Safety Assessment of *Equisetum arvense*-Derived Ingredients as Used in Cosmetics

Status: Draft Tentative Report for Panel Review
Release Date: August 20, 2021
Panel Meeting Date: September 13-14, 2021

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.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst/Writer, CIR.

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons

From: Wilbur Johnson, Jr.
Senior Scientific Analyst/Writer, CIR

Date: August 20, 2021

Subject: Safety Assessment of *Equisetum arvense*-Derived Ingredients as Used in Cosmetics

Enclosed is a Draft Tentative Report of the Safety Assessment of *Equisetum arvense*-Derived Ingredients as Used in Cosmetics (*equise092021rep*). At the December 2020 Panel meeting, the Panel issued an Insufficient Data Announcement (IDA) for these 5 ingredients with the following data requests:

Equisetum Arvense Juice, Equisetum Arvense Leaf Extract, Equisetum Arvense Leaf Powder, and Equisetum Arvense Powder

- Method of manufacture, impurities, and composition data

Equisetum Arvense Extract

- Skin irritation and sensitization data at maximum concentration of use

The following unpublished data, received from the Council in response to the IDA, are included in the Draft Tentative Report (highlighted in report text):

- Composition data on Equisetum Arvense Extract (*equise092021data1*)
- Mouse acute oral toxicity data on ~2% Equisetum Arvense Extract (*equise092021data1*)
- Rabbit ocular and skin irritation data on ~2% Equisetum Arvense Extract (*equise092021data1*)
- HRIPT on a nail polish containing 0.000049% Equisetum Arvense Extract (*equise092021data2)*
- In-use safety evaluation on a nail polish containing 0.000049% Equisetum Arvense Extract (*equise092021data2)*
- HRIPT on a product containing 0.60% Equisetum Arvense Extract (*equise092021data3)*

A draft Discussion, based on the Panel's deliberations at the December 2020 Panel meeting, an in vitro teratogenicity assay on *Equisetum arvense* (published in 2021), and 2021 FDA VCRP data (*equise092021FDA*) are also highlighted in the report for the Panel's consideration. Also included in this package for your review are the report history (*equise092021hist*), flow chart (*equise092021flow*), literature search strategy (*equise092021strat*), ingredient data profile (*equise092021prof*), and minutes from the December 2020 Panel meeting (*equise092021min).*

During one of the Team discussions at the December 2020 Panel meeting, it was suggested that the micronucleus assay involving X-radiated cultures should be deleted from the safety assessment because the test does not appear to be applicable to a genotoxicity evaluation of *Equisetum arvense*-derived ingredients. The Panel should determine whether or not this assay should remain.

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Report with a safe as used, safe with qualifications, unsafe, or split conclusion, and Discussion items should be identified. If the available data remain insufficient, the Panel should issue a Tentative Report with an insufficient data conclusion, specifying the data needs in the report Discussion.

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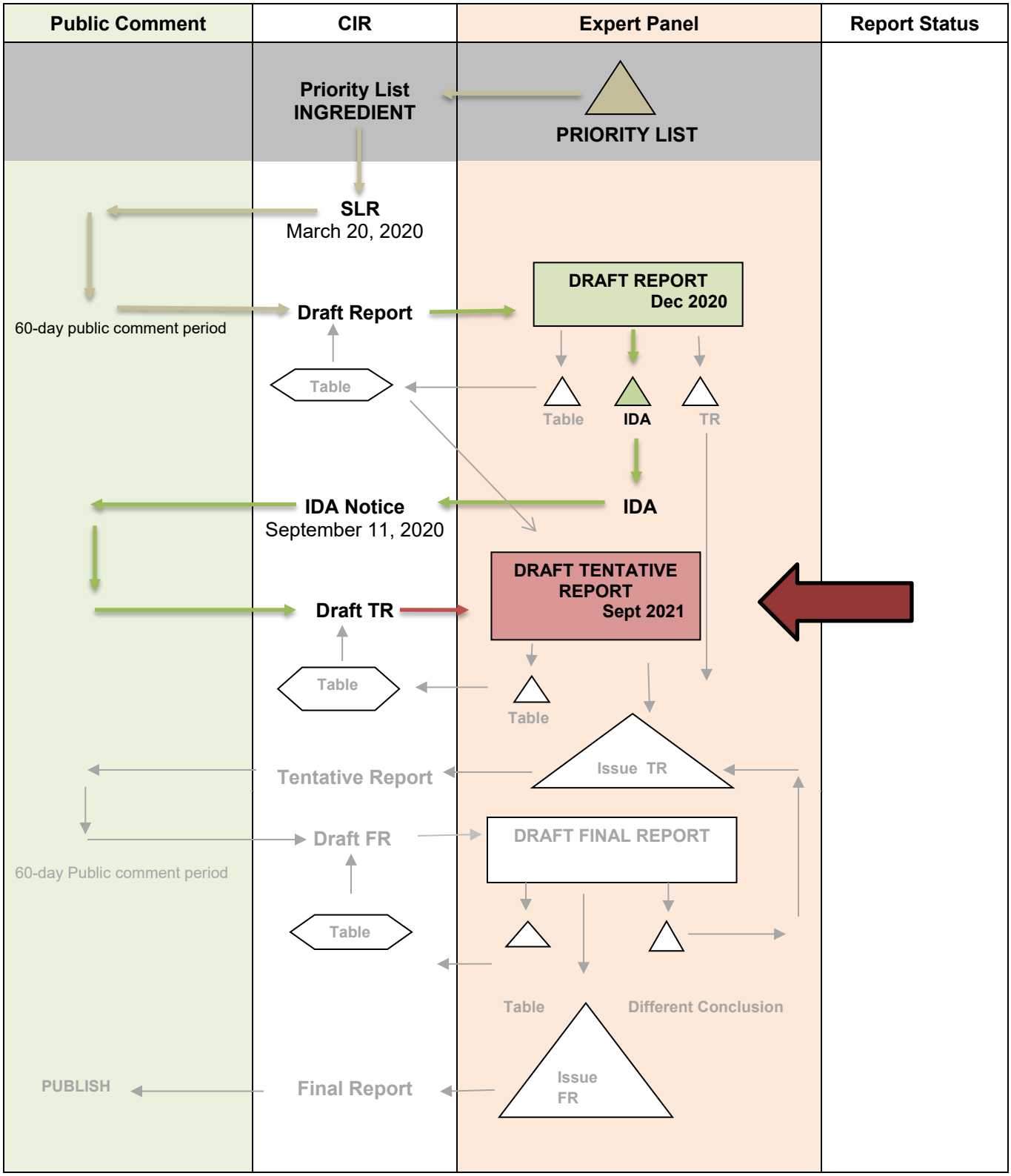
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY *Equisetum arvense*-derived ingredients

MEETING September 2021



CIR History of:

Equisetum arvense-derived Ingredients

A Scientific Literature Review (SLR) on *Equisetum arvense*-derived Ingredients was issued on March 20, 2020.

Draft Report, Teams/Panel: December 7-8, 2020

The draft report has been revised to include the Council's comments and the following unpublished data (received from the Council): use concentration data and data on Equisetum Arvense Extract relating to methods of production and skin irritation and sensitization potential.

The Panel issued an Insufficient Data Announcement (IDA) with the following data requests:

Equisetum Arvense Juice, Equisetum Arvense Leaf Extract, Equisetum Arvense Leaf Powder, and Equisetum Arvense Powder

- Method of manufacture, impurities, and composition data

Equisetum Arvense Extract

- Skin irritation and sensitization data at maximum concentration of use

During deliberations, the Panel noted that hair loss was observed in an oral dosing study in which Sprague-Dawley rats were fed 4% *Equisetum arvense* powder in a cholesterol diet for 14 d. However, they also noted no obvious clinical signs in another study in which F344 rats were fed *Equisetum arvense* (hot water extract of powder) at concentrations up to 3% in a basal diet for 13 wk.

Draft Tentative Report, Teams/Panel: September 13-14, 2021

The draft tentative report has been revised to address comments received from the Council prior to the December 2020 Panel meeting.

The following unpublished data were received from the Council in response to the IDA, and are included in the draft tentative report (highlighted in report text):

- Composition data on Equisetum Arvense Extract
- Mouse acute oral toxicity data on ~2% Equisetum Arvense Extract
- Rabbit ocular and skin irritation data on ~2% Equisetum Arvense Extract
- HRIPT on a nail polish containing 0.000049% Equisetum Arvense Extract
- In-use safety evaluation on a nail polish containing 0.000049% Equisetum Arvense Extract
- HRIPT on a product containing 0.60% Equisetum Arvense Extract

Equisetum arvense-derived Ingredients Data Profile* –September 13-14, 2021 – Wilbur Johnson, Jr.																														
						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	In Vitro	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Equisetum Arvense Extract	**186**		**X**	**X**					**X**							**X**					**X**	**X**		**X**	**X**			**X**		**X**
Equisetum Arvense Juice																														
Equisetum Arvense Leaf Extract	**12**																													
Equisetum Arvense Leaf Powder																														
Equisetum Arvense Powder	**1**											**X**																		
Equisetum arvense (horsetail)	**3**			**X**	**X**				**X**						**X**	**X**	**X**													**X**

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

[*Equisetum arvense*-derived Ingredients – 12/05/2019; 10/19/2020; 6/2/2021; 7/20/2021]

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	ECE-TOC	Web
Equisetum Arvense Extract	71011-23-9	Yes		0/0														
Equisetum Arvense Juice	71011-23-9	Yes		6/1														
Equisetum Arvense Leaf Extract	71011-23-9	Yes		1/0														
Equisetum Arvense Leaf Powder		Yes		1/0														
Equisetum Arvense Powder		Yes		0/0														
Horsetail		Yes		161/5														
Search: Equisetum Arvense OR Horsetail				499/32	127/23													

*ECHA – pre-registration process

Search Strategy
[*document search strategy used for PubMed, and Toxnet*]
[*identify total # of hits /# hits that were useful or examined for usefulness*]

LINKS

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

FDA databases – http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (CFR); then,
list of all databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm; then,
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
ECETOC (European Center for Ecotoxicology and Toxicology Database) - http://www.ecetoc.org/

IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/
NTIS (National Technical Information Service) - http://www.ntis.gov/
NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/ (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc

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

DECEMBER 2020 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito Team –December 7, 2020

Equisetum Arvense-derived Ingredients

Equisetum arvense, yes. Yeah. Equisetum arvense, so this is the first time that we're looking at this. And let me see if I can do a better job at finding my comments. Okay. My first comment was on -- so are we sure about the aerosols here? Because the products that are listed that it says cologne and toilet water, they don't necessarily have to be sprays, do they? Or are we just assuming they're going to be a spray when it's used in a cologne and a toilet water?

MR. JOHNSON: I think we've always had that assumption, Dr. Belsito.

DR. BELSITO: Okay. And Wilber, under Short-term Toxicity Studies, what are serum liver lipids? I've never heard of serum -- lipids I've heard of, but not liver lipids. Is that a misprint? This is PDF page 12 under Oral, the fourth line.

MR. JOHNSON: Which paragraph, which section?

DR. BELSITO: Under Short-term Toxicity on page 12.

MR. JOHNSON: Yeah.

DR. BELSITO: The oral study, the first oral study, right at the beginning of line four you say, "serum liver lipids?" Is that serum lipids?

MR. JOHNSON: I would have to check that, but I believe that that terminology is being used in the publication.

DR. BELSITO: Because I've never heard of measuring liver lipids.

DR. LIEBLER: Yeah. It doesn't make sense.

DR. BELSITO: And then on PDF page 13, the study on the extract, I just made a note. I don't understand the study. Irradiated with what, UV-ABC? This is a micronucleus test, and it was performed in unirradiated and irradiated samples? Curt or Paul or Dan, have you ever seen anything like that?

DR. SNYDER: I have not.

DR. LIEBLER: No.

DR. BELSITO: It doesn't say what it was irradiated with.

MR. JOHNSON: If you have time, I could check that publication, or I could wait on that.

DR. BELSITO: I would just check it and see what they irradiated it with. I'd be curious about what Tom has to say about this study. I've never heard of a genotox study being done irradiated and nonirradiated, particularly for a material that doesn't appear to absorb light.

DR. LIEBLER: Well, it probably does absorb light but --

DR. BELSITO: Right.

DR. LIEBLER: Where are you at actually, Don, on this? What page? I'm trying to find this.

