Safety Assessment of *Rosa centifolia*-Derived Ingredients as Used in Cosmetics

Status:	Draft Tentative Report for Panel Review
Release Date:	September 1, 2022
Panel Meeting Date:	September 26-27, 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.; Daniel C. Liebler, 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 member involved in this assessment: 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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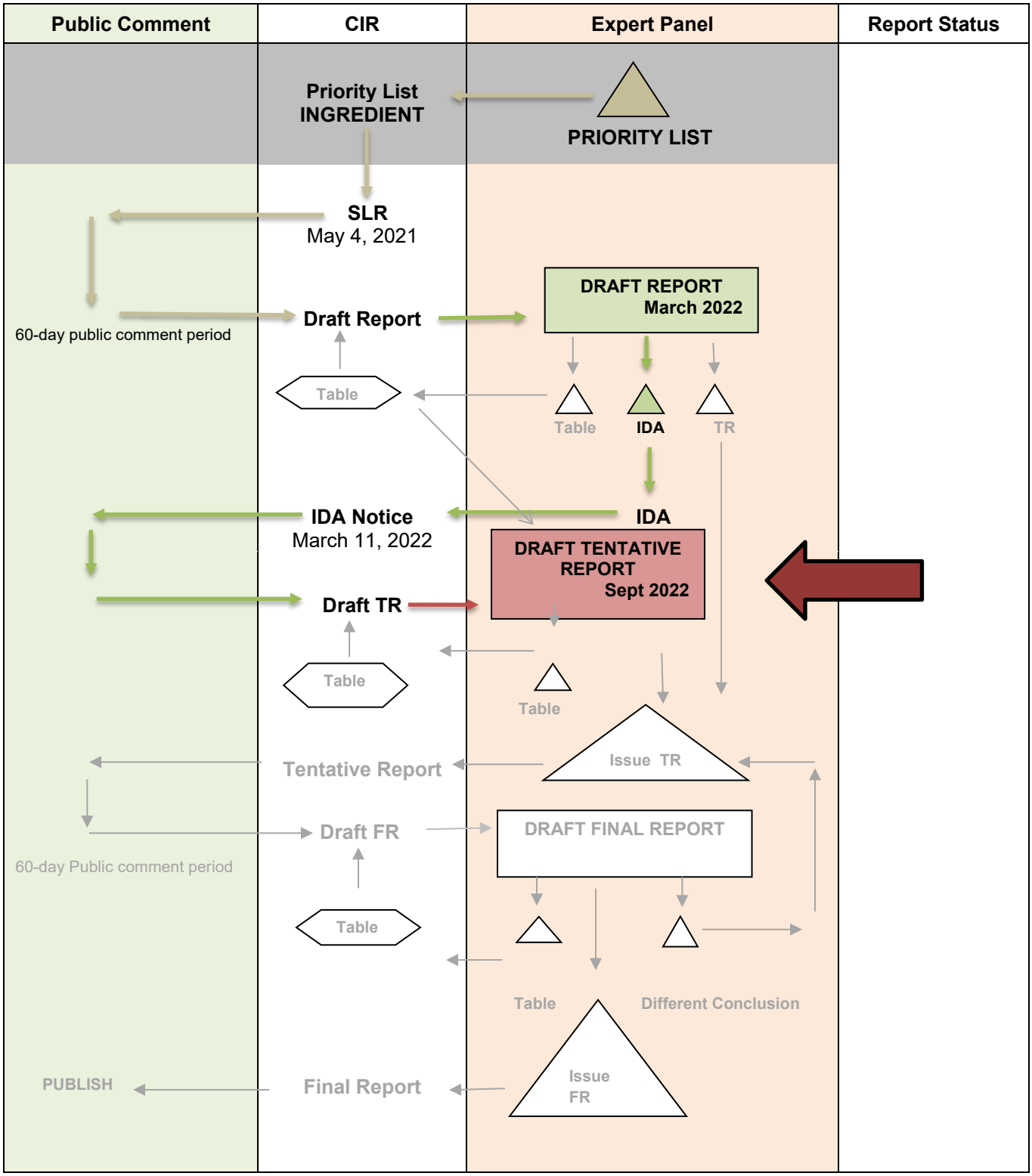
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY *Rosa centifolia*-derived ingredients

MEETING September 2022



Cosmetic Ingredient Review

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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: September 1, 2022
Subject: Safety Assessment of *Rosa centifolia*-Derived Ingredients as Used in Cosmetics

Enclosed is a Draft Tentative Report of the Safety Assessment of *Rosa centifolia*-Derived Ingredients as Used in Cosmetics. (It is identified in this report package as *report_RosaCentifolia_092022*.) After reviewing the Draft Report at the March 2022 meeting, an Insufficient Data Announcement (IDA) on the 12 *Rosa centifolia*-derived ingredients was issued with the following data needs:

- Method of manufacturing
- Composition and impurities data for all, except the flower and bud ingredients
- Dermal toxicity (28-day dermal)
    - If positive, other toxicological endpoints (e.g., developmental and reproductive toxicity, genotoxicity, carcinogenicity, etc.) may be needed

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

- Anonymous. 2014. Clinical safety evaluation repeated insult patch test (eye serum containing 0.1% Rosa Centifolia Flower Extract) *(data1_RosaCentifolia_092022)*.
- Unpublished data on Rosa Centifolia Stem Extract *(data2_RosaCentifolia_092022)*
    - Noveal. 2022. Method of manufacture Mexoryl SDA (Rosa Centifolia Stem Extract).
    - Noveal. 2022. Certificate of analytical composition Mexoryl SDA (Rosa Centifolia Stem Extract).
    - Anonymous. 2019. Mexoryl SDA (Rosa Centifolia Stem Extract): Bacterial reverse mutation assay.
    - Anonymous. 2019. Mexoryl SDA (Rosa Centifolia Stem Extract): In vitro human lymphocyte micronucleus assay.
    - Anonymous. 2019. EpiSkin™ Micronucleus assay Mexoryl SDA (Rosa Centifolia Stem Extract)

Also included in this package for your review are the report history (*history_RosaCentifolia_092022*), flow chart (*flow_RosaCentifolia_092022*), literature search strategy (*strategy_RosaCentifolia_092022*), data profile (*dataprofile_RosaCentifolia_092022*), transcripts from the March meeting (*transcripts_RosaCentifolia_092022*), and 2022 FDA VCRP data (*VCRP_RosaCentifolia_092022*).

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.

CIR History of:

Rosa centifolia-derived Ingredients

May 2021

A Scientific Literature Review (SLR) on Rose centifolia-derived ingredients was issued on May 4, 2021.

January 2022

Updated (2022) VCRP data were received and incorporated.

March 2022

Comments on the draft report were received from The Personal Care Products Council

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
- Composition and impurities data for all, except the flower and bud ingredients
- Dermal toxicity (28 day dermal)
    - If positive, other toxicological endpoints (e.g., developmental and reproductive toxicity, genotoxicity, carcinogenicity, etc.) may be needed

September 2022: Draft Tentative Report

The following unpublished data were received:

- Anonymous. 2014. Clinical safety evaluation Repeated insult patch test (eye serum containing 0.1% Rosa Centifolia Flower Extract).
- Method of manufacture Mexoryl SDA (Rosa Centifolia Stem Extract).
- Certificate of analytical composition Mexoryl SDA (Rosa Centifolia Stem Extract).
- Mexoryl SDA (Rosa Centifolia Stem Extract): Bacterial reverse mutation assay.
- Mexoryl SDA (Rosa Centifolia Stem Extract): In vitro human lymphocyte micronucleus assay.
- EpiSkin™ Micronucleus assay Mexoryl SDA (Rosa Centifolia Stem Extract)

Rosa centifolia-derived Ingredients Data Profile* –September 2022 – Wilbur Jonhnson/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
Rosa Centifolia Bud Extract		X																												
Rosa Centifolia Callus Culture Extract																														
Rosa Centifolia Extract			X																										X	
Rosa Centifolia Flower	14	X																				X			X					
Rosa Centifolia Flower Extract	174	X	X	X	X				X			X													X					X
Rosa Centifolia Flower Juice	1	X	X	X	X																									
Rosa Centifolia Flower Oil	25	X	X					X	X												X	X			X					
Rosa Centifolia Flower Powder	5	X	X																											
Rosa Centifolia Flower Water	99	X	X	X	X																									
Rosa Centifolia Flower Wax	10	X	X																											
Rosa Centifolia Leaf Cell Extract																														
Rosa Centifolia Stem Extract			X	X	X												X													

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

Rosa centifolia-derived Ingredients

Ingredient	CAS #	InfoBase	PubMed	TOXNET	FDA*	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	ECE-TOC	Web
Rosa Centifolia Bud Extract		Yes	0/0			No	No	No	No	No	No	No	No	No	No	No	Yes
Rosa Centifolia Callus Culture Extract		Yes	0/0		Yes*	No	No	No	No	No	No	No	No	No	No	No	Yes
Rosa Centifolia Extract		Yes	6/6		Yes*	No	No	No	No	No	No	No	No	No	No	No	Yes**
Rosa Centifolia Flower		Yes	4/4			No	No	No	No	No	No	No	No	No	No	No	No
Rosa Centifolia Flower Extract	84604-12-6	Yes	1/1		Yes*	No	No	No	No	No	No	No	No	No	No	No	Yes
Rosa Centifolia Flower Juice		Yes	0/0			No	No	No	No	No	No	No	No	No	No	No	Yes
Rosa Centifolia Flower Oil		Yes	1		Yes	No	No	No	No	No	No	No	No	No	No	No	Yes
Rosa Centifolia Flower Powder		Yes	0/0			No	No	No	No	No	No	No	No	No	No	No	Yes
Rosa Centifolia Flower Water		Yes	1/1			No	No	No	No	No	No	No	No	No	No	No	Yes
Rosa Centifolia Flower Wax		Yes	0/0			No	No	No	No	No	No	No	No	No	No	No	Yes
Rosa Centifolia Leaf Cell Extract		Yes	0/0		Yes*	No	No	No	No	No	No	No	No	No	No	No	Yes
Rosa Centifolia Stem Extract		Yes	0/0		Yes*	No	No	No	No	No	No	No	No	No	No	No	Yes
Rosa centifolia (genus and species, not an ingredient)			/22		Yes*	No	No	No	No	No	No	No	No	No	No	No	Yes

*Rose Absolute (can also be Rosa centifolia): Essential oil, oleoresins (solvent-free), and natural extractants (including distillates) GRAS for use in foods for human consumption (21 CFR 182.20). Same derivatives GRAS for use in foods, drugs, and related products for animal consumption (21 CFR 582.20) – Need to determine if any of other ingredients covered by 12 CFR 182.20 and 21 CFR 582.20.

**Search Rosa Centifolia Extract – Cosmetic Analysis

Dr. Duke's has composition data on Rosa centifolia

No IFRA standard in Standards Library

Rosa Centifolia Flower Extract has fragrance function also listed

Qualifiers

Absorption
Acute
Allergy
Allergic
Allergenic
Cancer
Carcinogen
Chronic
Development
Developmental
Excretion
Genotoxic
Irritation
Metabolism
Mutagen
Mutagenic
Penetration
Percutaneous
Pharmacokinetic
Repeated dose
Reproduction
Reproductive
Sensitization
Skin
Subchronic
Teratogen
Teratogenic
Toxic
Toxicity
Toxicokinetic
Toxicology
Tumor

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,
http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true (EAFUS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm (GRAS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm (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)- Chemical information | Australian Industrial Chemicals Introduction Scheme (AICIS)
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) - Flavor Extract Manufacturers Association (FEMA) (femaflavor.org) Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - http://www.ecetoc.org/

Botanical Websites, if applicable

Dr. Duke's https://phytochem.nal.usda.gov/phytochem/search
Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
Sigma Aldrich plant profiler http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html

Fragrance Websites, if applicable

IFRA (International Fragrance Association) – http://www.ifraorg.org/
RIFM (the Research Institute for Fragrance Materials) should be contacted

MARCH 2022 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT
Belsito's Team Meeting – March 7, 2022

Dr. Donald Belsito
Alright. Let me save this before I lose everything. Then we're going to Rosa Centifolia. So this is the first time we're seeing this. And a we've got a bunch of data, looks like a lot of it was sent in from the Cosmetic are from RIFM. Let me find it here. We have a wave, three comments from
Personal Care Products Council as well on this, so looks like we can clear the flower ingredients. It's grass and there's some sensitization data. Oh, no, we have an issue with flower oil, it absorbs and it's phototoxic. And there's no photo allergy data. Where he's going to go across all the flower because, right, I mean. Dan you're the chemist. Major components in the oil better photosensitizing could come out with other extraction methods or no?

Dr. Dan Liebler
The oil comes from the oil layer of steam distillation. So the that is separate from the juice. And, let's see. I would argue it's also separate from the act, the flower extract.

Dr. Donald Belsito
Or with photoallergy, even a small component could be an issue.

Dr. Dan Liebler
So what's the endpoint test endpoint for photoallergy?

Dr. Donald Belsito
We don't have one now.

Dr. Dan Liebler
Well, when we're in trouble for this ingredient.

Dr. Donald Belsito
Unless we want to do it on animals. But you know, that's probably why RIFM hasn't taken this up because there is no photo allergy data. There is very good photo toxicity data for the flower oil.

Dr. Dan Liebler
So the flat. Yeah. So you just said the flower oil has good photo tox data.

Dr. Donald Belsito
Yeah, showing it's quite phototoxic.

Dr. Dan Liebler
OK, so if we have photo tox data then we assume that we have a higher risk of photo allergenicity until proven otherwise?

Dr. Donald Belsito
Right.

Dr. Dan Liebler
OK.

Dr. Donald Belsito
We know it can absorb, so we and we don't have photo allergy data or.

Dr. Dan Liebler
There's something in it that was photo tox. I thought I heard. Carol starts to talk.

Carol Eisenmann (PCPC)
Yes, but they actually tested the concrete, not the oil. She's put the concrete under the oil, which really shouldn't be.

Dr. Dan Liebler
Ah.

Carol Eisenmann (PCPC)
It's called upon. It's the concrete that's positive photo tox.

Dr. Dan Liebler
K.

Dr. Donald Belsito
Yeah.

Dr. Dan Liebler
I was going to.

Dr. Donald Belsito
Does it really matter?

Dr. Dan Liebler
Well, the concrete is a is derivative of the oil.

Dr. Donald Belsito
Right.

Dr. Dan Liebler
And the process sort of bifurcates from steam distillation to give you the flower oil and then whatever becomes of it including a concrete, whereas the others are the aqueous layer, which have a different composition.

Dr. Donald Belsito
But not completely different. I mean, we don't know what the photo absorbing component is. Isn't it possible that there could be small amounts in the other factions?

Dr. Dan Liebler
Ah, it's of course it's always possible that there's a small amount. I mean they these fractions are very distinct in the compositions, but not in the absolute amounts. Ah, and so I guess the question it would be, do we have any photo tox, negative photo tox data for any other component of this Rosa centifolia?

Dr. Donald Belsito
No. Right. I'm trying to find the page with the photo tox.

Dr. Dan Liebler
Because, if we don't, then the logic that a teeny tiny will count something.

Dr. Paul Snyder
Page 18.

Dr. Dan Liebler
Yeah.

Dr. Donald Belsito
Excellent. Correct. You have the concrete. I mean, it was only at a very high dilution 33%, you know, I mean you can deal with, you know, as you know from experience with the RIFM panel Dan, that you can deal with phototoxicity by going 110th below the minimum phototoxic dose but photo allergy are a complete no, no. And we don't have any photo allergy data.

Dr. Dan Liebler
So the Rosa centifolia, flower oil was strongly phototoxic, but only at the highest concentration, 33% in benzene.

Dr. Donald Belsito
Right.

Dr. Dan Liebler
All responses.

Dr. Curtis Klaassen
Does it help us any? Does it help us in the non-cosmetic section? It says that, ah, the Rosebuds and rose flowers are generally recognized as safe as for use for food for human consumption.

Dr. Donald Belsito
Yeah, but you're not putting it on skin.

Dr. Curtis Klaassen
Yeah, he has also determined that these are grass for use in foods, drugs and related products for animal consumption.

Dr. Donald Belsito
Yeah, but it's consumption, not putting on your skin and getting exposed to sunlight.

Dr. Dan Liebler
Like lime.

Dr. Donald Belsito
Right.

Dr. Dan Liebler
So what does it mean when it says all responses were abolished as the result of binary dilution? Is that just mean a one to one delusion? Did you Regina, did you take that line verbatim out of the reference or did you rephrase that from something? Do you know what they mean by binary dilution here?

Regina Tucker (CIR)
No, I'm not certain what they meant by that.

Dr. Donald Belsito
Usually it's log dilutions, no?

Dr. Dan Liebler
I'm just unfamiliar with that term.

Dr. Donald Belsito
Yeah, me too.

Dr. Dan Liebler
I mean if there's log deletion via tenfold.

Dr. Donald Belsito
Right would be 3.3, which is a big difference.

Dr. Dan Liebler
Yeah. No, but there's still a whole lot more higher concentration that would be used in. Cosmetic ingredients, I think?

Dr. Donald Belsito
Yeah, I agree Dan. But the problem is that if it's photoallergy then concentration becomes less important.

Dr. Dan Liebler
Well, let's just cut to the chase and Don is this ingredient saveable period.

Dr. Donald Belsito
I don't know.

Dr. Dan Liebler
I mean, we've got the Guilty. Ah. This is the proof you're not guilty. Review approach when we get to photo allergenicity if there's a photo tox response.

Dr. Donald Belsito
Right. Yeah, Dan, to answer your question, just doing a quick Google search, it looks binary delusions or keep cutting it in half.

Dr. Dan Liebler
OK, so one to one. So if you went from 33 to you know?

Dr. Donald Belsito
16.5.

Dr. Dan Liebler
16 or so, yeah. Then you're the effect went away.

Dr. Donald Belsito
Right.

Dr. Dan Liebler
You know you're the dermatologist, or you're one of the dermatologists on the panel, but, it seems to me that the solution here might be in the concentration applied.

Dr. Donald Belsito
For Phototoxicity but. Yeah, it's just there's something that's absorbing.

Dr. Dan Liebler
Right, you know, but if it's absorbing it mean photo allergenicity still, at least our understanding of the adverse outcome pathway is something becomes photo excited and then reacts with some protein to form a Hampton.

Dr. Donald Belsito
Right.

Dr. Dan Liebler
That then sensitizes and so mechanistically it's very similar to the photoactive photo tox adverse outcome pathway or where an excited species either binds to a protein or produces oxidants that causes damage to some critical molecules. And if you're able to take that mechanism out by a one to one delusion, then it suggests that you could dramatically decrease the hapten formation. Similarly, in fact, you know, if you extrapolate that down too, I don't know how many logs we are above the maximum use concentration here, but if you're, you know, talking about 1000 fold, let's say, you may not realistically have a concern about photo allergenicity mechanistically.

Dr. Donald Belsito
OK, so I'm just looking at point 025.096 for the flower water. .002 for the flower oil. I mean, the concentrations are very low. Very low.

Dr. Dan Liebler
Yeah. So I think that you know, I think we have an approach we could take to assess the risk.
Without knocking all the ingredients out, you know as possible that we take the oil and the concrete out of, you know, out or we. Because the if it's. If it's something that's in the concrete, it's got to be really, really organic lipid soluble. So it's, you know, whatever that is its going to be present in the sort of the more aqueous ingredients and very negligible concentrations. And then we have a very low overall use concentration. So I think that provides the logic that we might employ. To consider you know the photo allergenicity issue. I'd love to hear what David and Wilma had to say about this as well, of course. But ah.

Dr. Donald Belsito
OK.

Dr. Dan Liebler
Let's see.

Dr. Donald Belsito
Yeah. OK. So.

Dr. Dan Liebler
So this is David is presenting first on this.

Dr. Donald Belsito
So centralization is cleared, photo tox was seen at 33% but not 16.5%.

Dr. Dan Liebler
And Regina, I just have one more question about the wording here in the paper. So did they actually show data that was negative at in a one to one delusion or it was just something that was said in the text or? You know. It'll be good to know what that actually was in that report, because that's a critical piece of information for our line of thinking is, you know, if you heard us talking about it.

Dr. Donald Belsito
You could send me that paper. Or it's not a paper, right? It's data from RIFM.

Regina Tucker (CIR)
It was, it was data. It was data from RIFM. And if you would like that data, yes, I will be able to send that over.

Dr. Donald Belsito
OK.

Monice Fiume (CIR)
You have the data it's PDF page that it where it says Davies. Or between PDF page 72 and 80 are the studies.

Dr. Donald Belsito
Temple University.

Monice Fiume (CIR)
But they are Davies and Forbes, I believe. It may actually be the one starting on page 7, PDF page 75.

Dr. Donald Belsito
So it it's a shoulder at 3:20. Right. So this is Rose Bulgari concrete is what we're talking about here. Was irritating at high concentrations when a phototoxic response not strongly dose related apparently superimposed on the irritant background. No clear photo toxic threshold.

Dr. Dan Liebler
So you're looking at PDF 77 here.

Dr. Donald Belsito
Well, I'm looking at page 79, which is even more. Says the Rose Bulger concrete had an unusual response, with had the appearance of a phototoxic reaction is localized in most cases to the light exposed area, but had the appearance of multiple petechiae rather than the can fluent edema or erythema normally observed. Moreover, the response was first seen prior to radiation.

Dr. Dan Liebler
Wow.

Dr. Donald Belsito
Maybe suspected localization was related to occlusion rather than light exposure.

Dr. Dan Liebler
Well, this is ambiguous then?

Dr. Donald Belsito
Yeah. And, they concluded, was mildly phototoxic, but some other reaction unrelated to light was a greater significance. Ah. Almost think that study, is a poor study. And shouldn't be, I mean, they were reporting it as to contact irritant at 33% and 16%. And you any radiating areas. I'm not even getting a dose response. It's two out of six and three out of six, for 33 and 16 and then it goes away.
I think this is a crummy study. And probably even should not be referenced. I mean, it's just very confusing it to what they're describing is more urgency than phototoxicity. I mean phototoxic reactions are more severe clinically than photoallergic reactions. Photoallergic reactions look like allergy phototoxic reactions very frequently cause blisters when severe. So I just think that this is a study that shouldn't be included? I mean, it was sent to us, but I just don't see the relevance of it. Looking at all the details. In which case all that concerned about sent Photosensitization goes away.

Dr. Dan Liebler
Yep. OK, so I you know defer to your judgment on this whether to include I think there are certainly big question marks about this study. It certainly isn't on him is not on ambiguous evidence for photo tox.

Dr. Donald Belsito
No, I mean not at all. The response was seen before light. The same response that is seen after light, which just gave it more time to develop, it was severely irritating at 16% and 33%. There was no dose response in the sense that two out of six at 33 and three out of six at 16 and then it all goes away at 8. So I. I think we just get rid of this study and don't even quote it.

Dr. Paul Snyder
What about the clinical studies on page 18, PDF page 18.

Dr. Donald Belsito
Which clinical study?

Dr. Paul Snyder
For the case reports, I'm sorry. Do yuppies 18?

Dr. Donald Belsito
Right. But that when patch tested, it was a positive patch test, not.

Dr. Donald Belsito
So, eptopic female patient with a history of polymorphous light eruption, two week history of a rash after using rose absolute and their non scented body lotion with Rosa Center folio. So let's talk about these reactions and then they patched, tested them. It wasn't a photo patch, so this was just patch test positive.

Dr. Paul Snyder
OK.

Dr. Donald Belsito
So one case report of an allergin. Not a photo allergy. Yeah, I mean, I, I, Regina, I would just get rid of that photo tox study. It's a very poor study and not interpretable.

Regina Tucker (CIR)
Yes, I can do that. So just to be clear. I will be getting rid of the photo tox, the photo tox study from your PDF, page 77.

Dr. Donald Belsito
Well, that's this. You'll be getting rid of that and then you'll be getting rid of in the documents itself that photosensitization phototoxicity that whole area will go away. Because it's.

Regina Tucker (CIR)
OK, thank you. I understand that now. So the whole section that whole section will be taken out of the report.

Dr. Donald Belsito
Yes, that's what I would recommend. That study is I mean to me what they're reporting is irritation, not phototoxicity.

Regina Tucker (CIR)
Thank you.

Dr. Donald Belsito
So having gotten rid of that.

Dr. Dan Liebler
So Don and with respect to the plant parts and the data on.

Dr. Donald Belsito
Right.

Dr. Dan Liebler
Chemistry. You know, method of manufacture impurities and so forth. I think we're OK on everything except. On the callous cell culture and the Leafs of cell culture extracts. Everything else, I think we've got covered. By appropriate by either direct at or appropriate inference from related plant perhaps.

Dr. Donald Belsito
OK, well I had come, looks like we can clear the flower ingredients, their grass, and we have the sensitization data. But the others I thought were insufficient for manufacturing, except the extract, composition impurities. And depending upon these photo tox endpoints.

Dr. Dan Liebler
Let's see, leaves all the Leafs , leaf cells, Akalis and stem extract. I think the buds OK because, that's flower.

Dr. Donald Belsito
Right.

Dr. Dan Liebler
And. So everything Rosa centifolia extract. Yeah, that's whole plant. Isn't that whole plant? Yeah, whole plant. So that's going to be leaves and stems and stuff. So. Yeah. OK. Let me just restate everything. Flower derived is OK. And then everything including the bud. So we're going to group that will flower, I think.

Dr. Donald Belsito
Yeah.

Dr. Dan Liebler
And then everything else is not, because.

Dr. Donald Belsito
We need.

Dr. Dan Liebler
Now we've got method of manufacture Rosa Centifolia, but no composition impurities.

Dr. Donald Belsito
Right. We have method of manufacture except the extract. So we have the extract?

Dr. Dan Liebler
Yep.

Dr. Donald Belsito
So we don't have method of manufacture for the stem, extract the leaf cell extract.

Dr. Dan Liebler
And the callus.

Dr. Donald Belsito
And the callus extract, we need those three.

Dr. Dan Liebler
Yep.

Dr. Donald Belsito
The leaf cell and stem. And then we need composition and impurities. For all except the flowering Bart, correct.

Dr. Dan Liebler
See I think the flower extract, a flower juice flower water collectively clears all the other flower related stuff.

Dr. Donald Belsito
Yeah, I think all the flowers stuff is fine. But I'm saying is composition and impurities for the non flower ones.

Dr. Dan Liebler
Yep. Correct. We don't have that.

Dr. Donald Belsito
So that would include the whole extract.

Dr. Dan Liebler
Correct.

Dr. Donald Belsito
That would be bud extract cell culture extract the extract, the leaf cell extract the stem extract.

Dr. Dan Liebler
Yeah. So I'm keeping the bud with the flowers. Because my understanding is, the bud is an unopened flower.

Dr. Donald Belsito
I mean, I'm fine with that.

Dr. Dan Liebler
I mean, it's perhaps a little bit less developed and you know, maybe depending on where you cut off, how old its bud is relative to flowering, but I'm just lumping the button with the flower.

Dr. Donald Belsito
I'm good with that, Curt, Paul.

Dr. Curtis Klaassen
Sure, go ahead.

Dr. Paul Snyder
Yeah, I'm fine with that.

Dr. Donald Belsito
OK.

Dr. Dan Liebler
Since we're reviewing it in the early spring.

Regina Tucker (CIR)
Yeah. OK. So just to be clear, so I just want to make sure I have this correct. So on the flower in the bud is OK, but everything else is not. So we need the composition and impurities, method of manufacture for the stem leaf callus cell extracts. Is that correct?

Dr. Dan Liebler
That's right.

Dr. Donald Belsito
Right. So we have we need manufacturing for Callus leaf cell and stem extract, then we need composition and impurities for those three plus the whole plant extract.

Regina Tucker (CIR)
OK. so you need the whole plant, extract the composition and impurities for the whole plant, extract the stem, the leaf, the callous in the cell extracts, yes. Thank you.

Dr. Donald Belsito
Yeah. And then in the discussion, so it's going to be formulated to be non sensitizing because these have sensitizing component. We have the botanical boilerplate. We have the respiratory boilerplate. We clearing Bay flowering bug based upon grass status and sensitization data. And the others, obviously we're going insufficient. This is our first go around so this is a really.

Dr. Dan Liebler
Yep.

Dr. Donald Belsito
Anything else with this? So Regina, you also have the sort of early discussion botanical respiratory and then the sensitization boilerplate for botanicals.

Regina Tucker (CIR)
Yes, I had it botanical respiratory and sensitization boilerplate.

Dr. Donald Belsito
Right.

Regina Tucker (CIR)
Yes, I have that.

Dr. Donald Belsito
And. OK. And are safe as used for the flour and butter based upon graph status and sensitization data that clears them.

Monice Fiume (CIR)
I'm sorry I missed. What were the constituents of concern, so we can make sure it makes it into the abstract and discussion.

Regina Tucker (CIR)
Yep.

Dr. Donald Belsito
I'm. Off the top of my head. Then once I'm remembering as citronellol geraniol. Let me just do a search for such an ally. I think they were all in the orgeraniol. They're all in the same.

Monice Fiume (CIR)
Yes, and phenethyl alcohol, would that also be one?

Dr. Donald Belsito
Phenethyl alcohol near all, I think was there right? A whole bunch of sensitizers. I don't think we need to list them all.

Dr. Dan Liebler
Table 3.

Dr. Donald Belsito
Yeah, I'm.

Monice Fiume (CIR)
And I would like to point to some examples. So I just wanted to make sure we had some.

Dr. Donald Belsito
Yeah. So citronellol, you know geraniol are the real big ones, but you have phenethyl alcohol, even pining's you have, wellimitinglula when they're oxidized, you have myrcene. So I would, you know, the 26 that need to be labeled in Europe would be citronellal, geraniol, eugenol, farnesol, among other potential sensitizers, I would just put those four.

Dr. Dan Liebler
Those are three of those are the top three by concentration in Table 3.

Dr. Donald Belsito
Yeah.

Monice Fiume (CIR)
Thank you.

Dr. Donald Belsito
Anything else on those? OK, that was quicker than I thought then. Let me just save this, and then we're moving to starch phosphates, which is also a first go around. Ah we had wave 3 for the Rosa centifolia that I was fine with the Council made some comments on placement of, concrete in the oil and also the extraction right, the extraction medium does not always need to be volatile. I think they're really pretty straightforward. Starch phosphates. And we have wave three comments here as well. And then, we have comments that were made before Wave 3. And that they they've been addressed on PDF page 5.

Cohen's Team Meeting - March 7, 2022

Dr. David Cohen
Alright, let's move on to Rosa. Rosa centifolia. So, Regina, this is yours as well. This is a draft report. This is the first time we've reviewing it. Of note, in 1990 the panel had a safety assessment on phenethyl alcohol. At up to 1%. As safe as used and they reaffirm that conclusion in 2008. We don't have method of manufacturing that is clear. Is this the whole plant or not on the extract? Although I guess that was in the setting of us getting that barley

information. But Bud callus, culture leaf and stem, I don't know if we have that and we have one maximization study. That showed that it can induce contact sensitization, as we might expect with this type of product. Ah. Tom, you want to comment?

