABORATORY PRACTICE

1.9 STATEMENT OF COMPLIANCE / GLP GUIDELINES

PROJECT NUMBER	[redacted]
TEST ARTICLE	[redacted] Phytosteryl octyldodecyl lauroyl glutamate
STUDY DIRECTOR	W.H. Braun
TITLE	Phytosteryl octyldodecyl lauroyl glutamate [redacted]: 28-Day Oral Toxicity (Gavage) Study in the Wistar Rat

The study described in this report was conducted in compliance with the following Good Laboratory Practice Standards:

Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March, 1986.

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

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

Study Director: W.H. Braun

date: 23 July 1999

Management: (for) T. R. Allen

date: 23-Jul-1999

2 SUMMARY

GENERAL

In this subacute toxicity study, Phytosteryl octyldodecyl lauroyl glutamate was administered daily by oral gavage to SPF-bred Wistar rats of both sexes at dose levels of 50, 200 or 1000 mg/kg body weight/day for a period of 28 days.

The groups comprised 5 animals per sex which were sacrificed after 28 days of treatment.

Clinical signs, outside cage observation, food consumption and body weights were recorded periodically during pretest and the treatment period. Functional observational battery, locomotor activity and grip strength were performed during week 4.

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

MORTALITY / VIABILITY

All animals survived until scheduled necropsy.

CLINICAL SIGNS

No test article-related clinical signs were ascertained during daily observation or during the weekly detailed clinical observations (weeks 1-3).

FUNCTIONAL OBSERVATIONAL BATTERY

No test article-related clinical signs were evident during examination at week 4.

GRIP STRENGTH

No test article-related differences in grip strength were ascertained at any dose level.

LOCOMOTOR ACTIVITY

The locomotor activity of the test article-treated animals compared favorably with those of the control animals.

FOOD CONSUMPTION

The mean daily food consumption and relative food consumption did not show test article-related differences wen compared with those of the control animals.

BODY WEIGHT

The mean body weight development of test article-treated and control animals compared favorably.

CLINICAL LABORATORY INVESTIGATIONS

Hematology

The hematology parameters of the test article-treated animals compared favorably with those of the control animals.

Clinical biochemistry

The clinical biochemistry parameters of the test article-treated animals compared favorably with those of the control animals.

ORGAN WEIGHTS

The organ weights of test article-treated animals were considered to be unaffected.

MACROSCOPIC / MICROSCOPIC FINDINGS

There were no macroscopic or microscopic findings considered to distinguish test article-treated rats from control rats. All macroscopic and microscopic findings noted in this study were considered to be incidental findings commonly occurring in rats of this strain and age.

RCC PROJECT

3 ASSESSMENT

Phytosteryl octyldodecyl lauroyl glutamate

Oral administration of to Wistar rats at doses of 50, 200 and 1000 mg/kg/day, for 28 days resulted in no deaths. The test article-treated animals were without clinical signs and there were no effects upon food consumption, body weight development, behavior, grip strength, locomotor activity or organ weights. There were no test article-related effects upon hematology or clinical biochemistry parameters, nor were test article-related macroscopic or microscopic findings observed.

Phytosteryl octyldodecyl lauroyl glutamate

Based on the results of this study, 1000 mg/kg body weight/day (group 4) of was established as the no-observed-adverse-effect-level (NOAEL) and as the no-observed-effect-level (NOEL).

4 OBJECTIVE

4.1 PURPOSE AND RATIONALE

Phytosteryl octyldodecyl lauroyl glutamate

The purpose of this oral toxicity study was to assess the cumulative toxicity of the test article, , when administered daily to rats by gavage for a period of 28 days.

This study should provide a rational basis for toxicological risk assessment in man. The results of this study should indicate potential target organs and should identify chemicals with neurotoxic potential.

5 MATERIALS AND METHODS

5.1 TEST SYSTEM

Test system	Rat, HanIbm:WIST (SPF)
Rationale	Recognized by the international guidelines as the recommended test system.
Source	RCC Ltd Biotechnology & Animal Breeding Division CH-4414 Füllinsdorf / Switzerland
Total number of animals per group	Groups 1 to 4: 5 males; 5 females
Total number of animals	20 males and 20 females
Age at delivery	6 weeks
Body weight range at acclimatization	Males: 137-150 grams (mean 142 grams) Females: 112-124 grams (mean 119 grams)
Identification	Cage card and individual ear tattoo
Randomization	Computer-generated random algorithm
Acclimatization	Under test conditions after health examination. Only animals without any visible signs of illness were used for the study.

5.2 ALLOCATION

Dose	Group 1* 0 mg/kg	Group 2 50 mg/kg	Group 3 200 mg/kg	Group 4 1000 mg/kg
Males	1 - 5	6 - 10	11 - 15	16 - 20
Females	21 – 25	26 - 30	31 – 35	36 - 40

* - Control animals were treated with the vehicle only (PEG 300)

5.3 HUSBANDRY

Room number	E 22
Conditions	Standard Laboratory Conditions. Air-conditioned with 10-15 air changes per hour, and continuously monitored environment with a target range for temperature of 22 ± 3 °C and for relative humidity between 40-70 %. 12 hours fluorescent light / 12 hours dark (light period between 06.00 and 18.00), music during the light period.
Accommodation	In groups of five in Makrolon type-4 cages with wire mesh tops and standardized softwood bedding ('Lignocel' Schill AG, CH-4132 Muttenz/Switzerland).
Diet	Pelleted standard Provimi Kliba 3433 (batch no. 33/98) rat maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst/ Switzerland) was available *ad libitum*. The feed batch was analyzed for contaminants (see Appendix III, pp. 183 - 184).
Water	Community tap-water from Itingen was available *ad libitum* in water bottles. Results of bacteriological assay, chemical and contaminant analyses of representative samples are attached to this report (see Appendix II, pp. 179 - 181 None of the contaminants analyzed in the water and diet is considered to have been present at a concentration which would have affected the validity of the results.

5.4 TEST ARTICLE

Identity	Phytosteryl octyldodecyl lauroyl glutamate
Description	pale yellow oily liquid
Batch number	808010
Purity	100%
Composition	Confidential information, available in Sponsor's file.
Stability of test article	Stable under storage conditions
Expiry date	March 31, 2000
Stability of test article formulation	up to seven days
Storage conditions	In the original container at room temperature (20°C) away from direct sunlight.
Safety precautions	Routine hygienic procedures (gloves, goggles, face mask)

The test article information was supplied by the sponsor.

5.5 TEST ARTICLE PREPARATION

The test article formulations were prepared weekly, and were stored at room temperature (17-23°C). Phytosteryl octyldodecyl lauroyl glutamate was weighed into a glass beaker on a tared Mettler balance and the vehicle added. The mixtures were prepared using a magnetic stirrer.

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

5.5.1 ANALYSIS OF DOSE FORMULATIONS

Concentration, homogeneity and stability (after 2 hours and 7 days) of the dose formulations were determined in samples taken during acclimatization. Concentration and homogeneity of the dose formulations were determined in samples taken during week 3 of the treatment. The analyses were performed by RCC Ltd (Environmental Chemistry & Pharmanalytics Division) according to a HPLC method supplied by the Sponsor. The results are presented in Appendix V.

See pp. 199-214

5.6 VEHICLE AND CONTROL ARTICLE

Identity	PEG 300
Batch number	395304/1 20199
Expiry date	December 29, 2003
Storage conditions	In the original container at room temperature (20°C) away from direct sunlight.
Safety precautions	Routine hygienic procedures (gloves, goggles, face mask)

5.7 TREATMENT

Method	Oral, by gavage.
Rationale	Accidental oral ingestion is a possible route of human exposure.
Daily dose levels	Group 1: 0 mg/kg body weight Group 2: 50 mg/kg body weight Group 3: 200 mg/kg body weight Group 4: 1000 mg/kg body weight Dose levels are expressed in terms of the test article as supplied.
Rationale for dose level selection	Based upon the results of a non-GLP 5-day dose-range-finding study (RCC Project in which was administered by gavage to 2 rats per group and sex. Animals showed no signs of toxicity.
Frequency of administration	Daily
Dose volume	5 ml/kg body weight
Duration of acclimatization period	7 days
Duration of treatment	28 days

Phytosteryl octyldodecyl lauroyl glutamate

5.8 OBSERVATIONS

5.8.1 MORTALITY / VIABILITY

Observations for mortality/viability were recorded twice daily.

5.8.2 GENERAL CAGESIDE OBSERVATIONS (DAILY)

The animals were observed for clinical signs once before commencement of administration; twice daily on days 1-3 (target range ca. 1 and 3 hours after administration); as well as once daily on days 4-28 (target range ca. 1 hour after administration).

5.8.3 DETAILED CLINICAL OBSERVATIONS (WEEKLY)

The animals were observed in their home cages, outside their home cages in a standard arena and in the hand. These observations were performed in random sequence once before commencement of administration and once weekly (weeks 1-3) thereafter.

5.8.4 FOOD CONSUMPTION

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

5.8.5 BODY WEIGHTS

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

5.8.6 FUNCTIONAL OBSERVATIONAL BATTERY

During week 4, relevant parameters (presented in Appendix I) from a modified Irwin screen test were evaluated in all animals.

The test was performed up to 3 hours after application.

NB. The results of the Functional Observational Battery are presented in the summary and individual tables of the Detailed Clinical Observations (Weekly) under week 4. This method of data presentation permits a clear evaluation and assessment of weekly clinical signs observed during the study.

GRIP STRENGTH

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

LOCOMOTOR ACTIVITY

Locomotor (decreased or increased) activity was measured quantitatively with Activity Monitor AM 1052 system (Benwick Electronic Equipment Design Manufacture, England). Animals were randomized monitored during the fourth treatment week for a 60-minute period and the total activity of this time period was recorded.

Low beams count was reported in 15-minute intervals as well as the total activity of the measuring period.

5.9 CLINICAL LABORATORY INVESTIGATIONS

Blood samples for hematology and clinical biochemistry were collected from all animals under light ether anesthesia. The animals were fasted for approximately 18 hours before blood sampling but allowed access to water *ad libitum*. Blood samples were collected early in the working day to reduce biological variation caused by circadian rhythms. Blood samples were drawn from the retro-orbital plexus using a micro-hematocrit glass capillary tube.

after 4 weeks: March 23, 1999

The assays were performed at RCC Ltd (Füllinsdorf) under internal laboratory quality control conditions to assure reliable test results.

In the summary and individual tables the names of some parameters have been abbreviated. Any abbreviation has been defined in this section.

General remarks:
Historical reference values for untreated Wistar Han-Ibm rats, see Appendix IV, pp. 186-197

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

Key to abbreviations of Units of Measure:

L	: liter	g	: gram	T	: tera (10^{12})	µ	: micro (10^{-6})
mol	: mole	kg	: kilogram	G	: giga (10^{9})	n	: nano (10^{-9})
sec	: second	kat	: katal (mol/sec)	m	: milli (10^{-3})	f	: femto (10^{-15})

5.9.1 HEMATOLOGY

The following anticoagulants were used during blood collection:

EDTA-K2 (hematology); Lithium heparin 30 I.U./ml (methemoglobin); Sodium citrate, 3.8% (coagulation; 1 part anticoagulant to 9 parts blood)

The following commercial reference controls were used to monitor the performance of the method:

Hematology: Eightcheck-3WP (normal range); Eightcheck-L-3WP (low abnormal range); Ret-check (reticulocyte control) (TOA Medical Electronics Co., Ltd. Kobe/Japan)

Methemoglobin: CalDye Reference Standard IL Multi-4 CO-Oximeter Control (Instrumentation Laboratory, Lexington, Ma/U.S.A.)

Coagulation: IL Control Plasma (normal range); IL Control Plasma (abnormal range) (Instrumentation Laboratory, SpA, Milano/Italy)

Hematology Parameter	Abbreviation	Unit	Instrumentation
Erythrocyte count	RBC	T/L	1
Hemoglobin	HB	mmol/L	1
Hematocrit	HCT	L/L	1
Mean corpuscular volume	MCV	fL	1
Mean corpuscular hemoglobin	MCH	fmol	1
Mean corpuscular hemoglobin concentration	MCHC	mmol/L	1
Platelet count	PLATELETS	G/L	1
Reticulocyte count	RETIC.	%(rel.) T/L(abs.)	2
Reticulocyte fluorescence ratios	HFR = high, MFR = middle, LFR = low	%	2
Nucleated erythrocytes (normoblasts)	NEN	NEN/100WBC	3
Heinz bodies	HEINZ-BOD.	‰	3

Hematology Parameter	Abbreviation	Unit / Score	Instrumentation
Methemoglobin	MET-HB	%	5
Total leukocyte count	WBC	G/L	1
Differential leukocyte count	Diff. WBC Count[4]	1(rel.) G/L(abs.)	3
Red blood cell morphology	TYPE CELL	normal/abnormal	3
Thromboplastin time (=prothrombin time)	PT	sec	6
Activated partial thromboplastin time	APTT	sec	6

Key:

1 Sysmex (TOA) E-4000 Multi-Parameter Automated Hematology Analyzer
2 Sysmex (TOA) R-1000 Automated Reticulocyte Analyzer
3 Leitz Laborlux 12 or Dialux 22 light microscope and Baumann –Medical Mediff 8000 Computer Printer Blood Cell Calculator
4 Cell Classification:

BAND.	= Band Neutrophil	LYMPH.	= Lymphocyte
SEG.	= Segmented Neutrophil	MONO.	= Monocyte
EO.	= Eosinophil	PLAS.	= Plasma Cell
BASO.	= Basophil	OTHER	= Blast Cell (undifferentiated)

5 IL 482 CO-Oximeter
6 Instrumentation Laboratory (IL) ACL 300 Coagulation System

5.9.2 CLINICAL BIOCHEMISTRY

The following anticoagulant was used during blood collection:

Lithium heparin (15 I.U./ml).

The following commercial reference controls were used to monitor the performance of the method:

Precinorm U (normal range); Precipath U (abnormal range) (Boehringer Mannheim GmbH, Mannheim/Germany)

Lyphochek, Level 1 (normal range); Lyphochek, Level 2 (abnormal range) (Bio-Rad Laboratories, ECS Division, Anaheim, California/USA)

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RCC PROJECT [redacted]

Clinical Biochemistry Parameter	Abbreviation	Unit	Instrumentation
Glucose		mmol/L	1
Urea		mmol/L	1
Creatinine		µmol/L	1
Uric Acid		µmol/L	1
Bilirubin, total	BILI. T	µmol/L	1
Cholesterol, total	CHOLEST. T	mmol/L	1
Triglycerides	TRIGL.	mmol/L	1
Phospholipids	PHOS.LIPID	mmol/L	1
Aspartate aminotransferase	ASAT/GOT	µkat/L (37°C)	1
Alanine aminotransferase	ALAT/GPT	µkat/L (37°C)	1
Lactate dehydrogenase	LDH	µkat/L (37°C)	1
Creatine kinase	CK	µkat/L (37°C)	1
Alkaline phosphatase	ALP	µkat/L (37°C)	1
Gamma-glutamyl transferase	G-GT	nkat/L (37°C)	1
Calcium		mmol/L	1
Phosphorus		mmol/L	1
Sodium		mmol/L	1
Potassium		mmol/L	1
Chloride		mmol/L	1
Albumin		g/L	1
Protein, total	PROTEIN T.	g/L	1
Globulin		g/L	2
Albumin/Globulin ratio	A/G RATIO	---	2

Key:

1 BM/Hitachi 917 Discrete Random-Access Analyzer (Boehringer Mannheim/Hitachi)
2 Calculated

5.10 PATHOLOGY

5.10.1 NECROPSY

after 4 weeks: March 23, 1999

All animals were weighed and necropsied. Descriptions of all macroscopic abnormalities were recorded. Necropsies were performed by experienced prosectors supervised by an experienced veterinary pathologist.

All animals were anesthetized by intraperitoneal injection of sodium pentobarbitone and killed by exsanguination.

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

Adrenal glands
Aorta
Bone (sternum, femur including joint)
Bone marrow (femur)
Brain (cerebrum, cerebellum, pons)
Cecum
Colon
Duodenum
Epididymides (fixed in Bouin's solution)
Esophagus
Eyes with optic nerve (fixed in Davidson's solution)
Harderian gland (fixed in Davidson's solution)
Heart
Ileum, with Peyer's patches
Jejunum with Peyer's patches
Kidneys
Larynx
Lacrimal gland (exorbital)
Liver
Lungs (infused with formalin at necropsy)
Lymph nodes (mesenteric, mandibular)
Mammary gland area
Nasal cavity
Ovaries
Pancreas
Pituitary gland
Prostate gland
Rectum
Salivary glands (mandibular, sublingual)
Sciatic nerve
Seminal vesicles
Skeletal muscle
Skin
Spinal cord (cervical, midthoracic, lumbar)
Spleen
Stomach
Testes (fixed in Bouin's solution)
Thymus
Thyroid (incl. parathyroid gland)
Tongue
Trachea
Urinary bladder (infused with formalin at necropsy)
Uterus
Vagina
Gross lesions

5.10.2 ABSOLUTE AND RELATIVE ORGAN WEIGHTS

The following organ weights were recorded on the scheduled date of necropsy:

Brain	Thymus	Spleen
Heart	Kidneys	Testes
Liver	Adrenals	Epididymides

The organ to terminal body weight ratios as well as organ to brain weight ratios were determined.

The determination of the terminal body weight was performed immediately prior to necropsy.

5.10.3 HISTOTECHNIQUE

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

5.10.4 HISTOPATHOLOGY

Slides of all organs and tissues listed in boldface type (see Necropsy, above) which were collected at scheduled sacrifices from the animals of control and high-dose groups as well as gross lesions from animals of groups 2 and 3, were examined by a pathologist.

5.11 DATA COMPILATION

5.11.1 GENERAL

The following data were recorded on-line: food consumption, body weights, organ weights and macroscopic findings at necropsy and histopathology.

Clinical Laboratory data were recorded on-line (Sysmex TOA E-4000 Multiparameter Automated Hematology Analyzer, Sysmex TOA R-1000 Automated Reticulocyte Analyzer, Instrumentation Laboratory ACL 300 Coagulation System) or on data sheets (Il 482 CO-Oximeter, BM/Hitachi 917 Discrete Random-Access Analyzer) and then transferred to the computer system.

Cageside and detailed clinical signs, data from the functional observational battery grip strength and locomotor activity were recorded on data sheets and transcribed for compilation and analysis into the computer system.

The computer-generated values which appear in the tables represent the rounded-off results of the raw data values or of calculations which used the exact raw data values.

Group means were calculated according to the definition of any mean value using the individual values per animal and the number of animals.

5.12 DATA CALCULATION

5.12.1 FOOD CONSUMPTION

The food consumption was calculated per rat and per food consumption interval. It expresses the average food consumed per animal and per day over the food consumption interval.

FC = C / AD

FC = Food consumption in grams of food per animal and day;
C = Measured food consumption in grams per cage over the consumption interval;
AD = Total consumption days over all animals in the cage during the consumption interval.

5.12.2 RELATIVE FOOD CONSUMPTION

The relative food consumption was calculated according to the following formula:

RFC = [FC / BW(i)] x 1000

RFC = Relative food consumption in grams of food per kg body weight and day;
FC = Food consumption in grams of food per animal and day;
BW(i) = Most ideal body weight in grams or the body weight (of the corresponding rats) recorded on the day most close to the middle of the food consumption interval. In cases of equal "closeness" of two body weight records the later one was chosen.

5.13 STATISTICAL ANALYSIS

The following statistical methods were used to analyze grip strength, locomotor activity, body weights, organ weights and all ratios, as well as clinical laboratory data :

- The Dunnett-test (many to one t-test) based on a pooled variance estimate was applied if the variables could be assumed to follow a normal distribution for the comparison of the treated groups and the control groups for each sex.
- The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution.
- Fisher's exact-test was applied to macroscopic findings.

References :

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

6 RESULTS

6.1 VIABILITY/MORTALITY

All animals survived until scheduled necropsy.

See pp. 36-43

6.2 OBSERVATIONS

6.2.1 GENERAL CAGESIDE OBSERVATIONS (DAILY)

No clinical signs were evident in any animal during daily observation.

See pp 44-51 and 83-122

6.2.2 DETAILED CLINICAL OBSERVATIONS (WEEKLY)

There were no test article-related findings evident in any animal during weeks 1-3.

Slight reduction of activity was noted during treatment week 3 in one male treated with 1000 mg/kg/day. This finding was not seen in other males at this dose level and was considered to be incidental.

See pp. 52-53 and 123-130

6.2.3 FUNCTIONAL OBSERVATIONAL BATTERY

No qualitative or quantitative differences to the control animals were noted in any test article-treated animal.

See pp. 52-53 and 131-132

Grip Strength

The mean forelimb grip strength was increased in the test article-treated males and females. In females, the difference attained statistical significance ($p<0.05$) when compared with the controls. This finding was not considered to be test article-related as similar significant differences were not evident in the males.

The hindlimb grip strength of test article-treated animals compared favorably with those of the controls.

See pp. 54-55 and 133-134

Locomotor Activity

The mean locomotor activity of males and females compared favorably with that of the control animals.

See pp. 56-57 and 135-136

6.3 FOOD CONSUMPTION

No test article-related differences in mean daily food consumption were noted when compared with the control values.

The relative food consumption of the test article-treated animals compared favorably with that of the control animals.

See pp. 29-30, 33-34, 58-61, 66-69, 137-138 ands 141-142

6.4 BODY WEIGHTS

No test article-related differences in body weight development were noted when compared with the control values.

See pp. 31-32, 62-65 and 139-140

6.5 CLINICAL LABORATORY INVESTIGATIONS

6.5.1 HEMATOLOGY

No test article-related differences in hematology parameters were noted when compared with the control values.

Slightly lower hemoglobin levels were noted in males treated with 1000 mg/kg/day when compared with the controls. The difference attained statistical significance, but was considered to be incidental.

The relative and absolute reticulocyte counts were slightly higher in animals treated with 1000 mg/kg/day (statistically significant in males), and coincided with a shift towards high fluorescence reticulocytes, but in the absence of concomitant differences in related red cell parameters and a clear dose-response relationship, these differences were considered to be incidental.

See pp. 70-71 and 143-150

6.5.2 CLINICAL BIOCHEMISTRY

No effects of toxicological significance upon the clinical biochemistry parameters were noted.

Creatinine levels were significantly higher in males ($p<0.05$) and females ($p<0.01$) treated with 1000 mg/kg/day when compared with the controls. Considering the relatively minor differences between the high-dose and control groups and in the absence of concomitant microscopic changes in the kidney, this finding was considered to be of no toxicological significance.

Triglyceride levels were lower in males ($p<0.05$) treated with 1000 mg/kg/day and in all treated females when compared with the controls. In the absence of a dose-response relationship in males, this finding was considered to be incidental.

Sodium levels in males ($p<0.05$) and females (not significant) treated with 200 mg/kg/day or 1000 mg/kg/day were marginally elevated when compared with the controls. This finding was not accompanied by similar or consistent changes in the remaining electrolytes, were within the ranges of historical control data and were therefore considered to be fortuitous.

All other differences to the control values were considered to be fortuitous and unrelated to the treatment with the test article.

See pp. 72-73 and 151-158

6.6 PATHOLOGY

6.6.1 ORGAN WEIGHTS

No test article-related differences to the organ weights of the control group were ascertained.

The absolute and relative thymus weights were marginally elevated in males and females treated with the test article at 1000 mg/kg/day. The difference to control values attained statistical significance in males ($p<0.05$). This finding was considered to be incidental as microscopic changes were not evident. All differences to the control values were considered to be fortuitous.

See pp. 76-81 and 167-175

6.6.2 MACROSCOPICAL FINDINGS

The macroscopic findings included renal pelvic dilation (one control male, one male at 50 mg/kg/day and one female at 1000 mg/kg/day) and foci on the thymus (one male and one female at 50 mg/kg/day, three females at 200 mg/kg/day and one female at 1000 mg/kg/day) were recorded. These findings did not distinguish test article-treated rats from controls and were within the range of spontaneous alterations which may be seen in rats of this age and strain.

See pp. 74-75 and 159-166

6.6.3 MICROSCOPICAL FINDINGS

A number of findings were noted in the organs/tissues examined. They were comparable to those occurring spontaneously in rats of the same strain and age, and their incidence, severity and morphologic appearance did not distinguish treated rats from control rats.

See pathology report pp. 216-277

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RCC PROJECT [redacted]

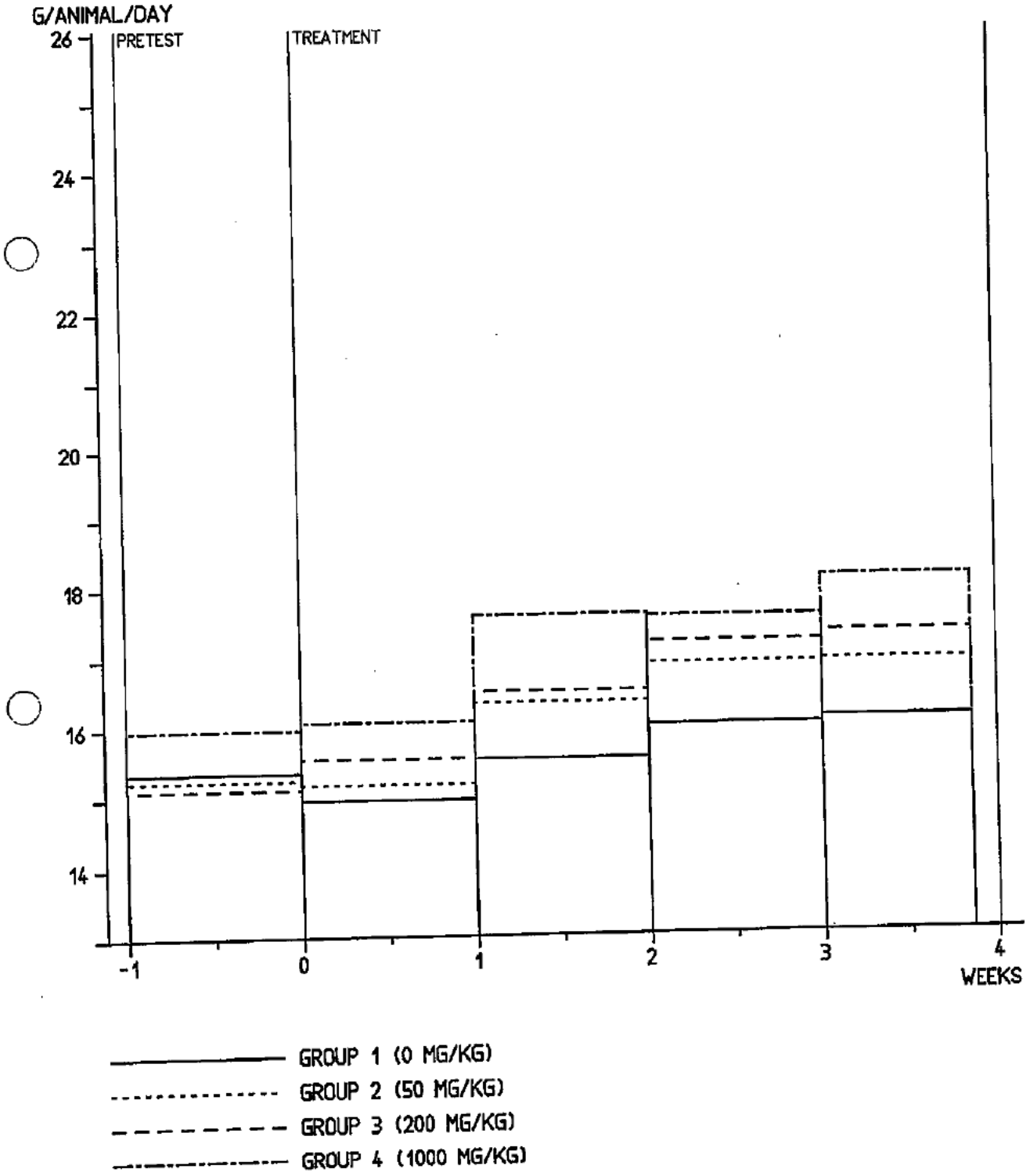
7 FIGURES

[redacted]

RCC PROJECT [redacted]

FC-SPLT - 2
23-JUL-99

FOOD CONSUMPTION
FEMALES



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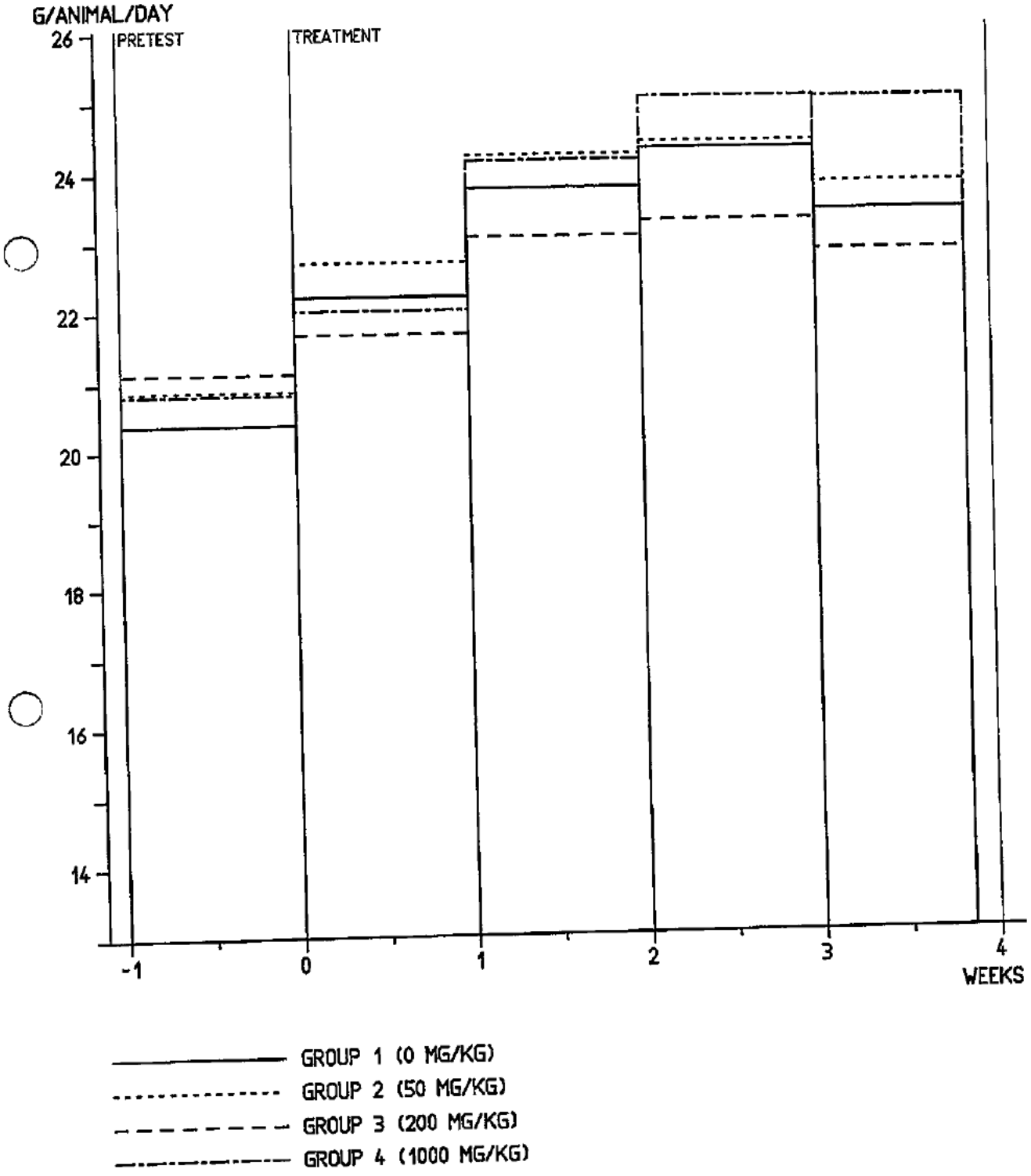
23-JUL-99

FOOD CONSUMPTION
MALES



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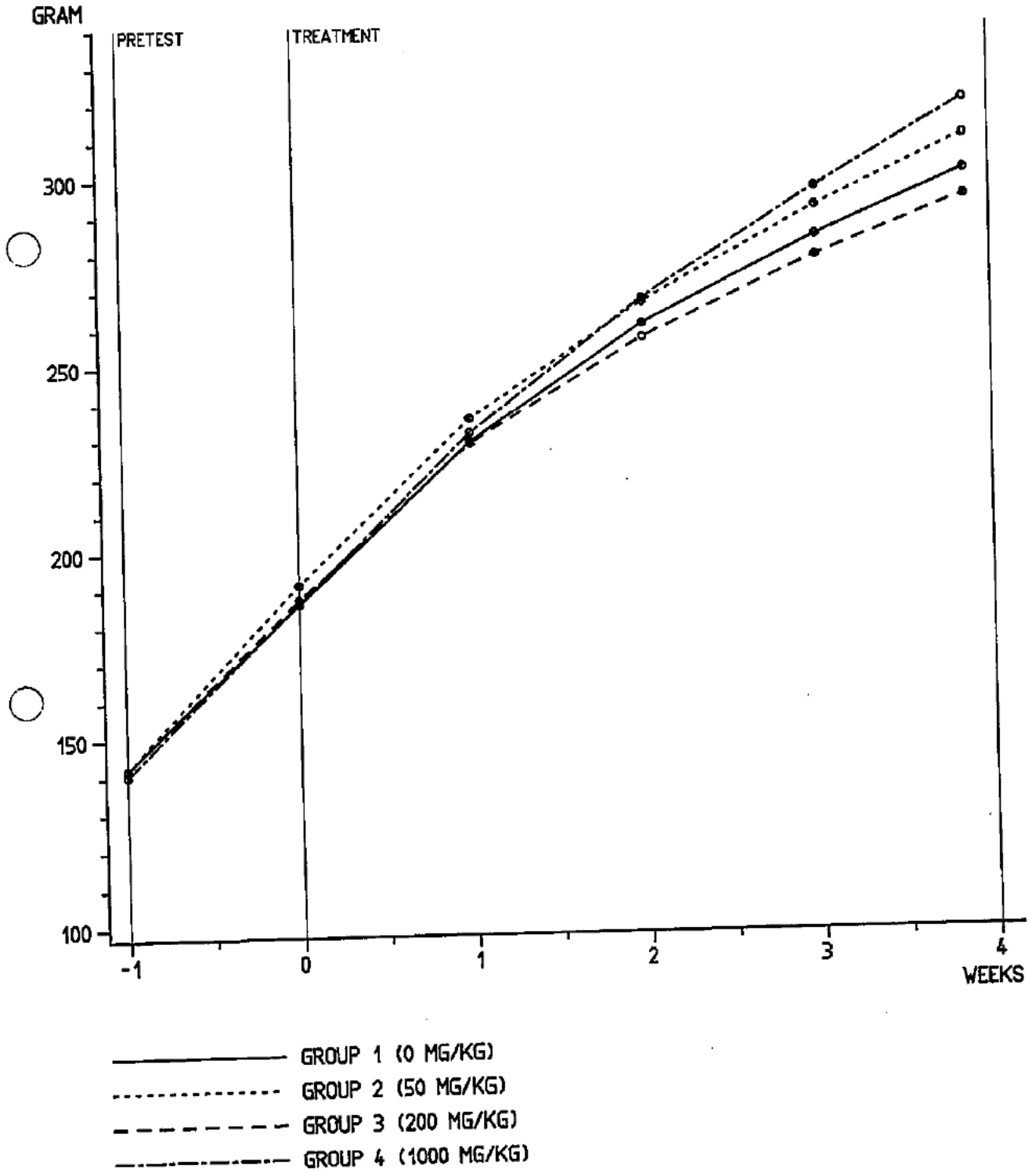
BW-SPLT - 1
23-JUL-99

BODY WEIGHTS
MALES



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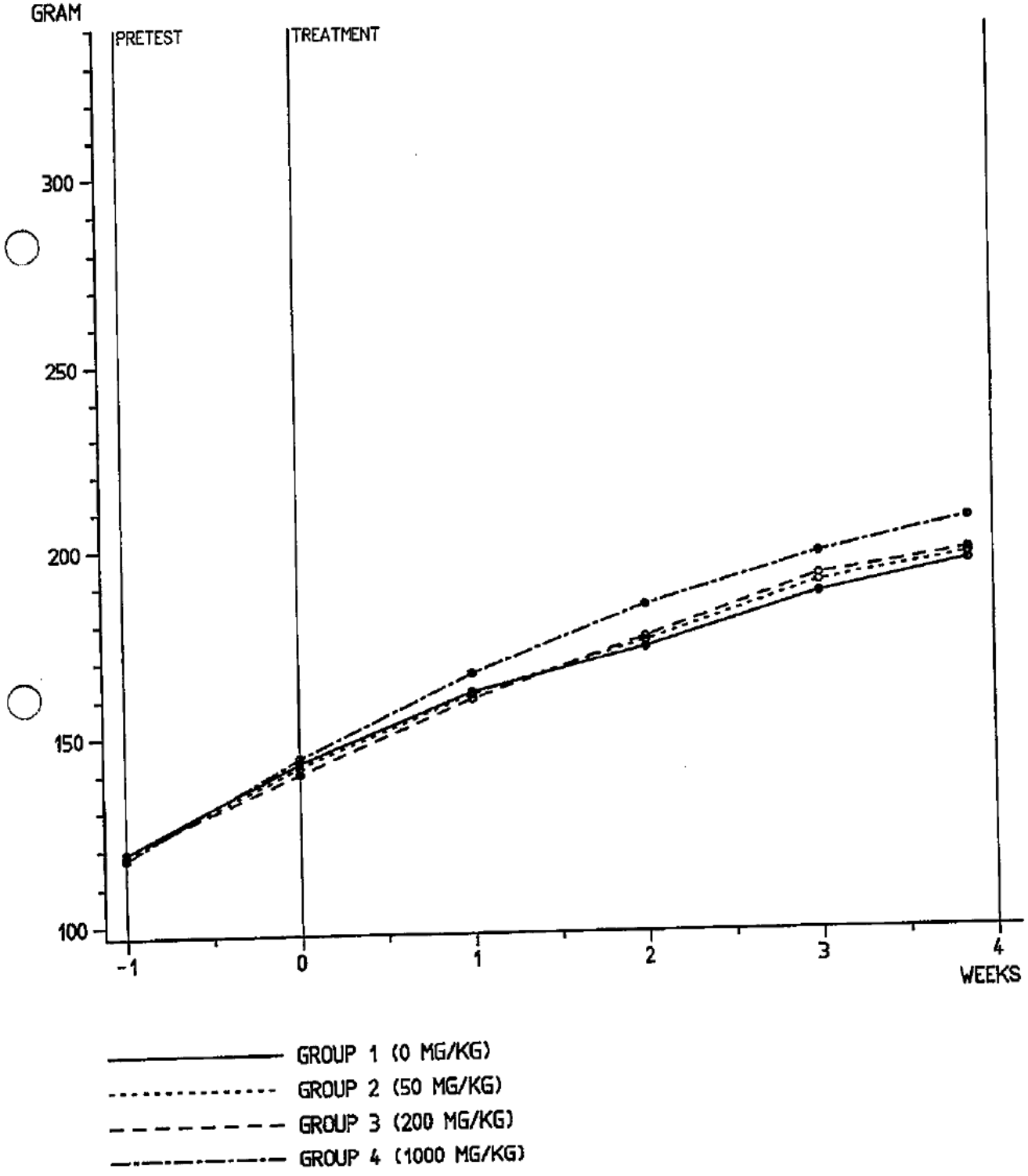
BW-SPET - 2
23-JUL-99

BODY WEIGHTS
FEMALES



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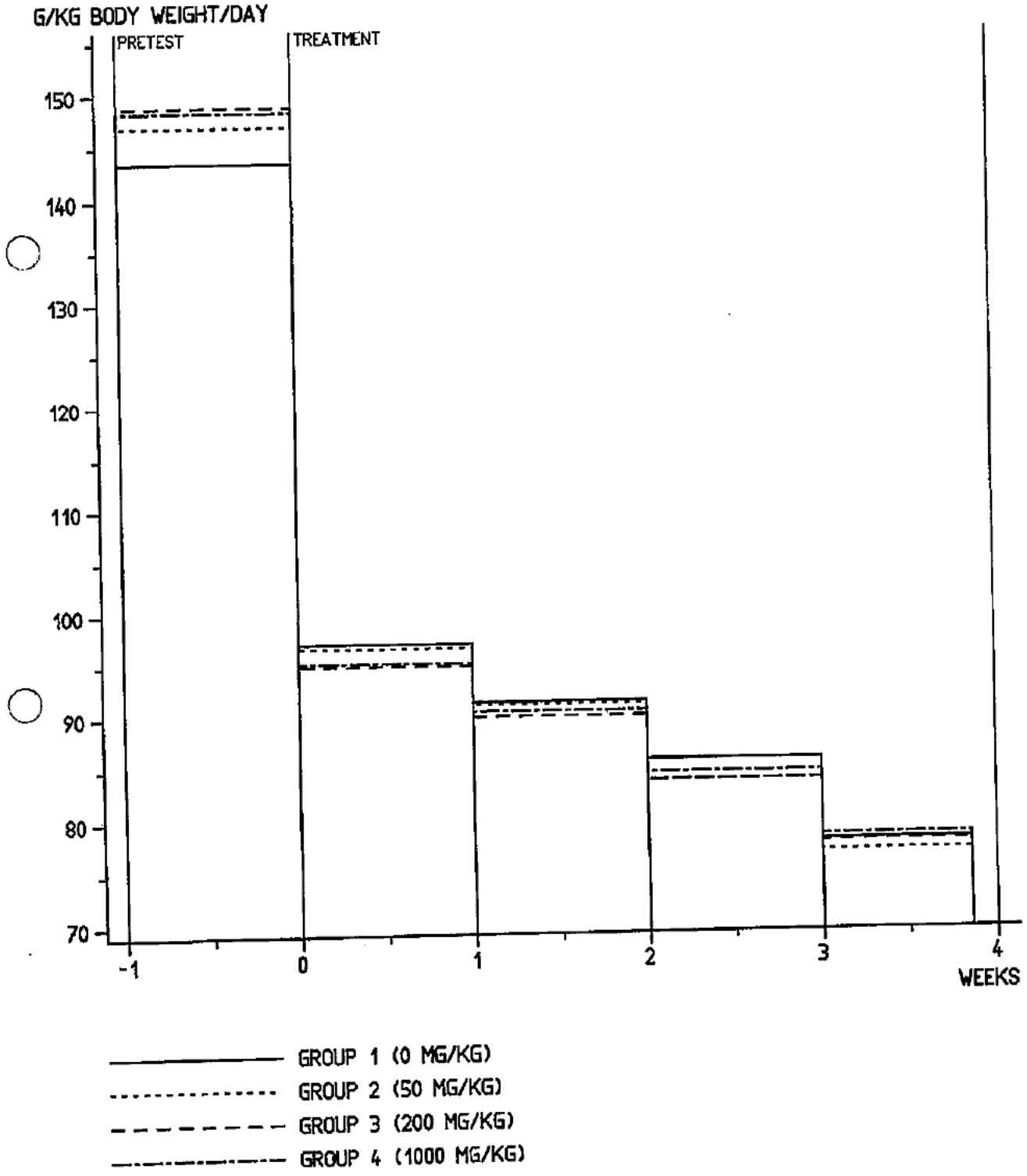
RCC PROJECT [redacted]

RFC-SPLT - 1
23-JUL-99

RELATIVE FOOD CONSUMPTION
MALES



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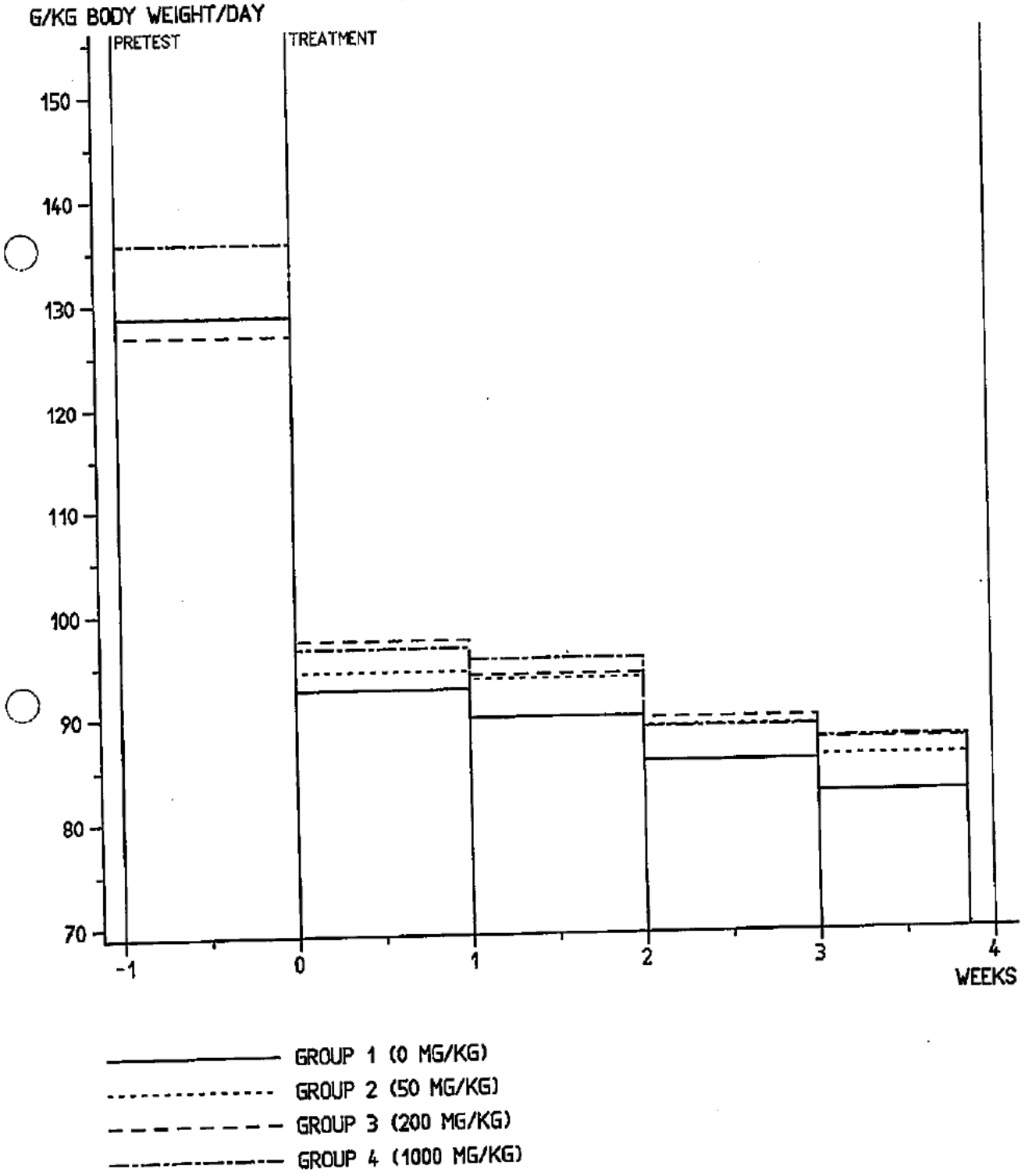
RFC-SPLT - 2
23-JUL-99

RELATIVE FOOD CONSUMPTION
FEMALES



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8 SUMMARY TABLES

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23-MAR-99

MORTALITY DATA
MALES
GROUP 1 (0 MG/KG)

ANIMAL	SCHEDULED NECROPSY	TREATMENT FROM	TO
1	23-MAR-99	23-FEB-99	22-MAR-99
2	23-MAR-99	23-FEB-99	22-MAR-99
3	23-MAR-99	23-FEB-99	22-MAR-99
4	23-MAR-99	23-FEB-99	22-MAR-99
5	23-MAR-99	23-FEB-99	22-MAR-99

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MORTALITY DATA
MALES
GROUP 2 (50 MG/KG)

ANIMAL	SCHEDULED NECROPSY	TREATMENT FROM	TO
6	23-MAR-99	23-FEB-99	22-MAR-99
7	23-MAR-99	23-FEB-99	22-MAR-99
8	23-MAR-99	23-FEB-99	22-MAR-99
9	23-MAR-99	23-FEB-99	22-MAR-99
10	23-MAR-99	23-FEB-99	22-MAR-99

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MORTALITY DATA
MALES
GROUP 3 (200 MG/KG)

ANIMAL	SCHEDULED NECROPSY	TREATMENT FROM	TO
11	23-MAR-99	23-FEB-99	22-MAR-99
12	23-MAR-99	23-FEB-99	22-MAR-99
13	23-MAR-99	23-FEB-99	22-MAR-99
14	23-MAR-99	23-FEB-99	22-MAR-99
15	23-MAR-99	23-FEB-99	22-MAR-99

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MORT-IND - 4
23-MAR-99

MORTALITY DATA
MALES
GROUP 4 (1000 MG/KG)

ANIMAL	SCHEDULED NECROPSY	TREATMENT FROM	TO
16	23-MAR-99	23-FEB-99	22-MAR-99
17	23-MAR-99	23-FEB-99	22-MAR-99
18	23-MAR-99	23-FEB-99	22-MAR-99
19	23-MAR-99	23-FEB-99	22-MAR-99
20	23-MAR-99	23-FEB-99	22-MAR-99

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MORT-IND - 5
23-MAR-99

MORTALITY DATA
FEMALES
GROUP 1 (0 MG/KG)

ANIMAL	SCHEDULED NECROPSY	TREATMENT FROM	TO
21	23-MAR-99	23-FEB-99	22-MAR-99
22	23-MAR-99	23-FEB-99	22-MAR-99
23	23-MAR-99	23-FEB-99	22-MAR-99
24	23-MAR-99	23-FEB-99	22-MAR-99
25	23-MAR-99	23-FEB-99	22-MAR-99

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RCC PROJECT [redacted]

23-MAR-99

MORTALITY DATA
FEMALES
GROUP 2 (50 MG/KG)

ANIMAL	SCHEDULED NECROPSY	TREATMENT FROM	TO
26	23-MAR-99	23-FEB-99	22-MAR-99
27	23-MAR-99	23-FEB-99	22-MAR-99
28	23-MAR-99	23-FEB-99	22-MAR-99
29	23-MAR-99	23-FEB-99	22-MAR-99
30	23-MAR-99	23-FEB-99	22-MAR-99

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RCC PROJECT [redacted]

MORT-IND - 7
23-MAR-99

MORTALITY DATA
FEMALES
GROUP 3 (200 MG/KG)

ANIMAL	SCHEDULED NECROPSY	TREATMENT FROM	TO
31	23-MAR-99	23-FEB-99	22-MAR-99
32	23-MAR-99	23-FEB-99	22-MAR-99
33	23-MAR-99	23-FEB-99	22-MAR-99
34	23-MAR-99	23-FEB-99	22-MAR-99
35	23-MAR-99	23-FEB-99	22-MAR-99

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RCC PROJECT [redacted]

MORT-IND - 8
23-MAR-99

MORTALITY DATA
FEMALES
GROUP 4 (1000 MG/KG)

ANIMAL	SCHEDULED NECROPSY	TREATMENT FROM	TO
36	23-MAR-99	23-FEB-99	22-MAR-99
37	23-MAR-99	23-FEB-99	22-MAR-99
38	23-MAR-99	23-FEB-99	22-MAR-99
39	23-MAR-99	23-FEB-99	22-MAR-99
40	23-MAR-99	23-FEB-99	22-MAR-99

RCC PROJECT [redacted]

DAILY CAGE-SIDE OBSERVATIONS, SUMMARY

GROUP 1 (CONTROL), MALES

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	No of animals in gr.		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
	No of animals in gr.		5	5	5	5	5	5	5
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No animals affected

n = Number of affected animals
n- = Mean severity between 1.00 and 1.67
n+ = Mean severity between 1.68 and 2.33
n! = Mean severity between 2.34 and 3.00

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, SUMMARY

GROUP 2 (LOW DOSE), MALES

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	No of animals in gr.		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
	No of animals in gr.		5	5	5	5	5	5	5
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No animals affected

n = Number of affected animals
n- = Mean severity between 1.00 and 1.67
n+ = Mean severity between 1.68 and 2.33
n! = Mean severity between 2.34 and 3.00

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, SUMMARY

GROUP 3 (MID DOSE), MALES

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	No of animals in gr.		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
	No of animals in gr.		5	5	5	5	5	5	5
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No animals affected

n = Number of affected animals
n- = Mean severity between 1.00 and 1.67
n+ = Mean severity between 1.68 and 2.33
n! = Mean severity between 2.34 and 3.00

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, SUMMARY

GROUP 4 (HIGH DOSE), MALES

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	No of animals in gr.		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
	No of animals in gr.		5	5	5	5	5	5	5
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No animals affected

n = Number of affected animals
n- = Mean severity between 1.00 and 1.67
n+ = Mean severity between 1.68 and 2.33
n! = Mean severity between 2.34 and 3.00

RCC PROJECT [redacted]

DAILY CAGE-SIDE OBSERVATIONS, SUMMARY

GROUP 1 (CONTROL), FEMALES

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	No of animals in gr.		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
	No of animals in gr.		5	5	5	5	5	5	5
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
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BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

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n! = Mean severity between 2.34 and 3.00

RCC PROJECT [redacted]

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DAILY CAGE-SIDE OBSERVATIONS, SUMMARY

GROUP 2 (LOW DOSE), FEMALES

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	No of animals in gr.		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
	No of animals in gr.		5	5	5	5	5	5	5
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

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BEH = Behavioral
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n! = Mean severity between 2.34 and 3.00

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DAILY CAGE-SIDE OBSERVATIONS, SUMMARY

GROUP 3 (MID DOSE), FEMALES

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	No of animals in gr.		5	5	5	5	5	5	5	6	5	5	5	5	5	5	5	5	5	5	5	5	5	5
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
	No of animals in gr.		5	5	5	5	5	5	5
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

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n! = Mean severity between 2.34 and 3.00

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[redacted]

DAILY CAGE-SIDE OBSERVATIONS, SUMMARY

GROUP 4 (HIGH DOSE), FEMALES

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	No of animals in gr.		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
	No of animals in gr.		5	5	5	5	5	5	5
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No animals affected

n = Number of affected animals
n- = Mean severity between 1.00 and 1.67
n+ = Mean severity between 1.68 and 2.33
n! = Mean severity between 2.34 and 3.00

RCC PROJECT [redacted]

[redacted]