DR. KLAASSEN: It's at 13.

DR. BELSITO: It's PDF page 13, under Genotox. It's the third paragraph. It says, "The acquired micronucleus formation in unirradiated and irradiated samples of human blood lymphocytes." It doesn't say irradiated with what.

DR. LIEBLER: Yeah. Okay. Okay. Yeah. That's odd.

MR. JOHNSON: I'm looking at this publication. And it says using a, I guess, cobalt gamma ray source, cobalt 60, a gamma ray source.

DR. BELSITO: So it was X irradiation.

DR. LIEBLER: Yeah.

DR. BELSITO: Which would presumably kill lymphocytes, no?

MR. JOHNSON: And the dose rate was 0.45 Gy per minute.

DR. BELSITO: Yeah.

DR. LIEBLER: Grays.

DR. BELSITO: Grays per minute. So it's X-rayed. That's really weird. Should we just strike that study?

DR. LIEBLER: I think so. I don't think it's at all applicable to a genotox evaluation of the ingredient.

DR. BELSITO: Yeah. So I would get rid of this one, Wilbur.

MR. JOHNSON: Okay. Will do.

DR. BELSITO: So, for the sensitization study here, we really don't have a lot of data. There was only five guinea pigs. Normally, you'd want 20 for a guinea pig maximization test. It was negative, but I don't know what other people thought about this.

DR. LIEBLER: I assumed you'd think it was inadequate. We didn't have human data.

DR. BELSITO: Yeah. I did. So at the end of the day after looking at this, I thought it was insufficient for manufacturing for all the ingredients except the extract. Composition and impurities, possibly all the details on one extract I thought were a little sketchy. The only extract with a concentration of use or -- only the extract has a concentration of use, right? The juice and the leaf powder are not used?

DR. SNYDER: Yeah. The extract's the only one reported to be used at 0.4 percent maximum concentration.

DR. BELSITO: Right. We have no DART. So do we need a 28-day dermal? And genotox is, I guess, okay for the extract, but it's insufficient for all the others? And sensitization and irritation for the extract at maximum concentration of use?

DR. LIEBLER: So the only thing I didn't really concur with you on, Don, was the method of manufacture. I felt that the method of manufacture for the extract will suffice as well as the composition and impurities. The manufacturing for the powders really appears self-evident from the definitions. And the data for the whole plant extract, which is everything in the plant, and the major ingredient use would cover the other ingredients.

DR. BELSITO: Okay. So you don't think we need manufacturing or composition and impurities for any of them?

DR. LIEBLER: I think we have enough data to cover all of the ingredients.

DR. BELSITO: Okay.

DR. LIEBLER: Because potentially the extract sort of contains everything.

DR. BELSITO: Okay.

DR. LIEBLER: And then the rest of these are sort of subsets of that. So that was my rationale. I also think, practically, we're unlikely to get anything else because none of these others are in use, right?

DR. BELSITO: Right. Correct.

DR. LIEBLER: We can go through the exercise, but I think at the end of the day, if we had the other data that we needed in the report we could go forward based on that. So that was my take. Let's see what the other team thinks.

DR. BELSITO: I guess my only concern, Dan, is we have it for the extract. But is it possible that the juice and the leaf powder would have higher concentrations of ingredients than the whole extract?

DR. LIEBLER: Well, the juice wouldn't really. I doubt the juice would. It's literally just you squeeze the stuff. We've seen other juices described that way. The powder likely would have lower concentration.

DR. BELSITO: Okay. So we'll get rid of our request for manufacturing and composition and impurities. What about the 28-day dermal?

DR. LIEBLER: I'm fine with all the rest of it, Don.

DR. BELSITO: Okay.

DR. LIEBLER: I'm fine with insufficient on all the rest of those items.

DR. BELSITO: Do we need genotox if -- do you think it's okay for the extract?

DR. LIEBLER: If we get it for the extract, I think it's okay.

DR. BELSITO: I think even with striking that study we have adequate genotox, do we not?

DR. LIEBLER: Let's look again, here.

DR. BELSITO: We have in vitro.

DR. LIEBLER: So we have -- yeah.

DR. BELSITO: And then we have --

DR. LIEBLER: And we have (audio skip) without the irradiation.

DR. BELSITO: Right. So even striking that study I think we have sufficient genotox.

DR. LIEBLER: Yeah. I mean that basically -- logic here, Don, is just using the extract to clear everything else.

DR. BELSITO: Okay. So we need a 28-day dermal on the extract, and we need sensitization and irritation for the extract. And you feel that given the composition, if we get sensitization and irritation at concentration of use for the extract, that would clear the powder and the juice water, correct?

DR. LIEBLER: I think so. I do think so. We don't have uses and use concentrations for all these other things. So the assumption, as would be in our typical language, is that if these others were in use, they would be at similar concentrations and uses.

DR. BELSITO: Okay.

DR. SNYDER: Now, we do have some tox data on the powder extract where there's a NOAEL up to three percent, the highest dose tested.

DR. BELSITO: Right.

DR. SNYDER: So this is not -- it doesn't appear to be -- I'm not certain that the dermal is going to give us anything more than that. Because that was an oral study and there was no effect up to three percent. And this is only used at 0.4 percent on leave on.

DR. BELSITO: Okay. So you think that -- but how long was that study for, Paul? It was 90 days, no?

DR. SNYDER: Thirteen weeks, yes.

DR. BELSITO: Is that sufficient?

DR. SNYDER: Yeah.

DR. BELSITO: Okay. So then we don't need a 28-day dermal.

DR. SNYDER: But, Don, this is one in the discussion I think we do have to talk about, and this was raised by Bart. About the regional influence of where it's grown, with the particulars of the impurities of flavonoids being different depending upon whether it was grown in different regions I believe. But I just wanted to raise that as an issue, regarding the need for composition and impurities for things other than the extract. That's on page ten.

DR. KLAASSEN: Are there some specific flavonoids that you're concerned about, or what are you saying, Paul?

DR. SNYDER: Well, it's just that there's this impurities data here that the composition is drastically different in plants from Taiwan versus China. And they do list this one glucopyranoside is a major flavonoid, comprising 50 to 60 percent of the total flavonoid content. So I don't know. We don't have this --

DR. KLAASSEN: Yes. That's what they're saying.

DR. SNYDER: Yeah.

DR. KLAASSEN: Most of these -- well, the flavonoids are polyhydroxylated compounds that generally don't go across the skin very well. I guess I wouldn't be overly concerned.

DR. BELSITO: I didn't see any compounds that were particularly of concern to me with those differences. I think if we saw a constituent that had significant variation depending upon where the plant was grown, and we had a toxicologic concern about it, then that would be an issue. But if we don't have a toxicologic concern, is that something we'd put in the discussion here? That we noted that there was variation in composition, depending upon where the plant was grown, but that variation did not raise any toxicologic concerns for us?

DR. SNYDER: No. But it'd be more of a sensitization issue, wouldn't it? If we clear it on sensitization, then we'd be okay, right?

DR. BELSITO: But I don't see any sensitizers here that vary in terms of the differences in composition. I mean flavonoids are not skin sensitizers. So we're saying we don't need a 28-day dermal. So then at this point we just need sensitization and irritation for the extract? And that will clear the juice and the powder?

DR. SNYDER: Yes.

DR. BELSITO: And then in the discussion, obviously, we need the botanical and the respiratory boilerplate, correct?

DR. SNYDER: Correct.

DR. BELSITO: Anything else that we need here?

MR. JOHNSON: Excuse me, Dr. Belsito, are the skin irritation and sensitization data on the extract, is that supposed to be an HRIPT?

DR. BELSITO: You know, I mean it -- so I hate to keep bringing up RIFM. It could be an adequately controlled guinea pig maximization test. It could be a LLNA if it's out there, which I doubt because it's a natural complex substance. Or an HRIPT would be the best. But currently, this guinea pig maximization test, first of all, it's poorly described. But clearly, the number is not adequate because you want at least 20 animals in a treatment group.

MR. JOHNSON: So just state skin irritation and sensitization data, and not specify the type of study?

DR. BELSITO: Yeah. Ideally, they can read the minute notes. An HRIPT would be nice, but an adequately-controlled guinea pig maximization test would be too. But again, normally for complex natural mixtures, you'd do an HRIPT. You wouldn't do an in vitro, you wouldn't do a LLNA, you wouldn't do a guinea pig maximization test.

MR. JOHNSON: And Dr. Belsito, you said that there is no need for genotoxicity data?

DR. BELSITO: That's correct.

MR. JOHNSON: Okay. I'm noticing upon PDF page 13, that there is an in vivo genotoxicity study on Equisetum arvense, but the plant part is not specified.

DR. BELSITO: This is 13.

DR. SNYDER: It's under the extract, isn't it?

MR. JOHNSON: Yes. This is just before the Carcinogenicity Studies section, under In Vivo. The plant part is not specified.

DR. LIEBLER: Oh, I see. I think it's okay to leave it in there.

MR. JOHNSON: Okay.

DR. LIEBLER: I don't think it really influences our decision one way or another.

MR. JOHNSON: Okay. So there's no need for additional in vivo genotoxicity data?

DR. SNYDER: I don't think so.

DR. KLAASSEN: No.

DR. BELSITO: So that's in vitro. And then we have genotox on the extract, in a micronucleus test, that was negative. So I think I'm fine with it. I mean, Dan, Paul, Curt?

DR. LIEBLER: I agree.

DR. SNYDER: Yes.

DR. BELSITO: So basically, Wilbur, insufficient for sensitization and irritation for the extract at concentration of use. And in the discussion, really the botanical and respiratory boilerplate, and the fact that we have this 13-week oral that really clears all of the other systemic toxin points.

MR. JOHNSON: Dr. Belsito, eventually, did you want a statement relating to that 13-week oral study, clearing the other systemic endpoints, included in the discussion?

DR. BELSITO: Yes.

MR. JOHNSON: Okay. Thank you.

DR. BELSITO: Anything else on this? Okay.

Cohen Team – December 7, 2020

Equisetum Arvense-derived Ingredients

DR. COHEN: Also, group, what's the typical protocol for when we break for lunch, and how long does that go? I want to be cognizant of that as we're going through this.

DR. HELDRETH: Typically, we break around noon and take an hour break.

DR. COHEN: Okay. Okay. Next is Equisetum arvense or horsetail or field horsetail. This is Wilbur's. This is a draft report, and this is the first time we're reviewing this. This safety assessment has five derived ingredients: the extract, juice, leaf extract, leaf powder, and powder. Its max use was reported at 0.00078 in a rinse off and 0.4 in a leave on.

We have method of manufacturing for the extract, but I don't think we have it for the others. And we had a late-breaking memorandum requiring some corrections in terminology and verbiage, which seemed fine. Comments from the group? Ron, do you want to start?

DR. SHANK: Well, I think we can use the data we have on the plant extract --

DR. BERGFELD: So from the chemistry pers- --

DR. COHEN: Ron, go ahead.

DR. SHANK: -- to cover the needs for any other ingredients. We would need on the whole plant extract --

DR. BERGFELD: Ron, I didn't hear that beginning.

DR. SHANK: Okay. We could use the data that we already have in the report on the plant extract to cover the needs for the other ingredients. But we still need, on the whole plant extract, 28-day dermal and DART.

It's an interesting thing, there was a 14-day oral study where there was hair loss and dermatitis, and a 13-week oral study, which is longer, where there was no hair loss. This is in a Japanese journal, and I don't have access to it, so I couldn't read to see how to explain that.

If there is hair loss from oral administration, we definitely need to do the 28-day dermal study. This extract is an herbal medicine, and it's used at low concentration as a cosmetic. So we may not need the developmental and reproductive toxicity, but we'd have to see what the 28 dermal says.

DR. COHEN: Okay.

DR. SHANK: Could anybody explain the hair loss in the oral study of 14 days, but not at 13 weeks?

DR. COHEN: Well, Wilma is a hair expert, internationally recognized so I might ask her.

DR. PETERSON: I thought the 13 week one was a different mode of --

DR. BERGFELD: I couldn't ex- --

DR. SHANK: Well, I didn't have access to the paper, so I couldn't tell.

DR. COHEN: Wilma, you said you could not explain it?

DR. BERGFELD: Usually, it's toxicity and --

DR. COHEN: It would endure if it was toxicity or worsen?