Dr. Thomas Slaga
Yeah. We got some data and you know all of flower parts are grass just to bring that out and we have data to go with that. So. They only thing we didn't have data on is this stem

Dr. Wilma Bergfeld
No leaf.

Dr. Thomas Slaga
In Leaf. The rest we have, you know irritation and sensitization, we have a good bit of data.
But and so, in a way, I think we can go for safe for all the flower parts. And insufficient for the rest.

Dr. Ron Shank
I agree.

Dr. Wilma Bergfeld
I do too.

Dr. Ron Shank
ICP says there's no supplier for the flower oil. Yet we list 25 uses.

Dr. David Cohen
25 yeah.

Dr. Thomas Slaga
Yeah.

Dr. Ron Shank
And we're going to say it's safe as used. This song sounds like a conundr. Or a difficulty.

Dr. Thomas Slaga
Yeah.

Dr. Bart Heldreth
Yeah.

Dr. Ron Shank
Is it used?

Dr. Bart Heldreth
But unfortunately we get our concentration of use and our frequency of use from two different sources. And so the 25 reported uses comes from FDA's voluntary cosmetic registration program. Talking with the someone that worked, there are just two years ago they've done a big clean up of their VCRP to make sure that things that aren't still in use, are not removed. They've made sure to pare down and we see that if you look at the VCRP numbers as a whole. Most of them have gone down to some extent, and that's because they've went through and cleaned them up. So my suspicion is that those 25, at least some of them, are real and that it is in use. It just may not be in use by member companies of the Council. Or member companies that want to report on it.

Dr. Ron Shank
OK. Thank you.

Dr. Bart Heldreth
As Carol mentioned, not everybody wants to respond.

Dr. David Cohen
So, I had safe as used when formulated to be non sensitizing.

Dr. Wilma Bergfeld
I agree.

Dr. David Cohen
But we are going to exclude leaf cell extract stem extract. Bud extract and callus culture extract?

Dr. Thomas Slaga
Yes.

Dr. Ron Shank
Yes.

Dr. David Cohen
Except. Bud callous leaf, cell and stem. What do we want? We want everything?

Dr. Thomas Slaga
Well, it's early in the game everything.

Dr. David Cohen
So we want that sort of manufacturing dermal tox.

Dr. Wilma Bergfeld
Chemical characterization.

Dr. Thomas Slaga
Yeah.

Dr. David Cohen
Composition and impurities, right?

Dr. Thomas Slaga
Yes.

Dr. David Cohen
Sensitization in irritation. Right.

Dr. Thomas Slaga
Right.

Dr. David Cohen
Got it.

Dr. Wilma Bergfeld
You don't need any tox data, you have enough there is Antimutagenic studies.

Dr. Thomas Slaga
Yeah. Well, like.

Dr. Wilma Bergfeld
Because it's the grass. Because it's a grass.

Dr. Thomas Slaga
Right. And if you have antimutagenic, it can't be mutagenics, so you know the.

Dr. Wilma Bergfeld
That's an assumption.

Dr. Thomas Slaga
Yeah.

Dr. David Cohen
Yeah. Yes, right.

Dr. Thomas Slaga
You can’t have both.

Dr. David Cohen
Right. Isn't the poison just by the dose?

Dr. Thomas Slaga
Yeah.

Dr. David Cohen
OK. All right. We'll move on to a starch phosphates.

Dr. Bart Heldreth
Alright Regina, did you get all of those presents efficiencies for the IDA?

Dr. Ron Shank
Before we move on, could somebody explain what is meant by absolute and concrete? In these extracts.
It's I couldn't find a net.

Dr. Wilma Bergfeld
And while you're doing that, I had the two, will you add need to I tried with any AG.

Dr. Ron Shank
Pardon, could you say that again?

Dr. Wilma Bergfeld
Add the word meat also meat at this first time this group of documents used it.

Dr. Ron Shank
OK, Meat usually means undiluted.

Dr. Bart Heldreth
OK, so.

Dr. Wilma Bergfeld
That's what I figured, but we never used it before.

Dr. Ron Shank
OK.

Dr. Bart Heldreth
Yeah, there there's a there's two common definitions for meat either one is undiluted and the other is water free.

Dr. David Cohen
Ah.

Dr. Bart Heldreth
I'm here if you go into a bar and you say I want this liquor neat. It means don't add any water to it, don't add any ice to it either.

Dr. Wilma Bergfeld
That's how I like my Scotch.

Dr. Bart Heldreth
Exactly.

Dr. David Cohen
Don't you call that straight? I thought that was straight.

Dr. Wilma Bergfeld
Yeah, I heard it straight.

Dr. Bart Heldreth
Yes. That was years ago, neat was the term of choice for that. The absolute and the concrete there are two, I guess extraction methodologies even the absolute is typically you're getting by one extraction method or another, you're getting the oil out. Not going to say with the essential oil, but something along the oil and wax line. And, whereas the concrete is usually you're going to get some sort of a solid residue out of the extraction process, but they're very general terms. They're not, they're not terms that are in the dictionary. And they're not terms that we use very frequently it's a, it's frustrating to try to put them into the terms of our ingredients, but because they're fairly brought in in terms, but.

Dr. Ron Shank
OK. Thank you.

Dr. Bart Heldreth
Alright, I can bring up the cosmetic dictionaries terms for those they do have it in their intro. Let me see if I can find that real quick.

Dr. Ron Shank
There was a question about sensitization. And on page 18. Something was tested. It was redacted out from the raw data. But it was a strong sensitizer.

Dr. David Cohen
Well, I think.

Dr. Ron Shank
Would be kind of nice and kind and nice to know what that was. And then there's a flower oil. Absolute. Rose French. And that was not a sensitizer. Does that help anything?

Dr. David Cohen
I think when you look at the Table 3, the chemical composition, you see sensitizers in there you see citronellol, you see geraniol. So I none of that surprised me, which is why we have the non sensitizing safe as used when formulated be non sensitizing.

Dr. Ron Shank
OK.

Dr. David Cohen
Right, I mean.

Dr. Ron Shank
But you asked for sensitization data, didn't you?

Dr. David Cohen
Well, on the on the parts that we don't know this represents right? Oh, right, because. It says whole plant leaf, well leaf we have but whole plant, we have not stated we don't. Ah. Well, the truth is, yeah, it says less than the stated PPMS for whole plant.

Dr. Ron Shank
So if we say formulated to be non sensitizing, that would cover the data in the.

Dr. David Cohen
Everything.

Dr. Wilma Bergfeld
Yeah.

Dr. Ron Shank
Would it not?

Dr. Wilma Bergfeld
Yes.

Dr. Bart Heldreth
Let me just remind the panel of our typical usage of formulated to be non sensitizing when it comes to botanicals. Typically we only say formulated to be non sensitizing for botanicals. One more concerned with cumulative effect. In other words you may put two or three or more botanical ingredients in one formulation, each containing the same constituents of concern and that the some of concentrations of that constitutional concern may go over a threshold of where we're concerned about it. Typically we only use when formulated to be non sensitizing and it's aimed at the specific ingredient as it's used by itself. When we're talking about discrete chemicals.

Dr. David Cohen
That helps, but there's a few discrete chemicals in here that at least know are a problem and that's a manufacturing

issue at that point, isn't it? It's, it's for the company to be aware, not to mix key Sensitizers that puts you over the threshold of concern, right?

Dr. Bart Heldreth
Well, in that that's why we put it in the conclusion by my one more worried about you know mixing those ingredients together and one formulation and taking the concentration of key sensitizer up to a level where could you know inducer illicit you know a response. But if you don't have enough data to say that that ingredient as used at the concentrations reported in the report, won't cause sensitization whether induction or licitation, then I would propose asking for that information.

Dr. David Cohen
Bart, can you translate that for me into what we're asking for? I think I understood it, but I'm not quite sure.

Dr. Bart Heldreth
Right. OK, so we try to look at botanicals as the whole mixture. So we try to say when we're looking at the safety of, let's say, the Rosa, Santa Foley a flower. We're not looking at, you know necessarily, the concentration of Citronelle or lemony or something in there specifically, we're typically looking at sensitization data on the whole mixture. We're typically looking at all the other tox endpoints on the whole mixture. Now we're aware of those constituents of concern. And that's why we have this cumulative type effect conclusion caveat when formulated to be non sensitizing because we're worried those levels might get too high. If the test data we have for the ingredient itself is showing sensitization. At those levels, then, that's a very different situation than the cumulative effect.

Dr. David Cohen
Yeah, but we had this issue with tea tree oil.

Dr. Bart Heldreth
Right, I mean, it's like if we don't know if the ingredient itself, if we don't have enough information to say the ingredient won't cause sensitization, then we should ask for that that information. That should be part of the insufficient data announcement that we put out saying we want to know. Can you give us a and HRIPT or can you give us one of these new methodologies that makes us feel confident that this ingredient, at least if it's not used with others that contain the stand constituents of concern, will not because it sensitization.

Dr. David Cohen
What would we have a maximization tests with sensitization? Although we don't have the concentration used we and maybe we should ask for that. So you're saying this early on, let's ask for more. Let's not go out with safe as used and say, what's the concentration of that maximization test that had 16 out of 25 people sensitized?

Dr. Bart Heldreth
Right.

Dr. Thomas Slaga
Right.

Dr. David Cohen
OK.

Dr. Bart Heldreth
Right because you know, what if it comes back and the concentration is, you know of that test is 85% and you know there way, way over the top of what we need, then we need a study that's closer to the maximum use concentration or worse. It comes back. And they were using it at 0.0005% and it cost sensitization then we have a different situation too.

Dr. David Cohen
Yeah, but wouldn't the discussion tomorrow then always lead back to, well, that's why we're saying safe as used when formulated to be non sensitizing. So at 85% were coming out with that and a .05% were coming out with that.

Dr. Bart Heldreth
I completely agree with you. However, if you looked at the discussion section of botanical reports, we always explain that the that the non sensitizing caveat is because of the cumulative effect of multiple ingredients sharing the same constituent of concern. And so that that language is always in there with the botanicals. If we're worried that a situation where a product just has one of these roses centifolia ingredients would cause sensitization, then that won't be covered in the discussion section we normally write for botanicals, so the panel has the, you know, the prerogative to come out with their botanical conclusion that says when formulated to be non sensitizing and have it not be a cumulative effect issue. But you will need to add something to the conclusion to alert the reader and the formulator to the fact that this ingredient alone may be a problem.

Dr. Wilma Bergfeld
Well, can't we say that in our discussion or we can call out this sensitizers that could be there?

Dr. Bart Heldreth
We can, but it would be it would be embarking on something different than that then the panel has been doing. It would be a change of a new conclusion *(inaudible).

Dr. Wilma Bergfeld
Well, we don't particularly have a threshold.

Dr. David Cohen
And I don't see any way we're going to know what constituent cause desensitization.

Dr. Wilma Bergfeld
Yeah.

Dr. David Cohen
I mean, there's like two dozen or three dozen listed here and at least a few of them are in a concentration that. Is significant enough I suppose to. Cause a problem.
Yeah, I mean we can ask for the concentration. Of use of that Max, you study that cause sensitization. I just suspect tomorrow I'm going to have a boomerang come back around me, and we're going to have. Is that's what the formulated to be non sensitizing and then Bart, you're going to have to j p in. Regarding the discussion component of it.

Dr. Bart Heldreth
Yep. I'll be happy to.

Dr. David Cohen
I guess one of the thing is if we have in Table 3 chemical composition of the whole plant, is it true? We have no then we're missing constituents. I guess there's other things like impurities and other components that we don't have. So. Yeah. And unfortunately with the botanicals and working with natural products chemist at pull, these things out, constituent data on botanicals is.

Dr. Bart Heldreth
Terribly, Inaccurate, it’s very hard to take, let's say, any botanical and separated into all the separate chemicals and identify them all up.

Dr. Thomas Slaga
Right.

Dr. Bart Heldreth
And that's why we rarely have very much of that kind of data. That's why you look at like what is it? Doctor Dukes? Information on what constituents are in botanicals and. They have a short list of what ingredients or what the constituents might be in there, but usually they don't say how much because they don't know, and it's really hard to it's really hard to make that separation and find out what it is.
Yes, that's why our approach has been to look at these botanicals as a whole instead of the separate constituents. If we can say that the. The you know the sensitization study at Maxis concentration didn't cause us any heartburn. Then it doesn't matter if you know. Citronella and laminin and MI and everything else was in there it's coming back as no sensitization. So that's why we not we normally ask for that specially at this stage we're in a draft report.

Dr. David Cohen
OK. So will it's an idea and we're asking for the concentrations we were asking for greater detail on the Max use studies. That were mentioned where concentration is not stated.

Carol Eisenmann (PCPC)
And if I believe correctly, those came from RIFM.

Dr. David Cohen
Yeah, and I don't think it would surprise anyone if we so different. Cultivars or locations of this will see all kinds of differences in the concentrations of. The chemical compositions and the phenethyl alcohol is nine, 100th of a percent, where there's known sensitizers in the mid teens. So I'm not sure that passed. Safety assessment on the phenethyl alcohol is all that. Comforting.

Dr. Bart Heldreth
Right.

Dr. David Cohen
Anyway, any other comments, Tom, Ron. I'm Wilma about. What we should ask for in the idea? We're going to ask for the full battery on the bud, callous Leafs cell and stem, and then we'll ask for further information about the Max use study protocols.

Dr. Wilma Bergfeld
On the oil. It's flower oil.

Dr. Ron Shank
Can't, can't we save the flower ingredients or safe?

Dr. David Cohen
You mean safe when formulated not to be sensitizing?

Dr. Thomas Slaga
Yeah.

Dr. Ron Shank
Well.

Dr. Thomas Slaga
The bud gives rise to the flowers, so to me. We could include the budcat way.

Dr. Ron Shank
Yeah, flower extract an oil, we have sensitization data.

Dr. David Cohen
And it's sensitizing.

Dr. Ron Shank
No.

Dr. David Cohen
The flower oil. Desensitizing, right?

Dr. Ron Shank
Oh, let me look.

Dr. Wilma Bergfeld
And looted. It's irritating. I mean. Rabbit.

Dr. Bart Heldreth
So. But if these ingredients are sensitizers themselves.

Dr. Thomas Slaga
16.

Dr. Bart Heldreth
Is it? But these instead be unsafe. Next ingredient itself is a sensitizer, and it's a botanical mixture and we don't really know the composition. How would we formulate it to be non sensitizing? It's showing that it's sensitizing it use concentration.

Dr. David Cohen
We don't know if it's it used concentration. It says concentration not stated. But more than half the people got sensitized.

Dr. Bart Heldreth
So then I went session.

Dr. David Cohen
Right. It's the same as the other botanical, particularly tea tree oil, which we know when it oxidizes, it becomes the sensitizer and but we were able to muscle through that. And come out with a very good report on it. I kind of look at this the same way. I think at the right concentration, it's probably can be safe as used as long as you don't. We don't know that. We don't know the concentration at that maximization test.

Dr. Bart Heldreth
Right.

Carol Eisenmann (PCPC)
Well, and actually the test is what was tested as the absolute in the concrete and those that absolute is not the same as the essential oil. And those are both RIFM tests.

Dr. David Cohen
So.

Dr. Thomas Slaga
Good.

Dr. David Cohen
It's absolute. It's absolute French flower oil. Is the test product but it doesn't say tested neat or?

Carol Eisenmann (PCPC)
Well. The court needs to be revised the absolute and concrete should not be presented under coil. It should be under extract. Their types of extracts or not, and then they're not the essential oil.

Dr. David Cohen
Right.

Carol Eisenmann (PCPC)
But yes, that was tested the absolute in the concrete and there was a RIFM studies and I suspect they were tested undiluted but Rep from needs to be contacted to clarify that.

Dr. Thomas Slaga
It.

Dr. David Cohen
Yeah. OK. So yeah, I. So we'll put that out and we'll wait for. A counter response.

Carol Eisenmann (PCPC)
But there are other sensitization study, so at 2% it was not sensitizing the flower oil which I think was actually the flower oil and not. The absolute.

Dr. David Cohen
Yeah, yeah. There's a flower extract at 20%.OK. Somehow I still think we wind up in the same place, but we'll have more information.

Dr. Thomas Slaga
Right.

Dr. David Cohen
OK. Any final comments before we close? This. Row center failure. Foliar. Kane will go to starch phosphates. We just close this other one.

Full Panel - March 8, 2022

Dr. David Cohen
So Rosa Centifolia, this is the first time we're reviewing this and it's a safety assessment on 12 derived ingredients or. Parts to use this as skin conditioning agent with some other additional uses as well described in the report. We have frequency of use and Max use of .096%. It has the potential for incidental exposure. As a side note, in in 1990 the panel published a safety assessment on phenethyl alcohol. However, keep in mind that phenethyl alcohol is a very small component of this plant, less than .1%. There is ample evidence of its sensitization potential with a list of constituent components that are known sensitizers, there's evidence of a mild phototoxicity, but this is at irritating concentrations. Well above Max use. We felt we needed some additional information before coming to a conclusion

with caveats, and we are issuing, we're proposing an idea. Asking for concentrations. Of the tested materials in the sensitization studies. Method of manufacturing dermal talks. Composition and impurities and sensitization and derotation for Bud Callis leaf cell and stem. So that's our motion and I'm sure there will be some discussion.

Dr. Wilma Bergfeld
Done.

Dr. Don Belsito
Yeah. So, we thought the data were sufficient for all the flowering bud ingredients in terms of sensitization, it has citronellol and has geraniol and has farnesol. So, it's going to have our botanical sensitization boilerplate.

Dr. Don Belsito
Then it's going to need to be formulated to be non-sensitive that we didn't feel that we needed sensitization data, we did think that it was insufficient for manufacturing for all except the extract. So again, flowering bud ingredients safe as used with the botanical boilerplates. Insufficient for manufacturing for the others except the extract and come composition and impurities. And depending upon these other talks endpoints for all other than the flower and the. Bart and that's where we were.

Dr. David Cohen
Where there were not too far apart, the reason we didn't clear with a safe formulated not to be sensitizing is the concentrations on in the sensitization. Protocols weren't mentioned and we just wanted more information on that. Of course, the logical conclusion is safe when formulated to be non-sensitizing it. And we flirted with that, but we wanted a little more information about. What concentrations were used to demonstrate this sensitization?

Dr. Don Belsito
I mean, we're both in agreement that this is going to go in as out as insufficient. So fine. I I'm not going to argue. I mean include more or we can always drop it.

Dr. David Cohen
Yeah.

Dr. Wilma Bergfeld
So you're seconding the motion. Thank you. Any further discussion, comma?

Dr. Don Belsito
Awesome.

Dr. Dan Liebler -
And justice, just to clarify, we through the bud in with the flower because we looked at the bud is an unopened flower that's my.

Dr. Wilma Bergfeld
Yeah.

Dr. Dan Liebler -
Chemist version of botany.

Dr. Thomas Slaga -
Yeah.

Dr. Don Belsito
So we can start being bud light.

Dr. David Cohen
Hey.

Dr. Thomas Slaga -
That's true.

Dr. Dan Liebler -
Yeah.

Dr. David Cohen
Boy, what Will ask for some more information, and I don't know if we'll get it, but it might be interesting to see what changes from a bud to a flower and its constituents but.

Dr. Wilma Bergfeld
Or sprout enough and a seed.

Dr. Ron Shank
That's similar to a seat in a sprout, isn't it?

Dr. Wilma Bergfeld
Right, right.

Dr. Dan Liebler -
Yeah.

Dr. Wilma Bergfeld
OK. Any other subsequent comments?

Dr. Dan Liebler -
I think we're fresh out.

Dr. Wilma Bergfeld
OK. Regina, are you clear on everything?

Regina Tucker (CIR)
Yes, I have everything. Thank you.

Dr. Wilma Bergfeld
Alright, alright, I'll call the question then. All in favor of an insufficient Rep conclusion here and a request for added information as stated, those opposed Abstaining.

Dr. Don Belsito
Never, ever hand up someplace in the 36.

Dr. Wilma Bergfeld
Wait a minute. Wait a minute. I can't.

Dr. Don Belsito
I think Monice has her hand up.

Dr. Wilma Bergfeld
Who else?

Monice Fiume
I do.

Dr. Wilma Bergfeld
How many is, OK? Good. OK.

Monice Fiume
Wasn't sure if you were moving on or if you wanted.

Dr. Wilma Bergfeld
No, we didn't. We didn't have a vote yet finished.

Monice Fiume
OK. I'll let you finish the belt and then I have a question.

Dr. Wilma Bergfeld
OK, OK, abstaining. So approved. Alright, thank you. Go ahead Monice.

Monice Fiume
I just wanted to make sure before moving on to the next ingredient that yesterday and the Belsito team, the discussion about the photo toxicity studies. If that was going to be brought up today about excluding them from the document.

Dr. Don Belsito
Well, David said it discussed it yet dated. I thought that study was so crummy and so confusing with the irritation, and there was no dose response. There were more. It actually going from 33 to 16 1/2 for the numbers increased a little bit and then they totally disappeared that.

Dr. David Cohen
Right. And they're so far from the concentration of use.

Dr. Don Belsito
Reality. Yeah, I just thought that study shouldn't even be quoted and should be deleted from the document.

Dr. Wilma Bergfeld
David, what do you think? I said her payment with that.

Dr. Don Belsito
Maybe even investigators said that they couldn't understand it, that it, I forget the language they used. It was quirky or something.

Dr. Don Belsito
And it's just not it's study.

Dr. David Cohen
I'd have to go back to the study. We don't have to make that determination now. It's going to swing around again, and I'll read this study.

Dr. Don Belsito
Yeah, look. Yeah. Look at it.

Dr. Ron Shank
It's a very poor study.

Dr. Don Belsito
Right. Even the principal investigators said that they couldn't interpret the data essentially.

Dr. David Cohen
It.

Dr. Wilma Bergfeld
It sounded like it to air it before they even did the photo talks.

Dr. Ron Shank
That tells you something, it's.

Dr. Don Belsito
Yes.
Dr. David Cohen
Yeah, I looked at it being so far out of range that I didn't.
Dr. Don Belsito
And the response? The responses were particular. They were seen before a photo or ration. It was just bizarre.

Dr. David Cohen
Yes.

Dr. Wilma Bergfeld
Yeah.

Dr. David Cohen
No, my inclination would be to take it out because I didn't. Put much in in with it. So, I'm OK with that. I'll go back if we want to discuss it and put it back in. But I doubt that will occur.

Dr. Wilma Bergfeld
OK, so we can move on then and keep that in eyes view that we might want to discuss it again. OK, moving on to the last in ingredient in this group and that's the starch phosphates Dr Belsito.

Safety Assessment of *Rosa centifolia*-Derived Ingredients as Used in Cosmetics

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ABBREVIATIONS

CFR	Code of Federal Regulations
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPA	Cyclophosphamide
EU	European Union
FCA	Freund's Complete Adjuvant
FDA	Food and Drug Administration
GRAS	generally recognized as safe
LA	Luria agar
LD_{50}	lethal dose, 50%
Panel	Expert Panel for Cosmetic Ingredient Safety
Rif^R	rifampicin-resistant
Rif^S	rifampicin-sensitive
*rpo*B	RNA polymerase B
RIFM	Research Institute for Fragrance Materials
s.c.	subcutaneous
US	United States
VCRP	Voluntary Cosmetic Registration Program
wINCI	web-based *International Cosmetic Ingredient Dictionary and Handbook*

DRAFT ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 12 *Rosa centifolia*-derived ingredients as used in cosmetic formulations. The majority of these ingredients are reported to function in cosmetics as skin conditioning agents; other functions associated with ingredients in this group include abrasives, antioxidants, fragrance ingredients, and skin protectants. Because final product formulations may contain multiple botanicals, each containing similar constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. With *Rosa centifolia*-derived ingredients, the Panel was concerned about the presence of citronellol and geraniol in cosmetics. Industry should use good manufacturing practices to limit impurities. The Panel considered the available data and concluded [TBD].

INTRODUCTION

The safety of the following 12 *Rosa centifolia*-derived ingredients as used in cosmetics is reviewed in this safety assessment.

Rosa Centifolia Bud Extract	Rosa Centifolia Flower Extract	Rosa Centifolia Flower Water
Rosa Centifolia Callus Culture Extract	Rosa Centifolia Flower Juice	Rosa Centifolia Flower Wax
Rosa Centifolia Extract	Rosa Centifolia Flower Oil	Rosa Centifolia Leaf Cell Extract
Rosa Centifolia Flower	Rosa Centifolia Flower Powder	Rosa Centifolia Stem Extract

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), most *Rosa centifolia*-derived ingredients are reported to function as skin conditioning agents in cosmetic products (Table 1).[1] Other functions associated with ingredients in this group include abrasives, antioxidants, fragrance ingredients, and skin protectants. Additionally, Rosa Centifolia Flower Oil is reported to function as a fragrance ingredient (only) in cosmetics. The Expert Panel for Cosmetic Ingredient Safety (Panel) does not review ingredients that function only as fragrance ingredients because, as fragrances, the safety of these ingredients is evaluated by the Research Institute for Fragrance Materials (RIFM). However, this ingredient is not currently scheduled for review by RIFM; thus, the Panel is reviewing the safety of this ingredient.

The Panel has previously reviewed the safety of one of the main volatile components of *Rosa centifolia*. In 1990, the Panel published a safety assessment of phenethyl alcohol, with the conclusion that phenethyl alcohol is safe in cosmetic products in the present practices of use at concentrations of up to 1%;[2] the Panel reaffirmed this conclusion in 2008.[3] The full report on this ingredient 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. Published data are 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 provided 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. A published RIFM monograph was available for "Rose Oil Moroccan,"[4] and unpublished studies were provided by RIFM to the CIR on Rosa Centifolia Flower Oil.[5-13] The unpublished studies were ascribed, typically, to an "absolute" or a "concrete;" these names are provided with the data.

Botanicals, such as *Rosa centifolia*-derived ingredients, may contain numerous constituents, some of which may have the potential to cause toxic effects; for example, citronellol and geraniol are potential sensitizers. In this assessment, the Panel is evaluating the potential toxicity of each of the *Rosa centifolia*-derived ingredients as a whole, complex mixture; toxicity from single components may not predict the potential toxicity of botanical ingredients.

The names of the ingredients in this report are written in accordance with the INCI naming conventions, i.e., capitalized without italics or abbreviations. When referring to the genus and species from which the ingredients are derived, the standard taxonomic practice of using italics is followed (e.g., *Rosa centifolia*). It is often not known how the substance being tested in a study compares to the cosmetic ingredient. In the report text, if it is known that the material being tested is a cosmetic ingredient, the INCI naming convention will be used (e.g., Rosa Centifolia Extract). However, if it is not known that the test substance is the same as the cosmetic ingredient, the taxonomic naming conventions (e.g., a *Rosa centifolia* extract) will be used.

CHEMISTRY

Definition and Plant Identification

Botanicals are cosmetic ingredients directly derived from plants.[1] Generally, these ingredients have not undergone chemical modification and some are classified as follows: extracts, juices, waters, powders, oils, and waxes. Definitions of the *Rosa centifolia*-derived ingredients reviewed in this safety assessment are presented in Table 1.

Cabbage rose is a common name for *Rosa centifolia*.[14] *Rosa centifolia* L. (Rosaceae), a perennial plant that is also commonly known as hundred-leaved rose or shatapatri or taruni, is available throughout India.[15] It is a complex hybrid that is bred from *Rosa gallica* L., *Rosa moschata* Herm., *Rosa canina* L., and *Rosa damascene* Mill.

According to another source, *Rosa centifolia* grows as a plant, shrub, bush, or thicket.[16] This plant is of Asiatic origin, and the countries where it is extensively cultivated for extractive purposes include: Bulgaria, Turkey, Morocco, France, and Italy. The parts used are the flowers, buds, leaves, and fruit (hips).

Chemical Properties

Rosa Centifolia Extract is a light-brown, viscous liquid, and Rosa Centifolia Flower Wax is a solid that is insoluble in water.[16,17] According to another source, Rosa Centifolia Bud Extract, Rosa Centifolia Callus Culture Extract, or Rosa Centifolia Flower Extract may be a solid or liquid, depending upon the components of the extract.[18-20] Also, the water solubility of the extract is related to components of the extract and the solvent that is used for extraction. Rosa Centifolia Flower Oil is miscible with chloroform.[21] UV absorption data indicate an absorption peak at 320 nm (shoulder) for Rosa Centifolia Flower Extract (rose absolute).[5] A flash point of ≥ 100ºC has been reported for a Rosa Centifolia Flower Extract trade name mixture.[22] Chemical properties data on *Rosa centifolia*-derived ingredients are presented in Table 2.

Method of Manufacture

Some of the following methods of manufacturing described below are general to the production of some of the *Rosa centifolia*-derived ingredients, and it is unknown whether these methods are used in the manufacture of these ingredients for use in cosmetics. Additionally, in some cases, the definition of the ingredients, as given in the *Dictionary*, provides insight as to the method of manufacture.[1]

Rosa Centifolia Extract

A whole plant extract of *Rosa centifolia* is prepared by extraction with volatile solvents, which are subsequently removed (usually under vacuum).[16] The removal of solvents is followed by redissolution in alcohol, chilling, filtration, and removal of the alcohol.

Rosa Centifolia Flower Extract

According to a supplier of Rosa Centifolia Flower Extract, a fraction of the petals of rose of Morocco (*Rosa centifolia*) is extracted by a mixture of propylene glycol + water.[23] This process is followed by filtration, yielding a Rosa Centifolia Flower Extract trade name mixture.

The production method for another Rosa Centifolia Flower Extract trade name mixture has also been described.[24] Dried raw material is extracted with hot water, and this step is followed by filtration and then concentration. The concentrated filtrate is dissolved in 1,3-butylene glycol (50 vol%) solution. The resulting solution is subjected to sedimentation and filtration, and the production sequence ends with adjustment, and packaging.

Rosa Centifolia Flower Juice

According to a supplier of Rosa Centifolia Flower Juice, petals of *Rosa centifolia* are rehydrated and then pressed.[25] This process is followed by stabilization with vegetal glycerin and then filtration, yielding a Rosa Centifolia Flower Juice trade name mixture. The supplier also stated that, in the method of manufacture of this trade name mixture, the *Rosa centifolia* petals are cold pressed without using any solvents.[26]

Rosa Centifolia Flower Oil

Rosa centifolia flower oil is produced by the steam distillation of the flowers of *Rosa centifolia*.[4,21]

Rosa Centifolia Flower Powder

Rosa Centifolia Flower Powder is obtained from the dried, ground flowers of *Rosa centifolia*.[1]

Rosa Centifolia Flower Water

Rosa Centifolia Flower Water is an aqueous extract obtained by steam distillation of rose petals from *Rosa centifolia*.[27] Another source states Rosa Centifolia Flower Water is manufactured by subjecting dried raw material to steam distillation, yielding a water-soluble fraction.[24] Ethanol (15 vol%) is then added to this fraction, and the production sequence ends with filtration and packaging.