WEEKLY OUTSIDE CAGE OBSERVATIONS, SUMMARY DATA

MALES

			GROUP 1					GROUP 2					GROUP 3					GROUP 4				
NUMBER OF ANIMALS IN GR.			5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
FINDINGS / STUDY WEEK		SCORE	P	1	2	3	4	P	1	2	3	4	P	1	2	3	4	P	1	2	3	4
APP	Piloerection	1-3																				
	Salivation	1-3																				
	Hunched posture	1																				
MOT	Ataxia	1-3																				
	Tremor/twitching	1-3																				
	Prostration	1																				
	Circling	1																				
	Spasm	1-3																				
BEH	Hyperactivity	1-3																				
	Somnolence	1-3																				
	Red. activity	1-3																			1-	
	Inc. exploration	1-3																				
	Red. grooming	1-3																				
	Vocalization	1-3																				
RES	Dyspnea	1																				
	Tachypnea	1																				
	Bradypnea	1																				
REF	Blink	1																				
	Pinna	1																				
	Iridic light reflex	1																				
	Push-off (hind legs)	1																				
	Pain response	1																				
	Startle / hearing	1																				
MIS	Lacrimation	1-3																				
	Limbs cyanotic	1																				
	Mydriasis	1																				
	Miosis	1																				
	Exophthalmos	1																				
	Red. muscle tone	1-3																				

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
MIS = Miscellaneous
REF = Reflexes
P = Pretest examination (during acclimatization)

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n! = Mean severity between 2.34 and 3.00

RCC PROJECT [redacted]

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WEEKLY OUTSIDE CAGE OBSERVATIONS, SUMMARY DATA

FEMALES

			GROUP 1					GROUP 2					GROUP 3					GROUP 4				
NUMBER OF ANIMALS IN GR.			5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
FINDING / STUDY WEEK		SCORE	P	1	2	3	4	P	1	2	3	4	P	1	2	3	4	P	1	2	3	4
APP	Piloerection	1-3																				
	Salivation	1-3																				
	Hunched posture	1																				
MOT	Ataxia	1-3																				
	Tremor/twitching	1-3																				
	Prostration	1																				
	Circling	1																				
	Spasm	1-3																				
BEH	Hyperactivity	1-3																				
	Somnolence	1-3																				
	Red. activity	1-3																				
	Inc. exploration	1-3																				
	Red. grooming	1-3																				
	Vocalization	1-3																				
RES	Dyspnea	1																				
	Tachypnea	1																				
	Bradypnea	1																				
REF	Blink	1																				
	Pinna	1																				
	Iridic light reflex	1																				
	Push-off (hind legs)	1																				
	Pain response	1																				
	Startle / hearing	1																				
MIS	Lacrimation	1-3																				
	Limbs cyanotic	1																				
	Mydriasis	1																				
	Miosis	1																				
	Exophthalmos	1																				
	Red. muscle tone	1-3																				

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REF = Reflexes
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n = Number of affected animals
n- = Mean severity between 1.00 and 1.67
n+ = Mean severity between 1.68 and 2.33
nI = Mean severity between 2.34 and 3.00

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23-MAR-99

GRIP STRENGTH SUMMARY
AT WEEK 4
MALES

		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
Forelimb Mean (g)	MEAN	607	631	630	631
	ST.DEV.	26	25	19	18
	N	5	5	5	5
Hindlimb Mean (g)	MEAN	531	535	533	536
	ST.DEV.	13	14	10	14
	N	5	5	5	5

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

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RCC PROJECT [redacted]

PAR-SUM - 2
23-MAR-99

GRIP STRENGTH SUMMARY
AT WEEK 4
FEMALES

		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
Forelimb Mean (g)	MEAN	479	521 *	516 *	514 *
	ST.DEV.	11	12	25	31
	N	5	5	5	5
Hindlimb Mean (g)	MEAN	432	455	445	438
	ST.DEV.	10	10	23	18
	N	5	5	5	5

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

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LOCOMOTOR ACTIVITY SUMMARY
LOW BEAMS COUNT
MALES

		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
15 min.	MEAN	860	932	981	539
	ST.DEV.	379	386	514	300
	N	5	5	5	5
30 min.	MEAN	450	538	530	416
	ST.DEV.	207	186	220	203
	N	5	5	5	5
45 min.	MEAN	305	416	352	277
	ST.DEV.	142	214	125	138
	N	5	5	5	5
60 min.	MEAN	378	382	521	351
	ST.DEV.	171	136	288	249
	N	5	5	5	5
Total	MEAN	1993	2267	2384	1584
	ST.DEV.	808	830	941	513
	N	5	5	5	5

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

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RCC PROJECT [redacted]

PAR-SUM - 2
07-APR-99

LOCOMOTOR ACTIVITY SUMMARY
LOW BEAMS COUNT
FEMALES

		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
15 min.	MEAN	814	970	1041	740
	ST.DEV.	286	313	356	184
	N	5	5	5	5
30 min.	MEAN	452	632	614	389
	ST.DEV.	126	188	252	217
	N	5	5	5	5
45 min.	MEAN	259	417	655 *	203
	ST.DEV.	141	160	270	198
	N	5	5	5	5
60 min.	MEAN	205	256	346	201
	ST.DEV.	184	280	259	158
	N	5	5	5	5
Total	MEAN	1730	2275	2656	1534
	ST.DEV.	400	725	813	588
	N	5	5	5	5

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

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FC-SUM - 1
23-MAR-99

FOOD CONSUMPTION (G/ANIMAL/DAY) SUMMARY
MALES

PRETEST		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-8	MEAN	20.4	20.9	21.1	20.8
WEEKS 1/2	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1

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FOOD CONSUMPTION (G/ANIMAL/DAY) SUMMARY
MALES

TREATMENT		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-8	MEAN	22.2	22.7	21.7	22.0
WEEKS 1/2	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
DAYS 8-15	MEAN	23.8	24.2	23.1	24.2
WEEKS 2/3	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
DAYS 15-22	MEAN	24.3	24.4	23.3	25.0
WEEKS 3/4	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
DAYS 22-28	MEAN	23.4	23.8	22.8	25.0
WEEK 4	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
MEAN OF MEANS OVER TREATMENT		23.4	23.8	22.7	24.1

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FC-SUM - 3
23-MAR-99

FOOD CONSUMPTION (G/ANIMAL/DAY) SUMMARY
FEMALES

PRETEST		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-8	MEAN	15.3	15.2	15.1	16.0
WEEKS 1/2	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1

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FC-SUM - 4
23-MAR-99

FOOD CONSUMPTION (G/ANIMAL/DAY) SUMMARY
FEMALES

TREATMENT		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-8 WEEKS 1/2	MEAN	15.0	15.2	15.5	16.1
	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
DAYS 8-15 WEEKS 2/3	MEAN	15.5	16.3	16.5	17.6
	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
DAYS 15-22 WEEKS 3/4	MEAN	16.0	16.9	17.2	17.6
	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
DAYS 22-28 WEEK 4	MEAN	16.1	16.9	17.3	18.1
	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
MEAN OF MEANS OVER TREATMENT		15.6	16.3	16.6	17.3

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[redacted]
23-JUL-99

BODY WEIGHTS (GRAM) SUMMARY
MALES

PRETEST		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
DAY 1	MEAN	142	142	142	140
WEEK 1	ST.DEV.	1.3	2.1	4.8	2.9
	N	5	5	5	5

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

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RCC PROJECT [redacted]

23-JUL-99

BODY WEIGHTS (GRAM) SUMMARY
MALES

TREATMENT		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
DAY 1 WEEK 1	MEAN	186	191	187	186
	ST.DEV.	6.2	3.4	9.5	6.3
	N	5	5	5	5
DAY 8 WEEK 2	MEAN	229	235	228	231
	ST.DEV.	12.7	3.5	10.4	8.8
	N	5	5	5	5
DAY 15 WEEK 3	MEAN	260	266	257	267
	ST.DEV.	17.5	3.5	12.0	14.6
	N	5	5	5	5
DAY 22 WEEK 4	MEAN	284	292	278	297
	ST.DEV.	21.9	8.7	12.5	14.4
	N	5	5	5	5
DAY 28 WEEK 4	MEAN	301	310	294	320
	ST.DEV.	25.9	12.3	10.1	16.2
	N	5	5	5	5

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

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RCC PROJECT [redacted]

23-JUL-99

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

PRETEST		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
DAY 1	MEAN	119	118	119	118
WEEK 1	ST.DEV.	2.0	4.8	3.7	3.1
	N	5	5	5	5

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

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RCC PROJECT [redacted]

BW-SUM # 4
23-JUL-99

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

TREATMENT		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
DAY 1	MEAN	143	142	140	144
WEEK 1	ST.DEV.	4.7	5.9	7.4	3.9
	N	5	5	5	5
DAY 8	MEAN	161	161	160	166
WEEK 2	ST.DEV.	5.4	10.3	9.4	8.5
	N	5	5	5	5
DAY 15	MEAN	173	175	176	184
WEEK 3	ST.DEV.	6.9	8.4	7.9	9.9
	N	5	5	5	5
DAY 22	MEAN	187	190	192	198
WEEK 4	ST.DEV.	10.0	13.6	8.1	10.5
	N	5	5	5	5
DAY 28	MEAN	195	197	198	207
WEEK 4	ST.DEV.	8.1	16.1	9.6	16.5
	N	5	5	5	5

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

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RCC PROJECT [redacted]

23-JUL-99

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

PRETEST		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-8	MEAN	144	147	149	148
WEEKS 1/2	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

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RCC PROJECT [redacted]

23-JUL-99

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

TREATMENT		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-8 WEEKS 1/2	MEAN	97	97	95	95
	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
DAYS 8-15 WEEKS 2/3	MEAN	91	91	90	90
	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
DAYS 15-22 WEEKS 3/4	MEAN	86	84	84	84
	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
DAYS 22-28 WEEK 4	MEAN	78	77	78	78
	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
MEAN OF MEANS OVER TREATMENT		88	87	86	87

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

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RCC PROJECT [redacted]

23-JUL-99

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

PRETEST		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-8	MEAN	129	129	127	136
WEEKS 1/2	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

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RCC PROJECT [redacted]

23-JUL-99

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

TREATMENT		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
DAYS 1-8 WEEKS 1/2	MEAN	93	94	97	97
	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
DAYS 8-15 WEEKS 2/3	MEAN	90	94	94	95
	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
DAYS 15-22 WEEKS 3/4	MEAN	86	89	90	89
	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
DAYS 22-28 WEEK 4	MEAN	82	86	87	88
	ST.DEV.	---	---	---	---
	N (CAGE)	1	1	1	1
MEAN OF MEANS OVER TREATMENT		88	91	92	92

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

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RCC PROJECT [redacted]

HEMATOLOGY SUMMARY
MALES

	RBC T/L	HB mmol/L	HCT L/L	MCV fL	MCH fmol	MCHC mmol/L	PLATELETS G/L
AFTER 4 WEEKS							
1 0 MG/KG	8.88	10.6	0.49	55.2	1.19	21.6	887
2 50 MG/KG	8.85	10.2	0.48	54.3	1.15	21.3	1033
3 200 MG/KG	8.97	10.4	0.48	54.0	1.16	21.4	1046
4 1000 MG/KG	8.78	10.0 *	0.47	53.6	1.13	21.2	1027

	RETICULOCYTE COUNT						
	RETIC. %	RETIC. T/L	HFR %	MFR %	LFR %	NEN /100 WBC	HEINZ BOD. o/oo
AFTER 4 WEEKS							
1 0 MG/KG	2.40	0.2106	7.8	37.5	54.7	0.0	0
2 50 MG/KG	2.71	0.2286	8.7	37.4	53.9	1.2	0
3 200 MG/KG	2.50	0.2166	7.9	35.2	56.9	0.6	0
4 1000 MG/KG	3.12 +	0.2662	12.7	37.8	49.6	1.0	0

			DIFF.WBC COUNT (REL.)				
	MET-HB %	WBC G/L	BAND 1	SEG. 1	EO. 1	BASO. 1	LYMPH. 1
AFTER 4 WEEKS							
1 0 MG/KG	0.4	9.3	0.00	0.09	0.01	0.00	0.91
2 50 MG/KG	0.6	8.2	0.00	0.13	0.01	0.00	0.86
3 200 MG/KG	0.5	9.1	0.00	0.13	0.00	0.00	0.87
4 1000 MG/KG	0.5	8.1	0.00	0.08	0.01	0.00	0.91

	DIFF.WBC COUNT (REL.)			DIFF.WBC COUNT (ABS.)			
	MONO. 1	PLAS. 1	OTHER 1	BAND G/L	SEG. G/L	EO. G/L	BASO. G/L
AFTER 4 WEEKS							
1 0 MG/KG	0.00	0.00	0.00	0.0	0.8	0.1	0.0
2 50 MG/KG	0.00	0.00	0.00	0.0	1.0	0.1	0.0
3 200 MG/KG	0.00	0.00	0.00	0.0	1.2	0.0	0.0
4 1000 MG/KG	0.00	0.00	0.00	0.0	0.7	0.0	0.0

	DIFF.WBC COUNT (ABS.)				COAGULATION	
	LYMPH. G/L	MONO. G/L	PLAS. G/L	OTHER G/L	PT sec	APTT sec
AFTER 4 WEEKS						
1 0 MG/KG	8.4	0.0	0.0	0.0	13.6	20.3
2 50 MG/KG	7.1	0.0	0.0	0.0	13.4	20.6
3 200 MG/KG	7.8	0.0	0.0	0.0	13.6	21.5
4 1000 MG/KG	7.4	0.0	0.0	0.0	13.7	20.8

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level. +: Steel-test sig. at 5% level.

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RCC PROJECT [redacted]

HEMATOLOGY SUMMARY
FEMALES

	RBC T/L	HB mmol/L	HCT L/L	MCV fL	MCH fmol	MCHC mmol/L	PLATELETS G/L
AFTER 4 WEEKS							
1 0 MG/KG	8.28	10.0	0.47	56.7	1.21	21.3	946
2 50 MG/KG	8.20	9.8	0.46	55.8	1.20	21.5	805
3 200 MG/KG	8.31	9.8	0.46	55.6	1.19	21.3	867
4 1000 MG/KG	8.25	9.8	0.46	55.5	1.20	21.5	974

	RETICULOCYTE COUNT						
	RETIC. %	RETIC. T/L	HFR %	MFR %	LFR %	NEN /100 WBC	HEINZ BOD. o/oo
AFTER 4 WEEKS							
1 0 MG/KG	2.60	0.2177	6.6	37.7	55.7	0.2	0
2 50 MG/KG	2.78	0.2308	8.2	34.0	57.8	0.8	0
3 200 MG/KG	3.08	0.2512	10.2	39.4	50.4	0.4	0
4 1000 MG/KG	3.11	0.2474	10.3	37.6	52.1	1.0	0

			DIFF.WBC COUNT (REL.)				
	MET-HB %	WBC G/L	BAND 1	SEG. 1	EO. 1	BASO. 1	LYMPH. 1
AFTER 4 WEEKS							
1 0 MG/KG	0.4	7.1	0.00	0.04	0.00	0.00	0.96
2 50 MG/KG	0.4	5.8	0.00	0.10	0.01	0.00	0.89
3 200 MG/KG	0.3	7.0	0.00	0.08	0.01	0.00	0.91
4 1000 MG/KG	0.4	6.9	0.00	0.06	0.00	0.00	0.94

	DIFF.WBC COUNT (REL.)			DIFF.WBC COUNT (ABS.)			
	MOND. 1	PLAS. 1	OTHER 1	BAND G/L	SEG. G/L	EO. G/L	BASO. G/L
AFTER 4 WEEKS							
1 0 MG/KG	0.00	0.00	0.00	0.0	0.2	0.0	0.0
2 50 MG/KG	0.00	0.00	0.00	0.0	0.5	0.0	0.0
3 200 MG/KG	0.00	0.00	0.00	0.0	0.6	0.1	0.0
4 1000 MG/KG	0.00	0.00	0.00	0.0	0.4	0.0	0.0

	DIFF.WBC COUNT (ABS.)				COAGULATION	
	LYMPH. G/L	MOND. G/L	PLAS. G/L	OTHER G/L	PT sec	APTT sec
AFTER 4 WEEKS						
1 0 MG/KG	6.9	0.0	0.0	0.0	13.4	20.6
2 50 MG/KG	5.2	0.0	0.0	0.0	13.6	22.0
3 200 MG/KG	6.4	0.0	0.0	0.0	13.8	21.1
4 1000 MG/KG	6.5	0.0	0.0	0.0	13.4	21.2

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level. +: Steel-test sig. at 5% level.

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RCC PROJECT [redacted]

CLINICAL BIOCHEMISTRY SUMMARY
MALES

	GLUCOSE mmol/L	UREA mmol/L	CREATININE µmol/L	URIC ACID µmol/L	BILI. T. µmol/L	CHOLEST.T. mmol/L	TRIGL. mmol/L
AFTER 4 WEEKS							
1 0 MG/KG	6.73	6.55	44.4	34.6	1.90	2.03	1.02
2 50 MG/KG	7.02	7.04	44.2	17.2 *	2.11	2.20	0.76
3 200 MG/KG	7.03	7.62	45.1	21.6	1.45	2.23	1.29
4 1000 MG/KG	5.98	7.46	48.7 *	25.8	1.88	1.86	0.53 *

	PHOS.LIPID mmol/L	ASAT(GOT) µkat/L	ALAT(GPT) µkat/L	LDH µkat/L	CK µkat/L	ALP µkat/L	G-GT nkat/L
AFTER 4 WEEKS							
1 0 MG/KG	1.99	1.19	0.82	3.48	3.54	6.56	23.54
2 50 MG/KG	2.05	1.29	0.75	2.79	2.81	8.05	23.44
3 200 MG/KG	2.11	1.22	0.77	3.23	3.01	6.24	23.74
4 1000 MG/KG	1.75	1.24	0.67	3.22	3.24	5.82	20.47

	CALCIUM mmol/L	PHOSPHORUS mmol/L	SODIUM mmol/L	POTASSIUM mmol/L	CHLORIDE mmol/L	ALBUMIN g/L	PROTEIN T. g/L
AFTER 4 WEEKS							
1 0 MG/KG	2.85	2.25	144.3	3.33	101.6	32.4	67.6
2 50 MG/KG	2.90	2.52	144.9	3.44	102.2	32.3	67.3
3 200 MG/KG	2.87	2.31	146.5 *	3.33	104.9 **	32.7	67.9
4 1000 MG/KG	2.86	2.54	146.9 *	3.28	103.1	32.1	67.0

	GLOBULIN g/L	A/G RATIO
AFTER 4 WEEKS		
1 0 MG/KG	35.1	0.92
2 50 MG/KG	35.0	0.92
3 200 MG/KG	35.2	0.93
4 1000 MG/KG	34.9	0.92

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level. +: Steel-test sig. at 5% level.

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RCC PROJECT [redacted]

CHE-SUM - 2
04-MAY-99

CLINICAL BIOCHEMISTRY SUMMARY
FEMALES

	GLUCOSE mmol/L	UREA mmol/L	CREATININE µmol/L	URIC ACID µmol/L	BILI. T. µmol/L	CHOLEST.T. mmol/L	TRIGL. mmol/L
AFTER 4 WEEKS							
1 0 MG/KG	4.87	7.74	47.7	21.7	1.72	1.71	0.87
2 50 MG/KG	5.75	7.76	48.0	28.4	2.24	1.61	0.55
3 200 MG/KG	5.58	8.05	49.7	25.9	1.73	1.75	0.50
4 1000 MG/KG	5.79	8.53	54.1 **	29.0	2.35	1.57	0.42

	PHOS.LIPID mmol/L	ASAT(GOT) µkat/L	ALAT(GPT) µkat/L	LDH µkat/L	CK µkat/L	ALP µkat/L	G-GT nkat/L
AFTER 4 WEEKS							
1 0 MG/KG	1.97	1.06	0.61	3.65	3.19	2.76	18.80
2 50 MG/KG	1.94	1.15	0.64	3.55	2.76	2.45	19.20
3 200 MG/KG	1.94	1.23 *	0.64	3.85	3.35	4.01 *	25.37
4 1000 MG/KG	1.84	1.19	0.63	3.29	3.06	2.74	21.04

	CALCIUM mmol/L	PHOSPHORUS mmol/L	SODIUM mmol/L	POTASSIUM mmol/L	CHLORIDE mmol/L	ALBUMIN g/L	PROTEIN T. g/L
AFTER 4 WEEKS							
1 0 MG/KG	2.87	2.09	145.9	3.21	104.1	33.7	67.1
2 50 MG/KG	2.78	1.85	144.9	3.20	104.5	35.2	68.6
3 200 MG/KG	2.76	2.01	146.9	3.02	106.0 *	32.3	64.4
4 1000 MG/KG	2.89	2.26	146.8	3.15	104.1	35.2	69.8

	GLOBULIN g/L	A/G RATIO
AFTER 4 WEEKS		
1 0 MG/KG	33.4	1.01
2 50 MG/KG	33.5	1.05
3 200 MG/KG	32.0	1.01
4 1000 MG/KG	34.6	1.02

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level. +: Steel-test sig. at 5% level.

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MACROSCOPICAL FINDINGS SUMMARY
MALES

	GROUP 1 0 MG/KG		GROUP 2 50 MG/KG		GROUP 3 200 MG/KG		GROUP 4 1000 MG/KG	
ANIMALS EXAMINED	5		5		5		5	
ANIMALS WITHOUT FINDINGS	4		3		5		5	
ANIMALS AFFECTED:								
KIDNEYS.....								
PELVIC DILATION	1	20%	1	20%	0	0%	0	0%
THYMUS.....								
FOCUS/FOCI	0	0%	1	20%	0	0%	0	0%

/ ## : Fisher's Exact Test based on counts significant at 5% (#) or 1% (##) level

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MACROSCOPICAL FINDINGS SUMMARY
FEMALES

	GROUP 1 0 MG/KG		GROUP 2 50 MG/KG		GROUP 3 200 MG/KG		GROUP 4 1000 MG/KG	
ANIMALS EXAMINED	5		5		5		5	
ANIMALS WITHOUT FINDINGS	5		4		2		3	
ANIMALS AFFECTED:								
KIDNEYS.....								
PELVIC DILATION	0	0%	0	0%	0	0%	1	20%
THYMUS.....								
FOCUS/FOCI	0	0%	1	20%	3	60%	1	20%

/ ## : Fisher's Exact Test based on counts significant at 5% (#) or 1% (##) level

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UN-SUM - 1
23-MAR-99

ORGAN WEIGHTS (GRAM) SUMMARY
AFTER 4 WEEKS
MALES

		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
BODY W.	MEAN	278.3	283.7	271.7	292.6
	ST.DEV.	26.5	12.5	10.4	15.2
	N	5	5	5	5
BRAIN	MEAN	1.95	1.91	1.93	1.95
	ST.DEV.	0.06	0.04	0.05	0.08
	N	5	5	5	5
HEART	MEAN	0.886	0.950	0.890	0.945
	ST.DEV.	0.089	0.066	0.025	0.095
	N	5	5	5	5
LIVER	MEAN	8.09	8.26	8.14	8.28
	ST.DEV.	1.13	0.37	0.60	0.64
	N	5	5	5	5
THYMUS	MEAN	0.38	0.42	0.40	0.49 *
	ST.DEV.	0.05	0.05	0.04	0.09
	N	5	5	5	5
KIDNEYS	MEAN	1.99	2.12	2.00	2.09
	ST.DEV.	0.13	0.06	0.16	0.18
	N	5	5	5	5
ADRENALS	MEAN	0.064	0.071	0.060	0.062
	ST.DEV.	0.013	0.013	0.008	0.014
	N	5	5	5	5
SPLEEN	MEAN	0.620	0.673	0.612	0.720
	ST.DEV.	0.067	0.083	0.076	0.122
	N	5	5	5	5
TESTES	MEAN	3.44	3.19	3.25	3.20
	ST.DEV.	0.20	0.05	0.28	0.23
	N	5	5	5	5
EPIDIDYMIDES	MEAN	0.986	0.919	0.943	0.960
	ST.DEV.	0.100	0.091	0.053	0.045
	N	5	5	5	5

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

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23-MAR-99

ORGAN/BODY WEIGHT RATIOS SUMMARY
AFTER 4 WEEKS
MALES

		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
BODY W. (GRAM)	MEAN	278.3	283.7	271.7	292.6
	ST.DEV.	26.5	12.5	10.4	15.2
	N	5	5	5	5
BRAIN (%)	MEAN	0.71	0.68	0.71	0.67
	ST.DEV.	0.05	0.04	0.01	0.02
	N	5	5	5	5
HEART (%)	MEAN	0.319	0.335	0.328	0.323
	ST.DEV.	0.015	0.022	0.006	0.018
	N	5	5	5	5
LIVER (%)	MEAN	2.90	2.91	3.00	2.83
	ST.DEV.	0.23	0.15	0.15	0.11
	N	5	5	5	5
THYMUS (%)	MEAN	0.14	0.15	0.15	0.17 *
	ST.DEV.	0.01	0.02	0.01	0.02
	N	5	5	5	5
KIDNEYS (%)	MEAN	0.72	0.75	0.73	0.71
	ST.DEV.	0.04	0.03	0.03	0.03
	N	5	5	5	5
ADRENALS (%)	MEAN	0.023	0.025	0.022	0.021
	ST.DEV.	0.004	0.004	0.004	0.005
	N	5	5	5	5
SPLEEN (%)	MEAN	0.223	0.238	0.225	0.246
	ST.DEV.	0.021	0.038	0.022	0.041
	N	5	5	5	5
TESTES (%)	MEAN	1.24	1.12 *	1.20	1.09 **
	ST.DEV.	0.08	0.06	0.08	0.04
	N	5	5	5	5
EPIDIDYMIDES (%)	MEAN	0.356	0.324	0.347	0.329
	ST.DEV.	0.043	0.035	0.020	0.025
	N	5	5	5	5

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

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UW-SUM - 3
23-MAR-99

ORGAN/BRAIN WEIGHT RATIOS SUMMARY
AFTER 4 WEEKS
MALES

		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
BRAIN (GRAM)	MEAN	1.95	1.91	1.93	1.95
	ST.DEV.	0.06	0.04	0.05	0.08
	N	5	5	5	5
HEART (%)	MEAN	45.340	49.683	46.015	48.360
	ST.DEV.	3.633	3.398	0.554	3.381
	N	5	5	5	5
LIVER (%)	MEAN	413.72	432.04	421.07	424.20
	ST.DEV.	50.80	25.39	25.13	29.96
	N	5	5	5	5
THYMUS (%)	MEAN	19.53	21.94	20.62	24.78 *
	ST.DEV.	2.41	3.11	1.95	3.53
	N	5	5	5	5
KIDNEYS (%)	MEAN	101.97	110.80	103.17	106.85
	ST.DEV.	5.76	5.45	5.75	6.20
	N	5	5	5	5
ADRENALS (%)	MEAN	3.267	3.720	3.086	3.166
	ST.DEV.	0.636	0.719	0.467	0.761
	N	5	5	5	5
SPLEEN (%)	MEAN	31.698	35.149	31.644	36.855
	ST.DEV.	2.839	3.918	3.497	5.773
	N	5	5	5	5
TESTES (%)	MEAN	176.04	166.53	168.22	163.55 *
	ST.DEV.	6.51	1.95	11.86	5.32
	N	5	5	5	5
EPIDIDYMIDES (%)	MEAN	50.481	48.024	48.744	49.239
	ST.DEV.	4.412	4.512	2.110	2.711
	N	5	5	5	5

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

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RCC PROJECT [redacted]

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OW-SUM - 4
23-MAR-99

ORGAN WEIGHTS (GRAM) SUMMARY
AFTER 4 WEEKS
FEMALES

		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
BODY W.	MEAN	177.3	177.8	176.8	187.1
	ST.DEV.	8.2	13.2	7.8	12.9
	N	5	5	5	5
BRAIN	MEAN	1.78	1.79	1.81	1.76
	ST.DEV.	0.06	0.10	0.04	0.09
	N	5	5	5	5
HEART	MEAN	0.648	0.691	0.670	0.703
	ST.DEV.	0.046	0.045	0.046	0.037
	N	5	5	5	5
LIVER	MEAN	5.44	5.36	5.29	5.53
	ST.DEV.	0.28	0.26	0.52	0.62
	N	5	5	5	5
THYMUS	MEAN	0.37	0.38	0.34	0.43
	ST.DEV.	0.09	0.08	0.04	0.10
	N	5	5	5	5
KIDNEYS	MEAN	1.39	1.42	1.38	1.41
	ST.DEV.	0.08	0.12	0.11	0.19
	N	5	5	5	5
ADRENALS	MEAN	0.079	0.080	0.080	0.081
	ST.DEV.	0.012	0.012	0.005	0.002
	N	5	5	5	5
SPLEEN	MEAN	0.451	0.479	0.460	0.470
	ST.DEV.	0.052	0.032	0.068	0.047
	N	5	5	5	5

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

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OW-SUM - 5
23-MAR-99

ORGAN/BODY WEIGHT RATIOS SUMMARY
AFTER 4 WEEKS
FEMALES

		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
BODY W.	MEAN	177.3	177.8	176.8	187.1
(GRAM)	ST.DEV.	8.2	13.2	7.8	12.9
	N	5	5	5	5
BRAIN	MEAN	1.01	1.01	1.02	0.94
(%)	ST.DEV.	0.06	0.08	0.04	0.07
	N	5	5	5	5
HEART	MEAN	0.366	0.390	0.380	0.377
(%)	ST.DEV.	0.031	0.037	0.034	0.023
	N	5	5	5	5
LIVER	MEAN	3.07	3.02	2.99	2.95
(%)	ST.DEV.	0.12	0.16	0.31	0.15
	N	5	5	5	5
THYMUS	MEAN	0.21	0.21	0.19	0.23
(%)	ST.DEV.	0.05	0.05	0.02	0.04
	N	5	5	5	5
KIDNEYS	MEAN	0.79	0.80	0.78	0.75
(%)	ST.DEV.	0.03	0.04	0.07	0.06
	N	5	5	5	5
ADRENALS	MEAN	0.045	0.045	0.045	0.043
(%)	ST.DEV.	0.008	0.008	0.004	0.003
	N	5	5	5	5
SPLEEN	MEAN	0.255	0.271	0.261	0.251
(%)	ST.DEV.	0.034	0.034	0.043	0.015
	N	5	5	5	5

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

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UW-SUM - 8
23-MAR-99

ORGAN/BRAIN WEIGHT RATIOS SUMMARY
AFTER 4 WEEKS
FEMALES

		GROUP 1 0 MG/KG	GROUP 2 50 MG/KG	GROUP 3 200 MG/KG	GROUP 4 1000 MG/KG
BRAIN (GRAM)	MEAN	1.78	1.79	1.81	1.76
	ST.DEV.	0.06	0.10	0.04	0.09
	N	5	5	5	5
HEART (%)	MEAN	36.457	38.538	37.089	40.024
	ST.DEV.	2.981	1.241	2.766	0.945
	N	5	5	5	5
LIVER (%)	MEAN	305.71	299.65	292.66	315.16
	ST.DEV.	22.28	13.79	30.56	36.10
	N	5	5	5	5
THYMUS (%)	MEAN	20.70	21.26	18.69	24.21
	ST.DEV.	5.76	5.25	1.93	4.60
	N	5	5	5	5
KIDNEYS (%)	MEAN	78.48	79.27	76.40	80.45
	ST.DEV.	6.48	4.86	4.92	9.70
	N	5	5	5	5
ADRENALS (%)	MEAN	4.424	4.488	4.430	4.620
	ST.DEV.	0.681	0.635	0.237	0.319
	N	5	5	5	5
SPLEEN (%)	MEAN	25.410	26.840	25.460	26.723
	ST.DEV.	3.436	2.753	3.922	1.806
	N	5	5	5	5

*/**: Dunnett-test based on pooled variance sig. at 5% or 1% level.

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RCC PROJECT [redacted]

9 INDIVIDUAL TABLES

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RCC PROJECT [redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 1 (CONTROL), MALES

ANIMAL No. 1

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1-3							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 1 (CONTROL), MALES

ANIMAL No. 2

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 1 (CONTROL), MALES

ANIMAL No. 3

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 1 (CONTROL), MALES

ANIMAL No. 4

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 1 (CONTROL), MALES

ANIMAL No. 5

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 2 (LOW DOSE), MALES

ANIMAL No. 6

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 2 (LOW DOSE), MALES

ANIMAL No. 7

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 2 (LOW DOSE), MALES

ANIMAL No. 8

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 2 (LOW DOSE), MALES

ANIMAL No. 9

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 2 (LOW DOSE), MALES

ANIMAL No. 10

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n. = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 3 (MID DOSE), MALES

ANIMAL No. 11

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 3 (MID DOSE), MALES

ANIMAL No. 12

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 3 (MID DOSE), MALES

ANIMAL No. 13

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 3 (MID DOSE), MALES

ANIMAL No. 14

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 3 (MID DOSE), MALES

ANIMAL No. 15

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 4 (HIGH DOSE), MALES

ANIMAL No. 16

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 4 (HIGH DOSE), MALES

ANIMAL No. 17

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 4 (HIGH DOSE), MALES

ANIMAL No. 18

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 4 (HIGH DOSE), MALES

ANIMAL No. 19

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 4 (HIGH DOSE), MALES

ANIMAL No. 20

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 1 (CONTROL), FEMALES

ANIMAL No. 21

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 1 (CONTROL), FEMALES

ANIMAL No. 22

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

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RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 1 (CONTROL), FEMALES

ANIMAL No. 23

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 1 (CONTROL), FEMALES

ANIMAL No. 24

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

[redacted]

BCC PROJECT [redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 1 (CONTROL), FEMALES

ANIMAL No. 25

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 2 (LOW DOSE), FEMALES

ANIMAL No. 26

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 2 (LOW DOSE), FEMALES

ANIMAL No. 27

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 2 (LOW DOSE), FEMALES

ANIMAL No. 28

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 2 (LOW DOSE), FEMALES

ANIMAL No. 29

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 2 (LOW DOSE), FEMALES

ANIMAL No. 30

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 3 (MID DOSE), FEMALES

ANIMAL No. 31

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 3 (MID DOSE), FEMALES

ANIMAL No. 32

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 3 (MID DOSE), FEMALES

ANIMAL No. 33

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

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n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 3 (MID DOSE), FEMALES

ANIMAL No. 34

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

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n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 3 (MID DOSE), FEMALES

ANIMAL No. 35

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 4 (HIGH DOSE), FEMALES

ANIMAL No. 36

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 4 (HIGH DOSE), FEMALES

ANIMAL No. 37

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
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RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 4 (HIGH DOSE), FEMALES

ANIMAL No. 38

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
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[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 4 (HIGH DOSE), FEMALES

ANIMAL No. 39

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

DAILY CAGE-SIDE OBSERVATIONS, INDIVIDUAL DATA

GROUP 4 (HIGH DOSE), FEMALES

ANIMAL No. 40

	PARAMETER	SCORE	P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
APP	Piloerection	1-3																						
	Salivation	1-3																						
	Hunched posture	1																						
MOT	Ataxia	1-3																						
	Tremor/twitching	1-3																						
	Prostration	1																						
BEH	Hyperactivity	1-3																						
	Somnolence	1-3																						
RES	Dyspnea	1																						
	Tachypnea	1																						
	Bradypnea	1																						

			Treatment						
	PARAMETER	SCORE	22	23	24	25	26	27	28
APP	Piloerection	1-3							
	Salivation	1-3							
	Hunched posture	1							
MOT	Ataxia	1-3							
	Tremor/twitching	1-3							
	Prostration	1							
BEH	Hyperactivity	1-3							
	Somnolence	1-3							
RES	Dyspnea	1							
	Tachypnea	1							
	Bradypnea	1							

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
P = Pretest examination (during acclimatization)

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

WEEKLY OUTSIDE CAGE OBSERVATIONS, INDIVIDUAL DATA

PRETEST

GROUP 1 (CONTROL) GROUP 2 (LOW DOSE)

			MALES					FEMALES					MALES					FEMALES				
FINDINGS / ANIMAL NO		SCORE	1	2	3	4	5	21	22	23	24	25	6	7	8	9	10	26	27	28	29	30
APP	Piloerection	1-3																				
	Salivation	1-3																				
	Hunched posture	1																				
MOT	Ataxia	1-3																				
	Tremor/twitching	1-3																				
	Prostration	1																				
	Circling	1																				
	Spasm	1-3																				
BEH	Hyperactivity	1-3																				
	Somnolence	1-3																				
	Red. activity	1-3																				
	Inc. Exploration	1-3																				
	Red. grooming	1-3																				
	Vocalization	1-3																				
RES	Dyspnea	1																				
	Tachypnea	1																				
	Bradypnea	1																				
REF	Blink	1																				
	Pinna	1																				
	Iridic light reflex	1																				
	Push-off (hind legs)	1																				
	Pain response	1																				
	Startle / hearing	1																				
MIS	Lacrimation	1-3																				
	Limbs cyanotic	1																				
	Mydriasis	1																				
	Miosis	1																				
	Exophthalmos	1																				
	Red. muscle tone	1-3																				

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
REF = Reflexes
MIS = Miscellaneous

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

WEEKLY OUTSIDE CAGE OBSERVATIONS, INDIVIDUAL DATA

PRETEST

GROUP 3 (MID DOSE) GROUP 4 (HIGH DOSE)

			MALES					FEMALES					MALES					FEMALES				
FINDINGS / ANIMAL NO		SCORE	11	12	13	14	15	31	32	33	34	35	16	17	18	19	20	36	37	38	39	40
APP	Piloerection	1-3																				
	Salivation	1-3																				
	Hunched posture	1																				
MOT	Ataxia	1-3																				
	Tremor/twitching	1-3																				
	Prostration	1																				
	Circling	1																				
	Spasm	1-3																				
BEH	Hyperactivity	1-3																				
	Somnolence	1-3																				
	Red. activity	1-3																				
	Inc. exploration	1-3																				
	Red. grooming	1-3																				
	Vocalization	1-3																				
RES	Dyspnea	1																				
	Tachypnea	1																				
	Bradypnea	1																				
REF	Blink	1																				
	Pinna	1																				
	Iridic light reflex	1																				
	Push-off (hind legs)	1																				
	Pain response	1																				
	Startle / hearing	1																				
MIS	Lacrimation	1-3																				
	Limbs cyanotic	1																				
	Mydriasis	1																				
	Miosis	1																				
	Exophthalmos	1																				
	Red. muscle tone	1-3																				

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
REF = Reflexes
MIS = Miscellaneous

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

WEEKLY OUTSIDE CAGE OBSERVATIONS, INDIVIDUAL DATA

WEEK 1

GROUP 1 (CONTROL) GROUP 2 (LOW DOSE)

			MALES					FEMALES					MALES					FEMALES				
FINDINGS / ANIMAL NO		SCORE	1	2	3	4	5	21	22	23	24	25	6	7	8	9	10	26	27	28	29	30
APP	Piloerection	1-3																				
	Salivation	1-3																				
	Hunched posture	1																				
MOT	Ataxia	1-3																				
	Tremor/twitching	1-3																				
	Prostration	1																				
	Circling	1																				
	Spasm	1-3																				
BEH	Hyperactivity	1-3																				
	Somnolence	1-3																				
	Red. activity	1-3																				
	Inc. exploration	1-3																				
	Red. grooming	1-3																				
	Vocalization	1-3																				
RES	Dyspnea	1																				
	Tachypnea	1																				
	Bradypnea	1																				
REF	Blink	1																				
	Pinna	1																				
	Iridic light reflex	1																				
	Push-off (hind legs)	1																				
	Pain response	1																				
	Startle / hearing	1																				
MIS	Lacrimation	1-3																				
	Limbs cyanotic	1																				
	Mydriasis	1																				
	Miosis	1																				
	Exophthalmos	1																				
	Red. muscle tone	1-3																				

Abbreviations:

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RCC PROJECT [redacted]

[redacted]

WEEKLY OUTSIDE CAGE OBSERVATIONS, INDIVIDUAL DATA

WEEK 1

			GROUP 3 (MID DOSE)										GROUP 4 (HIGH DOSE)									
			MALES					FEMALES					MALES					FEMALES				
FINDINGS / ANIMAL NO		SCORE	11	12	13	14	15	31	32	33	34	35	16	17	18	19	20	36	37	38	39	40
APP	Piloerection	1-3																				
	Salivation	1-3																				
	Hunched posture	1																				
MOT	Ataxia	1-3																				
	Tremor/twitching	1-3																				
	Prostration	1																				
	Circling	1																				
	Spasm	1-3																				
BEH	Hyperactivity	1-3																				
	Somnolence	1-3																				
	Red. activity	1-3																				
	Inc. exploration	1-3																				
	Red. grooming	1-3																				
	Vocalization	1-3																				
RES	Dyspnea	1																				
	Tachypnea	1																				
	Bradypnea	1																				
REF	Blink	1																				
	Pinna	1																				
	Iridic light reflex	1																				
	Push-off (hind legs)	1																				
	Pain response	1																				
	Startle / hearing	1																				
MIS	Lacrimation	1-3																				
	Limbs cyanotic	1																				
	Mydriasis	1																				
	Miosis	1																				
	Exophthalmos	1																				
	Red. muscle tone	1-3																				

Abbreviations:

APP = Appearance
MOT = Motor
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MIS = Miscellaneous

[ ] = No abnormal findings noted

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RCC PROJECT [redacted]

[redacted]

WEEKLY OUTSIDE CAGE OBSERVATIONS, INDIVIDUAL DATA

WEEK 2

GROUP 1 (CONTROL) GROUP 2 (LOW DOSE)

			MALES					FEMALES					MALES					FEMALES				
FINDINGS / ANIMAL NO		SCORE	1	2	3	4	5	21	22	23	24	25	6	7	8	9	10	26	27	28	29	30
APP	Piloerection	1-3																				
	Salivation	1-3																				
	Hunched posture	1																				
MOT	Ataxia	1-3																				
	Tremor/twitching	1-3																				
	Prostration	1																				
	Circling	1																				
	Spasm	1-3																				
BEH	Hyperactivity	1-3																				
	Somnolence	1-3																				
	Red. activity	1-3																				
	Inc. exploration	1-3																				
	Red. grooming	1-3																				
	Vocalization	1-3																				
RES	Dyspnea	1																				
	Tachypnea	1																				
	Bradypnea	1																				
REF	Blink	1																				
	Pinna	1																				
	Iridic light reflex	1																				
	Push-off (hind legs)	1																				
	Pain response	1																				
	Startle / hearing	1																				
MIS	Lacrimation	1-3																				
	Limbs cyanotic	1																				
	Mydriasis	1																				
	Miosis	1																				
	Exophthalmos	1																				
	Red. muscle tone	1-3																				

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
REF = Reflexes
MIS = Miscellaneous

[ ] = No abnormal findings noted

n = Severity of findings

RCC PROJECT [redacted]

[redacted]

WEEKLY OUTSIDE CAGE OBSERVATIONS, INDIVIDUAL DATA

WEEK 2

GROUP 3 (MID DOSE) GROUP 4 (HIGH DOSE)

			MALES					FEMALES					MALES					FEMALES				
FINDINGS / ANIMAL NO		SCORE	11	12	13	14	15	31	32	33	34	35	16	17	18	19	20	36	37	38	39	40
APP	Piloerection	1-3																				
	Salivation	1-3																				
	Hunched posture	1																				
MOT	Ataxia	1-3																				
	Tremor/twitching	1-3																				
	Prostration	1																				
	Circling	1																				
	Spasm	1-3																				
BEH	Hyperactivity	1-3																				
	Somnolence	1-3																				
	Red. activity	1-3																				
	Inc. exploration	1-3																				
	Red. grooming	1-3																				
	Vocalization	1-3																				
RES	Dyspnea	1																				
	Tachypnea	1																				
	Bradypnea	1																				
REF	Blink	1																				
	Pinna	1																				
	Iridic light reflex	1																				
	Push-off (hind legs)	1																				
	Pain response	1																				
	Startle / hearing	1																				
MIS	Lacrimation	1-3																				
	Limbs cyanotic	1																				
	Mydriasis	1																				
	Miosis	1																				
	Exophthalmos	1																				
	Red. muscle tone	1-3																				

Abbreviations:

APP = Appearance
MOT = Motor
BEH = Behavioral
RES = Respiration
REF = Reflexes
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RCC PROJECT [redacted]

[redacted]

WEEKLY OUTSIDE CAGE OBSERVATIONS, INDIVIDUAL DATA

WEEK 3

GROUP 1 (CONTROL) GROUP 2 (LOW DOSE)

			MALES					FEMALES					MALES					FEMALES				
FINDINGS / ANIMAL NO		SCORE	1	2	3	4	5	21	22	23	24	25	6	7	8	9	10	26	27	28	29	30
APP	Piloerection	1-3																				
	Salivation	1-3																				
	Hunched posture	1																				
MOT	Ataxia	1-3																				
	Tremor/twitching	1-3																				
	Prostration	1																				
	Circling	1																				
	Spasm	1-3																				
BEH	Hyperactivity	1-3																				
	Somnolence	1-3																				
	Red. activity	1-3																				
	Inc. exploration	1-3																				
	Red. grooming	1-3																				
	Vocalization	1-3																				
RES	Dyspnea	1																				
	Tachypnea	1																				
	Bradypnea	1																				
REF	Blink	1																				
	Pinna	1																				
	Iridic light reflex	1																				
	Push-off (hind legs)	1																				
	Pain response	1																				
	Startle / hearing	1																				
MIS	Lacrimation	1-3																				
	Limbs cyanotic	1																				
	Mydriasis	1																				
	Miosis	1																				
	Exophthalmos	1																				
	Red. muscle tone	1-3																				

Abbreviations:

APP = Appearance
MOT = Motor
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RES = Respiration
REF = Reflexes
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n = Severity of findings

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WEEKLY OUTSIDE CAGE OBSERVATIONS, INDIVIDUAL DATA

WEEK 3

GROUP 3 (MID DOSE) GROUP 4 (HIGH DOSE)

			MALES					FEMALES					MALES					FEMALES				
FINDINGS / ANIMAL NO		SCORE	11	12	13	14	15	31	32	33	34	35	16	17	18	19	20	36	37	38	39	40
APP	Piloerection	1-3																				
	Salivation	1-3																				
	Hunched posture	1																				
MOT	Ataxia	1-3																				
	Tremor/twitching	1-3																				
	Prostration	1																				
	Circling	1																				
	Spasm	1-3																				
BEH	Hyperactivity	1-3																				
	Somnolence	1-3																				
	Red. activity	1-3														1						
	Inc. exploration	1-3																				
	Red. grooming	1-3																				
	Vocalization	1-3																				
RES	Dyspnea	1																				
	Tachypnea	1																				
	Bradypnea	1																				
REF	Blink	1																				
	Pinna	1																				
	Iridic light reflex	1																				
	Push-off (hind legs)	1																				
	Pain response	1																				
	Startle / hearing	1																				
MIS	Lacrimation	1-3																				
	Limbs cyanotic	1																				
	Mydriasis	1																				
	Miosis	1																				
	Exophthalmos	1																				
	Red. muscle tone	1-3																				

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WEEKLY OUTSIDE CAGE OBSERVATIONS, INDIVIDUAL DATA

WEEK 4

GROUP 1 (CONTROL) GROUP 2 (LOW DOSE)

			MALES					FEMALES					MALES					FEMALES				
FINDINGS / ANIMAL NO		SCORE	1	2	3	4	5	21	22	23	24	25	6	7	8	9	10	26	27	28	29	30
APP	Piloerection	1-3																				
	Salivation	1-3																				
	Hunched posture	1																				
MOT	Ataxia	1-3																				
	Tremor/twitching	1-3																				
	Prostration	1																				
	Circling	1																				
	Spasm	1-3																				
BEH	Hyperactivity	1-3																				
	Somnolence	1-3																				
	Red. activity	1-3																				
	Inc. exploration	1-3																				
	Red. grooming	1-3																				
	Vocalization	1-3																				
RES	Dyspnea	1																				
	Tachypnea	1																				
	Bradypnea	1																				
REF	Blink	1																				
	Pinna	1																				
	Iridic light reflex	1																				
	Push-off (hind legs)	1																				
	Pain response	1																				
	Startle / hearing	1																				
MIS	Lacrimation	1-3																				
	Limbs cyanotic	1																				
	Mydriasis	1																				
	Miosis	1																				
	Exophthalmos	1																				
	Red. muscle tone	1-3																				

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WEEKLY OUTSIDE CAGE OBSERVATIONS, INDIVIDUAL DATA

WEEK 4

GROUP 3 (MID DOSE) GROUP 4 (HIGH DOSE)

			MALES					FEMALES					MALES					FEMALES				
FINDINGS / ANIMAL NO		SCORE	11	12	13	14	15	31	32	33	34	35	16	17	18	19	20	36	37	38	39	40
APP	Piloerection	1-3																				
	Salivation	1-3																				
	Hunched posture	1																				
MOT	Ataxia	1-3																				
	Tremor/twitching	1-3																				
	Prostration	1																				
	Circling	1																				
	Spasm	1-3																				
BEH	Hyperactivity	1-3																				
	Somnolence	1-3																				
	Red. activity	1-3																				
	Inc. exploration	1-3																				
	Red. grooming	1-3																				
	Vocalization	1-3																				
RES	Dyspnea	1																				
	Tachypnea	1																				
	Bradypnea	1																				
REF	Blink	1																				
	Pinna	1																				
	Iridic light reflex	1																				
	Push-off (hind legs)	1																				
	Pain response	1																				
	Startle / hearing	1																				
MIS	Lacrimation	1-3																				
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GRIP STRENGTH
MALES

ANIMAL NUMBER		Forelimb Mean (g)	Hindlimb Mean (g)
GROUP 1 (0 MG/KG)			
1	AT WEEK 4	587	520
2	AT WEEK 4	633	547
3	AT WEEK 4	580	520
4	AT WEEK 4	637	543
5	AT WEEK 4	600	527
GROUP 2 (50 MG/KG)			
6	AT WEEK 4	610	523
7	AT WEEK 4	627	540
8	AT WEEK 4	610	520
9	AT WEEK 4	670	553
10	AT WEEK 4	640	540
GROUP 3 (200 MG/KG)			
11	AT WEEK 4	620	520
12	AT WEEK 4	647	543
13	AT WEEK 4	653	543
14	AT WEEK 4	607	527
15	AT WEEK 4	623	530
GROUP 4 (1000 MG/KG)			
16	AT WEEK 4	603	523
17	AT WEEK 4	630	523
18	AT WEEK 4	637	533
19	AT WEEK 4	653	553
20	AT WEEK 4	633	547

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PAR-IND - 2
23-MAR-99

GRIP STRENGTH
FEMALES

ANIMAL NUMBER		Forelimb Mean (g)	Hindlimb Mean (g)
GROUP 1 (0 MG/KG)			
21	AT WEEK 4	467	440
22	AT WEEK 4	477	423
23	AT WEEK 4	487	420
24	AT WEEK 4	470	440
25	AT WEEK 4	493	437
GROUP 2 (50 MG/KG)			
26	AT WEEK 4	537	440
27	AT WEEK 4	527	460
28	AT WEEK 4	513	450
29	AT WEEK 4	507	457
30	AT WEEK 4	523	467
GROUP 3 (200 MG/KG)			
31	AT WEEK 4	520	463
32	AT WEEK 4	523	447
33	AT WEEK 4	537	460
34	AT WEEK 4	527	447
35	AT WEEK 4	473	407
GROUP 4 (1000 MG/KG)			
36	AT WEEK 4	520	443
37	AT WEEK 4	460	407
38	AT WEEK 4	527	450
39	AT WEEK 4	530	440
40	AT WEEK 4	533	450

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PAR-IND - I
07-APR-99

LOCOMOTOR ACTIVITY
MALES

ANIMAL		15 min.	30 min.	45 min.	60 min.	Total
GROUP 1 (0 MG/KG)						
1	LOW BEAMS COUNT	1118	594	144	534	2390
2	LOW BEAMS COUNT	509	282	376	245	1412
3	LOW BEAMS COUNT	982	449	340	381	2152
4	LOW BEAMS COUNT	1274	711	486	558	3029
5	LOW BEAMS COUNT	416	216	178	173	983
GROUP 2 (50 MG/KG)						
6	LOW BEAMS COUNT	817	456	424	432	2129
7	LOW BEAMS COUNT	609	375	382	389	1755
8	LOW BEAMS COUNT	617	471	350	274	1712
9	LOW BEAMS COUNT	1528	856	756	578	3718
10	LOW BEAMS COUNT	1088	530	168	237	2023
GROUP 3 (200 MG/KG)						
11	LOW BEAMS COUNT	561	219	263	227	1270
12	LOW BEAMS COUNT	608	572	548	410	2138
13	LOW BEAMS COUNT	1830	835	255	905	3825
14	LOW BEAMS COUNT	1051	544	291	738	2624
15	LOW BEAMS COUNT	853	479	404	325	2061
GROUP 4 (1000 MG/KG)						
16	LOW BEAMS COUNT	741	111	368	311	1531
17	LOW BEAMS COUNT	698	609	215	714	2236
18	LOW BEAMS COUNT	389	452	224	142	1207
19	LOW BEAMS COUNT	81	333	465	115	994
20	LOW BEAMS COUNT	785	576	115	474	1950

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PAR-IND - 2
07-APR-99

LOCOMOTOR ACTIVITY
FEMALES

ANIMAL		15 min.	30 min.	45 min.	60 min.	Total
GROUP 1 (0 MG/KG)						
21	LOW BEAMS COUNT	1298	621	289	7	2215
22	LOW BEAMS COUNT	713	435	430	388	1966
23	LOW BEAMS COUNT	677	444	329	328	1778
24	LOW BEAMS COUNT	821	492	189	7	1509
25	LOW BEAMS COUNT	560	269	60	295	1184
GROUP 2 (50 MG/KG)						
26	LOW BEAMS COUNT	980	378	260	135	1753
27	LOW BEAMS COUNT	1362	827	668	676	3533
28	LOW BEAMS COUNT	610	498	362	406	1876
29	LOW BEAMS COUNT	717	763	471	34	1985
30	LOW BEAMS COUNT	1183	692	325	29	2229
GROUP 3 (200 MG/KG)						
31	LOW BEAMS COUNT	879	751	1093	234	2957
32	LOW BEAMS COUNT	729	484	348	128	1689
33	LOW BEAMS COUNT	1649	830	595	629	3703
34	LOW BEAMS COUNT	1019	232	624	117	1992
35	LOW BEAMS COUNT	930	772	617	620	2939
GROUP 4 (1000 MG/KG)						
36	LOW BEAMS COUNT	642	423	126	162	1353
37	LOW BEAMS COUNT	661	445	230	1	1337
38	LOW BEAMS COUNT	878	73	16	336	1303
39	LOW BEAMS COUNT	538	332	115	121	1106
40	LOW BEAMS COUNT	983	673	530	384	2570