DR. SHANK: Pardon me?

DR. COHEN: No, I was asking Wilma.

DR. PETERSON: Well, one was a powder, and one was an extract. One is a pow- --

DR. COHEN: Go ahead, Lisa.

DR. PETERSON: Well, one's a powder, and one's an extract, which I think goes to the perhaps you can't read across without knowing what the different constituents are and the different formulations. So I actually would argue that, in order to do read across, you would want constituents for all of them so that you could justify that.

But, I mean, there is a difference in the oral -- the single dose was -- the short term was done with the powder, and the long-term one was done with a powder extracted with hot water, so it was an extract.

MR. JOHNSON: Excuse me, Dr. Cohen?

DR. COHEN: Yes.

MR. JOHNSON: Yes, I'd like to also call the Panel's attention to PDF Page 16, in the Clinical Studies section, the third paragraph.

DR. COHEN: Let me get to that. Yes. Yeah, actually, I have them in my comments right here. You mean for discussion, in general, are you talking about the report of hand and face dermatitis?

MR. JOHNSON: Yeah, the issue of hair loss.

DR. COHEN: Ah. Yeah, there was a case of hair loss and nail fragility, and someone using it, I think, as a diuretic. So the hair loss thing has come up in different venues, so to speak.

MR. JOHNSON: Yes. Mm-hmm.

DR. COHEN: So I guess Lisa's point to have composition/impurities in all of them in order to read across for other items. Is that acceptable to everyone?

DR. SHANK: Okay.

DR. PETERSON: Yes.

DR. COHEN: Tom?

MR. JOHNSON: Dr. Cohen, what about the method of manufacturing? Are those data needed on the others as well?

DR. PETERSON: Yes. Yes. Yeah, so I would say insufficient, and the needs are method of manufacturing for the juice, the leaf extract, the leaf powder, and the powder. And then constituents for the juice, the leaf extract, the leaf powder, and the plant powder. And impurities for the extract, the juice, the leaf extract, the leaf powder, and the plant powder.

DR. COHEN: Okay. I think I got that down.

DR. PETERSON: I think that there's --

MR. JOHNSON: Dr. Peterson, when you have a chance, will you just briefly go over that again?

DR. PETERSON: Sure. So I said method of manufacturing deficient for the juice, the leaf extract, the leaf powder, and the plant powder.

MR. JOHNSON: Okay. Thank you.

DR. PETERSON: And then, it's basically, the empty spaces on that table at the beginning.

MR. JOHNSON: Okay.

DR. COHEN: Composition for the --

DR. PETERSON: Then constituents on the juice, leaf extract, leaf powder, and plant powder. And then impurities for the extract, for the juice, the leaf extract, the leaf powder, and the plant powder. And then impurities for the extract, the juice, the leaf extract, the leaf powder, and the plant powder.

And, certainly, it seems like, you know -- you definitely would like for the ingredients that are in use, which are the leaf extract and plant powder. And it does seem like there's something different going on when it's given oral versus when it's given dermal. It seems safer. I don't know why I thought the dermal was -- I must have misread.

But I think the two studies that give conflicting results are with two different preparations, and there's not a lot of information given. But the animal studies seem to be relevant to the human studies, due to the fact that there are at least one human showing up with hair loss when he check this.

DR. COHEN: Okay.

DR. PETERSON: And, Wilbur, I want to add that I was really happy to have the number of reported uses in that summary table at the beginning. I'm going to -- actually, it would be nice if everyone could do that. Because I end up using that table and then writing those numbers in, so it was really nice to have them written in already.

MR. JOHNSON: Yes. Thank you. Yes.

DR. PETERSON: So thank you.

MR. JOHNSON: Thank you. You're welcome.

DR. COHEN: Tom, any remarks, comments, needs? Tom?

MR. JOHNSON: I have one comment, Dr. Cohen. So what was (audio skip) section?

DR. SHANK: So we're going insufficient and --

DR. COHEN: Tom, you broke up, and we'll go back to your remarks in one second.

MR. JOHNSON: Yes, on PDF Page 12, in the short-term toxicity section.

DR. COHEN: Tom, we couldn't hear you.

MR. JOHNSON: The third line.

DR. COHEN: Short term -- what was the question?

MR. JOHNSON: Are we waiting for Dr. Shank's comment? Right, Dr. Cohen? Are you waiting on Dr. Shank's comment?

DR. COHEN: Yeah, I thought I -- Tom, we couldn't hear what you were saying. I couldn't hear what it was.

DR. SLAGA : What was the question?

MR. JOHNSON: Oh, you couldn't hear me? Okay. There you go.

DR. COHEN: No, I couldn't hear Tom if he had any other comments or remarks or needs to proceed.

DR. HELDRETH: I see Tom as currently muted.

DR. COHEN: Oh, Tom, we can't hear you.

DR. BERGFELD: Right.

DR. COHEN: Yeah, Tom is -- Tom, you're muted. Okay.

DR. SLAGA : Now, can you hear me?

DR. COHEN: Yes.

MR. JOHNSON: Yes. Mm-hmm.

DR. SLAGA: Yeah, no additional needs or comment. I go back to the original, we do need that 28-day dermal that Ron mentioned.

DR. COHEN: Got it.

DR. PETERSON: Yes.

DR. BERGFELD: Yep.

DR. COHEN: I'm sorry, Wilbur, I didn't mean to cut you off. What was your comment?

MR. JOHNSON: No, no. Yes, I'm just calling the Panel's attention to PDF 12.

DR. BERGFELD: And you're going to do that on what -- on which one?

DR. COHEN: I think he was talking about short term.

MR. JOHNSON: Oh. I thought she was asking for the 28-day dermal tox study, which ingredients should be included in that data request.

DR. SHANK: Whole plant extract.

MR. JOHNSON: Okay. Okay. Thank you.

DR. COHEN: Thank you. Thank you for clarifying that, Wilbur. I didn't have that down either.

DR. PETERSON: Yes. Yes.

MR. JOHNSON: Okay. Okay. And on PDF Page 12, the Short-Term Toxicity section, the third line -- I'm sorry the fourth line down. It says, "serum liver lipids", that should be serum "or" liver lipids.

DR. SHANK: Okay.

DR. COHEN: There was a no effect on serum. Now, I understand that the term "serum lipids" or the liver function." What's the liver lipids?

MR. JOHNSON: That is language that is used in that particular publication. I could check to see if they specifically mentioned any names --

DR. COHEN: Okay.

MR. JOHNSON: -- if you'd like. Okay.

DR. COHEN: Okay. Look, there are also some discussion of a non-atopic patient having rhinoconjunctivitis after inhaling the steam of a vegetable mixture, and she had a positive prick test to Equisetum and had a positive conjunctival challenge. So there is some potential for immediate type hypersensitivity.

And just a question for the group to help me, is this mention of heavy metals like lead, cadmium, and mercury, are these at levels we need to have any verbiage in the report on?

DR. SHANK: We have a boilerplate to take care of that.

DR. BERGFELD: That appears in the discussion, the boilerplate impurities.

DR. COHEN: Ron, do we need to comment on that at all -- okay. So do we need to have that in there? Okay. I'm sorry. Okay. Thank you.

Okay. So we're going to have an IDA here. We need 28-day dermal tox and DART for whole plant and extract, whole plant extract. And we need method of manufacturing for the juice, leaf extract, leaf powder, plant powder, composition for those and impurities for the leaf extract, leaf powder, plant powder and juice.

DR. SHANK: Okay.

DR. SLAGA : Good.

DR. COHEN: Did I miss -- did I leave anything out?

DR. SHANK: No.

DR. COHEN: Okay.

DR. PETERSON: Well, it looks like we -- I'm sorry.

DR. COHEN: We're at 11:57, and I'm pretty sure we can't do Polyquaternium-6 in six minutes -- in three minutes so. Go ahead, Lisa.

DR. PETERSON: Well, there is some impurity information for the extract because it's in the --

DR. COHEN: Was there anything else? Did I miss something?

DR. PETERSON: I mean, there is some impurity information on the extract --

DR. COHEN: Okay. So --

DR. PETERSON: -- on PDF Page 11, second paragraph or the first full paragraph. Where we talked about the lead, cadmium, mercury, and that's for the water and method.

DR. COHEN: Yeah.

DR. PETERSON: I think that's for an ex- -- I don't know if that's for the plant -- or actually, that must be for the plants. Never mind. That's for the plant. Sorry. So it's actually stated as --

DR. BERGFELD: Okay. Before you close for lunch, David, can you just go over what we've done here. It's all insufficient?

DR. COHEN: Okay. So is there a change in what we're going to ask for in our IDA?

DR. PETERSON: No. I'm sorry I drove you to that con- --

DR. COHEN: I'm sorry.

DR. PETERSON: No. No, you had it right when you said it the first -- before I interup- -- before I got confused, so you had it right when you first --

DR. COHEN: I think it's all insufficient because we --

DR. BERGFELD: Yeah.

DR. COHEN: Well, that's a good question. Is it all insufficient?

DR. PETERSON: It's insufficient for the things you said it was insufficient for, which was the various methods of manufacturing, constit- --

DR. COHEN: (Inaudible).

DR. PETERSON: -- and you wanted dermal -- 28 dermal with the extract.

DR. COHEN: Yeah. Well, is that list okay to proceed for tomorrow's group meeting? I mean, do we need to have any other comments about non-insufficiencies?

DR. BERGFELD: No.

DR. COHEN: Okay. So all right. So we got through two-thirds of the list before lunch. We have about five more when we get back. Wilma, Bart, is there anything that we haven't done, that we need to do, before we break?

DR. BERGFELD: No.

DR. HELDRETH: No, I don't believe so.

DR. COHEN: Okay. So do we return at 1:00 p.m. Eastern time?

DR. BERGFELD: Yes.

DR. HELDRETH: That works.

DR. BERGFELD: Do we leave our computers on or do we re-enter, Bart?

DR. HELDRETH: It's up to you. Either way will work.

DR. BERGFELD: Okay.

DR. HELDRETH: Whatever's easier for you.

DR. COHEN: I'm just going to put my mic and camera off.

Full Panel – December 8, 2020

Equisetum Arvense-derived Ingredients

DR. COHEN: So, this is a draft report; it's the first time we're review this. This is the safety assessment for five derived ingredients for Equisetum arvense, horsetail or field horsetail, with a max use of 0.00078 in the rinse-off, and 0.4 in a leave-on.

We had incomplete data needs, method of manufacturing for the juice, leaf extract, leaf powder and plant powder, and composition and impurities of the juice, leaf extract, leaf powder and plant powder. And dermal tox on the whole plant extract.

DR. BERGFELD: Is there a second or a comment? An IDA is being requested for this ingredient, with the needs so stated by Dr. Cohen.

DR. SHANK: I'll second.

DR. BERGFELD: Okay. Further discussion or comment? Dr. Belsito team?

DR. BELSITO: Yeah, we had a somewhat different conclusion. We thought the 13-week oral cleared the other systemic endpoints for this. And we thought that there was a data need for sensitization and irritation for the extract, and that would clear the juice and the powder extract.

DR. COHEN: You broke up -- can you repeat that last part, Don?

DR. BELSITO: We thought we needed sensitization and irritation for the extract and that that would clear the juice and powder extract.

DR. COHEN: I'll ask the team; I think that's reasonable.

DR. BERGFELD: You want to ask your team now? Ron, Tom, Lisa?

DR. SHANK: Can anyone explain -- there was a 14-day oral study that showed hair loss and dermatitis, and a 13-week old study that did not. Does anybody have an explanation for that?

DR. BERGFELD: No.

DR. BELSITO: Paul?

DR. SHANK: The paper is a Japanese Journal, and I don't have access to that journal so I couldn't read the papers.

DR. SNYDER: Yeah, I didn't put much weight into that paper coming out of Japan, in lieu of the longer 13-week study, Ron, so.

DR. SHANK: So we just ignore it.

DR. BERGFELD: Curt.

DR. SHANK: For a cosmetic that would be a pretty important effect.

DR. SNYDER: Well, we should probably look at the study, but they also used some terminology that's -- like liver lipids and stuff. And so we asked for clarification of that. Was that liver enzyme activities, or is that liver lipids? And, get some more details regarding that particular study.

DR. SHANK: Liver lipids, what's wrong with that?

DR. BELSITO: Have you ever heard of serum liver lipids?

DR. SHANK: It's just the fat extraction of liver tissue.