According to another source, the distillation of *Rosa centifolia* (rose) yields the following 3 products: rose water, rose oil, and rose waste biomass.[28,29] The method of manufacture of a Rosa Centifolia Flower Water trade name material involves the steam distillation of *Rosa centifolia* petals, and this process is followed by filtration.[30]

Rosa Centifolia Flower Wax

The extraction process that is used to produce rose absolutes (aromatic oils) from *Rosa centifolia* also yields an intermediary product that contains resins, waxes, and other lipids.[31] After the volatile oils have been removed, the waxy components can be used to produce floral wax, also referred to as a concrete.

Rosa Centifolia Stem Extract

A production method for a Rosa Centifolia Stem Extract was provided by a supplier.[32] An extract was harvested using the epicormic, new, and old shoots of Rosa Centifolia through direct thermomechanical extraction in a water/ethanol solution. Following a series of 3 solid-liquid separations first to remove coarser solid fraction, second via centrifugation to remove fine particles, and third via filtration to remove the finest particles), the extract is then concentrated by vacuum distillation and spray-dried (both steps remove the solvent) to form a powder.

Composition/Impurities

The main volatile constituents of *Rosa centifolia* have been identified as citronellol, geraniol, and phenethyl alcohol.[16] Composition data relating to the essential oil, flower and leaf parts, stem, and whole plant of *Rosa centifolia* are presented in Table 3.[14,16,26,27,33-35]

Composition data on *Rosa centifolia* hydrosol were also found in the published literature.[36] Hydrosols are products of the hydro-distillation of aromatic herbs and plants and are basically saturated solutions of essential oils (volatile fraction) in water. Rose hydrosols (e.g., *Rosa centifolia*) contain 103 ± 4.1 mg/l of total volatile compounds. The major volatile compounds in *Rosa centifolia* hydrosol have been identified as: phenethyl alcohol (42 ± 2 mg/l), citronellol (22 ± 1 mg/l), geraniol (14 ± 1 mg/l).

Rosa Centifolia Flower Extract

A Rosa Centifolia Flower Extract trade mixture of propylene glycol, water, and Rosa Centifolia Flower Extract contains 2.8% to 3.8% dry extract.[37] The total aerobic microbial count is ≤ 100 colony forming units (CFU)/g. Additional data on composition indicate that another Rosa Centifolia Flower Extract trade name mixture contains flavonoid and tannin.[24]

Rosa Centifolia Flower Juice

A Rosa Centifolia Flower Juice trade name mixture consisting of glycerin and Rosa Centifolia Flower Juice is preserved with 0.2% potassium sorbate.[38] Additional data on this Rosa Centifolia Flower Juice trade name mixture indicate that the total aerobic microbial count is ≤ 100 CFU/g.[26]

Rosa Centifolia Flower Water

Rosa Centifolia Flower Water (aqueous extract of *Rosa centifolia* petals) is preserved with 1.5% phenoxyethanol.[27] The total aerobic mesophilic microorganisms count is ≤ 100 CFU/g. A bibliographical study on realized *Rosa centifolia* revealed the potential presence of citral (< 8 ppm), citronellol (< 100 ppm), eugenol (< 6 ppm), geraniol (< 150 ppm) and farnesol (< 4 ppm) in the plant. Composition data on another Rosa Centifolia Flower Water trade name material indicate that it contains β-phenylethyl alcohol and geraniol.[24]

Rosa Centifolia Stem Extract

According to a supplier, a Rosa Centifolia Stem Extract contains 2 - 6 % water, < 20% ash (determined by sulfuric ashes), ≤ 1 % lipids, and ≥ 20 % polyphenols (typical concentration ≤ 40 %).[35] Of the allergens listed in Annex III of European Union (EU) Regulation 1223/2009, ≤ 1 ppm limonene and ≤ 4ppm benzyl alcohol were present; the remaining 24 allergens, including geraniol and citronellol, were not detected.

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 VCRP data, Rosa Centifolia Flower Extract has the greatest frequency of use; it is reported to be used in 174 cosmetic products, 150 of which are leave-on formulations (Table 4).[39] The results of a concentration of use survey conducted by the Council in 2021 indicate that Rosa Centifolia Flower Water has the highest concentration of use; it is used at maximum use concentrations up to 0.096%, specifically in face and neck products (not spray), body and hand products (not spray), and moisturizing products (not spray).[40] According to both VCRP and Council survey data, 5 of the 12 *Rosa centifolia*-derived ingredients reviewed in this safety assessment are not currently in use in cosmetic products. These ingredients are listed in Table 5.[39]

Cosmetic products containing *Rosa centifolia*-derived ingredients may incidentally come in contact with the eyes (e.g., Rosa Centifolia Flower Extract is used in mascaras at up to 0.02%).[39] *Rosa centifolia*-derived ingredients are also being used in cosmetic products that may be incidentally ingested (e.g., Rosa Centifolia Flower Extract is used at up to 0.002% in lipstick formulations).

Additionally, some of these ingredients are reported to be used in cosmetic products that could possibly be inhaled; for example, Rosa Centifolia Flower Extract is reported to be used at up to 0.025% in spray fragrance preparations and at up to 0.0001% in face powders.[39,40] 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. 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 *Rosa centifolia*-derived ingredients are not restricted from use in any way under the rules governing cosmetic products in the EU.[41] However, it should be noted that 2 of the main volatile components of *Rosa centifolia*, citronellol and geraniol, are included in Annex III of the Cosmetics Regulation European Commission (EC) No. 1223/2009 (list of substances which cosmetic products must not contain except subject to the restrictions laid down) as fragrance allergens. These ingredients must be on the label if they exceed 0.001% in leave-on and 0.01% in rinse-off products.

Non-Cosmetic

According to the US FDA, essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of rose absolute (*Rosa alba* L., *Rosa centifolia* L., *Rosa damascena* Mill., *Rosa gallica* L., and vars. of these spp.), rose buds, and rose flowers are generally recognized as safe (GRAS) for use in foods for human consumption (21 CFR 182.20). The FDA has also determined that these are GRAS for use in foods, drugs, and related products for animal consumption (21 CFR 582.20).

Rosa centifolia is famous among oil-producing species of roses.[42] Additionally, it is used in the traditional systems of medicine for the management of inflammatory conditions, including arthritis, cough, asthma, bronchitis, wounds, and ulcers.[15,43] Specifically, therapeutic uses (as astringent) of the dried petals of rose flower (e.g., from *Rosa centifolia*) include treatment of mild inflammations of the oral and pharyngeal mucosa (dosage = 1 to 2 g of drug per cup (200 ml) of water, for tea).[44]

TOXICOKINETIC STUDIES

Toxicokinetics studies of the *Rosa centifolia*-derived ingredients reviewed in this safety assessment were neither found in the published literature, nor were these data submitted. In general, toxicokinetic data are not expected to be found on botanical ingredients because each botanical ingredient is a complex mixture of constituents.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

Rosa Centifolia Flower Extract

Rosa Centifolia Flower Extract (rose absolute; (a product of extraction of a concrete with ethanol[34]) was evaluated for acute dermal toxicity using 7 rabbits (strain not stated).[6] The test substance was administered (protocol not included) at single dermal doses of 0.8 g/kg (2 animals) and 5 g/kg (5 animals). Dosing was followed by a 14-d observation period. There were no mortalities at the 0.8 g/kg dose; moderate redness (2 rabbits) and slight edema (1 rabbit) were observed. All 5 animals dosed with 5 g/kg died on observation day 2; ataxia was reported. Moderate redness (5 rabbits), slight edema (2 rabbits), and moderate edema (3 rabbits) were also observed in the 5 g/kg dose group. An acute dermal LD_{50} of > 0.8 g/kg was reported.

Rosa Centifolia Flower Oil

An acute dermal LD_{50} of > 2.5 g/kg for Rosa Centifolia Flower Oil was reported in a study involving rabbits (number and strain not stated).[4] Details relating to the test protocol and study results were not included.

Oral

Rosa Centifolia Flower Extract

The acute oral toxicity of a *Rosa centifolia* flower extract (ethanol extract) was evaluated according to Organization for Economic Cooperation and Development (OECD) Test Guideline (TG) 425.[15] A limit test on a *Rosa centifolia* flower extract (ethanol extract; dose = 2 g/kg body weight; route of administration not stated) was performed using 5 male Wistar albino rats. Dosing was followed by a 14-d observation period. None of the animals died during the observation period, and the LD_{50} was established at > 2 g/kg body weight.

The acute oral toxicity of Rosa Centifolia Flower Extract (rose absolute) was evaluated using 10 rats (strain not stated).[6] The test substance was administered as a single oral dose of 5 g/kg. Dosing was followed by a 14-d observation period. Three of 10 animals died on day 2 of the observation period; piloerection and lethargy were observed. An LD_{50} of > 5 g/kg was reported.

Short-Term Toxicity Studies

Oral

Rosa Centifolia Flower Extract

The short-term oral toxicity of *Rosa centifolia* flower extract (ethanol extract) was evaluated according to OECD TG 407.[15] Two groups of 8 male Wistar rats were used. *Rosa centifolia* flower extract was administered orally (route of administration not stated; dose of 640 mg/kg) to one of the groups once daily for 28 d. The control group was dosed orally with normal saline (1 ml/kg). After day 28, the animals were killed, and the heart and liver were examined histologically. Repeated dosing resulted in a statistically significant decrease in hepatic transaminases and an increase in white blood cells. However, it was noted that these changes were within the physiological limits for the rat and not toxicologically relevant. When compared to the control group, no other physiological, biochemical, or histopathological changes were observed in the animals dosed with *Rosa centifolia* flower extract.

Subchronic Toxicity Studies

Data on the subchronic toxicity of the *Rosa centifolia*-derived ingredients reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

Chronic Toxicity Studies

Data on the chronic toxicity of *Rosa centifolia*-derived ingredients 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 *Rosa centifolia*-derived ingredients reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

GENOTOXICITY STUDIES

The genotoxicity studies summarized below are presented in Table 6.

The genotoxic potential of Rosa Centifolia Stem Extract was evaluated in an Ames test and in 2 in vitro micronucleus assays. Rosa Centifolia Stem Extract (at doses of 5 – 5000 µg/plate) was not mutagenic to *Salmonella typhimurium*, tested with and without metabolic activation.[45] Additionally, it was not genotoxic in a micronucleus assay using cultured human peripheral blood lymphocytes (at concentrations of 200 - 5000 µg/ml),[46] or in an EpiSkin™ micronucleus assay (at concentrations of 25 – 100 mg/ml),[47] with or without metabolic activation.

ANTI-MUTAGENICITY STUDIES

Rosa Centifolia Flower Extract

The anti-mutagenicity of aqueous extracts of petals from different cultivars ("passion," "pink noblesse," and "sphinx") of *Rosa centifolia* was studied using the *Escherichia coli* RNA polymerase B (*rpo*B)-based $Rif^S \rightarrow Rif^R$ (rifampicin sensitive to resistant) forward mutation assay against ethyl methanesulfonate-induced mutagenesis.[48] *E. coli* MG1655 cells were used. The cell suspension was mixed with a *Rosa centifolia* flower extract (aqueous extract) and ethyl methanesulfonate (133 mM) and the mixture was incubated. Later, the culture was serially diluted and spread-plated on Luria agar (LA)-rifampicin (100 µg/ml) plates for scoring Rif^R mutants and LA plates for enumerating viable cells. Mutation frequency was calculated as ratio of total number of Rif^R mutants per ml to the total number of viable cells in same culture volume. Spontaneous mutation frequency was determined by incubating the cell suspension in the absence of mutagen. The Rif^R mutation frequency in *E.*

coli cells exposed to ethyl methanesulfonate was approximately $1500/10^8$ cells, whereas the spontaneous mutation frequency was approximately $1/10^8$ cells. Aqueous extracts of rose petals of the 3 cultivars, "passion," "pink noblesse," and "sphinx" (1.5 mg/ml), resulted in reduction in the mutation frequency by 55, 19, and 4%, respectively. Thus, the "passion," cultivar was the most antimutagenic among the rose cultivars that were evaluated. The analysis of antimutagenicity indicated that the blue-colored anthocyanin(s) (for which concentration was maximum in the passion cultivar) was the major contributing bioactive constituent.

CARCINOGENICITY STUDIES

Data on the carcinogenicity of *Rosa centifolia*-derived ingredients reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

OTHER RELEVANT STUDIES

Anti-Inflammatory Activity

Because skin irritation is a sign of dermatitis (skin inflammation), data on anti-inflammatory activity may be useful in evaluating the safety of Rosa Centifolia Flower Extract in the absence of skin irritation data.

Rosa Centifolia Flower Extract

The anti-inflammatory activity of a *Rosa centifolia* flower extract (ethanol extract; doses of 32, 64, and 128 mg/kg) was evaluated using the carrageenan-induced paw edema and Freund's complete adjuvant (FCA)-induced arthritis model.[15] The study involved the following 5 groups of 6 male Wistar albino rats, dosed by gavage: group 1 (2 ml/kg of 1% gum acacia suspension; vehicle control), group 2 (3 mg/kg of indomethacin), group 3 (32 mg/kg of *Rosa centifolia* flower extract), group 4 (64 mg/kg of *Rosa centifolia* flower extract), and group 5 (128 mg/kg of *Rosa centifolia* flower extract). At 30 min post-administration, paw inflammation was induced by subcutaneous (s.c.) administration of 0.1 ml of 1% λ-carrageenan in saline into the subplantar surface of the left hind paw. Paw volume was measured at 1 , 3 , and 6 h after s.c. λ-carrageenan injection. The *Rosa centifolia* flower extract (64 and 128 mg/kg) statistically significantly ($p < 0.01$) inhibited carrageenan-induced paw edema at 1 , 3 , and 6 h post-carrageenan challenge and demonstrated statistically significant ($p < 0.01$) antiarthritic activity on days 3, 7, 14, and 21 after complete FCA immunization. Treatment with the *Rosa centifolia* flower extract (128 mg/kg) also caused a statistically significant decrease in circulating pro-inflammatory cytokine levels when compared to the control.

DERMAL IRRITATION AND SENSITIZATION STUDIES

The dermal irritation and sensitization studies summarized below are presented in Table 7.

Undiluted Rosa Centifolia Flower Oil was classified as moderately irritating to the skin when applied for 24 h to intact or abraded skin of rabbits (number and strain not stated) using occlusive patches.[4] In a study involving hairless mice (number and strain not stated), undiluted Rosa Centifolia Flower Oil was applied to the back for an unspecified duration; skin irritation was not observed. In human clinical studies, a face mask containing 0.8% Rosa Centifolia Flower (undiluted) was not irritating in a 24-h single insult occlusive patch test involving 20 subjects.[49] Rosa Centifolia Flower Oil (2% in petrolatum) was not irritating in a 48-h closed patch test (number of subjects not stated).[4]

A face mask containing 0.8% Rosa Centifolia Flower was not a sensitizer in a maximization study with sodium lauryl sulfate (SLS) pretreatment in 25 subjects.[50] In human repeated insult patch tests (HRIPT), an eye serum containing 0.1% Rosa Centifolia Flower Extract (49 subjects) [51] and a Rosa Centifolia Flower Extract trade name mixture (tested at 20% in 55 subjects) were not sensitizers.[24,52] Multiple maximization studies with SLS pretreatment were performed with Rosa Centifolia Flower Extract (test concentration not stated).[7,8,10-13] In 6 studies, involving 22 – 33 subjects per study, the only reaction reported was an incidence of contact sensitization in 1 subject (out of 25).[7] In a maximization test of Rosa Centifolia Flower Oil (2% in petrolatum) involving 24 subjects, no evidence of skin sensitization was found.[4]

OCULAR IRRITATION STUDIES

Data on the ocular irritation potential of *Rosa centifolia*-derived ingredients reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

CLINICAL STUDIES

Case Report

Rosa Centifolia Flower Extract and Rosa Centifolia Extract

A non-atopic female patient with a history of polymorphic light eruption presented with a 2-wk history of a rash after use of a *Rosa centifolia* flower extract (rose absolute eau de parfum) and a non-scented body lotion containing a *Rosa centifolia* extract.[53] Erythema, papules, and edematous plaques were observed on the neck (only perfume application site), upper chest, arms, shoulders, abdomen, and upper thighs. Patch testing (protocol not stated) was performed using van der Bend chambers,

and *Rosa centifolia* extract (5% in alcohol) and the body lotion induced the following positive reactions: + (on day 2), ++ (on day 4), and + (on day 7). Testing with the *Rosa centifolia* flower extract (rose absolute eau de parfum) did not cause a positive reaction on day 2 but did cause positive reactions on days 4 (+ reaction) and 7 (+ reaction).

Other Clinical Reports

Rosa Centifolia Flower Extract

A clinical evaluation (double-blind study) of a shampoo for seborrheic dermatitis was performed using 3 groups of up to 25 patients with this scalp condition.[54] The composition of the shampoo was as follows: 0.01% *Rosa centifolia* flower extract, 0.005% epigallocatechin gallate, 0.3% zinc pyrithione, and 0.45% climbazole. The study was classified as double-blind, and one group of 24 patients was treated with the *Rosa centifolia* flower extract shampoo. The other 2 groups were treated with a 2% ketoconazole shampoo (25 patients) and a 1% zinc pyrithione shampoo (23 patients), respectively. All patients in each group were instructed to massage their scalps for at least 5 min with the assigned shampoo. This was followed by rinsing with water 3 times per wk for 4 wk. A clinical severity score was determined at 2 and 4 wk after shampoo use. Irritation was assessed using a questionnaire, and photographs were taken using a folliscope. In all groups, the clinical severity score improved statistically significantly ($p < 0.05$) relative to baseline at weeks 2 and 4. However, the changes in the clinical severity score at weeks 2 and 4 did not differ statistically significantly between the 3 groups ($p = 0.39$ and $p = 0.63$, respectively). The changes in clinical severity sub-scores (i.e., for erythema, dandruff, and lesion extent) at weeks 2 and 4 did not differ statistically significantly between the 3 groups. Irritation did not differ statistically significantly between the 3 groups ($p = 0.63$). Of the 11 patients who complained of irritation, 9 reported pruritus and 4 reported erythema. These reactions were identified as mild, and the distribution of reactions among the groups was not stated.

Rosa Centifolia Flower Oil

A randomized, placebo-controlled aromatherapy trial was performed.[55] In the experimental group of 25 female subjects, treatment involved massage into abdominal skin (for 15 min after topical application) of a botanical mixture consisting of *Lavandula officinalis* (lavender, 2 drops), *Salvia sclarea* (clary sage, 1 drop), and a *Rosa centifolia* flower oil (rose, 1 drop) in 5 ml of almond oil. The subjects reported no treatment-related side effects.

SUMMARY

The safety of 12 *Rosa centifolia*-derived ingredients as used in cosmetics is reviewed in this safety assessment. According to the *Dictionary*, most *Rosa centifolia*-derived ingredients are reported to function as skin conditioning agents in cosmetic products. Other functions associated with ingredients in this group include abrasives, antioxidants, fragrance ingredients, and skin protectants.

The main volatile constituents of *Rosa centifolia* have been identified as citronellol, geraniol, and phenethyl alcohol. UV absorption data indicate an absorption peak at 320 nm (shoulder) for Rosa Centifolia Flower Extract (rose absolute).

According to 2022 VCRP data, Rosa Centifolia Flower Extract has the greatest frequency of use; it is reported to be used in 174 cosmetic products (150 leave-on, 23 rinse-off, and 1 diluted for bath use). The results of a concentration of use survey conducted by the Council in 2021 indicate that Rosa Centifolia Flower Water is has the highest concentration of use; it is used at maximum use concentrations up to 0.096%.

Two of the main volatile components of *Rosa centifolia*, citronellol and geraniol, are included in Annex III of Cosmetics Regulation European Commission (EC) No. 1223/2009 (list of substances which cosmetic products must not contain except subject to the restrictions laid down) as fragrance allergens. These ingredients must be on the label if they exceed 0.001% in leave-on and 0.01% in rinse-off products.

According to the US FDA, essential oil, oleoresins (solvent-free), and natural extractives (including distillates) of rose absolute (including *Rosa centifolia* L.), rose buds, and rose flowers are GRAS for use in foods for human consumption and for use in foods, drugs, and related products for animal consumption.

Rosa Centifolia Flower Extract (rose absolute) was evaluated for acute dermal toxicity using 7 rabbits (strain not stated). Single dermal doses of 0.8 g/kg (2 animals) and 5 g/kg (5 animals) were administered. At a dose of 0.8 g/kg, moderate erythema (2 rabbits) and slight edema (1 rabbit) were observed. At 5 g/kg, moderate erythema (5 rabbits), slight edema (2 rabbits), and moderate edema (3 rabbits) were observed. An acute dermal LD_{50} of > 0.8 g/kg was reported. An acute dermal LD_{50} of > 2.5 g/kg for Rosa Centifolia Flower Oil was reported in a study involving rabbits (number and strain not stated).

The acute oral toxicity of a *Rosa centifolia* flower extract (ethanol extract) was evaluated using 5 male Wistar rats. None of the animals died during the 14-d observation period, and the LD_{50} was > 2 g/kg body weight. An acute oral LD_{50} of > 5 g/kg was reported for Rosa Centifolia Flower Oil in a study involving rats (number and strain not stated). The acute oral toxicity of Rosa Centifolia Flower extract (rose absolute) was evaluated using 10 rats (strain not stated). Three of 10 rats died, and piloerection and lethargy were observed. An LD_{50} of > 5 g/kg was reported.

The short-term (28-d) oral toxicity of Rosa Centifolia Flower Extract (ethanol extract) was evaluated using groups of 8 male Wistar rats (route of administration not stated; dose of 640 mg/kg). When compared to the saline control group, no toxicologically relevant findings were observed after dosing with Rosa Centifolia Flower Extract.

The genotoxic potential of Rosa Centifolia Stem Extract was evaluated in an Ames test and in 2 invitro micronucleus assays. Rosa Centifolia Stem Extract (at doses of 5-5000 µg/plat) was not mutagenic to Salmonella typhimurium, tested with and without metabolic activation. Additionally, it was not genotoxic in a micronucleus assay using cultured human peripheral blood lymphocytes (at concentrations of 200-5000 µg/ml), or in an EpiSkin™ micronucleus assay (at concentrations of 25-100 mg/ml), with or without metabolic activation.

The anti-mutagenicity of aqueous extracts of petals from different cultivars ("passion," "pink noblesse," and "sphinx") of *Rosa centifolia* was studied using the *E. coli rpo* B-based Rif ^{S}→Rif ^{R} forward mutation assay against ethyl methanesulfonate-induced mutagenesis. The cell suspension was mixed with *Rosa centifolia* flower extract (aqueous extract) and ethyl methanesulfonate (133 mM). Aqueous extracts of rose petals of the 3 cultivars, "passion," "pink noblesse," and "sphinx" (1.5 mg/ml), resulted in reduction in the ethyl methanesulfonate mutation frequency by 55, 19, and 4%, respectively. An anthocyanin, peonidin 3-glucoside, was identified as the major bioactive contributing to rose antimutagenicity.

The anti-inflammatory activity of a *Rosa centifolia* flower extract (ethanol extract; doses of 32, 64, and 128 mg/kg) was evaluated using the carrageenan-induced paw edema and FCA- induced arthritis model. *Rosa centifolia* flower extract (64 and 128 mg/kg) statistically significantly ($p < 0.01$) inhibited carrageenan-induced paw edema at 1, 3, and 6 h post-carrageenan challenge and demonstrated statistically significant ($p < 0.01$) antiarthritic activity on days 3, 7, 14, and 21 after complete FCA immunization.

Undiluted Rosa Centifolia Flower Oil was classified as moderately irritating to the skin when applied for 24 h to intact or abraded skin of rabbits (number and strain not stated) using occlusive patches. In a study involving hairless mice (number and strain not stated), undiluted Rosa Centifolia Flower Oil was applied to the back for an unspecified duration; skin irritation was not observed. In human clinical studies, a face mask containing 0.8% Rosa Centifolia Flower (undiluted) was not irritating in a 24-h single insult occlusive patch test involving 20 subjects. Rosa Centifolia Flower Oil (2% in petrolatum) was not irritating in a 48-h closed patch test (number of subjects not stated).

A face mask containing 0.8% Rosa Centifolia Flower was not a sensitizer in a maximization study with SLS pretreatment in 25 subjects. In HRIPTs, an eye serum containing 0.1% Rosa Centifolia Flower Extract (49 subjects) and a Rosa Centifolia Flower Extract trade name mixture (tested at 20% in 55 subjects) were not sensitizers. Multiple maximization studies with SLS pretreatment were performed with Rosa Centifolia Flower Extract (test concentration not stated). In 6 studies, involving 22 – 33 subjects per study, the only reaction reported was an incidence of contact sensitization in 1 subject (out of 25). In a maximization test of Rosa Centifolia Flower Oil (2% in petrolatum) involving 24 subjects, no evidence of skin sensitization was found.

A non-atopic female patient presented with a rash after use of a *Rosa centifolia* flower extract (rose absolute eau de parfum) and a non-scented body lotion containing *Rosa centifolia.* Patch testing with *Rosa centifolia* extract (5% in alcohol) and the body lotion induced the following positive reactions: + (on day 2), ++ (on day 4), and + (on day 7). Testing with the *Rosa centifolia* flower extract (rose absolute eau de parfum) did not cause a positive reaction on day 2 but did cause positive reactions on days 4 (+ reaction) and 7 (+ reaction).

A 4-wk clinical evaluation of a shampoo for seborrheic dermatitis containing 0.01% *Rosa centifolia* flower extract was performed using 3 groups of up to 25 patients with this scalp condition; each group used a different shampoo. Of the 11 patients who complained of irritation, 9 reported pruritus and 4 reported erythema. These reactions were identified as mild, and the distribution of reactions among the groups was not stated. Irritation did not differ statistically significantly between the 3 groups.

No treatment-related side effects were observed in an aromatherapy trial involving 25 female subjects. A botanical mixture consisting of Lavandula *officinalis* (lavender, 2 drops), *Salvia sclarea* (clary sage, 1 drop), and *Rosa centifolia* (rose, 1 drop) in 5 ml of almond oil was massaged into abdominal skin for 15 min.

DISCUSSION

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

This assessment reviews the safety of *12 Rosa Centifolia-derived ingredients* The Panel reviewed the available data and concluded [TBD].

Because final product formulations may contain multiple botanicals, each possibly containing similar constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. For *Rosa centifolia*-derived ingredients, the Panel was concerned about the presence of citronellol and geraniol in cosmetics, which could result in sensitization reactions. Therefore, when formulating products, manufacturers should avoid reaching levels of plant constituents that may cause sensitization or other adverse health effects.

The Panel expressed concern about pesticide residues, heavy metals, and other plant species that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities.