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FC-IND - 1
23-MAR-99

FOOD CONSUMPTION (G/ANIMAL/DAY)
MALES

	PRETEST	TREATMENT			
DAYS	1-8	1-8	8-15	15-22	22-28
WEEKS	1/2	1/2	2/3	3/4	4
CAGE					
GROUP 1 (0 MG/KG)					
1	20.4	22.2	23.8	24.3	23.4
GROUP 2 (50 MG/KG)					
2	20.9	22.7	24.2	24.4	23.8
GROUP 3 (200 MG/KG)					
3	21.1	21.7	23.1	23.3	22.8
GROUP 4 (1000 MG/KG)					
4	20.8	22.0	24.2	25.0	25.0

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FC-IND - 2
23-MAR-99

FOOD CONSUMPTION (G/ANIMAL/DAY)
FEMALES

	PRETEST	TREATMENT			
DAYS	1-8	1-8	8-15	15-22	22-28
WEEKS	1/2	1/2	2/3	3/4	4
CAGE					
GROUP 1 (0 MG/KG)					
5	15.3	15.0	15.5	16.0	16.1
GROUP 2 (50 MG/KG)					
6	15.2	15.2	16.3	16.9	16.9
GROUP 3 (200 MG/KG)					
7	15.1	15.5	16.5	17.2	17.3
GROUP 4 (1000 MG/KG)					
8	16.0	16.1	17.6	17.6	18.1

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BW-IND - 1
23-JUL-99

BODY WEIGHTS (GRAM)
MALES

	PRETEST	TREATMENT				
DAYS	1	1	8	15	22	28
WEEKS	1	1	2	3	4	4
ANIMAL						
GROUP 1 (0 MG/KG)						
1	143	185	241	279	309	334
2	141	183	218	244	258	272
3	143	193	242	275	297	319
4	143	190	230	264	290	298
5	140	178	214	241	264	281
GROUP 2 (50 MG/KG)						
6	143	189	231	263	287	309
7	145	193	239	271	299	319
8	140	195	238	266	286	301
9	141	186	232	263	284	297
10	141	193	235	268	303	327
GROUP 3 (200 MG/KG)						
11	139	179	216	245	268	289
12	141	185	226	251	273	290
13	141	192	232	259	276	291
14	138	178	224	252	275	288
15	150	201	244	276	300	312
GROUP 4 (1000 MG/KG)						
16	137	183	228	258	289	312
17	138	177	218	248	277	299
18	140	192	235	273	305	333
19	144	191	237	270	300	316
20	142	186	240	286	313	339

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BW-IND - 2
23-JUL-99

BODY WEIGHTS (GRAM)
FEMALES

	PRETEST	TREATMENT				
DAYS	1	1	8	15	22	28
WEEKS	1	1	2	3	4	4
ANIMAL						
GROUP 1 (0 MG/KG)						
21	116	140	160	172	179	189
22	120	138	156	163	180	192
23	118	143	159	171	187	195
24	122	143	162	177	185	191
25	120	150	170	181	204	209
GROUP 2 (50 MG/KG)						
26	112	134	148	164	178	178
27	122	150	175	182	208	221
28	116	139	158	168	175	188
29	124	144	167	183	195	203
30	117	142	157	177	194	196
GROUP 3 (200 MG/KG)						
31	116	131	143	162	178	181
32	119	137	162	178	192	200
33	122	150	164	183	200	203
34	123	144	164	176	197	205
35	115	138	165	179	192	202
GROUP 4 (1000 MG/KG)						
36	119	148	173	192	208	232
37	119	143	165	178	190	200
38	115	141	158	175	189	192
39	121	148	177	198	211	215
40	114	140	158	179	192	195

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RFC-IND - 1
23-JUL-99

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

	PRETEST	TREATMENT			
DAYS	1-8	1-8	8-15	15-22	22-28
WEEKS	1/2	1/2	2/3	3/4	4
CAGE					
GROUP 1 (0 MG/KG)					
1	144	97	91	86	78
GROUP 2 (50 MG/KG)					
2	147	97	91	84	77
GROUP 3 (200 MG/KG)					
3	149	95	90	84	78
GROUP 4 (1000 MG/KG)					
4	148	95	90	84	78

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

	PRETEST	TREATMENT			
DAYS	1-8	1-8	8-15	15-22	22-28
WEEKS	1/2	1/2	2/3	3/4	4
CAGE					
GROUP 1 (0 MG/KG)					
5	129	93	90	86	82
GROUP 2 (50 MG/KG)					
6	129	94	94	89	86
GROUP 3 (200 MG/KG)					
7	127	97	94	90	87
GROUP 4 (1000 MG/KG)					
8	136	97	95	89	88

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HEM-IND - 1
04-MAY-99

HEMATOLOGY
AFTER 4 WEEKS
MALES
GROUP 1 (0 MG/KG)

ANIMAL NUMBER	REMARK	RBC T/L	HB mmol/L	HCT L/L	MCV fL	MCH fmol	MCHC mmol/L	PLATELETS G/L
1	---	8.52	10.1	0.47	54.7	1.19	21.7	984
2	---	8.42	10.4	0.48	57.2	1.23	21.5	626
3	---	9.28	11.0	0.50	54.2	1.19	22.0	1266
4	---	9.49	11.0	0.52	55.1	1.17	21.1	922
5	---	8.70	10.4	0.48	54.9	1.20	21.8	637

	RETICULOCYTE COUNT							
ANIMAL NUMBER	RETIC. %	RETIC. T/L	HFR %	MFR %	LFR %	NEN /100 WBC	HEINZ BOD. o/oo	MET-HB %
1	2.38	0.1959	10.0	32.6	57.4	0.0	0	1.0
2	2.39	0.2175	8.2	37.0	54.8	0.0	0	0.1
3	2.29	0.2024	8.8	40.6	50.6	0.0	0	0.0
4	2.35	0.2084	6.4	36.3	57.3	0.0	0	0.0
5	2.57	0.2290	5.6	41.2	53.2	0.0	0	0.7

		DIFF.WBC COUNT (REL.)						
ANIMAL NUMBER	WBC G/L	BAND 1	SEG. 1	EO. 1	BASO. 1	LYMPH. 1	MOND. 1	PLAS. 1
1	8.8	0.00	0.03	0.01	0.00	0.96	0.00	0.00
2	9.7	0.00	0.11	0.00	0.00	0.89	0.00	0.00
3	9.0	0.00	0.10	0.00	0.00	0.90	0.00	0.00
4	10.1	0.00	0.11	0.02	0.00	0.87	0.00	0.00
5	8.7	0.00	0.08	0.00	0.00	0.92	0.00	0.00

	DIFF.WBC COUNT (REL.)	DIFF.WBC COUNT (ABS.)					
ANIMAL NUMBER	OTHER 1	BAND G/L	SEG. G/L	EO. G/L	BASO. G/L	LYMPH. G/L	MONO. G/L
1	0.00	0.0	0.3	0.1	0.0	8.4	0.0
2	0.00	0.0	1.1	0.0	0.0	8.6	0.0
3	0.00	0.0	0.9	0.0	0.0	8.1	0.0
4	0.00	0.0	1.1	0.2	0.0	8.8	0.0
5	0.00	0.0	0.7	0.0	0.0	8.0	0.0

	DIFF.WBC COUNT (ABS.)		RED CELL MORPHOLOGY	COAGULATION	
ANIMAL NUMBER	PLAS. G/L	OTHER G/L	TYPE CELL	PT sec	APTT sec
1	0.0	0.0	NORMAL	14.5	22.3
2	0.0	0.0	NORMAL	13.0	19.9
3	0.0	0.0	NORMAL	13.5	18.0
4	0.0	0.0	NORMAL	13.6	19.9
5	0.0	0.0	NORMAL	13.2	21.6

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HEM-IND - 2
04-MAY-99

HEMATOLOGY
AFTER 4 WEEKS
MALES
GROUP 2 (50 MG/KG)

ANIMAL NUMBER	REMARK	RBC T/L	HB mmol/L	HCT L/L	MCV fL	MCH fmol	MCHC mmol/L	PLATELETS G/L
6	---	8.72	9.8	0.47	53.7	1.12	21.0	997
7	---	8.61	10.2	0.49	56.3	1.19	21.1	870
8	---	9.06	10.2	0.49	53.9	1.13	21.0	1027
9	---	9.32	11.0	0.50	53.1	1.18	22.2	1148
10	---	8.54	9.8	0.47	54.6	1.15	21.0	1124

	RETICULOCYTE COUNT							
ANIMAL NUMBER	RETIC. %	RETIC. T/L	HFR %	MFR %	LFR %	NEN /100 WBC	HEINZ BOD. o/oo	MET-HB %
6	2.00	0.1650	2.8	30.2	67.0	3.0	0	0.4
7	2.43	0.1995	8.2	37.1	54.7	1.0	0	0.4
8	2.75	0.2351	9.2	39.7	51.1	1.0	0	1.0
9	2.31	0.2081	5.8	38.5	55.7	0.0	0	0.5
10	4.08	0.3354	17.3	41.6	41.1	1.0	0	0.5

		DIFF.WBC COUNT (REL.)						
ANIMAL NUMBER	WBC G/L	BAND 1	SEG. 1	EO. 1	BASO. 1	LYMPH. 1	MONO. 1	PLAS. 1
6	7.2	0.00	0.08	0.00	0.00	0.92	0.00	0.00
7	10.4	0.00	0.11	0.00	0.00	0.89	0.00	0.00
8	8.5	0.00	0.13	0.02	0.00	0.85	0.00	0.00
9	7.5	0.00	0.13	0.00	0.00	0.87	0.00	0.00
10	7.4	0.00	0.18	0.03	0.00	0.79	0.00	0.00

	DIFF.WBC COUNT (REL.)	DIFF.WBC COUNT (ABS.)					
ANIMAL NUMBER	OTHER 1	BAND G/L	SEG. G/L	EO. G/L	BASO. G/L	LYMPH. G/L	MONO. G/L
6	0.00	0.0	0.6	0.0	0.0	6.6	0.0
7	0.00	0.0	1.1	0.0	0.0	9.3	0.0
8	0.00	0.0	1.1	0.2	0.0	7.2	0.0
9	0.00	0.0	1.0	0.0	0.0	6.5	0.0
10	0.00	0.0	1.3	0.2	0.0	5.8	0.0

	DIFF.WBC COUNT (ABS.)		RED CELL MORPHOLOGY	COAGULATION	
ANIMAL NUMBER	PLAS. G/L	OTHER G/L	TYPE CELL	PT sec	APTT sec
6	0.0	0.0	NORMAL	13.2	21.0
7	0.0	0.0	NORMAL	13.7	22.6
8	0.0	0.0	NORMAL	12.9	19.3
9	0.0	0.0	NORMAL	13.9	20.5
10	0.0	0.0	NORMAL	13.5	19.7

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HEM-IND - 3
04-MAY-99

HEMATOLOGY
AFTER 4 WEEKS
MALES
GROUP 3 (200 MG/KG)

ANIMAL NUMBER	REMARK	RBC T/L	HB mmol/L	HCT L/L	MCV fL	MCH fmol	MCHC mmol/L	PLATELETS G/L
11	---	8.62	9.9	0.47	54.4	1.14	21.0	896
12	---	9.23	10.4	0.49	53.2	1.13	21.2	1028
13	---	8.73	10.7	0.49	55.9	1.22	21.8	1044
14	---	9.28	10.3	0.49	53.0	1.11	20.9	1035
15	---	9.00	10.7	0.48	53.4	1.19	22.2	1228

	RETICULOCYTE COUNT							
ANIMAL NUMBER	RETIC. %	RETIC. T/L	HFR %	MFR %	LFR %	NEN /100 WBC	HEINZ BOD. o/oo	MET-HB %
11	2.67	0.2213	5.4	34.5	60.1	0.0	0	0.5
12	2.37	0.2090	7.9	31.3	60.8	1.0	0	0.7
13	1.90	0.1613	5.3	36.9	57.8	1.0	0	0.5
14	2.76	0.2448	9.1	36.6	54.3	0.0	0	0.3
15	2.79	0.2466	11.7	36.8	51.5	1.0	0	0.6

		DIFF.WBC COUNT (REL.)						
ANIMAL NUMBER	WBC G/L	BAND 1	SEG. 1	EO. 1	BASO. 1	LYMPH. 1	MONO. 1	PLAS. 1
11	9.1	0.00	0.06	0.00	0.00	0.94	0.00	0.00
12	10.7	0.00	0.21	0.00	0.00	0.79	0.00	0.00
13	6.3	0.00	0.13	0.00	0.00	0.87	0.00	0.00
14	9.5	0.00	0.18	0.01	0.00	0.81	0.00	0.00
15	9.8	0.00	0.07	0.01	0.00	0.92	0.00	0.00

	DIFF.WBC COUNT (REL.)	DIFF.WBC COUNT (ABS.)					
ANIMAL NUMBER	OTHER 1	BAND G/L	SEG. G/L	EO. G/L	BASO. G/L	LYMPH. G/L	MONO. G/L
11	0.00	0.0	0.5	0.0	0.0	8.6	0.0
12	0.00	0.0	2.2	0.0	0.0	8.5	0.0
13	0.00	0.0	0.8	0.0	0.0	5.5	0.0
14	0.00	0.0	1.7	0.1	0.0	7.7	0.0
15	0.00	0.0	0.7	0.1	0.0	9.0	0.0

	DIFF.WBC COUNT (ABS.)		RED CELL MORPHOLOGY	COAGULATION	
ANIMAL NUMBER	PLAS. G/L	OTHER G/L	TYPE CELL	PT sec	APTT sec
11	0.0	0.0	NORMAL	13.4	21.2
12	0.0	0.0	NORMAL	13.2	21.0
13	0.0	0.0	NORMAL	13.2	20.5
14	0.0	0.0	NORMAL	13.9	21.6
15	0.0	0.0	NORMAL	14.1	23.4

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HEM-IND - 4
04-MAY-99

HEMATOLOGY
AFTER 4 WEEKS
MALES
GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	REMARK	RBC T/L	HB mmol/L	HCT L/L	MCV fL	MCH fmol	MCHC mmol/L	PLATELETS G/L
16	---	9.10	9.9	0.47	51.4	1.09	21.1	1118
17	---	8.84	10.3	0.47	53.6	1.17	21.7	898
18	---	8.54	10.1	0.47	54.7	1.18	21.5	1183
19	---	9.08	10.1	0.49	53.4	1.10	20.7	940
20	---	8.36	9.5	0.46	54.8	1.14	20.7	994

	RETICULOCYTE COUNT							
ANIMAL NUMBER	RETIC. %	RETIC. T/L	HFR %	MFR %	LFR %	NEN /100 WBC	HEINZ BOD. o/oo	MET-HB %
16	3.51	0.3089	16.9	40.3	42.8	3.0	0	0.4
17	2.93	0.2546	13.1	37.7	49.2	2.0	0	0.4
18	3.01	0.2495	8.8	39.4	51.8	0.0	0	0.7
19	3.10	0.2688	11.1	34.1	54.8	0.0	0	0.3
20	3.06	0.2491	13.5	37.3	49.2	0.0	0	0.7

		DIFF.WBC COUNT (REL.)						
ANIMAL NUMBER	WBC G/L	BAND 1	SEG. 1	EO. 1	BASO. 1	LYMPH. 1	MONO. 1	PLAS. 1
16	7.7	0.00	0.12	0.00	0.00	0.88	0.00	0.00
17	6.9	0.00	0.09	0.01	0.00	0.90	0.00	0.00
18	9.3	0.00	0.08	0.01	0.00	0.91	0.00	0.00
19	8.3	0.00	0.06	0.00	0.00	0.94	0.00	0.00
20	8.3	0.00	0.06	0.01	0.00	0.93	0.00	0.00

	DIFF.WBC COUNT (REL.)	DIFF.WBC COUNT (ABS.)					
ANIMAL NUMBER	OTHER 1	BAND G/L	SEG. G/L	EO. G/L	BASO. G/L	LYMPH. G/L	MONO. G/L
16	0.00	0.0	0.9	0.0	0.0	6.8	0.0
17	0.00	0.0	0.6	0.1	0.0	6.2	0.0
18	0.00	0.0	0.7	0.1	0.0	8.5	0.0
19	0.00	0.0	0.5	0.0	0.0	7.8	0.0
20	0.00	0.0	0.5	0.1	0.0	7.7	0.0

	DIFF.WBC COUNT (ABS.)		RED CELL MORPHOLOGY	COAGULATION	
ANIMAL NUMBER	PLAS. G/L	OTHER G/L	TYPE CELL	PT sec	APTT sec
16	0.0	0.0	NORMAL	13.8	20.1
17	0.0	0.0	NORMAL	14.3	22.6
18	0.0	0.0	NORMAL	14.1	20.6
19	0.0	0.0	NORMAL	13.4	20.5
20	0.0	0.0	NORMAL	13.0	20.3

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HEM-IND - 5
04-MAY-99

HEMATOLOGY
AFTER 4 WEEKS
FEMALES
GROUP 1 (0 MG/KG)

ANIMAL NUMBER	REMARK	RBC T/L	HB mmol/L	HCT L/L	MCV fL	MCH fmol	MCHC mmol/L	PLATELETS G/L
21	---	8.52	9.8	0.47	55.0	1.15	20.9	898
22	---	7.75	9.6	0.45	57.8	1.24	21.5	821
23	---	8.84	10.4	0.48	54.6	1.18	21.6	996
24	---	8.34	10.1	0.48	57.0	1.20	21.2	1214
25	---	7.95	10.0	0.47	59.1	1.26	21.3	801

	RETICULOCYTE COUNT							
ANIMAL NUMBER	RETIC. %	RETIC. T/L	HFR %	MFR %	LFR %	NEN /100 WBC	HEINZ BOD. o/oo	MET-HB %
21	3.19	0.2613	12.9	39.0	48.1	0.0	0	0.4
22	2.37	0.1939	7.5	38.7	53.8	0.0	0	0.3
23	2.35	0.2012	3.8	38.1	58.1	1.0	0	0.6
24	2.69	0.2340	6.2	37.8	56.0	0.0	0	0.3
25	2.39	0.1981	2.8	34.7	62.5	0.0	0	0.3

		DIFF.WBC COUNT (REL.)						
ANIMAL NUMBER	WBC G/L	BAND 1	SEG. 1	EO. 1	BASO. 1	LYMPH. 1	MONO. 1	PLAS. 1
21	6.5	0.00	0.07	0.01	0.00	0.92	0.00	0.00
22	7.9	0.00	0.01	0.00	0.00	0.99	0.00	0.00
23	7.0	0.00	0.03	0.00	0.00	0.97	0.00	0.00
24	8.9	0.00	0.01	0.00	0.00	0.99	0.00	0.00
25	5.3	0.00	0.07	0.01	0.00	0.92	0.00	0.00

	DIFF.WBC COUNT (REL.)	DIFF.WBC COUNT (ABS.)					
ANIMAL NUMBER	OTHER 1	BAND G/L	SEG. G/L	EO. G/L	BASO. G/L	LYMPH. G/L	MONO. G/L
21	0.00	0.0	0.5	0.1	0.0	6.0	0.0
22	0.00	0.0	0.1	0.0	0.0	7.8	0.0
23	0.00	0.0	0.2	0.0	0.0	6.8	0.0
24	0.00	0.0	0.1	0.0	0.0	8.8	0.0
25	0.00	0.0	0.4	0.1	0.0	4.9	0.0

	DIFF.WBC COUNT (ABS.)		RED CELL MORPHOLOGY	COAGULATION	
ANIMAL NUMBER	PLAS. G/L	OTHER G/L	TYPE CELL	PT sec	APTT sec
21	0.0	0.0	NORMAL	13.3	21.6
22	0.0	0.0	NORMAL	13.4	20.4
23	0.0	0.0	NORMAL	13.4	20.0
24	0.0	0.0	NORMAL	13.6	20.8
25	0.0	0.0	NORMAL	13.4	20.3

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HEM-IND - 6
04-MAY-99

HEMATOLOGY
AFTER 4 WEEKS
FEMALES
GROUP 2 (50 MG/KG)

ANIMAL NUMBER	REMARK	RBC T/L	HB mmol/L	HCT L/L	MCV fL	MCH fmol	MCHC mmol/L	PLATELETS G/L
26	---	8.73	10.1	0.47	53.3	1.15	21.6	1055
27	---	7.65	9.9	0.46	60.4	1.30	21.5	575
28	---	7.85	9.5	0.44	56.1	1.21	21.6	808
29	---	7.87	9.6	0.44	56.4	1.22	21.7	572
30	---	8.89	9.9	0.47	52.8	1.12	21.2	1015

	RETICULOCYTE COUNT							
ANIMAL NUMBER	RETIC. %	RETIC. T/L	HFR %	MFR %	LFR %	NEN /100 WBC	HEINZ BOD. o/oo	MET-HB %
26	2.97	0.2507	4.7	30.1	65.2	1.0	0	0.3
27	2.42	0.1970	13.7	27.9	58.4	1.0	0	0.4
28	3.01	0.2519	7.9	40.4	51.7	0.0	0	0.4
29	2.90	0.2355	8.1	40.6	51.3	1.0	0	0.3
30	2.58	0.2190	6.7	31.1	62.2	1.0	0	0.5

		DIFF.WBC COUNT (REL.)						
ANIMAL NUMBER	WBC G/L	BAND 1	SEG. 1	EO. 1	BASO. 1	LYMPH. 1	MONO. 1	PLAS. 1
26	7.0	0.00	0.07	0.00	0.00	0.93	0.00	0.00
27	6.9	0.00	0.01	0.01	0.00	0.98	0.00	0.00
28	4.8	0.00	0.09	0.00	0.00	0.91	0.00	0.00
29	4.9	0.00	0.19	0.02	0.00	0.79	0.00	0.00
30	5.2	0.00	0.15	0.00	0.00	0.85	0.00	0.00

	DIFF.WBC COUNT (REL.)	DIFF.WBC COUNT (ABS.)					
ANIMAL NUMBER	OTHER 1	BAND G/L	SEG. G/L	EO. G/L	BASO. G/L	LYMPH. G/L	MONO. G/L
26	0.00	0.0	0.5	0.0	0.0	6.5	0.0
27	0.00	0.0	0.1	0.1	0.0	6.8	0.0
28	0.00	0.0	0.4	0.0	0.0	4.4	0.0
29	0.00	0.0	0.9	0.1	0.0	3.9	0.0
30	0.00	0.0	0.8	0.0	0.0	4.4	0.0

	DIFF.WBC COUNT (ABS.)		RED CELL MORPHOLOGY	COAGULATION	
ANIMAL NUMBER	PLAS. G/L	OTHER G/L	TYPE CELL	PT sec	APTT sec
26	0.0	0.0	NORMAL	13.5	22.4
27	0.0	0.0	NORMAL	13.9	21.7
28	0.0	0.0	NORMAL	13.4	21.2
29	0.0	0.0	NORMAL	13.0	22.5
30	0.0	0.0	NORMAL	14.0	22.0

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HEM-IND - 7
04-MAY-99

HEMATOLOGY
AFTER 4 WEEKS
FEMALES
GROUP 3 (200 MG/KG)

ANIMAL NUMBER	REMARK	RBC T/L	HB mmol/L	HCT L/L	MCV fL	MCH fmol	MCHC mmol/L	PLATELETS G/L
31	---	8.44	9.5	0.45	53.3	1.12	21.1	912
32	---	8.00	9.5	0.44	55.4	1.19	21.4	1045
33	---	7.52	9.7	0.45	59.4	1.28	21.7	643
34	---	8.88	10.3	0.49	54.8	1.16	21.2	1037
35	---	8.70	10.2	0.48	55.1	1.18	21.3	698

	RETICULOCYTE COUNT							
ANIMAL NUMBER	RETIC. %	RETIC. T/L	HFR %	MFR %	LFR %	NEN /100 WBC	HEINZ BOD. o/oo	MET-HB %
31	3.30	0.2676	9.2	41.8	49.0	0.0	0	0.3
32	3.56	0.2798	15.0	37.8	47.2	0.0	0	0.3
33	2.34	0.1881	5.5	37.6	56.9	1.0	0	0.4
34	2.76	0.2368	11.6	39.7	48.7	0.0	0	0.4
35	3.46	0.2837	9.7	40.3	50.0	1.0	0	0.3

		DIFF.WBC COUNT (REL.)						
ANIMAL NUMBER	WBC G/L	BAND 1	SEG. 1	EO. 1	BASO. 1	LYMPH. 1	MONO. 1	PLAS. 1
31	5.4	0.00	0.03	0.01	0.00	0.96	0.00	0.00
32	6.3	0.00	0.13	0.00	0.00	0.87	0.00	0.00
33	8.4	0.00	0.06	0.02	0.00	0.92	0.00	0.00
34	5.4	0.00	0.10	0.00	0.00	0.90	0.00	0.00
35	9.6	0.00	0.10	0.01	0.00	0.89	0.00	0.00

	DIFF.WBC COUNT (REL.)	DIFF.WBC COUNT (ABS.)					
ANIMAL NUMBER	OTHER 1	BAND G/L	SEG. G/L	EO. G/L	BASO. G/L	LYMPH. G/L	MONO. G/L
31	0.00	0.0	0.2	0.1	0.0	5.2	0.0
32	0.00	0.0	0.8	0.0	0.0	5.5	0.0
33	0.00	0.0	0.5	0.2	0.0	7.7	0.0
34	0.00	0.0	0.5	0.0	0.0	4.9	0.0
35	0.00	0.0	1.0	0.1	0.0	8.5	0.0

	DIFF.WBC COUNT (ABS.)		RED CELL MORPHOLOGY	COAGULATION	
ANIMAL NUMBER	PLAS. G/L	OTHER G/L	TYPE CELL	PT sec	APTT sec
31	0.0	0.0	NORMAL	13.8	21.2
32	0.0	0.0	NORMAL	13.7	21.4
33	0.0	0.0	NORMAL	13.9	22.5
34	0.0	0.0	NORMAL	14.2	21.8
35	0.0	0.0	NORMAL	13.5	18.4

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HEM-IND - B
04-MAY-99

HEMATOLOGY
AFTER 4 WEEKS
FEMALES
GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	REMARK	RBC T/L	HB mmol/L	HCT L/L	MCV fL	MCH fmol	MCHC mmol/L	PLATELETS G/L
36	---	7.89	9.7	0.45	56.5	1.23	21.7	1257
37	---	8.20	9.9	0.46	55.5	1.20	21.7	1082
38	---	8.14	9.7	0.45	55.5	1.20	21.5	814
39	---	8.85	9.9	0.48	53.7	1.12	20.9	858
40	---	8.17	10.0	0.46	56.5	1.22	21.6	859

	RETICULOCYTE COUNT							
ANIMAL NUMBER	RETIC. %	RETIC. T/L	HFR %	MFR %	LFR %	NEN /100 WBC	HEINZ BOD. o/oo	MET-HB %
36	3.72	0.2857	12.7	39.2	48.1	2.0	0	0.1
37	3.12	0.2508	13.8	38.7	47.5	1.0	0	0.3
38	2.61	0.2062	10.4	34.8	54.8	1.0	0	0.5
39	2.21	0.1834	6.8	35.0	58.2	0.0	0	0.3
40	3.89	0.3108	8.0	40.3	51.7	1.0	0	0.6

		DIFF.WBC COUNT (REL.)						
ANIMAL NUMBER	WBC G/L	BAND 1	SEG. 1	EO. 1	BASO. 1	LYMPH. 1	MONO. 1	PLAS. 1
36	4.5	0.00	0.09	0.00	0.00	0.91	0.00	0.00
37	7.3	0.00	0.13	0.00	0.00	0.87	0.00	0.00
38	5.6	0.00	0.03	0.00	0.00	0.97	0.00	0.00
39	9.0	0.00	0.03	0.01	0.00	0.96	0.00	0.00
40	8.2	0.00	0.03	0.00	0.00	0.97	0.00	0.00

	DIFF.WBC COUNT (REL.)	DIFF.WBC COUNT (ABS.)					
ANIMAL NUMBER	OTHER 1	BAND G/L	SEG. G/L	EO. G/L	BASO. G/L	LYMPH. G/L	MONO. G/L
36	0.00	0.0	0.4	0.0	0.0	4.1	0.0
37	0.00	0.0	0.9	0.0	0.0	6.4	0.0
38	0.00	0.0	0.2	0.0	0.0	5.4	0.0
39	0.00	0.0	0.3	0.1	0.0	8.6	0.0
40	0.00	0.0	0.2	0.0	0.0	8.0	0.0

	DIFF.WBC COUNT (ABS.)		RED CELL MORPHOLOGY	COAGULATION	
ANIMAL NUMBER	PLAS. G/L	OTHER G/L	TYPE CELL	PT sec	APTT sec
36	0.0	0.0	NORMAL	13.7	20.0
37	0.0	0.0	NORMAL	13.0	21.4
38	0.0	0.0	NORMAL	13.8	20.8
39	0.0	0.0	NORMAL	12.9	21.4
40	0.0	0.0	NORMAL	13.4	22.2

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CHE-IND - 1
04-MAY-99

CLINICAL BIOCHEMISTRY
AFTER 4 WEEKS
MALES
GROUP 1 (0 MG/KG)

ANIMAL NUMBER	REMARK	GLUCOSE mmol/L	UREA mmol/L	CREATININE µmol/L	URIC ACID µmol/L	BILI. T. µmol/L	CHOLEST.T. mmol/L	TRIGL. mmol/L
1	---	7.98	7.39	43.8	20.1	1.73	1.99	0.71
2	---	5.95	5.09	43.3	53.5	2.12	1.78	1.08
3	---	7.70	9.09	46.0	19.9	1.63	1.91	1.25
4	---	5.61	5.90	46.1	45.8	1.97	2.22	0.87
5	---	6.39	5.29	42.6	33.6	2.06	2.24	1.20

ANIMAL NUMBER	PHOS.LIPID mmol/L	ASAT(GOT) µkat/L	ALAT(GPT) µkat/L	LDH µkat/L	CK µkat/L	ALP µkat/L	G-GT nkat/L	CALCIUM mmol/L
1	1.90	1.12	0.97	3.32	3.40	7.75	23.00	2.88
2	1.84	1.08	0.63	3.68	3.01	5.34	22.17	2.78
3	2.01	1.22	1.01	3.55	3.45	6.63	24.84	2.89
4	2.05	1.31	0.69	3.69	4.86	8.99	20.50	2.85
5	2.16	1.22	0.79	3.15	2.98	4.07	27.17	2.87

ANIMAL NUMBER	PHOSPHORUS mmol/L	SODIUM mmol/L	POTASSIUM mmol/L	CHLORIDE mmol/L	ALBUMIN g/L	PROTEIN T. g/L	GLOBULIN g/L	A/G RATIO
1	2.56	143.4	3.45	102.5	32.1	66.3	34.2	0.94
2	2.07	146.0	3.26	101.4	32.0	67.1	35.1	0.91
3	2.28	144.5	3.34	102.3	32.1	68.0	35.9	0.89
4	2.09	144.8	3.25	102.4	32.6	66.8	34.2	0.95
5	2.24	143.0	3.35	99.4	33.3	69.6	36.3	0.92

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CHE-IND - 2
04-MAY-99

CLINICAL BIOCHEMISTRY
AFTER 4 WEEKS
MALES
GROUP 2 (50 MG/KG)

ANIMAL NUMBER	REMARK	GLUCOSE mmol/L	UREA mmol/L	CREATININE µmol/L	URIC ACID µmol/L	BILI. T. µmol/L	CHOLEST.T. mmol/L	TRIGL. mmol/L
6	---	6.65	6.01	42.0	12.7	2.53	2.57	0.92
7	---	7.53	7.69	46.2	15.8	2.73	1.85	0.43
8	---	7.75	6.81	43.4	14.2	2.82	2.31	0.98
9	---	5.97	6.52	41.5	18.8	0.98	2.06	0.78
10	---	7.19	8.18	47.7	24.6	1.49	2.19	0.70

ANIMAL NUMBER	PHOS.LIPID mmol/L	ASAT(GOT) µkat/L	ALAT(GPT) µkat/L	LDH µkat/L	CK µkat/L	ALP µkat/L	G-GT nkat/L	CALCIUM mmol/L
6	2.30	1.24	0.71	2.68	2.47	6.76	19.67	2.89
7	1.86	1.06	0.84	3.16	3.16	7.03	27.51	2.97
8	2.31	1.52	0.65	2.22	2.24	11.93	20.50	2.82
9	1.70	1.57	0.80	3.12	3.44	8.38	23.67	2.84
10	2.06	1.07	0.77	2.78	2.72	6.17	25.84	3.00

ANIMAL NUMBER	PHOSPHORUS mmol/L	SODIUM mmol/L	POTASSIUM mmol/L	CHLORIDE mmol/L	ALBUMIN g/L	PROTEIN T. g/L	GLOBULIN g/L	A/G RATIO
6	2.86	143.7	3.58	101.4	31.8	65.3	33.5	0.95
7	2.63	143.7	3.54	100.8	32.3	67.6	35.3	0.92
8	2.25	146.0	3.35	104.3	33.7	68.2	34.5	0.98
9	2.22	145.8	3.23	102.2	31.0	65.6	34.6	0.90
10	2.64	145.1	3.48	102.3	32.7	69.7	37.0	0.88

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CHE-IND - 3
04-MAY-99

CLINICAL BIOCHEMISTRY
AFTER 4 WEEKS
MALES
GROUP 3 (200 MG/KG)

ANIMAL NUMBER	REMARK	GLUCOSE mmol/L	UREA mmol/L	CREATININE µmol/L	URIC ACID µmol/L	BILI. T. µmol/L	CHOLEST.T. mmol/L	TRIGL. mmol/L
11	---	7.52	7.03	46.0	12.5	1.49	2.42	1.35
12	---	7.25	9.72	47.3	18.5	1.80	2.48	1.08
13	---	6.74	5.70	42.3	27.0	1.20	3.09	0.99
14	---	7.01	7.68	45.0	32.2	1.18	1.54	1.14
15	---	6.63	7.97	44.8	17.9	1.60	1.63	1.88

ANIMAL NUMBER	PHOS.LIPID mmol/L	ASAT(GOT) µkat/L	ALAT(GPT) µkat/L	LDH µkat/L	CK µkat/L	ALP µkat/L	G-GT nkat/L	CALCIUM mmol/L
11	2.17	1.47	0.88	3.51	3.25	4.97	24.84	2.84
12	2.39	1.14	0.92	2.61	2.56	10.78	20.17	2.92
13	2.40	1.14	0.68	3.66	3.27	4.75	22.00	2.91
14	1.74	1.21	0.67	2.91	2.95	5.52	25.17	2.79
15	1.87	1.14	0.68	3.48	3.04	5.16	26.51	2.89

ANIMAL NUMBER	PHOSPHORUS mmol/L	SODIUM mmol/L	POTASSIUM mmol/L	CHLORIDE mmol/L	ALBUMIN g/L	PROTEIN T. g/L	GLOBULIN g/L	A/G RATIO
11	2.37	146.0	3.39	104.9	31.3	64.4	33.1	0.95
12	2.54	145.7	3.40	102.7	33.4	68.8	35.4	0.94
13	2.00	146.6	3.30	104.2	33.1	69.0	35.9	0.92
14	2.26	149.4	3.19	108.4	31.9	66.3	34.4	0.93
15	2.39	144.9	3.39	104.1	33.9	71.0	37.1	0.91

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CHE-IND - 4
04-MAY-99

CLINICAL BIOCHEMISTRY
AFTER 4 WEEKS
MALES
GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	REMARK	GLUCOSE mmol/L	UREA mmol/L	CREATININE µmol/L	URIC ACID µmol/L	BILI. T. µmol/L	CHOLEST.T. mmol/L	TRIGL. mmol/L
16	---	5.44	7.77	52.8	40.3	2.56	1.79	0.54
17	---	6.17	7.27	52.0	18.8	1.63	1.94	0.33
18	---	4.82	5.64	44.6	18.6	1.86	1.90	0.48
19	---	6.92	9.61	46.1	24.3	1.79	1.87	0.68
20	---	6.53	7.03	48.0	27.2	1.58	1.80	0.63

ANIMAL NUMBER	PHOS.LIPID mmol/L	ASAT(GOT) µkat/L	ALAT(GPT) µkat/L	LDH µkat/L	CK µkat/L	ALP µkat/L	G-GT nkat/L	CALCIUM mmol/L
16	1.86	1.26	0.84	4.15	3.55	6.82	18.00	2.97
17	1.74	1.28	0.59	3.46	3.33	6.19	17.84	2.85
18	1.68	1.19	0.59	2.16	3.08	5.40	24.84	2.79
19	1.87	1.30	0.71	3.29	2.99	5.76	20.50	2.89
20	1.59	1.18	0.61	3.05	3.27	4.91	21.17	2.82

ANIMAL NUMBER	PHOSPHORUS mmol/L	SODIUM mmol/L	POTASSIUM mmol/L	CHLORIDE mmol/L	ALBUMIN g/L	PROTEIN T. g/L	GLOBULIN g/L	A/G RATIO
16	2.89	146.9	3.59	104.2	33.7	71.4	37.7	0.89
17	2.44	145.2	3.22	102.5	32.0	65.6	33.6	0.95
18	2.41	147.7	3.03	103.9	31.3	63.6	32.3	0.97
19	2.50	147.1	3.31	102.4	32.6	68.1	35.5	0.92
20	2.46	147.4	3.23	102.5	30.9	66.1	35.2	0.88

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CHE-IND - 5
04-MAY-99

CLINICAL BIOCHEMISTRY
AFTER 4 WEEKS
FEMALES
GROUP 1 (0 MG/KG)

ANIMAL NUMBER	REMARK	GLUCOSE mmol/L	UREA mmol/L	CREATININE µmol/L	URIC ACID µmol/L	BILI. T. µmol/L	CHOLEST.T. mmol/L	TRIGL. mmol/L
21	---	5.01	7.56	51.7	24.2	2.07	1.61	0.43
22	---	4.40	8.25	46.1	21.9	2.04	1.82	0.76
23	---	4.34	7.68	45.6	23.7	1.19	1.80	1.40
24	---	5.10	6.85	46.8	20.9	1.69	1.97	0.50
25	---	5.52	8.36	48.1	17.9	1.63	1.33	1.28

ANIMAL NUMBER	PHOS.LIPID mmol/L	ASAT(GOT) µkat/L	ALAT(GPT) µkat/L	LDH µkat/L	CK µkat/L	ALP µkat/L	G-GT nkat/L	CALCIUM mmol/L
21	1.85	1.16	0.53	4.23	3.27	4.21	20.17	2.79
22	2.10	0.99	0.56	3.69	3.46	1.88	16.17	2.89
23	2.10	1.20	0.90	3.49	3.94	3.00	16.50	2.81
24	2.15	0.97	0.55	3.47	2.41	2.30	17.17	2.88
25	1.67	1.00	0.49	3.38	2.85	2.43	24.00	2.97

ANIMAL NUMBER	PHOSPHORUS mmol/L	SODIUM mmol/L	POTASSIUM mmol/L	CHLORIDE mmol/L	ALBUMIN g/L	PROTEIN T. g/L	GLOBULIN g/L	A/G RATIO
21	2.23	145.7	3.08	104.3	32.2	64.2	32.0	1.01
22	2.06	145.8	3.40	104.2	36.2	70.8	34.6	1.05
23	2.10	146.7	3.44	104.1	30.1	61.0	30.9	0.97
24	2.05	146.1	3.18	105.7	34.2	68.1	33.9	1.01
25	2.02	145.3	2.95	102.0	35.6	71.4	35.8	0.99

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CHE-IND - 6
04-MAY-99

CLINICAL BIOCHEMISTRY
AFTER 4 WEEKS
FEMALES
GROUP 2 (50 MG/KG)

ANIMAL NUMBER	REMARK	GLUCOSE mmol/L	UREA mmol/L	CREATININE µmol/L	URIC ACID µmol/L	BILI. T. µmol/L	CHOLEST.T. mmol/L	TRIGL. mmol/L
26	---	5.40	8.96	48.0	26.8	1.40	1.56	0.55
27	---	5.90	7.84	52.0	31.2	1.59	1.99	0.37
28	---	5.11	7.18	48.4	29.7	3.33	1.42	0.75
29	---	6.37	7.20	46.6	34.1	2.64	1.12	0.59
30	---	5.96	7.60	44.8	20.3	2.23	1.97	0.51

ANIMAL NUMBER	PHOS.LIPID mmol/L	ASAT(GOT) µkat/L	ALAT(GPT) µkat/L	LDH µkat/L	CK µkat/L	ALP µkat/L	G-GT nkat/L	CALCIUM mmol/L
26	1.97	1.06	0.67	3.59	2.93	2.11	18.84	2.86
27	1.93	1.11	0.54	3.83	2.60	2.93	27.34	2.75
28	1.87	1.14	0.71	2.60	3.06	2.13	12.34	2.84
29	1.69	1.18	0.52	3.84	2.33	2.21	19.17	2.68
30	2.25	1.24	0.78	3.87	2.86	2.88	18.34	2.77

ANIMAL NUMBER	PHOSPHORUS mmol/L	SODIUM mmol/L	POTASSIUM mmol/L	CHLORIDE mmol/L	ALBUMIN g/L	PROTEIN T. g/L	GLOBULIN g/L	A/G RATIO
26	1.82	146.0	3.33	106.1	36.7	70.2	33.5	1.10
27	2.20	146.0	3.25	104.1	32.6	65.7	33.1	0.98
28	1.61	143.8	3.20	104.6	36.1	69.7	33.6	1.07
29	1.61	144.5	3.08	104.0	36.6	70.8	34.2	1.07
30	2.03	144.4	3.12	103.8	33.8	66.8	33.0	1.02

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CHE-IND - 7
04-MAY-99

CLINICAL BIOCHEMISTRY
AFTER 4 WEEKS
FEMALES
GROUP 3 (200 MG/KG)

ANIMAL NUMBER	REMARK	GLUCOSE mmol/L	UREA mmol/L	CREATININE µmol/L	URIC ACID µmol/L	BILI. T. µmol/L	CHOLEST.T. mmol/L	TRIGL. mmol/L
31	---	4.57	8.14	49.2	24.2	1.37	1.52	0.25
32	---	5.26	8.96	52.3	33.7	1.38	1.73	0.28
33	---	5.39	6.89	46.2	17.3	2.25	1.36	0.36
34	---	5.52	8.28	49.6	19.9	1.77	1.95	0.85
35	---	7.15	7.98	51.4	34.6	1.86	2.21	0.76

ANIMAL NUMBER	PHOS.LIPID mmol/L	ASAT(GOT) µkat/L	ALAT(GPT) µkat/L	LDH µkat/L	CK µkat/L	ALP µkat/L	G-GT nkat/L	CALCIUM mmol/L
31	1.70	1.23	0.48	3.76	2.82	3.60	22.50	2.77
32	1.74	1.35	0.79	4.11	4.27	5.72	21.17	2.60
33	1.65	1.08	0.48	3.56	2.78	3.08	26.67	2.78
34	2.24	1.21	0.69	3.83	3.61	4.43	26.67	2.87
35	2.36	1.26	0.75	3.97	3.27	3.20	29.84	2.76

ANIMAL NUMBER	PHOSPHORUS mmol/L	SODIUM mmol/L	POTASSIUM mmol/L	CHLORIDE mmol/L	ALBUMIN g/L	PROTEIN T. g/L	GLOBULIN g/L	A/G RATIO
31	2.17	146.6	2.87	106.4	33.4	65.8	32.4	1.03
32	2.01	147.6	2.91	106.4	30.8	60.2	29.4	1.05
33	1.91	145.6	3.10	105.7	32.6	64.8	32.2	1.01
34	2.02	146.4	3.29	105.8	32.9	66.0	33.1	0.99
35	1.95	148.1	2.94	105.6	32.0	65.0	33.0	0.97

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CHE-IND - 8
04-MAY-99

CLINICAL BIOCHEMISTRY
AFTER 4 WEEKS
FEMALES
GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	REMARK	GLUCOSE mmol/L	UREA mmol/L	CREATININE µmol/L	URIC ACID µmol/L	BILI. T. µmol/L	CHOLEST.T. mmol/L	TRIGL. mmol/L
36	---	5.77	9.47	49.2	33.0	2.52	2.03	0.50
37	---	5.94	6.97	52.6	37.1	2.22	1.54	0.23
38	---	5.26	7.78	58.0	34.0	2.94	1.30	0.42
39	---	6.66	8.98	55.4	21.7	1.97	1.67	0.60
40	---	5.31	9.44	55.3	19.1	2.12	1.30	0.36

ANIMAL NUMBER	PHOS.LIPID mmol/L	ASAT(GOT) µkat/L	ALAT(GPT) µkat/L	LDH µkat/L	CK µkat/L	ALP µkat/L	G-GT nkat/L	CALCIUM mmol/L
36	2.15	1.17	0.84	2.69	2.95	2.64	19.34	2.91
37	1.80	1.14	0.47	3.47	2.87	2.82	22.84	2.89
38	1.61	1.10	0.59	3.10	2.66	2.55	26.17	2.91
39	2.05	1.19	0.76	3.37	2.94	3.01	22.84	2.95
40	1.58	1.35	0.51	3.80	3.90	2.69	14.00	2.80

ANIMAL NUMBER	PHOSPHORUS mmol/L	SODIUM mmol/L	POTASSIUM mmol/L	CHLORIDE mmol/L	ALBUMIN g/L	PROTEIN T. g/L	GLOBULIN g/L	A/G RATIO
36	2.30	147.4	3.32	103.8	34.7	69.2	34.5	1.01
37	1.99	148.2	3.06	105.5	35.2	71.3	36.1	0.98
38	2.45	146.2	3.41	103.8	34.1	67.5	33.4	1.02
39	2.22	146.2	2.95	103.7	36.8	72.7	35.9	1.03
40	2.32	145.8	3.00	103.6	35.4	68.4	33.0	1.07

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MAC-IND - 1
23-MAR-99

MACROSCOPICAL FINDINGS
MALES
GROUP 1 (0 MG/KG)

ANIMAL 1 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 2 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 3 (SCHEDULED NECROPSY, 23-MAR-99)

KIDNEYS..... RIGHT SIDE: PELVIC DILATION.

ANIMAL 4 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 5 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

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MAC-IND - 2
23-MAR-99

MACROSCOPICAL FINDINGS
MALES
GROUP 2 (50 MG/KG)

ANIMAL 6 (SCHEDULED NECROPSY, 23-MAR-99)

KIDNEYS..... RIGHT SIDE: PELVIC DILATION.

ANIMAL 7 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 8 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 9 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 10 (SCHEDULED NECROPSY, 23-MAR-99)

THYMUS..... FOCUS/FOCI, D=2 MM, DARK RED.

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MAC-IND - 3
23-MAR-99

MACROSCOPICAL FINDINGS
MALES
GROUP 3 (200 MG/KG)

ANIMAL 11 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 12 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 13 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 14 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 15 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

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MAC-IND - 4
23-MAR-99

MACROSCOPICAL FINDINGS
MALES
GROUP 4 (1000 MG/KG)

ANIMAL 16 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 17 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 18 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 19 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 20 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

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MAC-IND - 5
23-MAR-99

MACROSCOPICAL FINDINGS
FEMALES
GROUP 1 (0 MG/KG)

ANIMAL 21 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 22 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 23 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 24 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 25 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

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MAC-IND - 6
23-MAR-99

MACROSCOPICAL FINDINGS
FEMALES
GROUP 2 (50 MG/KG)

ANIMAL 26 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 27 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 28 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 29 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 30 (SCHEDULED NECROPSY, 23-MAR-99)

THYMUS..... FOCUS/FOCI, D=2 MM, DARK RED.

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MAC-IND - 7
23-MAR-99

MACROSCOPICAL FINDINGS
FEMALES
GROUP 3 (200 MG/KG)

ANIMAL 31 (SCHEDULED NECROPSY, 23-MAR-99)

THYMUS..... FOCUS/FOCI, D=2 MM, DARK RED.

ANIMAL 32 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 33 (SCHEDULED NECROPSY, 23-MAR-99)

THYMUS..... FOCUS/FOCI, D=2 MM, DARK RED.

ANIMAL 34 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 35 (SCHEDULED NECROPSY, 23-MAR-99)

THYMUS..... FOCUS/FOCI, D=2 MM, DARK RED.

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MAC-IND - 8
23-MAR-99

MACROSCOPICAL FINDINGS
FEMALES
GROUP 4 (1000 MG/KG)

ANIMAL 36 (SCHEDULED NECROPSY, 23-MAR-99)

THYMUS..... FOCUS/FOCI, ISOLATED, D=1 MM, REDDISH.

ANIMAL 37 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 38 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 39 (SCHEDULED NECROPSY, 23-MAR-99)

NO FINDINGS NOTED

ANIMAL 40 (SCHEDULED NECROPSY, 23-MAR-99)

KIDNEYS..... LEFT SIDE: PELVIC DILATION.