MR. JOHNSON: Yes, I have a -- excuse me, I have --

DR. SHANK: Basically--

DR. BELSITO: But, Ron, it said serum liver lipids. It didn't say extracting the liver and looking at the bits in the liver. It said serum liver lipids.

DR. SHANK: But, well --

MR. JOHNSON: I have a comment Dr. --

DR. SHANK: I read that it's probably serum and liver lipids.

DR. COHEN: It was serum and.

DR. SHANK: I agree, serum liver lipids doesn't make any sense.

MR. JOHNSON: Yes.

DR. SHANK: So I just thought that was a typo.

MR. JOHNSON: Yes, may I make a comment?

DR. BERGFELD: Yes, Wilbur.

MR. JOHNSON: Yes, according to the publication, the hepatic and fecal lipids were extracted and purified, and those include hepatic triglycerides, phospholipid and cholesterol levels to determine (inaudible). And you had serum triglyceride, phospholipid, and cholesterol levels.

DR. SHANK: Right.

DR. BERGFELD: Curt, do you want to respond?

DR. KLAASSEN: Yes, in regard to the liver lipids, that's, you know, rather traditional to do. So I don't really have any problem with that. I think the point that Ron brought up, about the hair loss in the first study but not in the longer study, is difficult to understand how that could be.

And if someone could send us that first paper, the Japanese paper, to look at that methodology a little closer to see what they did. But, I have no explanation for that.

DR. SHANK: Okay.

DR. KLAASSEN: I didn't worry about it as much after I saw in the longer study it didn't happen, but that's not exactly scientifically satisfying.

DR. BERGFELD: Tom, do you have a comment?

DR. SLAGA: Yeah, Tom, generally, you know, the short term study like that showing effect, and the longer study not showing effect, it's very confusing. Because generally any kind of toxicological effect should show some kind of dose response, and even if it's a time effect with repeating dosage. So, the only to satisfy this for sure is to see the paper and see what's going on.

DR. BELSITO: Well, I mean, so what they were seeing in those animals was dermatitis and hair loss. It's entirely possible that they had a fungal infection that was causing that. You know, particularly in light of the fact that the longer term studies didn't show any of that.

DR. BERGFELD: I can also say, as a hair expert supposedly, that if you have a rash or dermatitis on the scalp, you will have hair loss irrespective of cause.

DR. SLAGA: It's more likely it's something like that, but it still would be nice to see the paper.

DR. BERGFELD: Absolutely. I think that's a given; everyone has stated that. So we'll ask Bart to proceed with that -- or Wilbur.

DR. BELSITO: And we will need Japanese translation.

DR. BERGFELD: Yeah, well, I think they have the means to do that at the CIR.

DR. BELSITO: Bart, do you?

DR. BERGFELD: They can hire it.

DR. HELDRETH: I can look into it. It's typically rather expensive to get a Japanese's one translated. But let me find out and see what it would be.

DR. SNYDER: Just send me the paper; I have a pathology colleague -- couple from Japan, I can ask them to translate.

DR. HELDRETH: Will do.

DR. BERGFELD: Okay. All right. So we've come to a point having discussed this particular ingredient as an IDA, and everyone agrees to that. The needs, though, need to be re-expressed, or discussed.

MR. JOHNSON: Dr. Bergfeld?

DR. BERGFELD: Wilbur?

MR. JOHNSON: Yes, I'd like to call the Panel's attention to PDF Page 16, under clinical studies, and the third paragraph.

DR. KLAASSEN: What page?

MR. JOHNSON: PDF Page 16.

DR. BERGFELD: 16, not 60, 16.

MR. JOHNSON: 16, yeah.

DR. COHEN: Clinical studies. The case reports, Wilbur?

MR. JOHNSON: Yes, the paragraph beginning with hair loss.

DR. COHEN: Yes that was in a person taking it as a supplement -- food supplement, right?

DR. BELSITO: Yeah.

MR. JOHNSON: Yes.

DR. BERGFELD: Okay. Anything else Wilbur?

MR. JOHNSON: That's it, Dr. Bergfeld.

DR. BERGFELD: Going back to Dr. Cohen, where do we stand with this IDA?

DR. COHEN: So, Don, did you have an issue with the method of manufacturing ask and the composition and impurities ask? When I agreed with you I added the dermal sensi-- and irritation for the whole plant extract. And, I had dermal tox and DART for the whole plant extract. So, after this back-and-forth are we still landing there?

DR. BELSITO: Well, I mean, we thought sensitization and irritation to the extract should clear the juice and powder.

DR. COHEN: I think that's right.

DR. BELSITO: And we thought the 13-week oral would clear the other systemic endpoints, but obviously we're having some issues based upon the Japanese study.

So, I would say, you know, it's insufficient for sensitization and irritation, and that -- I don't know, how do we proceed? Since we don't have the Japanese study, do we table this awaiting data for the translation for Japanese study? Where do we go with this?

DR. BERGFELD: Let's ask Bart. Bart?

DR. HELDRETH: You could conclude that the data are insufficient, or since this is a first pass, draft report, you could have an IDA for clarification on this information.

DR. BELSITO: Okay, so, you know, I think insufficient data announcement for sensitization and irritation for the extract, and for clarification of the Japanese study with hair loss. I think would be reasonable.

DR. COHEN: And, Lisa, what about method of manufacturing for the juice, leaf extract, leaf powder, plant powder, and composition and impurities?

DR. PETERSON: Why not ask for it since you're asking for other things too? I think it'd be useful.

DR. BELSITO: I'm fine with that.

DR. COHEN: Okay.

DR. BERGFELD: Okay. So, Wilbur, do you have the whole list, Wilbur? Can you read it back?

MR. JOHNSON: I just want to make sure that we need method of manufacture, composition and impurities data on the juice, leaf extract, leaf powder, and the powder.

DR. COHEN: Yes.

DR. BERGFELD: Okay. And then the human studies?

DR. COHEN: Dermal sensitization and irritation.

MR. JOHNSON: You need the skin sensitization, irritation study on the Equisetum arvense extract.

DR. BELSITO: At maximum concentration of use.

MR. JOHNSON: At maximum concentration of use.

DR. BERGFELD: Anything else in that list?

DR. BELSITO: Clarification of the Japanese study.

MR. JOHNSON: Yeah.

DR. BERGFELD: Okay.

DR. COHEN: Are we leaving a dermal tox and DART on the whole plant extract now?

DR. BELSITO: Well I think depending upon -- I mean, oftentimes, David, we raise it depending upon the data from the Japanese study, additional endpoints if we need them.

DR. BERGFELD: Did you hear that David?

DR. COHEN: Yeah. Okay, because we have another round to go on this where we can make that decision later. Okay.

DR. BERGFELD: Right.

DR. COHEN: That's acceptable.

DR. BERGFELD: So we have an IDA and we have now a list of needs that I think everyone has agreed upon. I'm going to ask for any other comments at this time, before I proceed with the vote.

DR. COHEN: Wilma, I'm sorry to interrupt. Ron, are you okay with not having the dermal tox and DART in here now? Would you want to keep it in? You're on mute, I think. You're on mute.

DR. LIEBLER: Ron Shank, you're on mute.

DR. COHEN: You're on mute.

DR. BERGFELD: Tom Slaga -- Tom, can you respond?

DR. SLAGA: I don't think we need it.

DR. BERGFELD: Okay. Curt, can you respond?

DR. SHANK: Okay, sorry.

DR. BERGFELD: Okay, Ron you can go. You want to respond to that DART?

DR. SHANK: Okay, I missed the conversation as to why you don't want it.

DR. BERGFELD: Okay. Dr. Cohen?

DR. SHANK: Sorry, my battery went dead, so I had to regroup here. You don't want DART, or dermal?

DR. COHEN: We were going to wait for clarification on the Japanese study before asking for it.

DR. SHANK: Okay, I agree with that, yes.

DR. COHEN: Okay.

DR. BERGFELD: Okay. All right, so I'm going to call the question. This is going out as an IDA with a list of needs. It's been motioned and seconded, and anyone opposing please indicate with your name. Seeing none then this is approved unanimously.

Safety Assessment of *Equisetum arvense*-Derived Ingredients as Used in Cosmetics

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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.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst/Writer, CIR.

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DRAFT ABSTRACT: The Expert Panel for Cosmetic Ingredient Safety (Panel) reviewed the safety of 5 *Equisetum arvense*-derived ingredients in cosmetic products; all of these ingredients are reported to function as skin-conditioning agents in cosmetics. Because final product formulations may contain multiple botanicals, each containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. Industry should use current good manufacturing practices to minimize impurities that could be present in botanical ingredients. The Panel reviewed data relevant to the safety of these ingredients in cosmetic formulations, and concluded [TBD]

INTRODUCTION

The safety of the following 5 *Equisetum arvense*-derived ingredients as used in cosmetics is reviewed in this assessment.

Equisetum Arvense Extract
Equisetum Arvense Juice
Equisetum Arvense Leaf Extract
Equisetum Arvense Leaf Powder
Equisetum Arvense Powder

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), all of the *Equisetum arvense*-derived ingredients are reported to function as skin conditioning agents in cosmetic products (Table 1).[1] Common names for the herb *Equisetum arvense* include horsetail and field horsetail.[2]

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Expert Panel for Cosmetic Ingredient Safety (Panel) typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data may be provided by the cosmetics industry, as well as by other interested parties.

Botanicals, such as *Equisetum arvense* -derived ingredients, may contain hundreds of constituents. However, in this assessment, the Panel is evaluating the potential toxicity of each botanical ingredient as a whole, complex substance; potential toxicity from exposures to mixtures of different chemical compounds may not replicate the biological activity of the individual components.

Also with botanicals, 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 is used (i.e., the names of cosmetic ingredients are capitalized, without italics (e.g., Equisetum Arvense Extract)). If it is not known that the test substance is the same as the cosmetic ingredient, the taxonomic naming conventions (i.e., with genus and species name italicized (e.g., an *Equisetum arvense extract*)) is used.

CHEMISTRY

Definition and Plant Identification

The ingredients in this report are related as derivatives from the same species, *Equisetum arvense.* The definitions of these *Equisetum arvense*-derived ingredients are presented in Table 1; the generic CAS number for 3 of these ingredients is 71011-23-9.[1]

Equisetum arvense (horsetail) has been described as a non-flowering weed (a perennial with hollow stems and shoots) that is found throughout parts of Europe, Asia, the Middle East, and North America.[3] *Equisetum arvense* is distributed throughout temperate and arctic areas of the northern hemisphere, growing typically in moist soils.[4] It has also been described as an herbaceous perennial relative of ferns consisting of 2 types of stems, namely, sterile non-reproductive and photosynthetic, and reproductive and non- photosynthetic. The latter, which is 10 to 25 cm long with brown scale leaves and a 10 to 40 mm long spore cone, emerges in spring and then withers, giving rise to the sterile, photosynthetic stems. These stems persist from summer until the first frost. According to another source, *Equisetum arvense* has aerial stems, branched with regular verticilies (2 - 23 mm in diameter) and terminal strobile in the branches and in the main stem (10 mm long and 4 mm in diameter).[5]

Physical and Chemicals Properties

Equisetum Arvense Extract

Equisetum arvense is available as a dried extract in powdered form or as a liquid extract.[5] As the plant dries, silica crystals form in the stems and branches.[3]

Method of Manufacture

Equisetum Arvense Extract

Equisetum Arvense Extract is an extraction directly into the solvent mixture (water/glycol) by maceration.[6] The method of production for an Equisetum Arvense Extract in ethanol and water (0.7% solids w/v) tradename material is described as follows: dried raw material ⇒ extract with ethanol ⇒ filtrate ⇒ concentration ⇒ adjustment ⇒ sedimentation ⇒ filtrate ⇒ adjustment ⇒ packaging.[7]

An Equisetum Arvense Extract in butylene glycol (0.54% solids w/v) tradename material is produced according to the following process: dried raw material ⇒ extract with 50 vol% 1,3-butylene glycolic solution ⇒ filtrate ⇒ sedimentation ⇒ filtrate ⇒ packaging.[7] The method of production of an Equisetum Arvense Extract in ethanol and water tradename material is described as follows: dried raw material ⇒ extract with 30 vol% ethanolic solution ⇒ filtrate ⇒ sedimentation ⇒ filtrate ⇒ packaging.