Finally, The Panel discussed the issue of incidental inhalation exposure resulting from these ingredients (for example, Rosa Centifolia Flower Extract is reported to be used at up to 0.025% in spray fragrance preparations and at up to 0.0001% in face powders). 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 & Structures	Function(s)
Rosa Centifolia Bud Extract	Rosa Centifolia Bud Extract is the extract of the buds of *Rosa centifolia*.	Skin-Conditioning Agents - Emollient
Rosa Centifolia Callus Culture Extract	Rosa Centifolia Callus Culture Extract is the extract of a culture of the callus of *Rosa centifolia*.	Skin Protectants
Rosa Centifolia Extract	Rosa Centifolia Extract is the extract of the whole plant, *Rosa centifolia*.	Skin-Conditioning Agents - Miscellaneous
Rosa Centifolia Flower	Rosa Centifolia Flower are the flowers of *Rosa centifolia*.	Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous
Rosa Centifolia Flower Extract 84604-12-6	Rosa Centifolia Flower Extract is the extract of the flowers of *Rosa centifolia*.	Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous
Rosa Centifolia Flower Juice	Rosa Centifolia Flower Juice is the juice expressed from the flower of *Rosa centifolia*.	Skin-Conditioning Agents - Miscellaneous
Rosa Centifolia Flower Oil	Rosa Centifolia Flower Oil is the volatile oil obtained from the flowers of *Rosa centifolia*.	Fragrance Ingredients
Rosa Centifolia Flower Powder	Rosa Centifolia Flower Powder is the powder obtained from the dried, ground flowers of *Rosa centifolia*.	Abrasives
Rosa Centifolia Flower Water	Rosa Centifolia Flower Water is an aqueous solution of the steam distillate obtained from the flowers of the rose, *Rosa centifolia*.	Skin-Conditioning Agents - Miscellaneous
Rosa Centifolia Flower Wax	Rosa Centifolia Flower Wax is a wax obtained from the flower of *Rosa centifolia*.	Skin-Conditioning Agents - Miscellaneous
Rosa Centifolia Leaf Cell Extract	Rosa Centifolia Leaf Cell Extract is the extract of a culture of the leaf cells of *Rosa centifolia*.	Antioxidants; Skin Protectants
Rosa Centifolia Stem Extract	Rosa Centifolia Stem Extract is the extract of the stems of *Rosa centifolia*.	Skin-Conditioning Agents - Emollient

Table 2. Chemical properties

Property	Value/Results	Reference
Rosa Centifolia Bud Extract		
Form	Solid or liquid; appearance is related to components of the extract	18
Solubility	Solubility is related to components of extract and solvent used for extraction	18
Rosa Centifolia Callus Culture Extract		
Form	Solid or liquid; appearance is related to components of the extract	19
Solubility	Solubility is related to components of extract and solvent used for extraction	19
Rosa Centifolia Extract		
Form	Yellowish to light-brown viscous liquid	16
Rosa Centifolia Flower Extract		
Form	Solid or liquid; appearance is related to components of the extract	20
Solubility	Solubility is related to components of extract and solvent used for extraction	20
Rosa Centifolia Flower Extract (trade mixture)		
Form (at 20°C)	translucent solution with possibly a slight precipitate (brown, orange color)	37
Density (at 20°C)	1.053 – 1.065	37
Refractive index (at 20°C)	1.412 – 1.423	37
Solubility	Miscible in water and alcohol (50% v/v); immiscible in mineral oils and vegetable oils	37
Flash point	≥ 100°C	22
Rosa Centifolia Flower Juice (trade mixture)		
Form (20°C)	liquid to opalescent liquid with an orange to brown color	26
Density (at 20°C)	1.130 – 1.150	26
Refractive index (at 20°C)	1.390 – 1.410	26
Solubility	Miscible in water and alcohol (50% v/v); immiscible in mineral oils and vegetable oils	26
Rosa Centifolia Flower Oil		
Form	Colorless or yellow liquid	21
Solubility	Miscible with chloroform	21
Specific gravity (at 30º C/15º C)	Between 0.848 and 0.863	21
Refractive index (at 30º C)	Between 1.457 and 1.463	21
Rosa Centifolia Flower Extract (rose absolute)		
UV absorption peak (nm)	320 (shoulder)	5
Rosa Centifolia Flower Water (trade name material)		
Form (at 20°C)	Colorless, transparent liquid.	27
Density (at 20°C)	0.999 – 1.002	27
Refractive index (at 20°C)	1.332 – 1.339	27
Solubility	Miscible in water and alcohol (50% v/v) and immiscible in mineral oils and vegetable oils; soluble in propylene glycol	27,56
Rosa Centifolia Flower Wax		
Form	Solid	17
Solubility	Insoluble in water	17

Table 3. Constituents of *Rosa centifolia*

Constituents	Concentration
Essential Oil	
α-pinene	not stated.[14]
β-phenethyl alcohol	0.09%.[34]
β-pinene	not stated.[14]
cis-rose oxide	0.07%.[34]
citral	not stated.[14]
citronellol	1200 ppm.[14]
citronellol	9.22%.[34]
n-eicosane C_{20}	0.55%.[34]
eugenol	0.74%.[34]
farnesol	3.48%.[34]
geranic acid	not stated.[14]
geraniol	17.60%.[34]
geraniol aldehyde	not stated.[14]
n-heneicosane C_{21}	6.31%.[34]
n-heptacosane C_{27}	1.79%.[34]
n-heptadecane	1.07%.[34]
limonene	0.05%.[34]
linalool	1.03%.[34]
methyl eugenol	0.56%.[34]
myrcene	not stated.[14]
nerol	4.36%.[34]
n-nonadecane C_{19}	8.10%.[34]
nonadecene $C_{19:1}$	2.28%.[34]
n-pentacosane C_{25}	2.86%.[34]
trans-rose oxide	0.04%.[34]
n-tricosane C_{23}	5.90%.[34]
Flower	
cyanin	not stated.[14]
EO (undefined)	2000 ppm.[14]
eusupinin A	not stated.[33]
gallic acid	not stated.[14]
malic acid	not stated.[14]
methionine sulfoxide	not stated.[14]
pectin	not stated.[14]
quercitrin	not stated.[14]
resin	not stated.[14]
rugosin A	not stated.[33]
rugosin B	not stated.[33]
rugosin D	not stated.[33]
saponin	13,000 ppm.[14]
shisonin-A	not stated.[14]
sugar	not stated.[14]
tannins	100,000 to 240,000 ppm.[14]
tartaric acid	not stated.[14]
tellimagrandin I	not stated.[33]
wax	not stated.[14]
Leaf	
saponin (in leaf)	85,000 ppm[14]
Stem	
ash content	< 20%[35]
benzyl alcohol	< 4 ppm[35]
limonene	< 1 ppm[35]
lipid content	< 1%[35]
polyphenols	> 20%[35]
water content	2 - 6 %[35]
Whole plant (main volatile constituents)	
citronellol	not stated[16]
geraniol	not stated[16]
phenethyl alcohol	not stated[16]
Whole plant (constituent levels potentially present)	
citral	< 8 ppm.[27]
citronellol	< 250 ppm.[26]
citronellol	< 100 ppm.[27]
eugenol	< 6 ppm.[27]
geraniol	< 250 ppm.[26]
geraniol	< 150 ppm.[27]
farnesol	< 4 ppm.[27]

Table 4. Frequency (2022) and concentration (2021) of use according to duration and type of exposure.[39,40]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Rosa Centifolia Flower		Rosa Centifolia Flower Extract		Rosa Centifolia Flower Juice	
Totals*	**14**	**NR**	**174**	**0.0001-0.025**	**1**	**NR**
Duration of Use						
Leave-On	*6*	*NR*	*150*	*0.0001-0.025*	*1*	*NR*
Rinse-Off	*2*	*NR*	*23*	*0.0001-0.002*	*NR*	*NR*
Diluted for (Bath) Use	*6*	*NR*	*1*	*0.0001-0.002*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	5	0.0005-0.02	NR	NR
Incidental Ingestion	NR	NR	7	0.002	NR	NR
Incidental Inhalation-Spray	4[a]; 2[b]	NR	5; 50[a]; 71[b]	0.0005-0.025; 0.01[b]	1[a]	NR
Incidental Inhalation-Powder	4[a]	NR	50[a]; 1[c]	0.0001; 0.00013-0.002[c]	1[a]	NR
Dermal Contact	13	NR	158	0.0001-0.025	1	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	9	0.001-0.002	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	7	NR	11	0.0001-0.002	NR	NR
Baby Products	NR	NR	1	NR	NR	NR

	Rosa Centifolia Flower Oil		Rosa Centifolia Flower Powder		Rosa Centifolia Flower Water	
Totals*	**25**	**0.001-0.002**	**5**	**NR**	**99**	**0.0000096-0.096**
Duration of Use						
Leave-On	*17*	*0.001-0.002*	*3*	*NR*	78	*0.000096-0.096*
Rinse Off	*6*	*NR*	*1*	*NR*	21	*0.0000096-0.023*
Diluted for (Bath) Use	*2*	*NR*	*1*	*NR*	NR	*0.0048*
Exposure Type						
Eye Area	NR	NR	NR	NR	10	NR
Incidental Ingestion	1	0.001	NR	NR	3	NR
Incidental Inhalation-Spray	4[a]; 8[b]	NR	2[a];1[b]	NR	1; 30[a]; 33[b]	0.00096; 0.00096[b]
Incidental Inhalation-Powder	4[a]	0.001-0.002[c]	2[a]	NR	30[a]	0.096[c]
Dermal Contact	20	0.001-0.002	5	NR	93	0.0000096-0.096
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	3	NR	NR	NR	2	0.00096-0.023
Hair-Coloring	NR	NR	NR	NR	NR	0.0096
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	5	0.001	1	NR	10	0.0048
Baby Products	NR	NR	NR	NR	NR	NR

	Rosa Centifolia Flower Wax	
Totals*	**10**	**NR**
Duration of Use		
Leave-On	*9*	*NR*
Rinse Off	*1*	*NR*
Diluted for (Bath) Use	*NR*	*NR*
Exposure Type		
Eye Area	1	NR
Incidental Ingestion	3	NR
Incidental Inhalation-Spray	3[a]; 1[b]	NR
Incidental Inhalation-Powder	3[a]	NR
Dermal Contact	6	NR
Deodorant (underarm)	NR	NR
Hair - Non-Coloring	NR	NR
Hair-Coloring	NR	NR
Nail	NR	NR
Mucous Membrane	4	NR
Baby Products	NR	NR

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[a]Not specified that 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
[b]It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays
[c]It is possible that these products may be powders, but it is not specified whether the reported uses are powders
NR = not reported

Table 5. *Rosa centifolia*-derived ingredients with no reported uses.[39]

Rosa Centifolia Bud Extract
Rosa Centifolia Callus Culture Extract
Rosa Centifolia Extract
Rosa Centifolia Leaf Cell Extract
Rosa Centifolia Stem Extract

Table 6. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle/Solvent	Test System	Procedure	Results	Reference
IN VITRO						
Rosa Centifolia Stem Extract	5 – 5000 200	Vehicle – water	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA102)	OECD TG 471; Ames test, with and without metabolic activation. Vehicle and appropriate positive controls were used.	not mutagenic Positive control caused statistically significant increase	[45]
Rosa Centifolia Stem Extract	1000 – 5000 µg/ml (3 h exposure) and 200 – 800 µg/ml (24 h exposure) without activation 2000 – 5000 µg/ml with activation (3 h)	Vehicle – water	cultured human peripheral blood lymphocytes	In vitro mammalian cell micronucleus test; cells were exposed to the test article for 3 or 24 hand for 3 h without metabolic activation	not genotoxic Positive control induced statistically significant increases	[46]
Rosa Centifolia Stem Extract	25 – 100 mg/ml	normal saline	12 reconstructed epidermal units	EpiSkin™ micronucleus assay Mitomycin was used as the positive control	not genotoxic Positive control caused statistically significant increase	[47]

Table 7. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION					
ANIMAL					
Rosa Centifolia Flower Oil	Undiluted	Hairless mice (number and strain not stated)	Applied to the back for an unspecified duration. Additional study details not included	No evidence of skin irritation	4
Rosa Centifolia Flower Oil	Undiluted	Rabbits (number and strain not stated)	Applied for 24 h to intact or abraded skin using occlusive patches. Additional study details not included	Test substance classified as moderately irritating to the skin	4
HUMAN					
Face mask containing 0.8% Rosa Centifolia Flower	Undiluted	20 subjects	Single-insult occlusive patch test; 24 h patch. Irritation scores determined at time of patch removal	No evidence of skin irritation	49
Rosa Centifolia Flower Oil	2% in petrolatum	number of subjects not stated	48-h closed patch test	No evidence of skin irritation	4
SENSITIZATION					
HUMAN					
Face mask containing 0.8% Rosa Centifolia Flower	tested neat (0.05 ml)	25 subjects (20 females, 5 males)	Maximization test. Product (0.05 ml) applied under 15 mm occlusive patch to SLS (0.25%) pretreated site on upper outer arm or back. Procedure involved five 48-h induction patches (72 h on weekends). After a 10 - 14 d non-treatment period,a -h occlusive patch with 5% aq. SLS was applied to a previously untreated site, and an occlusive patch with the test substance was applied for 48 h Challenge site evaluated for reactions at time of patch removal and 24 h later	No adverse or unexpected reactions during induction phase. No evidence of contact allergy at time of challenge patch removal or 24 later. Concluded that product does not possess a detectable contact-sensitizing potential and, hence, is not likely to cause contact sensitivity reactions under normal use conditions	50
Eye serum containing 0.1% Rosa Centifolia Flower Extract	tested neat (0.1 – 0.15 g)] approximately 25 – 38 mg/cm^2 test material	49 subjects	HRIPT. Occlusive patches were applied 3x/wk for 3 wk, for a total of 9 induction applications. (The test material was volatilized for 30 – 90 min on the patch prior to application.) After a 2-wk non-treatment period, a challenge patch was applied to a new site, and 24 to 72 h after, the site was scored.	No reactions were observed during induction or challenge and the researchers concluded that the test article was not associated with skin irritation or allergic contact dermatitis.	51
Rosa Centifolia Flower Extract trade name mixture	20%	55 subjects (45 females, 10 males)	HRIPT (modified Shelanski method). Total of 9 induction patches (occlusive patches) applied over 3-wk period. Induction phase followed by 10- to 21-d non-treatment period. Occlusive challenge patch applied to new site on lower back.	No dermal reactions observed during induction or challenge phase. Test substance did not induce delayed contact sensitization	24,52
Rosa Centifolia Flower Extract (concrete rose)	Concentration not stated	28 subjects	Maximization test. Test substance applied, under occlusion, to volar aspect of forearm for 5 alternate-day 48-h periods. Test site pretreated for 24 h with 5% aqueous SLS (under occlusion). After 10- to 14-d non-treatment period, challenge phase. Single challenge application preceded by 30-min application of SLS (under occlusion). Another challenge application (different site, no pretreatment) also made	Moderate degree of irritation observed at SLS-treated site. No other significant or allergic reactions observed.	8
Rosa Centifolia Flower Extract (concrete rose)	Concentration not stated	25 subjects	Modified maximization test procedure. Test substance applied, under occlusion, to volar aspect of forearm for 5 alternate 48-h periods. Initial patch test site pretreated for 24 h with 5% aqueous SLS (under occlusion). After 10- to 14-d non-treatment period, test substance (under occlusive challenge patch) applied for 48 h to new test site. Challenge applications preceded by 30-min application of 5% aqueous SLS (under occlusion). Additional challenge site not pretreated with SLS.	Approximately 1/3 of subjects tested developed irritation at SLS-treated site. No other significant irritation or allergic reactions observed. Test substance produced no reactions that were considered significantly irritating or allergic in nature	10

Table 7. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Rosa Centifolia Flower Extract (concrete rose)	Concentration not stated	22 subjects	Modified maximization test procedure, as described above	Test substance produced no reactions that were considered significantly irritating or allergic in nature	[11]
Rosa Centifolia Flower Extract (rose centifolia concrete)	Concentration not stated	33 subjects	Modified maximization test procedure, as described above.	Sweat retention response observed in 1 subject. Test substance produced no reactions that were considered significantly irritating or allergic in nature	[12]
Rosa Centifolia Flower Extract (rose absolute)	Concentration not stated	24 subjects	Modified maximization test procedure, as described above, except, challenge applications preceded by 30-min application of 2% aqueous SLS (under occlusion). Additional challenge site not pretreated with SLS.	A 3+ reaction observed in 1 subject after initial patch application. Retesting of subject did not yield positive reaction. Test substance did not induce skin sensitization	[13]
Rosa Centifolia Flower Extract (rose absolute)	Concentration not stated	25 subjects	Maximization test. Test substance applied, under occlusion, to volar forearm for 5 alternate-day 48-h periods. Patch test sites pretreated for 24 h with 5% aqueous SLS (under occlusion). After 10-d non-treatment period, test substance, under occlusive challenge patch, applied for 48 h to new test site. Challenge applications preceded by 1-h application of 10% aqueous SLS (under occlusion). Challenge sites evaluated at time of patch removal and 24 h later.	Test substance induced contact sensitization (mild reaction) in 1 subject; therefore, the researcher concluded the test material is a mild sensitizer	[7]
Rosa Centifolia Flower Oil	2% in petrolatum	24 subjects	Maximization test. Protocol details not included	No evidence of skin sensitization	[4]

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: April 18, 2022

SUBJECT: Rosa Centifolia Flower Extract

Anonymous. 2014. Clinical safety evaluation Repeated insult patch test (eye serum containing 0.1% Rosa Centifolia Flower Extract).

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[redacted]

FINAL REPORT

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

Eye serum contains 0.1%
Rosa Centifolia Flower Extract

Sponsor

[redacted]

Sponsor Representatives

[redacted]

Clinical Testing Facility

[redacted]

[redacted]

Date of Final Report

3-17-14

[redacted]

[redacted]

SIGNATURE PAGE

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

[redacted]

Laboratory Manager
Study Director

3/13/14
Date

[redacted]

Scientific Director
Principal Investigator

3/17/14
Date

[redacted]

Board-Certified Dermatologist
Medical Investigator

Date _____

[redacted] _____

QUALITY ASSURANCE STATEMENT

This study [redacted] was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in 21 CFR Part 50 (Protection of Human Subjects – Informed Consent) and the Standard Operating Procedures o[redacted]

For purposes of this clinical study:

_X_	Informed Consent was obtained.
____	Informed Consent was not obtained.
_X_	An IRB review was not required.
____	An IRB review was conducted and approval to conduct the proposed clinical research was granted.

To assure compliance with the study protocol, the Quality Assurance Unit completed an audit of the applicable study records and report. This report is considered a true and accurate reflection of the testing methods and source data.

[redacted]

Manager, Quality Assurance

14 Mar 2014
Date

[redacted]

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TABLE OF CONTENTS

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

1.0 OBJECTIVE

The objective of this study was to determine the irritation and/or sensitization potential of the test article after repeated application under occlusive patch test conditions to the skin of human subjects (exclusive panel).

2.0 SPONSOR

2.1 Sponsor Representatives

3.0 CLINICAL TESTING FACILITY

The study was conducted by:

4.0 CLINICAL INVESTIGATORS

Study Director:
Principal Investigator:
Medical Investigator:

5.0 STUDY DATES

Study initiation: January 29, 2014

Final evaluation: March 7, 2014

6.0 ETHICS

6.1 Ethical Conduct of the Study

This study was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in Title 21 of the U.S. Code of Federal Regulations (CFR), the Declaration of [redacted] Standard Operating Procedures.

6.2 Subject Information and Consent

This study was conducted in compliance with CFR Title 21, Part 50 (Informed Consent of Human Subjects). Informed Consent was obtained from each subject in the study and documented in writing before participation in the study. A copy of the Informed Consent was provided to each subject.

7.0 TEST MATERIAL

The test article used in this study was provided by:

[redacted]

It was received on January 17, 2014 and identified as follows:

[redacted]

Description
White Lotion*

*The test article was volatilized at least 30 minutes, but less than 90 minutes, on the patch prior to application to the skin.

8.0 TEST SUBJECTS

Approximately 50 male or female subjects ranging in age from 18 to 79 years were to be empanelled for this test. Subject demographics are listed in Table 1.

The subjects chosen were to be dependable and able to read and understand instructions. The subjects were not to exhibit any physical or dermatological condition that would have precluded application of the test article or determination of potential effects of the test article.

[redacted]

9.0 TEST PROCEDURE

The 9 Repeated Insult (occlusive) Patch Test (9-RIPT)[1] was conducted as follows:

9.1 Induction Phase

A sufficient amount of the test article (approximately 0.1 g – 0.15 g) was placed onto a Parke-Davis Readi-Bandage® occlusive patch (approximately 25 - 38 mg/cm^2 of test material) and applied to the back of each subject between the scapulae and waist, adjacent to the spinal mid-line. This procedure was performed by a trained technician/examiner and repeated every Monday, Wednesday and Friday until 9 applications of the test article had been made.

The subjects were instructed to remove the patch 24 hours after application. Twenty-four hour rest periods followed the Tuesday and Thursday removals and 48-hour rest periods followed each Saturday removal. Subjects returned to the Testing Facility and the site was scored by a trained examiner just prior to the next patch application.

If a subject developed a positive reaction of a level 2 erythema or greater during the Induction phase or if, at the discretion of the Study Director, the skin response warranted a change in site, the patch was applied to a previously unpatched, adjacent site for the next application. If a level 2 reaction or greater occurred at the new site, no further applications were made. However, any reactive subjects were subsequently Challenge patch tested.

9.2 Challenge Phase

After a rest period of approximately 2 weeks (no applications of the test article), the Challenge patch was applied to a previously unpatched (virgin) test site. The site was scored 24 and 72 hours after application. All subjects were instructed to report any delayed skin reactivity that occurred after the final Challenge patch reading. When warranted, selected test subjects were called back to the Clinic for additional examinations and scoring to determine possible increases or decreases in Challenge patch reactivity.

Dermal responses for both the Induction and Challenge phases of the study were scored according to the following 6-point scale:

0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

All other observed dermal sequelae (eg, edema, dryness, hypo- or hyperpigmentation) were appropriately recorded on the data sheet and described as mild, moderate or severe.

[1] Marzulli FN, Maibach HI. (1976) Contact allergy: predictive testing in man. *Contact Dermatitis*. 2, 1-17.

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9.0 TEST PROCEDURE (CONT'D)

9.3 Data Interpretation

Edema, vesicles, papules and/or erythema that persist or increase in intensity either during the Induction and/or Challenge phase may be indicative of allergic contact dermatitis. Allergic responses normally do not resolve or improve markedly at 72-96 hours.

Exceptions to typical skin reactions may occur. These may include, but not be limited to, symptoms of allergic contact sensitivity early in the Induction period to one or more test products. When this occurs in one subject, such a reaction usually suggests either an idiosyncratic response or that the subject had a pre-exposure/sensitization to the test material or component(s) of the test material or a cross-reactivity with a similar product/component. Data for such reactions will be included in the study report but will not be included in the final study analysis/conclusion of sensitization.

10.0 RESULTS AND DISCUSSION

(See Table 2 for Individual Scores)

A total of 55 subjects (6 males and 49 females ranging in age from 20 to 74 years) were empanelled for the testing procedure. Forty-nine (49/55) subjects satisfactorily completed the test procedure on Test Article Six (6/55) subjects discontinued for personal reasons unrelated to the conduct of the study. Discontinued subject data are shown up to the point of discontinuation, but are not used in the Conclusions section of this final report.

Induction Phase Summary

Test Article	Induction Scores (Number of Responses)						Evidence of Irritation
	0.5	1	2	3	4	Other	
	0	0	0	0	0	0	No

Challenge Phase Summary

Test Article	Challenge Scores (Number of Responses)						Evidence of Sensitization
	0.5	1	2	3	4	Other	
	0	0	0	0	0	0	No

There was no skin reactivity observed at any time during the course of the study.

11.0 CONCLUSIONS

Under the conditions of a repeated insult (occlusive) patch test procedure conducted in 49 subjects, Test Article: was "Dermatologist-Tested" and was not associated with skin irritation or allergic contact dermatitis in human subjects.

TABLE 1

SUBJECT DEMOGRAPHICS

Subject No.	Initials	Age	Sex	Race	Subject No.	Initials	Age	Sex	Race
1	[redacted]	59	F	CA	29	[redacted]	39	F	CA
2		51	F	BA	30		47	F	CA
3		27	F	CA	31		38	F	CA
4		56	F	CA	32		32	F	CA
5		48	F	CA	33		65	F	CA
6		53	F	BA	34		45	F	BA
7		36	F	BH	35		74	F	CA
8		30	M	BA	36		60	F	CA
9		56	M	BA	37		47	F	BA
10		70	F	BA	38		54	F	CA
11		56	F	CA	39		72	F	CA
12		24	F	CA	40		60	F	CA
13		38	F	CA	41		46	F	CA
14		44	F	CA	42		37	F	HS
15		46	F	BA	43		68	F	CA
16		50	F	HS	44		40	F	CA
17		39	F	CA	45		66	F	CA
18		51	M	CA	46		47	F	BH
19		22	M	CA	47		56	F	CA
20		38	F	CA	48		48	M	CA
21		34	F	CA	49		59	F	BH
22		41	F	HS	50		71	F	CA
23		67	F	CA	51		58	F	BA
24		44	F	CA	52		40	F	CA
25		43	F	CA	53		36	F	BA
26		20	M	CA	54		66	F	CA
27		51	F	CA	55		31	F	CA
28		49	F	BA					

BA = Black African American
BH = Black Hispanic
CA = Caucasian
HS = Hispanic

Shaded area = Discontinued subject

TABLE 2

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST – OCCLUSIVE

Subj. No.	Induction Evaluation Number 1	2	3	4	5	6	7	8	9	Challenge Virgin Site 24hr	72hr
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	Discontinued					
10	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0
18	0	Discontinued									
19	0	Discontinued									
20	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

TABLE 2 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - OCCLUSIVE

Subj. No.	Induction Evaluation Number 1	2	3	4	5	6	7	8	9	Challenge Virgin Site 24hr	72hr
31	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0
35	0	Discontinued									
36	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0
46	0	Discontinued									
47	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0
53	0	Discontinued									
54	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: May 10, 2022

SUBJECT: Rosa Centifolia Stem Extract

Noveal. 2022. Method of manufacture Mexoryl SDA (Rosa Centifolia Stem Extract).

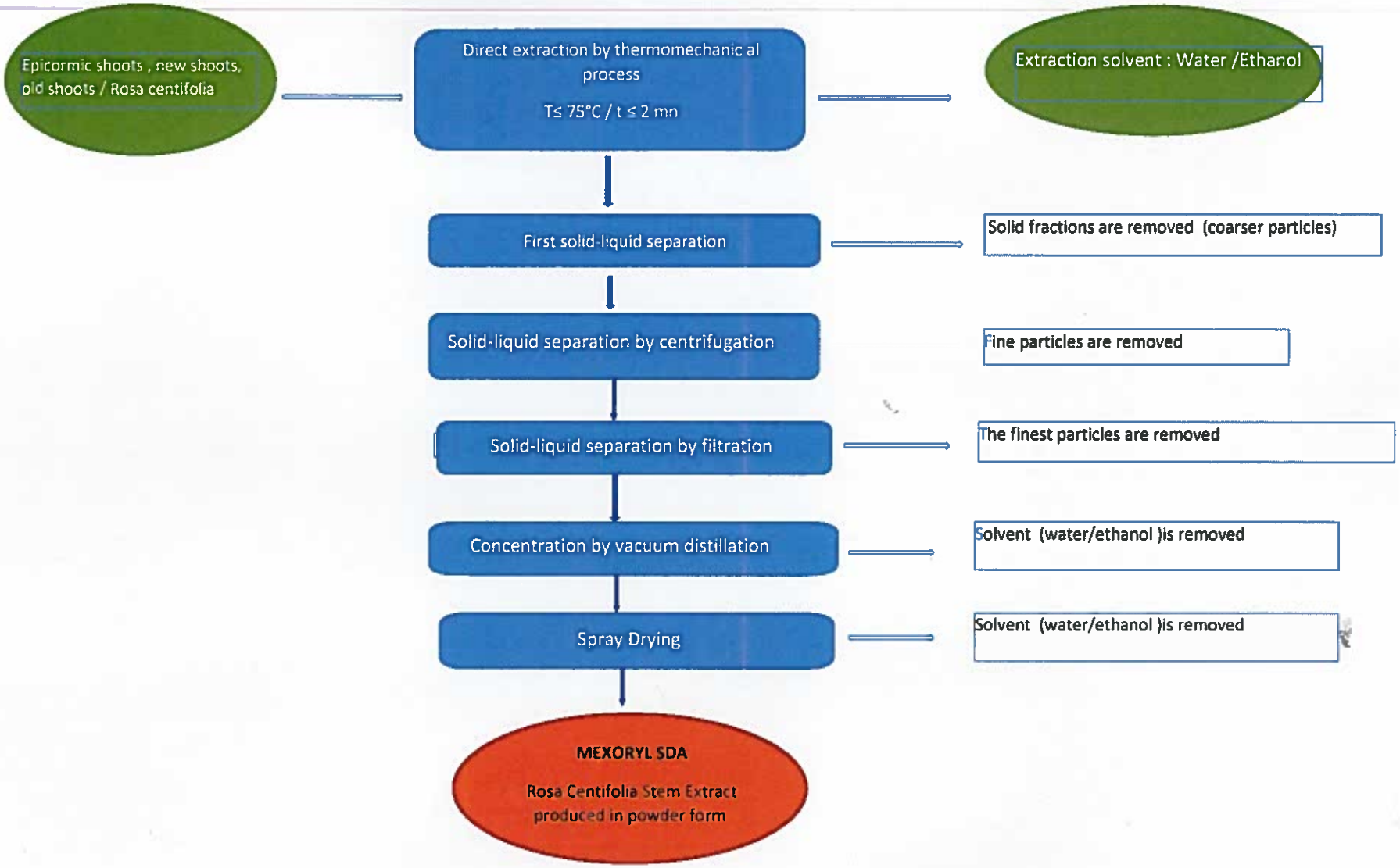
Noveal. 2022. Certificate of analytical composition Mexoryl SDA (Rosa Centifolia Stem Extract).

Anonymous. 2019. Mexoryl SDA (Rosa Centifolia Stem Extract): Bacterial reverse mutation assay.

Anonymous. 2019. Mexoryl SDA (Rosa Centifolia Stem Extract): *In vitro* human lymphocyte micronucleus assay.

Anonymous. 2019. EpiSkin™ Micronucleus assay Mexoryl SDA (Rosa Centifolia Stem Extract).

May 2022



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[redacted]

CERTIFICATE ON ANALYTICAL COMPOSITION

Product name:	**MEXORYL SDA**
INCI name	**Rosa Centifolia Stem Extract**
Manufacturer:	**NOVEAL**

By the document, we, the undersigned company NOVEAL, declare that:

Identification and Composition MEXORYL SDA:

Identification substance : UVCB

Appearance : beige powder

Water Content 2 ≥/ ≤ 6 %

Ash content < 20% (determined by sulfuric ashes)

Lipid content ≤ 1 %

Polyphenols ≥ 20 % and *Typical concentration* ≤ *40 %* (determined by Follin method)

Allergens listed in Regulation 1223/2009.

- Geraniol and Citronellol not detected
- Limonene ≤ 1ppm
- Benzyl Alcohol ≤ 4ppm
- The other twenty-two allergens listed in the Annexe III of the Regulation 1223/2009 are not detected.

Place: **Le Thillay** Date : 21/04/2022

Company representative : **Eric DUFOUR**

Function : **Regulatory Manager** Signature :

[redacted]

[redacted]

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[redacted]

Final Report

Study Title	Mexoryl SDA: Bacterial Reverse Mutation Assay Rosa Centifolia Stem Extract
Study Director	[redacted]
Test Facility	[redacted]
[redacted] Study Number	8403662
[redacted] Client Identifier	1008632
Sponsor	[redacted]
Report Issue Date	31 May 2019
Page Number	1 of 29

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COMPLIANCE STATEMENT

I, the Study Director, hereby declare that the work was performed under my supervision and that the findings provide a true and accurate record of the results obtained.

Compilation of the historical control range was performed outside of the scope of this study.

This study was conducted in accordance with the following:

- The United Kingdom Good Laboratory Practice Monitoring Authority, Medicines and Healthcare products Regulatory Agency (MHRA): Good Laboratory Practice Regulations 1999, Statutory Instrument 1999 No.3106 as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations, 2004, Statutory Instrument 2004, No. 994

- The OECD Principles on Good Laboratory Practice ENV/MC/CHEM (98) 17 (Revised in 1997, Issued January 1998).

The stability, homogeneity and achieved concentration of test article formulations were not analysed in this study. Although stability of the test article formulation was not determined in this study, formulations were used on the day of preparation.

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QUALITY ASSURANCE STATEMENT

Mexoryl SDA: Bacterial Reverse Mutation Assay

Critical procedures performed routinely in an operational area may be audited as part of a process inspection programme. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included in the following.

In addition to the inspection programme detailed in the following, a facility inspection programme is also operated. Details of this programme, which covers all areas of the facility annually (at a minimum), are set out in standard operating procedures.

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
13 Feb 2019	13 Feb 2019	Protocol Review	13 Feb 2019
08 Apr 2019	09 Apr 2019	Draft Report and Data Review	09 Apr 2019
23 May 2019	23 May 2019	Sign off Inspection Record	23 May 2019
24 May 2019	24 May 2019	Final Report Review	24 May 2019

Process

Inspection Dates		Phase	Date Reported to Management
From	To		
18 Feb 2019	25 Feb 2019	Set-up and Treatment of Test Systems	27 Feb 2019
18 Feb 2019	27 Feb 2019	Assessment	27 Feb 2019
12 Mar 2019	14 Mar 2019	Dispensary Procedures	18 Mar 2019
19 Mar 2019	21 Mar 2019	Set-up and Treatment of Test Systems	21 Mar 2019
20 Mar 2019	21 Mar 2019	Assessment	21 Mar 2019
20 Mar 2019	22 Mar 2019	Study Direction	22 Mar 2019

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RESPONSIBLE PERSONNEL

Study Monitor

Study Director

Genetic Toxicology Operations

Lead Quality Assurance Contact

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1. SUMMARY

Mexoryl SDA was assayed for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9), in two separate experiments.

All Mexoryl SDA treatments in this study were performed using formulations prepared in purified water.

Mutation Experiment 1 treatments of all the tester strains were performed in the absence and in the presence of S-9, using final concentrations of Mexoryl SDA at 5, 16, 50, 160, 500, 1600 and 5000 µg/plate, plus vehicle and positive controls. Following these treatments, evidence of toxicity was observed at 5000 µg/plate in strains TA102 in the absence of S-9, and TA98 in the presence of S-9.