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OW-IND - 1
23-MAR-99

ORGAN WEIGHTS (GRAM)
AFTER 4 WEEKS
MALES

GROUP 1 (0 MG/KG)

ANIMAL NUMBER	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
1	313.4	1.99	1.024	9.38	0.47	2.07	0.085	0.630
2	249.7	1.85	0.780	7.05	0.34	1.88	0.053	0.533
3	294.3	1.98	0.903	9.19	0.38	2.18	0.059	0.715
4	278.4	1.97	0.855	7.08	0.36	1.97	0.053	0.586
5	255.6	1.97	0.870	7.75	0.36	1.86	0.070	0.635

ANIMAL NUMBER	TESTES	EPIDIDYMID
1	3.48	0.932
2	3.15	0.878
3	3.70	1.125
4	3.44	0.947
5	3.43	1.051

GROUP 2 (50 MG/KG)

ANIMAL NUMBER	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
6	280.7	1.92	0.966	7.67	0.38	2.08	0.051	0.598
7	296.5	1.92	1.012	8.28	0.41	2.09	0.086	0.639
8	270.8	1.95	0.986	8.29	0.43	2.10	0.068	0.767
9	273.4	1.92	0.841	8.36	0.37	2.09	0.072	0.756
10	297.1	1.85	0.946	8.69	0.50	2.23	0.079	0.604

ANIMAL NUMBER	TESTES	EPIDIDYMID
6	3.19	0.810
7	3.19	0.996
8	3.27	1.027
9	3.15	0.858
10	3.13	0.904

GROUP 3 (200 MG/KG)

ANIMAL NUMBER	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
11	265.2	1.88	0.862	7.67	0.36	1.84	0.056	0.646
12	267.1	1.91	0.895	8.54	0.44	1.93	0.072	0.530
13	272.1	1.94	0.890	8.25	0.36	2.11	0.057	0.568
14	264.4	1.93	0.875	7.42	0.39	1.89	0.062	0.593
15	289.6	2.01	0.927	8.85	0.45	2.21	0.051	0.725

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OW-IND - 2
23-MAR-99

ORGAN WEIGHTS (GRAM)
AFTER 4 WEEKS
MALES

GROUP 3 (200 MG/KG)

ANIMAL NUMBER	TESTES	EPIDIDYMID
11	3.20	0.857
12	2.99	0.943
13	3.02	0.940
14	3.41	0.994
15	3.65	0.978

GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
16	289.0	1.91	0.868	8.45	0.41	2.11	0.048	0.578
17	269.7	1.88	0.829	7.32	0.40	1.88	0.056	0.689
18	302.5	2.07	1.037	8.19	0.61	2.21	0.065	0.869
19	292.2	1.90	0.960	8.35	0.48	1.94	0.085	0.822
20	309.5	2.00	1.033	9.09	0.53	2.30	0.055	0.644

ANIMAL NUMBER	TESTES	EPIDIDYMID
16	3.06	1.008
17	2.98	0.961
18	3.46	1.003
19	3.07	0.915
20	3.42	0.915

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DW-IND - 3
23-MAR-99

ORGAN/BODY WEIGHT RATIOS (%)
AFTER 4 WEEKS
MALES

GROUP 1 (0 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
1	313.4	0.64	0.327	2.99	0.15	0.66	0.027	0.201
2	249.7	0.74	0.312	2.82	0.13	0.75	0.021	0.213
3	294.3	0.67	0.307	3.12	0.13	0.74	0.020	0.243
4	278.4	0.71	0.307	2.54	0.13	0.71	0.019	0.211
5	255.6	0.77	0.340	3.03	0.14	0.73	0.027	0.248

ANIMAL NUMBER	TESTES	EPIDIDYMID
1	1.11	0.297
2	1.26	0.352
3	1.26	0.382
4	1.24	0.340
5	1.34	0.411

GROUP 2 (50 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
6	280.7	0.68	0.344	2.73	0.14	0.74	0.018	0.213
7	296.5	0.65	0.341	2.79	0.14	0.71	0.029	0.216
8	270.8	0.72	0.364	3.06	0.16	0.77	0.025	0.283
9	273.4	0.70	0.308	3.06	0.13	0.77	0.026	0.277
10	297.1	0.62	0.319	2.92	0.17	0.75	0.027	0.203

ANIMAL NUMBER	TESTES	EPIDIDYMID
6	1.14	0.288
7	1.08	0.336
8	1.21	0.379
9	1.15	0.314
10	1.05	0.304

GROUP 3 (200 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
11	265.2	0.71	0.325	2.89	0.14	0.69	0.021	0.244
12	267.1	0.71	0.335	3.20	0.16	0.72	0.027	0.198
13	272.1	0.71	0.327	3.03	0.13	0.78	0.021	0.209
14	264.4	0.73	0.331	2.81	0.15	0.72	0.024	0.224
15	289.6	0.69	0.320	3.06	0.15	0.76	0.018	0.250

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OW-IND - 4
23-MAR-99

ORGAN/BODY WEIGHT RATIOS (%)
AFTER 4 WEEKS
MALES

GROUP 3 (200 MG/KG)

ANIMAL NUMBER	TESTES	EPIDIDYMID
11	1.21	0.323
12	1.12	0.353
13	1.11	0.346
14	1.29	0.376
15	1.26	0.338

GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
16	289.0	0.66	0.300	2.92	0.14	0.73	0.017	0.200
17	269.7	0.70	0.307	2.71	0.15	0.70	0.021	0.255
18	302.5	0.68	0.343	2.71	0.20	0.73	0.021	0.287
19	292.2	0.65	0.329	2.86	0.16	0.66	0.029	0.281
20	309.5	0.65	0.334	2.94	0.17	0.74	0.018	0.208

ANIMAL NUMBER	TESTES	EPIDIDYMID
16	1.06	0.349
17	1.10	0.356
18	1.15	0.332
19	1.05	0.313
20	1.10	0.296

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OW-IND - 5
23-MAR-99

ORGAN/BRAIN WEIGHT RATIOS (%)
AFTER 4 WEEKS
MALES

GROUP 1 (0 MG/KG)

ANIMAL NUMBER	BRAIN (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN	TESTES
1	1.99	51.426	471.37	23.75	104.17	4.254	31.642	174.60
2	1.85	42.072	380.13	18.10	101.31	2.864	28.735	169.64
3	1.98	45.595	464.07	19.28	110.04	2.955	36.113	187.04
4	1.97	43.434	359.65	18.43	100.01	2.713	29.779	174.96
5	1.97	44.170	393.37	18.10	94.34	3.548	32.222	173.96

ANIMAL NUMBER	EPIDIDYMID
1	46.791
2	47.336
3	56.830
4	48.087
5	53.363

GROUP 2 (50 MG/KG)

ANIMAL NUMBER	BRAIN (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN	TESTES
6	1.92	50.276	399.19	20.00	108.17	2.638	31.132	166.04
7	1.92	52.616	430.78	21.56	108.81	4.463	33.252	165.85
8	1.95	50.520	424.54	21.91	107.45	3.499	39.286	167.34
9	1.92	43.826	435.85	19.12	109.07	3.732	39.427	164.08
10	1.85	51.179	469.83	27.11	120.48	4.267	32.647	169.33

ANIMAL NUMBER	EPIDIDYMID
6	42.127
7	51.805
8	52.590
9	44.733
10	48.864

GROUP 3 (200 MG/KG)

ANIMAL NUMBER	BRAIN (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN	TESTES
11	1.88	45.945	408.88	19.14	98.02	2.970	34.460	170.84
12	1.91	46.893	447.42	22.96	101.14	3.789	27.772	156.54
13	1.94	45.761	424.32	18.40	108.67	2.917	29.201	155.26
14	1.93	45.397	384.78	20.40	98.09	3.231	30.759	177.04
15	2.01	46.080	439.93	22.21	109.93	2.525	36.029	181.41

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OW-IND - 6
23-MAR-99

ORGAN/BRAIN WEIGHT RATIOS (%)
AFTER 4 WEEKS
MALES

GROUP 3 (200 MG/KG)

ANIMAL NUMBER	EPIDIDYMID
11	45.716
12	49.434
13	48.379
14	51.574
15	48.616

GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	BRAIN (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN	TESTES
16	1.91	45.451	442.33	21.61	110.70	2.518	30.266	160.09
17	1.88	44.035	388.58	21.04	99.75	2.948	36.568	158.03
18	2.07	50.126	395.50	29.54	106.66	3.141	41.979	167.36
19	1.90	50.588	440.14	25.15	102.16	4.463	43.300	161.52
20	2.00	51.599	454.47	26.55	114.99	2.758	32.161	170.77

ANIMAL NUMBER	EPIDIDYMID
16	52.738
17	51.036
18	48.468
19	48.222
20	45.733

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OW-IND - 7
23-MAR-99

ORGAN WEIGHTS (GRAM)
AFTER 4 WEEKS
FEMALES

GROUP 1 (0 MG/KG)

ANIMAL NUMBER	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
21	169.5	1.84	0.698	5.25	0.29	1.34	0.099	0.426
22	173.2	1.83	0.572	5.10	0.33	1.28	0.069	0.453
23	178.7	1.76	0.661	5.80	0.33	1.46	0.073	0.521
24	174.3	1.68	0.652	5.39	0.51	1.42	0.083	0.474
25	190.7	1.79	0.659	5.64	0.37	1.48	0.070	0.383

GROUP 2 (50 MG/KG)

ANIMAL NUMBER	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
26	160.9	1.68	0.665	5.13	0.46	1.23	0.084	0.523
27	194.9	1.75	0.673	5.60	0.42	1.48	0.084	0.487
28	168.8	1.82	0.704	5.19	0.38	1.38	0.081	0.469
29	180.7	1.94	0.762	5.69	0.26	1.52	0.092	0.482
30	183.6	1.78	0.649	5.21	0.36	1.48	0.061	0.435

GROUP 3 (200 MG/KG)

ANIMAL NUMBER	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
31	164.7	1.78	0.655	4.78	0.33	1.36	0.080	0.449
32	178.0	1.82	0.713	5.53	0.39	1.46	0.086	0.517
33	185.4	1.86	0.615	4.79	0.37	1.50	0.084	0.364
34	181.3	1.77	0.646	5.32	0.29	1.21	0.072	0.436
35	174.6	1.80	0.722	6.01	0.32	1.37	0.079	0.532

GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	BODY W.	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
36	205.2	1.88	0.765	6.33	0.58	1.70	0.079	0.545
37	175.7	1.75	0.691	5.28	0.37	1.37	0.079	0.422
38	177.5	1.80	0.700	4.84	0.42	1.19	0.083	0.479
39	196.0	1.66	0.663	6.03	0.44	1.47	0.084	0.462
40	181.2	1.69	0.698	5.18	0.32	1.35	0.081	0.442

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OW-IND - 8
23-MAR-99

ORGAN/BODY WEIGHT RATIOS (%)
AFTER 4 WEEKS
FEMALES

GROUP 1 (0 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
21	169.5	1.09	0.412	3.10	0.17	0.79	0.058	0.251
22	173.2	1.05	0.330	2.95	0.19	0.74	0.040	0.261
23	178.7	0.99	0.370	3.24	0.19	0.82	0.041	0.292
24	174.3	0.96	0.374	3.10	0.29	0.81	0.048	0.272
25	190.7	0.94	0.346	2.96	0.19	0.77	0.037	0.201

GROUP 2 (50 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
26	160.9	1.04	0.414	3.19	0.29	0.77	0.052	0.325
27	194.9	0.90	0.345	2.88	0.21	0.76	0.043	0.250
28	168.8	1.08	0.417	3.08	0.23	0.82	0.048	0.278
29	180.7	1.07	0.422	3.15	0.14	0.84	0.051	0.267
30	183.6	0.97	0.353	2.84	0.20	0.81	0.033	0.237

GROUP 3 (200 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
31	164.7	1.08	0.398	2.90	0.20	0.83	0.048	0.272
32	178.0	1.02	0.401	3.11	0.22	0.82	0.048	0.291
33	185.4	1.01	0.332	2.58	0.20	0.81	0.045	0.196
34	181.3	0.98	0.356	2.94	0.16	0.67	0.040	0.241
35	174.6	1.03	0.413	3.44	0.18	0.79	0.045	0.305

GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	BODY W. (GRAM)	BRAIN	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
36	205.2	0.92	0.373	3.08	0.28	0.83	0.038	0.265
37	175.7	0.99	0.393	3.00	0.21	0.78	0.045	0.240
38	177.5	1.02	0.394	2.73	0.24	0.67	0.047	0.270
39	196.0	0.85	0.338	3.08	0.22	0.75	0.043	0.236
40	181.2	0.93	0.385	2.86	0.18	0.74	0.044	0.244

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DW-IND - 9
23-MAR-99

ORGAN/BRAIN WEIGHT RATIOS (%)
AFTER 4 WEEKS
FEMALES

GROUP 1 (0 MG/KG)

ANIMAL NUMBER	BRAIN (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
21	1.84	37.893	284.87	15.53	72.71	5.349	23.128
22	1.83	31.288	279.33	17.99	70.29	3.787	24.776
23	1.76	37.560	329.21	18.84	82.74	4.157	29.599
24	1.68	38.797	320.86	30.46	84.43	4.925	28.187
25	1.79	36.745	314.30	20.66	82.23	3.903	21.364

GROUP 2 (50 MG/KG)

ANIMAL NUMBER	BRAIN (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
26	1.68	39.667	305.80	27.68	73.38	5.020	31.177
27	1.75	38.498	320.47	23.88	84.91	4.810	27.865
28	1.82	38.651	285.09	21.07	75.94	4.421	25.773
29	1.94	39.378	294.05	13.44	78.78	4.770	24.898
30	1.78	36.497	292.86	20.23	83.35	3.420	24.487

GROUP 3 (200 MG/KG)

ANIMAL NUMBER	BRAIN (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
31	1.78	36.776	268.52	18.56	76.53	4.479	25.180
32	1.82	39.124	303.78	21.22	79.98	4.698	28.399
33	1.86	33.011	257.01	19.83	80.75	4.519	19.541
34	1.77	36.403	300.07	16.23	68.33	4.054	24.595
35	1.80	40.133	333.94	17.62	76.41	4.398	29.586

GROUP 4 (1000 MG/KG)

ANIMAL NUMBER	BRAIN (GRAM)	HEART	LIVER	THYMUS	KIDNEYS	ADRENALS	SPLEEN
36	1.88	40.577	335.60	30.91	90.04	4.170	28.894
37	1.75	39.551	302.16	21.25	78.20	4.539	24.154
38	1.80	38.779	268.24	23.48	65.87	4.590	26.566
39	1.66	39.966	363.59	26.30	88.58	5.041	27.882
40	1.69	41.244	306.20	19.10	79.56	4.760	26.118

[redacted]

DIRECT PEPTIDE REACTIVITY ASSAY DATA FOR **L-GLUTAMIC ACID, N-(1-OXODODECYL)-, MIXED (3.BETA.,24R)-ERGOST-5-EN-3-YL AND 2-OCTYLDODECYL AND (3.BETA.,22E)-STIGMASTA-5,22-DIEN-3-YL AND (3.BETA.)-STIGMAST-5-EN-3-YL ESTERS**

Report Date:	July 31,2015
[redacted]	
Study Reference No.:	[redacted]
Submitting toxicologist:	[redacted]
[redacted]	
Request#:	

Test Materials

Chemical	CAS
L-GLUTAMIC ACID, N-(1-OXODODECYL)-, MIXED (3.BETA.,24R)-ERGOST-5-EN-3-YL AND 2-OCTYLDODECYL AND (3.BETA.,22E)-STIGMASTA-5,22-DIEN-3-YL AND (3.BETA.)-STIGMAST-5-EN-3-YL ESTERS [redacted]	[220465-88-3]

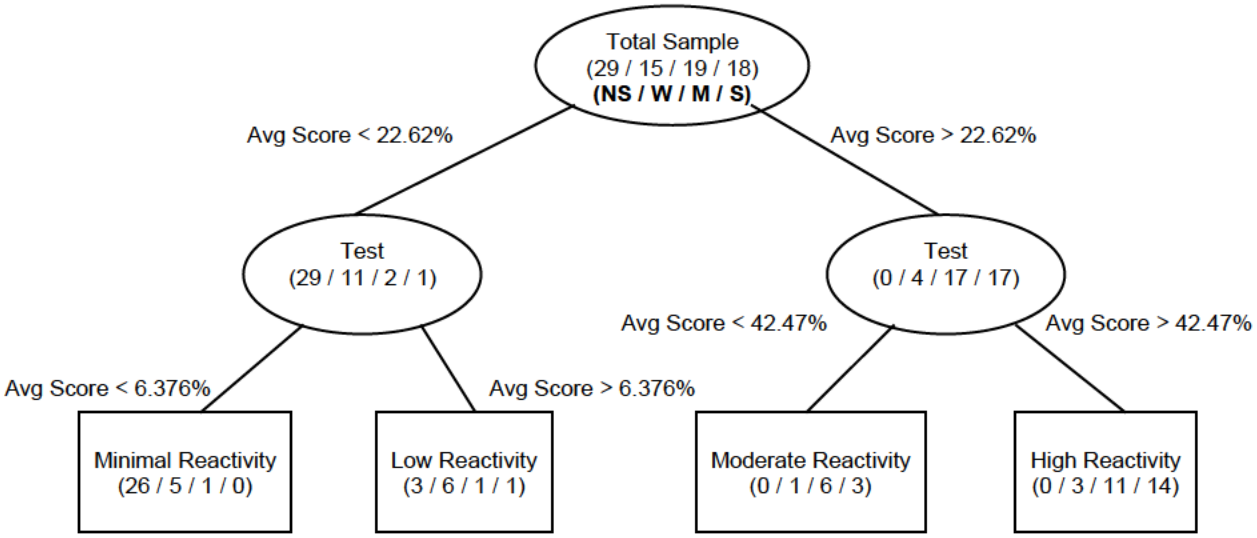
The chemical of interest was tested according to the standard Direct Peptide Reactivity Assay SOP. All materials were dissolved in acetonitrile, to prepare the 100mM test chemical stock solution for testing.

Raw Data

	Cysteine Depletion	Lysine Depletion
L-GLUTAMIC ACID, N-(1-OXODODECYL)-, MIXED (3.BETA.,24R)-ERGOST-5-EN-3-YL AND 2-OCTYLDODECYL AND (3.BETA.,22E)-STIGMASTA-5,22-DIEN-3-YL AND (3.BETA.)-STIGMAST-5-EN-3-YL ESTERS [220465-88-3]		
rep 1	-3.2%	0.0%
rep 2	3.7%	0.6%
rep 3	5.0%	-0.2%
Mean	**1.8%**	**0.1%**
Stand. Dev.	**4.4**	**0.5**

QC data for this run was met. The positive control is in the appropriate range for both peptides (Cys: 60.8% < mean < 100%; Lys: 40.2% < mean < 69.4%). All standard deviations are less than 14.9.

Prediction Model



Test chemical	CAS	% Cysteine Depletion	% Lysine Depletion	Reactivity Prediction
L-GLUTAMIC ACID, N-(1-OXODODECYL)-, MIXED (3.BETA.,24R)-ERGOST-5-EN-3-YL AND 2-OCTYLDODECYL AND (3.BETA.,22E)-STIGMASTA-5,22-DIEN-3-YL AND (3.BETA.)-STIGMAST-5-EN-3-YL ESTERS	[220465-88-3]	**1.8%**	**0.1%**	**Minimal reactivity**

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HUMAN CUMULATIVE IRRITATION PATCH TEST

TKL STUDY NO. [redacted]

[redacted]

CONDUCTED FOR:

[redacted]

DATE OF REPORT:

January 24, 2013

201.587.0500 • www.tklresearch.com

TKL Research, Inc. 365 West Passaic Street, Rochelle Park, NJ 07662

Version 1.0

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SIGNATURES

This study was conducted in compliance with the requirements of the protocol and TKL's Standard Operating Procedures, and in the spirit of GCP ICH Topic E6.[1] The report accurately reflects the raw data for this study.

Jonathan S. Dosik, MD
Principal Investigator

1/22/13
Date

Kathleen Georgeian
Kathleen Georgeian
Director, Dermatologic Safety Testing

1/23/13
Date

Michelle Medina
Manager, Dermatologic Safety Testing

1/23/13
Date

STATEMENT OF QUALITY ASSURANCE

This report has been reviewed by the TKL Research, Inc (TKL) Corporate Quality Assurance Department and the report accurately reflects the raw data for this study.

Quality Assurance

1/24/13
Date

[1] ICH Topic E6 "note for the guidance on Good Clinical Practices (CPMP/ICH/135/95)" – ICH Harmonised Tripartite Guideline for Good Clinical Practices having reached Step 5 of the ICH Process at the ICH Steering Committee meeting on 1 May 1996.
Version 1.0

TITLE OF STUDY

Human Cumulative Irritation Patch Test

SPONSOR

STUDY MATERIALS

Facial Essence with 1.5% phytosteryl/octyldodecyl lauroyl glutamate

DATE STUDY INITIATED

October 22, 2012

DATE STUDY COMPLETED

October 29, 2012

DATE OF REPORT

January 24, 2013

INVESTIGATIVE PERSONNEL

Jonathan S. Dosik, MD - Dermatologist
Principal Investigator

Kathleen Georgeian
Director, Dermatologic Safety Testing

Michelle Medina
Manager, Dermatologic Safety Testing

CLINICAL SITE

TKL RESEARCH, INC
1 Palmer Terrace
Carlstadt, NJ 07072

SUMMARY

one of which is a facial essence with 1.5% phytosteryl/octyldodecyl lauroyl glutamate

Eight (8) study materials, [redacted] were evaluated neat to determine their ability to cause irritation to the skin of volunteer subjects with normal skin using a 7-day semi-occlusive cumulative irritation patch study. Distilled water served as a negative control and 0.75% SLS served as a positive control. Thirty-eight (38) subjects completed the study.

The dermatologist was present on the final study day for evaluation.

This study determined the following irritation scores and associated classifications:

[redacted]	Irritation Scores: Total	Irritation Scores: Overall Average Skin Grade (N=39 Enrolled)	Cumulative Irritation Index
SLS 0.75%	117.5	0.4361	0.1090
[redacted]			
Facial essence with 1.5% phytosteryl/octyldodecyl lauroyl glutamate [redacted]	4.5	0.0169	0.0042
[redacted]			
Distilled Water	1.0	0.0038	0.0009
[redacted]			

Source: Table 4, Appendix 1, and Data Listing 3, Appendix II

Under the conditions employed in this study, the subjects showed no evidence of irritation to [redacted] Facial essence with 1.5% phytosteryl/octyldodecyl lauroyl glutamate

1.0 OBJECTIVE

The objective of this study was to assess the potential of the test substances to elicit human skin irritation by repetitive topical application.

2.0 RATIONALE

Cumulative irritancy patch testing is a modified primary irritancy patch test that can detect weak irritants, which require multiple applications to cause a skin reaction. These reactions are due to direct damage to the epidermal cells and no immunologic (allergic) mechanism is involved. This procedure may detect so-called "fatiguing substances" which are mild irritants that cause more strongly positive reactions with successive multiple skin exposure.

3.0 STUDY DESIGN

3.1 STUDY POPULATION

A sufficient number of healthy subjects were enrolled to provide a minimum of 30 completed subjects.

3.1.1 Inclusion Criteria

Individuals eligible for inclusion in the study were those who:

1. Were male or female, between the ages of 18 and 65;
2. Were in general good health as determined by the Medical and Dermatological History Questionnaire (Appendix A of Protocol, see Appendix IV);
3. Read, understood, and signed an informed consent (IC) agreement after being advised of the nature of the study;
4. Were willing to refrain from using lotions, creams, powders, or other skin preparations on the skin in the test area for the duration of the study;
5. Were willing to refrain from exposing skin sites to the sun or going to tanning beds for the duration of the study; and
6. Were willing to refrain from swimming and using hot tubs for the duration of the study.

3.1.2 Exclusion Criteria

Individuals excluded from participation in the study were those who:

1. Had clinically significant active dermatitis or skin disease anywhere on the body (excluding facial acne);
2. Had a history of psoriasis, eczema, or skin cancer;
3. Had a condition or were taking medication(s) which, in the judgment of the Investigator or Designate, made the subject ineligible or placed the subject at undue risk;
4. Had received treatment (chemotherapy, radiation, immune suppressant medications) for any type of cancer within the last 6 months;
5. Had a mastectomy or axillary lymph nodes removed;
6. Had an autoimmune or immune deficiency disease (eg, lupus, myositis, Crohns disease, autoimmune thyroid diseases, autoimmune hepatitis);

7. Were taking any immunosuppressant medication;
8. Had insulin-dependent diabetes;
9. Had asthma or any other chronic respiratory condition requiring daily therapy;
10. Were taking or using any antihistamines or systemic/topical anti-inflammatory medications (eg, ibuprofen, corticosteroid) on a routine or frequent basis. Maximum acceptable dosage should be determined by written laboratory guidelines;
11. Used a topical anti-inflammatory in the patch area within the last 2 weeks;
12. Were receiving allergy injections, expected to start injections before the conclusion of the study, or had the final injection within a week of the study start;
13. Were participating in another dermal study of any kind;
14. Were participating in any clinical study, which, in the judgment of the Investigator, would have potentially affected responses in either study;
15. Had a confirmed skin allergy as a result of participation in a patch study;
16. Had a known sensitivity or allergy relating to the substance(s) being evaluated;
17. Had a known sensitivity or allergy to adhesives, surgical tapes, bandages, etc; and/or
18. Had scars, moles, sunburn, tattoos, etc. in the patch area.

3.1.3 Informed Consent

A properly executed IC document was obtained from each subject prior to entering the study. The signed IC document is maintained in the study file. In addition, the subject was provided with a copy of the IC document (see Appendix III).

3.2 Description of Study

3.2.1 Outline of Study Procedures

The study extended over a 7-consecutive day period with 4 product applications and evaluations. On Day 1, the study material was applied to the back under conditions described in Section 3.3.4. Twenty-three hours (±1 hour) later the patches were removed. Twenty to 40 minutes after patch removal the sites were evaluated, the responses recorded, and identical patches applied to the same sites. This was repeated daily for a total of 7 days, including Saturdays and Sundays.

3.2.2 Study Flow Chart

Day	Activities
1	Obtained informed consent, reviewed completed medical screening form, applied patches
2, 3, 4, 5, 6	Staff removed patches, graded, applied patches
7	Staff removed patches, graded

3.2.3 Definitions Used for Grading Responses

Responses were graded using the following numeral equivalents.

Response	Numerical Equivalent
No apparent cutaneous involvement	0

Greater than 0, less than 1 (Faint, barely perceptible erythema OR slight dryness [glazed appearance])	0.5
Faint but definite erythema, no eruptions or broken skin OR no erythema but definite dryness; may have epidermal fissuring	1.0
Greater than 1, less than 2 (Well-defined erythema OR faint erythema with definite dryness, may have epidermal fissuring)	1.5
Moderate erythema, may have a few papules OR deep fissures, moderate-to-severe erythema in the cracks (Cut-off grade, patches are not reapplied)	2.0
Greater than 2 less than 3 (Moderate erythema with barely perceptible edema OR severe erythema not involving a significant portion of the patch [halo effect around the edges], may have a few papules OR moderate-to-severe erythema	2.5
Severe erythema (beet redness), may have generalized papules OR moderate-to-severe erythema with slight edema (edges well defined by raising)	3.0
Greater than 3, less than 4 (Moderate-to-severe erythema with moderate edema(confined to patch area) OR moderate-to-severe erythema with isolated eschar formations or vesicles)	3.5
Generalized vesicles or eschar formations OR moderate-to-severe erythema and/or edema extending beyond the area of the patch.	4.0

The maximum obtainable individual score was a 4.0. Should a reaction of 2.0 have occurred at any point during the study, further patch application on that subject would have been terminated with respect to the product involved. An "NP" symbol and a score of 2.0 would be assigned to all subsequent days.

For each test substance, the average of all inclusive scores was calculated for each completed grade day and reported as the average skin grade for that particular day. An overall average skin grade was calculated (ie, the sum of the daily average skin grades divided by the number of study days) for each test substance.

The Cumulative Irritation Index (CII) was calculated by dividing the total score by the sums of the highest possible score multiplied by the number of subjects multiplied by the number of days.

3.2.4 Evaluation of Responses

All responses were graded by a trained dermatologic evaluator meeting TKL's strict certification requirements to standardize the assignment of response grades.

3.3 STUDY MATERIAL

3.3.1 Storage, Handling, and Documentation of Study Material

Receipt of the material used in this study was documented in a general logbook, which serves as a permanent record of the receipt, storage, and disposition of all study material received by TKL. On the basis of information provided by the sponsor, the study material was considered reasonably safe for evaluation on human subjects. A sample of the study material was reserved and will be stored for a period of 6 months. All study material is kept in a locked product storage room accessible to

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clinical staff members only. At the conclusion of the clinical study, the remaining study material was discarded or returned to the Sponsor and the disposition documented in the logbook.

3.3.2 Nature of Study Material

[redacted]

Identification : [redacted] Facial Essence
Amount Applied : 0.2 mL Facial essence with 1.5% phytosteryl/octyldodecyl lauroyl glutamate

[redacted]

Special Instructions : Prior to the first application of product, the sites were wiped with 70% isopropyl alcohol. All Patches were prepared upon the subjects' arrival and were used within 15 minutes from patch preparation.

3.3.3 Application of Study Material

Study material was applied to patches as instructed. Patches were applied in a randomized schedule to the infrascapular area of the back, either to the right or left of the midline, or to the upper arm.

3.3.4 Description of Patch Conditions

Material evaluated under occlusive patch conditions is applied to a 2 cm x 2 cm Webril™ pad attached to a non-porous, plastic film adhesive bandage (3M medical tape). The patch is secured with hypoallergenic tape (Micropore), as needed.

Material evaluated under semi-occlusive patch conditions is applied to a 2 cm x 2 cm Webril™ pad. The pad is affixed to the skin with hypoallergenic tape (Micropore).

4.0 INTERPRETATION

Cutaneous irritation accounts for the majority of cases of contact dermatitis. Reactions consist of local inflammatory responses characterized by erythema and/or edema, or an erosive reaction characterized by local tissue destruction or necrosis. These reactions are due to direct damage to the

epidermal cells and require no prior sensitization. No immunologic (allergic) mechanism is involved.

To qualify as an "irritant", a substance should evoke inflammation on initial exposure (primary irritation) or on repeated exposure to an identical site (cumulative irritation). An irritant substance will cause dermatitis if it is permitted to act in sufficient concentration for a sufficient length of time. Irritant reactions may develop in all subjects, although individual susceptibility varies greatly.

Cumulative irritancy patch testing can detect weak irritants that require multiple applications to produce skin irritation. During and after first contacts with weak irritants, no visible skin alterations are observed. After repeated contact, the skin gradually becomes erythematous; drying and cracking occur; and later, oozing, crusting, and erosion may develop. An eczematous reaction with papules, vesicles, and edema may also develop.

The procedure employed is a modification of that described by Dr. B. M. Lanman[1] at the Joint Conference on Cosmetic Sciences, April 21-23, 1968 in Washington, DC, and further modified by Phillips, et al[2] and Berger, et al.[3]

5.0 PROTOCOL

See Protocol - Appendix IV.

6.0 DOCUMENTATION AND RETENTION OF DATA

The case report forms (CRFs) are designed to identify each subject by subject number and initials, and to record demographics, examination results, adverse events (AEs), and end of study status. Originals or copies of all CRFs, correspondence, study reports, and all source data will be kept on hard-copy file for a minimum of 20 years from completion of the study. Storage is maintained either at a TKL facility in a secured room accessible only to TKL employees, or at an offsite location which provides a secure environment with burglar/fire alarm systems, camera detection and controlled temperature and humidity. Documentation will be available for the Sponsor's review on the premises of TKL.

7.0 RESULTS & DISCUSSION

Thirty-nine (39) subjects between the ages of 20 and 65 were enrolled and 38 subjects completed the study (see Tables 1 and 2 in Appendix I and Data Listings 1 and 2 in Appendix II). The following table summarizes subject enrollment and disposition.

Number enrolled:		39
Number discontinued:		1
Lost to follow-up:	1	
Number completed:		38

Source: Table 1, Appendix I

The dermatologist was present on the final study day for evaluation.

There were no AEs reported.

The Sponsor's Test Substance Information contained incorrect product descriptions. A protocol amendment was issued on January 8, 2013 with instruction to change the incorrect product descriptions to the correct descriptions provided in the protocol amendment. A copy of the protocol amendment is included in Appendix IV.

A summary of response data are provided in Table 3, Appendix I. A Cumulative Irritation Index by product is provided in Table 4. Individual dermatological response grades are provided in Data Listing 3, Appendix II.

This study determined the following irritation scores and associated classifications:

[redacted]	Irritation Scores: Total	Irritation Scores: Overall Average Skin Grade (N=39 Enrolled)	Cumulative Irritation Index
SLS 0.75%	117.5	0.4361	0.1090
[redacted]			
Facial essence with 1.5% phytosteryl/octyldodecyl lauroyl glutamate [redacted]	4.5	0.0169	0.0042
[redacted]			
Distilled Water	1.0	0.0038	0.0009
[redacted]			

Source: Table 4, Appendix 1, and Data Listing 3, Appendix II

8.0 CONCLUSION

Under the conditions employed in this study, the subjects showed no evidence of irritation to [redacted]

Facial essence with 1.5% phytosteryl/octyldodecyl lauroyl glutamate

9.0 REFERENCES

1 B.M. Lanman, E.B. Elvers and C.J. Howard. "The Role of Human Patch Testing in a Product Development Program" Joint Conference on Cosmetic Sciences, The Toilet Goods Association, Washington, D.C., April 21-23, 1968.

2 L. Philips, M. Steinberg, H.I. Maibach and W.A. Akers. "Comparison of Rabbit and Human Skin Response to Certain Irritants". Toxicol. Appl. Pharmacol. 21.369, 1972.

3 R.S. Berger and J.P. Bowman. "A Reappraisal of the 21-day Cumulative Irritation Test in Man" J. Toxicol. - Cut. & Ocular Toxical. 1 (2). 109-115, 1982.

[redacted]

APPENDIX I

SUMMARY TABLES

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Table 1: Summary of Subject Enrollment and Disposition

	N (%)
Subjects enrolled	39
Subjects completed all phases	38 (97.4)
Total subjects discontinued	1 (2.6)
Lost to follow-up	1 (2.6)

Note: All percentages are relative to total subjects enrolled.

See data listing 1 for further detail.

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TKL Study No. [redacted]

Table 2: Summary of Subject Demographics
All Enrolled Subjects

Age	
N (%) 18 to 44	16 (41.0)
N (%) 45 to 64	21 (53.8)
N (%) 65 and up	2 (5.1)
Mean (SD)	47.3 (12.8)
Median	48.4
Range	20.9 to 65.7
Gender	
N (%) Male	14 (35.9)
N (%) Female	25 (64.1)
Race	
Black	3 (7.7)
Caucasian	21 (53.8)
Hispanic	15 (38.5)

See data listing 2 for further detail.

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PRODUCT = R

[redacted]

TKL Study No. [redacted]

Table 3: Summary of Dermatologic Response Grades
Number of Subjects by Product

Product = [redacted] Facial essence with 1.5% phytosteryl/octyldodecyl lauroyl glutamate

	Reading No.						
Response	**1**	**2**	**3**	**4**	**5**	**6**	**7**
0	38	38	38	38	37	38	38
0.5	0	0	0	0	1	0	0
1	1	1	1	1	0	0	0
Average skin grade	0.03	0.03	0.03	0.03	0.01	0.00	0.00
Total evaluable	39	39	39	39	38	38	38
Number absent	0	0	0	0	0	0	0
Number discontinued	0	0	0	0	1	1	1

Overall Average Skin Grade: 0.02

Note: 'Total evaluable' includes subjects with no patch applied.
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PRODUCT = R

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TKL Study No. [redacted]

Table 3: Summary of Dermatologic Response Grades
Number of Subjects by Product

Product = DISTILLED WATER

	Reading No.						
Response	**1**	**2**	**3**	**4**	**5**	**6**	**7**
0	39	39	39	38	37	38	38
0.5	0	0	0	1	1	0	0
Average skin grade	0.00	0.00	0.00	0.01	0.01	0.00	0.00
Total evaluable	39	39	39	39	38	38	38
Number absent	0	0	0	0	0	0	0
Number discontinued	0	0	0	0	1	1	1

Overall Average Skin Grade: 0.00

Note: 'Total evaluable' includes subjects with no patch applied.
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PRODUCT = R

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TKL Study No. [redacted]

Table 3: Summary of Dermatologic Response Grades
Number of Subjects by Product

Product = SLS 0.75% LOT# CS71350001

Response	Reading No. 1	2	3	4	5	6	7
0	35	33	21	15	11	11	11
0.5	4	6	10	14	9	2	2
1	0	0	8	8	16	23	23
2	0	0	0	2	0	0	0
Average skin grade	0.05	0.08	0.33	0.49	0.64	0.74	0.74
Total evaluable	39	39	39	39	38	38	38
Number absent	0	0	0	0	0	0	0
Number discontinued	0	0	0	0	1	1	1
Patch not applied	0	0	0	0	2	2	2

Overall Average Skin Grade: 0.45

Note: 'Total evaluable' includes subjects with no patch applied.
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PRODUCT = R

Table 3.1: [redacted] Uniform Laboratory Patch Test Grading Scale

Score or Symbol	Description
0	No apparent cutaneous involvement.
0.5	Greater than 0, less than 1.
1	Faint but definite erythema, no eruptions or broken skin or no erythema but definite dryness; may have epidermal fissuring.
1.5	Greater than 1, less than 2.
2	Moderate erythema, may have a few papules or deep fissures, moderate-to-severe erythema in the cracks. **Cut-off Grade - Patches are not re-applied.**
2.5	Greater than 2, less than 3.
3	Severe erythema (beet redness), may have generalized papules or moderate-to-severe erythema with slight edema (edges well defined by raising.)
3.5	Greater than 3, less than 4.
4	Generalized vesicles or eschar formations or moderate-to-severe erythema and/or edema extending beyond the area of the patch.
NA	Not applied
NP	Not patched due to reaction achieved
-	Reading not performed due to missed visit or subject discontinuation

TKL STUDY NO.[redacted]

Table 4: Cumulative Irritation Index, by Study Product

Product Index	Product Name or ID	Cumulative Irritation Index	Average Skin Grade
1	[redacted]	[redacted]	[redacted]
2			
3			
4			
5			
6			
7	[redacted] Facial essence with 1.5% phytosteryl/	0.0042	0.0169
8	[redacted] octyldodectyl lauroyl glutamate	[redacted]	[redacted]
9	DISTILLED WATER	0.0009	0.0038
10	SLS 0.75% LOT# CS71350001	0.1090	0.4361

Note: Cumulative Irritation Index = Total Irritation Score (sum of all days for all completed subjects)/[Highest possible score (=4) x Number of completed subjects x Number of study days (=7)].

Overall average skin grade is calculated as the sum of the daily average skin grades divided by the number of study days.

[redacted]

APPENDIX II

DATA LISTINGS

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Data Listing 1: Subject Enrollment and Disposition

Subject No.	Screened	Study Dates 1st Applic	Ended	Last Reading #	Completion Status	Days in Study
001	10/22/12	10/22/12	10/29/12	8	C	8
002	10/22/12	10/22/12	10/29/12	8	C	8
003	10/22/12	10/22/12	10/29/12	8	C	8
004	10/22/12	10/22/12	10/29/12	8	C	8
005	10/22/12	10/22/12	10/29/12	8	C	8
006	10/22/12	10/22/12	10/29/12	8	C	8
007	10/22/12	10/22/12	10/29/12	8	C	8
008	10/22/12	10/22/12	10/29/12	8	C	8
009	10/22/12	10/22/12	10/29/12	8	C	8
010	10/22/12	10/22/12	10/29/12	8	C	8
011	10/22/12	10/22/12	10/29/12	8	C	8
012	10/22/12	10/22/12	10/29/12	8	C	8
013	10/22/12	10/22/12	10/29/12	8	C	8
014	10/22/12	10/22/12	10/29/12	8	C	8
015	10/22/12	10/22/12	10/29/12	8	C	8
016	10/22/12	10/22/12	10/29/12	8	C	8
017	10/22/12	10/22/12	10/29/12	8	C	8
018	10/22/12	10/22/12	10/29/12	8	C	8
019	10/22/12	10/22/12	10/29/12	8	C	8
020	10/22/12	10/22/12	10/29/12	8	C	8
021	10/22/12	10/22/12	10/27/12	4	L	6
022	10/22/12	10/22/12	10/29/12	8	C	8
023	10/22/12	10/22/12	10/29/12	8	C	8
024	10/22/12	10/22/12	10/29/12	8	C	8
025	10/22/12	10/22/12	10/29/12	8	C	8
026	10/22/12	10/22/12	10/29/12	8	C	8
027	10/22/12	10/22/12	10/29/12	8	C	8
028	10/22/12	10/22/12	10/29/12	8	C	8
029	10/22/12	10/22/12	10/29/12	8	C	8
030	10/22/12	10/22/12	10/29/12	8	C	8
031	10/22/12	10/22/12	10/29/12	8	C	8
032	10/22/12	10/22/12	10/29/12	8	C	8
033	10/22/12	10/22/12	10/29/12	8	C	8
034	10/22/12	10/22/12	10/29/12	8	C	8
035	10/22/12	10/22/12	10/29/12	8	C	8
036	10/22/12	10/22/12	10/29/12	8	C	8
037	10/22/12	10/22/12	10/29/12	8	C	8
038	10/22/12	10/22/12	10/29/12	8	C	8
039	10/22/12	10/22/12	10/29/12	8	C	8

Key: Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
001	38.5	Female	Caucasian
002	51.9	Female	Hispanic
003	43.0	Female	Hispanic
004	20.9	Male	Black
005	36.3	Male	Hispanic
006	58.4	Female	Caucasian
007	46.6	Female	Hispanic
008	64.2	Male	Caucasian
009	62.8	Male	Caucasian
010	56.6	Female	Caucasian
011	41.1	Female	Caucasian
012	32.1	Male	Caucasian
013	56.7	Male	Caucasian
014	37.6	Female	Caucasian
015	54.0	Female	Caucasian
016	41.1	Female	Black
017	48.2	Female	Hispanic
018	22.4	Female	Caucasian
019	40.9	Male	Hispanic
020	43.2	Female	Hispanic
021	47.1	Female	Caucasian
022	48.4	Female	Caucasian
023	55.8	Female	Caucasian
024	24.5	Male	Hispanic
025	59.7	Female	Hispanic
026	42.9	Female	Hispanic
027	60.4	Female	Hispanic
028	39.7	Female	Caucasian
029	49.1	Female	Caucasian
030	65.7	Male	Caucasian
031	57.5	Male	Hispanic
032	52.0	Female	Caucasian
033	55.8	Male	Caucasian
034	65.6	Female	Hispanic
035	57.2	Male	Hispanic
036	23.1	Female	Hispanic
037	54.0	Male	Caucasian
038	63.7	Female	Caucasian
039	26.4	Male	Black

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[redacted]

TKL Study No. [redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted]
Facial essence with 1.5% phytosteryl/octyldodectyl lauroyl glutamate

Subject Number	Reading No. 1	2	3	4	5	6	7	Total Score
001	0	0	0	0	0	0	0	0
002	0	0	0	0	0	0	0	0
003	0	0	0	0	0	0	0	0
004	0	0	0	0	0	0	0	0
005	0	0	0	0	0	0	0	0
006	0	0	0	0	0	0	0	0
007	0	0	0	0	0	0	0	0
008	1	1	1	1	0.5	0	0	4.5
009	0	0	0	0	0	0	0	0
010	0	0	0	0	0	0	0	0
011	0	0	0	0	0	0	0	0
012	0	0	0	0	0	0	0	0
013	0	0	0	0	0	0	0	0
014	0	0	0	0	0	0	0	0
015	0	0	0	0	0	0	0	0
016	0	0	0	0	0	0	0	0
017	0	0	0	0	0	0	0	0
018	0	0	0	0	0	0	0	0
019	0	0	0	0	0	0	0	0
020	0	0	0	0	0	0	0	0
021	0	0	0	0	-	-	-	0
022	0	0	0	0	0	0	0	0
023	0	0	0	0	0	0	0	0
024	0	0	0	0	0	0	0	0
025	0	0	0	0	0	0	0	0
026	0	0	0	0	0	0	0	0
027	0	0	0	0	0	0	0	0
028	0	0	0	0	0	0	0	0
029	0	0	0	0	0	0	0	0
030	0	0	0	0	0	0	0	0
031	0	0	0	0	0	0	0	0
032	0	0	0	0	0	0	0	0
033	0	0	0	0	0	0	0	0
034	0	0	0	0	0	0	0	0
035	0	0	0	0	0	0	0	0
036	0	0	0	0	0	0	0	0
037	0	0	0	0	0	0	0	0
038	0	0	0	0	0	0	0	0
039	0	0	0	0	0	0	0	0

Total Score: 4.5
Normalized Total Score: 1.2

Please see table 3.1 for key to scores and symbols.

[redacted]

TKL RESEARCH
TKL Study No. [redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = DISTILLED WATER

Subject Number	Reading No. 1	2	3	4	5	6	7	Total Score
001	0	0	0	0	0	0	0	0
002	0	0	0	0	0	0	0	0
003	0	0	0	0	0	0	0	0
004	0	0	0	0	0	0	0	0
005	0	0	0	0	0	0	0	0
006	0	0	0	0	0	0	0	0
007	0	0	0	0	0	0	0	0
008	0	0	0	0	0	0	0	0
009	0	0	0	0	0	0	0	0
010	0	0	0	0	0	0	0	0
011	0	0	0	0	0	0	0	0
012	0	0	0	0	0	0	0	0
013	0	0	0	0	0	0	0	0
014	0	0	0	0	0	0	0	0
015	0	0	0	0	0	0	0	0
016	0	0	0	0	0	0	0	0
017	0	0	0	0	0	0	0	0
018	0	0	0	0	0	0	0	0
019	0	0	0	0	0	0	0	0
020	0	0	0	0	0	0	0	0
021	0	0	0	0	-	-	-	0
022	0	0	0	0	0	0	0	0
023	0	0	0	0	0	0	0	0
024	0	0	0	0	0	0	0	0
025	0	0	0	0	0	0	0	0
026	0	0	0	0	0	0	0	0
027	0	0	0	0	0	0	0	0
028	0	0	0	0	0	0	0	0
029	0	0	0	0	0	0	0	0
030	0	0	0	0	0	0	0	0
031	0	0	0	0.5	0.5	0	0	1
032	0	0	0	0	0	0	0	0
033	0	0	0	0	0	0	0	0
034	0	0	0	0	0	0	0	0
035	0	0	0	0	0	0	0	0
036	0	0	0	0	0	0	0	0
037	0	0	0	0	0	0	0	0
038	0	0	0	0	0	0	0	0
039	0	0	0	0	0	0	0	0

Total Score: 1.0
Normalized Total Score: 0.3

Please see table 3.1 for key to scores and symbols

[redacted]

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Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = SLS 0.75% LOT# CS71350001

Subject Number	Reading No. 1	2	3	4	5	6	7	Total Score
001	0	0	0	0	0	0	0	0
002	0	0	0.5	0.5	1	1	1	4
003	0	0	0	0	0.5	1	1	2.5
004	0.5	0.5	1	2	NP	NP	NP	10
005	0	0	1	2	NP	NP	NP	9
006	0	0	0.5	0.5	1	1	1	4
007	0	0	0	0	0.5	1	1	2.5
008	0	0.5	1	1	1	1	1	5.5
009	0	0	0.5	0.5	1	1	1	4
010	0	0	0	0	0	0	0	0
011	0	0	0	0.5	0.5	0.5	0.5	2
012	0	0	0	0	0.5	1	1	2.5
013	0	0	0.5	0.5	1	1	1	4
014	0	0	0	0	0	0	0	0
015	0.5	0.5	1	1	1	1	1	6
016	0	0	0	0	0	0	0	0
017	0	0	0	0	0.5	0.5	0.5	1.5
018	0	0	0	0	0	0	0	0
019	0	0	0.5	0.5	1	1	1	4
020	0	0.5	1	1	1	1	1	5.5
021	0	0	0.5	1	-	-	-	1.5
022	0	0	0	0.5	0.5	1	1	3
023	0	0	0.5	1	1	1	1	4.5
024	0	0	0.5	0.5	1	1	1	4
025	0.5	0.5	1	1	1	1	1	6
026	0	0	0.5	0.5	1	1	1	4
027	0	0	1	1	1	1	1	5
028	0	0	0	0	0	0	0	0
029	0	0	0	0	0	0	0	0
030	0	0	0	0	0	0	0	0
031	0	0	0	0.5	1	1	1	3.5
032	0	0	0.5	0.5	1	1	1	4
033	0	0	0	0.5	0.5	1	1	3
034	0	0	0	0	0	0	0	0
035	0	0	0	0.5	0.5	1	1	3
036	0.5	0.5	1	1	1	1	1	6
037	0	0	0	0	0	0	0	0
038	0	0	0	0	0	0	0	0
039	0	0	0	0.5	0.5	1	1	3

Total Score: 117.5
Normalized Total Score: 34.6

Please see table 3.1 for key to scores and symbols.

[redacted]

TKL RESEARCH
TKL STUDY NO. [redacted]

Data Listing 3A: Residual Readings
by Product and Subject
SLS 0.75% LOT# CS71350001

Subject Number	Reading No. 1	2	3	4	5	6	7
004					2	2	1.5
005					2	2	1.5

Please see table 3.1 for key to scores and symbols.

[redacted]

APPENDIX III

INFORMED CONSENT DOCUMENT

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INFORMED CONSENT

8-DAY CUMULATIVE IRRITATION PATCH STUDY

STUDY NO.: [redacted]

PURPOSE

The purpose of this research is to determine if the study material can be put on human skin without causing some degree of irritation. The study will extend over an eight-day period and will involve a minimum of 25 participants in good health.

STUDY MATERIALS

The study materials include or may be components of cosmetics, skin care products, shampoos, shower gel/body wash, antiperspirants/deodorants, disinfectants, antibacterial, topical drugs, fragrances, soaps, sunscreens, fibers, medications, insect repellents, antimicrobials, and/or any other materials which are intended for and/or may come into contact with human skin.

PROCEDURE

The study material will be put on your back with a "patch" (i.e., a small adhesive square with a cotton pad). The patches will remain on your back for 24 hours. You will return to TKL Research, Inc. for eight consecutive days to have patches removed, sites evaluated by a clinical evaluator and product reapplied. On your final visit patches will be removed and final evaluation of the study sites will be conducted.

If you are a female of childbearing potential (i.e., not surgically sterile or have not experienced menopause), you must agree to prevent pregnancy throughout this study by using an accepted form of birth control [e.g., oral contraceptive pill, IUD, condom/diaphragm with spermicide, abstinence (no sexual relations)].

If you are nursing a child, you will not be permitted to participate in this study. Pregnancy and nursing are prohibited to prevent any unforeseen risk to an unborn or nursing child.

SUBJECT REQUIREMENTS

You must agree to make all your scheduled visits to TKL Research. You must not apply products such as creams, lotions and moisturizers on or near the test sites. You must avoid sun exposure or the use of tanning beds on your back (including the rest period). You must agree to refrain from swimming during the course of the study. You must agree to minimize water exposure on the patch area while showering or bathing by taking a low tub bath or frontal shower. You will receive written instructions for this study.

POTENTIAL RISKS

Some of the study products may be irritating under certain conditions but the degree of irritation is not expected to be greater than that described below. Individuals participating in this study may experience side effects such as redness, swelling, itching, cracking, peeling, or in rare cases, small blisters or sores. Reactions usually occur only where the study products or patch products (such as the patch tape adhesive) touch the skin. On rare occasions, the reactions may spread beyond the patch. A reaction may result in localized lightening or darkening of the skin, which may persist in an occasional individual. Reactions may be due to either skin irritation or allergy to either study products or patch products (e.g., patch tape adhesive).

It may be necessary to do additional application (rechallenge) to determine if an allergic reaction has occurred. If you should prove to be allergic, you can expect to react to this product if you encounter it at a later date. Whenever possible, you will be informed as to the identity of the product in order that you may avoid contact with it in the future.

For any significant reactions that may occur as a direct result of your participation in this study, appropriate and reasonable medical treatment will be provided by TKL Research, Inc. at no cost to you to resolve the immediate problem. Provision of such medical care is not an admission of legal liability or responsibility for the condition being treated. If such reactions occur, TKL personnel should be contacted immediately at (201) 587-0505 day, night or weekends. Extended medical care will not be provided.

POTENTIAL BENEFITS

You may receive no direct benefit from being in this study. However, taking part in this study may benefit society by gaining new knowledge.

SIGNIFICANT NEW FINDINGS

You will be informed of any significant new findings that may affect your willingness to continue your participation.

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INFORMED CONSENT

8-DAY CUMULATIVE IRRITATION PATCH STUDY

STUDY NO.: [redacted]

ALTERNATIVE TREATMENT
Since this study is for research only, the only alternative is for you not to participate.

WITHDRAWAL FROM STUDY
Participation in the study is voluntary and you may refuse to participate or may withdraw at any time. Voluntary withdrawal from the study for reasons unrelated to the study or failure to follow test procedures will result in some loss of payment based on the number of visits completed. Subjects will be paid [redacted] per visit for early withdrawal. Your participation may also be discontinued at any time without your consent by the study doctor, or the study sponsor(s) (the company(s) that makes the product(s) being evaluated). If you fail to comply with study procedures, your participation may be terminated.

COST
Your participation in the study will not incur any cost to you.

FINANCIAL INCENTIVE
Your participation is voluntary. You may discontinue participation at any time without prejudice. You will be compensated for you participation. A payment of [redacted] will be made only upon completion of all phases of the study. If in the judgment of the investigating personnel, it is best to discontinue your participation in this study due to an adverse experience or severe reaction you will be paid in full for your participation. Voluntary withdrawal from the study for reasons unrelated to the study or failure to follow test procedures will result in some loss of payment based on the number of visits completed. Subjects will be paid [redacted] per visit. Other than the compensation described above, you will not directly benefit from this study. This study is for scientific information. Not participating in the study would be your alternative.

CONFIDENTIALITY AND AUTHORIZATION
TKL Research will protect information about you and your taking part in this research study to the best of our ability. If information about this study is published, your identity will remain confidential. Reports prepared by TKL Research will utilize statistical information only and at no time will your name be used. However, the U.S. Food and Drug Administration (FDA), the sponsor and TKL Research, Inc may sometimes inspect the research record and study information of those who take part in this study. By signing this consent form, you are authorizing such access. A court of law could also order research records shown to other people, but that is unlikely. Therefore, absolute confidentiality cannot be guaranteed.

WHO TO CALL
Additional information regarding this research is available either before or during the course of this study. If you have any questions or research related side effect or injury, you may contact the study coordinator, Michelle Medina, at (201) 587-0500 x2230 or Dr. Philip LaStella, at 201-587-0500. The emergency 24 hr. phone number is 201-587-0500.

A copy of this consent form will be given to you.
****
I have read and understand the information given in this consent form. I have had an opportunity to ask questions and my questions have been answered. I voluntarily consent to participate. By signing this form I have not given up any of my legal rights which I would otherwise have as a research subject.

ENTRY NO. _____ PRINT NAME _____ SIGNATURE _____ DATE _____

SIGNATURE OF WITNESS _____ DATE _____

APPENDIX IV

PROTOCOL

PROTOCOL AMENDMENT 1

Testing Facility: TKL Research, Inc.
Study Type: Human Cumulative Irritation Patch Test
Sponsor's Ref. #: [redacted]
Study Number:
Date: 08-Jan-2013

Change:
Protocol Attachment I, Sponsor's Test Substance Information, Page 1. The lined out entries will be deleted. Revised wording is indicated.

A. Test Substance Information and Handling:

[redacted]	A) [redacted] B) C) D) E) F) G) [redacted] Facial essence with 1.5% phytosteryl/octyldodectyl lauroyl glutamate H) [redacted]

Product Type: (e.g. moisturizer, shampoo, AP/DO, etc.)	[redacted] **Facial Essence (G, [redacted])**
Physical Form: (e.g. liquid, gel, paper, cream, solid, etc.)	[redacted] **~~Viscous Liquid Serum (G)~~** [redacted]

Reason for Change:
Incorrect descriptions were provided.

1/8/13

Jonathan S. Dosik, M.D.
Principal Investigator
TKL Research, Inc.

Date

[redacted]

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HUMAN CUMULATIVE IRRITATION PATCH TEST

Sponsor's Reference Number:	[redacted]
[redacted]	[redacted] Facial essence with 1.5% phytosteryl/octyldodectyl lauroyl glutamate Distilled Water and Sodium Lauryl Sulfate to be supplied by TKL Research, Inc.
Investigator's Study #:	[redacted]
Principal Investigator/Dermatologist:	Jonathan S. Dosik, M.D.
Investigator's Facility - Address/Telephone Number:	TKL Research, Inc. 4 Forest Avenue Paramus NJ 07652 UNITED STATES Phone #: 1-201-587-0500 FAX #: 1-201-881-8729
Site of Investigation:	To be documented in study records
Sponsor and Address:	[redacted]
Sponsor Representative: Telephone Number:	[redacted]
Number of Subjects to Complete:	35
Special Recruiting Criteria:	None
Number of Patch Application Days:	7 Days
Cumulative Irritation Calculation to be Used (see page 11):	#1
Minimum Time Between Patch Test Participation:	4 Weeks
Report Preparation Instructions:	Single Report
Anticipated Study Start Date/End Date:	22-Oct-2012 / 29-Oct-1012

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18.0 ATTACHMENTS
- 1. Sponsor's Test Substance Information

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1.0 SIGNATURES

1.1 INVESTIGATOR APPROVAL

I have reviewed the attached protocol and concur with the study design. I agree to participate as the Investigator and to follow the protocol, as written.

10/22/12

Jonathan S. Dosik, M.D.
Principal Investigator
TKL Research, Inc.
Investigator's Study #: DS360112

Date

1.2 SPONSOR APPROVAL

The Sponsor's approval is represented by the signature of the sponsoring toxicologist on the Sponsor's Test Substance Information sheets (Attachment 1).

[redacted]

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2.0 STUDY OBJECTIVE

The purpose of this study is to assess the potential of test substances to elicit human skin irritation by repetitive topical application.

3.0 INCLUSION / EXCLUSION CRITERIA

Individuals will be enrolled in the study based on the following inclusion and exclusion criteria.

3.1 Inclusion Criteria:

In order to be eligible for participation, subjects must be:

1. males or females between the ages of 18 and 65
2. in general good health as determined by the Medical and Dermatological History Questionnaire (Appendix A)
3. able to read, understand and sign an informed consent agreement after being advised of the nature of the study
4. willing to refrain from using lotions, creams, powders or other skin preparations on the skin in the test area for the duration of the study
5. willing to refrain from exposing skin sites to the sun or going to tanning beds for the duration of the study
6. willing to refrain from swimming and using hot tubs for the duration of the study

3.2 Exclusion Criteria:

Subjects are not eligible for participation if any one of the following applies:

1. have a clinically significant active dermatitis or skin disease anywhere on the body (excluding facial acne)
2. have a history of psoriasis, eczema or skin cancer
3. have a condition or are taking medication(s) which, in the judgment of the Investigator or Designate, makes the subject ineligible or places the subject at undue risk
4. have received treatment (chemotherapy, radiation, immune suppressant medications) for any type of cancer within the last 6 months
5. have had a mastectomy or axillary lymph nodes removed
6. have an autoimmune or immune deficiency disease (e.g. lupus, myositis, Crohns disease, autoimmune thyroid diseases, autoimmune hepatitis, etc.)
7. are currently taking any immunosuppressant medication
8. have insulin-dependent diabetes
9. have asthma or any other chronic respiratory condition requiring daily therapy
10. are currently taking/using any antihistamines or systemic/topical anti-inflammatory medications (e.g. ibuprofen, corticosteroid, etc.) on a routine or frequent basis. Maximum acceptable dosage should be determined by written laboratory guidelines.
11. have used a topical anti-inflammatory in the patch area within the last 2 weeks
12. are currently receiving allergy injections, expects to start injections before the conclusion of the study or has had the final injection within a week of the study start

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13. are currently participating in another dermal study of any kind
14. are currently participating in any clinical study, which in the judgment of the Investigator, could potentially affect responses in either study.
15. have a confirmed skin allergy as a result of participation in a patch study
16. have a known sensitivity or allergy relating to the substance(s) being evaluated
17. have a known sensitivity or allergy to adhesives, surgical tapes, bandages, etc.
18. have scars, moles, sunburn, tattoos, etc. in the patch area

The test facility may, with concurrence of the Sponsor, include additional criteria that determine eligibility.