A method of manufacture relating to the preparation of three different extracts of *Equisetum arvense* (sterile stems) is also available in the published literature.[8] Air-dried and powdered plant material (100 g) was macerated with petroleum ether overnight, and afterwards with 70% methanol (24 h). After filtration, the methanolic extract was concentrated to dryness. The dry residue was dissolved in hot water and then separated by liquid-liquid extraction into the chloroform, ethyl acetate, and *n*-butanol extracts.

Composition/Impurities

The following composition data could be characterized as general information relating to *Equisetum arvense* or *Equisetum arvense* extracts, and it is unknown if it applies to the cosmetic ingredients that are being reviewed in this safety assessment.

Data on flavonoid composition reveal the existence of 2 chemotypes of *Equisetum arvense*, one in Asia and North America, and the other in Europe.[8] *Equisetum arvense* from Asia and North America contains luteolin-5-*O*-glucoside (quantitative information absent) and its malonyl ester (quantitative information absent), but these compounds are not found in *Equisetum arvense* from Europe. The dominant compounds in *Equisetum arvense* from Europe are quercetin 3-*O*-glucoside, apigenin 5-*O*-glucoside, and dicaffeoyl-*meso*-tartaric acid (quantitative information absent). Di-*E*-caffeoyl-*meso*-tartaric acid (quantitative information absent) is a marker for both chemotypes. According to another source, quercetin 3-*O*-(6"-*O*-malonyl-β-D-glucopyranoside) has been found to be the major flavonoid in European plants (*Equisetum arvense*), comprising between 28% and 50% of the total flavonoid content.[9] In plants from Taiwan and China, luteolin 5-*O*-β-D-glucopyranoside has been reported as the major flavonoid, comprising 50% to 60% of the total flavonoid content.

Additional data on the composition of *Equisetum arvense* indicate that it contains more than 10% inorganic substances (two-thirds of which are silicic acid and potassium salts). Specifically, the aerial parts of *Equisetum arvense* contain flavonoids, saponins, caffeic acid and other phenolic compounds, alkaloids, sterols (β-sitosterol, campesterol, and isofucosterol), and minerals (primarily silicon and potassium salts).[2,8] According to other sources, acids that have been isolated from *Equisetum arvense* include aconitic acid (tricarboxylic acid), ascorbic acid (ketolactone), malic acid (dicarboxylic acid), oxalic acid (dicarboxylic acid), and the following phenolic acids: caffeic acid, cinnamic acids, *p*-coumaric acid, gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, and vanillic acid.[8,10] Other components include polyenic acids, rare dicarboxylic acids (equisetolic acid), flavonoids, and styrylpyrones.[8]

The concentration ranges for some essential elements in *Equisetum arvense* have been determined to be: iron (193.4 - 1757.9 µg/g), manganese (23.6 - 143.7 µg/g), zinc (15.4 - 32.7 µg/g), selenium (0.13 - 0.92 µg/g), and copper (11.3 - 21.8 µg/g).[11] Among the components (oligo-β-glucans) of the *Equisetum arvense* cell wall are the tetrasaccharide, β-glucosyl-(1→4)-β-glucosyl-(1→4)-β-glucosyl(1→3)-glucose and the trisaccharide, mixed-linkage (1→3, 1→4)-β-D-glucan.[12] The enzyme thiaminase (which breaks down vitamin B_1) also occurs in *Equisetum arvense*.[13]

Equisetum Arvense Extract

According to a cosmetics industry source, a tradename mixture of Equisetum Arvense Extract has the following composition: Equisetum Arvense Extract (~ 2% dry extract), propylene glycol (~ 66%), phenoxyethanol (~ 0.36%), methylparaben (~ 0.08%), ethylparaben (~ 0.02%), propylparaben (~ 0.04%), and water (qsp 100%).[14]

An Equisetum Arvense Extract in ethanol and water (approximately 0.7% solids w/v) tradename mixture contains tannin and saponin.[7] Tannin and flavonoid are present in an Equisetum Arvense Extract in butylene glycol (approximately 0.54% solids w/v) tradename mixture and in an Equisetum Arvense Extract in ethanol and water tradename mixture.

Whether or not flavonoids or phenolic acids are the predominant compounds in *Equisetum arvense* extracts is dependent upon the extractant that is used. In one publication, flavonoids were the main compounds in ethyl acetate and butanol *Equisetum arvense* extracts, and phenolic acids were the major constituents in the aqueous *Equisetum arvense* extracts.[8] These data are summarized in Table 2. According to another source, the following water-soluble acids have been detected in an aqueous extract of *Equisetum arvense* (whole, air-dried plant): aconitic acid, arabinonic acid, citric acid,

ferulic acid, fumaric acid, gluconic acid, glyceric acid, malic acid, malonic acid, phosphoric acid, quinic acid, and threonic acid.[10]

Composition data on a methanol extract of *Equisetum arvense* (aerial parts) indicate the presence of 2 phenolic petrosins, namely onitin and onitin-9-*O*-glucoside, and the following 4 flavonoids: apigenin, luteolin, kaempferol-3-*O*-glucoside, and quercetin-3-*O*-glucoside.[15] The following % composition values for flavonoid and caffeic acid derivatives of the hydro-alcoholic (20:80, v/v) extract of *Equisetum arvense* stems have been calculated (computed from the high performance liquid chromatography peak areas): quercetin (21.1%), quercetin 3-*O*-glucoside (49.6%), quercetin 3-*O*-(6"-*O*-malonylglucoside) (8.8%), 5-*O*-caffeoyl shikimic acid (4.4%), monocaffeoyl *meso*-tartaric acid (3%), and dicaffeoyl *meso*-tartaric acid (1.6%).[16]

Composition data on *Equisetum arvense* (water and methanol extract) grown in Asia versus *Equisetum arvense* grown in Europe are presented in Table 3.[17] In addition to these data, the researchers presented an accumulation profile (graph) of quercetin glucosides (absolute content, % dry weight) in *Equisetum arvense* during 2 growing seasons which indicated that development of the total amount (% dry weight) of the main flavonoids quercetin 3-*O*-glucoside and quercetin 3-*O*-(6"-*O*-malonylglucoside) was different over several years of observation. An accumulation profile (graph) of quercetin glucosides (proportional content, % total flavonoids) in *Equisetum arvense* during 2 growing seasons indicated that few differences were found in the proportional content (% of flavonoid content) of the 2 main flavonoids in several years of observation. Also, it was found that there was a decrease in quercetin 3-*O*-(6"-*O*-malonylglucoside) and a simultaneous increase in quercetin 3-*O*-glucoside toward the end of the growing period.

One source indicates the following mean values for 3 toxic metals in *Equisetum arvense*: lead (14.07 mg/kg), cadmium (0.139 mg/kg), and mercury (0.014 mg/kg).[18] In this analysis, *Equisetum arvense* herb (above ground plant parts, dried raw material) was studied.

Nicotine has been detected in British species of *Equisetum arvense*.[19] The amount of nicotine obtained from 5 g of dried plant material (British *Equisetum arvense*) has been estimated, using ultraviolet (UV) spectrophotometry, to be not more than 2 mg.

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. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database.[20] Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.[21]

According to 2021 FDA VCRP data, Equisetum Arvense Extract is reported to be used in 186 cosmetic products (125 leave-on products, 59 rinse-off products, and 2 products that are diluted for (bath) use; Table 4).[20] Of the *Equisetum arvense*-derived ingredients that are being reviewed in this safety assessment, this is the greatest reported use frequency. The results of a concentration of use survey completed in 2018 and provided by the Council in 2019 indicate that Equisetum Arvense Extract is being used at maximum use concentrations up to 0.4% in leave-on products (body and hand products [not spray]), and at maximum use concentrations up to 0.00078% in rinse-off products (skin cleansing products).[21] Equisetum Arvense Extract is the only *Equisetum arvense*-derived ingredient in this safety assessment for which use concentration data were provided in response to the Council survey. Additionally, according to both VCRP and Council survey data, Equisetum Arvense Juice and Equisetum Arvense Leaf Powder are not reported to be used in cosmetic products.

It should be noted that frequency of use data on *Equisetum arvense* (horsetail), from 2021 FDA VCRP, are also included in Table 4. Because neither the plant part(s) associated with the name *Equisetum arvense* nor whether the name corresponds to a plant part extract is stated, it is not possible to specifically associate the frequency of use data on *Equisetum arvense* with any of the 5 *Equisetum arvense*-derived ingredients that are reviewed in this safety assessment.

Cosmetic products containing *Equisetum arvense*-derived ingredients may be applied to the skin/hair, or incidentally, may come in contact with the eyes (e.g., Equisetum Arvense Extract and Equisetum Arvense Leaf Extract). Equisetum Arvense Extract is used in products that come in contact with mucous membranes during product use (e.g., mouth washes and breath fresheners (concentrations up to 0.0002%); thus, Equisetum Arvense Extract may be incidentally ingested. Products containing *Equisetum arvense*-derived ingredients may be applied as frequently as several times per day and may come in contact with the skin for variable periods following application. Daily or occasional use may extend over many years.

Equisetum Arvense Extract is reported to be used in cologne and toilet waters, and in other fragrance preparations (concentrations unknown).[20] Equisetum Arvense Leaf Extract is reported to be used in hair spray (aerosol fixatives) (concentrations unknown). In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have

aerodynamic equivalent diameters > 10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm, compared with pump sprays.[22-25] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[22,23] Equisetum Arvense Extract is reported to be used in face powders (concentrations unknown).[20] 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.[26-28]

The *Equisetum arvense*-derived ingredients are not restricted from use in any way under the rules governing cosmetic products in the European Union.[29]

Non-Cosmetic

Equisetum arvense (horsetail) is an herbal remedy that dates back to ancient Rome and Greece.[3] Traditionally, it was used to stop bleeding, heal ulcers and wounds, and for the treatment of tuberculosis and kidney problems. The aboveground parts of this plant are used for medicinal purposes. Because *Equisetum arvense* contains silicon, which strengthens bone, some practitioners recommend it as a treatment for osteoporosis (not an FDA-approved use). It is also used as a diuretic, and the diuretic effects of *Equisetum arvense* may enhance the toxic effects of certain medications, such as digoxin (used to treat congestive heart failure), phenytoin (for seizures), anticoagulants, and others.[30] Thus, individuals taking prescription medications should not take *Equisetum arvense* without first consulting a health care provider.

In Japan, *Equisetum arvense* (field horsetail) sporophyte (*tsukushi*) is consumed as food in sweetened vinegar, cooked food, and chopped fish.[16] Furthermore, in Asian traditional medicine, the aerial parts of *Equisetum arvense* have been used to treat hemorrhage, urethritis, jaundice, and hepatitis.[31,32] Sterile stems of *Equisetum arvense* are used in herbal medicine in various countries, constituting the "Equiseti herba" of European Pharmacopeias.[8] According to another source, *Equisetum arvense* is used mainly for its diuretic properties, and also has the following uses: analgesic, hemostatic, astringent, and treatment for digestive disorders and kidney/bladder stones.[33]

According to the US FDA, *Equisetum arvense* is among the ingredients that have been present in over-the-counter (OTC) drug products for use as a digestive aid (21 CFR 310.545). However, based on evidence currently available, there are inadequate data to establish general recognition of safety and effectiveness of this ingredient for this specified use.

TOXICOKINETIC STUDIES

No relevant toxicokinetic studies on *Equisetum arvense*-derived ingredients were found in the published literature. In general, toxicokinetics 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

Oral

Equisetum Arvense

A single-dose, oral toxicity study on *Equisetum arvense* was performed using groups of male and female rats (strain and number per group not stated).[34] Doses of 800 mg/kg, 2000 mg/kg, and 5000 mg/kg were administered orally (method not stated). No deaths or abnormal changes in body weight occurred, and no toxicity signs were observed at necropsy. The LD_{50} was > 5000 mg/kg.

Equisetum Arvense Extract

In an acute oral toxicity study involving 10 mice (strain not stated), an LD_0 of ≥ 20 ml/kg was reported for Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract).[6] Data on the composition of this extract are included in the section on Composition/Impurities. Details relating to the test protocol are not included.