Mutation Experiment 2 treatments of all the tester strains were performed in the absence and in the presence of S-9. The maximum test concentration of 5000 µg/plate was retained for all strains. Narrowed concentration intervals were employed covering the range 156.25-5000 µg/plate, in order to examine more closely those concentrations of Mexoryl SDA approaching the maximum test concentration and considered therefore most likely to provide evidence of any mutagenic activity. In addition, all treatments in the presence of S-9 were further modified by the inclusion of a pre-incubation step. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected using this assay system. Following these treatments, evidence of toxicity was observed at 5000 µg/plate in strain TA102 in the absence and presence of S-9.

The test article was completely soluble in the aqueous assay system at all concentrations treated, in each of the experiments performed. A slight colouration of the test agar was observed at 5000 µg/plate.

Vehicle and positive control treatments were included for all strains in both experiments. The mean numbers of revertant colonies fell within acceptable ranges for vehicle control treatments, and were elevated by positive control treatments.

Following Mexoryl SDA treatments of all the test strains in the absence and presence of S-9, no increases in revertant numbers were observed that were ≥1.5-fold (in strain TA102), ≥2-fold (in strains TA98 or TA100) or ≥3-fold (in strains TA1535 or TA1537) the concurrent vehicle control. This study was considered therefore to have provided no evidence of any Mexoryl SDA mutagenic activity in this assay system.

It was concluded that Mexoryl SDA did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at concentrations up to 5000 µg/plate (the maximum recommended concentration according to current regulatory guidelines, and a toxic concentration) in the absence and in the presence of a rat liver metabolic activation system (S-9).

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2. GENERAL STUDY INFORMATION

2.1 Objective

The objective of this study was to evaluate the ability of Mexoryl SDA to induce reverse mutations in histidine-requiring strains of *Salmonella typhimurium* in the absence and presence of a rat liver metabolising system (S-9).

2.2 Introduction

When the bacterial strains are exposed to a mutagen, some of the bacteria in the treated population undergo genetic changes which cause them to revert to a prototrophic state and thus grow in the absence of exogenous amino acids. Different tester strains have different sensitivities and responses to known mutagens, therefore, using a range of tester strains increases the sensitivity of the assay to detect any mutagenic activity.

The following bacterial strains were used in this study:

Organism	Strain	Type of Mutation	Mutant Gene
S. typhimurium	TA98	frame-shift	histidine
S. typhimurium	TA100	base-pair substitution	histidine
S. typhimurium	TA1535	base-pair substitution	histidine
S. typhimurium	TA1537	frame-shift	histidine
S. typhimurium	TA102	base-pair substitution	histidine

2.3 Study Timetable

Study Initiation Date:	08 February 2019
Experimental Start Date:	20 February 2019
Experimental Completion Date:	04 March 2019
Study Completion Date:	Is the date the final report is signed by the Study Director

2.4 Regulatory Test Guidelines

OECD Guideline 471 (OECD, 1997).

2.5 Protocol Adherence

This study was conducted according to the Protocol.

2.6 Major Computer Systems

Application Name	Application Function
REES*	Monitoring of facility storage conditions
eNotes*	Electronic communication system
Pristima*	Formulations
Ames Study Manager*/Sorcerer*	In-life data collection
Documentum	Document management system for generation of study-related documents and electronic signatures

2.7 Archive Statement

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3. MATERIALS

3.1 Test Article

Mexoryl SDA (CAS number 84604-12-6), also known as Rosebush watersprout solid extract, batch number E 14, was a beige powder. It was received on 07 February 2019 and stored at 15-25°C protected from light. The test article was a UVCB, therefore purity was considered to be 100% and the material was tested as supplied. The retest date (based on the Sponsor's knowledge of the test article) was given as 22 January 2020, see Appendix 9-3. The test article information and certificate of analysis provided by the Sponsor are considered an adequate description of the characterisation, purity and stability of the test article. Determinations of stability and characteristics of the test article were the responsibility of the Sponsor. No retention of the test article was performed.

Preliminary solubility data indicated that Mexoryl SDA was soluble in purified water at concentrations up to at least 50 mg/mL. A maximum concentration of 5000 µg/plate was selected for Experiment 1, in order that initial treatments were performed up to this maximum recommended concentration according to current regulatory guidelines (OECD, 1997). A maximum concentration of 5000 µg/plate was also selected for Experiment 2.

Test article stock solutions were prepared by formulating Mexoryl SDA under subdued lighting in purified water with the aid of vortex mixing, ultrasonication and warming at 37°, where required, to give the maximum required treatment concentration. The Sponsor advised that the test article was non-sterile, stock solutions were therefore membrane filter-sterilised (Pall Acrodisc 32 mm filter, 0.2 µm pore size). Subsequent dilutions were made using purified water. The test article solutions were protected from light and used within approximately 5.5 hours of initial formulation. The following concentrations were tested:

Experiment	S-9	Concentration of Treatment Solution (mg/mL)	Final Concentration (µg/plate)
Mutation Experiment 1	- and +	0.05	5
		0.16	16
		0.5	50
		1.6	160
		5	500
		16	1600
		50	5000
Mutation Experiment 2	- and +	1.5625	156.25
		3.125	312.5
		6.250	625
		12.5	1250
		25	2500
		50	5000

0.1 mL volume additions of test article solution were used for all treatments.

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3.2 Formulations Analysis

In accordance with the regulatory test guidelines applicable for this study (see Section 2.4), no analyses of the stability of the test article in administered formulations or dilutions was undertaken as fresh preparation of test article were employed.

Following discussions with the Sponsor, analyses for achieved concentration and homogeneity (where appropriate) of test article formulations were not conducted as part of this study, as this is not a requirement of the regulatory test guidelines.

The absence of such analyses is noted in the Study Director's statement of GLP compliance.

3.3 Controls

Vehicle controls comprised treatments with the vehicle purified water using the same 0.1 mL additions per plate as the test article treatments. Positive controls comprised treatments with the appropriate stock positive control solution using 0.05 mL additions. The positive control chemicals were supplied and used according to the following table:

Chemical [a]	Stock [b] Concentration (µg/mL)	Final Concentration (µg/plate)	Strain(s)	S-9
2-nitrofluorene (2NF)	100	5	TA98	–
Sodium azide (NaN_3)	40	2	TA100, TA1535	–
9-aminoacridine (AAC)	1000	50	TA1537	–
Mitomycin C (MMC)	4	0.2	TA102	–
Benzo[a]pyrene (B[a]P)	200	10	TA98	+
2-aminoanthracene (AAN)	100	5	TA100, TA1535, TA1537	+
	400	20	TA102	+

[a] Obtained from Sigma-Aldrich.

[b] Stock solutions were formulated in purified water (NaN_3 and MMC), or in anhydrous analytical grade dimethyl sulphoxide (DMSO) (2NF, AAC, AAN and B[a]P). All stock solutions were stored in aliquots protected from light at 2-8°C, with the exception of B[a]P which was stored in aliquots at <-50°C and MMC which was prepared freshly on the day of use or stored in aliquots at <-50°C.

3.4 Metabolic Activation System

The mammalian liver post-mitochondrial fraction (S-9) used for metabolic activation was obtained from Molecular Toxicology Incorporated, USA where it was prepared from male Sprague Dawley rats induced with Aroclor 1254. The S-9 was supplied as lyophilized S-9 mix (Mutazyme™), stored frozen at <-20°C, and thawed and reconstituted with purified water to provide a 10% S-9 mix just prior to use. Each batch was checked by the manufacturer for sterility, protein content, ability to convert ethidium bromide and cyclophosphamide to bacterial mutagens, and cytochrome P-450-catalysed enzyme activities (alkoxyresorufin-O-dealkylase activities). See Appendix 9-4.

Treatments were carried out both in the absence and presence of S-9 by addition of either buffer solution or 10% S-9 mix respectively. The composition of the Mutazyme™ 10% S-9 mix and buffer solution are described in the following table:

Ingredient	Final Content per mL in:	
	10% S-9 mix	Buffer Solution
Sodium phosphate buffer pH 7.4 (SPB)	100 µmoles	100 µmoles
Glucose-6-phosphate (disodium) (G-6-P)	5 µmoles	-
β-Nicotinamide adenine dinucleotide phosphate (NADP) (disodium)	4 µmoles	-
Magnesium chloride ($MgCl_2$)	8 µmoles	-
Potassium chloride (KCl)	33 µmoles	-
Water	To volume	To volume
S-9	100 µL	-

3.5 Supplements

L-histidine HCl (in 250 mM $MgCl_2$) and D-biotin were added at the time of plating, by supplementing the top agar. Quantities of each supplement were as follows:

Supplement	Final Quantity
L-histidine HCl	20 µg
D-biotin	24.4 µg

3.6 Bacteria

Five strains of *Salmonella typhimurium* bacteria (TA98, TA100, TA1535, TA1537 and TA102) were used in this study. Strains TA98, TA1535 and TA1537 were originally obtained from the UK NCTC. Strains TA100 and TA102 were derived from cultures originally obtained from [redacted]. For all assays, bacteria were cultured at 37±1°C for 10 hours in nutrient broth, containing ampicillin (TA98, TA100) or ampicillin and tetracycline (TA102) as appropriate, to provide bacterial cultures in the range of approximately 10^8 to 10^9 cells/mL, based on cell density assessments for each culture. Incubation was carried out with shaking in an anhydric incubator, set to turn on using a timer switch. All treatments were completed within 6 hours of the end of the incubation period.

The inocula were taken from master plates or vials of frozen cultures, which had been checked for strain characteristics (histidine dependence, *rfa* character, *uvrB* character, if applicable and resistance to ampicillin or ampicillin plus tetracycline).

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4. METHODS

4.1 Test System

The test system was suitably labelled to clearly identify the study number, bacterial strain, test article concentration (where appropriate), positive and vehicle controls, in the absence or presence of S-9 mix.

4.2 Mutation Experiments

Mexoryl SDA was tested for mutation (and toxicity) in five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102), in two separate experiments, at the concentrations detailed previously, using triplicate plates without and with S-9 for test article, vehicle and positive controls. These platings were achieved by the following sequence of additions to 2 mL supplemented molten top agar at 45±1°C:

- 0.1 mL bacterial culture
- 0.1 mL of test article solution/vehicle control or 0.05 mL of positive control
- 0.5 mL 10% S-9 mix or buffer solution

followed by rapid mixing and pouring on to Vogel-Bonner E agar plates. When set, the plates were inverted and incubated at 37±1°C protected from light for 3 days. Following incubation, these plates were examined for evidence of toxicity to the background lawn, and where possible revertant colonies were counted (see Colony Enumeration Section 4.4).

As the results of Experiment 1 were negative, treatments in the presence of S-9 in Experiment 2 included a pre-incubation step. Quantities of test article, vehicle control or positive control, bacteria and S-9 mix detailed above, were mixed together and incubated for 20 minutes at 37±1°C, with shaking, before the addition of 2 mL molten agar at 45±1°C. Plating of these treatments then proceeded as for the normal plate-incorporation procedure. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected in the assay.

4.3 Toxicity Assessment

The background lawns of the plates were examined for signs of toxicity. Revertant plate count data were also assessed as a marked reduction in revertants compared to the concurrent vehicle controls would also be considered as evidence of toxicity.

4.4 Colony Enumeration

Colonies were counted electronically using a Sorcerer Colony Counter (Perceptive Instruments) or manually where confounding factors such as bubbles or splits in the agar affected the accuracy of the automated counter.

4.5 Analysis of Results

4.5.1 Treatment of Data

Individual plate counts were recorded separately and the mean and standard deviation of the plate counts for each treatment were determined. Control counts were compared with the laboratory's historical control ranges (Appendix 9-1 and

Appendix 9-2). Data were considered acceptable if the vehicle control counts fell within the calculated historical control ranges and the positive control plate counts were comparable with the historical control ranges.

The presence or otherwise of a concentration response was checked by non-statistical analysis, up to limiting levels (for example toxicity, precipitation or 5000 µg/plate). However, adequate interpretation of biological relevance was of critical importance.

4.5.2 Acceptance Criteria

The assay was considered valid if all the following criteria were met:

1. The vehicle control counts fell within the laboratory's historical control ranges as defined in Appendix 9-1

2. The positive control chemicals induced increases in revertant numbers of ≥1.5-fold (in strain TA102), ≥2-fold (in strains TA98 and TA100) or ≥3-fold (in strains TA1535 and TA1537) the concurrent vehicle control confirming discrimination between different strains, and an active S-9 preparation.

4.5.3 Evaluation Criteria

For valid data, the test article was considered to be mutagenic if:

1. A concentration related increase in revertant numbers was ≥1.5-fold (in strain TA102), ≥2-fold (in strains TA98 or TA100) or ≥3-fold (in strains TA1535 or TA1537) the concurrent vehicle control values

2. Any observed response described above was reproducible.

The test article was considered positive in this assay if both of the above criteria were met.

The test article was considered negative in this assay if neither of the above criteria were met.

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5. RESULTS

5.1 Toxicity, Solubility and Concentration Selection

Details of all treatment solution concentrations and final Mexoryl SDA concentrations are provided in the Test Article Section 3.1.

Mutation Experiment 1 treatments of all the tester strains were performed in the absence and in the presence of S-9, using final concentrations of Mexoryl SDA at 5, 16, 50, 160, 500, 1600 and 5000 µg/plate, plus vehicle and positive controls. Following these treatments, evidence of toxicity in the form of a slight thinning of the background bacterial lawn and/or a reduction in revertants was observed at 5000 µg/plate in strains TA102 in the absence of S-9, and TA98 in the presence of S-9.

Mutation Experiment 2 treatments of all the tester strains were performed in the absence and in the presence of S-9. The maximum test concentration of 5000 µg/plate was retained for all strains. Narrowed concentration intervals were employed covering the range 156.25-5000 µg/plate, in order to examine more closely those concentrations of Mexoryl SDA approaching the maximum test concentration and considered therefore most likely to provide evidence of any mutagenic activity. In addition, all treatments in the presence of S-9 were further modified by the inclusion of a pre-incubation step. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected using this assay system. Following these treatments, evidence of toxicity in the form of a slight thinning of the background bacterial lawn was observed at 5000 µg/plate in strain TA102 in the absence and presence of S-9.

The test article was completely soluble in the aqueous assay system at all concentrations treated, in each of the experiments performed. A slight colouration of the test agar was observed at 5000 µg/plate.

5.2 Data Acceptability and Validity

The individual mutagenicity plate counts were averaged to give mean values, which are presented in Section 8. From the data it can be seen that vehicle control counts fell within the laboratory's historical ranges (Appendix 9-1). The positive control chemicals all induced increases in revertant numbers of ≥1.5-fold (in strain TA102), ≥2-fold (in strains TA98 and TA100) or ≥3-fold (in strains TA1535 and TA1537) the concurrent vehicle controls confirming discrimination between different strains, and an active S-9 preparation. The study therefore demonstrated correct strain and assay functioning and was accepted as valid.

5.3 Mutation

Following Mexoryl SDA treatments of all the test strains in the absence and presence of S-9, no increases in revertant numbers were observed that were ≥1.5-fold (in strain TA102), ≥2-fold (in strains TA98 and TA100) or ≥3-fold (in strains TA1535 and TA1537) the concurrent vehicle control. This study was considered therefore to have provided no evidence of any Mexoryl SDA mutagenic activity in this assay system.

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6. CONCLUSION

It was concluded that Mexoryl SDA did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at concentrations up to 5000 µg/plate (the maximum recommended concentration according to current regulatory guidelines, and a toxic concentration) in the absence and in the presence of a rat liver metabolic activation system (S-9).

7. ASSOCIATED STUDY INFORMATION

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7.1 References

OECD (1997). "Bacterial Reverse Mutation Test", in: OECD Guideline for the Testing of Chemicals, Test Guideline 471.

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7.2 Abbreviations

Abbreviation	Description
AAC	9-Aminoacridine
AAN	2-Aminoanthracene
B[a]P	Benzo[a]pyrene
DMSO	Dimethyl sulphoxide
GLP	Good Laboratory Practice
G6P	Glucose-6-phosphate
KCl	Potassium chloride
$MgCl_2$	Magnesium chloride
MMC	Mitomycin C
NADP	β-Nicotinamide adenine dinucleotide phosphate (disodium)
NaN_3	Sodium azide
2NF	2-Nitrofluorene
OECD	Organisation for Economic Co-operation and Development
S-9	Rat liver metabolic activation system
SOP	Standard Operating Procedure
SPB	Sodium phosphate buffer pH 7.4
UK NCTC	United Kingdom National Collection of Type Cultures
Units of Measure	
°C	Degrees Celsius
µg	Microgram
mg	Milligram
mL	Millilitre
µL	Microlitre
µmoles	Micromoles
mM	Millimolar
mm	Millimeter
Footnotes to Tables	
B	Bubbles or split in agar
M	Plate counted manually
S	Slight thinning of background bacterial lawn

8. TABLES

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Table 8.1: Raw Plate Counts and Calculated Mutagenicity Data, Mutation Experiment 1, -S-9

Strain	Compound	Conc. Level (µg/plate)	Mean	Standard Deviation	Fold Increase	Revertant Numbers Per Plate
TA98	Purified water	-	20.0	5.3	-	16, 26, 18
	Mexoryl SDA	5	20.3	6.8	1.0	28, 18, 15
		16	20.7	6.0	1.0	15, 20, 27
		50	16.0	4.6	0.8	17, 20, 11
		160	19.7	5.5	1.0	20, 25, 14
		500	24.7	8.5	1.2	15, 28, 31
		1600	25.7	4.7	1.3	24, 31, 22
		5000	20.7	4.2	1.0	24, 16, 22
	2NF	5	789.0	63.9	39.5	862, 762, 743
TA100	Purified water	-	112.7	9.5	-	102, 120, 116
	Mexoryl SDA	5	110.3	5.5	1.0	113, 104, 114
		16	110.3	6.5	1.0	117, 110, 104
		50	108.3	6.5	1.0	115, 108, 102
		160	116.0	11.5	1.0	115, 128, 105
		500	99.7	14.8	0.9	96, 116, 87
		1600	104.7	11.0	0.9	101, 96, 117
		5000	104.7	24.1	0.9	102, 130, 82
	NaN_3	2	1066.3	61.0	9.5	1067, 1127, 1005
TA1535	Purified water	-	13.7	4.0	-	9, 16, 16
	Mexoryl SDA	5	16.0	3.6	1.2	20, 13, 15
		16	12.0	7.0	0.9	7, 20, 9
		50	13.0	2.0	1.0	13, 15, 11
		160	11.0	3.6	0.8	10, 15, 8
		500	10.0	3.0	0.7	7, 13, 10
		1600	8.3	0.6	0.6	8, 8, 9
		5000	12.7	4.2	0.9	16, 14, 8 M B
	NaN_3	2	809.0	39.7	59.2	814, 767, 846
TA1537	Purified water	-	7.3	2.3	-	6, 6, 10
	Mexoryl SDA	5	9.7	5.7	1.3	8, 5, 16
		16	4.7	2.5	0.6	2, 5, 7
		50	7.0	6.1	1.0	14, 3, 4
		160	9.0	1.0	1.2	9, 10 M B, 8
		500	10.7	5.0	1.5	6, 10, 16
		1600	7.3	1.2	1.0	8, 8, 6 M B
		5000	5.3	0.6	0.7	5, 5, 6
	AAC	50	950.7	110.3	129.6	890, 884, 1078
TA102	Purified water	-	229.3	14.4	-	221, 221, 246
	Mexoryl SDA	5	238.3	14.0	1.0	225, 237, 253
		16	231.0	13.0	1.0	218, 231, 244
		50	239.0	14.7	1.0	223, 242, 252
		160	250.0	10.4	1.1	238, 256, 256
		500	235.3	26.8	1.0	265, 228, 213
		1600	258.0	17.3	1.1	249, 278, 247
		5000	270.0	24.2	1.2	296 S, 266 S, 248 S
	MMC	0.2	854.7	88.7	3.7	884, 925, 755

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Table 8.2: Raw Plate Counts and Calculated Mutagenicity Data, Mutation Experiment 1, +S-9

Strain	Compound	Conc. Level (µg/plate)	Mean	Standard Deviation	Fold Increase	Revertant Numbers Per Plate
TA98	Purified water	-	35.3	5.0	-	36, 30, 40
	Mexoryl SDA	5	36.7	4.7	1.0	33, 35, 42
		16	40.3	2.5	1.1	40, 38, 43
		50	36.0	9.6	1.0	29, 32, 47
		160	32.7	7.0	0.9	26, 32, 40
		500	31.7	0.6	0.9	32, 31, 32
		1600	25.0	4.4	0.7	28, 20, 27
		5000	16.7	1.5	0.5	17, 15, 18 M B
	B[a]P	10	324.3	21.8	9.2	342, 300, 331
TA100	Purified water	-	126.7	9.6	-	125, 137, 118
	Mexoryl SDA	5	138.0	18.7	1.1	121, 158, 135
		16	138.3	5.0	1.1	143, 139, 133
		50	134.3	19.6	1.1	129, 118, 156
		160	137.7	6.8	1.1	143, 140, 130
		500	115.7	2.1	0.9	115, 114, 118
		1600	103.7	4.9	0.8	107, 98, 106
		5000	107.7	4.7	0.9	104, 113, 106
	AAN	5	2554.0	211.7	20.2	2318, 2617, 2727
TA1535	Purified water	-	15.0	7.5	-	8, 14, 23
	Mexoryl SDA	5	13.3	6.0	0.9	7, 19, 14
		16	15.7	4.2	1.0	11, 17, 19
		50	17.3	2.9	1.2	14, 19, 19
		160	11.3	3.5	0.8	8, 11, 15
		500	16.3	2.9	1.1	13, 18, 18
		1600	16.0	0.0	1.1	16, 16, 16
		5000	17.0	9.5	1.1	23, 6, 22
	AAN	5	274.3	3.5	18.3	278, 274, 271
TA1537	Purified water	-	18.0	3.6	-	17, 15, 22
	Mexoryl SDA	5	13.7	0.6	0.8	14, 14, 13
		16	18.7	4.6	1.0	16, 24, 16
		50	15.3	2.1	0.9	16, 17, 13
		160	17.0	5.6	0.9	22, 18, 11
		500	12.3	8.1	0.7	18, 16, 3
		1600	9.7	3.8	0.5	8, 7, 14
		5000	13.0	5.3	0.7	19, 11, 9
	AAN	5	285.7	14.5	15.9	269, 295, 293
TA102	Purified water	-	273.7	32.1	-	243, 271, 307
	Mexoryl SDA	5	304.3	7.0	1.1	297, 305, 311
		16	298.7	13.0	1.1	298, 312, 286
		50	328.7	11.0	1.2	320, 341, 325
		160	327.7	28.0	1.2	313, 360, 310
		500	316.0	18.4	1.2	308, 337, 303
		1600	280.7	12.6	1.0	279, 269, 294
		5000	299.0	17.3	1.1	319, 290, 288
	AAN	20	2559.3	415.4	9.4	2993, 2165, 2520

Table 8.3: Raw Plate Counts and Calculated Mutagenicity Data, Mutation Experiment 2, -S-9

Strain	Compound	Conc. Level (µg/plate)	Mean	Standard Deviation	Fold Increase	Revertant Numbers Per Plate
TA98	Purified water	-	31.0	1.0	-	32, 30, 31
	Mexoryl SDA	156.25	25.0	3.6	0.8	24, 22, 29
		312.5	31.0	2.6	1.0	33, 28, 32
		625	26.0	3.5	0.8	24, 24, 30
		1250	23.0	4.4	0.7	18, 26, 25
		2500	21.3	8.5	0.7	30, 21, 13
		5000	23.7	8.6	0.8	22, 33, 16
	2NF	5	1241.7	83.5	40.1	1325, 1158, 1242
TA100	Purified water	-	127.3	10.8	-	135, 115, 132
	Mexoryl SDA	156.25	124.0	18.1	1.0	107, 122, 143
		312.5	118.3	3.8	0.9	120, 114, 121
		625	120.7	19.6	0.9	123, 139, 100
		1250	120.0	6.2	0.9	125, 122, 113
		2500	131.7	4.9	1.0	134, 126, 135
		5000	122.0	5.6	1.0	127, 123, 116
	NaN_3	2	1113.0	38.4	8.7	1107, 1154, 1078
TA1535	Purified water	-	14.7	3.5	-	15, 18, 11
	Mexoryl SDA	156.25	14.3	6.4	1.0	18, 18, 7
		312.5	15.0	1.7	1.0	16, 16, 13
		625	16.0	2.0	1.1	18, 16, 14
		1250	16.0	5.0	1.1	11, 21, 16
		2500	13.0	4.6	0.9	17, 8, 14
		5000	8.0	1.7	0.5	6, 9, 9
	NaN_3	2	785.3	3.5	53.5	782, 785, 789
TA1537	Purified water	-	12.3	2.1	-	14, 10, 13
	Mexoryl SDA	156.25	12.0	2.6	1.0	13, 14, 9
		312.5	11.3	1.5	0.9	11, 13, 10
		625	12.7	4.0	1.0	8, 15, 15
		1250	10.3	3.2	0.8	9, 14, 8
		2500	11.0	2.6	0.9	14, 9, 10
		5000	7.0	2.6	0.6	5, 6, 10
	AAC	50	603.3	49.7	48.9	547, 622, 641
TA102	Purified water	-	274.7	3.1	-	272, 278, 274
	Mexoryl SDA	156.25	275.0	3.5	1.0	271, 277, 277
		312.5	269.7	13.8	1.0	280, 275, 254
		625	269.3	16.4	1.0	257, 288, 263
		1250	253.3	10.2	0.9	265, 246, 249
		2500	256.0	16.6	0.9	249, 275, 244
		5000	239.7	7.6	0.9	245 S, 231 S, 243 S
	MMC	0.2	963.3	190.7	3.5	1090, 1056, 744

Table 8.4: Raw Plate Counts and Calculated Mutagenicity Data, Mutation Experiment 2, +S-9

Strain	Compound	Conc. Level (µg/plate)	Mean	Standard Deviation	Fold Increase	Revertant Numbers Per Plate
TA98	Purified water	-	32.3	5.1	-	38, 28, 31
	Mexoryl SDA	156.25	39.0	12.5	1.2	27, 38, 52
		312.5	35.0	7.9	1.1	41, 38, 26
		625	29.0	1.0	0.9	28, 29, 30
		1250	23.7	5.5	0.7	21, 30, 20
		2500	23.0	4.0	0.7	19, 27, 23
		5000	26.7	5.5	0.8	24, 33, 23
	B[a]P	10	339.3	21.6	10.5	337, 362, 319
TA100	Purified water	-	158.0	4.4	-	156, 163, 155
	Mexoryl SDA	156.25	135.0	12.5	0.9	125, 149, 131
		312.5	140.0	9.0	0.9	131, 149, 140
		625	132.3	5.5	0.8	127, 138, 132
		1250	135.0	3.6	0.9	139, 132, 134
		2500	118.7	8.3	0.8	116, 112, 128
		5000	122.3	4.6	0.8	117, 125, 125
	AAN	5	2579.3	254.4	16.3	2287, 2701, 2750
TA1535	Purified water	-	16.0	1.0	-	17, 16, 15
	Mexoryl SDA	156.25	15.0	5.3	0.9	19, 17, 9
		312.5	14.7	3.5	0.9	11, 18, 15
		625	14.3	1.5	0.9	16, 14, 13
		1250	12.7	4.9	0.8	7, 15, 16
		2500	16.7	2.1	1.0	19, 16, 15
		5000	12.7	5.7	0.8	19, 8, 11
	AAN	5	236.3	13.7	14.8	230, 252, 227
TA1537	Purified water	-	17.7	7.0	-	11, 25, 17
	Mexoryl SDA	156.25	22.0	0.0	1.2	22, 22, 22
		312.5	18.3	2.5	1.0	21, 16, 18
		625	19.0	10.0	1.1	29, 19, 9
		1250	16.7	4.9	0.9	11, 20, 19
		2500	13.7	2.5	0.8	14, 11, 16
		5000	10.3	4.0	0.6	15, 8, 8
	AAN	5	232.7	8.1	13.2	227, 242, 229
TA102	Purified water	-	326.7	31.6	-	291, 351, 338
	Mexoryl SDA	156.25	391.3	12.0	1.2	392, 379, 403
		312.5	378.0	18.2	1.2	369, 399, 366
		625	378.7	10.0	1.2	371, 375, 390
		1250	368.0	28.5	1.1	396, 339, 369
		2500	336.7	19.3	1.0	359, 325, 326
		5000	215.0	3.6	0.7	214 S, 219 S, 212 S
	AAN	20	2694.3	628.6	8.2	3215, 2872, 1996

9. APPENDICES

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Appendix 9-1: Historical Vehicle Control Values for *S. typhimurium* Strains

Data generated from studies performed within the GLP laboratory, by GLP trained staff, whether a claim of GLP compliance was made or not, were included in the compilation of the historical control ranges without bias.

Strain	S-9	No of Studies	No of Plates	Mean	99% Reference Range		Date Range	
					Lower	Higher	From	To
TA98	-	108	412	22.3	9	47	16 Oct 17	26 Feb 18
TA98	+	107	391	34.6	15	58	16 Oct 17	26 Feb 18
TA100	-	103	377	101.8	56	168	16 Oct 17	26 Feb 18
TA100	+	100	370	108.7	72	166	16 Oct 17	26 Feb 18
TA1535	-	78	287	19.6	7	35	25 Jun 18	28 Sep 18
TA1535	+	76	281	18.8	5	37	25 Jun 18	28 Sep 18
TA1537	-	76	299	10.0	1	22	16 Oct 17	26 Feb 18
TA1537	+	76	292	13.9	5	29	16 Oct 17	26 Feb 18
TA102	-	72	266	290.4	220	403	16 Oct 17	26 Feb 18
TA102	+	72	274	315.7	193	411	16 Oct 17	26 Feb 18

Ranges calculated using data selected without bias from studies scored between the stated periods.

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Appendix 9-2: Historical Positive Control Values for *S. typhimurium* Strains

Data generated from studies performed within the GLP laboratory, by GLP trained staff, whether a claim of GLP compliance was made or not, were included in the compilation of the historical control ranges without bias.

Strain	S-9	No of Studies	No of Plates	Mean	99% Reference Range		Date Range	
					Lower	Higher	From	To
TA98	-	106	408	922.7	421	2365	16 Oct 17	26 Feb 18
TA98	+	103	372	317.9	100	603	16 Oct 17	26 Feb 18
TA100	-	102	372	650.2	431	1470	16 Oct 17	26 Feb 18
TA100	+	98	351	1524.3	455	2884	16 Oct 17	26 Feb 18
TA1535	-	78	287	668.1	234	927	25 Jun 18	28 Sep 18
TA1535	+	76	278	190.2	37	614	25 Jun 18	28 Sep 18
TA1537	-	75	294	303.3	84	885	16 Oct 17	26 Feb 18
TA1537	+	76	276	286.3	41	550	16 Oct 17	28 Feb 18
TA102	-	72	264	936.9	454	2148	16 Oct 17	26 Feb 18
TA102	+	71	255	1559.8	368	3566	16 Oct 17	26 Feb 18

Ranges calculated using data selected without bias from studies scored between the stated periods.