4.0 SUBJECT ENROLLMENT

4.1 Medical and Dermatological History

Each subject will complete a Medical and Dermatological History Questionnaire (Appendix A). Individuals will be enrolled based on the medical and dermatological history information provided and the pre-enrollment interview. The Investigator or Designate may exclude an individual for any reason if the exclusion is in the best interest of the individual.

4.2 Informed Consent

All subjects eligible for enrollment will complete a written Informed Consent. The consent form will contain all elements required by Federal Register, 21 CRF 50 or the equivalent specific to each country. The original signed copy for each subject participating on the study will be retained in the Investigator's records. Each subject will receive a copy of his/her signed statement.

4.3 Subject Identification

Once eligibility has been confirmed, each subject will be assigned a subject number according to test facility guidelines. This number will uniquely identify each subject and will remain with the subject throughout the study.

5.0 PATCH TYPES AND DESCRIPTION

The patch type(s) to be used for this study is indicated on the Sponsor's Test Substance Information (Attachment 1). The patch/application options are as follows:

1. **Completely Occlusive Patch:** consisting of a nonwoven cotton pad (e.g. Webril®) measuring ~4 cm^2 (2 cm x 2 cm) covered by and held securely to the skin on all sides with an occlusive, hypoallergenic tape (e.g. Blenderm®).

2. **Completely Occlusive Patch with Plastic Chamber:** available in 2 sizes, as described below:
   a. **25 mm chamber:** A cotton pad (e.g. Webril®) measuring 19 mm in diameter (2.54 cm^2) is inserted into a plastic chamber measuring 25 mm in diameter.
   b. **19 mm chamber:** A cotton pad (e.g. Webril®) measuring 12 mm in diameter (1.13 cm^2) is inserted into a plastic chamber measuring 19 mm in diameter.

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3. **Semi-Occlusive Patch:** consisting of a nonwoven cotton pad (e.g. Webril®) measuring ~4 cm^2 (2 cm x 2 cm) covered by and held securely to the skin on all sides with a porous, hypoallergenic tape (e.g. Scanpor®, Micropore®, perforated Blenderm®, etc.)

4. **Semi-open Patch:** consisting of a nonwoven cotton pad (e.g. Webril®) measuring ~ 4 cm^2 (2 cm x 2 cm) held to the skin on two opposing sides by a strip of porous, hypoallergenic tape (e.g. Scanpor®, Micropore®, perforated Blenderm®) (pad is open on two sides similar to a Band-Aid®).

5. **Open Application:** consisting of direct placement of the test substance on the skin and covering the application site with a loosely woven covering (e.g. cotton gauze) held in place with hypoallergenic tape (e.g. Blenderm®, Dermacil®, Micropore®, Tenderskin®) along two opposing edges.

6.0 TEST SUBSTANCE ACCOUNTING AND PREPARATION RECORDS

The test substance(s) will be provided by the Sponsor and shipped directly to the test facility. The substance(s) will be identified by Sponsor identification numbers. The following will be documented in the study records:

- container label information
- date of receipt
- weight upon receipt, after each dose preparation and final weight
- final disposition of the test substance – disposed of or returned to Sponsor
- procedures required to prepare the test substance for patching (e.g. cutting into pieces, grinding into powder, making a dilution, heating, etc.)

Additional information on the test substance(s) is provided in the Sponsor's Test Substance Information (Attachment 1).

7.0 STUDY DESIGN

This study is designed to assess the irritation potential of test substances by repetitive, consecutive daily patch application to the skin of human subjects.

7.1 Duration

Patch applications are made on consecutive days (including Saturday and Sunday) for the number of days specified on page 1 of the protocol. A final grade is taken 24 hours following the last patch application. For example, a 7 Day Cumulative Irritation Study runs over a period of 8 days. Patches are applied for 7 consecutive days. A final grade is taken on the 8th and final day of the study, 24 hours after the 7th patch application. Follow-up evaluations may be necessary, see page 8 (Section 8, Grading of Test Sites) of the protocol.

7.2 Randomization

Test substances will be randomized among all test sites to minimize possible site variability. Each substance should be assigned so that it occupies each test site within the group of subjects approximately an equal number of times. Unless provided by the Sponsor, the randomization should be determined according to the procedures of the test facility. Randomizations provided by the Sponsor will be Appendix C of the protocol.

7.3 Control Materials and Benchmark Products

Control materials and benchmark products eliminate many of the variables (e.g. skin condition, weather conditions, grader variability, etc.) inherent in irritation patch testing and should always be included. Distilled water or saline are usually used as the negative irritant control. Sodium lauryl sulfate is used as the positive irritant control. Generally, the negative and positive irritant controls are provided by the test facility [indicated on page 1, "Test Substance Identification Number(s) [redacted]. Benchmark products are generally products with known irritation potential which are provided by the Sponsor. The irritation potential of the test substances can be

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positioned by comparing the irritation elicited by the test substances to the irritation produced by the positive, negative and/or benchmark materials.

7.4 Procedure

Up to a maximum of 10 patches can be applied (5 on each arm or 10 on the back). Patches containing the test substances are applied to the lateral surface of the upper arm(s), midway between the shoulder and the elbow, or to the upper (shoulder blade area) and/or lower back (between the waistline and the lower edge of the shoulder blade) on either side of the spine. Patches are applied to the area specified in Attachment I (Sponsor's Test Substance Information). Areas routinely covered by tight fitting clothing or underclothing should be avoided. Patches are applied in a vertical row on the arm or in a vertical or horizontal row(s) on the back.

Skin sites should be wiped with ethanol or isopropyl alcohol and air-dried prior to the first patch application. Alcohol removes any oils or lotions that may be present and increases adherence of the patch to the skin.

The site of application will be marked in such a way that the location of the patch pad is clearly indicated for scoring. To assure good adhesion, patches may be reinforced with a hypoallergenic tape (e.g. Dermacil®, Micropore®, etc.). If extra tape is used, it should be applied only over existing tape, not directly over the patch pad.

The test substances will be applied to the surface of the patch pad as specified in the Sponsor's Test Substance Information (Attachment 1). Applications are made every day for the number of consecutive days specified on page 1 of the protocol ("Number of Patch Application Days"). All applications for individual test substances are made to the same site (the site used for the initial patch application) unless reactions become so strong (grade of 2.0 or greater) as to make continued application inadvisable.

Patches are worn for approximately 23 hours (± 1 hour). Subjects will be instructed to keep their patches dry. Showering and bathing restrictions are at the discretion of the Investigator or Designate. Subjects will return to the test facility for patch removal and disposal by test facility personnel. Skin sites will be gently wiped with water and patted dry to remove excess test substance immediately following patch removal. Skin sites will be graded ~approximately 20-40 minutes following patch removal. Subjects should not replace any patches that fall off. If a patch falls off or comes loose, the time the event happened should be reported to test facility personnel at the next visit. Subjects will be provided with instructions and a schedule of the study dates on the first day of the study. A telephone number(s) will also be provided to report adverse events or answer questions on a 24-hour basis.

Subjects must be present on all patching/grading days to complete all aspects of the study requirements. Any subject who is absent on one of the patch application days or at the final reading will be dropped from the study. A sufficient number of subjects should be enrolled in the study to ensure that the number of subjects completing meets or exceeds the number indicated under "Number of Subjects to Complete" on page 1.

8.0 GRADING OF TEST SITES

An artificial light source, preferably a lamp with an incandescent, 60-100 watt, daylight bulb, is used to illuminate the patch area. Sites should be graded using the grading scale in Appendix B. Any visible response should be palpated to aid in detecting signs of elevation.

Patches are not applied to any skin site exhibiting a grade of 2.0 or greater. Subjects with grades of 2.0 or greater will not have the causative test substance applied for the remainder of the study. Grades for sites where patching has been discontinued will continue to be scored as "residual grades" for the remainder of the study. Residual grades for those sites where patching has been discontinued will be included in the report but will not be used in the calculations of average grades. The highest score

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observed is carried over to all subsequent grading days. This grade can either be the grade observed at the time patching was discontinued or a residual grade, whichever is higher.

Any site with an erythema grade of 2.0 or greater and/or any elevated response observed at the final regularly scheduled grading will be followed until the response(s) has regressed to a 1.5 or less. If the subject cannot return to the test facility for evaluation, the subject should be contacted by phone. Test facility personnel must establish that the subject's reaction(s) is resolving and has not increased in intensity. Any subject exhibiting a grade of 3.5 or 4.0 on any test site will be offered the opportunity to receive medical treatment from the Investigator's consulting dermatologist.

The Sponsor should be notified within 1-2 business days of any erythema score of 3 or greater or any score that includes edema, papules, vesicles, bullae and/or spreading beyond the patch site, regardless of the degree of erythema.

The same qualified skin grader should be used throughout the study. The grader will be blinded as to the treatment assignments.

9.0 ADDITIONAL STUDY INFORMATION

9.1 Adverse Events

Anticipated adverse events in this study are visible responses of the skin at or immediately surrounding the site(s) of test substance application. Those most likely to occur are mild-to-occasionally moderate erythema. On occasion, strong irritant responses may include cracking and peeling of the skin at the application site. It is possible that responses will include edema, papules and/or vesicles in addition to erythema. On rare occasions, strong reactions may spread beyond the area of the patch pad. Hypo- or hyper-pigmentation may occur at the site of a strong reaction in some individuals which could persist for several months. The skin of some subjects may become irritated by the tape and, on occasion, an allergic reaction to the tape may be seen. Some subjects may experience itching, burning and/or stinging in the test area.

Any unanticipated adverse events reported to or noted by test facility personnel should be investigated, documented and followed to resolution by the Investigator or Designate and/or consulting dermatologist. The Investigator or Designate will ascertain whether the reported symptom(s):

- is related to study participation
- warrants the subject be dropped from the study
- warrants follow-up evaluation and/or treatment

All information related to the assessment and treatment of unanticipated, potentially study related adverse events, including presenting signs, assessment of the relationship to the study, prescribed course of action, follow-up visits and final resolution will be documented in the study records.

All unanticipated, potentially study related adverse events should be reported to the Sponsor within 1-2 business days of the date initially reported or noted by the test facility.

9.2 Subject Termination

A subject may voluntarily withdraw from the study at any time for any reason. The Investigator or Designate may elect to discontinue a subject's participation at any time if deemed necessary or if it is in the best interest of the subject. If a subject experiences an adverse event/condition, the Investigator and the Sponsor will decide whether the subject should be dropped from the study. The reason for a subject's discontinuation in the study will be recorded in the study records and included in the final report.

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10.0 ADDITIONAL STUDY DOCUMENTATION

10.1 Protocol Amendments

If it becomes necessary to modify this protocol, the modification will be documented by a protocol amendment signed by the Investigator and a representative of the Sponsor. Amendments to the final protocol will be consecutively numbered and will describe any change and the rationale for making the change. All amendments will be included in the final report.

10.2 Protocol Deviations

If a deviation from the protocol occurs, it is the responsibility of the Investigator or Designee to notify the Sponsor Representative as soon as possible. All deviations will be documented appropriately and included in the final report.

10.3 Documentation of Data

All data generated in this study will be recorded in blue or black ink on the data collection forms. Data entries will be initialed and dated by the individual responsible for the entry. If subsequent corrections are required, the original entry will be crossed out with a single pen stroke so that it remains clearly legible; the correction will be entered close to the original entry and initialed and dated by the individual making the correction. If the reason for making the correction is not obvious, it should be stated or referenced near the entry. All spaces on each data collection form should be filled in or crossed through if not applicable. If a response is unknown, it should be marked as "unk". An example of one or more data collection form is appended to the protocol. The test facility may use this or an equivalent form(s) as long as all required data are collected.

11.0 INSTITUTIONAL REVIEW BOARD (IRB) / INDEPENDENT ETHICS COMMITTEE (IEC) (if applicable)

If IRB/IEC approval is required for this study, the Board/Committee will review this protocol, the informed consent statement, subject instructions, subject recruitment, safety data for the test substance(s) and any other relevant documents. The study will not begin until the IRB/IEC has given approval. The Investigator will provide the Sponsor with a copy of the IRB/IEC approval letter.

Approval procedures for protocol modifications, informing the Board/Committee of adverse events and providing a final report will be carried out according to the guidelines of the IRB/IEC.

12.0 STUDY MONITORING

The Investigator will permit representatives of the Sponsor to visit the test site during the course of the study. The Investigator will permit the representatives to observe all study procedures and review all study related documents. The representative will notify the Investigator in advance of the date and time of the visit. The monitoring visit will be documented in the study records.

13.0 RESULTS

13.1 Average Skin Grade

For each test substance, the average of all inclusive scores (see below) is calculated for each completed grade day and reported as the average skin grade for that particular day. An overall average skin grade is calculated (i.e. the sum of the daily average skin grades divided by the number of study days) for each test substance.

Subject scores **included** in the calculations:

- For any patch worn for 12 hours or more
- For any patch worn less than 12 hours when the skin site exhibits a grade greater than zero

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Subject scores **not included** in the calculations:

- For any patch worn less than 12 hours and the skin site exhibits a grade of zero
- For any subject dropped from the study for any reason
- Following discontinuation of patch application due to tape reaction

Scores from all subjects, including those dropped from the study will be included in the report.

13.2 Cumulative Irritation Index

The cumulative irritation index will be calculated for each test substance using one of the following examples, as specified under "Cumulative Irritation Calculation to be Used" on page 1:

Example #1:

$$\textbf{Irritation Index} = \frac{\text{Total Irritation Score (add all scores on all days for all subjects)}}{\text{Highest } \textbf{possible} \text{ score} \times \text{Number of subjects} \times \text{Number of study days}}$$

Example: $\frac{280}{4 \times 30 \times 7} = \frac{280}{840} = 0.33$

Example #2:

$$\textbf{Irritation Index} = \frac{\text{Total Irritation Score (add all scores on all days for all subjects)}}{\text{Highest } \textbf{anticipated} \text{ score} \times \text{Number of subjects} \times \text{Number of study days}}$$

Example: $\frac{75}{2 \times 30 \times 7} = \frac{75}{420} = 0.18$

13.3 Draft Report

An audited draft report, including all test and control substances, should be issued within 4 weeks of work being completed. The report will include the following:

- Purpose of the study
- Brief study design/overview
- Identification of study personnel and responsibilities
- Identification and concentration of test substance(s)
- **Description of the patch used - including dimensions of patch pad and the amount of test substance applied to the pad**
- Site of investigation
- Study dates
- Number of subjects enrolled and completing the study
- Discontinued subjects with reasons for withdrawal
- Protocol deviations
- Test substance preparation procedures
- Test substance accounting record
- Procedures/methods for applying test substance/solution to patch pads

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- Adverse events and resolution of theses events
- Complete tabulations of all data
- Summary of test results
- Data interpretation/conclusions
- QA statement
- Copy of the protocol and all protocol amendments

13.4 Final Report

A final report should be completed within 4 weeks of receiving the Sponsor's comments on the draft report.

14.0 STUDY RECORDS RETENTION

The Investigator will follow the procedure outlined in "Section K. Data Retention" of the Sponsor's Test Substance Information document (Attachment 1).

15.0 CONFIDENTIALITY

The Investigator and employees of the test facility are obligated to keep any information confidential regarding the test substances and all aspects of the study, as subject to the terms and conditions of the Master Laboratory Services Agreement between the test facility and the Sponsor.

16.0 REGULATORY COMPLIANCE

This study will be conducted in compliance with the protocol, ICH (International Conference on Harmonization) Guidelines for Good Clinical Practice, Sections 4.1, 4.2, 4.4 (when applicable), 4.5, 4.6, 4.7, 4.8, 4.9, 4.11 and 4.12, and any applicable Federal, state or local regulatory requirements or standards.

Appendix A

Medical and Dermatological History Questionnaire

Subject Initials: ___/___/___ (F M L) Sex: [ ]M [ ]F Age: _____ Subject #: _____ Study #: _____

If you are uncertain about any of the following questions, please ask for help.

Have you signed the informed consent document? No _____ Yes _____

I. Medical Conditions Now

Do You Currently Have or Have a History of:	NO	YES	
Psoriasis			
Eczema			
Skin cancer			
Other skin problems (not including facial acne)			Describe:
Scars, moles, or other blemishes at or near skin test sites			
Redness from sunburn or other source at or near skin test sites			
Asthma requiring daily medication			
Any other chronic respiratory condition requiring daily therapy			Describe:
Diabetes requiring insulin therapy			
A disease affecting your immune system (e.g. lupus, thyroiditis, myositis, Crohn's Disease, autoimmune hepatitis, etc.)			Describe:

II. Medical Conditions in the Past

Have You Had:	NO	YES
Treatment for a malignancy (cancer) within the last 6 months		
A mastectomy		
Axillary lymph nodes removed		

III. Medications

Are You Currently Taking or Have You Used Any of the Following:	NO	YES	
An antihistamine or anti-inflammatory drug (for example; aspirin, ibuprofen, corticosteroid or other steroid medication)			Drug/dose:
An anti-inflammatory medication on your skin within the last 2 weeks			
An immunosuppressive drug (e.g. Cyclosporin A)			

Appendix A – Continued

Subject Initials: ___/___/___ (F M L) Subject #:_____ Study #: _____

IV. Allergy Injections

	NO	YES
Are you currently receiving allergy injections?		
Have you received a final injection within the last week?		
Do you expect to begin injections during the study?		

V. Allergies

Are you allergic to any of the following:	NO	YES	Specify
detergents/cleaning products			
cosmetics/perfumes			
personal cleansing products (soap, shampoo, etc.)			
skin creams/lotions			
anti-perspirants/deodorants			
adhesives, surgical tape, bandages			
medicines			
other materials			

VI. Miscellaneous Questions

	NO	YES	SPECIFY
Have you ever participated in a patch test?			Date of last participation:
Have you ever had a skin allergy confirmed as the result of participation in a patch test			
Are you currently participating in another study of any kind?			
Are you willing to refrain from exposing skin sites to the sun or going to tanning beds for the duration of the study			
Are you willing to refrain from using lotions, creams, powders, etc. on your skin in the test area for the duration of the study			

Appendix A – Continued

Subject Initials: ___/___/___ (F M L) Subject #:_____ Study #: _____

VI. Miscellaneous Questions - Continued

	NO	YES	SPECIFY
Do you have any other medical condition we should know about?			
Do you regularly use any medication on your skin?			
Are you currently seeing a doctor about:			
allergies			
skin problems			
another reason			
Do you regularly take any prescription or OTC medication?			

Signature of person providing information: _____ **Date:** _____

FOR INTERVIEWER'S USE ONLY (Please review this form with the subject for completeness and accuracy.)

Answers to questions indicate the subject is: [ ] Acceptable
[ ] Not Acceptable - Reason: _____(use Key for Subject Exclusion)

Comments: _____

Interviewer's Signature: _____ Date: _____

Appendix A – Continued

Key for Subject Exclusion

01	Psoriasis	015	Immunosuppressive Drug
02	Eczema	016	Allergy Injections
03	Skin Cancer	017	Other Study Participation
04	Other Active Dermatitis	018	Confirmed Skin Allergy
05	Skin Abnormality – arms or back	019	Relevant Allergy
06	Sunburn	020	Other Disqualifying Medication
07	Severe Asthmatic	021	Patch Test Within Minimum Time Limit
08	Other Chronic Respiratory Condition	022	Unwilling to Comply with Requirements
09	Insulin Dependent Diabetic	023	Other Disqualifying Condition
10	Immune System Disease	024	Declined Participation
11	Treatment for Cancer	025	Other – See Report
12	Mastectomy		
13	Axillary Nodes Removed		
14	Anti-inflammatory Drug		

Appendix B

[redacted] Uniform Laboratory Patch Test Grading Scale

0	No apparent cutaneous involvement.
0.5	Greater than 0, less than 1.
1	Faint but definite erythema, no eruptions or broken skin or no erythema but definite dryness; may have epidermal fissuring.
1.5	Greater than 1, less than 2.
2	Moderate erythema, may have a few papules or deep fissures, moderate-to-severe erythema in the cracks. **Cut-off Grade - Patches are not reapplied**
2.5	Greater than 2, less than 3.
3	Severe erythema (beet redness), may have generalized papules or moderate-to-severe erythema with slight edema (edges well defined by raising).
3.5	Greater than 3, less than 4.
4	Generalized vesicles or eschar formations or moderate-to-severe erythema and/or edema extending beyond the area of the patch.

NOTE: The degree of reaction expressed by such descriptive terms as "moderate" and "severe" is, in itself, subjective. Such terminology can be accurately understood only through experience.

NOTE: Any reaction of greater severity than Grade 4 should be described in detail. Unusual reactions not described by the scale should also be described.

TYPICAL EXAMPLES OF HALF-GRADE SCORES

0.5	Faint, barely perceptible erythema or slight dryness (glazed appearance).
1.5	Well-defined erythema or faint erythema with definite dryness, may have epidermal fissuring.
2.5	Moderate erythema with barely perceptible edema or severe erythema not involving a significant portion of the patch (halo effect around the edges), may have a few papules or moderate-to-severe erythema.
3.5	Moderate-to-severe erythema with moderate edema (confined to patch area) or moderate-to-severe erythema with isolated eschar formations or vesicles.

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	A) [redacted] B) C) D) E) F) G) [redacted] Facial essence with 1.5% phytosteryl/octyldodectyl lauroyl glutamate H) [redacted]	**Sponsor's Reference Number:**	[redacted]

A. Test Substance Information and Handling:

Investigator's Sample Code: **[X] Assigned by Investigator, to be documented in study records – Insert "N/A" in box to right** [ ] Assigned by Sponsor – specify sample code in box to right	**N/A**
Product Type: (e.g. moisturizer, shampoo, AP/DO, etc.)	[redacted] Facial essence
Color: (if no color, indicate "colorless")	[redacted] **White to slight yellow (G)** [redacted]
Physical Form: (e.g. liquid, gel, paper, cream, solid, etc.)	[redacted] **Viscous Liquid (G)** [redacted]
Hazards:	**Non-hazardous** [redacted]
Storage: Stock Test Substance	**Room Temperature** [redacted]
Test Concentration:	**100%-Neat (A-H)**
Vehicle:	**N/A**
Storage: Test Solution – if applicable (solution prepared by test facility using stock substance and vehicle)	**N/A**

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	[redacted] G) [redacted] Facial essence with 1.5% phytosteryl/octyldodectyl lauroyl glutamate	Sponsor's Reference Number:	[redacted]

How to Prepare Test Substance for Testing:

[X] **Apply as provided**
[ ] Prepare test solution (Use space below for detailed instructions to the lab)
[ ] Cut into pieces (Use space below for detailed instructions to the lab)
[ ] Other – describe:

Special Instructions for Preparation of Test Substance/Test Solution (e.g. describe how test solution should be prepared, test substance should be cut, etc.) **and/or Special Instructions for Application of Test Substance to Patch Pad (e.g. Webril®)** (e.g. describe procedure for applying AP/DOs, paper materials, etc. to patch pad):
(If instructions are not specified, the SOPs or written test facility guidelines should be used.)

NONE

Test Solution Preparation Frequency:

[X] **Not applicable**
[ ] Daily
[ ] Weekly
[ ] Other – describe:

Mixing/Stirring Requirements:

[X] **Not applicable**
[ ] Mix/stir stock test substance before each withdrawal for preparation of test solution.
[ ] Mix/stir stock test substance/test solution immediately before applying to patches.
[ ] Stir test solution continuously during patch preparation.
[ ] Other (describe):

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	[redacted] G) [redacted] Facial essence with 1.5% phytosteryl/octyldodectyl lauroyl glutamate [redacted]	**Sponsor's Reference Number:**	[redacted]

Open-to-Air Requirements:

[X] **Apply test substance/solution/vehicle to patch pad no longer than _15____ minutes before application**

[ ] Allow test substance/solution/vehicle to remain on patch pad, open to the air, for at least _____ minutes, but no longer than _____ minutes, before application

[ ] Other (describe):

Disposition of Test Substance/Test Solutions [check all that apply]:

[X] **Dispose of unused stock test substance after the final report is issued.**

[ ] All unused stock test substance should be returned to the Sponsor after the final report has been issued. *(Use the address provided below.)

[ ] Dispose of all test solutions following use.

[ ] All test solutions should be returned to the Sponsor at the completion of the study. (Use address provided below.

* **Return to**: ***(*Note to Lab: Please include study number, Sponsor's Ref. Number and [redacted] with shipment.)***

B. Patch Type or Type of Application to be Used:

[ ] Completely occlusive patch
[ ] Completely occlusive patch with plastic chamber
[X] **Semi-occlusive patch**
[ ] Semi-open patch
[ ] Open application
[ ] Other (describe below):

C. Amount to be Applied per Patch:

[ ] To be determined by contract laboratory * (must be documented in study records and final report)
[X] **_0.2__ ml/gm per patch**
[ ] _____ layer(s) per patch
[ ] _____ square inches **OR** _____ square centimeters per **layer**
[ ] Other (describe below):

*Test substance/solution to be applied to each patch in an amount sufficient to cover/saturate the pad without

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	[redacted]	Sponsor's Reference Number:	[redacted]
	G) [redacted] Facial essence with 1.5% phytosteryl/octyldodectyl lauroyl glutamate [redacted]		

extruding or extending from it when applied to the skin.

D. Patch Location:

[X] **No preference – arm or back (to be determined by contract laboratory and documented in the study records and final report)**

[ ] Other (describe below):

E. Number of Subjects to Complete: 35

F. *Special* Inclusion/Exclusion Criteria (e.g. sensitive skin subjects, ethnic population, etc.)

[X] **None**

[ ] Special criteria required as specified below:

G. Dermatologist Required:

[ ] No

[X] **Yes, at final grade day only**

If dermatologist is required on additional days, please specify when: N/A

H. IRB Required:

(typically an IRB is not required; however, if in doubt, check with Sponsor Regulatory personnel)

[X] **No**

[ ] Yes

I. Data Updates/Topline Data Required:

[ ] Not required

[X] **Required as specified below:**

Topline results should be sent to [redacted] as soon as available following study completion.

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	[redacted]	**Sponsor's Reference Number:**	[redacted]
	G) [redacted] Facial essence with 1.5% phytosteryl/octyldodectyl lauroyl glutamate [redacted]		

J. Report Information:

Please send the following to [redacted]:

- Audited DRAFT report in Word Format, along with the final, signed Protocol and any Amendments
- Audited FINAL report in Adobe Portable Document Format (.pdf)
- [redacted]

K. Data Retention:

Upon Issuing the Final Report:

- The original Final Report and Protocol and any amendments, all raw data, and records for this study should be shipped to the following address:

  [redacted]

- Prior to shipping, please notify [redacted] anticipates receipt of the study records.

L. Safety/Sponsor's Risk Assessment:

Check the appropriate box(es):

[ ] This formula(s)/substance(s) is regulated by _____.
{specify agency(ies)}

[X] Relevant safety testing has been conducted on this formula(s)/substance(s).
[X] Relevant safety testing has been conducted on a similar formula(s)/substance(s).
[X] This formula(s) is similar to currently marketed or previously approved formula(s).
[X] Consumer exposure to product has been assessed and does not present any safety concerns under the conditions of this test.

Additional safety information:

All ingrediants to be tested are considered acceptable at the levels in the formulation. Similar formulas have been tested previously in either consumer and/or safety studies up to 4 weeks under typical use condictions. Overall there is a minimal human safety risk associated with participation in this 7-day study.

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	[redacted] G) [redacted] Facial essence with 1.5% phytosteryl/octyldodectyl lauroyl glutamate	Sponsor's Reference Number:	[redacted]

Sponsor's Toxicologists: [redacted]	[redacted]
[redacted]	
Alternate Contact	

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	N/A Distilled Water	Sponsor's Reference Number:	[redacted]

A. Test Substance Information and Handling:

Investigator's Sample Code: [X] **Assigned by Investigator, to be documented in study records – insert "N/A" in box to right** [ ] Assigned by Sponsor – specify sample code in box to right	**N/A**
Product Type: (e.g. moisturizer, shampoo, AP/DO, etc.)	**Distilled Water – Negative Irritant Control**
Color: (if no color, indicate "colorless")	**Colorless**
Physical Form: (e.g. liquid, gel, paper, cream, solid, etc.)	**Liquid**
Hazards:	**None**
Storage: Stock Test Substance	**Room temperature**
Test Concentration:	**100%, Neat**
Vehicle:	**N/A**
Storage: Test Solution – if applicable (solution prepared by test facility using stock substance and vehicle)	**N/A**

How to Prepare Test Substance for Testing:

[X] **Not applicable**
[ ] Apply as provided
[ ] Prepare test solution (Use space below for detailed instructions to the lab)
[ ] Cut into pieces (Use space below for detailed instructions to the lab)
[ ] Other – describe:

Special Instructions for Preparation of Test Substance/Test Solution (e.g. describe how test solution should be prepared, test substance should be cut, etc.) **and/or Special Instructions for Application of Test Substance to Patch Pad (e.g. Webril®)** (e.g. describe procedure for applying AP/DOs, paper materials, etc. to patch pad):
(If instructions are not specified, the SOPs or written test facility guidelines should be used.)

None

Test Solution Preparation Frequency:

[X] **Not applicable**
[ ] Daily
[ ] Weekly
[ ] Other – describe:

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	N/A Distilled Water	Sponsor's Reference Number:	[redacted]

Mixing/Stirring Requirements:
[X] **Not applicable**
[ ] Mix/stir stock test substance before each withdrawal for preparation of test solution.
[ ] Mix/stir stock test substance/test solution immediately before applying to patches.
[ ] Stir test solution continuously during patch preparation.
[ ] Other (describe):

Open-to-Air Requirements:
[X] **Apply test substance/solution/vehicle to patch pad no longer than __15__ minutes before application**
[ ] Allow test substance/solution/vehicle to remain on patch pad, open to the air, for at least _____ minutes, but no longer than _____ minutes, before application
[ ] Other (describe):

Disposition of Test Substance/Test Solutions [check all that apply]:
[X] **Not applicable**

[ ] Dispose of unused stock test substance after the final report is issued.
[ ] All unused stock test substance should be returned to the Sponsor after the final report has been issued. *(Use the address provided below.)

[ ] Dispose of all test solutions following use.
[ ] All test solutions should be returned to the Sponsor at the completion of the study. (Use address provided below.

* **Return to**: *(*Note to Lab: Please include study number, Sponsor's Ref. Number and [redacted] with shipment.)*

B. Patch Type or Type of Application to be Used:
[ ] Completely occlusive patch
[ ] Completely occlusive patch with plastic chamber
[X] Semi-occlusive patch
[ ] Semi-open patch
[ ] Open application
[ ] Other (describe below):

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	N/A Distilled Water	Sponsor's Reference Number:	[redacted]

C. Amount to be Applied per Patch:

[ ] To be determined by contract laboratory * (must be documented in study records and final report)
[X] 0.3 ml per patch
[ ] _____ layers per patch
[ ] _____ square inches **OR** _____ square centimeters per **layer**
[ ] Other (describe below):

*Test substance/solution to be applied to each patch in an amount sufficient to cover/saturate the pad without extruding or extending from it when applied to the skin.

D. Patch Location:

[X] No preference – arm or back (to be determined by contract laboratory and documented in the study records and final report)
[ ] Other (describe below):

E. Number of Subjects to Complete: 35

F. *Special* Inclusion/Exclusion Criteria (e.g. sensitive skin subjects, ethnic population, etc.)

[X] None
[ ] Special criteria required as specified below:

G. Dermatologist Required:

[ ] No
[X] Yes, at final grade day only
If dermatologist is required on additional days, please specify when: N/A

H. IRB Required:

(typically an IRB is not required; however, if in doubt, check with Sponsor Regulatory personnel)
[X] No
[ ] Yes

I. Data Updates/Topline Data Required:

[ ] Not required
[X] Required as specified below:

Topline results should be sent to [redacted] as soon as available following study completion.

J. Report Information:

Please send the following to [redacted]:

- Audited DRAFT report in Word Format, along with the final, signed Protocol and any Amendments
- Audited FINAL report in Adobe Portable Document Format (.pdf)
- [redacted]

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	N/A Distilled Water	Sponsor's Reference Number:	[redacted]

K. Data Retention:

Upon issuing the Final Report:

- The original Final Report and Protocol and any amendments, all raw data, and records for this study should be shipped to the following address:

  [redacted]

- Prior to shipping, please notify [redacted] anticipates receipt of the study records.

L. Safety/Sponsor's Risk Assessment:

Check the appropriate box(es):

[ ] This formula(s)/substance(s) is regulated by _____.
[specify agency(ies)]

[X] Relevant safety testing has been conducted on this formula(s)/substance(s).

[ ] Relevant safety testing has been conducted on a similar formula(s)/substance(s).

[ ] This formula(s) is similar to currently marketed or previously approved formula(s).

[ ] Consumer exposure to product has been assessed and does not present any safety concerns under the conditions of this test.

Additional safety information:

No safety issues are anticipated with the use of distilled water in this study. Distilled water is often used as the negative irritant control in skin irritancy testing. No irritation to mild irritation is anticipated from the proposed testing.

Sponsor's Toxicologist:	[redacted]
Alternate Contact	

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	N/A Sodium Lauryl Sulfate	Sponsor's Reference Number:	[redacted]

A. Test Substance Information and Handling:

Investigator's Sample Code: [X] **Assigned by Investigator, to be documented in study records – insert "N/A" in box to right** [ ] Assigned by Sponsor – specify sample code in box to right	**N/A**
Product Type: (e.g. moisturizer, shampoo, AP/DO, etc.)	**Sodium Lauryl Sulfate (SLS) – Positive Irritant Control**
Color: (if no color, indicate "colorless")	**White**
Physical Form: (e.g. liquid, gel, paper, cream, solid, etc.)	**Powder**
Hazards:	**Potential skin, eye and respiratory irritant**
Storage: Stock Test Substance	**Room temperature**
Test Concentration:	wrong concentration ~~**0.5%** w/v~~ 0.75 % w/v [redacted] 9/19/12
Vehicle:	**Distilled water**
Storage: Test Solution – if applicable (solution prepared by test facility using stock substance and vehicle)	**N/A**

How to Prepare Test Substance for Testing:

[ ] Apply as provided
[X] **Prepare test solution (Use space below for detailed instructions to the lab)**
[ ] Cut into pieces (Use space below for detailed instructions to the lab)
[ ] Other – describe:

Special Instructions for Preparation of Test Substance/Test Solution (e.g. describe how test solution should be prepared, test substance should be cut, etc.) **and/or Special Instructions for Application of Test Substance to Patch Pad (e.g. Webril®)** (e.g. describe procedure for applying AP/DOs, paper materials, etc. to patch pad):
(If instructions are not specified, the SOPs or written test facility guidelines should be used.)

None

Test Solution Preparation Frequency:

[ ] Not applicable
[X] **Daily**
[ ] Weekly
[ ] Other – describe:

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	N/A Sodium Lauryl Sulfate	Sponsor's Reference Number:	[redacted]

Mixing/Stirring Requirements:
[ ] Not applicable
[ ] Mix/stir stock test substance before each withdrawal for preparation of test solution.
[X] Mix/stir stock test substance/test solution immediately before applying to patches.
[ ] Stir test solution continuously during patch preparation.
[ ] Other (describe):

Open-to-Air Requirements:
[X] Apply test substance/solution/vehicle to patch pad no longer than ___15___ minutes before application
[ ] Allow test substance/solution/vehicle to remain on patch pad, open to the air, for at least _____ minutes, but no longer than _____ minutes, before application
[ ] Other (describe):

Disposition of Test Substance/Test Solutions [check all that apply]:
[ ] Dispose of unused stock test substance after the final report is issued.
[ ] All unused stock test substance should be returned to the Sponsor after the final report has been issued. *(Use the address provided below.)
[X] Not applicable

[X] Dispose of all test solutions following use.
[ ] All test solutions should be returned to the Sponsor at the completion of the study. (Use address provided below.

* **Return to:** ***(*Note to Lab: Please include study number, Sponsor's Ref. Number and [redacted] with shipment.)***

B. Patch Type or Type of Application to be Used:
[ ] Completely occlusive patch
[ ] Completely occlusive patch with plastic chamber
[X] Semi-occlusive patch
[ ] Semi-open patch
[ ] Open application
[ ] Other (describe below):

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	N/A Sodium Lauryl Sulfate	Sponsor's Reference Number:	[redacted]

C. Amount to be Applied per Patch:

[ ] To be determined by contract laboratory * (must be documented in study records and final report)
[X] 0.3 ml per patch
[ ] _____ layers per patch
[ ] _____ square inches **OR** _____ square centimeters per **layer**
[ ] Other (describe below):

*Test substance/solution to be applied to each patch in an amount sufficient to cover/saturate the pad without extruding or extending from it when applied to the skin.

D. Patch Location:

[X] No preference – arm or back (to be determined by contract laboratory and documented in the study records and final report)
[ ] Other (describe below):

E. Number of Subjects to Complete: 35

F. *Special* Inclusion/Exclusion Criteria (e.g. sensitive skin subjects, ethnic population, etc.)

[X] None
[ ] Special criteria required as specified below:

G. Dermatologist Required:

[ ] No
[X] Yes, at final grade day only
If dermatologist is required on additional days, please specify when: N/A

H. IRB Required:

(typically an IRB is not required; however, if in doubt, check with Sponsor Regulatory personnel)
[X] No
[ ] Yes

I. Data Updates/Topline Data Required:

[ ] Not required
[X] Required as specified below:

Topline results should be sent to [redacted] as soon as available following study completion.

J. Report Information:

Please send the following to [redacted]:

- Audited DRAFT report in Word Format, along with the final, signed Protocol and any Amendments
- Audited FINAL report in Adobe Portable Document Format (.pdf)
- [redacted]

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	N/A Sodium Lauryl Sulfate	Sponsor's Reference Number:	[redacted]

K. Data Retention:

Upon issuing the Final Report:

- The original Final Report and Protocol and any amendments, all raw data, and records for this study should be shipped to the following address:

[redacted]

- Prior to shipping, please notify [redacted] anticipates receipt of the study records.

L. Safety/Sponsor's Risk Assessment:

Check the appropriate box(es):

[ ] This formula(s)/substance(s) is regulated by _____.
[specify agency(ies)]

[X] Relevant safety testing has been conducted on this formula(s)/substance(s).

[ ] Relevant safety testing has been conducted on a similar formula(s)/substance(s).

[ ] This formula(s) is similar to currently marketed or previously approved formula(s).

[ ] Consumer exposure to product has been assessed and does not present any safety concerns under the conditions of this test.

Additional safety information:

Sodium lauryl sulfate is a commonly used cosmetic surfactant. It is employed in numerous marketed personal cleansing products at concentrations much higher than the level proposed in this study. It maintains a favorable safety database for its use in the proposed context, and is commonly used at comparable levels as an irritation control in studies of this type. Although a minor degree of erythema may be expected, the testing risk under the proposed conditions of the study protocol is judged to be acceptable.

Sponsor's Toxicologist:	[redacted]
Alternate Contact	

Memorandum

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

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

DATE: August 17, 2022

SUBJECT: Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate

TKL Research. 2005. Human repeated insult patch test face cream containing 5% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate.

Institute for In Vitro Sciences, Inc. 2014. Tissue equivalent assay with Epiocular™ cultures (1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate in a face cream).

North Cliff Consultants, Inc. 2014. Human cumulative irritation patch test ((1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate in a face cream).

Distributed for Comment Only -- Do Not Cite or Quote

HUMAN REPEATED INSULT PATCH STUDY

TKL STUDY NO. [redacted]

[redacted]

CONDUCTED FOR:

[redacted]

DATE OF REPORT:

April 7, 2005

TKL Research, Inc., 4 Forest Avenue, Paramus, NJ 07652 Tel (201) 587-0500 Fax (201) 587-0878 Email: info@tklresearch.com

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SIGNATURES

Kathleen Georgeian — 4/6/05

Kathleen Georgeian, Clinical Research Coordinator and Manager, Dermatologic Safety Testing — Date

4/5/05

Jonathan S. Dosik, MD
Principal Investigator
Dermatologist — Date

STATEMENT OF QUALITY ASSURANCE

This report has been reviewed by the TKL Research, Inc. (TKL) Corporate Quality Assurance Department and the report accurately reflects the raw data for this study.

Clinical research studies are performed by TKL in accordance with all applicable federal regulations and proposed guidelines for Good Clinical Practices, which include:

21 CFR Part 312,	Investigational New Drug Application
21 CFR Part 50,	Protection of Human Subjects
21 CFR Part 56,	Institutional Review Boards

Henry Boisvoe — 4/7/05

Quality Assurance — Date

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TITLE OF STUDY

Human Repeated Insult Patch Study

SPONSOR

[redacted]

STUDY MATERIAL

[redacted] 5% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream

DATE STUDY INITIATED

January 3, 2005

DATE STUDY COMPLETED

February 10, 2005

DATE OF REPORT

April 7, 2005

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INVESTIGATIVE PERSONNEL

Jonathan S. Dosik, MD
Principal Investigator
Dermatologist

Kathleen Georgeian
Clinical Research Coordinator and Manager, Dermatologic Safety Testing

Tina Kelly
Assistant Manager, Dermatologic Safety Testing

CLINICAL SITE

TKL RESEARCH, INC.
71 Franklin Turnpike
Waldwick, NJ 07463

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SUMMARY

5% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream

One study material, [redacted], was evaluated neat to determine its ability to sensitize the skin of normal volunteer subjects using an occlusive repeated insult patch study. One hundred two subjects completed the study.

Under the conditions employed in this study, there was no evidence of sensitization to study material [redacted]. 5% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream

1.0 OBJECTIVE

The objective of this study was to determine the ability of the study material to cause sensitization by repeated applications to the skin of humans under controlled patch study conditions.

2.0 RATIONALE

Substances that come into contact with human skin need to be evaluated for their propensity to irritate and/or sensitize. Once an appropriate pre-clinical safety evaluation has been performed, a reproducible, standardized, quantitative patch evaluation procedure must be used to demonstrate that a particular material can be applied safely to human skin without significant risk of adverse reactions. The method herein employed is generally accepted for such a purpose.

Repeated insult patch evaluation is a modified predictive patch study that can detect weak sensitizers that require multiple applications to induce a cell-mediated (Type IV) immune response sufficient to cause an allergic reaction. Irritant reactions may also be detected using this evaluation method, although this is not the primary purpose of this procedure. Results are interpreted according to interpretive criteria based upon published works, as well as the clinical experience of TKL Research, Inc. These interpretive criteria are periodically reviewed and amended as new information becomes available.

3.0 STUDY DESIGN

3.1 STUDY POPULATION

A sufficient number of subjects were to be enrolled to provide 100 completed subjects.

3.1.1 Inclusion Criteria

Individuals eligible for inclusion in the study were those who:

1. were males or females, 18 years of age or older, in general good health;
2. were free of any systemic or dermatologic disorder which, in the opinion of the investigative personnel, would have interfered with the study results or increased the risk of adverse events;
3. were of any skin type or race, providing the skin pigmentation would allow discernment of erythema;
4. had completed a medical screening procedure; and
5. had read, understood, and signed an informed consent agreement.

3.1.2 Exclusion Criteria

Individuals excluded from participation in the study were those who:

1. had any visible skin disease at the study site which, in the opinion of the investigative personnel, would have interfered with the evaluation;

2. were receiving systemic or topical drugs or medication which, in the opinion of the investigative personnel, would have interfered with the study results;

3. had psoriasis and/or active atopic dermatitis/eczema;

4. were females who were pregnant, planning to become pregnant during the study, or breast-feeding; and/or

5. had a known sensitivity to cosmetics, skin care products, or topical drugs as related to the material being evaluated.

3.1.3 Informed Consent

A properly executed informed consent document in compliance with FDA regulations (21 CFR Part 50) was obtained from each subject prior to entering the study. The signed informed consent document is maintained in the study file. In addition, the subject was provided with a copy of the informed consent document (see Appendix IV).

3.2 DESCRIPTION OF STUDY

3.2.1 Outline of Study Procedures

Subjects participated in the study over a 6-week period involving 3 phases: (1) Induction, (2) Rest, and (3) Challenge. Prior to study entry, the subjects were screened to assure that they met the inclusion/exclusion criteria. Informed consent was obtained. Each subject was provided with a schedule of the study activities. All subjects were told to avoid wetting the patches and were asked not to engage in activities that caused excessive perspiration. They were instructed to notify the staff if they experienced any discomfort beyond mild itching or observed any adverse changes at the patch sites, while on the study or within 2 weeks of completing the study.

The Induction Phase consisted of 9 consecutive applications of the study material and subsequent evaluations of the patch sites. Prior to application of the patches, the sites were outlined with a skin marker, eg, gentian violet. The subjects were required to remove the patches approximately 24 hours after application. They returned to the facility at 48-hour intervals to have the sites evaluated and identical patches applied to the same sites. Patches applied on Friday were removed by subjects after 24 hours. The sites were evaluated on the following Monday, ie, 72 hours after patch application*.

* A Monday or Friday holiday could result in evaluation at 96 hours after patch application.

Following the ninth evaluation, the subjects were dismissed for a rest period of approximately 10-15 days.

Subjects who were absent once during the induction phase received a make-up (MU) patch at the last induction visit. The MU applications were graded 48 hours later at the MU visit, or were recorded as N9G (no ninth grading).

The Challenge Phase was initiated during the sixth week of the study. Identical patches were applied to sites previously unexposed to the study material. The patches were removed by subjects after 24 hours and the sites graded after additional 24-hour and 48-hour periods (ie, 48 and 72 hours after application). Rechallenge was performed whenever there was evidence of possible sensitization.

To be considered a completed case, a subject must have had 9 applications and no fewer than 8 subsequent readings during induction, and a single application and 2 readings during challenge. Only completed cases were used to assess sensitization.

3.2.2 Definitions Used for Grading Responses

The symbols found in the scoring scales below were used to express the response observed at the time of examination:

- = No reaction
? = Minimal or doubtful response, slightly different from surrounding normal skin
+ = Definite erythema, no edema
++ = Definite erythema, definite edema
+++ = Definite erythema, definite edema and vesiculation

SPECIAL NOTATIONS

E = Marked/severe erythema
S = Spreading of reaction beyond patch site (ie, reaction where material did not contact skin)
p = Papular response > 50%
pv = Papulovesicular response > 50%
D = Damage to epidermis: oozing, crusting and/or superficial erosions
I = Itching
X = Subject absent
PD = Patch dislodged
NA = Not applied
NP = Not patched (due to reaction achieved)
N9G = No ninth grading

3.2.3 Evaluation of Responses

All responses were graded by a trained dermatologic evaluator meeting TKL's strict certification requirements to standardize the assignment of response grades.

4.0 NATURE OF STUDY MATERIAL

4.1 STUDY MATERIAL SPECIFICATIONS

		5% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream
Identification	:	[redacted] Facial Moisturizer
Amount Applied	:	0.2 mL
Special Instructions	:	Applied to patch pad no longer than 15 minutes prior to patch application.

4.2 STORAGE, HANDLING, AND DOCUMENTATION OF STUDY MATERIAL

Receipt of the material used in this study was documented in a general logbook, which serves as a permanent record of the receipt, storage, and disposition of all study material received by TKL. On the basis of information provided by the sponsor, the study material was considered reasonably safe for evaluation on human subjects. A sample of the study material was reserved and will be stored for a period of 6 months. At the conclusion of the clinical study, the remaining study material was discarded or returned to the sponsor and the disposition documented in the logbook. All information regarding the receipt, storage, and disposition of the study material was also recorded on a Clinical Material Record form (see Appendix III), which is incorporated in this study report. All study material is kept in a locked product storage room accessible to clinical staff members only.

4.3 APPLICATION OF STUDY MATERIAL

Study material was applied to the patch as instructed. The patch was applied to the infrascapular area of the back, either to the right or left of the midline, or to the upper arm.

4.4 DESCRIPTION OF PATCH CONDITIONS

Material evaluated under occlusive patch conditions was applied to a 2-cm x 2-cm Webril pad attached to a non-porous, plastic film adhesive bandage (3M medical tape). The patch was secured with hypoallergenic tape (Micropore), as needed.

Material evaluated under semi-occlusive patch conditions was applied to a 2-cm x 2-cm Webril pad. The pad was affixed to the skin with hypoallergenic tape (Micropore).

5.0 INTERPRETATION

Sensitization is characterized by an acute allergic contact dermatitis. Typical sensitization reactions begin with an immunologic response in the dermis resulting in erythema, edema formation, and secondary epidermal damage (vesiculation), sometimes extending beyond the patch site and often accompanied by itching. Sensitization reactions tend to be delayed. The reaction typically becomes evident between 24 and 48 hours, peaks at 48-72 hours and subsequently subsides. The reaction is often greater at 72 hours than at 48 hours. The severity of the reaction is generally greater during the challenge phase of a Repeated Insult Patch Test (RIPT) than that seen during induction.

Irritant reactions are characterized as a non-immunologic, localized, superficial, exudative, inflammatory response of the skin due to an externally applied material. The typical initial reaction does not develop much edema or vesiculation but results in scaling, drying, cracking, oozing, crusting, and erosions. The reaction is usually sharply delineated, not spreading beyond the patch site. Irritant reactions are typically evident by 24 hours and diminish over the next 48-72 hours. Removal of the offending agent results in gradual improvement of the epidermal damage. The reaction seen at 72 hours is, therefore, less severe than that seen at 48 hours. Finally, the severity of the reaction experienced in the challenge phase is generally similar to that seen during induction.

If the results of the study indicate the likelihood of sensitization, the recommended practice is to rechallenge the subjects who have demonstrated sensitization-like reactions to confirm that these reactions are, indeed, associated with the product. Our preferred rechallenge procedure involves the application of the product to naïve sites, under both occlusive and semi-occlusive patch conditions. Use of the semi-occlusive patch condition helps to differentiate irritant and sensitization reactions. Generally speaking, if a product is a sensitizer it will produce a similar reaction under both occlusion and semi-occlusion. Whereas, if the product has caused an irritant reaction, the reactions will be less pronounced under the semi-occlusive condition.

6.0 PROTOCOL

See Protocol - Appendix V.

7.0 DOCUMENTATION AND RETENTION OF DATA

The case report forms (CRFs) are designed to identify each subject by subject number and initials, and to record demographics, examination results, adverse events, and end of study status. Originals or copies of all CRFs, correspondence, study reports, and all source data will be kept on hard-copy file for a minimum of 5 years from completion of the study. Storage is maintained either at a TKL facility in a secured room accessible only to TKL employees, or at an offsite location that provides a secure environment with burglar/fire alarm systems, camera detection and controlled temperature and humidity. Documentation will be available for the sponsor's review on the premises of TKL.

8.0 RESULTS AND DISCUSSION

One hundred ten subjects between the ages of 20 and 71 were enrolled and 102 completed the study (see Tables 1 and 2 in Appendix I and Data Listings 1 and 2 in Appendix II).

The following table summarizes subject enrollment and disposition.

Number enrolled:		110
Number discontinued:		8
Lost to follow-up:	6	
Voluntary withdrawal:	2	
Number completed:		102

Source: Table 1, Appendix I

There were no adverse events.

A summary of response data is provided in Table 3, Appendix I. Individual dermatological response grades are provided in Data Listing 3, Appendix II.

9.0 CONCLUSION

Under the conditions employed in this study, there was no evidence of sensitization to study material [redacted]. 5% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream

10.0 REFERENCES

Kligman AM. The identification of contact allergens by human assay II. A critique of standard methods. J Invest Dermatol 1966; 47:369.

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Fisher AA. 3rd ed. Contact Dermatitis. Philadelphia:Lea & Feiberger, 1986.

Shelanski HA, Shelanski MV. A new technique of human patch tests. Proc Sci Sect Toilet Goods Assoc 1953; 204:107-110.

Jordan WP, King SF. Related hypersensitivity in families. Contact Dermatitis 1977; 3:19-26.

Kligman AM, Epstein W. Updating the maximization test for identifying contact allergens. Contact Dermatitis 1975; 1:231-239.

Stotts, J. Planning, conduct and interpretation of human predictive sensitization patch tests. In: Drill VA, Lazar P, eds. Current Concepts In Cutaneous Toxicity. New York:Academic Press, 1980:41-53.