Intraperitoneal

The acute toxicity of an *Equisetum arvense* extract (hydroalcoholic extract) was evaluated using groups of 8 male Wistar rats.[35] The groups received intraperitoneal (i.p.) doses of 1000 mg/kg, 2000 mg/kg, and 5000 mg/kg. Control animals were dosed with saline. The number of survivors was recorded on the following day. Mortalities were observed in the 2000 mg/kg group (12.5% of the animals) and in the 5000 mg/kg group (37.5% of the animals). In all 3 dose groups, transitory respiratory depression and elevated sedation were observed. Both signs persisted to the end of the 240 min observation period, and were dose-dependent.

Short-Term Toxicity Studies

Oral

Equisetum Arvense Powder

In a short-term study, male Sprague-Dawley rats (groups of 6) were fed an *Equisetum arvense* powder (0.4% or 4%) in a 20% casein diet, with and without cholesterol (0.5% cholesterol and 0.15% sodium cholate), for 14 d.[36] At a concentration of 0.4% or 4% in either diet, the test material did not influence food intake or growth. There also were no apparent effects on serum or liver lipids after feeding with either concentration in both diets. However, on days 9 to 12 of feeding with 4% of a *Equisetum arvense* powder in the cholesterol diet, 4 of 6 rats lost their hair, and dermatitis was observed on the neck, head, nose, and back. At microscopic examination, dense infiltration of neutrophils and lymphocytes was observed in the dermis and subcutaneous tissue. At the center of the eruption, the dermis was ulcerated. The number of mast cells was also increased. These changes at microscopic examination were diagnosed as nonspecific inflammatory lesion of the skin. Reversal of the dermatitis was noted when the diet was changed to commercial pellets. Serum immunoglobulin E (IgE) levels, measured by enzyme-linked immunoassay, indicated that the induction of IgE may not necessarily be involved in the dermatitis caused by *Equisetum arvense* intake. In 2 additional experiments (21 rats total), rats were fed a *Equisetum arvense* powder (concentration not stated) in a cholesterol diet (composition not stated) for 4 wk and 6 wk. Six of 21 rats from the 2 experiments had dermatitis on the neck and back. The incidence of dermatitis after feeding for 4 wk and 6 wk was approximately 20% and 30%, respectively.

Intraperitoneal

Equisetum Arvense Extract

An *Equisetum arvense* extract (dried stem, ethanol and water extract) was administered to 10 male Wistar rats, at a daily i.p. dose of 50 mg/kg for 8 wk.[37] Signs of toxicity were not observed during the treatment period.

Subchronic Toxicity Studies

Oral

The subchronic oral toxicity of *Equisetum arvense* (powder extracted with hot water; plant part not stated) was evaluated in a study involving groups of 10 male and 10 female F344 rats.[5] The groups were fed *Equisetum arvense* at a concentration of 0.3%, 1%, or 3% in the diet (powdered basal diet) for 13 wk. Animals of the control group received diet only. Test and control animals were killed at the end of the study, and histopathological examinations of internal organs were performed. If lesions were frequently found in the 3% dietary group, then histopathological examination was extended to all tissues of the 0.3% and 1% dietary groups. None of the animals died and no obvious clinical signs were observed in any of the animals during the study. Body weights and cumulative body weight gains in all dietary groups were similar to control values. Additionally, there were no differences in food consumption between the groups. Urinalyses revealed no significant differences in any of the parameters evaluated among the groups. However, the protein levels in males of the 1% and 3% dietary groups were decreased. Additional results are summarized below.

Statistically significant alterations in hematological parameters (e.g., mean corpuscular hemoglobin and platelet count) were observed, but no dose-dependence was apparent. However, a trend toward a dose-dependent decrease in the white blood cell count was noted in females. There were no statistically significant differences in organ weights. However, a tendency toward a decrease in absolute adrenal weights was observed in males. No treatment-related macroscopic changes were observed at necropsy. However, the following histopathological changes (minimal grade changes) were observed in treated animals: spontaneous inflammatory/proliferative lesions in the liver (3% dietary group, 2 males), spontaneous inflammatory and proliferative lesions in the pancreas (1 female, 3% dietary group), liver microgranulomas (3% dietary group, 1 male and 2 females), kidney atrophy (3 males [0.3% group], 2 males [1% group], and 1 male [3% group]; 1 female [3% group]), and ovarian cysts (3% dietary group, 1 female). Eosinophilic bodies and alpha 2u-globulin expression in the proximal tubules of the kidney were observed in all male rats, including the control group. No treatment-related findings were observed in other tissues and organs of male or female rats. The no-observed-adverse-effect level (NOAEL) for *Equisetum arvense* was determined to be more than 3% in male and female rats (> 1.79 g/kg bw/d males; > 1.85 g/kg bw/d females).

Chronic Toxicity Studies

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

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In Vitro

Equisetum arvense

A study was performed to determine the effect of *Equisetum arvense* and thymol during early development of the zebrafish.[38] Embryos resulting from the natural spawning of adult wild-type zebrafish (*Danio rerio*, AB strain) were used.

The source of *Equisetum arvense* (natural extract) was a commercial formulation containing horsetail extract (*Equisetum arvense*) decoction (95.2% decoction of horsetail (*Equisetum arvense* 7%). Stock solutions of *Equisetum arvense* (500 mg/l, 6250 mg/l, and 80,000 mg/l) were prepared. Exposure solutions were freshly prepared in embryo water (200 mg/l instant ocean salt and 100 mg/l sodium bicarbonate, UV sterilized) prepared from filtered tap water. The lethal concentration that causes 50% mortality (LC_{50}) was determined using a modification of Organization for Economic Co-operation and Development (OECD) Test Guideline 236. The 96-h LC_{50} and 95% confidence limits for *Equisetum arvense* were 1.98 mg/l (0.50 - 4.13). Based on this LC_{50} value, 3 sublethal concentrations (0.00625 mg/l, 0.0625 mg/l, and 0.625 mg/l) were selected for the assay. The incubation period for tested embryo cultures was 96 h. Embryo cultures incubated in embryo water served as the blank control. The experiment was repeated independently 5 times. Lethal parameters such as failure of somites, eye and otolith development, missing heartbeat, and non-detached tail and head were recorded at 24, 48, 72, and 98 h post fertilization (hpf). The spontaneous movements at 24 hpf, pigmentation formation and heart rate at 48 hpf, and hatching rate at 72 hpf were evaluated as sublethal endpoints. Morphological abnormalities (body length, area of egg yolk, area of heart and eye, and head to body angle) were screened at 98 hpf in 10 randomly 3% methylcellulose-immobilized eleutheroembryo. The authors noted that the results of this study demonstrated no teratogenic potential of *Equisetum arvense* at sublethal concentrations during the early development of zebrafish. At 98 hpf, embryo development in control cultures was, as expected, around 80% with normal development. Thymol was tested at concentrations of 0.008 mg/l, 0.08 mg/l, and 0.8 mg/l in this assay, based on 96-h LC_{50} and 95% confidence limits of 2.35 mg/l (0.78 - 5.55). At 98 hpf, malformations were observed at the highest concentration of thymol (0.8 mg/l), namely, pericardial edema, yolk and eye deformations, and decreased body length. Increased lethality was also noted.

GENOTOXICITY STUDIES

In Vitro

The genotoxicity potential of *Equisetum arvense* (plant part, method of preparation, and doses not stated) was evaluated in a reverse mutation test using the following bacterial strains: *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2uvrA.[34] Details relating to the test protocol were not included. The number of revertant colonies per plate was not increased in any bacterial strain, and *Equisetum arvense* was non-genotoxic in this assay.

A chromosomal aberration test on *Equisetum arvense* (plant part, method of preparation, and doses not stated) was performed using Chinese hamster lung cells.[34] Details relating to the test protocol were not included. However, it was stated that the short treatment method and the continuous treatment method were used. Using both methods, the incidence of cells with chromosomal aberrations was less than 5%. It was concluded that *Equisetum arvense* did not have any potential for inducing chromosomal aberrations.

Equisetum Arvense Extract

The acquired micronucleus formation in unirradiated and irradiated (X-radiation) samples of human blood lymphocytes cultured with an *Equisetum arvense* extract (ethanol extract of whole, or cut, dried sterile aerial parts; 0.025, 0.05, 0.1, and 0.2 mg/ml) was evaluated using the cytochalasin block micronucleus test.[39] Centromere-positive micronuclei were identified by fluorescence in situ hybridization, using a DNA probe labeled with alpha-satellite digoxigenin. The yield of micronuclei increased in unirradiated samples in a concentration-dependent manner. A reduction in the level of radiation-induced micronuclei in a concentration-dependent manner was also reported. In the control (unirradiated samples), 36.8% of micronuclei were centromere positive (MNC+). In irradiated samples, the percentage of MNC+ ranged from 10.8% to 15.3%. These results were indicative of a clastogenic mechanism for micronuclei formation. The authors noted that this *Equisetum arvense* extract had weak clastogenic properties.

The genotoxicity of an *Equisetum arvense* extract (stem hydro-alcoholic (20:80, v/v) extract) was evaluated in the micronucleus test.[16] Human blood samples were cultured with the extract (62.5 µg/ml) for a total of 67 h. Cell cultures without the extract (also contained cytochalasin B, 6 µg/ml) served as negative controls. Blood samples were also incubated with quercetin (1.3 µg/ml) for comparative purposes. Test results consisted of the number of micronuclei-containing cells per 1000 scored cells and as the incidence of micronuclei formation relative to the incidence of micronuclei formation in the control sample. The incidence (21%, mean of 5 measurements) of micronucleus formation in the sample treated with the extract was higher than that of the control sample. This incidence of micronucleus formation was also comparable to that caused by quercetin alone (20% incidence).

In Vivo

Equisetum arvense (doses, plant part, and method of derivation not stated) was evaluated for genotoxicity potential in the rat (strain not stated) micronucleus test.[34] Details relating to the test protocol were not included. The incidence of micronucleated polychromatic erythrocytes (MNPCEs) was not significantly increased, and *Equisetum arvense* was classified as non-genotoxic in this assay.

CARCINOGENICITY STUDIES

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

OTHER RELEVANT STUDIES

Hepatotoxicity

The hepatotoxicity of *Equisetum arvense* (plant part, method of preparation, and dose not stated) was evaluated using Wistar rats (number not stated).[40] Details relating to the test protocol were not included. Repeated oral dosing for 7 d induced the following important changes in hepatic structure: decrease in the number of hepatocytes, increase in the cytoplasmic volume, increase in the production of nuclear volume in hepatic cells, and coagulative necrosis in central areas.

Equisetum Arvense Extract

The hepatotoxicity of an *Equisetum arvense* extract (aqueous extract of shade-dried and powdered *Equisetum arvense*) was evaluated using groups of 10 adult male Wistar rats.[40] The animals received graded doses of the extract (30 mg/kg, 50 mg/kg, and 100 mg/kg; 1 dose per group) by gavage for 14 d. The control group was dosed with distilled water. Blood samples were collected (schedule not stated) to determine hepatic enzyme (aspartate amino transferase (AST), alanine amino transferase (ALT), and gamma glutamyl transferase (γ-GT)) activities in the serum. Hepatic tissue fragments were obtained for histological analysis. None of the animals in either of the 3 dose groups died. Additionally, when compared to the control group, dosing did not change serum activities of hepatic enzymes. Only benign changes in the hepatic morphology were observed in the 3 dose groups as well as in the control group. Centrilobular steatosis and cellular tumefaction (hydropic degeneration) were observed in the 3 dose groups. However, only centrilobular steatosis was observed in the control group. The authors concluded that oral treatment with graded doses of *Equisetum arvense* extract was not able to produce significant hepatic changes, when compared to the control group. They also noted that further studies are necessary in order to evaluate the chronic hepatotoxicity of *Equisetum arvense* in rats. In another study, an *Equisetum arvense* extract (methanol extract) had a hepatoprotective effect in human hepatoma Hep G2 cells incubated with tacrine (hepatotoxin).[32]

Cytotoxicity

Equisetum Arvense Extract

An *Equisetum arvense* extract (water and ethanol extract) was evaluated for anti-proliferative activity using mouse melanoma B16 cells.[41] This cell line is derived from a spontaneous skin tumor in C57BI/6 mice. Test concentrations ranged from < 0.25 mg/ml to > 0.5 mg/ml. After a 2-d incubation period with the extract, cell counts were made with a hemicytometer and cell viability was assessed by trypan blue exclusion. Each test was performed 6 times, and the extract concentration that caused 50% growth inhibition (IC_{50}) was determined. A cytotoxic effect was not observed (i.e., no effect on cell proliferation) at low concentrations (< 0.25 mg/ml). This *Equisetum arvense* extract caused a significant (statistical significance not stated) cytotoxic (antiproliferative) effect at high concentrations (> 0.5 mg/ml). An IC_{50} of 1.5 mg/ml was reported for *Equisetum arvense* extract.