Appendix 9-3: Certificate of Analysis

Laboratoire de Développement Analytique Qualité

CoA

E 510341
MEXORYL SDA
(Batch : E 14)

	Results
Appearance	beige powder
pH (Solution at 25 % in water)	5.4
Dry extract	96.9 %
Turbidity (1 % in water)	66 NTU
Dosage HPLC (w/w)	
Catechine content	3.0 %
HPLC profile	
Equivalent catechines	5.1 %
Procyanidine	0.8 %
Hyperoside	0.6 %
Equivalent Hyperosides	1.0 %
Kaempferol-3-O-glucoside	Not detected
Kaempferol	Not detected
Ethanol content	2500 ppm
Polyphenols (method Folin)	
(expressed in gallique acid)	33.4 %

Date of fabrication 23/01/2019
Validity of analysis 22/01/2020

04/02/2019

Appendix 9-4: Quality Control Statement for S-9

[redacted]

[redacted]

To: Moltzyme

QUALITY CONTROL & PRODUCTION CERTIFICATE

Animal Information	Part Number Information	PREP DATE: November 02, 2018
SPECIES: Rat	LOT NO.: 4022	EXPIRY: November 02, 2020
STRAIN: Sprague Dawley	PART NO.: 11-4021	INDUCING AGENT: Aroclor 1254, (Monsanto KL615), 500 mg/kg i.p.
SEX: Male	VOLUME: 20 mL	
AGE: 5 – 6 weeks	STORAGE: At or below -20°C	
WEIGHT: 175 – 199 g		
TISSUE: Liver		
REFERENCE: Maron, D & Ames, B., *Mutat Res*, 113: 173, 1983.		For Research Purposes Only

BIOCHEMISTRY:

- PROTEIN: 4.0 mg/ml

Assayed according to the method of Lowry et al., JBC 193:265, 1951 using bovine serum albumin as the standard. Protein concentration of reconstituted S9 mix was mathematically derived from the concentration of S9 used in production.

- ALKOXYRESORUFIN-O-DEALKYLASE ACTIVITIES

Activity	P450	Fold-Induction
BROD	2B1, 2B2	34.5
EROD	1A1, 1A2	66.1
MROD	1A1, 1A2	33.5
PROD	2B1, 2B2	24.7

Assays for ethoxyresorufin-O-deethylase (EROD), pentoxy-, benzyl- and methoxyresorufin-O-dealkylases (PROD, BROD, & MROD) were conducted using a modification of the methods of Burke, et al., *Biochem Pharm* 34:3337, 1985. Fold-Inductions were calculated as the ratio of the sample vs. uninduced specific activities (SA's). Control SA's (pmoles/min/ mg protein) were 158.6, 125.3, 49.4 & 55.2 for BROD, EROD, MROD and PROD, respectively.

BIOASSAY:

- TEST FOR THE PRESENCE OF ADVENTITIOUS AGENTS

Samples of S-9 were assayed for the presence of contaminating microorganisms by plating 1.0 ml volumes on Nutrient Agar and Minimal Glucose (Vogel-Bonner E, supplemented with 0.05 mM L-histidine and D-biotin) media. Duplicate plates were read after 40 - 48 h incubation at 35 ± 2°C. The tested samples met acceptance criteria.

- PROMUTAGEN ACTIVATION

No. His+ Revertants

TA98	TA1535
128.8	878

The ability of the sample to activate ethidium bromide (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lesca, et al., *Mutation Res* 129: 299, 1984. Data were expressed as revertants per µg EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.6 – 10% in S9 mix, were tested for their ability to activate benzo(a)pyrene (BP) and 2-aminoanthracene (2-AA) to metabolites mutagenic to TA100. Assays were conducted as described by Maron & Ames, (*Mutat Res* 113: 173, 1983.

	µl S9 per plate/number *his*+ revertants per plate					
Promutagen	0	3.1	6.3	12.5	25	50
BP (5 µg))	85	224	368	473	628	737
2-AA (2.5 µg)	110	687	1384	1779	1765	1228

[redacted]

Electronic Signatures

Date(GMT)	Justification
31-May-2019 11:03:29	QA Approval
31-May-2019 11:39:13	Study Director Approval

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[redacted]

Final Report

Study Title	Mexoryl SDA: *In Vitro* Human Lymphocyte Micronucleus Assay Rosa Centifolia Stem Extrac
Study Director	[redacted]
Test Facility	[redacted]
	8403663
Sponsor	[redacted]
Report Issue Date	31 July 2019
Page Number	1 of 41

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COMPLIANCE STATEMENT AND SIGNATURE PAGE

I, the undersigned, hereby declare that the work was performed under my supervision and that the findings provide a true and accurate record of the results obtained.

Compilation of the historical control range was performed outside of the scope of this study.

This study was conducted in accordance with the following:

- The United Kingdom Good Laboratory Practice Monitoring Authority, Medicines and Healthcare products Regulatory Agency (MHRA): Good Laboratory Practice Regulations 1999, Statutory Instrument 1999 No.3106 as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations, 2004, Statutory Instrument 2004, No. 994

- The OECD Principles on Good Laboratory Practice ENV/MC/CHEM (98) 17 (Revised in 1997, Issued January 1998).

The stability, homogeneity and achieved concentration of test article formulations were not analysed in this study. Although stability of the test article formulation was not determined in this study, formulations were used on the day of preparation.

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QUALITY ASSURANCE STATEMENT

Mexoryl SDA: *In Vitro* Human Lymphocyte Micronucleus Assay

This study has been reviewed by the Quality Assurance Unit of Covance, and the report accurately reflects the raw data. The following study-specific inspections were conducted and findings reported to the Study Director (SD) and associated management.

Critical procedures performed routinely in an operational area may be audited as part of a process inspection programme. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included in the following.

In addition to the inspection programme detailed in the following, a facility inspection programme is also operated. Details of this programme, which covers all areas of the facility annually (at a minimum), are set out in standard operating procedures.

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
14 Feb 2019	14 Feb 2019	Protocol Review	14 Feb 2019
24 Apr 2019	26 Apr 2019	Draft Report and Data Review	26 Apr 2019
24 Jul 2019	24 Jul 2019	Final Report Review	24 Jul 2019

Process

Inspection Dates		Phase	Date Reported to Management
From	To		
18 Feb 2019	25 Feb 2019	Set-up and Treatment of Test Systems	27 Feb 2019
18 Feb 2019	27 Feb 2019	Assessment	27 Feb 2019
12 Mar 2019	14 Mar 2019	Dispensary Procedures	18 Mar 2019
19 Mar 2019	21 Mar 2019	Set-up and Treatment of Test Systems	21 Mar 2019
20 Mar 2019	21 Mar 2019	Assessment	21 Mar 2019
20 Mar 2019	22 Mar 2019	Study Direction	22 Mar 2019
09 Apr 2019	09 Apr 2019	Assessment	09 Apr 2019
16 Apr 2019	26 Apr 2019	Historical Control Ranges	26 Apr 2019
16 Apr 2019	25 Apr 2019	Set-up and Treatment of Test Systems	30 Apr 2019

RESPONSIBLE PERSONNEL

Study Monitor

Study Director

Genetic Toxicology Operations

Quality Assurance Contact

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1. SUMMARY

Mexoryl SDA was tested in an *in vitro* micronucleus assay using duplicate human lymphocyte cultures prepared from the pooled blood of two male donors in a single experiment. Treatments covering a broad range of concentrations, separated by narrow intervals, were performed both in the absence and presence of metabolic activation (S-9) from Aroclor 1254-induced rats. The test article was formulated in purified water and the highest concentrations tested in the Micronucleus Experiment were determined following a preliminary cytotoxicity Range-Finder Experiment.

Treatments were conducted (as detailed in the following summary table) 48 hours following mitogen stimulation by phytohaemagglutinin (PHA). The test article concentrations for micronucleus analysis were selected by evaluating the effect of Mexoryl SDA on the replication index (RI). Micronuclei were analysed at three concentrations and a summary of the data is presented in the following table:

Treatment	Concentration (µg/mL)	Cytotoxicity (%) [$]	Mean MN Cell Frequency (%)	Historical Control Range (%) [#]	Statistical Significance
3+21 -S-9	Vehicle [a]	-	0.43	0.00 to 0.70	-
	1000	4	0.80		$p \leq 0.05$
	3000	31	0.80		$p \leq 0.05$
	5000	55	0.88		$p \leq 0.01$
	*MMC, 0.30	27	7.55		$p \leq 0.001$
3+21 +S-9	Vehicle [a]	-	0.50	0.10 to 0.90	-
	2000	11	0.75		NS
	3500	35	0.78		NS
	5000	53	0.85		$p \leq 0.05$
	*CPA, 5.00	52	1.45		$p \leq 0.001$
24+24 -S-9	Vehicle [a]	-	0.45	0.00 to 0.80	-
	200.0	15	0.65		NS
	400.0	33	1.00		$p \leq 0.05$
	800.0	54	0.80		NS
	*VIN, 0.04	51	2.60		$p \leq 0.001$

[a] Vehicle control was water
* Positive control
[#] 95th percentile of the observed range
[$] Based on RI
MN Micronucleated
NS Not significant

Appropriate negative (vehicle) control cultures were included in the test system under each treatment condition. The proportion of micronucleated binucleate (MNBN) cells in these cultures fell within (or very close to) the 95th percentile of the current observed historical vehicle control (normal) ranges. Mitomycin C (MMC) and Vinblastine (VIN) were employed as clastogenic and aneugenic positive control chemicals, respectively, in the absence of rat liver S-9. Cyclophosphamide (CPA) was employed as a clastogenic positive control chemical in the presence of rat liver S-9. Cells receiving these were sampled in the Micronucleus Experiment at 24 hours (CPA, MMC) or 48 hours (VIN) after the start of treatment. All positive control compounds induced statistically significant increases in the proportion of cells with micronuclei.

All acceptance criteria were considered met and the study was accepted as valid.

Treatment of cells for 3+21 hours in the absence of S-9 resulted in frequencies of MNBN cells that were significantly higher ($p \leq 0.05$), compared to those observed in the concurrent vehicle controls, at all three test article concentrations analysed (1000, 3000 and 5000 µg/mL, giving 4%, 31% and 55% cytotoxicity, respectively). The MNBN cell frequencies exceeded the normal range (0 to 0.7%) in single cultures at 1000 µg/mL (0.95%) and 3000 µg/mL (0.9%) and in both cultures at 5000 µg/mL (0.95% and 0.8%), with a weakly significant linear trend ($p \leq 0.05$). The data fulfilled all of the evaluation criteria and were indicative of a weak positive result, but the increases in MNBN cell frequency were small in magnitude and were considered of questionable biological relevance.

Treatment of cells for 3+21 hours in the presence of S-9 resulted in frequencies of MNBN cells that were significantly higher ($p \leq 0.05$), compared to those observed in the concurrent vehicle controls, at the highest concentration analysed (5000 µg/mL, giving 53% cytotoxicity). However, the MNBN cell frequency exceeded the normal range (0.1 to 0.9%) in only one culture analysed at 5000 µg/mL (1.15%) and the mean MNBN cell frequency at this concentration (0.85%) was within the normal range, although there was a weakly significant linear trend ($p \leq 0.05$). The isolated increase in MNBN cell frequency in a single culture at 5000 µg/mL was considered not biologically relevant.

Treatment of cells for 24+24 hours in the absence of S-9 resulted in frequencies of MNBN cells that were significantly higher ($p \leq 0.05$), compared to those observed in the concurrent vehicle controls, at the intermediate concentration analysed (400 µg/mL, giving 33% cytotoxicity). However, the MNBN cell frequency exceeded the normal range (0 to 0.8%) in only one culture analysed at 400 µg/mL (1%). There was a weakly significant linear trend ($p \leq 0.05$) but no clear evidence of a concentration-related relationship over the concentrations analysed. The isolated increase in MNBN cell frequency in the single culture at 400 µg/mL was considered not biologically relevant.

It is concluded that Mexoryl SDA showed evidence of weak induction of micronuclei in cultured human peripheral blood lymphocytes when tested for 3+21 hours in the absence of a rat liver metabolic activation system (S-9), but the increases in the frequency of micronuclei were small in magnitude and were considered of questionable biological relevance. In the same test system, Mexoryl SDA did not induce biologically relevant increases in the frequency of micronuclei when tested up to toxic concentrations for 3+21 hours in the presence of S-9 and for 24+24 hours in the absence of S-9 under the experimental conditions described.

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2. GENERAL STUDY INFORMATION

2.1 Objective

The objective of this study was to evaluate the clastogenic and aneugenic potential of Mexoryl SDA by examining its effects on the frequency of micronuclei in cultured human peripheral blood lymphocytes treated in the absence and presence of a rat liver metabolising system (S-9).

2.2 Introduction

Chromosome defects are recognised as the basis of a number of human genetic diseases (Mitelman, 1991). Assays for the detection of chromosome damage in mammalian cells are recommended in regulatory guidelines as a complement to Ames tests in a genotoxicity test battery. There is a large database on the use of chromosomal assays for screening purposes (Preston *et al.*, 1981; Fenech, 1998; Fenech *et al.*, 2003). The use of human peripheral blood lymphocytes is recommended because the cells are only used in short-term culture and maintain a stable karyotype (Evans & O'Riordan, 1975). Experiments with these cells can also be performed in conjunction with a rat liver metabolising system (S-9) since, for short incubation periods, no toxicity is induced by the liver homogenate itself.

An alternative to measuring structural aberrations in mitotic cells is to measure micronuclei. These are produced from whole chromosomes or acentric fragments that are unable to attach to the spindle at mitosis and appear during the next interphase as small darkly staining bodies adjacent to the main daughter nucleus. Cytochalasin B (Cyto-B), if added to cultures, inhibits cytokinesis (cell division) but not karyokinesis (nuclear division) resulting in the formation of binucleate cells (Fenech & Morley, 1985). If micronuclei are counted in binucleate cells, then a measurement of micronucleus induction resulting from cell division can be obtained.

Theoretical considerations, together with published data (Lorge *et al.*, 2006), indicate that most aneugens and clastogens will be detected by a short term treatment period of 3-6 hours in the presence and absence of S-9 followed by removal of the test article and a growth period of 1.5-2.0 cell cycles (Fenech & Morley, 1986).

The most efficient approach is to test lymphocytes 44-48 hours post-mitogen stimulation by PHA, when cycle synchronisation will have dissipated (Fenech, 2007).

The test article was added at approximately 48 hours following culture initiation (stimulation by PHA). Cells were exposed to the test article for 3 hours in the absence and presence of S-9 (from rats induced with Aroclor 1254) (Maron & Ames, 1983). These cultures were sampled 24 hours after the beginning of treatment (i.e. 72 hours after culture initiation). In addition, an extended 24 hour treatment (equivalent to approximately 1.5 to 2 times the average generation time of cultured lymphocytes from the panel of donors used in this laboratory) in the absence of S-9 was included. These cultures were sampled 48 hours after the beginning of treatment (i.e. 96 hours after culture initiation).

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2.3 Study Timetable

Study Initiation Date:	11 February 2019
Experimental Start Date:	20 February 2019
Experimental Completion Date:	09 April 2019
Study Completion Date:	Is the date the final report is signed by the Study Director

2.4 Regulatory Test Guidelines

OECD Guideline 487 (OECD, 2016), superseded where appropriate by ICH S2(R1) (2011) and accepted scientific/regulatory principles described in current guidelines for clastogenicity testing *in vitro* (Aardema *et al.*, 1998; Elhajouji *et al.*, 1998; Fenech, 1998; Fenech *et al.*, 1999; Fenech *et al.*, 2003; Galloway *et al.*, 1994; Migliore & Nieri, 1991; Miller *et al.*, 1998; Rosefort *et al.*, 2004; Thybaud *et al.*, 2007).

2.5 Protocol Adherence

This study was conducted according to the Protocol, with the exception of the Protocol Deviations (Section 7.3). None of the deviations affected the integrity or interpretation of the results of the study.

2.6 Major Computer Systems

Application Name	Application Function
REES	Monitoring of facility storage conditions
eNotes	Electronic communication system
Pristima	Formulations
Cyto Study Manager	Slide coding, data generation and collation
Documentum	Document management system used for protocol production and electronic signatures

Version numbers of the applications are maintained on file at Covance.

2.7 Archive Statement

The raw data, including documentation, study protocol, final report, study correspondence and specimens resulting from this study will be retained in the test facility archives for at least ten years from the date of report finalisation. After completion of this period, the Sponsor will be contacted in order to determine their requirements for further retention, transfer or disposal of the archived materials (excluding facility records, non-transferable electronic data and facility copies of protocol/final report, which will be retained by Covance in accordance with test facility SOPs). Where continued retention is requested, the archived materials may subsequently be transferred to alternative Covance Archive locations. In this event, the Sponsor will be informed, and documented chain of custody records will be maintained.

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3. MATERIALS

3.1 Test Article

Mexoryl SDA (CAS number 84604-12-6), also known as Rosebush watersprout solid extract, batch number E 14, was a beige powder. It was received on 07 February 2019 and stored at 15-25°C, protected from light. The test article was a Chemical Substance of Unknown or Variable Composition, Complex Reaction Product or Biological Material (UVCB). Purity was considered to be 100% (dry content 96.9%) and the material was tested as supplied, without correction for purity. The retest date was given as 22 January 2020, based on the Sponsor's knowledge of the test article, see Certificate of Analysis. The test article information and certificate of analysis provided by the Sponsor are considered an adequate description of the characterisation, purity and stability of the test article.

Preliminary solubility data indicated that Mexoryl SDA was soluble in purified water at a concentration of at least 50.00 mg/mL. The solubility limit in culture medium was in excess of 5000 µg/mL as indicated by the absence of visible precipitation at this concentration approximately 24 hours after test article addition, with warming at 37°C. A maximum concentration of 5000 µg/mL was selected for the cytotoxicity Range-Finder Experiment, in order that treatments were performed up to the maximum recommended concentration according to current regulatory test guidelines (OECD, 2016). Concentrations selected for the Micronucleus Experiment were based on the results of this cytotoxicity Range-Finder Experiment.

Test article stock solutions were prepared by formulating Mexoryl SDA under subdued lighting in purified water, with the aid of vortex mixing, ultrasonication and warming at 37°C (as required), to give the maximum required concentration. Subsequent dilutions were made using purified water. The test article solutions were protected from light and used within approximately 3 hours of initial formulation. The following concentration ranges were tested:

Experiment	Treatment	Concentration Range (mg/mL)			Final Concentration Range (µg/mL)		
Range-Finder	3+21, -S-9	0.1814	to	50.00	18.14	to	5000
	3+21, +S-9	0.1814	to	50.00	18.14	to	5000
	24+24, -S-9	0.1814	to	50.00	18.14	to	5000
Micronucleus	3+21, -S-9	2.500	to	50.00	250.0	to	5000
Experiment	3+21, +S-9	2.500	to	50.00	250.0	to	5000
	24+24, -S-9	0.500	to	15.00	50.00	to	1500

3.2 Formulations Analysis

In accordance with the regulatory test guidelines applicable for this study (see Section 2.4), no analyses of the stability of the test article in administered formulations or dilutions was undertaken as fresh preparation of test article were employed.

Following discussions with the Sponsor, analyses for achieved concentration and homogeneity (where appropriate) of test article formulations were not conducted as part of this study, as this is not a requirement of the regulatory test guidelines.

The absence of such analyses is noted in the Study Director's statement of GLP compliance.

3.3 Controls

Sterile purified water was added to cultures designated as vehicle controls as described in the methods section of this report. The positive control chemicals were supplied and used according to the following table:

Chemical [a]	Stock Concentration (mg/mL) [b]	Final Concentration (µg/mL)	Treatment Regime
Mitomycin C (MMC)	0.03	0.3	3+21 –S-9
Cyclophosphamide (CPA)	0.3	3.0	3+21 +S-9
	0.5	5.0	3+21 +S-9
	0.7	7.0	3+21 +S-9
Vinblastine (VIN)	0.004	0.04	24+24 –S-9

[a] Obtained from Sigma-Aldrich.

[b] In the Micronucleus Experiment, CPA was dissolved in anhydrous analytical grade dimethyl sulphoxide (DMSO), frozen (<-50°C) and thawed immediately prior to use. VIN and MMC were dissolved in purified water immediately prior to use.

3.4 Metabolic Activation System

The mammalian liver post-mitochondrial fraction (S-9) used for metabolic activation was obtained from Molecular Toxicology Incorporated, USA where it was prepared from male Sprague Dawley rats induced with Aroclor 1254. The S-9 was supplied as lyophilized S-9 mix (Mutazyme^{TM}), stored frozen at <-20°C, and thawed and reconstituted with purified water to provide a 10% S-9 mix just prior to use. Each batch was checked by the manufacturer for sterility, protein content, ability to convert ethidium bromide and cyclophosphamide to bacterial mutagens, and cytochrome P-450-catalysed enzyme activities (alkoxyresorufin-O-dealkylase activities). See Quality Control Statement for S-9.

Treatments were carried out both in the absence and presence of S-9 by addition of either 150 mM KCl or 10% S-9 mix respectively. The final S-9 volume in the test system was 1% (v/v).

Ingredient	Final Content per mL in:
	10% S-9 mix
Sodium phosphate buffer pH 7.4 (SPB)	100 µmoles
Glucose-6-phosphate (disodium) (G-6-P)	5 µmoles
β-Nicotinamide adenine dinucleotide phosphate (NADP) (disodium)	4 µmoles
Magnesium chloride ($MgCl_2$)	8 µmoles
Potassium chloride (KCl)	33 µmoles
Water	To volume
S-9	100 µL

3.5 Blood Cultures

Blood from two healthy, non-smoking male volunteers from a panel of donors at [redacted] was used for each experiment as follows:

Experiment	Donor Sex	Donor Age (years)	Donor Identity
Range-Finder	Male	33, 32	9817, 8517
Micronucleus Experiment	Male	33, 28	8595, 7943

No donor was suspected of any virus infection or exposed to high levels of radiation or hazardous chemicals. All donors are non-smokers and are not heavy drinkers of alcohol. Donors were not taking any form of medication. The measured cell cycle time of the donors used at [redacted] falls within the range 13±2 hours. For each experiment, an appropriate volume of whole blood was drawn from the peripheral circulation into heparinised tubes on the day of culture initiation. Blood was stored refrigerated and pooled using equal volumes from each donor prior to use.

Whole blood cultures were established in sterile disposable centrifuge tubes by placing 0.4 mL of pooled heparinised blood into 7.6 mL pre-warmed (in an incubator set to 37±1°C) HEPES-buffered RPMI medium containing 10% (v/v) heat inactivated foetal calf serum and 0.52% penicillin / streptomycin, so that the final volume following addition of S-9 mix/KCl and the test article in its chosen vehicle was 10 mL. The mitogen Phytohaemagglutinin (PHA, reagent grade) was included in the culture medium at a concentration of approximately 2% of culture to stimulate the lymphocytes to divide. Blood cultures were incubated at 37±1°C for approximately 48 hours and rocked continuously.

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4. METHODS

4.1 Test System

The test system was suitably labelled (using a colour-coded procedure) to clearly identify the study number, assay type, experiment number, treatment time, donor sex, test article concentration (if applicable), positive and vehicle controls, in the absence and presence of S-9 mix.

4.2 Cytotoxicity Range-Finder

S-9 mix or KCl (1 mL per culture) was added appropriately. Cultures were treated with the test article or vehicle control (1 mL per culture). Positive control treatments were not included.

The final culture volume was 10 mL. Cultures were incubated at 37±1°C for the designated exposure time.

4.3 Micronucleus Experiment

Immediately prior to treatment, all positive control cultures had 0.9 mL culture medium added to give a final pre-treatment volume of 8.9 mL.

S-9 mix or KCl (1 mL per culture) was added appropriately. Cultures were treated with the test article or vehicle (1 mL per culture) or positive controls (0.1 mL per culture).

The final culture volume was 10 mL. Cultures were incubated at 37±1°C for the designated exposure time.

This scheme is illustrated as follows:

Treatment	S-9	Number of Cultures			
		Cytotoxicity Range-Finder		Micronucleus Experiment	
		3+21*	24+24*	3+21*	24+24*
Vehicle control	-	2	2	4	4
	+	2		4	
Test article	-	1	1	2	2
	+	1		2	
Positive controls	-			2	2
	+			2	

* Hours treatment + hours recovery

For removal of the test article, cells were pelleted (approximately 300 *g*, 10 minutes), washed twice with sterile saline (pre-warmed in an incubator set to 37±1°C), and resuspended in fresh pre-warmed medium containing foetal calf serum and penicillin / streptomycin. Cyto-B (formulated in DMSO) was added to post wash-off culture medium to give a final concentration of 6 µg/mL per culture.

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Duration of Treatment (hours)	S-9	Hours after Culture Initiation*			
		Addition of Test Article	Removal of Test Article	Addition of Cyto-B	Harvest Time
3	-	48	51	52**	72
24	-	48	72	73**	96
3	+	48	51	52**	72

* Approximate times

** Assuming approximately 1 hour for removal of test article at the post treatment wash-phase

Changes in osmolality of more than 50 mOsm/kg and fluctuations in pH of more than one unit may be responsible for an increase in chromosome aberrations (Scott *et al.*, 1991; Brusick, 1986). Osmolality and pH measurements on post-treatment incubation medium were taken in the cytotoxicity Range-Finder Experiment.

4.4 Harvesting

At the defined sampling time, cultures were centrifuged at approximately 300 *g* for 10 minutes, the supernatant removed and discarded and cells resuspended in 4 mL (hypotonic) 0.075 M KCl at 37±1°C for 4 minutes to allow cell swelling to occur. Cells were fixed by dropping the KCl suspension into fresh, cold methanol/glacial acetic acid (7:1, v/v). The fixative was changed by centrifugation (approximately 300 *g*, 10 minutes) and resuspension. This procedure was repeated as necessary (centrifuging at approximately 1250 *g*, 2-3 minutes) until the cell pellets were clean.

4.5 Slide Preparation

Lymphocytes were kept in fixative at 2-8°C prior to slide preparation for a minimum of 3 hours to ensure that cells were adequately fixed. Cells were centrifuged (approximately 1250 *g*, two to three minutes) and resuspended in a minimal amount of fresh fixative (if required) to give a milky suspension. Several drops of cell suspension were gently spread onto multiple clean, dry microscope slides. Slides were air-dried and stored protected from light at room temperature prior to staining (see Section 7.3). Slides were stained by immersion in 12.5 µg/mL Acridine Orange in phosphate buffered saline (PBS), pH 6.8 for approximately 10 minutes and washed with PBS (with agitation) for a few seconds. The quality of the staining was checked. Slides were air-dried and stored protected from light at room temperature. Immediately prior to analysis 1-2 drops of PBS were added to the slides before mounting with glass coverslips.

4.6 Selection of Concentrations for the Micronucleus Experiment

Slides from the cytotoxicity Range-Finder Experiment were examined, uncoded, for proportions of mono-, bi- and multinucleate cells, to a minimum of 200 cells per concentration. From these data the replication index (RI) was determined.

RI, which indicates the relative number of nuclei compared to vehicle controls was determined using the formula as follows:

$$RI = \frac{\textit{number binucleate cells} + 2\ \textit{(number multinucleate cells)}}{\textit{total number of cells in treated cultures}}$$

Relative RI (expressed in terms of percentage) for each treated culture was calculated as follows:

$$Relative\ RI\ (\%) = \frac{RI\ of\ treated\ cultures}{RI\ of\ vehicle\ controls} \times 100$$

Cytotoxicity (%) is expressed as (100 – Relative RI).

A selection of random fields was observed from enough treatments to determine whether chemically induced cell cycle delay or cytotoxicity had occurred.

A suitable range of concentrations was selected for the Micronucleus Experiment based on these toxicity data.

4.7 Selection of Concentrations for Micronucleus Analysis (Micronucleus Experiment Only)

Slides were examined, uncoded, for RI to a minimum of 500 cells per culture to determine whether chemically induced cell cycle delay or toxicity had occurred.

The highest concentrations selected for micronucleus analysis following all treatment conditions (up to a maximum of 5000 µg/mL for the 3+21 hour treatments) gave 50-60% cytotoxicity (OECD, 2016). Analysis of slides from highly toxic concentrations was avoided.

Slides from the highest selected concentration and two lower concentrations were taken for microscopic analysis, such that a range of cytotoxicity from maximum to little was covered.

The positive control concentrations analysed did not exceed the cytotoxicity limits for the test article concentration selection.

4.8 Slide Analysis

Scoring was carried out using fluorescence microscopy.

Binucleate cells were only included in the analysis if all of the following criteria were met:

1. The cytoplasm remained essentially intact, and
2. The daughter nuclei were of approximately equal size.

A micronucleus was only recorded if it met the following criteria:

1. The micronucleus had the same staining characteristics and a similar morphology to the main nuclei, and
2. Any micronucleus present was separate in the cytoplasm or only just touching a main nucleus, and
3. Micronuclei were smooth edged and smaller than approximately one third the diameter of the main nuclei.

For each treatment regime, two vehicle control cultures were analysed for micronuclei. It was subsequently deemed necessary to analyse the two remaining vehicle control cultures for the 3+21 hour treatments in the absence and presence of S-9, to aid data interpretation.

Slides from the positive control treatments were checked to ensure that the system was operating satisfactorily. One concentration from each positive control, which gave satisfactory responses in terms of quality and quantity of binucleated cells and numbers of micronuclei, was analysed. This pre-analysis slide check was conducted under non-blinded conditions.

All slides for analysis were coded by an individual not connected with the scoring of the slides, such that analysis was conducted under blind conditions. Labels with only the study number, assay type, experiment number, the sex of the donor and the code were used to cover treatment details on the slides.

A minimum of one thousand binucleate cells from each culture were analysed for micronuclei. For the 3+21 hour treatments in the absence and presence of S-9, 2000 binucleate cells were analysed per culture for the vehicle and test article control cultures selected for analysis, to aid data interpretation. The number of cells containing micronuclei was recorded.

Nucleoplasmic bridges (NPBs) between nuclei in binucleate cells were recorded during micronucleus analysis to provide an indication of chromosome rearrangement. Various mechanisms may lead to NPB formation following DNA misrepair of strand breaks in DNA (Thomas *et al.*, 2003). In this assay, binucleate cells with NPBs were recorded as part of the micronucleus analysis.

Micronucleus analysis was not conducted on slides generated from the Range-Finder treatments.

of analysis.

4.9 Analysis of Results

4.9.1 Treatment of Data

After completion of scoring and decoding of slides, the numbers of binucleate cells with micronuclei (MNBN cells) in each culture were obtained.

The proportions of MNBN cells in each replicate were used to establish acceptable heterogeneity between replicates by means of a binomial dispersion test (Richardson *et al.*, 1989).