[redacted]

APPENDIX I

SUMMARY TABLES

TKL STUDY NO.[redacted]

TABLE 1: SUMMARY OF SUBJECT ENROLLMENT AND DISPOSITION

	N	(%)
SUBJECTS ENROLLED	110	
SUBJECTS COMPLETED INDUCTION PHASE	103	(93.6)
SUBJECTS COMPLETED ALL PHASES	102	(92.7)
TOTAL SUBJECTS DISCONTINUED	8	(7.3)
LOST TO FOLLOW-UP	6	(5.5)
VOLUNTARY WITHDRAWAL	2	(1.8)

NOTE: ALL PERCENTAGES ARE RELATIVE TO TOTAL SUBJECTS ENROLLED

SEE DATA LISTING 1 FOR FURTHER DETAIL

PROGRAM: DISPSMY.SAS/USES: FINAL/17FEB05:10:39:53

TKL STUDY NO. [redacted]
TABLE 2: SUMMARY OF SUBJECT DEMOGRAPHICS
ALL ENROLLED SUBJECTS

AGE	
N (%) 18 TO 44	46 (41.8)
N (%) 45 TO 64	57 (51.8)
N (%) 65 AND UP	7 (6.4)
MEAN (SD)	47.6 (11.0)
MEDIAN	47.0
RANGE	20.0 TO 71.1
GENDER	
N (%) MALE	11 (10.0)
N (%) FEMALE	99 (90.0)
RACE	
N (%) ASIAN	1 (0.9)
N (%) CAUCASIAN	103 (93.6)
N (%) HISPANIC	5 (4.5)
N (%) OTHER	1 (0.9)

SEE DATA LISTING 2 FOR FURTHER DETAIL

PROGRAM: DEMOSMY.SAS/USES: DEMOGS/17FEB05:10:39:55

[redacted]

TABLE 3: SUMMARY OF DERMATOLOGIC RESPONSE GRADES
NUMBER OF SUBJECTS BY PRODUCT

PRODUCT= [redacted] 5% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream

	INDUCTION READING									MAKE-	CHALLENGE PHASE		
RESPONSE	1	2	3	4	5	6	7	8	9	UP	48HR	72HR	96HR(*)
-	106	104	102	103	100	101	103	98	101	15	101	102	
?	0	0	1	1	0	0	0	0	0	0	1	0	
+	1	1	0	0	1	1	1	1	1	0	0	0	
TOTAL EVALUABLE	107	105	103	104	101	102	104	99	102	15	102	102	
NUMBER ABSENT	1	1	3	2	4	3	0	4	1		0	0	
NUMBER DISCONTINUED	2	4	4	4	5	5	6	7	7		8	8	

MAXIMUM ELICITED RESPONSE DURING INDUCTION
ALL SUBJECTS COMPLETING INDUCTION (N=103)

RESPONSE	N(%) SUBJECTS
-	102 (99.0%)
+	1 (1.0%)

(*) WHEN REQUIRED

KEY TO SYMBOLS:
- = NO REACTION
? = MINIMAL OR DOUBTFUL RESPONSE, SLIGHTLY DIFFERENT FROM SURROUNDING NORMAL SKIN
+ = DEFINITE ERYTHEMA, NO EDEMA
++ = DEFINITE ERYTHEMA, DEFINITE EDEMA
+++ = DEFINITE ERYTHEMA, DEFINITE EDEMA AND VESICULATION
D = DAMAGE TO EPIDERMIS: OOZING, CRUSTING AND/OR SUPERFICIAL EROSIONS
P = PAPULAR RESPONSE >50%

PROGRAM: SUMMARY.SAS/USES: RESPONSE, PRODLIST, FINAL/17FEB05:10:40:04

APPENDIX II

DATA LISTINGS

TKL STUDY NO.[redacted]
DATA LISTING 1: SUBJECT ENROLLMENT AND DISPOSITION

SUBJECT NO.	STUDY DATES SCREENED	1ST APPLIC	CHALL APPLIC	ENDED	LAST READING #	COMPLETION STATUS	DAYS ON STUDY
1	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
2	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
3	01/03/05	01/03/05		01/17/05	I4	S	15
4	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
5	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
6	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
7	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
8	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
9	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
10	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
11	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
12	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
13	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
14	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
15	01/03/05	01/03/05		01/07/05	I0	L	5
16	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
17	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
18	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
19	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
20	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
21	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
22	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
23	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
24	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
25	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
26	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
27	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
28	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
29	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
30	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
31	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
32	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
33	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
34	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
35	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
36	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
37	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
38	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
39	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39

KEY: LAST READING # (I=INDUCTION PHASE, C=CHALLENGE PHASE)
COMPLETION STATUS (C=COMPLETED, L=LOST TO FOLLOW-UP, S=VOLUNTARY WITHDRAWAL
V=PROTOCOL VIOLATION, AE=ADVERSE EVENT, O=OTHER)

PROGRAM: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/23FEB05:16:09:04

TKL STUDY NO.[redacted]
DATA LISTING 1: SUBJECT ENROLLMENT AND DISPOSITION

SUBJECT NO.	STUDY DATES SCREENED	1ST APPLIC	CHALL APPLIC	ENDED	LAST READING #	COMPLETION STATUS	DAYS ON STUDY
40	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
41	01/03/05	01/03/05	02/07/05	02/09/05	I9	L	38
42	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
43	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
44	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
45	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
46	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
47	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
48	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
49	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
50	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
51	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
52	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
53	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
54	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
55	01/03/05	01/03/05		01/10/05	I1	L	8
56	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
57	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
58	01/03/05	01/03/05		01/10/05	I1	S	8
59	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
60	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
61	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
62	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
63	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
64	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
65	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
66	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
67	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
68	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
69	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
70	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
71	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
72	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
73	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
74	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
75	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
76	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
77	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
78	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39

KEY: LAST READING # (I=INDUCTION PHASE, C=CHALLENGE PHASE)
COMPLETION STATUS (C=COMPLETED, L=LOST TO FOLLOW-UP, S=VOLUNTARY WITHDRAWAL
V=PROTOCOL VIOLATION, AE=ADVERSE EVENT, O=OTHER)

PROGRAM: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/23FEB05:16:09:04

TKL STUDY NO.[redacted]
DATA LISTING 1: SUBJECT ENROLLMENT AND DISPOSITION

SUBJECT NO.	STUDY DATES SCREENED	1ST APPLIC	CHALL APPLIC	ENDED	LAST READING #	COMPLETION STATUS	DAYS ON STUDY
79	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
80	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
81	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
82	01/03/05	01/03/05		01/21/05	I7	L	19
83	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
84	01/03/05	01/03/05		01/05/05	I0	L	3
85	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
86	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
87	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
88	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
89	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
90	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
91	01/03/05	01/03/05	02/07/05	02/10/05	C2	C	39
92	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
93	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
94	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
95	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
96	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
97	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
98	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
99	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
100	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
101	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
102	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
103	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
104	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
105	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
106	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
107	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
108	01/05/05	01/05/05		01/24/05	I6	L	20
109	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37
110	01/05/05	01/05/05	02/07/05	02/10/05	C2	C	37

KEY: LAST READING # (I=INDUCTION PHASE, C=CHALLENGE PHASE)
COMPLETION STATUS (C=COMPLETED, L=LOST TO FOLLOW-UP, S=VOLUNTARY WITHDRAWAL
V=PROTOCOL VIOLATION, AE=ADVERSE EVENT, O=OTHER)

PROGRAM: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/23FEB05:16:09:04

TKL STUDY NO.[redacted]
DATA LISTING 2: SUBJECT DEMOGRAPHICS

SUBJECT NO.	AGE	GENDER	RACE
1	69.7	FEMALE	CAUCASIAN
2	60.4	FEMALE	CAUCASIAN
3	47.4	FEMALE	CAUCASIAN
4	42.2	FEMALE	CAUCASIAN
5	65.7	FEMALE	CAUCASIAN
6	50.2	FEMALE	CAUCASIAN
7	41.3	FEMALE	CAUCASIAN
8	40.8	FEMALE	HISPANIC
9	56.2	FEMALE	CAUCASIAN
10	64.3	FEMALE	CAUCASIAN
11	46.3	FEMALE	CAUCASIAN
12	40.3	FEMALE	CAUCASIAN
13	36.8	FEMALE	CAUCASIAN
14	50.8	FEMALE	CAUCASIAN
15	56.6	FEMALE	CAUCASIAN
16	52.3	FEMALE	CAUCASIAN
17	37.8	MALE	CAUCASIAN
18	44.3	FEMALE	CAUCASIAN
19	38.7	FEMALE	HISPANIC
20	50.9	FEMALE	CAUCASIAN
21	70.4	MALE	CAUCASIAN
22	55.3	FEMALE	CAUCASIAN
23	58.5	FEMALE	CAUCASIAN
24	66.3	FEMALE	CAUCASIAN
25	50.7	MALE	CAUCASIAN
26	39.9	FEMALE	CAUCASIAN
27	20.7	FEMALE	CAUCASIAN
28	38.8	FEMALE	CAUCASIAN
29	45.5	FEMALE	CAUCASIAN
30	49.6	FEMALE	CAUCASIAN
31	46.7	FEMALE	CAUCASIAN
32	45.1	FEMALE	CAUCASIAN
33	35.4	FEMALE	CAUCASIAN
34	48.6	FEMALE	CAUCASIAN
35	68.5	FEMALE	CAUCASIAN
36	51.4	FEMALE	CAUCASIAN
37	39.9	FEMALE	CAUCASIAN
38	43.7	FEMALE	CAUCASIAN
39	43.3	FEMALE	BI-RACIAL
40	27.9	FEMALE	CAUCASIAN

TKL STUDY NO. [redacted]
DATA LISTING 2: SUBJECT DEMOGRAPHICS

SUBJECT NO.	AGE	GENDER	RACE
41	35.5	FEMALE	CAUCASIAN
42	63.5	FEMALE	CAUCASIAN
43	54.4	FEMALE	CAUCASIAN
44	39.7	FEMALE	CAUCASIAN
45	57.8	FEMALE	CAUCASIAN
46	43.4	FEMALE	CAUCASIAN
47	32.9	FEMALE	CAUCASIAN
48	40.0	FEMALE	CAUCASIAN
49	52.8	FEMALE	CAUCASIAN
50	56.6	FEMALE	CAUCASIAN
51	27.8	FEMALE	CAUCASIAN
52	35.2	FEMALE	CAUCASIAN
53	42.2	FEMALE	CAUCASIAN
54	52.8	MALE	CAUCASIAN
55	60.2	MALE	CAUCASIAN
56	53.2	MALE	CAUCASIAN
57	60.8	FEMALE	CAUCASIAN
58	38.3	MALE	CAUCASIAN
59	40.9	FEMALE	CAUCASIAN
60	37.0	FEMALE	CAUCASIAN
61	47.1	FEMALE	CAUCASIAN
62	57.7	FEMALE	CAUCASIAN
63	20.5	FEMALE	CAUCASIAN
64	59.6	FEMALE	CAUCASIAN
65	44.3	FEMALE	CAUCASIAN
66	52.5	FEMALE	CAUCASIAN
67	38.7	FEMALE	ASIAN
68	58.1	FEMALE	CAUCASIAN
69	58.8	MALE	CAUCASIAN
70	71.1	FEMALE	CAUCASIAN
71	35.8	FEMALE	CAUCASIAN
72	44.1	FEMALE	CAUCASIAN
73	46.6	FEMALE	CAUCASIAN
74	42.2	FEMALE	CAUCASIAN
75	41.3	FEMALE	CAUCASIAN
76	51.9	FEMALE	CAUCASIAN
77	57.2	FEMALE	CAUCASIAN
78	34.6	FEMALE	CAUCASIAN
79	51.6	FEMALE	CAUCASIAN
80	51.0	FEMALE	CAUCASIAN

TKL STUDY NO. [redacted]
DATA LISTING 2: SUBJECT DEMOGRAPHICS

SUBJECT NO.	AGE	GENDER	RACE
81	57.9	FEMALE	CAUCASIAN
82	48.8	FEMALE	CAUCASIAN
83	68.7	FEMALE	CAUCASIAN
84	45.7	FEMALE	CAUCASIAN
85	56.7	MALE	CAUCASIAN
86	43.5	FEMALE	CAUCASIAN
87	32.4	FEMALE	CAUCASIAN
88	58.2	FEMALE	CAUCASIAN
89	42.6	FEMALE	CAUCASIAN
90	36.9	FEMALE	HISPANIC
91	30.3	FEMALE	HISPANIC
92	53.5	FEMALE	CAUCASIAN
93	46.9	FEMALE	CAUCASIAN
94	46.2	FEMALE	CAUCASIAN
95	37.7	MALE	CAUCASIAN
96	57.5	FEMALE	CAUCASIAN
97	48.8	FEMALE	CAUCASIAN
98	52.0	MALE	CAUCASIAN
99	55.4	FEMALE	CAUCASIAN
100	59.2	FEMALE	CAUCASIAN
101	59.8	FEMALE	CAUCASIAN
102	46.6	FEMALE	CAUCASIAN
103	42.7	FEMALE	CAUCASIAN
104	37.3	FEMALE	CAUCASIAN
105	54.6	FEMALE	CAUCASIAN
106	49.9	FEMALE	CAUCASIAN
107	32.4	FEMALE	CAUCASIAN
108	20.0	FEMALE	HISPANIC
109	29.8	FEMALE	CAUCASIAN
110	58.0	FEMALE	CAUCASIAN

[redacted]

DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT= [redacted] 5% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream

SUBJECT	INDUCTION READING										CHALLENGE PHASE		
NO.	1	2	3	4	5	6	7	8	9	MU	48HR	72HR	96HR(*)
1	-	-	-	-	-	-	-	-	-		-	-	
2	-	-	-	-	-	-	-	-	-		-	-	
3	-	-	-	-	X	X	X	X	X		X	X	
4	-	-	-	X	-	-	-	-	-	-	-	-	
5	-	-	-	-	-	-	-	-	-		-	-	
6	-	-	-	-	-	-	-	-	-		-	-	
7	-	-	-	-	-	-	-	-	-		-	-	
8	-	-	-	-	-	-	-	-	-		-	-	
9	-	-	-	-	-	-	-	-	-		-	-	
10	-	-	-	-	-	-	-	-	-		-	-	
11	-	-	-	-	-	-	-	-	-		-	-	
12	-	-	-	-	-	-	-	-	-		-	-	
13	-	-	-	-	-	-	-	-	-		-	-	
14	-	-	-	-	-	-	-	-	-		-	-	
15	X	X	X	X	X	X	X	X	X		X	X	
16	-	-	-	-	-	-	-	-	-		-	-	
17	-	-	-	-	-	-	-	-	-		-	-	
18	-	-	-	-	-	-	-	-	-		-	-	
19	-	-	-	-	-	-	-	-	-		-	-	
20	-	-	-	-	-	X	-	-	-	-	-	-	

KEY TO SYMBOLS:

- = NO REACTION
? = MINIMAL OR DOUBTFUL RESPONSE, SLIGHTLY DIFFERENT FROM SURROUNDING NORMAL SKIN

+ = DEFINITE ERYTHEMA, NO EDEMA
++ = DEFINITE ERYTHEMA, DEFINITE EDEMA

+++ = DEFINITE ERYTHEMA, DEFINITE EDEMA AND VESICULATION

N9G = NO NINTH GRADING NA=NOT APPLIED NP=NOT PATCHED DUE TO REACTION ACHIEVED
X = READING NOT PERFORMED DUE TO MISSED VISIT OR SUBJECT DISCONTINUATION
D = DAMAGE TO EPIDERMIS: OOZING, CRUSTING AND/OR SUPERFICIAL EROSIONS
P = PAPULAR RESPONSE >50% NR=DATA NOT RECORDED
MU = MAKE-UP READING FOR MISSED INDUCTION VISIT

(*) WHEN REQUIRED

PROGRAM: DETAIL.SAS/USES: RESPONSE, PRODLIST/23FEB05:16:09:07

[redacted]

DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT= [redacted] 5% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream

SUBJECT	INDUCTION READING										CHALLENGE PHASE		
NO.	1	2	3	4	5	6	7	8	9	MU	48HR	72HR	96HR(*)
21	-	-	-	-	-	-	-	-	-		-	-	
22	-	-	-	-	X	-	-	-	-	-	-	-	
23	-	-	-	-	-	-	-	-	-		-	-	
24	-	-	-	-	-	-	-	-	-		-	-	
25	-	-	-	-	-	-	-	-	-		-	-	
26	-	-	-	-	-	-	-	X	-	-	-	-	
27	-	-	-	-	-	-	-	-	-		-	-	
28	-	-	-	-	-	-	-	-	-		-	-	
29	-	-	-	-	-	-	-	-	-		-	-	
30	-	-	-	-	-	-	-	-	-		-	-	
31	-	-	-	-	-	-	-	-	-		-	-	
32	-	-	-	-	-	-	-	-	-		-	-	
33	-	-	-	-	-	-	-	-	-		-	-	
34	-	-	-	-	-	-	-	-	-		-	-	
35	-	-	-	-	-	-	-	-	-		-	-	
36	-	-	-	-	-	-	-	-	-		-	-	
37	X	-	-	-	-	-	-	-	-	-	-	-	
38	-	-	-	-	-	-	-	-	-		-	-	
39	-	-	-	-	-	-	-	-	-		-	-	
40	-	-	-	-	-	-	-	-	-		-	-	
41	-	-	-	-	-	-	-	-	-		X	X	
42	-	-	-	-	-	-	-	-	-		-	-	
43	-	-	-	-	-	-	-	-	-		-	-	
44	-	-	-	-	-	-	-	-	-		-	-	
45	-	-	X	-	-	-	-	-	-	-	-	-	
46	-	-	-	-	-	-	-	-	-		-	-	
47	-	-	-	-	-	-	-	-	-		-	-	
48	-	-	-	-	-	-	-	-	-		-	-	
49	-	-	-	-	-	-	-	-	-		-	-	
50	-	-	-	-	-	-	-	-	-		-	-	
51	-	-	X	-	-	-	-	-	-	-	-	-	
52	-	X	-	-	-	-	-	-	-	-	-	-	

(*) WHEN REQUIRED

PROGRAM: DETAIL.SAS/USES: RESPONSE, PRODLIST/23FEB05:16:09:07

[redacted]

DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT= [redacted] 5% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream

SUBJECT	INDUCTION READING										CHALLENGE PHASE		
NO.	1	2	3	4	5	6	7	8	9	MU	48HR	72HR	96HR(*)
53	-	-	-	-	-	-	-	-	-		-	-	
54	-	-	-	-	-	-	-	-	-		-	-	
55	-	X	X	X	X	X	X	X	X		X	X	
56	-	-	-	-	-	-	-	-	-		-	-	
57	-	-	-	-	-	-	-	-	-		-	-	
58	-	X	X	X	X	X	X	X	X		X	X	
59	-	-	-	-	-	X	-	-	-	-	-	-	
60	-	-	-	-	X	-	-	-	-	N9G	-	-	
61	-	-	-	-	-	-	-	-	-		-	-	
62	-	-	-	-	-	-	-	-	-		-	-	
63	-	-	-	-	-	-	-	-	-		-	-	
64	-	-	-	-	X	-	-	-	-	N9G	-	-	
65	-	-	-	-	-	-	-	-	-		-	-	
66	-	-	-	-	-	-	-	-	-		-	-	
67	-	-	-	-	-	-	-	-	-		-	-	
68	-	-	-	-	-	-	-	-	-		-	-	
69	-	-	-	-	-	-	-	-	-		-	-	
70	-	-	-	-	-	-	-	-	-		-	-	
71	-	-	-	-	-	-	-	-	-		-	-	
72	-	-	-	-	-	-	-	X	-	-	-	-	
73	-	-	-	-	-	-	-	-	-		-	-	
74	-	-	-	-	-	-	-	X	-	-	-	-	
75	-	-	-	-	-	-	-	-	-		-	-	
76	-	-	-	-	-	-	-	-	-		-	-	
77	-	-	-	-	-	-	-	-	-		-	-	
78	-	-	-	-	-	-	-	-	-		-	-	
79	-	-	-	-	-	-	-	-	-		-	-	
80	-	-	-	-	-	X	-	-	-	-	-	-	
81	-	-	-	-	-	-	-	X	-	-	-	-	
82	-	-	-	-	X	-	-	X	X		X	X	
83	-	-	-	-	-	-	-	-	-		-	-	
84	X	X	X	X	X	X	X	X	X		X	X	

(*) WHEN REQUIRED

PROGRAM: DETAIL.SAS/USES: RESPONSE, PRODLIST/23FEB05:16:09:07

[redacted]

DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT= [redacted] 5% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream

SUBJECT	INDUCTION READING										CHALLENGE PHASE		
NO.	1	2	3	4	5	6	7	8	9	MU	48HR	72HR	96HR(*)
85	-	-	-	-	-	-	-	-	-		-	-	
86	-	-	X	-	-	-	-	-	-	-	-	-	
87	-	-	-	-	-	-	-	-	-		-	-	
88	-	-	-	-	-	-	-	-	-		-	-	
89	-	-	-	-	-	-	-	-	-		-	-	
90	-	-	-	-	-	-	-	-	-		-	-	
91	-	-	-	-	-	-	-	-	-		-	-	
92	-	-	-	-	-	-	-	-	-		-	-	
93	-	-	-	-	-	-	-	-	-		-	-	
94	-	-	-	-	-	-	-	-	-		-	-	
95	-	-	-	-	-	-	-	-	-		-	-	
96	-	-	-	-	-	-	-	-	-		-	-	
97	-	-	-	-	-	-	-	-	-		-	-	
98	-	-	-	-	-	-	-	-	-		-	-	
99	+	+	?	?	+	+	+	+	+		?	-	
100	-	-	-	-	-	-	-	-	-		-	-	
101	-	-	-	-	-	-	-	-	-		-	-	
102	-	-	-	-	-	-	-	-	-		-	-	
103	-	-	-	-	-	-	-	-	-		-	-	
104	-	-	-	-	-	-	-	-	-		-	-	
105	-	-	-	X	-	-	-	-	-	-	-	-	
106	-	-	-	-	-	-	-	-	-		-	-	
107	-	-	-	-	-	-	-	-	-		-	-	
108	-	-	-	-	-	-	X	X	X		X	X	
109	-	-	-	-	-	-	-	-	-		-	-	
110	-	-	-	-	-	-	-	-	N9G		-	-	

(*) WHEN REQUIRED

PROGRAM: DETAIL.SAS/USES: RESPONSE, PRODLIST/23FEB05:16:09:07

APPENDIX III

CLINICAL MATERIAL RECORD

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TKL RESEARCH, INC. 4 FOREST AVENUE PARAMUS, NJ 07652

CLINICAL MATERIAL RECORD

TKL Study No.: [redacted]

PRODUCT ID CODE	PRODUCT DESCRIPTION	EXPIRATION DATE	RECEIPT	ADDITIONAL RECEIPT	STORAGE CONDITIONS *see code below	RETAINED SAMPLE
5% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream [redacted]	MOISTURIZER/ YELLOWISH WHITE LIQUID	4/25/05	DATE: 12/7/04 AMOUNT: 1 X GROSS WT. 557.67g TARE WT. 66.26g	DATE: AMOUNT:	A	DATE: AMOUNT: NA
			DATE: AMOUNT:	DATE: AMOUNT:		DATE: AMOUNT:
			DATE: AMOUNT:	DATE: AMOUNT:		DATE: AMOUNT:
			DATE: AMOUNT:	DATE: AMOUNT:		DATE: AMOUNT:

DISPOSITION DATE: _____ CARRIER: _____

DISPOSITION: ❑ RETURN X ❑ DISCARD (AFTER ISSUANCE OF FINAL REPORT)

SPONSOR: [redacted]

RECEIPT RECORDED BY: _____

DISPOSITION RECORDED BY: _____

LOG BOOK PAGE NUMBER: _____

*STORAGE CONDITIONS: A = AMBIENT (Room Temperature) CONDITIONS W = WARMER (Temp. Req._____) R = REFRIGERATED (4-8°C) F = FROZEN (Temp. Req._____)
O = OTHER (EXPLAIN): _____

STORAGE INFORMATION: Generally, a 20 ml or g sample or a full (pre-packaged) product unit or device will be retained for a period of six months.

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FINAL REPORT

Study Title

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Test Articles

[redacted] 1% phytosteryl/behenyl/octyldodectyl lauroyl glutamate in a face cream

Authors
Greg Mun, B.A.
Dana Wolfinger, B.S.

Study Completion Date
9 October 2014

Performing Laboratory
Institute for In Vitro Sciences, Inc.
30 West Watkins Mill Road, Suite 100
Gaithersburg, MD 20878

Study Number
[redacted]

Laboratory Project Number
[redacted]

[redacted]

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TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

SUMMARY

IIVS Test Article Number	Sponsor's Designation	Conc.	t_{50} (hours)		pH
			Preliminary (2 July 2014)	Trial 1 (9 July 2014)	
[redacted]	[redacted]	Neat	>16	>24	5.0-5.5
[redacted]					
Positive Control	0.3% Triton®-X-100	NA	<15 minutes*	23.3 minutes	NA

1% phytosteryl/behenyl/ octyldodectyl lauroyl glutamate in a face cream

NA - Not Applicable

*- The t_{50} result was not within the acceptable range. However, the results were sufficient to select appropriate test article exposure times for the definitive assay.

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STATEMENT OF COMPLIANCE

1% phytosteryl/behenyl/octyldodectyl lauroyl glutamate in a face cream

The Tissue Equivalent Assay With EpiOcular™ cultures of the test articles, [redacted] was conducted in compliance with the U.S. FDA Good Laboratory Practice Regulations as published in 21 CFR 58 and the principles presented in the OECD series on Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test articles have not been determined by the testing facility. The certificates of analysis were not provided by the Sponsor.

The stability of the test articles under the storage conditions at the testing facility and under the actual test conditions has not been determined by the testing facility and is not included in the final report.

Greg Mun, B.A.
Study Director

9 October 2014
Date

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QUALITY ASSURANCE STATEMENT

Study Title: Tissue Equivalent Assay with EpiOcular™ Cultures

Study Number: [redacted]

Study Director: Greg Mun, B.A.

This study was divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitored each of these phases over a series of studies. Procedures, documentation, equipment records, etc., were examined in order to assure that the study was performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58) and the OECD Principles of Good Laboratory Practice and to assure that the study was conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected and report dates of QA inspections of this study:

Phase Inspected	Audit Date(s)	Reported to Study Director	Reported to Management
Protocol and Initial Paperwork	30-Jun-14	30-Jun-14	8-Jul-14
Treatment of Tissues with Test Article and Control	2-Jul-14	3-Jul-14	8-Jul-14
Draft Report and Data	18-21-Aug-14	28-Aug-14	2-Sept-14
Final Report	6-Oct-14	6-Oct-14	6-Oct-14

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Renee Forde, Ph.D., RQAP-GLP
Quality Assurance

7 October 2014
Date

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SIGNATURE PAGE

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Initiation Date:	25 June 2014
Completion Date:	9 October 2014
Sponsor:	[redacted]
Sponsor's Representative:	[redacted]
Testing Facility and Study Director Address:	Institute for In Vitro Sciences, Inc. 30 West Watkins Mill Road, Suite 100 Gaithersburg, MD 20878
Archive Location:	[redacted]
Study Director:	9 October 2014 Greg Mun, B.A. Date
Laboratory Manager:	Nathan R. Wilt, B.S.
Laboratory Supervisor:	Allison Hilberer, M.S.

TEST ARTICLE RECEIPT

IIVS Test Article Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions*
1% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream [redacted]	[redacted]	off-white cream	24 June 2014	room temperature
[redacted]				

* - Protected from exposure to light

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

INTRODUCTION

The EpiOcular™ Human Cell Construct (MatTek Corporation) was used to assess the potential ocular irritancy of the test articles. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) conversion assay, which measures the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, the succinate dehydrogenase reduction of MTT) to a blue formazan precipitate, was used to assess cellular metabolism after exposure to a test article for various exposure times[1]. The duration of exposure resulting in a 50% decrease in MTT conversion in test article-treated EpiOcular™ human cell constructs, relative to control cultures, was determined (t_{50}).

The purpose of this study was to evaluate the potential toxicity of the test articles, supplied by [redacted] as measured by the conversion of MTT by EpiOcular™ human cell constructs after exposure to a test article for various exposure times. The laboratory phase of the study was conducted from 1 July 2014 to 10 July 2014 at the Institute for In Vitro Sciences, Inc. After a time range finding assay, the test article was tested in a valid definitive assay to determine the time of exposure to a test article, which resulted in the t_{50} endpoint.

[1] Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. **Biochemica** 4:14-19.

MATERIALS AND METHODS

Receipt of the EpiOcular™ Human Cell Construct Model

Upon receipt of the EpiOcular™ Human Cell Construct Kit (MatTek Corporation), the solutions were stored as indicated by the manufacturer. The EpiOcular™ human cell constructs were stored at 2-8°C until used. On the day of dosing an appropriate volume of EpiOcular™ human cell construct assay medium was removed and warmed to approximately 37°C. Nine-hundred µL of assay medium were aliquoted into the wells of 6-well plates. The six-well plates were labeled to indicate test article and exposure time. The samples were inspected for air bubbles between the agarose gel and cell culture insert prior to opening the sealed package. Cultures with air bubbles covering greater than 50% of the cell culture area were not used. The 24-well shipping containers were removed from the plastic bag and their surfaces were disinfected with 70% ethanol. The EpiOcular™ human cell constructs were transferred aseptically into the 6-well plates. The EpiOcular™ human cell constructs were then incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for at least one hour. The medium was then aspirated and 0.9 mL of fresh medium were added to each assay well below the EpiOcular™ human cell construct. The plates were returned to the incubator until treatment was initiated. Upon opening the shipping bag, any remaining unused tissues were briefly gassed with an atmosphere of 5% CO_2/95% air and placed back at 2-8°C for later use.

Test Article Preparation

As instructed by the Sponsor, each test article was administered to the test system without dilution.

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) supplemented with 2 mM L-glutamine (MTT Addition Medium) to assess its ability to directly reduce MTT. Approximately 100 µL of each test article were added to 1 mL of the MTT solution and the mixtures were incubated in the dark at 37°C for one to three hours. 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 below in the section "Killed Controls (KC) for Assessment of Residual Test Article Reduction of MTT".

The test articles were not observed to reduce MTT in the absence of viable cells.

pH Determination

The pH of each liquid test article was measured using pH paper (EMD Millipore Corporation). Initially, each test article was added to pH paper with 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. The pH values obtained from the narrower range pH paper are presented in Table 1.

Time Range Finding Assay

A time range finding assay was performed to establish an appropriate exposure time range to be used in the definitive assay for each test article. Four exposure times of 1, 4, 8, and 16 hours were tested in the time range finding assay. One culture was treated per exposure time with 100 µL of the appropriate test article or control. The negative control, 100 µL of sterile, deionized water (Quality Biological), was exposed for 16 hours. The positive control, 100 µL of 0.3% Triton®-X-100 (Fisher), was exposed for 15 and 45 minutes (one culture per exposure time). The exposed cultures were then incubated for the appropriate amount of time at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air.

After the appropriate exposure time, the EpiOcular™ cultures were extensively rinsed with Calcium and Magnesium-Free Dulbecco's Phosphate Buffered Saline ($Ca^{++}Mg^{++}$-Free DPBS) and the wash medium was decanted. After rinsing, the tissue was transferred to 5 mL of Assay Medium for a 10 to 20 minute soak at room temperature to remove any test article absorbed into the tissue. A 1.0 mg/mL solution of MTT in warm MTT Addition Medium was prepared no more than 2 hours before use. Three-hundred µL of MTT solution were added to designated wells in a prelabeled 24-well plate. The EpiOcular™ constructs were transferred to the appropriate wells after rinsing with $Ca^{++}Mg^{++}$-Free DPBS. The trays were incubated at 37±1°C for approximately three hours in a humidified atmosphere of 5±1% CO_2 in air.

After the incubation period with MTT solution, the EpiOcular™ cultures were blotted on absorbent paper, cleared of excess liquid, and transferred to a prelabeled 24-well plate containing 2.0 mL of isopropanol in each designated well. The plates were sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time was harvested. The plates were then shaken for at least two hours at room temperature.

At the end of the extraction period, the liquid within the cell culture inserts was decanted into the well from which the cell culture insert was taken. The extract solution was mixed and 200 µL were transferred to the appropriate wells of a 96-well plate. Two hundred µL of isopropanol were added to the two wells designated as the blanks. The absorbance at 550 nm (OD_{550}) of each well was measured with a Molecular Devices Vmax plate reader.

Definitive Assay

1% phytosteryl/behenyl/octyldodectyl lauroyl glutamate in a face cream

Based on the results of the time range finding assay, three to five exposure times were chosen for the definitive assay. The exposure times for the test articles, [redacted] were 8, 16, 20, and 24 hours. [redacted] The negative control (100 µL of sterile, deionized water) was exposed for 0.25, 4, 8, and 24 hours. The positive control (100 µL of 0.3% Triton®-X-100) was exposed for 15 and 45 minutes. The procedures used to conduct the definitive assay were essentially the same as for the time range finding assay with the exception that at least duplicate cultures were dosed per exposure time.

Presentation of Data

The raw absorbance values were captured. The mean OD_{550} value of the blank wells was calculated. The corrected mean OD_{550} values of the negative controls were determined by subtracting the mean OD_{550} values of the blank wells from their mean OD_{550} values. The corrected OD_{550} value of the individual test article exposure times and the positive control exposure times were determined by subtracting the mean OD_{550} value of the blank control from their OD_{550} values. The individual % of Control values were averaged to get the mean % of Control value. All calculations were performed using an Excel spreadsheet. The following percent of control calculations were made:

$$\% \text{ of Control} = \frac{\text{corrected } OD_{550} \text{ of Test Article or Positive Control Exposure Time}}{\text{appropriate corrected mean } OD_{550} \text{ of Negative Control}} \times 100$$

Exposure time response curves were plotted with the % of Control on the ordinate and the test article or positive control exposure time on the abscissa. The t_{50} value was interpolated from each plot. To determine the t_{50}, the two consecutive points were selected, where one exposure time resulted in a relative survival greater than 50%, and one exposure time resulted in less than 50% survival. Two select points were used to determine the slope and the y-intercept for the equation $y=m(x) + b$. Finally, to determine the t_{50}, the equation was solved for $y=50$. When all of the exposure time points show greater than 50% survival, the t_{50} value is presented as greater than the longest test article exposure time.

Criteria for a Valid Test

The assay results were accepted when: 1) The t_{50} value of the positive control fell within two standard deviations of the historical mean (updated every three months) and 2) The corrected mean OD_{550} value for the minimum negative control exposure time was within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time (up to 4 hours).

RESULTS AND DISCUSSION

Time Range Finding Assay

A time range finding assay was performed, consisting of four exposure times of 1, 4, 8, and 16 hours for the test articles supplied by [redacted]. The exposure time response curves are included in Appendix B. Based upon the results of the time range finding assay, three to five exposure times were selected for each test article for the definitive assay (see Materials and Methods). The t_{50} results for the time range finding assay are reported in Table 1, under "Preliminary".

1% phytosteryl/behenyl/octyldodectyl lauroyl glutamate in a face cream

The test articles, [redacted], were not observed to reduce MTT directly in the absence of viable tissue.

[redacted]

Definitive Assay

1% phytosteryl/behenyl/octyldodectyl lauroyl glutamate in a face cream

Three to five exposure times were treated in duplicate for each test article. The exposure times for the test articles, [redacted], were 8, 16, 20, and 24 hours. [redacted] The negative control was also exposed in duplicate for 0.25, 4, 8, and 24 hours. Table 1 summarizes the t_{50} results of the definitive Tissue Equivalent Assay With EpiOcular™ Cultures for the test articles and the positive control, 0.3% Triton®-X-100, under "Trial 1". The exposure time response curves are included in Appendix B. Since the positive control fell within two standard deviations of the historical mean (18.5-36.2 minutes), and the corrected mean OD_{550} value for the minimum negative control exposure time (1.438) was within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time (up to 4 hours) (1.520), the assay results were accepted. Finally, none of the test articles were observed to directly reduce MTT in the absence of viable tissue.

Table 1

IIVS Test Article Number	Sponsor's Designation	Conc.	t_{50} (hours)		pH
			Preliminary (2 July 2014)	Trial 1 (9 July 2014)	
1% phytosteryl/behenyl/ octyldodectyl lauroyl glutamate in a face cream [redacted]	[redacted]	Neat	>16	>24	5.0-5.5
[redacted]					
Positive Control	0.3% Triton®-X-100	NA	<15 minutes*	23.3 minutes	NA

NA - Not Applicable
*- The t_{50} result was not within the acceptable range. However, the results were sufficient to select appropriate test article exposure times for the definitive assay.

APPENDIX A

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TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

1.0 PURPOSE

The purpose of this study is to evaluate the potential toxicity of the test article. In the Tissue Equivalent Assay, stratified human epithelial cell cultures (MatTek EpiOcular™) are exposed to topically applied test articles to evaluate potential ocular toxicity. Cell viability is determined by conversion of 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) in the treated cultures, and is expressed as a percentage relative to negative control-treated cultures. The endpoint of the Tissue Equivalent Assay, the t_{50} value, is the time of exposure to test article required to reduce cell viability (MTT metabolism) to 50% of negative control levels as calculated from time-response curves.

2.0 SPONSOR

See Protocol Attachment 1

3.0 IDENTIFICATION OF TEST ARTICLE(S) AND ASSAY CONTROLS

3.1 Test Article(s): See Protocol Attachment 1

3.2 Assay Controls:
- Positive: 0.3% Triton®-X-100
- Negative: Sterile, deionized water (or other solvent as appropriate)

3.3 Determination of Strength, Purity, etc.

3.3.1 For GLP studies only, the Institute for In Vitro Sciences, Inc. (IIVS) will attempt to secure documentation of the analytical purity and composition of the test article and the stability and strength of the dosing solutions from the Sponsor. If the Sponsor is unable to provide such information, IIVS will retain documentation supporting attempts to obtain this information with the study file and an exception will be noted in the Statement of Compliance in the Final Report.

3.3.2 IIVS will be responsible for the documentation of the analytical purity and composition of the negative control and the Triton®-X-100 used for the stock dilution of the positive control. This may be accomplished by maintaining a certificate of analysis from the supplier.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1 Name: Institute for In Vitro Sciences, Inc.

4.2 Address: 30 West Watkins Mill Road, Suite 100

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Gaithersburg, MD 20878

4.3 Study Director: Greg Mun, B.A.

5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 2 July 2014

5.2 Proposed Experimental Completion Date: 17 July 2014

5.3 Proposed Report Date: 25 September 2014

6.0 TEST SYSTEM

The EpiOcular™ human cell model, obtained from the MatTek Corporation, will be used in this study. The use of EpiOcular™ tissues offers features appropriate for a model for ocular irritation. First, the model is composed of stratified human keratinocytes in a three-dimensional structure. Secondly, test articles can be applied topically to the model so that water insoluble materials may be tested. Prior to use, each plate (6, 12, 24, and 96-well) will be uniquely identified with a number written in permanent marker, on the plate and its cover, the test article number, and the exposure time.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The experimental design of this study consists of a determination of the direct MTT reduction potential and the pH followed by a time range finding assay and a definitive assay. The toxicity of the test article will be evaluated by the exposure time required to reduce cell viability to 50% of controls (t_{50}). Viability will be determined by the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, by the succinate dehydrogenase reduction of MTT) in control and test article-treated cultures (Berridge, et al., 1996). Data will be presented in the form of relative survival (relative MTT conversion) versus test article exposure time.

One of two exposure time ranges may be used. The standard exposure time range extends up to four hours. For materials considered to be mild, such as those that might be applied around or in the eyes, exposure times of up to 24 hours may be used (long term exposure assay). In general, the standard exposure range will be used, unless the Sponsor specifies an alternative exposure time range, or if the Study Director determines that the class of test articles warrants the use of an alternative exposure time range.

7.1 Media and Reagents

7.1.1 Assay Medium: supplied by MatTek Corporation

7.1.2 EpiOcular™ Tissue: OCL-200_2.0 supplied by MatTek Corporation

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7.1.3 Dulbecco's Modified Eagle's Medium (DMEM) containing 2mM L-glutamine (MTT Addition Medium)

7.1.4 3-[4,5 - dimethylthiazol-2-yl] - 2,5 - diphenyltetrazolium bromide (MTT) 10X stock solution: 10 mg/mL MTT in PBS

7.1.5 Ca^{++} and Mg^{++}-Free Dulbecco's Phosphate Buffered Saline – ($Ca^{++}Mg^{++}$-Free DPBS) (pH 7.0 ± 0.5)

7.1.6 Extraction Solvent: Isopropanol

7.1.7 Sterile Deionized Water (Quality Biological or equivalent)

7.2 Preparation and Delivery of Test Article

Test articles will generally be tested neat. End use concentrations or other forms may be used as directed by the Sponsor. Alternate volumes of the test article may be used as specified by the Sponsor. See Protocol Attachment 1.

One hundred µL of pipettable substances, such as liquids, gels, creams, and foams, will be applied directly on the tissue so as to cover the upper surface. To aid in filling the pipet for pipettable materials that are viscous, the test article may first be transferred to a syringe. The pipet tip of the positive displacement pipet will be inserted into the dispensing tip of the syringe so that the material can be loaded into the displacement tip under pressure. Simultaneously, the syringe plunger is depressed as the pipet piston is drawn upwards. If air bubbles appear in the pipet tip, the test article should be removed (expelled) and the process repeated until the tip is filled without air bubbles. This method should be used for any materials that cannot be easily drawn into the pipet such as gels (e.g., toothpastes, mascaras, and face creams), and solid test articles that are creamed, like lipsticks and antiperspirants/deodorant sticks. A dosing device (a flatheaded cylinder of slightly less diameter than the inner diameter of the tissue insert) may be placed over the test article to assure even spreading, if required. Materials which are too viscous to spread over the tissue will first be spread onto the flat end of a dosing device. The dosing device will be placed into the cell culture insert to bring the test article in contact with the tissue. When the test article must first be applied to a dosing device, approximately 30 µL or 30 ±1 mg of material will be applied to the dosing device so as to cover the dosing surface. The sample should be spread to form a relatively smooth even layer on the surface of the dosing device to maximize uniform tissue contact. Solids such as lipsticks or antiperspirant/deodorant sticks can be presoftened by creaming a portion in a weigh boat. The softened portion can be transferred to a syringe affixed with a three way stopcock attached to a second syringe. The sample is pushed from syringe to syringe until it is of a consistency which can be pipetted. Dry powders will be ground with a mortar and pestle, if needed, and passed through a #40 copper sieve. Powders will be placed directly onto the culture at 30 ±1 mg /culture. The exact exposure conditions used for other test article forms will be

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determined after consultation with the Sponsor and/or the Study Director. All exposure conditions will be documented in the study workbook.

The stability of the test article under the actual experimental conditions will not be determined by IIVS.

7.3 Route of Administration

Test article will be administered by topical application to the cell cultures.

7.4 pH Determination

The pH of the neat liquid test article (and/or dosing solution as appropriate) will be determined, if possible. The pH will be determined using pH paper (for example, with a pH range of 0 – 14 to estimate, and/or a pH range of 5 – 10 to determine a more precise value). The typical pH increments on the pH paper used to report the pH are approximately 0.3 to 0.5 pH units. The maximum increment on the pH paper is 1.0 pH units.

7.5 Controls

Two types of control treatments will be used in this assay. The tissues designated for the negative control cultures will be treated with sterile, deionized water or other solvent (if employed in its assay). Negative control tissues will be dosed and exposed in parallel with the test article and positive control tissues. The exposure times used for the negative controls will be selected to address the range of exposure times used for the test article and positive control cultures. The tissues designated for the positive control will be treated with 0.3% Triton®-X-100 prepared in sterile, deionized water and exposed for 15 and 45 minutes.

Time range finding assay: The assay will include a negative and positive control. . Each test condition will be tested with at least a single tissue. The negative control will be tested for one or more exposure times, which will generally be chosen to address the longest test article or positive control exposure time. At least one tissue will be used for each of the two positive control exposure times.

Definitive assay: Generally, at least two negative control exposure times will be tested in duplicate tissues. One negative control exposure time will be selected to fit the range of the shortest test article or positive control exposure times (the minimum negative control exposure time will be 15 minutes). The second negative control exposure time will be selected to match the longest test article or positive control exposure time (whichever is longer, up to 240 minutes). On occasion, the second negative control exposure time may be selected to fit the longest test article exposure time of a test article run concurrently, but from an independent study. For the long term exposure assay (exposures of greater than 240 minutes), multiple negative control exposure times may be selected to fit the range of test article exposure times.

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If all exposure times are one hour and less, a single negative control exposure time may be used. Additional negative control exposure times may be selected at the discretion of the Study Director. At least two tissues will be used for each of the two positive control exposure times.

7.6 Assessment of Direct Test Article Reduction of MTT

It is necessary to assess the ability of each test article to directly reduce MTT. A 1.0 mg/mL MTT solution will be prepared in warm MTT Addition Medium as described in §7.8. Approximately 100 µL (liquid test articles) or 30 ±1 mg (solid test articles) will be added to 1 mL of the MTT solution and the mixture incubated in the dark at 37 ±1°C for one to three hours. If the MTT solution color turns blue/purple, the test article is 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.

7.7 Receipt of the EpiOcular™ Model

Upon receipt of the EpiOcular™ assay materials, the solutions will be stored as indicated by the manufacturer. The tissues will be stored at 2-8°C until used. Tissues should generally be used within 2 days of receipt from the manufacturer.

On the day of dosing, an appropriate volume of EpiOcular™ Assay Medium will be removed and warmed to approximately 37°C. Nine tenths (0.9) mL of Assay Medium will be aliquoted into the wells of 6-well plates. Each tissue will be inspected for air bubbles between the agarose gel and cell culture insert prior to opening the sealed package. Tissues with air bubbles under greater than 50% of the cell culture insert area will not be used. The 24-well shipping containers will be removed from the plastic bag and the surface disinfected with 70% ethanol. An appropriate number of tissues will be transferred aseptically from the 24-well shipping containers into the 6-well plates. The EpiOcular™ tissues will be incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air (standard culture conditions) for at least one hour prior to dosing. The medium will be aspirated and 0.9 mL of fresh medium will be added to each assay well below the tissue prior to dosing. Note: The refeeding step may occur at less than 1 hour but the tissue should be allowed at least one hour of incubation to become fully metabolically active before dosing. Upon opening the bag, any unused tissues remaining on the shipping agar at the time of tissue transfer will be briefly gassed with an atmosphere of 5% CO_2/95% air, and the bag will be sealed and stored at 2-8°C for subsequent use.

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7.8 Time Range Finding Assay

At least four exposure times will be evaluated for each test article. For the standard assay, the exposure times for the time range finding assay will generally be 10, 30, 60 and 180 minutes unless the Sponsor requests other specific exposure times. The maximum exposure time will be 240 minutes. For the long term exposure assay, the exposure times for the time range finding assay will generally be 1, 4, 8, and 16 hours.

Each test article and control exposure time will be tested by treating at least one tissue. One hundred µL of each pipettable test article will be delivered with a positive displacement pipet onto the tissue. Powders will be placed directly onto the tissue at 30 ±1 mg/culture. Those test articles which are too viscous to spread directly on the tissue may be spread onto a dosing device. Approximately 30 µL or 30 ±1 mg will be applied to the dosing device for each tissue, as indicated in §7.2. Exposure times of ten minutes or greater will be incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air. Generally, exposure times of less than 10 minutes will be incubated at room temperature.

At the end of the treatment time, the test article will be removed by extensively rinsing both sides of the culture with $Ca^{++}Mg^{++}$ Free-DPBS. The process will be performed until the tissue appears free of test article. If it is not possible to remove all of the visible test article, a notation will be recorded in the study workbook. After rinsing, the tissue will be transferred to 5 mL of Assay Medium for a 10 to 20 minute soak at room temperature. This soak is intended to remove any test article absorbed into the tissue.

A 10X stock of MTT prepared in PBS (filtered at time of batch preparation) will be thawed and diluted in warm MTT Addition Medium to produce the 1.0 mg/mL solution no more than two hours before use. Alternatively, a 1.0 mg/mL MTT solution will be prepared in warm MTT Addition Medium and filtered through a 0.45 µm filter to remove undissolved crystals. Three hundred µL of the MTT solution will be added to each designated well of a prelabeled 24-well plate. Excess Assay Medium will be removed and then the tissues will be transferred to the appropriate wells of the MTT plate. The 24-well plates will be incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for 3 ±0.1 hours.

After the three hour incubation, the tissues will be blotted on absorbent paper, cleared of excess liquid, and transferred to a prelabeled 24-well plate containing 2.0 mL of isopropanol in each designated well. The plates will be sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time is harvested. If necessary, plates may be stored overnight (or up to 20 hours after the last exposure time is harvested) in the refrigerator prior to extracting the MTT. The plates will then be shaken for at least 2 hours at room temperature. At the end of the extraction period, the liquid within the cell culture inserts will be decanted into the well from which the cell culture insert was taken. The extract solution will be mixed and 200

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µL transferred to the appropriate wells of a 96-well plate. Two hundred µL of isopropanol will be added to two wells designated as blanks. The absorbance at 550 nm (OD_{550}) of each well will be measured with a Molecular Devices Vmax plate reader.

The range of exposure times for the definitive assay will be chosen by the Study Director or other qualified person to determine the t_{50} (the exposure time to the test article that reduces MTT metabolism by 50%). Based on the results of the time range finding assay, two exposure times will be chosen that should result in expected survivals lower than 50%, and two exposure times will be chosen that should result in expected survivals greater than 50%. If a test article fails to cause 50% toxicity in the time range finding assay, the maximum exposure time will be 240 minutes, or 24 hours, depending on the assay selected. In some cases, the exposure times for the definitive assay may be selected based on visual observations of the relative MTT reduction in the tissues.

7.9 Killed Controls for Assessment of Residual Test Article Reduction of MTT

In cases where the test article is shown to directly reduce MTT, only test articles that remain bound to the tissue after rinsing, resulting in a false MTT reduction signal, present a problem. To demonstrate that possible residual test article is not acting to directly reduce the MTT, a functional check will be performed in the preliminary assay to show that the test material is not binding to the tissue and leading to a false MTT reduction signal.

To determine whether residual test article is acting to directly reduce the MTT, a freeze-killed control tissue will be used. Freeze-killed tissue is prepared at IIVS by placing untreated tissues in the -20°C freezer at least over night, thawing to room temperature, and then refreezing. Once refrozen, the tissue may be stored indefinitely in the freezer. Freeze killed tissues may be received already prepared from MatTek, and stored in the -20°C freezer until use. To test for residual test article reduction, killed tissues will be treated with the test article in the normal fashion. Generally, each test article will be evaluated for at least the shortest and longest exposure times (or longest exposure time if all exposure times are one hour and less) in single replicate killed tissues. All assay procedures will be performed as for the viable tissue. At least one killed control treated with sterile deionized water (negative killed control) will be tested in parallel since a small amount of MTT reduction is expected from the residual NADH and associated enzymes within the killed tissue.

If little or no MTT reduction is observed in the test article-treated killed control, the MTT reduction observed in the test article-treated viable tissue may be ascribed to the viable cells. If there is appreciable MTT reduction in the treated killed control, additional steps will be taken to account for the chemical reduction in the Definitive Assay or the test article may be judged untestable in this system.

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If required in the Definitive Assay, the killed control testing will be similar to the procedure used in the time range finding assay. Additional test article-treated killed control tissues may be used to better cover the range of exposure times. The data from the killed controls will be analyzed as described in §7.11.

7.10 Definitive Assay

The definitive assay with generally three to five exposure times will be performed exactly like the time range finding assay with the exception that tissues will be tested in duplicate for each exposure time. If the test article(s) were found to be non-toxic in the time range finding assay, then fewer than four exposure times may be chosen for the definitive assay. At least duplicate tissues will be tested at each of the positive control exposures. At least duplicate tissues will be treated with negative or solvent control for each exposure time (see §7.5). The determination of the t_{50} will be based upon the results of the definitive assay. At the Study Director's option, a second definitive assay may be performed.

7.11 Presentation of Data

The raw absorbance values will be captured, and the following calculations made:

The mean OD_{550} value of the blank wells will be calculated. The corrected mean OD_{550} value of the negative control(s) will be determined by subtracting the mean OD_{550} value of the blank wells from their mean OD_{550} values. The corrected OD_{550} value of the individual test article exposure times and the positive control exposure times will be determined by subtracting the mean OD_{550} value of the blank wells from their OD_{550} values. Generally, all calculations will be performed using an Excel spreadsheet.

Corr. test article exposure time OD_{550} = Test article exp. time OD_{550} – Blank mean OD_{550}

If killed controls (KC) are used, the following additional calculations will be performed to correct for the amount of MTT reduced directly by test article residues. The OD_{550} value for the negative control killed control will be subtracted from the OD_{550} values for each of the test article-treated killed controls (at each exposure time), to determine the net OD_{550} values of the test article-treated killed controls.

Net OD_{550} for each test article KC = Raw OD_{550} test article KC – Raw OD_{550} negative control KC

The net OD_{550} values represent the amount of reduced MTT due to direct reduction by test article residues at each exposure time tested. When the net OD_{550} values are significant (> 0.150), the net OD_{550} values will be subtracted from the corrected mean OD_{550} values of the viable test article-treated tissues, at each corresponding exposure time, to obtain a final corrected OD_{550} value. These final corrected OD_{550} values will then be used to determine the % of Control viabilities at each exposure time.

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Final Corrected OD_{550} = Corrected test article OD_{550} (viable) – Net OD_{550} test article (KC)

Finally, the following % of Control calculations will be made:

$$\% \text{ of Control} = \frac{\text{corrected } OD_{550} \text{ of each Test Article or Positive Control exposure time}}{\text{corrected mean } OD_{550} \text{ of Negative Control}} \times 100$$

The individual % of Control values are then averaged to get the mean % of Control per exposure time. Viability calculations for test articles treated in the long exposure time assay may be performed by comparing the corrected OD_{550} values of each test article exposure time to the appropriate negative control(s).

Exposure time response curves may be plotted with the % of control on the ordinate and the test article exposure time on the abscissa. Other plot forms may be used as requested by the Sponsor. The t_{50} will be mathematically interpolated from each plot. To determine the t_{50}, two adjacent points will be selected, one that shows greater than 50% survival and one that shows less than 50% survival. The two selected points will be used to determine the slope and the y-intercept for the equation $y = m(x) + b$. Finally, to determine the t_{50}, the equation will be solved for $y = 50$. If all of the exposure time points show greater than 50% survival, the t_{50} will be listed as greater than the longest exposure time. If the shortest test article exposure time shows less than 50% relative survival, the plot will be extended to include the t_0 point which will be given a value of 100%. In this case, the t_{50} will be determined between the t_0 and the shortest exposure time. At the Study Director's option, additional assays may be performed to produce the final t_{50} value.

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

The assay will be accepted if the positive control, 0.3% Triton®-X-100, causes an t_{50} within two standard deviations of the historical mean. The historical mean is updated every three months. The corrected mean OD_{550} value for the minimum negative control exposure time must be within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time (up to 240 minutes).

9.0 REPORT

A report of the results of this study will be prepared by the Testing Facility and will accurately describe all methods used for generation and analysis of the data. A summary will be prepared reporting the t_{50} values for each assay with each test article as well as the positive control data. A copy of the protocol used for the study and any significant deviation(s) from the protocol and SOPs of the Testing Facility will appear as a part of the final report.

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0.0 RECORDS AND ARCHIVES

A separate working notebook will be used to record the materials and procedures used to perform this study. The archival requirements for the study are detailed in the Protocol Attachment 1.

1.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

The regulatory compliance requirements for this study are detailed in the Protocol Attachment 1.

The Quality Assurance Unit will review the study protocol, perform at least one in-process laboratory inspection, and audit the raw data workbook and all reports of the study to assure compliance with the appropriate regulations specified in the Protocol Attachment 1.

2.0 PROTOCOL AMENDMENTS

When it becomes necessary to change the approved protocol for a specific study, verbal agreement to make this change should be made between the Study Director and Sponsor. As soon as practical, this change and the reason for it should be put in writing and signed by the Study Director and provided to the Sponsor. This document is then attached to the protocol as an amendment.

3.0 REFERENCES

MTT Effective Time 50 (ET-50) Protocol, MatTek Corporation

Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. **Biochemica** 4:14-19.