The cytotoxicity of an *Equisetum arvense* extract (water extract; drug extract ratio[DER] 1:20) against human leukemia cells (U 937 cells) in vitro was evaluated.[42] Cultures were incubated with the extract at concentrations of 124, 248, and 496 µg dry matter/ml for 48 h. Cytotoxicity was increased in a dose-dependent manner. Whether or not the cell death was due to apoptosis was investigated. Test material concentrations of 124 µg/ml and 248 µg/ml did not influence the apoptotic process. However, the highest concentration of this *Equisetum arvense* extract (496 µg/ml) induced early and late apoptosis, when compared to the control (cells cultured without the extract).

The growth inhibitory activities of several different *Equisetum arvense* extracts (aerial parts; ethyl acetate, chloroform, petroleum ether, n-butanol, and water extracts) were evaluated using 3 histologically different human cancer cell lines (HeLa (human cervix epidermoid tumor cell line), MCF7 (human breast adenocarcinoma cell line), and HT-29 (human colon adenocarcinoma cell line)).[31] The extracts (20 µl per well) were added in order to achieve final concentrations for each extract of 0.0625 to 1 mg/ml. The HeLa human cervix epidermoid tumor cells were found to be the most sensitive to all of the extracts. Ethyl acetate, chloroform, and petroleum ether extracts exhibited a statistically significant ($p < 0.01$) antiproliferative effect in the HeLa cell line (in 0.125 to 1 mg/ml concentration range), with IC_{50} values ranging from 0.23 to 0.76 mg/ml. The *n*-butanol extract did not induce 50% inhibition of HeLa cell growth in the 0.0625 to 1 mg/ml concentration range, but growth inhibition effects at 0.5 to 1 mg/ml were statistically significantly different ($p < 0.01$) when compared to the control (not stated). Except for the *n*-butanol extract, all of the extracts statistically significantly decreased MCF-7 cell growth over the entire concentration range. The effects of the ethyl acetate and chloroform extracts were most prominent ($p < 0.01$) in the 0.125 to 0.5 mg/ml concentration range. However, in this concentration range, no extract caused 50% inhibition of MCF-7 cell growth. Both ethyl acetate and petroleum ether extracts caused a statistically significant ($p < 0.01$) antiproliferative effect in the HT-29 cell line, with IC_{50} values ranging from 0.32 to 0.53 mg/ml. Based on the IC_{50} values, the antiproliferative activity of the extracts decreased in the following order: ethyl acetate > chloroform > petroleum ether.

Morphological changes that resembled necrosis were observed in all cell lines. The most prominent morphological changes were observed in HeLa cells treated with ethyl acetate, chloroform, and n-butanol extracts of *Equisetum arvense*.

Equisetum Arvense Leaf Extract

The ability of an *Equisetum arvense* leaf extract (ethanol extract) to induce apoptosis was studied using A549 lung carcinoma cells.[43] The extract was evaluated at concentrations of 100 µg/ml and 150 µg/ml using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay. Acridine orange staining was used to access apoptosis. The development of an orange or orange-red color was indicative of disruption of the cell membrane. Following treatment with both concentrations, the cells were floating (sign of early apoptosis). Additionally, the edges of many cells were not clear and the cytoplasm was not as transparent when compared to untreated control cells. Overall, the cell structure was completely desegregated, with a hard-shelled appearance. Fifty percent of the cells treated with 100 µg/ml developed orange fluorescence. More than 70% of the cells developed orange florescence after treatment with 150 µg/ml. The results of this study indicated that this *Equisetum arvense* leaf extract manifested cytotoxicity and decreased the cell viability of A549 cells in a concentration-dependent manner.

Antimicrobial Activity

Equisetum Arvense Extract

The in vitro antimicrobial activity of an *Equisetum arvense* extract (stem; hydro-alcoholic (20:80, v/v) extract) against the following bacterial/fungal strains was evaluated: *Staphylococcus aureus*, *E. coli* 95, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *S. enteritidis* (all bacterial strains), and the fungal strains *Aspergillus niger* and *Candida albicans*.[16] Disks containing the extract (5 µg per disk) and a bacterial strain were incubated for 24 h. The incubation period for plates containing a fungal strain and the extract (5 µg per disk) was 48 h. For each disk, the diameter (mm) of the inhibition zone was measured. Disks containing ampicillin and nystatine (30 µg per disk) served as positive controls, and disks containing methanol served as negative controls. *Staphylococcus aureus* was found to be the strain that was most resistant to this *Equisetum arvense* extract. The most sensitive strain was *Pseudomonas aeruginosa.* Results indicated that the antimicrobial activity of this *Equisetum arvense* extract (5 µg per disk) was comparable to the antimicrobial activity of the positive controls (30 µg per disk).

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

Equisetum Arvense Extract

In a non-occlusive, skin irritation test involving 4 rabbits (strain not stated), Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract) was classified as non-irritating.[6] Data on the composition of this extract are included in the section on Composition/Impurities. Details relating to the test protocol are not included.

The skin irritation potential of Equisetum Arvense Extract (100%) was evaluated using 3 rabbits (strain not stated).[7] Details relating to the test protocol were not included. Skin irritation was not observed in the animals tested. Equisetum Arvense Extract (in butylene glycol) was evaluated for skin irritation potential using 3 rabbits (strain not stated).[7] The test material was applied to the skin at a concentration of 100%. Details relating to the test protocol were not included. Equisetum Arvense Extract (in butylene glycol) was slightly irritating to the skin.

Sensitization

Animal

Equisetum Arvense Extract

The skin sensitization potential of Equisetum Arvense Extract was evaluated in a maximization test involving 5 guinea pigs (strain not stated).[7] The first and second induction concentrations were 12.5% and 100%, respectively. The challenge concentration was 100%. Additional details relating to the test protocol were not included. The test substance did not induce skin sensitization in any of the animals tested. Equisetum Arvense Extract (in butylene glycol) was evaluated in another maximization test involving 5 guinea pigs (strain not stated).[7] The first and second induction concentrations were 25% and 100%, respectively. The challenge concentration was 100%. Additional details relating to the test protocol were not included. Test results were negative.

Human

Equisetum Arvense Extract

A human repeated insult patch test (HRIPT) on a nail polish containing 0.000049% Equisetum Arvense Extract was performed using 209 subjects.[44] During induction, the test substance (~ 0.2 ml) was applied for 24 h to the upper back (between the scapulae) using a 1" x 1" semi-occlusive patch. The application frequency was 3 times per week (Mondays,

Wednesdays, and Fridays) for a total of 9 applications. Tuesday and Thursday patch removals were followed by a 1-d non-treatment period, and Saturday removals were followed by a 2-d non-treatment period. After induction, the challenge phase was preceded by a 2-wk non-treatment period. A challenge patch was applied for 24 h to a new site that was adjacent to the induction site. Reactions were scored at days 1 and 3 post-application. Results indicated no potential for dermal irritation or allergic contact sensitization.

The skin sensitization potential of a product (mask) containing 0.6% Equisetum Arvense Extract was evaluated in an HRIPT involving 100 subjects.[45] During induction, the test substance (0.02 ml) was applied for 48 h to the lower or upper back using a 0.64 cm^2 occlusive patch. The test substance was application over a 3-wk period, which consisted of nine 48-h exposures. Following a 2-wk non-treatment period, the challenge phase was initiated. Challenge patches were applied to a new site and to the induction site. Challenge reactions were evaluated at 30 min and at 48 h, 72 h, and 96 h post-removal. Results indicated no evidence of skin irritation or sensitization.

OCULAR IRRITATION STUDIES

Equisetum Arvense Extract

In an ocular irritation test involving 4 rabbits (strain not stated), Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract) was classified as slightly irritating.[6] Data on the composition of this extract are included in the section on Composition/Impurities. Details relating to the test protocol are not included.

CLINICAL STUDIES

Case Reports

A dermatitis patient was regularly in contact with *Equisetum arvense* (plant part(s) not stated) in the proximity of his house.[19] In the hour after exposure, he developed dermatitis (resembled seborrheic dermatitis) of the right hand and face after passive inhalation of tobacco smoke. Additionally, a fresh exposure to *Equisetum arvense* induced a more rapid reaction, which necessitated local application of epinephrine and oral antihistamines. The authors noted that this patient's history of atopic reactions with nicotine as a hapten in tobacco smoke correlated with the possible presence of nicotine in *Equisetum arvense*. It was also noted that nicotine has been detected in British species of *Equisetum arvense*.

A woman with no history of atopy developed rhinoconjunctivitis symptoms (dyspnea and general malaise) and contact dermatitis after inhaling steam while cooking green beans, potatoes, and carrots.[46] The patient also used *Equisetum arvense* to lose weight. Prick test results for *Equisetum arvense* (1/1 (w/v) concentration in isotonic saline solution) were positive; the same was true for celery and carrots. Conjunctival challenge with *Equisetum arvense* (1/10 dilution) also yielded a positive response. Additionally, conjunctival challenge with celery (1/10 dilution) and carrot (1/1000 dilution) yielded positive responses. The authors noted that *Equisetum arvense* contains a protein that is similar to a protein that is found in carrots.

Hair loss and fragile nails were observed in a male consumer who took *Equisetum arvense* (3 units/d) for 12 mo.[47] It was noted that the hair loss could have been associated with the reported effect of *Equisetum arvense* in reducing the bioavailability of thiamine after chronic consumption.

Equisetum Arvense Extract

Hand and facial swelling were observed in a female patient after 2 d of oral consumption of an herbal diuretic containing an *Equisetum arvense* extract (ethanol extract).[15] The diuretic was taken 3 times daily and consisted of 225 mg of *Equisetum arvense* dry extract (DER 7.5 - 10.5:1; extraction solvent = ethanol (70% v/v)). Recovery was noted after treatment of symptoms.

In-Use Safety

Equisetum Arvense Extract

An in-use safety evaluation of 3 nail polish products (different shades) containing 0.000049% Equisetum Arvense Extract was performed using 31 female subjects.[48] They were instructed to use a product daily for 4 wk. Nail polish remover was used between applications. Nail plates and cuticles were examined by a trained clinical technician. No adverse reactions were observed after repeated use.

SUMMARY

The safety of 5 *Equisetum arvense*-derived ingredients as used in cosmetics is reviewed in this safety assessment. *Equisetum arvense* (horsetail) has been described as a non-flowering weed that is found throughout parts of Europe, Asia, the Middle East, and North America.

The method of production of an Equisetum Arvense Extract in ethanol and water (0.7% solids w/v) tradename mixture involves extraction of dried raw material with an unknown concentration of ethanol. Preparation of another Equisetum Arvense Extract in ethanol and water tradename mixture involves the extraction of dried raw material with 30 vol% ethanolic

solution. In the production of an Equisetum Arvense Extract in butylene glycol (0.54% solids w/v) tradename mixture, the dried raw material is extracted with 50 vol% 1,3-butylene glycolic solution.

The preparations of different extracts of *Equisetum arvense* have also been described in the published literature. Air dried and powdered plant material was macerated with petroleum ether overnight, and afterwards with 70% methanol. After filtration, the methanolic extract was concentrated to dryness. The dry residue was dissolved in hot water and then separated by liquid-liquid extraction into the chloroform, ethyl acetate, and *n*-butanol extracts.

According to a cosmetics industry source, a tradename mixture Equisetum Arvense Extract has the following composition: Equisetum Arvense Extract (~ 2% dry extract), propylene glycol (~ 66%), phenoxyethanol (~ 0.36%), methylparaben (~ 0.08%), ethylparaben (~ 0.02%), propylparaben (~ 0.04%), and water (qsp 100%). Data on flavonoid composition reveal the existence of 2 chemotypes of *Equisetum arvense*, one in Asia and North America, and the other in Europe. *Equisetum arvense* from Asia and North America contains luteolin-5-*O*-glucoside and its malonyl ester, but these compounds are not found in *Equisetum arvense* from Europe. The dominant compounds in *Equisetum arvense* from Europe are quercetin 3-*O*-glucoside, apigenin 5-*O*-glucoside, and dicaffeoyl-*meso*-tartaric acid. Di-*E*-caffeoyl-*meso*-tartaric acid is a marker for both chemotypes. Whether or not flavonoids or phenolic acids are the predominant compounds in *Equisetum arvense* extracts is dependent upon the extractant that is used.