The proportions of MNBN cells for each treatment condition were compared with the proportion in vehicle controls by using Fisher's exact test (Richardson *et al.*, 1989). A

Cochran-Armitage trend test was applied to each treatment condition. Probability values of $p \leq 0.05$ were accepted as significant.

4.9.2 Acceptance Criteria

The assay was considered valid if the following criteria were met:

1. The binomial dispersion test demonstrated acceptable heterogeneity (in terms of MNBN cell frequency) between replicate cultures, particularly where no positive responses were seen

2. The frequency of MNBN cells in vehicle controls fell within the current 95th percentile of the observed historical vehicle control (normal) ranges

3. The positive control chemicals induced statistically significant increases in the proportion of cells with micronuclei. Both replicate cultures at the positive control concentration analysed under each treatment condition demonstrated MNBN cell frequencies that clearly exceeded the normal range

4. A minimum of 50% of cells had gone through at least one cell division (as measured by binucleate + multinucleate cell counts) in vehicle control cultures at the time of harvest

5. The maximum concentration analysed under each treatment condition met the criteria specified in Section 4.7.

4.9.3 Evaluation Criteria

For valid data, the test article was considered to induce clastogenic and/or aneugenic events if:

1. A statistically significant increase in the frequency of MNBN cells at one or more concentrations was observed

2. An incidence of MNBN cells at such a concentration that exceeded the normal range in both replicates was observed

3. A concentration-related increase in the proportion of MNBN cells was observed (positive trend test).

The test article was considered positive in this assay if all of the above criteria were met.

The test article was considered negative in this assay if none of the above criteria were met.

Results which only partially satisfied the above criteria were dealt with on a case-by-case basis. Evidence of a concentration-related effect was considered useful but not essential in the evaluation of a positive result (Scott *et al.*, 1990). Biological relevance was taken into account, for example consistency of response within and between concentrations, or effects occurring only at very toxic concentrations (Thybaud *et al.*, 2007).

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5. RESULTS

5.1 Selection of Concentrations for Micronucleus Analysis

The results of the RI determinations from the cytotoxicity Range-Finder Experiment were as follows:

Text Table 1: Data for 3+21 Hour Treatments -S-9, Range-Finder - Male Donors

Treatment (µg/mL)	Replicate	Mono	Bi	Multi	Total	RI	Cytotoxicity Based on RI (%)
Vehicle	A	32	145	23	200	0.96	
	B	38	143	19	200	0.91	
	Total	70	288	42	400	0.93	-
18.14	A	0 NSc	0	0	0		-
30.23	A	0 NSc	0	0	0		-
50.39	A	0 NSc	0	0	0		-
83.98	A	0 NSc	0	0	0		-
140.0	A	37	143	20	200	0.92	2
233.3	A	25	175	14	214	0.95	0
388.8	A	28	148	24	200	0.98	0
648.0	A	41	148	11	200	0.85	9
1080	A	43	147	10	200	0.84	10
1800	A	62	134	4	200	0.71	24
3000	A	99	100	1	200	0.51	45 P
5000	A	123	77	0	200	0.39	59 P

Text Table 2: Data for 3+21 Hour Treatments +S-9, Range-Finder - Male Donors

Treatment (µg/mL)	Replicate	Mono	Bi	Multi	Total	RI	Cytotoxicity Based on RI (%)
Vehicle	A	37	140	23	200	0.93	
	B	37	148	15	200	0.89	
	Total	74	288	38	400	0.91	-
18.14	A	0 NSc	0	0	0		-
30.23	A	0 NSc	0	0	0		-
50.39	A	0 NSc	0	0	0		-
83.98	A	0 NSc	0	0	0		-
140.0	A	0 NSc	0	0	0		-
233.3	A	30	148	22	200	0.96	0
388.8	A	31	153	16	200	0.93	0
648.0	A	30	156	14	200	0.92	0
1080	A	46	141	13	200	0.84	8
1800	A	50	143	7	200	0.79	14
3000	A	85	113	2	200	0.59	36 P
5000	A	116	83	1	200	0.43	53 P

NSc = Not scored
P = Precipitation observed at treatment
Mono = Mononucleate Bi = Binucleate Multi = Multinucleate
RI = Replication index

Text Table 3: Data for 24+24 Hour Treatments -S-9, Range-Finder – Male Donors

Treatment (µg/mL)	Replicate	Mono	Bi	Multi	Total	RI	Cytotoxicity Based on RI (%)
Vehicle	A	26	135	39	200	1.07	
	B	30	132	38	200	1.04	
	Total	56	267	77	400	1.05	-
18.14	A	0 NSc	0	0	0		-
30.23	A	0 NSc	0	0	0		-
50.39	A	25	123	52	200	1.14	0
83.98	A	24	133	43	200	1.10	0
140.0	A	27	143	30	200	1.02	4
233.3	A	40	146	14	200	0.87	17
388.8	A	57	129	14	200	0.79	25
648.0	A	81	105	14	200	0.67	37
1080	A	104	91	5	200	0.51	52
1800	A	157	43	0	200	0.22	80
3000	A	151	49	0	200	0.25	77 P
5000	A	139	57	4	200	0.33	69 P

NSc = Not scored
P = Precipitation observed at treatment
Mono = Mononucleate
Bi = Binucleate
Multi = Multinucleate
RI = Replication index

No marked changes in osmolality or pH were observed at the highest concentration tested in the Range-Finder (5000 µg/mL), compared to the concurrent vehicle controls (individual data not reported).

The results of the cytotoxicity Range-Finder Experiment were used to select suitable maximum concentrations for the Micronucleus Experiment.

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The results of the RI determinations from the Micronucleus Experiment were as follows:

Text Table 4: Data for 3+21 Hour Treatments -S-9, Micronucleus Experiment – Male Donors

Treatment (µg/mL)	Replicate	Mono	Bi	Multi	Total	RI	Cytotoxicity Based on RI (%)
Vehicle	A	148	334	18	500	0.74	
	B	119	348	33	500	0.83	
	C	127	355	18	500	0.78	
	D	107	367	26	500	0.84	
	Total	501	1404	95	2000	0.80	-
250.0	A	96	364	40	500	0.89	
	B	92	358	50	500	0.92	
	Total	188	722	90	1000	0.90	0
500.0	A	144	321	35	500	0.78	
	B	139	320	41	500	0.80	
	Total	283	641	76	1000	0.79	1
1000	**A**	**141**	**327**	**32**	**500**	**0.78**	
	B	**149**	**331**	**20**	**500**	**0.74**	
	Total	**290**	**658**	**52**	**1000**	**0.76**	**4 #**
2000	A	197	293	10	500	0.63	
	B	168	326	6	500	0.68	
	Total	365	619	16	1000	0.65	18
2500	A	213	285	2	500	0.58	
	B	212	282	6	500	0.59	
	Total	425	567	8	1000	0.58	27
3000	**A**	**235**	**263**	**2**	**500**	**0.53**	
	B	**224**	**273**	**3**	**500**	**0.56**	
	Total	**459**	**536**	**5**	**1000**	**0.55**	**31 #**
3500	A	261	238	1	500	0.48	
	B	257	239	4	500	0.49	
	Total	518	477	5	1000	0.49	39
4000	A	257	243	0	500	0.49	
	B	265	232	3	500	0.48	
	Total	522	475	3	1000	0.48	40
4500	A	271	225	4	500	0.47	
	B	266	232	2	500	0.47	
	Total	537	457	6	1000	0.47	41 P
5000	**A**	**316**	**183**	**1**	**500**	**0.37**	
	B	**327**	**171**	**2**	**500**	**0.35**	
	Total	**643**	**354**	**3**	**1000**	**0.36**	**55 P #**
MMC, 0.30	**A**	**203**	**293**	**4**	**500**	**0.60**	
	B	**227**	**269**	**4**	**500**	**0.55**	
	Total	**430**	**562**	**8**	**1000**	**0.58**	**27 #**

P = Precipitation observed at treatment
Mono = Mononucleate
Bi = Binucleate
Multi = Multinucleate
RI = Replication index
# Highlighted concentrations selected for analysis

Text Table 5: Data for 3+21 Hour Treatments +S-9, Micronucleus Experiment - Male Donors

Treatment (µg/mL)	Replicate	Mono	Bi	Multi	Total	RI	Cytotoxicity Based on RI (%)
Vehicle	A	212	283	5	500	0.59	
	B	213	280	7	500	0.59	
	C	197	298	5	500	0.62	
	D	176	314	10	500	0.67	
	Total	798	1175	27	2000	0.61	-
250.0	A	219	275	6	500	0.57	
	B	192	298	10	500	0.64	
	Total	411	573	16	1000	0.61	2
500.0	A	204	288	8	500	0.61	
	B	186	303	11	500	0.65	
	Total	390	591	19	1000	0.63	0
1000	A	200	285	15	500	0.63	
	B	180	310	10	500	0.66	
	Total	380	595	25	1000	0.65	0
2000	**A**	**233**	**260**	**7**	**500**	**0.55**	
	B	**232**	**263**	**5**	**500**	**0.55**	
	Total	**465**	**523**	**12**	**1000**	**0.55**	**11 #**
2500	A	324	174	2	500	0.36	
	B	255	240	5	500	0.50	
	Total	579	414	7	1000	0.43	30
3000	A	314	186	0	500	0.37	
	B	320	180	0	500	0.36	
	Total	634	366	0	1000	0.37	40
3500	**A**	**301**	**198**	**1**	**500**	**0.40**	
	B	**305**	**190**	**5**	**500**	**0.40**	
	Total	**606**	**388**	**6**	**1000**	**0.40**	**35 #**
4000	A	307	192	1	500	0.39	
	B	330	169	1	500	0.34	
	Total	637	361	2	1000	0.37	41
4500	A	341	159	0	500	0.32	
	B	320	178	2	500	0.36	
	Total	661	337	2	1000	0.34	45 P
5000	**A**	**372**	**124**	**4**	**500**	**0.26**	
	B	**346**	**151**	**3**	**500**	**0.31**	
	Total	**718**	**275**	**7**	**1000**	**0.29**	**53 E,P #**
CPA, 3.00	A	301	199	0	500	0.40	
	B	308	191	1	500	0.39	
	Total	609	390	1	1000	0.39	36
CPA, 5.00	**A**	**359**	**140**	**1**	**500**	**0.28**	
	B	**345**	**154**	**1**	**500**	**0.31**	
	Total	**704**	**294**	**2**	**1000**	**0.30**	**52 #**
CPA, 7.00	A	359	139	2	500	0.29	
	B	328	169	3	500	0.35	
	Total	687	308	5	1000	0.32	48

P = Precipitation observed at treatment
E = Precipitation observed at the end of treatment incubation
Mono = Mononucleate Bi = Binucleate Multi = Multinucleate
RI = Replication index
# Highlighted concentrations selected for analysis

Text Table 6: Data for 24+24 Hour Treatments -S-9, Micronucleus Experiment - Male Donors

Treatment (µg/mL)	Replicate	Mono	Bi	Multi	Total	RI	Cytotoxicity Based on RI (%)
Vehicle	A	52	345	103	500	1.10	
	B	30	336	134	500	1.21	
	C	44	359	97	500	1.11	
	D	38	341	121	500	1.17	
	Total	164	1381	455	2000	1.15	-
50.00	A	38	345	117	500	1.16	
	B	34	347	119	500	1.17	
	Total	72	692	236	1000	1.16	0
100.0	A	39	338	123	500	1.17	
	B	49	357	94	500	1.09	
	Total	88	695	217	1000	1.13	1
200.0	**A**	**67**	**382**	**51**	**500**	**0.97**	
	B	**79**	**357**	**64**	**500**	**0.97**	
	Total	**146**	**739**	**115**	**1000**	**0.97**	**15 #**
400.0	**A**	**148**	**330**	**22**	**500**	**0.75**	
	B	**137**	**337**	**26**	**500**	**0.78**	
	Total	**285**	**667**	**48**	**1000**	**0.76**	**33 #**
600.0	A	189	287	24	500	0.67	
	B	202	283	15	500	0.63	
	Total	391	570	39	1000	0.65	43
800.0	**A**	**253**	**227**	**20**	**500**	**0.53**	
	B	**263**	**219**	**18**	**500**	**0.51**	
	Total	**516**	**446**	**38**	**1000**	**0.52**	**54 #**
900.0	A	312	182	6	500	0.39	
	B	320	171	9	500	0.38	
	Total	632	353	15	1000	0.38	67
1000	A	303	183	14	500	0.42	
	B	306	185	9	500	0.41	
	Total	609	368	23	1000	0.41	64
1100	A	295	193	12	500	0.43	
	B	321	169	10	500	0.38	
	Total	616	362	22	1000	0.41	65
1200	A	320	172	8	500	0.38	
	B	326	168	6	500	0.36	
	Total	646	340	14	1000	0.37	68
1500	A	370	128	2	500	0.26	
	B	343	156	1	500	0.32	
	Total	713	284	3	1000	0.29	75
VIN, 0.04	**A**	**273**	**187**	**40**	**500**	**0.53**	
	B	**248**	**204**	**48**	**500**	**0.60**	
	Total	**521**	**391**	**88**	**1000**	**0.57**	**51 #**

Mono = Mononucleate
Bi = Binucleate
Multi = Multinucleate
RI = Replication index
# Highlighted concentrations selected for analysis

5.2 Micronucleus Analysis

5.2.1 Raw Data

The raw data for the observations on the test article plus positive and vehicle controls are retained by [redacted]. A summary of the number of cells containing micronuclei is given in Table 8.1 to Table 8.3.

5.2.2 Validity of Study

The data in Table 8.1 to Table 8.6, ATTACHMENTS and Text Table 4 to Text Table 6 confirm that:

1. The binomial dispersion test demonstrated acceptable heterogeneity (in terms of MNBN cell frequency) between replicate cultures (Table 8.4 to Table 8.6).

2. The frequency of MNBN cells in vehicle controls fell within the normal range (ATTACHMENTS) with the exception of one culture one vehicle control culture for the 3+21 hour -S-9 treatment, which gave a MNBN cell frequency of 0.8%. However, this value was within the observed historical vehicle control range (0 to 0.8%) and the mean vehicle MNBN cell frequency for the four replicate cultures (0.43%) was within the normal range, therefore the data were considered acceptable and valid.

3. The positive control chemicals induced statistically significant increases in the proportion of MNBN cells. Both replicate cultures at the positive control concentration analysed under each treatment condition demonstrated MNBN cell frequencies that clearly exceeded the normal range (Table 8.1 to Table 8.3).

4. A minimum of 50% of cells had gone through at least one cell division (as measured by binucleate + multinucleate cell counts) in vehicle control cultures at the time of harvest (Text Table 4 to Text Table 6).

5. The maximum concentration analysed under each treatment condition met the criteria specified in Section 4.7.

5.2.3 Analysis of Data

Treatment of cells for 3+21 hours in the absence of S-9 resulted in frequencies of MNBN cells that were significantly higher ($p \leq 0.05$), compared to those observed in the concurrent vehicle controls (Table 8.1 and Table 8.4), at all three test article concentrations analysed (1000, 3000 and 5000 µg/mL, giving 4%, 31% and 55% cytotoxicity, respectively). The MNBN cell frequencies exceeded the normal range of 0 to 0.7% (ATTACHMENTS) in single cultures at 1000 µg/mL (0.95%) and 3000 µg/mL (0.9%) and in both cultures at 5000 µg/mL (0.95% and 0.8%), with a weakly significant linear trend ($p \leq 0.05$). The data fulfilled all of the evaluation criteria and were indicative of a weak positive result, but the increases in MNBN cell frequency were small in magnitude and were considered of questionable biological relevance.

Treatment of cells for 3+21 hours in the presence of S-9 resulted in frequencies of MNBN cells that were significantly higher ($p \leq 0.05$), compared to those observed in the concurrent vehicle controls (Table 8.2 and Table 8.5), at the highest concentration analysed (5000 µg/mL, giving 53% cytotoxicity). However, the MNBN cell frequency exceeded the normal range of 0.1 to 0.9% (ATTACHMENTS) in only one culture analysed at 5000 µg/mL (1.15%) and the mean MNBN cell frequency at this concentration (0.85%) was within the normal range, although there was a weakly significant linear trend ($p \leq 0.05$). The isolated increase in MNBN cell frequency in a single culture at 5000 µg/mL was considered not biologically relevant.

Treatment of cells for 24+24 hours in the absence of S-9 resulted in frequencies of MNBN cells that were significantly higher ($p \leq 0.05$), compared to those observed in the concurrent vehicle controls (Table 8.3 and Table 8.6), at the intermediate concentration analysed (400 µg/mL, giving 33% cytotoxicity). However, the MNBN cell frequency exceeded the normal range of 0 to 0.8% (ATTACHMENTS) in only one culture analysed at 400 µg/mL (1%). There was a weakly significant linear trend ($p \leq 0.05$) but no clear evidence of a concentration-related relationship over the concentrations analysed. The isolated increase in MNBN cell frequency in the single culture at 400 µg/mL was considered not biologically relevant.

No test article related increases in cells with NPBs were observed (data not reported).

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6. CONCLUSION

It is concluded that Mexoryl SDA showed evidence of weak induction of micronuclei in cultured human peripheral blood lymphocytes when tested for 3+21 hours in the absence of a rat liver metabolic activation system (S-9), but the increases in the frequency of micronuclei were small in magnitude and were considered of questionable biological relevance. In the same test system, Mexoryl SDA did not induce biologically relevant increases in the frequency of micronuclei when tested up to toxic concentrations for 3+21 hours in the presence of S-9 and for 24+24 hours in the absence of S-9 under the experimental conditions described.

7. ASSOCIATED STUDY INFORMATION

7.1 References

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7.2 Abbreviations

Abbreviation	Description
CPA	Cyclophosphamide
Cyto-B	Cytochalasin B
DMSO	Dimethyl sulphoxide
G6P	Glucose-6-phosphate
GLP	Good Laboratory Practice
ICH	International Conference on Harmonisation
HEPES	Hydroxythyl piperazineethane sulphonic acid
KCl	Potassium chloride
MMC	Mitomycin C
MNBN	Micronucleated binucleate cells
NADP	β-Nicotinamide adenine dinucleotide phosphate
NPBs	Nucleoplasmic bridges
OECD	Organization for Economic Cooperation and Development
PHA	Phytohaemagglutanin
PBS	Phosphate buffered saline
QA	Quality Assurance
RI	Replication index
RPMI	Roswell Park Memorial Institute
S-9	Rat liver metabolic activation system
SOP	Standard Operating Procedure
VIN	Vinblastine
Units of Measure	
µg	Microgram
°C	Degrees Celsius
mOsm/kg	Milliosmole per kilogram
mg	Milligram
mL	Millilitre
mM	Millimolar

7.3 Protocol Deviations

Procedure	Protocol Deviations
Filter-sterilisation of test article formulations	The protocol stated that aqueous stock test article formulations would be filter-sterilised before dilution or before use unless otherwise directed by the Sponsor. An attempt was made to filter-sterilise the stock formulation prior to use in the Range-Finder but this would not pass readily through a filter, therefore the formulation was used without filter-sterilisation. There was no contamination, therefore this did not affect the conduct of the study.
Slide preparation	The protocol stated that slides would be stored protected from light at room temperature prior to staining. This was true for the 3+21 hour treatments, but the slides from the 24+24 hour treatments in the absence of S-9 were not protected from light. This is not an absolute requirement and did not affect the conduct of the study.

These study deviations neither affected the overall interpretation of study findings nor compromised the integrity of the study.

8. TABLES

Table 8.1: Binucleate Cells with Micronuclei: Mexoryl SDA, 3+21 Hour Treatments in the Absence of S-9, Micronucleus Experiment – Male Donors

Treatment (µg/mL)	Rep	Total Cells Scored	Total MN Cells Scored	MN Cell Frequency (%)	Fisher's Exact Significance §	Cytotoxicity (%)
Vehicle	A	1000	5	0.50		
	B	1000	3	0.30		
	C	1000	1	0.10		
	D	1000	8	**0.80 #**		
	Total	4000	17	0.43	-	-
1000	A	2000	13	0.65		
	B	2000	19	**0.95 #**		
	Total	4000	32	0.80	p≤0.05	4
3000	A	2000	14	0.70		
	B	2000	18	**0.90 #**		
	Total	4000	32	0.80	p≤0.05	31
5000	A	2000	19	**0.95 #**		
	B	2000	16	**0.80 #**		
	Total	4000	35	0.88	p≤0.01	55 P
MMC, 0.30	A	1000	70	**7.00 #**		
	B	1000	81	**8.10 #**		
	Total	2000	151	7.55	p≤0.001	27

P = Precipitation observed at treatment
MN = Micronucleated
§ = Statistical significance (Table 8.4)
NS = Not significant # = Numbers exceed historical vehicle control range (ATTACHMENTS)

Table 8.2: Binucleate Cells with Micronuclei: Mexoryl SDA, 3+21 Hour Treatments in the Presence of S-9, Micronucleus Experiment – Male Donors

Treatment (µg/mL)	Rep	Total Cells Scored	Total MN Cells Scored	MN Cell Frequency (%)	Fisher's Exact Significance §	Cytotoxicity (%)
Vehicle	A	1000	4	0.40		
	B	1000	5	0.50		
	C	1000	5	0.50		
	D	1000	6	0.60		
	Total	4000	20	0.50	-	-
2000	A	2000	12	0.60		
	B	2000	18	0.90		
	Total	4000	30	0.75	NS	11
3500	A	2000	14	0.70		
	B	2000	17	0.85		
	Total	4000	31	0.78	NS	35
5000	A	2000	23	**1.15 #**		
	B	2000	11	0.55		
	Total	4000	34	0.85	p≤0.05	53 E,P
CPA, 5.00	A	1000	15	**1.50 #**		
	B	1000	14	**1.40 #**		
	Total	2000	29	1.45	p≤0.001	52

P = Precipitation observed at treatment
E = Precipitation observed at the end of treatment incubation
MN = Micronucleated
§ = Statistical significance (Table 8.5)
NS = Not significant # = Numbers exceed historical vehicle control range (ATTACHMENTS)

Table 8.3: Binucleate Cells with Micronuclei: Mexoryl SDA, 24+24 Hour Treatments in the Absence of S-9, Micronucleus Experiment – Male Donors

Treatment (µg/mL)	Rep	Total Cells Scored	Total MN Cells Scored	MN Cell Frequency (%)	Fisher's Exact Significance §	Cytotoxicity (%)
Vehicle	A	1000	4	0.40		
	B	1000	5	0.50		
	Total	2000	9	0.45	-	-
200.0	A	1000	5	0.50		
	B	1000	8	0.80		
	Total	2000	13	0.65	NS	15
400.0	A	1000	12	**1.20 #**		
	B	1000	8	0.80		
	Total	2000	20	1.00	$p \leq 0.05$	33
800.0	A	1000	8	0.80		
	B	1000	8	0.80		
	Total	2000	16	0.80	NS	54
VIN, 0.04	A	1000	27	**2.70 #**		
	B	1000	25	**2.50 #**		
	Total	2000	52	2.60	$p \leq 0.001$	51

MN = Micronucleated
§ = Statistical significance (Table 8.6)
NS = Not significant
# = Numbers exceed historical vehicle control range (ATTACHMENTS)

Table 8.4: Statistical Analysis of Test Article Data: Mexoryl SDA, 3+21 Hour Treatments in the Absence of S-9, Micronucleus Experiment – Male Donors

Treatment (µg/mL)	Cells	MN Cells	Frequency (%)	Fisher's Exact Test	Significance
Vehicle	4000	17	0.43	-	-
1000	4000	32	0.80	0.0219	p≤0.05
3000	4000	32	0.80	0.0219	p≤0.05
5000	4000	35	0.88	0.0086	p≤0.01
MMC, 0.30	2000	151	7.55	<0.0001	p≤0.001
Binomial Dispersion Test Chi-squared: 8.2185			DF: 6	p-value: 0.2225	NS
Cochran-Armitage Linear Trend				p-value: 0.0122	p≤0.05

Table 8.5: Statistical Analysis of Test Article Data: Mexoryl SDA, 3+21 Hour Treatments in the Presence of S-9, Micronucleus Experiment – Male Donors

Treatment (µg/mL)	Cells	MN Cells	Frequency (%)	Fisher's Exact Test	Significance
Vehicle	4000	20	0.50	-	-
2000	4000	30	0.75	0.1006	NS
3500	4000	31	0.78	0.0797	NS
5000	4000	34	0.85	0.0375	p≤0.05
CPA, 5.00	2000	29	1.45	0.0002	p≤0.001
Binomial Dispersion Test Chi-squared: 6.1753			DF: 6	p-value: 0.4038	NS
Cochran-Armitage Linear Trend				p-value: 0.0360	p≤0.05

Table 8.6: Statistical Analysis of Test Article Data: Mexoryl SDA, 24+24 Hour Treatment in the Absence of S-9, Micronucleus Experiment – Male Donors

Treatment (µg/mL)	Cells	MN Cells	Frequency (%)	Fisher's Exact Test	Significance
Vehicle	2000	9	0.45	-	-
200.0	2000	13	0.65	0.2612	NS
400.0	2000	20	1.00	0.0302	p≤0.05
800.0	2000	16	0.80	0.1140	NS
VIN, 0.04	2000	52	2.60	<0.0001	p≤0.001
Binomial Dispersion Test Chi-squared: 1.6165			DF: 4	p-value: 0.8058	NS
Cochran-Armitage Linear Trend				p-value: 0.0495	p≤0.05

NS = Not significant DF = Degrees of freedom

ATTACHMENTS

Historical Vehicle Control Ranges for the Human Peripheral Blood Lymphocyte Micronucleus Assay

Data generated from studies performed within the GLP laboratory, by GLP trained staff, whether a claim of GLP compliance was made or not, were included in the compilation of the historical control ranges without bias.

Male Donors, 3+21 hour -S-9

Control	Statistic	Micronucleated Binucleates Observed in 1000 Binucleates Scored	Frequency of MNBN Cells/Cells Scored (%)
Vehicle	Number of Expts	17	17
	Number of cultures	40	40
	Mean	3.45	0.35
	Standard deviation	1.87	0.19
	Observed range	0 to 8	0.00 to 0.80
	95% reference range	0 to 7.03	0.00 to 0.70

Reference ranges are calculated from percentiles of the observed distributions.
Ranges calculated in April 2018 for studies conducted between 08 February 2017 and 06 December 2017.

Male Donors, 3+21 hour +S-9

Control	Statistic	Micronucleated Binucleates Observed in 1000 Binucleates Scored	Frequency of MNBN Cells/Cells Scored (%)
Vehicle	Number of Expts	18	18
	Number of cultures	40	40
	Mean	3.55	0.36
	Standard deviation	2.01	0.20
	Observed range	0 to 10	0.00 to 1.00
	95% reference range	0.98 to 9.03	0.10 to 0.90

Reference ranges are calculated from percentiles of the observed distributions.
Ranges calculated in April 2018 for studies conducted between 08 February 2017 and 18 December 2017.

Male Donors, 24+24 hour -S-9

Control	Statistic	Micronucleated Binucleates Observed in 1000 Binucleates Scored	Frequency of MNBN Cells/Cells Scored (%)
Vehicle	Number of Expts	16	16
	Number of cultures	40	40
	Mean	3.40	0.34
	Standard deviation	2.32	0.23
	Observed range	0 to 9	0.00 to 0.90
	95% reference range	0 to 8.03	0.00 to 0.80

Reference ranges are calculated from percentiles of the observed distributions.
Ranges calculated in April 2018 for studies conducted between 24 July 2017 and 10 January 2018.

Historical Positive Control Ranges for the Human Peripheral Blood Lymphocyte Micronucleus Assay

Data generated from studies performed within the GLP laboratory, by GLP trained staff, whether a claim of GLP compliance was made or not, were included in the compilation of the historical control ranges without bias.

Male Donors, 3+21 hour -S-9

Control	Statistic	Micronucleated Binucleates Observed in 1000 Binucleates Scored	Frequency of MNBN Cells/Cells Scored (%)
MMC	Number of Expts	21	21
0.3 µg/mL	Number of cultures	40	40
	Mean	55.65	5.57
	Standard deviation	17.39	1.74
	Observed range	15 to 92	1.50 to 9.20
	95% reference range	25.73 to 85.18	2.57 to 8.52

Reference ranges are calculated from percentiles of the observed distributions.
Ranges calculated in April 2018 for studies conducted between 10 February 2016 and 27 November 2017.

Male Donors, 3+21 hour +S-9

Control	Statistic	Micronucleated Binucleates Observed in 1000 Binucleates Scored	Frequency of MNBN Cells/Cells Scored (%)
CPA	Number of Expts	22	22
3 µg/mL	Number of cultures	40	40
	Mean	22.10	2.21
	Standard deviation	8.48	0.85
	Observed range	10 to 47	1.00 to 4.70
	95% reference range	10 to 36.28	1.00 to 3.63

Reference ranges are calculated from percentiles of the observed distributions.
Ranges calculated in April 2018 for studies conducted between 08 February 2017 and 18 December 2017.

There is currently no historical control range for CPA (5 µg/mL), the concentration analysed in this study, therefore the range for the highest CPA concentration normally analysed (3 µg/mL) has been included for comparative purposes.

Male Donors, 24+24 hour -S-9

Control	Statistic	Micronucleated Binucleates Observed in 1000 Binucleates Scored	Frequency of MNBN Cells/Cells Scored (%)
VIN	Number of Expts	20	20
0.04 µg/mL	Number of cultures	41	41
	Mean	64.29	6.43
	Standard deviation	23.79	2.38
	Observed range	25 to 136	2.50 to 13.60
	95% reference range	28 to 135	2.80 to 13.50

Reference ranges are calculated from percentiles of the observed distributions.
Ranges calculated in April 2018 for studies conducted between 08 February 2017 and 10 January 2018.

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Certificate of Analysis

Laboratoire de Développement Analytique Qualité

CoA

E 510341
MEXORYL SDA
(Batch : E 14)

	Results
Appearance	beige powder
pH (Solution at 25 % in water)	5.4
Dry extract	96.9 %
Turbidity (1 % in water)	66 NTU
Dosage HPLC (w/w)	
Catechine content	3.0 %
HPLC profile	
Equivalent catechines	5.1 %
Procyanidine	0.8 %
Hyperoside	0.6 %
Equivalent Hyperosides	1.0 %
Kaempferol-3-0-glucoside	Not detected
Kaempferol	Not detected
Ethanol content	2500 ppm
Polyphenols (method Folin)	
(expressed in gallique acid)	33.4 %

Date of fabrication 23/01/2019
Validity of analysis 22/01/2020

04/02/2019

REPORT

EpiSkin™ Micronucleus Assay ----- MEXORYL SDA

Rosa centifolia Stem Extract

ABSTRACT

Purpose: Evaluate the genotoxic potential of test article [redacted] by assessing induction of micronuclei in the reconstructed skin micronucleus assay (EpiSkin™ Micronucleus Assay*)

Tissue: EpiSkin™ reconstructed epidermis model (Cat#: EpiSkin™-MNT)

Test substance: [redacted] (batch number: [redacted]0012)

Batch number of skin model: 19ER321111L1, 19ER331118L1, 19ER341125L1

Solvent: Normal saline (0.9% NaCl in deionized water)

Treatment: 72 hours' treatment

Replicates: 3 tissues/concentration in 2 independent experiments

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OBJECTIVE OF THE STUDY

- Evaluate the genotoxic potential of test article [ ] by assessing induction of micronuclei in the Reconstructed Skin Micronucleus Assay (EpiSkin™ Micronucleus Assay).