4.0 APPROVAL

(See Sponsor's Protocol Attachment 1)
SPONSOR REPRESENTATIVE

IVS STUDY DIRECTOR — 25 June 2014
DATE

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In Vitro Eye Irritation Tests
Cytosensor Microphysiometer/Tissue Equivalent Assay
Protocol Design Specifications and Sponsor Information

Sponsor:
[redacted]

Testing Facility:
INSTITUTE FOR IN VITRO SCIENCES, INC.
30 West Watkins Mill Road, Suite 100
Gaithersburg, MD 20878
TEL. (301) 947- 6523
FAX. (301) 947 - 6538

Sponsor's Representative: [redacted]

Alternate Sponsor's Representative: [redacted]

[redacted] **IIVS Project # (Completed by IIVS):** [redacted]
Study File # (Completed by IIVS):

Please Indicate Test Requirements:

		IIVS Protocol Number	Test Article Required Solid	Test Article Required Liquid
[ ]	Cytosensor Microphysiometer Assay (standard assay)	SP200019	30g	50 ml
[ ]	Cytosensor Microphysiometer Assay for EPA Anti-Microbial Compliance	SP200050	30g	50 ml
[X]	Tissue Equivalent Assay, EpiOcular [X] Long Term Exposure Assay (up to 24 hours) option for TEA, EpiOcular	SP015008	5g	10 ml

For the TEA only:
If any residual test article (RTA) is observed on the tissues following rinsing in the Time Range Finding Assay *and* a) the t_{50} is <4 hours, *or* b) the projected t_{50} is <4hours based on a visual observation of the rinsed tissues in MTT by the Study Director, the Sponsor's Representative will be notified prior to conducting the Definitive Assay test. A decision will be made, documented in the study records, and reported on whether to conduct an alternate rinse procedure utilizing 37°C $Ca^{++}Mg^{++}$ Free-DPBS and a 10-20 minute soak in 5 mL of assay media warmed to ~37°C. If attempts to contact the Sponsor or Alternate Sponsor Representative are not successful, it is acceptable to proceed directly to the alternate rinse with 37°C $Ca^{++}Mg^{++}$ Free-DPBS and document the study records accordingly.

Study design to meet the scientific requirements of the following authorities (mark all that apply):
[X] FDA [ ] EPA-TSCA [ ] EPA-FIFRA [ ] CPSC [ ] DOT [X] OECD [ ] EU [ ] CEPA
[ ] JMHW [ ] MAFF [ ] MITI [ ] None [ ] Other (please specify) _____

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PROTOCOL ATTACHMENT -1 (Page 2 of 3)
Cytosensor Microphysiometer and Tissue Equivalent Assay
Protocol Design Specifications and Sponsor Information

[redacted] **IIVS Project #** [redacted]

GLP Compliance:

Study will be conducted in accordance with the GLP requirements of the following agency(s)*:
[ ] EPA/FIFRA [ ] EPA/TSCA [X] FDA [X] OECD [ ] Other (list): _____
*If none specified, OECD GLPs will be followed.

[ ] This study is non-GLP.

Test Article Information

Test Article(s):

	Color	Physical Form
[redacted]	[redacted]	[redacted]
[redacted]	Light yellow	Viscous cream

1% phytosteryl/behenyl/octyldodectyl lauroyl glutamate in a face cream

Note: Lot/Batch # and Expiration Date to be documented in the study records

Test Article Storage Conditions: (Note: all test articles stored protected from white light)

[X] Room Temperature (approximately 20 to 25°C)
[ ] Refrigerated (approximately 4°C)
[ ] Frozen (approximately -20°C)
[ ] Other _____

Classified as: (Check all that apply) [X] Non-hazardous - No Special Instructions
[ ] Flammable [ ] Poison [ ] Other

_____ _____ _____
_____ _____ _____

Special Instructions for Test Article Preparation:

[X] None
[ ] As Follows: _____

Test Article Disposition:

[X] Dispose of Unused Test Article using facility SOPs
[ ] Return Unused Test Article to: (approximately one month after submission of final report)

_____
_____
_____

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PROTOCOL ATTACHMENT -1 (Page 3 of 3)
Cytosensor Microphysiometer and Tissue Equivalent Assay
Protocol Design Specifications and Sponsor Information

[redacted] **IIVS Project #** [redacted]

Test Article-Vehicle Mixture(s)

Concentration Analysis of Test Article-Vehicle Mixture(s):

[X] Not required
[ ] Required
Return ____ ml of highest and lowest concentration test article solutions to Sponsor's Representative
Storage conditions [ ] Room temperature [ ] Refrigerated [ ] Frozen [ ] Other _____
Shipping conditions [ ] Frozen [ ] Ambient [ ] Other _____

Test Article-Vehicle Mixture Disposition (if applicable):
[ ] Dispose of Unused Concentration Analysis Samples using facility SOP's.
[ ] Return Unused Concentration Analysis Samples To: (approx. one month after submission of final report)

_____
_____
_____
_____

Report Distribution:

An audited draft report will be prepared for this study by the Test Facility. The audited draft report will be sent electronically (wfw version) to [redacted] along with the final protocol and any protocol amendments. Interim tables may be submitted when more than one test article is being evaluated per Protocol.

One PDF copy of the entire Final Report and [redacted] eport in Excel format will be submitted to the Sponsor electronically to the [redacted]

Archiving of Materials:

Upon completion of the Final Report, all raw data, documentation, records, protocol, protocol amendments (if applicable) and original final report will be archived as indicated below:

Archive materials will be shipped to the following address:

[redacted]

Please notify [redacted] prior to shipment.

Sponsor's Representative: [redacted] **Date:** 24 Jun 2014

APPENDIX B

EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 2-Jul-14 Study No. [redacted]
TEST MATERIAL: [redacted] 1% phytosteryl/behenyl/octyldodectyl lauroyl glutamate in a face cream
TEST ARTICLE: [redacted]

t_{50} = >16 Hours

PRELIMINARY
CONCENTRATION: 100%

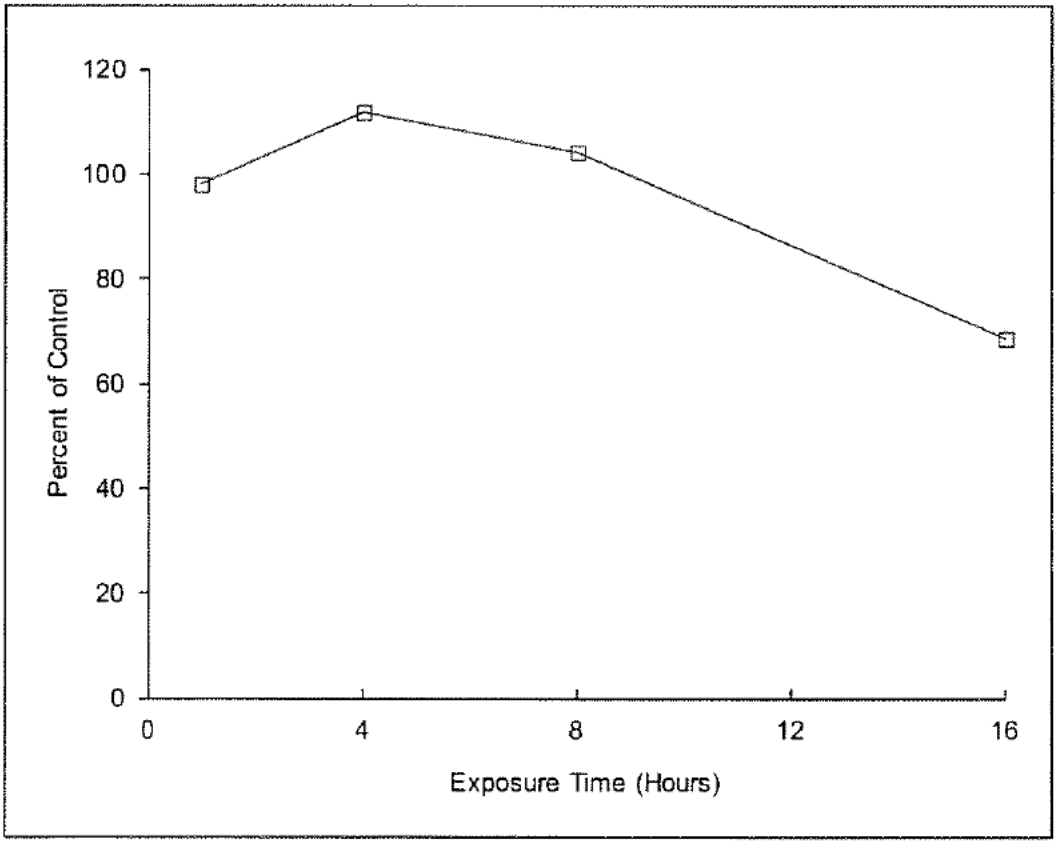
TIME EXPOSURE (Hours)	PERCENT VIABLE
1	98.1
4	111.9
8	104.1
16	68.7

y = Percent Viable
x = Exposure Time
slope=rise/ run=(y1-y2)/ (x1-x2)
y intercept=y-(slope*x)

	X		Y
1	16.0	1	68.7
2	16.0	2	68.7
3	#DIV/ 0!	3	50
slope =			#DIV/ 0!
y intercept =			#DIV/ 0!

[redacted]

CONCENTRATION: 100%
PRELIMINARY



Study No. [redacted]

EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 2-Jul-14

TEST MATERIAL: 0.3% TRITON® -X-100

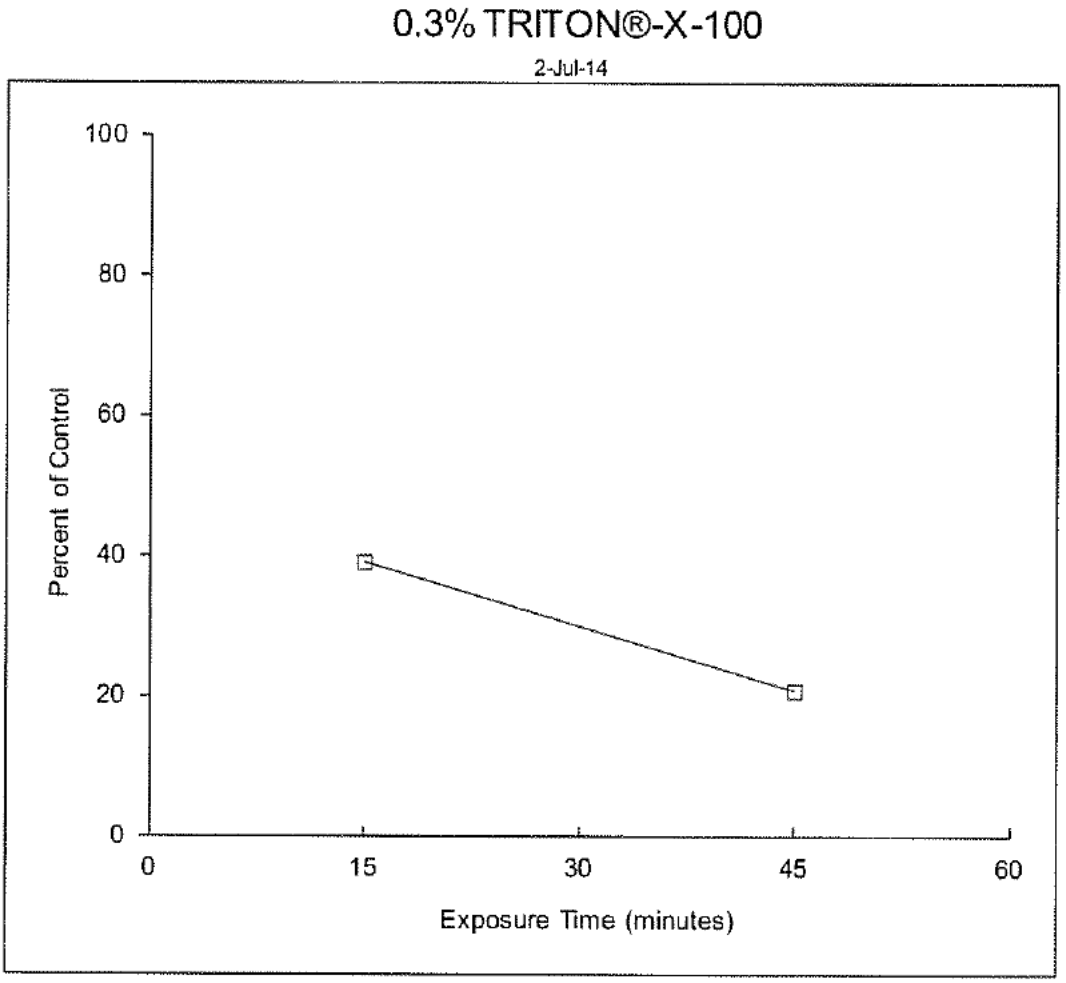
t_{50}= <15 Minutes

TIME EXPOSURE (Minutes)	PERCENT VIABLE
15	39.0
45	20.8

y = Percent Viable
x = Exposure Time
slope=rise/ run=(y1-y2)/ (x1-x2)
y intercept=y-(slope*x)

	X		Y
1	15.0	1	39
2	15.0	2	20.8
3	#DIV/ 0!	3	50

slope = #DIV/ 0!
y intercept = #DIV/ 0!



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 9-Jul-14 Study No. [redacted]
TEST MATERIAL: [redacted] 1% phytosteryl/behenyl/octyldodectyl lauroyl glutamate in a face cream
TEST ARTICLE:

t_{50} = >24 Hours

TRIAL 1
CONCENTRATION: 100%

TIME EXPOSURE (Hours)	PERCENT VIABLE
8	107.5
16	84.2
20	112.8
24	94.2

y = Percent Viable
x = Exposure Time
slope=rise/ run=(y1-y2)/ (x1-x2)
y intercept=y-(slope*x)

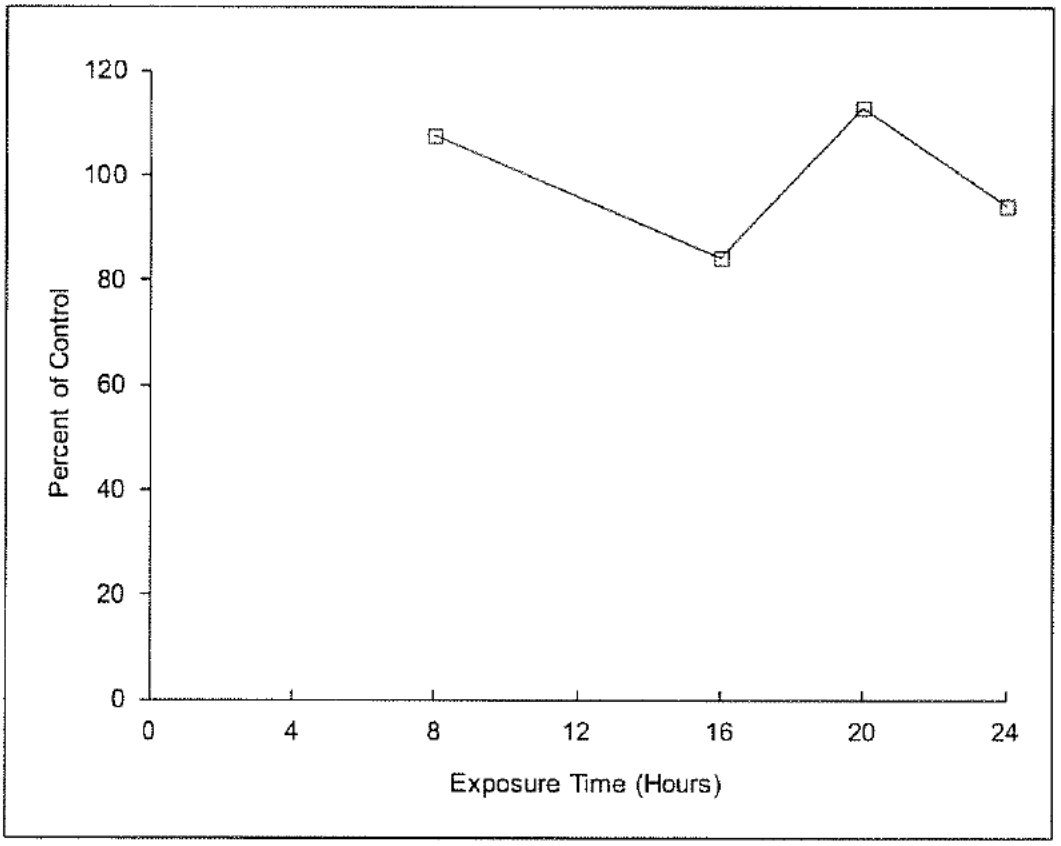
	X		Y
1	24.0	1	94.2
2	24.0	2	94.2
3	#DIV/ 0!	3	50

slope = #DIV/ 0!
y intercept = #DIV/ 0!

1% phytosteryl/behenyl/octyldodectyl lauroyl glutamate in a face cream

[redacted]

CONCENTRATION: 100%
TRIAL 1



Study No. [redacted]

EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 9-Jul-14

TEST MATERIAL: 0.3% TRITON® -X-100

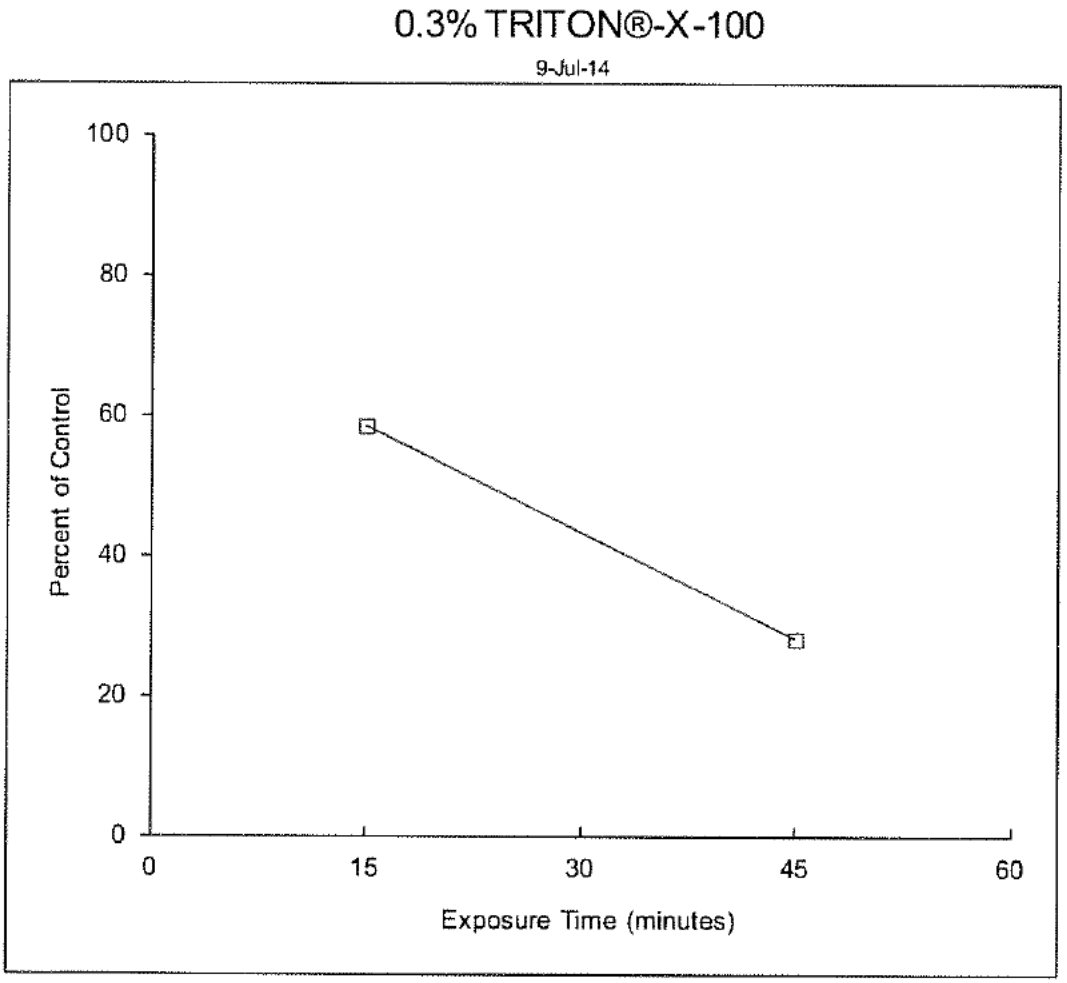
t_{50} = 23.3 Minutes

TIME EXPOSURE (Minutes)	PERCENT VIABLE
15	58.3
45	28.2

y = Percent Viable
x = Exposure Time
slope=rise/ run=(y1-y2)/ (x1-x2)
y intercept=y-(slope*x)

	X		Y
1	15.0	1	58.3
2	45.0	2	28.2
3	23.272425	3	50

slope = -1.003333
y intercept = 73.35



APPENDIX C

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Fisher Scientific

1 Reagent Lane
Fair Lawn, NJ 07410
201.796.7100 tel
201.796.1329 fax

Certificate of Analysis

Fisher Scientific's Quality System has been found to conform to Quality Management System Standard ISO9001:2008 standard by SAI Global Certificate Number CERT - 0064970

This is to certify that units of the above mentioned lot number were tested and found to comply with the specifications of the grade listed. Certain data have been supplied by third parties. Fisher Scientific expressly disclaims all warranties, expressed or implied, including the implied warranties of merchantability and fitness for a particular purpose. Certain products (USP/FCC/NF/EP/BP/JP grades) are sold for use in food, drug, or medical device manufacturing. Fisher does not claim regulatory coverage under 21 CFR nor maintain DMF's with the FDA. The following are the actual analytical results obtained.

Catalog Number	BP151	Quality Test / Release Date	12/5/2013
Lot Number	136597	Expiration Date	Dec/15
Description	TRITON X-100(R)		
Country of Origin	United States		
Chemical Origin	Organic - synthetic		
BSE/TSE Comment	No animal products are used as starting raw material ingredients, or used in processing, including lubricants, processing aids, or any other material that might migrate to the finished product.		

Result name	Units	Specifications	Test Value
APPEARANCE		REPORT	CLEAR, COLORLESS LIQUID
CLOUD POINT	DEGREES CENTIGRADE	Inclusive Between 63 - 69	66.0
IDENTIFICATION	PASS/FAIL	= PASS TEST	PASS TEST
PH 5% SOLUTION @ 25 DEG C		Inclusive Between 6 - 8	6.0
SOLUBILITY	PASS/FAIL	= Colorless, clear to slightly hazy solution	COLORLESS CLEAR

CERTIFIED BY

Lab Manager BPF

Note: The data listed is valid for all package sizes of this lot of this product, expressed as a extension of this catalog number listed above. If there are any questions with this certificate, please call Chemical Services at (800) 227-6701.
*Based on suggested storage condition

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PRODUCT: WATER – Ultra Pure (Cell Culture Grade)

CATALOG#: 118-162-131 Size: 1000ml

LOT#: 720328

CERTIFICATE OF ANALYSIS

This product is de-ionized by reverse osmosis and sterile filtered.

	Specifications	Result
RESISTIVITY:	15 – 20 Meg Ohms/cm	18.40 Meg Ohms/cm
ENDOTOXIN:	Less than 0.06 Eu/ml	pass
APPEARANCE:	Clear colorless fluid.	pass
STERILITY:	No evidence of bacterial or fungal contamination after 14 days testing on Fluid Thioglycollate and Sabouraud dextrose broth.	pass

RECOMMENDED STORAGE CONDITIONS: 15 – 30°C

MANUFACTURED DATE: 11/2013

EXPIRATION DATE: 11/2015

Ronald L. Brown 1/13/14

Ronald L. Brown, Ph.D. Date
Director of Quality Assurance

FOR RESEARCH USE ONLY
NOT FOR IN-VITRO DIAGNOSTIC USE.

7581 Lindbergh Drive • Gaithersburg, MD 20879
(301) 840-9331 • Toll Free (800) 443-9331 • Fax (301) 840-0743
Email: customerservice@qualitybiological.com • Web Site: www.qualitybiological.com

HUMAN CUMULATIVE IRRITATION PATCH TEST

NCCI STUDY [redacted]

[redacted]

[redacted]:

[redacted] 1% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream
Distilled Water – I
Sodium Lauryl Sulfate – J

[redacted]

PERFORMED BY:

NORTH CLIFF CONSULTANTS, INC.
3747 WARSAW AVENUE
CINCINNATI, OH 45205 USA

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STUDY OBJECTIVE: To assess the potential of test substances to elicit human skin irritation by repetitive topical application.

INVESTIGATIVE FACILITY: North Cliff Consultants, Inc. (NCCI)
3747 Warsaw Avenue
Cincinnati, OH 45205 USA

PRINCIPAL INVESTIGATOR: Mary S. Bailey, Ph.D.

CONSULTING DERMATOLOGIST: William Cohen, M.D.

STUDY MANAGER: Sheila Garnett

SITE OF INVESTIGATION: North Cliff Consultants, Inc. (NCCI)
3747 Warsaw Avenue
Cincinnati, OH 45205 USA

STUDY COORDINATOR: Cheryl Davis

SKIN GRADER: Dellene Arnold

SPONSOR: [redacted]

SPONSOR REPRESENTATIVE: [redacted]

STUDY DATES: 10-July-2014 – 17-July-2014

NUMBER OF SUBJECTS COMPLETING THE STUDY: 25

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QUALITY ASSURANCE STATEMENT

STUDY NUMBER: [redacted]

TYPE OF STUDY: HUMAN CUMULATIVE IRRITATION PATCH TEST

SITE OF STUDY: NORTH CLIFF CONSULTANTS, INC.
3747 Warsaw Ave. Cincinnati, Ohio 45205

This study was monitored in accordance with the Standard Operating Procedures of North Cliff Consultants, Inc. Completed study records and the final report were also audited.

Reviewed By:

9-19-14
Christopher J. Meyer / Date
Quality Assurance

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STUDY OVERVIEW

The study was conducted according to the Investigator's protocol, Human Cumulative Irritation Patch Test, approved and signed by the Sponsor's representative on May 12, 2014 and by the Principal Investigator on July 8, 2014.

A copy of the protocol is presented beginning on page 32 of this report.

TEST SUBJECT ACCOUNTABILITY

Total Number Visited Site:	42	Number not offered consent (Study Full):	10
		Number refused consent:	0
Number Read and Signed Consent:	32	Number screened and excluded (screen fails): (See Report of Excused Panelists and Reasons, page 30.)	4
Number enrolled in study:	28	Number dropped: (See Reasons For Failure to Complete Test, page 31.)	3
Number completed study: M: F:	**25** 6 19		

Twenty-five (25) healthy male and female subjects, ages 18 through 65 years, completed all aspects of the study. Originals of each subject's informed consent, medical history and skin sensitivity questionnaires are located in the Investigator's study records. A record of each subject's visits to the laboratory for evaluation was maintained and is also located in the Investigator's study records.

STUDY PROCEDURES:

Study Day 1, Screening: Prospective subjects arriving at the testing facility provided written informed consent prior to any other study procedures. Subjects answered questions regarding their medical and dermatological health history and had the skin on their backs visually examined for exclusionary conditions (scars, moles, tattoos, etc.) to determine eligibility for the study. Enrolled subjects were then patched with ten (10) semi-occlusive patches containing the test substances (five [5] on each side). Subjects were instructed to keep their patches dry and not use any sunscreens, moisturizers, or any other personal care products, including skin lotion, creams or oils on the patch sites during the entire study. Also, subjects were instructed to not attempt to reapply patches should they fall off, and were scheduled to return the following day to have the patches removed after 23 hours (+/- 1 hour). Subjects were provided with a copy of their signed Informed Consent Document and written instructions for care of their patch sites, including contact telephone numbers.

Study Days 2-6, Patch Wear and Grading: Upon return to the testing facility, the patches were removed and discarded by the study staff. The skin sites were gently wiped with water and patted dry to remove excess test substance immediately following patch removal. Thirty (30) minutes (+/- 10 minutes) following patch removal the skin sites were graded by a qualified skin grader (D. Arnold) using a lamp with a 60w daylight blue bulb, according to the grading scale presented in Protocol Appendix B. Following the grading procedure, patches were again applied to the same area on the subjects' back, and subjects were given instructions to return for the next visit.

Study Day 7, Final Grading Day: Subjects returned to the testing facility, had their patches removed and the skin sites were graded as described above. A Board Certified Dermatologist (W. Cohen) was present to evaluate the sites for the final grading procedure. Following the final grading, the subjects' participation in the study was considered complete.

Any site exhibiting a grade of 2.0 or greater was excluded from further treatment through the end of the study. The site continued to be graded and the grades were recorded through the duration of the study. On the final day of the study, five (5) subjects exhibited grades of 2.0 or above and were required to report to the testing facility for follow-up grading.

One (1) subject was unable to return for follow-up grading; subject was followed up with by phone. Four (4) subjects returned to the site for follow-up grading; all elevated grades were resolving. The subjects' participation was then considered complete. A record of each subject's grading measurements was maintained and is located in the Investigator's study records.

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DEVIATIONS FROM PROTOCOL:
No deviations to the protocol were reported.

PATCH WEAR TIME DEVIATIONS:

Subject	Test Date (Day)	Description
1003, 1030 1031	7/11/14 (2)	All patches worn >24 hours.
1028	7/14/14 (5)	Patch L1 (Code E) worn <12 hours.
1028	7/14/14 (5)	Patch L2 (Code F) worn >12 hours - <22 hours.

SUMMARY OF DATA
Grades were recorded all seven days for twenty-five (25) subjects. Daily scores for each subject, average daily skin grades, cumulative irritation index and the overall average skin grade for each test material are presented in Tables A through J, pages 20 - 29.

For each test material, the 'Group Total Irritation Score' is the sum total of skin grades of all subjects. The 'Cumulative Irritation Index' is the 'Group Total Irritation Score' divided by: 'highest anticipated score' (4) X 'number of subjects completing the study' (25) X 'number of days' (7). The 'Overall Average Skin Grade' is the sum of the 'Average Daily Skin Grade' (from the Table of Subject Scores) divided by the 'number of scoring days.'

The table below summarizes, in ascending order, the group total irritation score, cumulative irritation index, range of scores and overall average skin grade for each test material.

Test Material	Group Total Irritation Score	Cumulative Irritation Index	Range of Scores	Overall Average Skin Grade
I: DISTILLED WATER	82.5	0.12	0.0 – 2.0	0.47
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] 1% phytosteryl/behenyl/ octyldodecyl lauroyl glutamate in a face cream	147.0	0.21	0.0 – 2.0	0.84
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
J: SODIUM LAURYL SULFATE	327.5	0.47	0.0 – 3.0	1.87

The Consulting Dermatologist, W. Cohen, M.D., was present at the study site for the final subject evaluations and reported no unexpected skin conditions observed.

Do Not Cite or Quote

ADVERSE EVENTS:

Three (3) adverse events occurred during the course of this study; all AE's were related to study procedures, but not to study test materials. A summary of these events is provided below. Complete details can be found in the Adverse Event Reporting Forms beginning on page 14 of this report.

Subject	Event	Dates	Relationship to Test Material or Study Procedures	Withdrawn/Continued
1013	Tape irritation	07/17/14 – 07/21/14	Definitely Related	Continued
1016	Tape irritation	07/15/14 – 07/21/14	Definitely Related	Withdrawn
1023	Tape irritation	07/16/14 – 07/21/14	Definitely Related	Continued

1% phytosteryl/behenyl/
octyldodecyl lauroyl glutamate
in a face cream

CONCLUSIONS:

Based on the Cumulative Irritation Index for each test material under conditions of the 7-Day Human Cumulative Irritation Patch Test, Test Material J (Positive irritant control) elicited the greatest skin irritation responses; Test Materials [redacted] elicited skin responses similar to the negative irritant control (Test Material I); [redacted]

NORTH CLIFF CONSULTANTS, INC.

Mary S. Bailey 09/19/2014

Mary S. Bailey, Ph.D. / Date
Principal Investigator

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ATTACHMENTS TO THE STUDY REPORT

TEST MATERIAL INFORMATION

PERSONNEL RECORD

VISITORS TO THE STUDY SITE

PRODUCT RANDOMIZATION

PRODUCT CODE DESCRIPTION

ADVERSE EVENT REPORTING FORMS

GRADES

REPORT OF EXCUSED PANELISTS AND REASONS

REASONS FOR FAILURE TO COMPLETE TEST

PROTOCOL

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TEST MATERIAL INFORMATION:

[redacted]

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TEST MATERIAL INFORMATION:

Sponsor Sample Code/Identification Numbers: [redacted]

Color: [redacted] - Light Yellow

1% phytosteryl/behenyl/ octyldodecyl lauroyl glutamate in a face cream

Physical Form (Product Type): [redacted] - Moisturizer Cream

Date Received: 6/19/14

Product Weight as Received: [redacted] - 603.0 g

Weight After Subject Dosing: [redacted] - 496.0 g

Storage: Stock Test Substance: Room temperature

Test Concentration: 100%, neat

Vehicle: N/A

Storage: Test Solution: N/A

How to Prepare Test Substance for Testing: Apply as provided

Test Solution Preparation Frequency: Not applicable

Mixing/Stirring Requirements: Not applicable

Open-to-Air Requirements: Apply test substance to patch pad no longer than 15 minutes before application.

Disposition of Test Substance/Test Solutions: Dispose of unused stock test substance after the final report is issued.

Patch Type: Semi-occlusive patch. An individual patch made with a 2cm by 2cm square of Webril affixed to a strip of Micropore[R] tape.

Amount to be Applied per Patch: 0.2 ml per patch

Special Inclusion/Exclusion Criteria: None

Dermatologist Required: Yes, at final grade day only

TEST MATERIAL INFORMATION:

Sponsor Sample Code/Identification Numbers: [redacted] N/A (Distilled Water)

Color: Colorless

Physical Form (Product Type): Liquid (Negative Irritant Control)

Date Received: N/A (Supplied by NCCI)

Amount Received: N/A **Weight After Subject Dosing:** N/A

Storage: Stock Test Substance: Room temperature

Test Concentration: 100%, Neat

Vehicle: N/A

Storage: Test Solution: N/A

How to Prepare Test Substance for Testing: Not applicable.

Test Solution Preparation Frequency: Not applicable.

Mixing/Stirring Requirements: Not applicable.

Open-to-Air Requirements: Apply test substance to patch pad no longer than 15 minutes before application.

Disposition of Test Substance/Test Solutions: Not applicable.

Patch Type: Semi-occlusive patch. An individual patch made with a 2cm by 2cm square of Webril affixed to a strip of Micropore[R] tape.

Amount to be Applied per Patch: 0.3ml per patch

Special Inclusion/Exclusion Criteria: None

Dermatologist Required: Yes, at final grade day only

DISTILLED WATER INFORMATION: Kroger Brand Distilled Water, 19:10
B 18-1080, Best by 03/30/15

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TEST MATERIAL INFORMATION:

Sponsor Sample Code/Identification Numbers: [redacted] N/A (Sodium Lauryl Sulfate [SLS])

Color: White

Physical Form (Product Type): Powder (Positive Irritant Control)

Date Received: N/A (Supplied by NCCI)

Amount Received: N/A **Weight After Subject Dosing:** N/A

Storage: Stock Test Substance: Room temperature

Test Concentration: 0.8% w/v

Vehicle: Distilled water

Storage: Test Solution: N/A

How to Prepare Test Substance for Testing: N/A

Test Solution Preparation Frequency: Daily

Mixing/Stirring Requirements: Mix/stir immediately before applying to patches.

Open-to-Air Requirements: Apply test solution to patch pad no longer than 15 minutes before application.

Disposition of Test Substance: Not applicable.

Patch Type: Semi-occlusive patch. An individual patch made with a 2cm by 2cm square of Webril affixed to a strip of Micropore[R] tape.

Amount to be Applied per Patch: 0.3ml per patch

Special Inclusion/Exclusion Criteria: None

Dermatologist Required: Yes, at final grade day only

SODIUM LAURYL SULFATE INFORMATION: Sodium Dodecyl Sulfate, Sigma Aldrich, L6026-50G, Lot #SLBF8724V
PC Code 1001601043; Opened 02/25/14.

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STUDY PERSONNEL RECORD

Name:	Duties:	Name:	Duties:
Sheila Garnett	3	Lisa DeStazio	25 b
Cheryl Davis	1 b 4 5 a b 6 8 b 24 c 25 a	Linda Geil	7 8 a 24 c
Dellene Arnold	17 a b	Keith Hamilton	35
Mary Bailey, Ph. D	1 a	Cathy Holloway	6 (7 8 a training) 24 c
Susan Banfield	6 24 c	Sharon King	24 a b
Jeanne Bianco	24 a b	Kristen Kluener	35
Madonna Brehm	35	Cathy Knue	25 a
Kathy Brissie	6 7 8a 24 c	Kimberly Roark	5 a 6 8 b 24 c
Patti Brothers	5 a 6 7 24 c	Nancy Selm	6 24 c 25 b
William Cohen	2b	Courtney Sutton	35
Susan Crawford	7 8 a	Patricia Wilson	35

1 a) Investigator b) Designee	14 Product Interviewer / Diary Review	26 Confidentiality	38 Urine Testing
2 Medical a) Investigator b) Consultant	15 Water Conditions Check	27 Subjective Quest'naire Review	39 HR a) Instructor b) Monitor
3 Study Manager	16 Env'mntl Room a) Entry b) Conditions	28 Sensitivity Quest'naire Review	40 HR Soap Solution Prep
4 Study Coordinator	17 Visual a) Grader b) Recorder	29 Monitor	41 Initial Pad/Vial Weights
5 Sample a) Weight b) Count	18 Corneometer a) Operator b) Recorder	30 Tape Stripping	42 Final Pad/Vial Weights
6 Sample Preparation	19 DermaLab a) Operator b) Recorder	31 D-Squame Samples	
7 Patch Preparation	20 Vapometer a) Operator b) Recorder	32 Scalp Sample a) technician	
8 Patch a) Application b) Removal	21 Chromameter a) Operator b) Recorder	b) assistant c) slide preparation	
9 Treatment a) Application b) Removal	22 Novameter a) Operator b) Recorder	33 Still Photographer	
10 Wash Technician	23 Ski-Con a) Operator b) Recorder	34 Video Photographer	
11 Drill Sergeant	24 a) Entry b) Pay c) Witness	35 Recruiter	
12 Product Placement	25 a) Informed Consent Process	36 Visual Acuity	
13 Product a) Distribution b) Return	25 b) Medical/Screening Interview	37 Vital Signs	

RECORD OF MONITORING VISITS/VISITORS TO STUDY SITE

VISITORS	DATE	TIMES	REASON
William Cohen, M.D.	07/17/14	7:59 a.m. – 11:36 a.m.	Consulting Dermatologist

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NORTH CLIFF CONSULTANTS, INC.

3747 WARSAW AVENUE
CINCINNATI, OHIO 45205
PHONE: (513) 251-4930
FAX: (513) 557-3732

6831 COLERAIN AVENUE
CINCINNATI, OHIO 45239
PHONE: (513) 245-5483
FAX: (513) 245-5485

CONSULTING PHYSICIANS' STUDY MONITORING RECORD

On July 17, 2014 (date) I attended Study # 01-1402 as indicated in the study protocol. In my opinion, all aspects of the study protocol design and execution were being observed and adhered.

Observations during this monitoring visit were as follows:

☒ There were no adverse cutaneous conditions observed on any of the participant test (skin, eye, gynecological, etc.) subjects which would be characterized as unexpected from participating in this type study.

☐ The following test subjects exhibited responses which may or may not be related to exposure to one or more of the test products and specific comments regarding these observations follow:

Test Subject No.(s) _____ _____ _____ _____ _____ _____ _____

Comments: _____

_____

_____

_____

_____

William Cohen 7/17/2014
(Consulting Physician's Signature / Date)

William Cohen 7/17/2014
(Consulting Physician's Printed Name)

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PRODUCT RANDOMIZATION

PATCH SITE – UPPER BACK

RANDOMIZATION	LEFT					RIGHT				
NUMBER	1	2	3	4	5	6	7	8	9	10
001	A	B	C	D	E	F	G	H	I	J
002	B	C	D	E	F	G	H	I	J	A
003	C	D	E	F	G	H	I	J	A	B
004	D	E	F	G	H	I	J	A	B	C
005	E	F	G	H	I	J	A	B	C	D
006	F	G	H	I	J	A	B	C	D	E
007	G	H	I	J	A	B	C	D	E	F
008	H	I	J	A	B	C	D	E	F	G
009	I	J	A	B	C	D	E	F	G	H
010	J	A	B	C	D	E	F	G	H	I
011	A	B	C	D	E	F	G	H	I	J
012	B	C	D	E	F	G	H	I	J	A
013	C	D	E	F	G	H	I	J	A	B
014	D	E	F	G	H	I	J	A	B	C
015	E	F	G	H	I	J	A	B	C	D
016	F	G	H	I	J	A	B	C	D	E
017	G	H	I	J	A	B	C	D	E	F
018	H	I	J	A	B	C	D	E	F	G
019	I	J	A	B	C	D	E	F	G	H
020	J	A	B	C	D	E	F	G	H	I
021	A	B	C	D	E	F	G	H	I	J
022	B	C	D	E	F	G	H	I	J	A
023	C	D	E	F	G	H	I	J	A	B
024	D	E	F	G	H	I	J	A	B	C
025	E	F	G	H	I	J	A	B	C	D
026	F	G	H	I	J	A	B	C	D	E
027	G	H	I	J	A	B	C	D	E	F
028	H	I	J	A	B	C	D	E	F	G

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PRODUCT CODE DESCRIPTION

SAMPLE CODE	TEST SUBSTANCE INFORMATION	PREPARATION	PATCH TYPE
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	1% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream	0.2ml - 100% neat	Semi-Occlusive
i	Distilled Water	0.3ml distilled water	Semi-Occlusive
J	Sodium Lauryl Sulfate	0.3ml of 0.8% w/v SLS in distilled water	Semi-Occlusive

NCC Study# [redacted]

ADVERSE EVENT REPORTING FORM

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N/A (RE) CO 7-17-14

Subject #: 1013 Subject Initials: B J M Product #: ~~F, G, I~~

Date Reported: 7-17-14 Date of Onset: 7-17-14 Date Resolved: 7-21-14

- Questions #1 and 2 are to be completed by the Investigator after review of complete event history.
- If event is not related to test products or procedures, questions #4, 5, 6 & 7 are not answered.

SECTION A: SUMMARY OF EVENT

Describe event: tape irritation may be contributing to infiltration in to webril sites.

1. Was adverse event related to treatment or other study procedure(s)?

   ☐ Not related ☐ Possibly related ☐ Probably related ☐ Definitely related ☐ Relationship unknown

2. Severity (greatest severity during event): ☐ Mild ☐ Moderate ☐ Severe ☐ Serious

3. Subject Outcome: *(check all that apply)*

   ☑ Continued on Study ☐ Consulted physician ☐ Medication taken (Complete Section B on Page 2)

   ☐ Withdrawn from Study ☐ Hospitalized ☐ Other (explain in #8)

4. Test site(s) or body part affected: 6, 7, 9 back

5. Product Treatments at affected test site(s) or body part: ☐ No Change

   ☐ Treatment Temporarily Stopped: Stop-_____ Restart-_____

   ☐ Treatment Stopped Permanently: Beginning _____(date)

6. If event occurred at a test site, how long did the symptoms last: _____hrs/days/wks/persists/unknown

7. If event is possibly an allergic reaction:

   Has subject experienced similar symptoms with similar products? ☐ NO ☐ YES

   Does subject have an allergy to any foods, cosmetics, household products or medications? ☐ NO ☐ YES

   Were any new foods, cosmetics, household products or medications used since the study began? ☐ NO ☐ YES

   If yes to any of the above, give details: _____

8. Comments: Today was first day that we actually saw any tape irritation. 7-17-14 CD

[signature] 8-28-14

Person Reporting Initial Event Date

NCC Study# [redacted]

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ADVERSE EVENT REPORTING FORM

(RE) N/A 7-17-14

Subject #: 1013 Subject Initials: B J M Product #: F, G, I

SECTION B: MEDICATION TAKEN DUE TO EVENT

Medication	Total Daily Dose	Start Date mm/dd/yy	Stop Date mm/dd/yy	Indication
Cortisone Cream	As directed	7 / 17 / 14	7 / 21 / 14	
		/ /	/ /	
		/ /	/ /	
		/ /	/ /	

Comments:

SECTION C: FOLLOW-UP ACTION TAKEN

Date/ Initials	Action Taken (e.g. went to physician, used medication, telephone conversation)	Comments (results of action)
7/21/14 @RA	returned to site for	
	post study follow-up	healing very well - to continue
		cortisone crm. application for several
		days. No follow-up needed. @RA (grader) 7/21/14

Comments: excoriated edges

Tape reaction. Dr. Cohen advised panelist to apply cortisone cream to sites 2x/day. Will return on Monday to see grader (@RA) 7/17/14 CII (co-ordinator) for @RA (grader) 7/21/14.

AE related to study procedure, not to study test materials. MSB 09/02/2014

Mary S. Bailey 09/02/2014

Investigator Date

NCC Study# [redacted]

ADVERSE EVENT REPORTING FORM

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N/A (RE) CD 7-17-14

Subject #: 1016 Subject Initials: C A K Product #: ~~AH~~

Date Reported: 7-15-14 Date of Onset: 7-15-14 Date Resolved: 7-21-14

- Questions #1 and 2 are to be completed by the Investigator after review of complete event history.
- If event is not related to test products or procedures, questions #4, 5, 6 & 7 are not answered.

SECTION A: SUMMARY OF EVENT

Describe event: Tape irritation on all patched areas. Some open sore areas due to tape. Grader's (DPA) decision not to retape - OK'd per S.G./study manager.

1. Was adverse event related to treatment or other study procedure(s)?

   ☐ Not related ☐ Possibly related ☐ Probably related ☐ Definitely related ☐ Relationship unknown

2. Severity (greatest severity during event): ☐ Mild ☐ Moderate ☐ Severe ☐ Serious

3. Subject Outcome: *(check all that apply)*

   ☐ Continued on Study ☐ Consulted physician ☐ Medication taken (Complete Section B on Page 2)

   ☑ Withdrawn from Study ☐ Hospitalized ☐ Other (explain in #8)

4. Test site(s) or body part affected: all, back

5. Product Treatments at affected test site(s) or body part: ☐ No Change

   ☐ Treatment Temporarily Stopped: Stop-_____ Restart-_____

   ☑ Treatment Stopped Permanently: Beginning 7-15-14 (date)

6. If event occurred at a test site, how long did the symptoms last: _____hrs/days/wks/persists/unknown

7. If event is possibly an allergic reaction:

   Has subject experienced similar symptoms with similar products? ☐ NO ☐ YES

   Does subject have an allergy to any foods, cosmetics, household products or medications? ☐ NO ☐ YES

   Were any new foods, cosmetics, household products or medications used since the study began? ☐ NO ☐ YES

   If yes to any of the above, give details: _____

8. Comments: _____

Cheryl Davis 7-16-14

Person Reporting Initial Event Date

NCC Study# [redacted]

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ADVERSE EVENT REPORTING FORM

(RE) 7-17-14 CD

Subject #: 1016 Subject Initials: C A K Product #: AH

SECTION B: MEDICATION TAKEN DUE TO EVENT

Medication	Total Daily Dose	Start Date mm/dd/yy	Stop Date mm/dd/yy	Indication
hydrocortisone cream	As directed	7/17/14	7/21/14	
		/ /	/ /	
		/ /	/ /	
		/ /	/ /	

Comments:

SECTION C: FOLLOW-UP ACTION TAKEN

Date/ Initials	Action Taken (e.g. went to physician, used medication, telephone conversation)	Comments (results of action)
7/21/14 [illegible]	returned to site for	#6, 7 tape reaction resolving well
	followup grading	#9 will require follow-up by MSB
		(investigator) on 7/23/14. Continue cortisone. 7-21-14 [illegible]

Comments:

Tape reaction - all sites are healing well except #9.
7-17-14 Dr. Cohen advised panelist to use cortisone to areas 2x/day x 7 days. Will see grader (DRA) on Monday 7-21-14
AE related to procedures, not to study test materials.
Subject lost to follow-up after visit 7/21/14. MSB 9/3/14

Mary S. Bailey 09/03/2014
Investigator Date

NCC Study# [redacted]

ADVERSE EVENT REPORTING FORM

Distributed for Comment Only -- Do Not Use or Quote

N/A (RE) CD 7-17-14

Subject #: 1023 Subject Initials: H J R Product #: ~~E, G, H~~

Date Reported: 7-17-14 Date of Onset: 7-16-14 Date Resolved: 7-21-14

- Questions #1 and 2 are to be completed by the Investigator after review of complete event history.
- If event is not related to test products or procedures, questions #4, 5, 6 & 7 are not answered.