PROTOCOL OF EPISKIN™ MICRONUCLEUS ASSAY

1. SKIN MODEL PROCUREMENT

The EpiSkin™- MNT kit contains 12 reconstructed epidermis units and necessary culture media (maintenance media) were purchased from S[ ] The EpiSkin™- MNT batch was controlled by the manufacturer. Results of quality controls were supplied by manufacturer.

2. CONTROLS

Vehicle Control:

Normal saline (NS, 0.9% NaCl in deionized water) was used as vehicle for [ ] based on test article solubility data (showed in below).

Positive Control:

Results obtained from the positive controls are used to assure responsiveness of the test system.

Mitomycin C (MMC), known clastogen was used as the positive control in this study (the concentration of MMC for 72 hours' protocol was 1.5 µg/ml in Acetone).

3. TREATMENT WITH CHEMICALS

On the day of receipt, epidermal tissues were incubated with 2 mL of fresh maintenance medium containing defined concentration of Cytochalasin B (cyto B) every 24 hours. Test chemicals were dissolved in solvent, and a volume of 15 µL for each chemical was deposited three times (72 hours' protocol) on the surface of the epidermis at 24 hours' intervals.

4. SAMPLE COLLECTION

72 ± 3 hours after the initial exposure to test chemicals, cells were harvested from the EpiSkin™ tissues by treatment of warm (~37 °C) 0.25% trypsin-EDTA solution twice. A sample of cell suspension was diluted with trypan blue solution and counted using a hemacytometer.

5. FIXATION

Samples were treated with warm (37 °C) KCL (0.075 mol/L) solution, and then fixed in cold (4 °C) fresh methanol/acetic acid fixative.

6. SLIDE PREPARATION AND STAINING

After fixation, cell suspension was gently dropped onto a microscope slide. Slides were stained by freshly prepared acridine orange (AO) solution (40 µg/mL). After staining, the slides were scored using a fluorescent microscope with 20X or 40X objectives and equipped with a blue filter.

7. *Cytotoxicity Assessment*

At least 500 cells were scored per tissue to determine the percentage of mononucleated (1N), bi-nucleated (2N), and tri or more nucleated cells. Cytotoxicity was calculated with the percentage of bi-nucleated cells in treated tissues compared with solvent control.

Calculation to determine the % Binucleation of each sample:

$$\text{\% Binucleation of the sample} = \frac{\text{Number of binucleated cells x 100}}{(\#\,1N + \#\,2N + \#\,>2 \text{ nucleated cells})}$$

Use the following calculation to determine the % relative binucleation for each sample:

$$\text{\% Relative Binucleation of the sample} = \frac{\text{\% Binucleation of the Treatment sample x 100}}{\text{average \% Binucleation of solvent controls}}$$

Cytotoxicity = 100 - % Relative Binucleation of the sample

8. *Statistical Analysis and Predictive Model*

After cytotoxicity assessment, 1000 binucleated cells per tissue (or at least 500 binucleated cells if binucleated cells could not reach 1000) were scored to determine the frequency of micronucleated cells in the bi-nucleated cell population.

One-tailed Fisher's Exact Test was used to determine the statistical significance ($p<0.05$) of differences between solvent control and chemical treated groups. Five to six (5~6) concentrations of test chemical were picked out according to the cytotoxicity (0%, 10 ± 10%, 30 ± 10% and 55 ± 5%). If two or more than two concentrations of a test chemical induced a statistical significant increase of micronucleated cells, the test chemical was classified as positive chemical. Meanwhile, if no concentration of a test chemical was considered significant, the test chemical was classified as negative. If only one concentration of a test chemical was

considered significant, another statistical method named Cochran-Armitage test was performed to evaluate the trend of micronucleus formation at different concentrations. If $p<0.05$, the test chemical was classified as positive chemical.

The statistical analysis in this study was performed by "micronucleus" application on Mystat platform.

9. *Criteria for Determination of a Valid Test*

The assay will be accepted if the positive control compound, MMC, causes a statistically significant increase in the micronucleus frequency based on the Fisher's Exact Test. The cytotoxicity of the positive control compound should be below 30% and the percent of binucleated cells in the solvent control should be at least 25%.

Reference: Chen, L. Z., Li, N., Liu, Y. F., Faquet, B., Alépée, N., Ding, C. M., Eilstein, J., Zhong, L. Y., Peng, Z. G., Ma, J., Cai, Z. Z., Ouedraogo, G. (2020) A new 3D model for genotoxicity assessment: EpiSkin™ Micronucleus Assay. *Mutagenesis*, doi: 10.1093/mutage/geaa003. [Epub ahead of print]

Evaluation Design

Schedule:

*: If the conclusions of definitive micronucleus assay run 1 and run 2 are not consistent, a third run will be performed.

Runs 1, 2 and 3 are conducted under the same conditions, except for dose selection (The doses of testing chemical might be slightly changed based on the results of the previous run).

Experimental phase for [ ](initiated-completed)

- Solubility determination: 15/07/2019
- Dose finding assay: 12/11/2019-18/11/2019
- Definitive Micronucleus Assay Run 1: 19/11/2019-06/12/2019
- Definitive Micronucleus Assay Run 2: 26/11/2019-12/12/2019

RESULTS

1. SOLUBILITY DETERMINATION

Code	Solvent 1	Solubility	Note	Solvent 2	Solubility	Note
MEXORYL SDA	Acetone	< 1mg/ml		Normal saline	> 100 mg/ml	

For [ ], normal saline was used as solvent.

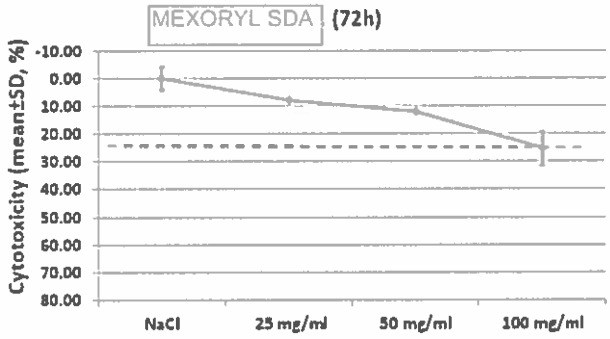
During the chemical treatment, the skin model should be covered by a circular nylon mesh ø=11.3 mm to promote the uniform distribution of chemical.

2. *DOSE FINDING ASSAY*

For dose finding assay, 10 concentrations of the RM were used (100, 50, 25, 10, 5, 1, 0.5, 0.1, 0.05, 0.01 mg/mL) to detect the cytotoxicity.

Data Summary:

Treatment	Cytotoxicity (Mean, %)	Cytotoxicity (SD, %)
Normal saline	0.00	4.0
25 mg/ml	7.91	1.9
50 mg/ml	12.09	0.6
100 mg/ml	25.49	5.2



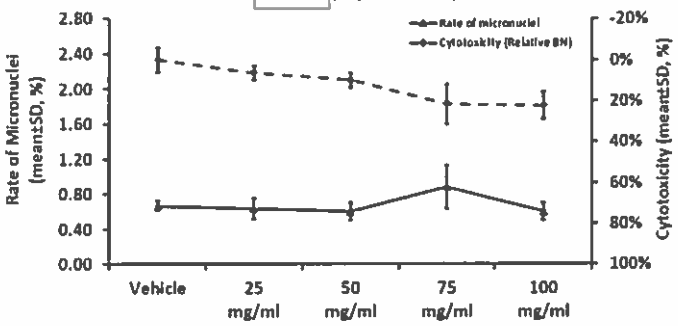
There was no high cytotoxicity even in the highest concentration (100 mg/ml).

Concentrations: 100, 75, 50, 25 mg/ml were used in definitive micronucleus assay.

[redacted]

3. *Definitive Micronucleus Assay Run 1*

[redacted] (Experiment 1)



Data summary:

	Rate of relative bi-nucleated cells (Mean, %)	Cytotoxicity (Mean, %)	Cytotoxicity (SD, %)	Rate of cells with micronuclei (Mean, %)	Rate of cells with micronuclei (SD, %)	Rate of cells with micronuclei (95% Confidence interval)	
Normal saline	100.00	0.00	5.91	0.67	0.06	0.601	0.732
MMC	80.55	19.45	3.90	1.53	0.06	1.468	1.599
100 mg/ml	77.64	22.36	6.48	0.60	0.10	0.487	0.713
75 mg/ml	78.41	21.59	9.68	0.88	0.24	0.604	1.159
50 mg/ml	90.05	9.95	3.59	0.60	0.10	0.487	0.713
25 mg/ml	93.57	6.43	3.45	0.63	0.12	0.503	0.764

Statistical analysis:

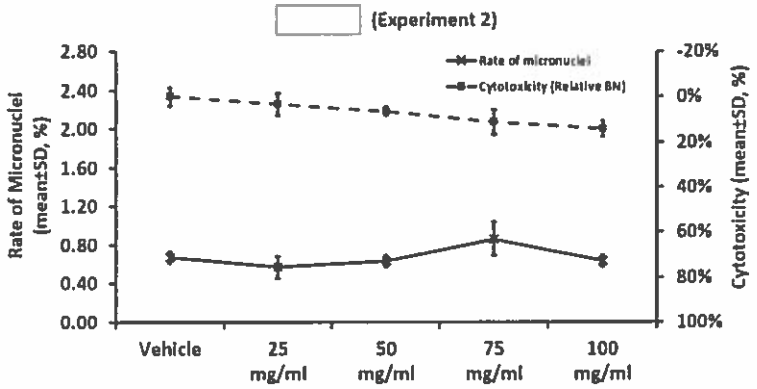
Treatment	number of cells WITHOUT micronuclei	number of cells WITH micronuclei	Total
NS	2980	20	3000
MMC	2954	46	3000
100 mg/ml	2982	18	3000
75 mg/ml	2251	19	2270
50 mg/ml	2982	18	3000
25 mg/ml	2981	19	3000

	P value One-tailed Fisher's Exact Test
MMC vs NS	< 0.01
100 mg/ml vs NS	0.69
75 mg/ml vs NS	0.29
50 mg/ml vs NS	0.69
25 mg/ml vs NS	0.63

As showed in the result, the positive control compound, MMC, caused a statistically significant increase in the micronucleus frequency based on the Fisher's Exact Test. The cytotoxicity of the positive control compound was below 30% and the percent of binucleated cells in the solvent control was over 25%. The data was qualified.

No concentration showed significantly different yield of micronuclei, the test chemical was classified as negative.

4. *Definitive Micronucleus Assay Run 2*



Data summary:

	Rate of relative bi-nucleated cells (Mean, %)	Cytotoxicity (Mean, %)	Cytotoxicity (SD, %)	Rate of cells with micronuclei (Mean, %)	Rate of cells with micronuclei (SD, %)	Rate of cells with micronuclei (95% Confidence interval)	
Normal saline	100.00	0.00	3.91	0.67	0.06	0.601	0.732
MMC	80.67	19.33	3.90	1.47	0.06	1.401	1.532
100 mg/ml	85.89	14.11	3.45	0.63	0.06	0.568	0.699
75 mg/ml	88.65	11.35	5.57	0.86	0.17	0.663	1.056
50 mg/ml	93.56	6.44	1.86	0.63	0.06	0.568	0.699
25 mg/ml	96.63	3.37	4.85	0.57	0.12	0.436	0.697

Statistical analysis:

Treatment	number of cells WITHOUT micronuclei	number of cells WITH micronuclei	Total
Normal saline	2980	20	3000
MMC	2956	44	3000
100 mg/ml	2981	19	3000
75 mg/ml	2201	19	2220
50 mg/ml	2981	19	3000
25 mg/ml	2983	17	3000

	P value One-tailed Fisher's Exact Test
MMC vs NS	< 0.01
100 mg/ml vs NS	0.63
75 mg/ml vs NS	0.27
50 mg/ml vs NS	0.63
25 mg/ml vs NS	0.74

As showed in the result, the positive control compound, MMC, caused a statistically significant increase in the micronucleus frequency based on the Fisher's Exact Test. The cytotoxicity of the positive control compound was 30% and the percent of binucleated cells in the solvent control was over 25%. The data was qualified.

No concentration showed significantly different yield of micronuclei, the test chemical was classified as negative.

The results from run 1 and run 2 were consistent.

CONCLUSION

MEXORYL SDA showed non genotoxic potential in EpiSkin™ Micronucleus Assay.

RAW DATA

1. DOSE FINDING ASSAY

Tissue #	Treatment	1N	2N	>2N	%Bi	Ave% Bi	% Rel Bi	Ave% Rel Bi	Cytotoxicity
1	Nacl	266	234		46.8%		102.9%		
2	Nacl	279	221		44.2%	45.5%	97.1%	100.0%	0.00
3									
4									
5	0.01 mg/ml	500			0.0%		0.0%		
6	0.01 mg/ml	500			0.0%	0.0%	0.0%	0.0%	100.00
7	0.05 mg/ml	500			0.0%		0.0%		
8	0.05 mg/ml	500			0.0%	0.0%	0.0%	0.0%	100.00
9	0.1 mg/ml	500			0.0%		0.0%		
10	0.1 mg/ml	500			0.0%	0.0%	0.0%	0.0%	100.00
11	0.5 mg/ml	500			0.0%		0.0%		
12	0.5 mg/ml	500			0.0%	0.0%	0.0%	0.0%	100.00
13	1 mg/ml	500			0.0%		0.0%		

14	1 mg/ml	500			0.0%	0.0%	0.0%	0.0%	100.00
15	5 mg/ml	500			0.0%		0.0%		
16	5 mg/ml	500			0.0%	0.0%	0.0%	0.0%	100.00
17	10 mg/ml	500			0.0%		0.0%		
18	10 mg/ml	500			0.0%	0.0%	0.0%	0.0%	100.00
		500							
19	25 mg/ml	300	200		40.0%		87.9%		
20	25 mg/ml	281	219		43.8%	41.9%	96.3%	92.1%	7.91
21	50 mg/ml	299	201		40.2%		88.4%		
22	50 mg/ml	301	199		39.8%	40.0%	87.5%	87.9%	12.09
21	100 mg/ml	322	178		35.6%		78.2%		
22	100 mg/ml	339	161		32.2%	33.9%	70.8%	74.5%	25.49

Batch of skin model: 19ER321111L1

2. *Definitive Micronucleus Assay Run 1*

Treatment	1N	2N	>2N	%Bi	Ave% Bi	% Rel Bi	Cytotoxicity	Ave% Rel Bi	Total 2N	µnuc	%µnuc	Ave%µnuc
NaCl	272	227	1	45.4%		104.3%	-4.3%		1000	7	0.7	
NaCl	297	203	0	40.6%		93.3%	6.7%		1000	7	0.7	
NaCl	277	223	0	44.6%	43.5%	102.5%	-2.5%	100.0%	1000	6	0.6	0.67
									2980	20		
MMC	334	165	1	33.0%		75.8%	24.2%		1000	15	1.5	
MMC	323	177	0	35.4%		81.3%	18.7%		1000	16	1.6	
MMC	316	184	0	36.8%	35.1%	84.5%	15.5%	80.6%	1000	15	1.5	1.53
									2954	46		

100 mg/ml	318	182	0	36.4%		83.6%	16.4%		1000	5	0.5	
100 mg/ml	328	171	1	34.2%		78.6%	21.4%		1000	6	0.6	
100 mg/ml	345	154	1	30.8%	33.8%	70.8%	29.2%	77.6%	1000	7	0.7	0.60
									2982	18		
75 mg/ml	347	153	0	30.6%		70.3%	29.7%		670	7	1.0	
75 mg/ml	306	194	0	38.8%		89.1%	10.9%		1000	6	0.6	
75 mg/ml	334	165	1	33.0%	34.1%	75.8%	24.2%	78.4%	600	6	1.0	0.88
									2251	19		
50 mg/ml	299	201	0	40.2%		92.3%	7.7%		1000	7	0.7	
50 mg/ml	300	200	0	40.0%		91.9%	8.1%		1000	5	0.5	
50 mg/ml	313	187	0	37.4%	39.2%	85.9%	14.1%	90.0%	1000	6	0.6	0.60
									2982	18		
25 mg/ml	289	211	0	42.2%		96.9%	3.1%		1000	5	0.5	
25 mg/ml	303	196	1	39.2%		90.0%	10.0%		1000	7	0.7	
25 mg/ml	296	204	0	40.8%	40.7%	93.7%	6.3%	93.6%	1000	7	0.7	0.63
									2981	19		

Batch of skin model: 19ER331118L1

3. *Definitive Micronucleus Assay Run 2*

Treatment	1N	2N	>2N	%Bi	Ave% Bi	% Rel Bi	Cytotoxicity	Ave% Rel Bi	Total 2N	µnuc	%µnuc	Ave%µnuc
NaCl	284	214	2	42.8%		96.5%	1.5%		1000	7	0.7	
NaCl	272	227	1	45.4%		104.4%	-4.4%		1000	6	0.6	
NaCl	289	211	0	42.2%	43.5%	97.1%	2.9%	100.0%	1000	7	0.7	0.67
									2980	20		
MMC	322	177	1	35.4%		81.4%	18.6%		1000	14	1.4	
MMC	334	165	1	33.0%		75.9%	24.1%		1000	15	1.5	
MMC	316	184	0	36.8%	35.1%	84.7%	15.3%	80.7%	1000	15	1.5	1.47
									2956	44		

100 mg/ml	313	187	0	37.4%		86.0%	14.0%		1000	6	0.6	
100 mg/ml	305	194	1	38.8%		89.3%	10.7%		1000	6	0.6	
100 mg/ml	321	179	0	35.8%	37.3%	82.4%	17.6%	85.9%	1000	7	0.7	0.63
									2981	19		
75 mg/ml	321	179	0	35.8%		82.4%	17.6%		750	7	0.9	
75 mg/ml	298	202	0	40.4%		92.9%	7.1%		720	7	1.0	
75 mg/ml	303	197	0	39.4%	38.5%	90.6%	9.4%	88.7%	750	5	0.7	0.86
									2201	19		
50 mg/ml	293	207	0	41.4%		95.2%	4.8%		1000	7	0.7	
50 mg/ml	295	204	1	40.8%		93.9%	6.1%		1000	6	0.6	
50 mg/ml	301	199	0	39.8%	40.7%	91.6%	8.4%	93.6%	1000	6	0.6	0.63
									2981	19		
25 mg/ml	289	211	0	42.2%		97.1%	2.9%		1000	5	0.5	
25 mg/ml	280	220	0	44.0%		101.2%	-1.2%		1000	5	0.5	
25 mg/ml	301	199	0	39.8%	42.0%	91.6%	8.4%	96.6%	1000	7	0.7	0.57
									2983	17		

Batch of skin model: 19ER341125L1

Mononucleated (1N), bi-nucleated (2N), and tri or more nucleated (>2N) cells

% Rel Bi: Rate of relative bi-nucleated cells

µnuc: Cells with micronuclei

SUPPLEMENTARY MATERIALS ---- SOP OF EPISKIN™ MICRONUCLEUS ASSAY

1. PREPARATIONS

Work in ventilated cabinets: to prevent accidental contact wear protective gloves, and if necessary a mask and/or safety glasses.

Sterilization: clear all materials (forceps, biopsy punch…) before the application.

1.1 Mitomycin C (MMC) solutions preparation

Mitomycin C (MMC) is the positive control. A stock solution of the positive control is prepared and frozen.

Make a 0.5 mg/mL stock solution of MMC by adding 4.0 ml of room temperature, sterile, tissue culture grade water to the vial containing 2 mg of MMC. Vortex until complete solubility is achieved. Using a calibrated micropipette, dispense a volume of MMC stock into sterile labeled micro-tubes that will allow dilutions to be easily made to achieve work solution. Cap each vial tightly and store aliquots at -15 to -25°C.

On the day of use, remove a vial of MMC stock from the freezer and bring it to room temperature, do not thaw in a 37°C water bath. Dilute it in acetone to get work solution. Prepare the MMC dose(s) fresh each day of dosing the tissues.

1.2 Cytochalasin b (cyto b) solutions preparation

Add 2.5 mL DMSO to a 5 mg vial of Cytochalasin B and vortex until completely solubilized. Using a calibrated micropipette, aliquot 100 µL (or other appropriate volume) of CytoB into appropriately labeled cryovials. Cap tightly and store at -15 to -25°C.

Thaw a 2 mg/mL aliquot of Cytochalasin B on each day of dosing. Prepare fresh CytoB in maintenance medium by adding 1µl of 2 mg/mL CytoB stock per mL maintenance medium, the final concentration is 2 µg (cytoB)/mL maintenance medium.

2. Treatment with chemicals

The tissues will be sent out on Monday morning and received on Tuesday morning. On the day of receipt, epidermal tissues will be incubated with 2 mL of fresh maintenance medium containing a defined concentration of cyto B every 24 hours. Test chemicals will be dissolved in pure acetone, and a dosing volume of 15 µL of each chemical will be applied twice (48-hour protocol) or three times (72-hour protocol) to the surface of the epidermis at 24-hour intervals.

For the dose finding assay, 10 concentrations of test chemical will be tested (100, 50, 25, 10, 5, 1, 0.5, 0.1, 0.05, 0.01 mg/mL) to determine the cytotoxicity (the cytotoxicity assessment is described in Section 2.4.5). Two tissues/treatment will be used in dose finding assay.

For the micronucleus assay, 6 concentrations of test chemical will be selected according to the cytotoxicity (0%, 10 ± 10%, 30 ± 10% and 55 ± 5%). Three tissues/treatment will be used in micronucleus assay.

3. Sample collection

In a 12-well plate, each tissue insert will be placed first in 4 mL DPBS at room temperature for 5-10 minutes. Each insert will be taken out of DPBS well, decanted and blotted on paper towel to remove excess DPBS and then placed in 4 mL of EDTA (0.1%, 1 g/L) at room temperature for 15 minutes. Each tissue insert will again be taken out, decanted and blotted to remove excess EDTA, and exposed to warm (~37 °C) trypsin-EDTA solution for 15 minutes at 37 °C. This initial exposure to the Trypsin-EDTA will be performed by placing the tissue in a well of a 12-well plate containing 1.5 mL of warm (~37 °C) trypsin-EDTA and adding 0.5 mL of warm trypsin inside the insert.

The tissue will be carefully separated from the supporting membrane by gently lifting the edge of the tissue with fine forceps while holding the insert with another forceps. Both the detached tissue will be transferred to a new well, and exposed to fresh 1 mL of warm (~37 °C) trypsin-EDTA for 15 minutes at 37 °C.

One mL of warm maintenance medium was added to neutralize the trypsin and the tissues were agitated with trypsin-EDTA/maintenance medium to release additional cells. Cell clumps were dissociated and the cell suspension was transferred into a 15 mL conical tube. A sample of cell suspension was diluted with trypan blue solution and counted using a hemocytometer. Cytotoxicity was defined as a decrease in cell viability compared with solvent control (pure acetone).

4. Fixation

The cell suspension will be centrifuged (100-150g for 5 minutes) and the supernatant will be carefully removed. The cell pellet will be loosened with gentle flicking of the base of the centrifuge tube and 1 mL of warm (~37 °C) KCl solution will be slowly added down the side of the tube while gently shaking the cell suspension. After ~3 minutes, 3 mL of fresh (prepared on day of use), cold (at ~4°C) methanol/acetic acid (3:1) fixative will be added slowly to fix the cells, and the cell suspension will be centrifuged at 100-150g for 5 minutes. Each "slow" addition process will take ~10 seconds, which will be kept identical so that in the harvest of multiple tissues, all cell suspensions received identical treatment periods.

An optional second fixation can be used if the above method results in significant salt crystals on the slide. Salt crystals on the slide will often interfere with the microscopic slide evaluation after the first fixation and centrifugation, the supernatant will be removed, the pellet loosened, and 2-3 mL of cold fresh methanol/acetic acid (99:1) fixative will be added. The cell suspension will be centrifuged at 100-150g for 5 minutes. This process will result in far fewer instances of salt crystallization on the slide.

5. Slide preparation and staining

After centrifugation, all but a small portion (less than 200 µL) of the supernatant will be removed, the cell pellet loosened by gentle flicking of the centrifuge tube, and a single drop (20-25 µL) of the cell suspension will be gently dropped onto a flat or slightly tilted clean, dry microscope slide. Two slides will be prepared for each tissue, if possible. After the slides are completely dry, they will be immersed in freshly prepared AO solution (40 µg/ml) for 2-3 minutes, immediately rinsed 3 times with DPBS (each rinse for at least 1 minute). Stained slides will

be stored in the dark at 2-8°C. Prior to analysis, a drop of PBS will be put onto the slide, a coverslip will be added and the slides scored using a fluorescent microscope with 40X or 60X objectives and equipped with a blue filter.

6. Cytotoxicity assessment

At least 500 cells will be scored per tissue to determine the percentage of mono-, bi-, tri- or multi-nucleated cells. Cytotoxicity will be calculated according to the percentage of binucleated cells in treated tissue compared with solvent control.

7. Micronucleus assessment

After cytotoxicity assessment, 1000 binucleated cells per tissue (or at least 500 binucleated cells if there will be less than 1000) will be scored to determine the frequency of micronuclei in the binucleated cell population. Highly differentiated cells with green cytoplasm will be excluded from this analysis. Only results from tissues that had at least 500 analyzable binucleated cells will be used for analysis.

8. Statistical analysis and predictive model

The one-tailed Fisher's Exact Test will be used to determine the statistical significance ($p<0.05$) of differences between solvent control and chemical treated groups. Six concentrations of test chemical will be selected according to the cytotoxicity (0%, 10 ± 10%, 30 ± 10% and 55 ± 5%). If two or more concentrations of a test chemical will be considered significant, the test chemical will be classified as positive. Conversely, if none of the concentrations of a test chemical will be considered significant, the test chemical will be classified as negative. If only one concentration of a test chemical will be considered significant, another statistical method, the Cochran-Armitage test, will be performed to evaluate the trend of micronucleus formation at different concentrations. If $p<0.05$, the test chemical will be classified as positive.

Calculation to determine the % Binucleation of each slide:

$$\textbf{\% Binucleation of the slide} = \frac{\textbf{Number of binucleated cells x 100}}{\textbf{(\# 1N + \# 2N + \# >2 nucleated cells)}}$$

Use the following calculation to determine the % relative binucleation for each slide:

$$\text{\% Relative Binucleation of the slide} = \frac{\text{\% Binucleation of the Treatment slide x 100}}{\text{average \% Binucleation of solvent controls}}$$

Cytotoxicity based on % BN cells = 100 - % Relative Binucleation of the slide

Use the following calculation to determine the % micronucleus of a slide:

$$\text{\% micronucleus} = \frac{\text{\# of BN cells with at least one micronucleus x 100}}{\text{Total \# of binucleated cells}}$$

9. Criteria for determination of a valid test

The assay will be accepted as valid if the positive control compound, MMC, caused a statistically significant increase in the micronucleus frequency based on the Fisher's Exact Test. The cytotoxicity of the positive control compound should be below 30% and the percent binucleation of the solvent control should be at least 25%.

2022 FDA-VCRP Data-Rosa Centifolia

ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER	02A	Bath Oils, Tablets, and Salts	4
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER	02D	Other Bath Preparations	2
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER	10C	Douches	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER	12C	Face and Neck (exc shave)	4
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER	12F	Moisturizing	2
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER	12H	Paste Masks (mud packs)	1

Total 14

ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	01B	Baby Lotions, Oils, Powders, and Creams	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	02B	Bubble Baths	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	03D	Eye Lotion	2
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	03E	Eye Makeup Remover	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	03G	Other Eye Makeup Preparations	2
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	04B	Perfumes	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	04E	Other Fragrance Preparation	4
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	05A	Hair Conditioner	4
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	05F	Shampoos (non-coloring)	3
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	05G	Tonics, Dressings, and Other Hair Grooming Aids	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	05I	Other Hair Preparations	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	07E	Lipstick	7
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	07F	Makeup Bases	2
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	07I	Other Makeup Preparations	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	10A	Bath Soaps and Detergents	3
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	12A	Cleansing	8
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	12C	Face and Neck (exc shave)	35
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	12D	Body and Hand (exc shave)	15
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	12F	Moisturizing	65
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	12H	Paste Masks (mud packs)	4
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	12I	Skin Fresheners	5
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER EXTRACT	12J	Other Skin Care Preps	8

Total 174

CENTIFOLIA (CABBAGE ROSE) FLOWER JUICE	12C	Face and Neck (exc shave)	1

Total 1

ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	02A	Bath Oils, Tablets, and Salts	2
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	05A	Hair Conditioner	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	05E	Rinses (non-coloring)	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	05F	Shampoos (non-coloring)	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	07E	Lipstick	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	10A	Bath Soaps and Detergents	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	10C	Douches	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	12A	Cleansing	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	12C	Face and Neck (exc shave)	2
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	12D	Body and Hand (exc shave)	2

ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	12F	Moisturizing	7
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	12H	Paste Masks (mud packs)	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER OIL	12J	Other Skin Care Preps	4
Total 25			
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER POWDER	02A	Bath Oils, Tablets, and Salts	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER POWDER	12C	Face and Neck (exc shave)	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER POWDER	12D	Body and Hand (exc shave)	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER POWDER	12F	Moisturizing	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER POWDER	12H	Paste Masks (mud packs)	1
Total 5			
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	03D	Eye Lotion	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	03E	Eye Makeup Remover	4
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	03G	Other Eye Makeup Preparations	5
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	04E	Other Fragrance Preparation	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	05A	Hair Conditioner	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	05F	Shampoos (non-coloring)	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	07E	Lipstick	3
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	10A	Bath Soaps and Detergents	5
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	10C	Douches	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	10E	Other Personal Cleanliness Products	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	12A	Cleansing	7
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	12C	Face and Neck (exc shave)	25
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	12D	Body and Hand (exc shave)	5
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	12F	Moisturizing	23
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	12G	Night	3
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	12H	Paste Masks (mud packs)	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	12I	Skin Fresheners	7
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WATER	12J	Other Skin Care Preps	5
Total 99			
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WAX	03F	Mascara	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WAX	07E	Lipstick	3
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WAX	10A	Bath Soaps and Detergents	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WAX	12C	Face and Neck (exc shave)	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WAX	12D	Body and Hand (exc shave)	2
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WAX	12F	Moisturizing	1
ROSA CENTIFOLIA (CABBAGE ROSE) FLOWER WAX	12J	Other Skin Care Preps	1
Total 10			