SECTION A: SUMMARY OF EVENT

Describe event: Tape irritation

1. Was adverse event related to treatment or other study procedure(s)?

   ☐ Not related ☐ Possibly related ☐ Probably related ☐ Definitely related ☐ Relationship unknown

2. Severity (greatest severity during event): ☐ Mild ☐ Moderate ☐ Severe ☐ Serious

3. Subject Outcome: *(check all that apply)*

   ☑ Continued on Study ☐ Consulted physician ☐ Medication taken (Complete Section B on Page 2)

   ☐ Withdrawn from Study ☐ Hospitalized ☐ Other (explain in #8)

4. Test site(s) or body part affected: 7, 9, 10 back

5. Product Treatments at affected test site(s) or body part: ☐ No Change

   ☐ Treatment Temporarily Stopped: Stop-_____ Restart-_____

   ☐ Treatment Stopped Permanently: Beginning _____(date)

6. If event occurred at a test site, how long did the symptoms last: _____hrs/days/wks/persists/unknown

7. If event is possibly an allergic reaction:

   Has subject experienced similar symptoms with similar products? ☐ NO ☐ YES

   Does subject have an allergy to any foods, cosmetics, household products or medications? ☐ NO ☐ YES

   Were any new foods, cosmetics, household products or medications used since the study began? ☐ NO ☐ YES

   If yes to any of the above, give details: _____

8. Comments: initial onset was treated with "special" patches to relieve tape adhesive of patches causing more skin irritation / overlaying sticky tape of patch with Micropore tape & taping beyond with tape to adhere beyond irritation. DEA 7/16/14

[signature] 7-17-14

Person Reporting Initial Event Date

NCC Study# [redacted]

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ADVERSE EVENT REPORTING FORM

RE CD 7-17-14

Subject #: 1023 Subject Initials: H J R Product #: ~~E, G, H~~ N/A

SECTION B: MEDICATION TAKEN DUE TO EVENT

Medication	Total Daily Dose	Start Date mm/dd/yy	Stop Date mm/dd/yy	Indication
cortisone Cream	as directed	7/17/14	7/21/14	
		/ /	/ /	
		/ /	/ /	
		/ /	/ /	

Comments:

SECTION C: FOLLOW-UP ACTION TAKEN

Date/ Initials	Action Taken (e.g. went to physician, used medication, telephone conversation)	Comments (results of action)

Comments:

Dr. Cohen told panelist to put cortisone on irritated areas 2x/daily until better. Returning to see grader (DRA) on Monday 7-21-14 CH (co-ordinators)

7/21/14 Returned to site/open areas have healed with application of cortisone cream. Sub. c/o itching of L-10 but has dissipated with cortisone application. DRA 7/21/14

AE related to study procedure, not to study test materials. 09/02/2014

Mary S. Bailey 09/02/2014

Investigator Date

HUMAN CUMULATIVE IRRITATION PATCH TEST

[redacted]: H[redacted] 1% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream

I: Distilled Water (negative control); J: Sodium Lauryl Sulfate (positive control)

NCCI STUDY [redacted]

PRODUCT H: [redacted] 1% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream

RANDOMIZATION NUMBER	SUBJECT NUMBER	SITE	PRODUCT	1	2	2 R	3	3 R	4	4 R	5	5 R	6	6 R	7	7 R	TOTAL SCORE	IRRITANCY INDEX
001	1002	8	H	0.0	0.0		0.0		0.0		0.0		0.0		0.0		0.0	0.00
002	1003	7	H	2.0	2.0	2.0	2.0	1.0	2.0	1.0	2.0	0.0	2.0	0.0	2.0	0.0	14.0	0.50
004	1005	5	H	0.0	0.0		0.0		0.0		0.5		0.5		0.0		1.0	0.04
005	1006	4	H	0.0	0.5		0.5		1.0		0.5		0.5		1.0		4.0	0.14
006	1007	3	H	0.0	0.0		0.0		0.5		1.0		1.0		1.0		3.5	0.13
007	1008	2	H	0.5	0.5		0.5		1.0		1.5		1.5		1.0		6.5	0.23
008	1009	1	H	0.5	0.0		0.0		1.0		0.5		1.0		1.5		4.5	0.16
009	1011	10	H	1.0	1.0		1.5		1.0		1.0		1.5		1.5		8.5	0.30
010	1010	9	H	0.0	0.5		0.0		1.0		1.0		0.5		1.0		4.0	0.14
011	1013	8	H	0.0	0.0		1.0		1.5		1.0		1.0		1.0		5.5	0.20
013	1014	6	H	0.5	1.5		1.5		1.5		1.5		1.5		1.5		9.5	0.34
015	1018	4	H	0.5	0.5		0.5		1.5		1.5		1.5		1.0		7.0	0.25
016	1017	3	H	0.0	1.0		0.5		1.0		1.5		1.0		1.0		6.0	0.21
017	1019	2	H	1.0	1.5		1.0		1.0		1.5		1.0		1.0		8.0	0.29
018	1021	1	H	1.0	0.0		1.0		1.5		1.5		1.0		1.0		7.0	0.25
019	1023	10	H	0.5	1.0		1.5		1.0		1.0		1.5		1.0		7.5	0.27
020	1022	9	H	0.5	0.0		1.0		0.5		1.0		0.5		0.5		4.0	0.14
021	1024	8	H	0.5	0.5		1.0		1.0		1.0		1.0		1.0		6.0	0.21
022	1025	7	H	0.0	0.0		1.5		1.0		1.0		1.0		1.0		5.5	0.20
023	1026	6	H	0.5	1.5		1.0		1.5		1.0		1.0		1.0		7.5	0.27
024	1027	5	H	0.0	0.5		1.0		1.0		1.5		1.0		1.0		6.0	0.21
025	1028	4	H	0.0	2.0		2.0	1.0	2.0	0.0	2.0	0.0	2.0	0.0	0.0		10.0	0.36
026	1029	3	H	0.0	0.0		0.5		0.0		0.5		0.5		0.5		2.0	0.07
027	1030	2	H	0.0	0.0		0.5		1.0		0.5		1.0		0.0		3.0	0.11
028	1031	1	H	0.0	0.5		1.0		1.5		1.5		1.0		1.0		6.5	0.23
	H AVERAGE DAILY SKIN GRADE			**0.36**	**0.60**		**0.84**		**1.04**		**1.10**		**1.04**		**0.90**			

GROUP TOTAL IRRITATION SCORE 147.0

CUMULATIVE IRRITATION INDEX 0.21

OVERALL AVERAGE SKIN GRADE 0.84

HUMAN CUMULATIVE IRRITATION PATCH TEST

[redacted]or Quote

I: Distilled Water (negative control); J: Sodium Lauryl Sulfate (positive control)

NCCI STUDY [redacted]

PRODUCT I: DISTILLED WATER

RANDOMIZATION NUMBER	SUBJECT NUMBER	SITE	PRODUCT	1	2	2 R	3	3 R	4	4 R	5	5 R	6	6 R	7	7 R	TOTAL SCORE	IRRITANCY INDEX
001	1002	9	I	0.0	0.0		0.0		0.0		0.0		0.0		0.0		0.0	0.00
002	1003	8	I	0.0	0.0		0.5		1.5		0.5		1.0		1.0		4.5	0.16
004	1005	6	I	0.0	0.0		0.0		0.5		1.0		0.0		0.5		2.0	0.07
005	1006	5	I	0.0	0.0		0.0		0.5		0.5		0.5		0.5		2.0	0.07
006	1007	4	I	0.0	0.0		0.0		0.0		0.5		0.5		0.0		1.0	0.04
007	1008	3	I	0.0	0.0		0.0		1.0		1.5		1.0		1.0		4.5	0.16
008	1009	2	I	0.0	0.0		0.0		0.5		1.0		0.5		1.5		3.5	0.13
009	1011	1	I	0.5	0.0		0.0		1.0		1.0		1.0		0.0		3.5	0.13
010	1010	10	I	0.5	1.0		1.0		1.0		0.5		0.5		1.0		5.5	0.20
011	1013	9	I	1.0	0.5		1.5		0.5		0.5		0.5		1.0		5.5	0.20
013	1014	7	I	1.0	0.0		1.0		0.5		1.0		1.0		1.5		6.0	0.21
015	1018	5	I	0.0	0.0		0.5		0.5		0.0		0.5		0.5		2.0	0.07
016	1017	4	I	0.0	0.0		0.0		0.5		0.5		0.0		0.5		1.5	0.05
017	1019	3	I	0.0	0.0		0.0		0.0		1.5		0.5		0.0		2.0	0.07
018	1021	2	I	0.0	0.0		0.0		0.0		0.5		0.0		0.0		0.5	0.02
019	1023	1	I	1.5	1.5		2.0		2.0	1.0	2.0	1.0	2.0	1.0	2.0	1.0	13.0	0.46
020	1022	10	I	0.0	0.0		0.5		0.5		0.5		0.0		0.0		1.5	0.05
021	1024	9	I	0.0	0.5		0.0		1.0		1.0		0.5		1.5		4.5	0.16
022	1025	8	I	0.0	0.0		0.0		0.5		1.0		0.5		0.5		2.5	0.09
023	1026	7	I	0.0	0.5		0.0		1.0		0.5		0.5		1.0		3.5	0.13
024	1027	6	I	0.0	0.0		0.0		0.0		0.5		0.5		1.0		2.0	0.07
025	1028	5	I	0.0	0.5		1.0		0.5		0.5		0.5		0.5		3.5	0.13
026	1029	4	I	0.0	0.0		0.5		0.5		0.0		0.0		0.0		1.0	0.04
027	1030	3	I	0.0	0.0		0.5		0.5		0.5		1.0		0.5		3.0	0.11
028	1031	2	I	0.0	0.0		0.0		1.5		1.0		0.5		1.0		4.0	0.14
	I AVERAGE DAILY SKIN GRADE			**0.18**	**0.18**		**0.36**		**0.64**		**0.72**		**0.54**		**0.68**			

GROUP TOTAL IRRITATION SCORE	82.5
CUMULATIVE IRRITATION INDEX	0.12
OVERALL AVERAGE SKIN GRADE	0.47

HUMAN CUMULATIVE IRRITATION PATCH TEST

[redacted] r Quote

I: Distilled Water (negative control); J: Sodium Lauryl Sulfate (positive control)

NCCI STUDY [redacted]

PRODUCT J: SODIUM LAURYL SULFATE

RANDOMIZATION NUMBER	SUBJECT NUMBER	SITE	PRODUCT	1	2	2 R	3	3 R	4	4 R	5	5 R	6	6 R	7	7 R	TOTAL SCORE	IRRITANCY INDEX
001	1002	10	J	0.0	1.0		1.5		2.0		2.0	2.0	2.0	2.0	2.0	1.5	10.5	0.38
002	1003	9	J	1.0	2.5		2.5	2.0	2.5	2.0	2.5	1.5	2.5	1.5	2.5	1.5	16.0	0.57
004	1005	7	J	0.0	0.0		0.5		1.0		2.5		2.5	1.5	2.5	1.5	9.0	0.32
005	1006	6	J	0.0	0.0		0.5		1.0		3.0		3.0	2.0	3.0	2.0	10.5	0.38
006	1007	5	J	1.5	2.0		2.0	1.5	2.0	1.0	2.0	1.0	2.0	0.0	2.0	0.5	13.5	0.48
007	1008	4	J	0.5	1.5		3.0		3.0	3.0	3.0	2.5	3.0	2.5	3.0	2.0	17.0	0.61
008	1009	3	J	0.0	0.0		1.0		2.0		2.0	1.5	2.0	1.5	2.0	1.5	9.0	0.32
009	1011	2	J	0.5	0.5		2.0		3.0		3.0	2.5	3.0	1.5	3.0	1.0	15.0	0.54
010	1010	1	J	0.0	0.0		1.5		2.5		2.5	2.5	2.5	2.5	2.5	1.5	11.5	0.41
011	1013	10	J	1.5	2.0		2.0	2.0	2.0	1.5	2.0	1.5	2.0	1.0	2.0	0.5	13.5	0.48
013	1014	8	J	0.5	0.0		1.5		2.5		2.5	2.0	2.5	2.5	2.5	1.5	12.0	0.43
015	1018	6	J	0.0	2.0		2.5		2.5	2.5	2.5	1.5	2.5	1.5	2.5	1.5	14.5	0.52
016	1017	5	J	1.0	1.5		2.5		2.5	2.5	2.5	2.0	2.5	1.5	2.5	1.5	15.0	0.54
017	1019	4	J	0.0	1.5		2.5		2.5	2.0	2.5	2.0	2.5	1.5	2.5	1.5	14.0	0.50
018	1021	3	J	0.5	1.0		1.5		2.5		2.5	2.5	2.5	2.5	2.5	2.0	13.0	0.46
019	1023	2	J	1.0	2.0		2.5		2.5	2.0	2.5	1.5	2.5	1.5	2.5	1.5	15.5	0.55
020	1022	1	J	1.5	1.5		2.0		2.5		2.5	2.5	2.5	2.0	2.5	1.5	15.0	0.54
021	1024	10	J	0.5	1.5		1.5		1.5		2.0		2.0	2.0	2.0	1.5	11.0	0.39
022	1025	9	J	0.0	1.5		3.0		3.0	2.0	3.0	2.0	3.0	1.5	3.0	1.5	16.5	0.59
023	1026	8	J	0.0	0.5		2.0		2.0	2.0	2.0	2.0	2.0	1.5	2.0	1.5	10.5	0.38
024	1027	7	J	1.5	3.0		3.0	3.0	3.0	2.5	3.0	2.0	3.0	1.5	3.0	1.5	19.5	0.70
025	1028	6	J	0.0	1.0		1.5		2.0		2.5		2.5	2.5	2.5	2.0	12.0	0.43
026	1029	5	J	0.5	1.0		1.5		1.5		2.0		2.0	2.0	2.0	1.5	10.5	0.38
027	1030	4	J	0.5	0.5		1.5		2.0		2.0		2.0	1.5	2.0	1.5	10.5	0.38
028	1031	3	J	0.0	1.0		1.5		2.5		2.5	2.0	2.5	1.5	2.5	1.5	12.5	0.45
	I AVERAGE DAILY SKIN GRADE			**0.50**	**1.16**		**1.88**		**2.24**		**2.44**		**2.44**		**2.44**			

GROUP TOTAL IRRITATION SCORE 327.5

CUMULATIVE IRRITATION INDEX 0.47

OVERALL AVERAGE SKIN GRADE 1.87

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REPORT OF EXCUSED PANELISTS AND REASONS

SUBJECT NUMBER	DATE	REASON
1001	07/10/14	05 – Excess moles
1012	07/10/14	05 – Acne
1020	07/10/14	05 – Excess moles and freckles
1032	07/10/14	025 - Study full

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REASONS FOR FAILURE TO COMPLETE TEST

SUBJECT NUMBER	DATE	REASON
1004	07/11/14	No show.
1015	07/11/14	Schedule conflict.
1016	07/15/14	Tape irritation (AE).

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HUMAN CUMULATIVE IRRITATION PATCH TEST

Sponsor's Reference Number:	[redacted]
Test Substance Identification [redacted] 1% phytosteryl/behenyl/octyldodecyl lauroyl glutamate in a face cream	[redacted] Sodium Lauryl Sulfate: provided by North Cliff Consultants, Inc. Distilled Water: provided by North Cliff Consultants, Inc.
Investigator's Study #:	[redacted]
Principal Investigator:	Mary S. Bailey, Ph.D.
Consulting Dermatologist:	William Cohen, M.D.
Investigator's Facility - Address/Telephone Number:	North Cliff Consultants, Inc. 3747 Warsaw Avenue Cincinnati, OH 45205 USA Phone: +1-513-251-4930
Site of Investigation:	To be documented in study records
Sponsor and Address:	[redacted]
Sponsor Representative: Telephone Number:	[redacted]
Number of Subjects to Complete:	25
Special Recruiting Criteria:	See Attached Sponsor Test Substance Information Sheet
Number of Patch Application Days:	7 consecutive days
Cumulative Irritation Calculation to be Used (see page 11):	# 1
Minimum Time Between Patch Test Participation:	4 Weeks
Report Preparation Instructions: (e.g. dermatologist should sign final report, etc.)	Single Report
Anticipated Study Start Date:	10-July-2014
Anticipated Study End Date:	17-July-2014

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- C. Skin Sensitivity Questionnaire (if applicable)

18.0 ATTACHMENTS
- 1. Sponsor's Test Substance Information

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1.0 SIGNATURES

1.1 INVESTIGATOR APPROVAL

I have reviewed the attached protocol and concur with the study design. I agree to participate as the Investigator and to follow the protocol, as written.

Mary S. Bailey 07/08/2014

Mary S. Bailey, Ph.D.
Principal Investigator
North Cliff Consultants, Inc.
Investigator's Study #: 01-1402

Date

1.2 SPONSOR APPROVAL

The Sponsor's approval is represented by the signature of the sponsoring toxicologist on the Sponsor's Test Substance Information sheets (Attachment 1).

(See Attachment 1)

[redacted]

2.0 STUDY OBJECTIVE

The purpose of this study is to assess the potential of test substances to elicit human skin irritation by repetitive topical application.

3.0 INCLUSION / EXCLUSION CRITERIA

Individuals will be enrolled in the study based on the following inclusion and exclusion criteria.

3.1 Inclusion Criteria:

In order to be eligible for participation, subjects must be:

1. males or females between the ages of 18 and 65
2. in general good health as determined by the Medical and Dermatological History Questionnaire (Appendix A)
3. able to read, understand and sign an informed consent agreement after being advised of the nature of the study
4. willing to refrain from using lotions, creams, powders or other skin preparations on the skin in the test area for the duration of the study
5. willing to refrain from exposing skin sites to the sun or going to tanning beds for the duration of the study
6. willing to refrain from swimming and using hot tubs for the duration of the study

3.2 Exclusion Criteria:

Subjects are not eligible for participation if any one of the following applies:

1. have a clinically significant active dermatitis or skin disease anywhere on the body (excluding facial acne)
2. have a history of psoriasis, eczema or skin cancer
3. have a condition or are taking medication(s) which, in the judgment of the Investigator or Designate, makes the subject ineligible or places the subject at undue risk
4. have received treatment (chemotherapy, radiation, immune suppressant medications) for any type of cancer within the last 6 months
5. have had a mastectomy or axillary lymph nodes removed
6. have an autoimmune or immune deficiency disease (e.g. lupus, myositis, Crohns disease, autoimmune thyroid diseases, autoimmune hepatitis, etc.)
7. are currently taking any immunosuppressant medication
8. have insulin-dependent diabetes
9. have asthma or any other chronic respiratory condition requiring daily therapy
10. are currently taking/using any antihistamines or systemic/topical anti-inflammatory medications (e.g. ibuprofen, corticosteroid, etc.) on a routine or frequent basis. Maximum acceptable dosage should be determined by written laboratory guidelines.
11. have used a topical anti-inflammatory in the patch area within the last 2 weeks
12. are currently receiving allergy injections, expects to start injections before the conclusion of the study or has had the final injection within a week of the study start

13. are currently participating in another dermal study of any kind

14. are currently participating in any clinical study, which in the judgment of the Investigator, could potentially affect responses in either study.

15. have a confirmed skin allergy as a result of participation in a patch study

16. have a known sensitivity or allergy relating to the substance(s) being evaluated

17. have a known sensitivity or allergy to adhesives, surgical tapes, bandages, etc.

18. have scars, moles, sunburn, tattoos, etc. in the patch area

The test facility may, with concurrence of the Sponsor, include additional criteria that determine eligibility.

4.0 SUBJECT ENROLLMENT

4.1 Medical and Dermatological History

Each subject will complete a Medical and Dermatological History Questionnaire (Appendix A). Individuals will be enrolled based on the medical and dermatological history information provided and the pre-enrollment interview. The Investigator or Designate may exclude an individual for any reason if the exclusion is in the best interest of the individual.

4.2 Informed Consent

All subjects eligible for enrollment will complete a written Informed Consent. The consent form will contain all elements required by Federal Register, 21 CRF 50 or the equivalent specific to each country. The original signed copy for each subject participating on the study will be retained in the Investigator's records. Each subject will receive a copy of his/her signed statement.

4.3 Subject Identification

Once eligibility has been confirmed, each subject will be assigned a subject number according to test facility guidelines. This number will uniquely identify each subject and will remain with the subject throughout the study.

5.0 PATCH TYPES AND DESCRIPTION

The patch type(s) to be used for this study is indicated on the Sponsor's Test Substance Information (Attachment 1). The patch/application options are as follows:

1. **Completely Occlusive Patch:** consisting of a nonwoven cotton pad (e.g. Webril®) measuring ~4 cm^2 (2 cm x 2 cm) covered by and held securely to the skin on all sides with an occlusive, hypoallergenic tape (e.g. Blenderm®).

2. **Completely Occlusive Patch with Plastic Chamber:** available in 2 sizes, as described below:
   a. **25 mm chamber:** A cotton pad (e.g. Webril®) measuring 19 mm in diameter (2.54 cm^2) is inserted into a plastic chamber measuring 25 mm in diameter.
   b. **19 mm chamber:** A cotton pad (e.g. Webril®) measuring 12 mm in diameter (1.13 cm^2) is inserted into a plastic chamber measuring 19 mm in diameter.

3. **Semi-Occlusive Patch:** consisting of a nonwoven cotton pad (e.g. Webril®) measuring ~4 cm^2 (2 cm x 2 cm) covered by and held securely to the skin on all sides with a porous, hypoallergenic tape (e.g. Scanpor®, Micropore®, perforated Blenderm®, etc.)

4. **Semi-open Patch:** consisting of a nonwoven cotton pad (e.g. Webril®) measuring ~ 4 cm^2 (2 cm x 2 cm) held to the skin on two opposing sides by a strip of porous, hypoallergenic tape (e.g. Scanpor®, Micropore®, perforated Blenderm®) (pad is open on two sides similar to a Band-Aid®).

5. **Open Application:** consisting of direct placement of the test substance on the skin and covering the application site with a loosely woven covering (e.g. cotton gauze) held in place with hypoallergenic tape (e.g. Blenderm®, Dermacil®, Micropore®, Tenderskin®) along two opposing edges.

6.0 TEST SUBSTANCE ACCOUNTING AND PREPARATION RECORDS

The test substance(s) will be provided by the Sponsor and shipped directly to the test facility. The substance(s) will be identified by Sponsor identification numbers. The following will be documented in the study records:

- container label information
- date of receipt
- weight upon receipt, after each dose preparation and final weight
- final disposition of the test substance -- disposed of or returned to Sponsor
- procedures required to prepare the test substance for patching (e.g. cutting into pieces, grinding into powder, making a dilution, heating, etc.)

Additional information on the test substance(s) is provided in the Sponsor's Test Substance Information (Attachment 1).

7.0 STUDY DESIGN

This study is designed to assess the irritation potential of test substances by repetitive, consecutive daily patch application to the skin of human subjects.

7.1 Duration

Patch applications are made on consecutive days (including Saturday and Sunday) for the number of days specified on page 1 of the protocol. A final grade is taken 24 hours following the last patch application. For example, a 7 Day Cumulative Irritation Study runs over a period of 8 days. Patches are applied for 7 consecutive days. A final grade is taken on the 8th and final day of the study, 24 hours after the 7th patch application. Follow-up evaluations may be necessary, see page 8 (Section 8, Grading of Test Sites) of the protocol.

7.2 Randomization

Test substances will be randomized among all test sites to minimize possible site variability. Each substance should be assigned so that it occupies each test site within the group of subjects approximately an equal number of times. Unless provided by the Sponsor, the randomization should be determined according to the procedures of the test facility.

7.3 Control Materials and Benchmark Products

Control materials and benchmark products eliminate many of the variables (e.g. skin condition, weather conditions, grader variability, etc.) inherent in irritation patch testing and should always be included. Distilled water or saline are usually used as the negative irritant control. Sodium lauryl sulfate is used as the positive irritant control. Generally, the negative and positive irritant controls are provided by the test facility [indicated on page 1, "Test Substance Identification Number(s) (TSIN)]. Benchmark products are generally products with known irritation potential which are provided by the Sponsor. The irritation potential of the test substances can be

positioned by comparing the irritation elicited by the test substances to the irritation produced by the positive, negative and/or benchmark materials.

7.4 Procedure

Up to a maximum of 10 patches can be applied (5 on each arm or 10 on the back). Patches containing the test substances are applied to the lateral surface of the upper arm(s), midway between the shoulder and the elbow, or to the upper (shoulder blade area) and/or lower back (between the waistline and the lower edge of the shoulder blade) on either side of the spine. Patches are applied to the area specified in Attachment I (Sponsor's Test Substance Information). Areas routinely covered by tight fitting clothing or underclothing should be avoided. Patches are applied in a vertical row on the arm or in a vertical or horizontal row(s) on the back.

Skin sites should be wiped with ethanol or isopropyl alcohol and air-dried prior to the first patch application. Alcohol removes any oils or lotions that may be present and increases adherence of the patch to the skin.

The site of application will be marked in such a way that the location of the patch pad is clearly indicated for scoring. To assure good adhesion, patches may be reinforced with a hypoallergenic tape (e.g. Dermacil®, Micropore®, etc.). If extra tape is used, it should be applied only over existing tape, not directly over the patch pad.

The test substances will be applied to the surface of the patch pad as specified in the Sponsor's Test Substance Information (Attachment 1). Applications are made every day for the number of consecutive days specified on page 1 of the protocol ("Number of Patch Application Days"). All applications for individual test substances are made to the same site (the site used for the initial patch application) unless reactions become so strong (grade of 2.0 or greater) as to make continued application inadvisable.

Patches are worn for approximately 23 hours (± 1 hour). Subjects will be instructed to keep their patches dry. Showering and bathing restrictions are at the discretion of the Investigator or Designate. Subjects will return to the test facility for patch removal and disposal by test facility personnel. Skin sites will be gently wiped with water and patted dry to remove excess test substance immediately following patch removal. Skin sites will be graded ~approximately 20-40 minutes following patch removal. Subjects should not replace any patches that fall off. If a patch falls off or comes loose, the time the event happened should be reported to test facility personnel at the next visit. Subjects will be provided with instructions and a schedule of the study dates on the first day of the study. A telephone number(s) will also be provided to report adverse events or answer questions on a 24-hour basis.

Subjects must be present on all patching/grading days to complete all aspects of the study requirements. Any subject who is absent on one of the patch application days or at the final reading will be dropped from the study. A sufficient number of subjects should be enrolled in the study to ensure that the number of subjects completing meets or exceeds the number indicated under "Number of Subjects to Complete" on page 1.

8.0 GRADING OF TEST SITES

An artificial light source, preferably a lamp with an incandescent, 60-100 watt, daylight bulb, is used to illuminate the patch area. Sites should be graded using the grading scale in Appendix B. Any visible response should be palpated to aid in detecting signs of elevation.

Patches are not applied to any skin site exhibiting a grade of 2.0 or greater. Subjects with grades of 2.0 or greater will not have the causative test substance applied for the remainder of the study. Grades for sites where patching has been discontinued will continue to be scored as "residual grades" for the remainder of the study. Residual grades for those sites where patching has been discontinued will be included in the report but will not be used in the calculations of average grades. The highest score

observed is carried over to all subsequent grading days. This grade can either be the grade observed at the time patching was discontinued or a residual grade, whichever is higher.

Any site with an erythema grade of 2.0 or greater and/or any elevated response observed at the final regularly scheduled grading will be followed until the response(s) has regressed to a 1.5 or less. If the subject cannot return to the test facility for evaluation, the subject should be contacted by phone. Test facility personnel must establish that the subject's reaction(s) is resolving and has not increased in intensity. Any subject exhibiting a grade of 3.5 or 4.0 on any test site will be offered the opportunity to receive medical treatment from the Investigator's consulting dermatologist.

The Sponsor should be notified within 1-2 business days of any erythema score of 3 or greater or any score that includes edema, papules, vesicles, bullae and/or spreading beyond the patch site, regardless of the degree of erythema.

The same qualified skin grader should be used throughout the study. The grader will be blinded as to the treatment assignments.

9.0 ADDITIONAL STUDY INFORMATION

9.1 Adverse Events

Anticipated adverse events in this study are visible responses of the skin at or immediately surrounding the site(s) of test substance application. Those most likely to occur are mild-to-occasionally moderate erythema. On occasion, strong irritant responses may include cracking and peeling of the skin at the application site. It is possible that responses will include edema, papules and/or vesicles in addition to erythema. On rare occasions, strong reactions may spread beyond the area of the patch pad. Hypo- or hyper-pigmentation may occur at the site of a strong reaction in some individuals which could persist for several months. The skin of some subjects may become irritated by the tape and, on occasion, an allergic reaction to the tape may be seen. Some subjects may experience itching, burning and/or stinging in the test area.

Any unanticipated adverse events reported to or noted by test facility personnel should be investigated, documented and followed to resolution by the Investigator or Designate and/or consulting dermatologist. The Investigator or Designate will ascertain whether the reported symptom(s):

- is related to study participation
- warrants the subject be dropped from the study
- warrants follow-up evaluation and/or treatment

All information related to the assessment and treatment of unanticipated, potentially study related adverse events, including presenting signs, assessment of the relationship to the study, prescribed course of action, follow-up visits and final resolution will be documented in the study records.

All unanticipated, potentially study related adverse events should be reported to the Sponsor within 1-2 business days of the date initially reported or noted by the test facility.

9.2 Subject Termination

A subject may voluntarily withdraw from the study at any time for any reason. The Investigator or Designate may elect to discontinue a subject's participation at any time if deemed necessary or if it is in the best interest of the subject. If a subject experiences an adverse event/condition, the Investigator and the Sponsor will decide whether the subject should be dropped from the study. The reason for a subject's discontinuation in the study will be recorded in the study records and included in the final report.

10.0 ADDITIONAL STUDY DOCUMENTATION

10.1 Protocol Amendments

If it becomes necessary to modify this protocol, the modification will be documented by a protocol amendment signed by the Investigator and a representative of the Sponsor. Amendments to the final protocol will be consecutively numbered and will describe any change and the rationale for making the change. All amendments will be included in the final report.

10.2 Protocol Deviations

If a deviation from the protocol occurs, it is the responsibility of the Investigator or Designee to notify the Sponsor Representative as soon as possible. All deviations will be documented appropriately and included in the final report.

10.3 Documentation of Data

All data generated in this study will be recorded in blue or black ink on the data collection forms. Data entries will be initialed and dated by the individual responsible for the entry. If subsequent corrections are required, the original entry will be crossed out with a single pen stroke so that it remains clearly legible; the correction will be entered close to the original entry and initialed and dated by the individual making the correction. If the reason for making the correction is not obvious, it should be stated or referenced near the entry. All spaces on each data collection form should be filled in or crossed through if not applicable. If a response is unknown, it should be marked as "unk". An example of one or more data collection form is appended to the protocol. The test facility may use this or an equivalent form(s) as long as all required data are collected.

11.0 INSTITUTIONAL REVIEW BOARD (IRB) / INDEPENDENT ETHICS COMMITTEE (IEC) (If applicable)

If IRB/IEC approval is required for this study, the Board/Committee will review this protocol, the informed consent statement, subject instructions, subject recruitment, safety data for the test substance(s) and any other relevant documents. The study will not begin until the IRB/IEC has given approval. The Investigator will provide the Sponsor with a copy of the IRB/IEC approval letter.

Approval procedures for protocol modifications, informing the Board/Committee of adverse events and providing a final report will be carried out according to the guidelines of the IRB/IEC.

12.0 STUDY MONITORING

The Investigator will permit representatives of the Sponsor to visit the test site during the course of the study. The Investigator will permit the representatives to observe all study procedures and review all study related documents. The representative will notify the Investigator in advance of the date and time of the visit. The monitoring visit will be documented in the study records.

13.0 RESULTS

13.1 Average Skin Grade

For each test substance, the average of all inclusive scores (see below) is calculated for each completed grade day and reported as the average skin grade for that particular day. An overall average skin grade is calculated (i.e. the sum of the daily average skin grades divided by the number of study days) for each test substance.

Subject scores **included** in the calculations:

- For any patch worn for 12 hours or more
- For any patch worn less than 12 hours when the skin site exhibits a grade greater than zero

Subject scores **not included** in the calculations:

- For any patch worn less than 12 hours and the skin site exhibits a grade of zero
- For any subject dropped from the study for any reason
- Following discontinuation of patch application due to tape reaction

Scores from all subjects, including those dropped from the study will be included in the report.

13.2 Cumulative Irritation Index

The cumulative irritation index will be calculated for each test substance using one of the following examples, as specified under "Cumulative Irritation Calculation to be Used" on page 1:

Example #1:

$$\textbf{Irritation Index} = \frac{\text{Total Irritation Score (add all scores on all days for all subjects)}}{\text{Highest } \textbf{possible} \text{ score} \times \text{Number of subjects} \times \text{Number of study days}}$$

Example: $\frac{280}{4 \times 30 \times 7} = \frac{280}{840} = 0.33$

Example #2:

$$\textbf{Irritation Index} = \frac{\text{Total Irritation Score (add all scores on all days for all subjects)}}{\text{Highest } \textbf{anticipated} \text{ score} \times \text{Number of subjects} \times \text{Number of study days}}$$

Example: $\frac{75}{2 \times 30 \times 7} = \frac{75}{420} = 0.18$

13.3 Draft Report

An audited draft report, including all test and control substances, should be issued within 4 weeks of work being completed. The report will include the following:

- Purpose of the study
- Brief study design/overview
- Identification of study personnel and responsibilities
- Identification and concentration of test substance(s)
- **Description of the patch used - including dimensions of patch pad and the amount of test substance applied to the pad**
- Site of investigation
- Study dates
- Number of subjects enrolled and completing the study
- Discontinued subjects with reasons for withdrawal
- Protocol deviations
- Test substance preparation procedures
- Test substance accounting record
- Procedures/methods for applying test substance/solution to patch pads

- Adverse events and resolution of theses events
- Complete tabulations of all data
- Summary of test results
- Data interpretation/conclusions
- QA statement
- Copy of the protocol and all protocol amendments

13.4 Final Report

A final report should be completed within 4 weeks of receiving the Sponsor's comments on the draft report.

14.0 STUDY RECORDS RETENTION

The Investigator will follow the procedure outlined in "Section K. Data Retention" of the Sponsor's Test Substance Information document (Attachment 1).

15.0 CONFIDENTIALITY

The Investigator and employees of the test facility are obligated to keep any information confidential regarding the test substances and all aspects of the study, as subject to the terms and conditions of the Master Laboratory Services Agreement between the test facility and the Sponsor.

16.0 REGULATORY COMPLIANCE

This study will be conducted in compliance with the protocol, ICH (International Conference on Harmonization) Guidelines for Good Clinical Practice, Sections 4.1, 4.2, 4.4 (when applicable), 4.5, 4.6, 4.7, 4.8, 4.9, 4.11 and 4.12, and any applicable Federal, state or local regulatory requirements or standards.

NORTH CLIFF CONSULTANTS, INC.
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CONSENT TO PARTICIPATE IN A 7-DAY CUMULATIVE IRRITATION PATCH STUDY (#01-1402)

INTRODUCTION AND OBJECTIVES

We are asking you to participate in a 7-Day Cumulative Irritation Patch Study that involves currently marketed products and/or products with ingredients similar to marketed products. This research study will help us to learn more about these materials, especially their potential to cause skin irritation. Results of this test will aid in the development of products that are safe for millions of people to use.

Approximately twenty five (25) male and female subjects will participate in the study. Each subject will receive the same ten (10) product treatments. If you would like to participate, we will first ask you to answer questions about your skin and your medical history. A qualified skin grader will evaluate the skin on your back. It is possible that you may not be able to participate, based on your answers to the medical questionnaire or the condition of the skin on your back. If you are excused you will receive [redacted] for your time today.

PROCEDURE

This study will last a total of eight (8) days and require one (1) visit each day. Your visit today will last approximately 45 to 60 minutes; the rest of the visits will last approximately forty-five (45) minutes each. If you are enrolled in this study, a total of ten (10) patches containing the test products will be applied to your upper back after gently wiping the skin with rubbing alcohol. After wearing this set of patches for approximately 23 hours, you will return to the test facility where a study technician will remove the patches from your back. You will wait approximately thirty (30) minutes before a qualified skin grader examines the test sites on your back; then another set of patches will be applied to the same area on your back. This procedure will be followed for eight (8) consecutive days. If you miss grading and re-application of patches on <u>any</u> day, you will be dropped from the study and will not be compensated for partial participation. On the final day of the study (Day 8, Thursday, July 17, 2014), the patches will be removed. After approximately thirty (30) minutes, the skin grader and a Board Certified Dermatologist (skin doctor) will evaluate the sites, but no additional patches will be applied. This will conclude your participation in the study.

You will not be permitted to use any sunscreens, moisturizers, or any other personal care products, including skin lotions, creams or oils on the patch sites during the entire study. You must avoid exposing the skin sites to the sun or going to tanning beds for the duration of the study. While wearing the patches, you must keep the patches dry; you may not swim or use a hot tub. You may shower or bathe in your normal fashion <u>one hour</u> prior to your visits to the test facility; however, do not let water or soap come into direct contact with the sites. Be careful not to irritate the sites with excessive rubbing or remove the patch application site marks. Do not attempt to replace a patch if it falls off; however, note the time and report it to test facility personnel.

RISKS

You should expect to experience skin reactions resembling mild sunburn from some of the test materials. Skin reactions are usually confined to the area covered by the surgical tape. The most severe reaction anticipated during the test would be redness with some swelling and small watery blisters. Skin reactions may include soreness and itching. Reactions normally begin to recover within two or three days.

Sometimes irritation can cause changes in skin color. For individuals with light skin, the patch area may become darker, similar to sun tanning; the skin usually will recover within a few days, however, in some rare instances, localized discoloration of the skin has persisted for a longer time.

For individuals who have deeply pigmented skin, the areas of skin where patches are applied may lose color (depigmentation). In many instances, this effect is temporary and may last several months; however, in some individuals, the loss of skin pigment is permanent.

If, in the course of the study, significant new findings relating to your risk of participation occur, this information will be shared with you. Should an unexpected skin problem arise, you should contact us immediately. During business hours (8:00 am - 4:30 pm) call 513-251-4930. In case of emergency <u>only</u>, contact the Study Manager at 513-728-2575.

Professional medical care to treat any skin problem associated with this study will be made available at no expense to you. Other financial compensation for such things as lost wages, disability, or discomfort due to this type of injury is not routinely available. However, you do not waive any of your legal rights by signing this form.

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BENEFITS

There are no expected personal benefits from using the test products in this study. This study is for research purposes only. The alternative is not to participate.

CONFIDENTIALITY

Any information that is obtained in connection with this study and that can be identified with you will be kept confidential with the study records, to the extent permitted by law. If requested, however, the results may be submitted to the Sponsor or an agency of the United States Government (for example, the Food and Drug Administration) for examination. If any information about the study is published or presented, you will be identified by your study identification number only, and not by name or initials.

The products you are using and any information you will be given concerning them are proprietary to the Sponsor. They should be considered confidential and should not be discussed with other subjects or with others outside the test facility.

OBLIGATIONS AND COMPENSATION

If you complete all aspects of this study, you will receive [redacted] for your time and travel during this study. You will not incur any study-related expenses. If you discontinue this study on your own accord for personal reasons or are dismissed for refusal to obey rules or follow directions, you will not be compensated. If, in the judgment of the Investigator or Investigator's designate, it is deemed best to discontinue your participation in the study for other reasons, you will either be compensated in full or for that portion of the study already completed. All payments, partial or full, will be made at the end of the study.

TERMINATION OF PARTICIPATION

Your participation in this study may be terminated without your consent if, in the opinion of the Investigator: (1) clinical observations suggest it would be unwise for you to continue; (2) you fail to report to the test facility as agreed; or (3) any other terms of this agreement are violated.

RIGHT TO WITHDRAW

Your participation in this research study is voluntary. You may withdraw your consent and discontinue participation in this study at any time without penalty or loss of benefits, other than monetary compensation, to which you are otherwise entitled. This study is for research purposes only. The alternative is not to participate.

AVAILABILITY OF INFORMATION

You are encouraged to ask us any questions that you may have about the nature of the study or any of the information contained on this form at any time during the course of the study. The products you will be using and any information you are told about the products is confidential and your participation in the study indicates your agreement not to disclose them to others. If you have any questions about this research study and your rights as a research subject, or in the event of a research-related injury, you may contact Paul McOsker, President (513-245-2800). . During business hours (8:00 am - 4:30 pm), or if you are running late for your scheduled time, you can contact us at 513-251-4930. In case of emergency only, contact the Study Manager at 513-728-2575. You will be provided with a copy of this informed consent.

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE. YOUR SIGNATURE INDICATES THAT YOU HAVE DECIDED TO PARTICIPATE, HAVING READ THE INFORMATION PROVIDED ABOVE. YOU HAVE ALSO BEEN GIVEN AN OPPORTUNITY TO ASK QUESTIONS, AND ANSWERS TO YOUR QUESTIONS HAVE BEEN SATISFACTORY. YOU WILL RECEIVE A COPY OF THIS SIGNED CONSENT FORM.

_____
Participant Name (PRINT)

_____ _____
Signature of Participant Date Signature of Witness Date

01-1402 / _____
Study Number Subject Number

Appendix A

Medical and Dermatological History Questionnaire

Subject Initials: ___/___/___ (F M L) Sex: [ ]M [ ]F Age: _____ Subject #: _____ Study #: _____

If you are uncertain about any of the following questions, please ask for help.

Have you signed the informed consent document? No _____ Yes _____

I. Medical Conditions Now

Do You Currently Have or Have a History of:	NO	YES	
Psoriasis			
Eczema			
Skin cancer			
Other skin problems (not including facial acne)			Describe:
Scars, moles, or other blemishes at or near skin test sites			
Redness from sunburn or other source at or near skin test sites			
Asthma requiring daily medication			
Any other chronic respiratory condition requiring daily therapy			Describe:
Diabetes requiring insulin therapy			
A disease affecting your immune system (e.g. lupus, thyroiditis, myositis, Crohn's Disease, autoimmune hepatitis, etc.)			Describe:

II. Medical Conditions in the Past

Have You Had:	NO	YES
Treatment for a malignancy (cancer) within the last 6 months		
A mastectomy		
Axillary lymph nodes removed		

III. Medications

Are You Currently Taking or Have You Used Any of the Following:	NO	YES	
An antihistamine or anti-inflammatory drug (for example; aspirin, ibuprofen, corticosteroid or other steroid medication)			Drug/dose:
An anti-inflammatory medication on your skin within the last 2 weeks			
An immunosuppressive drug (e.g. Cyclosporin A)			

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Subject Initials: ___/___/___ (F M L) Subject #:_____ Study #: _____

IV. Allergy Injections

	NO	YES
Are you currently receiving allergy injections?		
Have you received a final injection within the last week?		
Do you expect to begin injections during the study?		

V. Allergies

Are you allergic to any of the following:	NO	YES	Specify
detergents/cleaning products			
cosmetics/perfumes			
personal cleansing products (soap, shampoo, etc.)			
skin creams/lotions			
anti-perspirants/deodorants			
adhesives, surgical tape, bandages			
medicines			
other materials			

VI. Miscellaneous Questions

	NO	YES	SPECIFY
Have you ever participated in a patch test?			Date of last participation:
Have you ever had a skin allergy confirmed as the result of participation in a patch test			
Are you currently participating in another study of any kind?			
Are you willing to refrain from exposing skin sites to the sun or going to tanning beds for the duration of the study			
Are you willing to refrain from using lotions, creams, powders, etc. on your skin in the test area for the duration of the study			

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Subject Initials: ___/___/___ (F M L) Subject #:_____ Study #: _____

VI. Miscellaneous Questions - Continued

	NO	YES	SPECIFY
Do you have any other medical condition we should know about?			
Do you regularly use any medication on your skin?			
Are you currently seeing a doctor about:			
allergies			
skin problems			
another reason			
Do you regularly take any prescription or OTC medication?			

Signature of person providing information: _____ **Date:** _____

FOR INTERVIEWER'S USE ONLY (Please review this form with the subject for completeness and accuracy.)

Answers to questions indicate the subject is: [ ] Acceptable
[ ] Not Acceptable - Reason: _____(use Key for Subject Exclusion)

Comments: _____

Interviewer's Signature: _____ Date: _____

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Appendix A – Continued

Key for Subject Exclusion

01	Psoriasis
02	Eczema
03	Skin Cancer
04	Other Active Dermatitis
05	Skin Abnormality – arms or back
06	Sunburn
07	Severe Asthmatic
08	Other Chronic Respiratory Condition
09	Insulin Dependent Diabetic
10	Immune System Disease
11	Treatment for Cancer
12	Mastectomy
13	Axillary Nodes Removed
14	Anti-inflammatory Drug
015	Immunosuppressive Drug
016	Allergy Injections
017	Other Study Participation
018	Confirmed Skin Allergy
019	Relevant Allergy
020	Other Disqualifying Medication
021	Patch Test Within Minimum Time Limit
022	Unwilling to Comply with Requirements
023	Other Disqualifying Condition
024	Declined Participation
025	Other – See Report

Appendix B

[redacted] Uniform Laboratory Patch Test Grading Scale

0	No apparent cutaneous involvement.
0.5	Greater than 0, less than 1.
1	Faint but definite erythema, no eruptions or broken skin or no erythema but definite dryness; may have epidermal fissuring.
1.5	Greater than 1, less than 2.
2	Moderate erythema, may have a few papules or deep fissures, moderate-to-severe erythema in the cracks. **Cut-off Grade - Patches are not reapplied**
2.5	Greater than 2, less than 3.
3	Severe erythema (beet redness), may have generalized papules or moderate-to-severe erythema with slight edema (edges well defined by raising).
3.5	Greater than 3, less than 4.
4	Generalized vesicles or eschar formations or moderate-to-severe erythema and/or edema extending beyond the area of the patch.

NOTE: The degree of reaction expressed by such descriptive terms as "moderate" and "severe" is, in itself, subjective. Such terminology can be accurately understood only through experience.

NOTE: Any reaction of greater severity than Grade 4 should be described in detail. Unusual reactions not described by the scale should also be described.

TYPICAL EXAMPLES OF HALF-GRADE SCORES

0.5	Faint, barely perceptible erythema or slight dryness (glazed appearance).
1.5	Well-defined erythema or faint erythema with definite dryness, may have epidermal fissuring.
2.5	Moderate erythema with barely perceptible edema or severe erythema not involving a significant portion of the patch (halo effect around the edges), may have a few papules or moderate-to-severe erythema.
3.5	Moderate-to-severe erythema with moderate edema (confined to patch area) or moderate-to-severe erythema with isolated eschar formations or vesicles.

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ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	[redacted]	**Sponsor's Reference Number:**	[redacted]

A. Test Substance Information and Handling:

Investigator's Sample Code: **[X] Assigned by Investigator, to be documented in study records – insert "N/A" in box to right** [ ] Assigned by Sponsor – specify sample code in box to right	**N/A**
Product Type: (e.g. moisturizer, shampoo, AP/DO, etc.)	[redacted] **(H) Moisturizer cream**
Color: (if no color, indicate "colorless")	[redacted] **(H) Light Yellow**
Physical Form: (e.g. liquid, gel, paper, cream, solid, etc.)	[redacted] **(H) Cream**
Hazards:	**(A-H) Non-Hazordous**
Storage: Stock Test Substance	**(A-H) Room Temperature**
Test Concentration:	**(A-H) Neat (100%)**
Vehicle:	**N/A (A-H)**
Storage: Test Solution – if applicable	**N/A (A-H)**

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	[redacted]	Sponsor's Reference Number:	[redacted]

How to Prepare Test Substance for Testing:

[X] **Apply as provided**
[ ] Prepare test solution (Use space below for detailed instructions to the lab)
[ ] Cut into pieces (Use space below for detailed instructions to the lab)
[ ] Other – describe:

Special Instructions for Preparation of Test Substance/Test Solution (e.g. describe how test solution should be prepared, test substance should be cut, etc.) **and/or Special Instructions for Application of Test Substance to Patch Pad (e.g. Webril®)** (e.g. describe procedure for applying AP/DOs, paper materials, etc. to patch pad):
(If instructions are not specified, the SOPs or written test facility guidelines should be used.)

NONE

Test Solution Preparation Frequency:

[X] **Not applicable**
[ ] Daily
[ ] Weekly
[ ] Other – describe:

Mixing/Stirring Requirements:

[X] **Not applicable**
[ ] Mix/stir stock test substance before each withdrawal for preparation of test solution.
[ ] Mix/stir stock test substance/test solution immediately before applying to patches.
[ ] Stir test solution continuously during patch preparation.
[ ] Other (describe):

Open-to-Air Requirements:

[X] **Apply test substance/solution/vehicle to patch pad no longer than __15__ minutes before application**
[ ] Allow test substance/solution/vehicle to remain on patch pad, open to the air, for at least _____ minutes, but no longer than _____ minutes, before application
[ ] Other (describe):

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ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	[redacted]	**Sponsor's Reference Number:**	[redacted]

Disposition of Test Substance/Test Solutions [check all that apply]:

[X] **Dispose of unused stock test substance after the final report is issued.**

[ ] All unused stock test substance should be returned to the Sponsor after the final report has been issued. *(Use the address provided below.)

[ ] Dispose of all test solutions following use.

[ ] All test solutions should be returned to the Sponsor at the completion of the study. (Use address provided below.

* **Return to**: ***(*Note to Lab: Please include study number, Sponsor's Ref. Number and [redacted] with shipment.)***

B. Positive/Negative Control Information and Handling

Positive Control

Positive Irritant Control:	**Sodium Lauryl Sulfate (SLS)**
Color: (if no color, indicate "colorless")	**White**
Physical Form: (e.g. liquid, gel, paper, cream, solid, etc.)	**Powder**
Hazards:	**Potential skin, eye and respiratory irritant**
Storage: Stock Test Substance	**Room temperature**
Test Concentration:	**0.8% w/v**
Vehicle:	**Distilled water**
Storage: Test Solution – if applicable (solution prepared by test facility using stock substance and vehicle)	**N/A**
Preparation Frequency:	**Daily**
Mixing/Stirring Requirements:	**Mix/stir immediately before applying to patches**

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	[redacted]	**Sponsor's Reference Number:**	[redacted]

Open-to-Air Requirements:

[X] **Apply test substance/solution/vehicle to patch pad no longer than __15____ minutes before application**

[ ] Allow test substance/solution/vehicle to remain on patch pad, open to the air, for at least _____ minutes, but no longer than _____ minutes, before application

[ ] Other (describe):

Negative Control

Negative Irritant Control:	**Distilled Water**
Color: (if no color, indicate "colorless")	**Colorless**
Physical Form: (e.g. liquid, gel, paper, cream, solid, etc.)	**Liquid**
Hazards:	**None**
Storage: Stock Test Substance	**Room temperature**
Test Concentration:	**100%, Neat**
Vehicle:	**N/A**
Storage: Test Solution – if applicable (solution prepared by test facility using stock substance and vehicle)	**N/A**

Open-to-Air Requirements:

[X] **Apply test substance/solution/vehicle to patch pad no longer than __15____ minutes before application**

[ ] Allow test substance/solution/vehicle to remain on patch pad, open to the air, for at least _____ minutes, but no longer than _____ minutes, before application

[ ] Other (describe):

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ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	[redacted]	**Sponsor's Reference Number:**	[redacted]

Study Parameters

C. Patch Type or Type of Application to be Used:

[ ] Completely occlusive patch
[ ] Completely occlusive patch with plastic chamber
[X] Semi-occlusive patch
[ ] Semi-open patch
[ ] Open application
[ ] Other (describe below):

D. Amount to be Applied per Patch:

[ ] To be determined by contract laboratory * (must be documented in study records and final report)
[redacted]
[X] 0.2 ml/gm per patch ([redacted] H)
[ ] _____ layer(s) per patch
[ ] _____ square inches **OR** _____ square centimeters per **layer**
[ ] Other (describe below):

*Test substance/solution to be applied to each patch in an amount sufficient to cover/saturate the pad without extruding or extending from it when applied to the skin.

E. Patch Location:

[X] No preference – arm or back (to be determined by contract laboratory and documented in the study records and final report)
[ ] Other (describe below):

F. Number of Subjects to Complete: 25

F. *Special* Inclusion/Exclusion Criteria (e.g. sensitive skin subjects, ethnic population, etc.)

[X] None
[ ] Special criteria required as specified below:

G. Dermatologist Required:

[ ] No
[X] Yes, at final grade day only
If dermatologist is required on additional days, please specify when: N/A

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	[redacted]	**Sponsor's Reference Number:**	[redacted]

H. IRB Required:
(typically an IRB is not required; however, if in doubt, check with Sponsor Regulatory personnel)
[X] **No**
[ ] Yes

I. Data Updates/Topline Data Required:
[ ] Not required
[X] **Required as specified below:**

Topline results should be sent to [redacted] as soon as available following study completion.

J. Report Information:

Please send the following to [redacted]:
- Audited DRAFT report in Word Format, along with the final, signed Protocol and any Amendments
- Audited FINAL report in Adobe Portable Document Format (.pdf)
- [redacted]

K. Data Retention:

Upon issuing the Final Report:
- The original Final Report and Protocol and any amendments, all raw data, and records for this study should be shipped to the following address:

  [redacted]

- Prior to shipping, please notify [redacted] anticipates receipt of the study records.

L. Safety/Sponsor's Risk Assessment:

Test Material:
Check the appropriate box(es):
[ ] This formula(s)/substance(s) is regulated by _____.
[specify agency(ies)]
[X] Relevant safety testing has been conducted on this formula(s)/substance(s).
[X] Relevant safety testing has been conducted on a similar formula(s)/substance(s).
[X] This formula(s) is similar to currently marketed or previously approved formula(s).
[X] Consumer exposure to product has been assessed and does not present any safety concerns under the

ATTACHMENT 1

Sponsor's Test Substance Information

[redacted]	[redacted]	Sponsor's Reference Number:	[redacted]

conditions of this test.

Additional safety information:

[redacted]H: The safety of these formulas have been reviwed and contain common Skin Care ingredients which are not anticipated to pose a safety risk. In addition, these formulae are very similar to currently marketed skin care products that have been previously been tested for dermal and eye compatibility.
[redacted]

Sodium Lauryl Sulfate:

Check the appropriate box(es):

[ ] This formula(s)/substance(s) is regulated by _____.
[specify agency(ies)]

[X] Relevant safety testing has been conducted on this formula(s)/substance(s).

[ ] Relevant safety testing has been conducted on a similar formula(s)/substance(s).

[ ] This formula(s) is similar to currently marketed or previously approved formula(s).

[ ] Consumer exposure to product has been assessed and does not present any safety concerns under the conditions of this test.

Additional safety information:

Sodium lauryl sulfate is a commonly used cosmetic surfactant. It is employed in numerous marketed personal cleansing products at concentrations much higher than the level proposed in this study. It maintains a favorable safety database for its use in the proposed context, and is commonly used at comparable levels as an irritation control in studies of this type. Although a minor degree of erythema may be expected, the testing risk under the proposed conditions of the study protocol is judged to be acceptable.

Distilled Water:

Check the appropriate box(es):

[ ] This formula(s)/substance(s) is regulated by _____.
[specify agency(ies)]

[X] Relevant safety testing has been conducted on this formula(s)/substance(s).

[ ] Relevant safety testing has been conducted on a similar formula(s)/substance(s).

[ ] This formula(s) is similar to currently marketed or previously approved formula(s).

[ ] Consumer exposure to product has been assessed and does not present any safety concerns under the conditions of this test.

Additional safety information:

No safety issues are anticipated with the use of distilled water in this study. Distilled water is often used as the negative irritant control in skin irritancy testing. No irritation to mild irritation is anticipated from the proposed testing.

[redacted]

[redacted]	Ingredient Name
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate [redacted]
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]

Memorandum

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

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

DATE: August 24, 2022

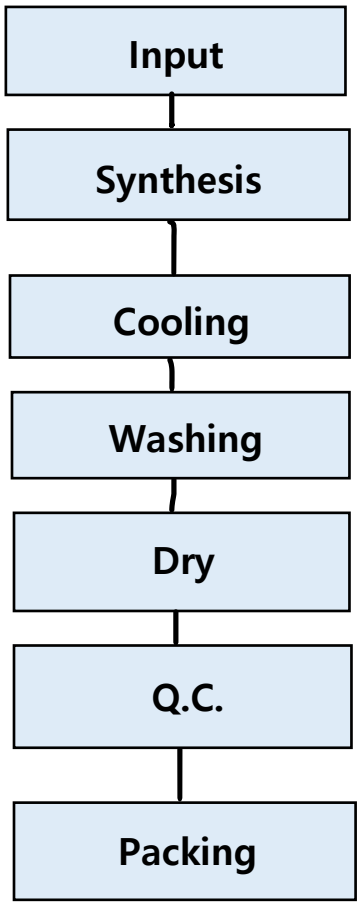
SUBJECT: Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate

Anonymous. 2022. Summary information – Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate.

August 2022

Summary Information – Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate

Manufacturing flow chart



Physical Chemical Properties – Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate

Property	Value
Appearance	White solid
Acid Value	0.16 mg KOH/g
Loss on drying (105°C, 1 hour)	0.1%

Gel Permeation Chromatography of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate - Results

Peak No.	Mn	Mw	Mz	Mp	Mw/Mn	Area %
1	1,344	1,372	1,402	1,389	1.021	74.2
2	746	757	768	765	1.015	13.8
3	383	396	409	388	1.034	12.0

Solvent: tetrahydrofuran (THF) Standard: Polystyrene

Mn: Number average molecular weight

Mw: Weight average molecular weight

Mz: Size average molecular weight

Mp: Molecular weight at the highest peak

Mw/Mn: Degree of dispersion

Arsenic and Lead Analysis of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate

Arsenic – not detected (detection limit 1 µg/g)

Lead – not detected (detection limit 2 µg/g)

Summary of Human Skin Compatibility Evaluation of Phytosteryl/Behneyl/Octyldodecyl Lauroyl Glutamate

Sample form: Cream (used "as is")

Treatment: 24 hours occlusive patch (application of 15 µl of the test sample to Finn chamber on the back of the subjects and fix it with a plaster); subjects were also tested with a blank

Number of subjects: 31 (3 male; 28 female)

Skin reactions were classified as follows:

0 (-)	No signs of inflammation, normal skin
0.5 (±)	Doubtful or slight reaction
1 (+)	Slight erythema
2 (++)	Moderate erythema with or without partial edema or papules
3 (+++)	Moderate erythema with diffuse edema
4 (++++)	Intense erythema with diffuse edema with vesicles

Reactions were scored 30 minutes after removing the patch, and at 24 and 48 hours after patch removal

Results: one subject had a 0.5 score 30 minutes after patch removal with scores of 0 24 and 48 hours after patch removal. Another subject had a score of 0.5 only at 48 hours after patch removal. All other subjects had scores of 0 at all time points.

The skin irritation index was determined to be 0.011

Scores were 0 for the blank for all 31 subjects at all time points.

Conclusion: No irritancy on human skin

2022 VCRP Data Phytosterol Glutamates

PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE

PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	03C	Eye Shadow	14
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	03D	Eye Lotion	5
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	03E	Eye Makeup Remover	1
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	03G	Other Eye Makeup Preparations	6
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	05A	Hair Conditioner	3
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	05B	Hair Spray (aerosol fixatives)	1
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	05E	Rinses (non-coloring)	1
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	05F	Shampoos (non-coloring)	2
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	05G	Tonics, Dressings, and Other Hair Grooming Aids	5
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	05I	Other Hair Preparations	1
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	07C	Foundations	12
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	07E	Lipstick	133
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	07G	Rouges	1
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	07I	Other Makeup Preparations	3
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	08G	Other Manicuring Preparations	1
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	12A	Cleansing	5
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	12C	Face and Neck (exc shave)	30
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	12D	Body and Hand (exc shave)	10
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	12F	Moisturizing	80
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	12G	Night	3
PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	12H	Paste Masks (mud packs)	2

PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE	12J	Other Skin Care Preps	6

Total 325

PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE

PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE	03D	Eye Lotion	4
PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE	07E	Lipstick	1
PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE	07G	Rouges	12
PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE	12C	Face and Neck (exc shave)	4
PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE	12F	Moisturizing	2
PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE	12G	Night	2

Total 25

PHYTOSTERYL/BEHENYL/OCTYLDODECYL/ISOSTEARYL LAUROYL GLUTAMATE

PHYTOSTERYL/BEHENYL/OCTYLDODECYL/ISOSTEARYL LAUROYL GLUTAMATE	07E	Lipstick	1

Total 1