According to 2021 VCRP data, Equisetum Arvense Extract is reported to be used in 186 cosmetic products (125 leave-on products, 59 rinse-off products, and 2 products that are diluted for (bath) use). Of the *Equisetum arvense*-derived ingredients that are being reviewed in this safety assessment, this is the greatest reported use frequency. The results of a concentration of use survey submitted by the Council in 2019 indicate that Equisetum Arvense Extract is being used at maximum use concentrations up to 0.4% in leave-on products (body and hand products (not spray)), and at maximum use concentrations up to 0.00078% in rinse-off products (skin cleansing products). Equisetum Arvense Extract is the only *Equisetum arvense*-derived ingredient in this safety assessment for which use concentration data were provided in response to the Council survey. According to VCRP and Council survey data, Equisetum Arvense Juice and Equisetum Arvense Leaf Powder are not being used in cosmetic products.

In Asian traditional medicine, the aerial parts of *Equisetum arvense* have been used to treat hemorrhage, urethritis, jaundice, and hepatitis. According to the US FDA, *Equisetum arvense* is among the ingredients that have been present in OTC drug products for use as a digestive aid. However, based on evidence currently available, there are inadequate data to establish general recognition of safety and effectiveness of this ingredient for this specified use.

In an acute oral toxicity study involving 10 mice (strain not stated), an LD_0 of ≥ 20 ml/kg was reported for Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract). A single-dose, oral toxicity study on *Equisetum arvense* was performed using groups of male and female rats (strain and number per group not stated). The approximate LD_{50} value was > 5000 mg/kg. None of the animals died and there were no signs of toxicity at necropsy.

An *Equisetum arvense* extract (hydroalcoholic extract) was evaluated for acute toxicity using groups of 8 male Wistar rats. The groups received i.p. doses of 1000 mg/kg, 2000 mg/kg, and 5000 mg/kg. Mortalities were observed in the 2000 mg/kg group (12.5% of the animals) and in the 5000 mg/kg group (37.5% of the animals). Transitory respiratory depression and elevated sedation (dose-dependent) were observed in all 3 dose groups.

In a short-term study, male Sprague-Dawley rats (groups of 6) were fed an *Equisetum arvense* powder (0.4% or 4%) in a 20% casein diet with and without cholesterol (0.5% cholesterol and 0.15% sodium cholate) for 14 d. At a concentration of 0.4% or 4% in either diet, *Equisetum arvense* powder did not influence food intake or growth, or have an effect on serum or liver lipids. However, on days 9 to 12 of feeding with 4% *Equisetum arvense* powder in the cholesterol diet, 4 of 6 rats lost their hair and dermatitis was observed on the neck, head, nose, and back. At microscopic examination, these changes were diagnosed as nonspecific inflammatory lesion of the skin. Reversal of the dermatitis was noted when the diet was changed to a commercial diet. In 2 additional experiments, rats were fed an *Equisetum arvense* powder (concentration not stated) in a cholesterol diet (composition not stated) for 4 wk and 6 wk. Six of 21 rats from the 2 experiments had dermatitis on the neck and back. The incidence of dermatitis after feeding for 4 wk and 6 wk was approximately 20% and 30%, respectively.

An *Equisetum arvense* extract (dried stem, ethanol and water extract) was administered to 10 male Wistar rats, at a daily i.p. dose of 50 mg/kg for 8 wk. Signs of toxicity were not observed.

The subchronic oral toxicity of *Equisetum arvense* (powder extracted with hot water; plant part not stated) was evaluated in a study involving groups of 10 male and female F344 rats fed *Equisetum arvense* at a concentration of 0.3%, 1%, or 3% in the diet (no further information on test substance composition or plant part(s) included) for 13 wk. None of the animals died, and no obvious clinical signs were observed in any of the animals during the study. Statistically significant alterations in hematological parameters (e.g., mean corpuscular hemoglobin and platelet count) were observed, but no dose dependence was apparent. Histopathological changes (not treatment-related) were observed in the liver, pancreas, kidneys, and ovaries. The NOAEL for *Equisetum arvense* was determined to be more than 3% in male and female rats (> 1.79 g/kg bw/d, males; > 1.85 g/kg bw/d, females).

The results of an in vitro teratogenicity assay on *Equisetum arvense* involving zebrafish (*Danio rerio*, AB strain) embryos demonstrated no teratogenic potential of this botanical (test concentrations of 0.00625 mg/l, 0.0625 mg/l, and 0.625 mg/l) during development of the zebrafish.

Equisetum arvense (plant part, method of preparation, and doses not stated) was evaluated in a reverse mutation test using *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, and *E. coli* strain WP2uvrA. Results were negative. A chromosomal aberration test on *Equisetum arvense* (plant part, method of preparation, and doses not stated) was performed using Chinese hamster lung cells. It was concluded that *Equisetum arvense* did not have any potential for inducing chromosomal aberrations. The acquired micronucleus formation in unirradiated and irradiated samples of human blood lymphocytes cultured with *Equisetum arvense* extract (ethanol extract, 0.025, 0.05, 0.1, and 0.2 mg/ml) was evaluated using the cytochalasin block micronucleus test in vitro. *Equisetum arvense* extract (ethanol extract) had weak clastogenic properties in this test. The genotoxicity of an *Equisetum arvense* extract (stem hydro-alcoholic (20:80, v/v) extract) was evaluated in another in vitro micronucleus test. Human blood samples were cultured with the extract (62.5 µg/ml). The incidence (21%, mean of 5 measurements) of micronucleus formation in the sample treated with the extract was higher than that of the control sample.

Equisetum arvense (plant part, method of preparation, and doses not stated) was evaluated for genotoxicity potential in the rat (strain not stated) micronucleus test in vivo. The incidence of MNPCEs was not significantly increased, and *Equisetum arvense* was classified as non-genotoxic in this assay.

The hepatotoxicity of an *Equisetum arvense* extract (aqueous extract of shade-dried and powdered *Equisetum arvense*) was evaluated using groups of 10 adult male Wistar rats. The animals received graded doses of the extract (30 mg/kg, 50 mg/kg, and 100 mg/kg; 1 dose per group) by gavage for 14 d. None of the animals died, and significant hepatic changes were not observed.

An *Equisetum arvense* extract (water and ethanol extract) caused a significant (statistical significance not stated) cytotoxic (antiproliferative) effect in mouse melanoma B16 cells at high concentrations (> 0.5 mg/ml). An IC_{50} of 1.5 mg/ml was reported. The cytotoxicity of an *Equisetum arvense* extract (water extract; DER 1:20) against human leukemia cells (U 937 cells) in vitro was evaluated. Concentrations of 124 µg/ml and 248 µg/ml did not influence the apoptotic process. However, the highest concentration of *Equisetum arvense* extract (496 µg/ml) induced early and late apoptosis, when compared to the control (cells cultured without *Equisetum arvense* extract). The growth inhibitory activity of *Equisetum arvense* extracts (aerial parts; ethyl acetate, chloroform, and petroleum ether, *n*-butanol, and water extracts) was evaluated using 3 histologically different human cancer cell lines (HeLa, MCF7, and HT-29 cells). The HeLa human cervix epidermoid tumor cells were found to be the most sensitive to all of the extracts. Ethyl acetate, chloroform, and petroleum ether extracts exhibited a statistically significant ($p < 0.01$) antiproliferative effect in the HeLa cell line (in 0.125 to 1 mg/ml concentration range), with IC_{50} values ranging from 0.23 to 0.76 mg/ml.

The ability of an *Equisetum arvense* leaf extract (ethanol extract) to induce apoptosis was studied using A549 lung carcinoma cells.[43] The extract was evaluated at concentrations of 100 µg/ml and 150 µg/ml using the MTT cytotoxicity assay. *Equisetum arvense* leaf extract manifested cytotoxicity and decreased the cell viability of A549 cells in a concentration-dependent manner.

The in vitro antimicrobial activities of an *Equisetum arvense* extract (stem hydro-alcoholic (20:80, v/v) extract) against *Staphylococcus aureus*, *E. coli* 95, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *S. enteritidis* (all bacterial strains), and the fungal strains *Aspergillus niger* and *Candida albicans* were evaluated. Results indicated that the antimicrobial activities of *Equisetum arvense* extract (5 µg per disk) were comparable to the antimicrobial activities of the positive controls (ampicillin and nystatine, 30 µg per disk).

In a non-occlusive, skin irritation test involving 4 rabbits (strain not stated), Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract) was classified as non-irritating. Skin irritation was not observed in a study in which Equisetum Arvense Extract (100%) was applied to the skin of 3 rabbits (strain not stated). Equisetum Arvense Extract (in butylene glycol) was also evaluated for skin irritation potential in a study involving 3 rabbits (strain not stated). The extract was applied at a concentration of 100%, and was classified as slightly irritating to the skin.

The skin sensitization potential of Equisetum Arvense Extract was evaluated in a maximization test involving 5 guinea pigs (strain not stated). A challenge concentration of 100% did not induce skin sensitization in any of the animals tested. Equisetum Arvense Extract (in butylene glycol) was evaluated in another maximization test involving 5 guinea pigs (strain not stated). Results were negative for a challenge concentration of 100%. An HRIPT on a nail polish containing 0.000049% Equisetum Arvense Extract was performed using 209 subjects. Results indicated no potential for dermal irritation or allergic contact sensitization. The skin sensitization potential of a product (mask) containing 0.6% Equisetum Arvense Extract was evaluated in an HRIPT involving 100 subjects. Results indicated no evidence of skin irritation or sensitization.

In an ocular irritation test involving 4 rabbits (strain not stated), Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract) was classified as slightly irritating.

Hand and facial swelling were observed in a female patient after 2 d of oral consumption of an herbal diuretic containing an *Equisetum arvense* extract (225 mg of *Equisetum arvense* dry extract (DER 7.5 - 10.5:1; extraction solvent =

ethanol (70% v/v)). A dermatitis patient who was regularly in contact with *Equisetum arvense* developed dermatitis of the right hand and face after passive inhalation of tobacco smoke; a fresh exposure to *Equisetum arvense* induced a more rapid reaction. A female patient with rhinoconjunctivitis and contact dermatitis had inhaled steam while cooking vegetables and also consumed *Equisetum arvense* for weight loss. Prick test results for *Equisetum arvense* (1/1 (w/v) concentration in isotonic saline solution) were positive. Conjunctival challenge with *Equisetum arvense* (1/10 dilution) also yielded a positive response. Hair loss and fragile nails were observed in a male consumer who took *Equisetum arvense* (3 units/d) for 12 mo. An in-use safety evaluation of 3 nail polish products (different shades) containing 0.000049% Equisetum Arvense Extract was performed using 31 female subjects. After 4 wk of daily use, no adverse reactions were observed.

DRAFT DISUSSION

This assessment reviews the safety of 5 Equisetum arvense-derived ingredient as used in cosmetic formulations. The Panel concluded [TBD]. Based on negative HRIPT data on products containing 0.000049% (209 subjects) and 0.6% (100 subjects) Equisetum Arvense Extract and a negative in-use safety evaluation (31 subjects) on nail products containing 0.000049% Equisetum Arvense Extract, the Panel agreed that the skin irritation and sensitization potential of this ingredient at the maximum reported use concentration of 0.4% in cosmetics is not a concern.

The Panel noted that hair loss was observed in an oral dosing study in which Sprague-Dawley rats were fed 4% *Equisetum arvense* powder in a cholesterol diet for 14 d. However, they also noted no obvious clinical signs in another study in which F344 rats were fed *Equisetum arvense* (hot water extract of powder) at concentrations up to 3% in a basal diet for 13 wk. The Panel acknowledged that *Equisetum arvense* was not definitively identified as the causative agent in the 14-d study. They also agreed that the negative results in the 13-wk rat oral toxicity study on *Equisetum arvense* (aqueous extract) obviate any concerns relating to the systemic toxicity of *Equisetum arvense*-derived ingredients.

Concern about pesticide residues, heavy metals, and other plant species that may be present in botanical ingredients was expressed by the Panel. According to one source, mean values for 3 toxic metals in *Equisetum arvense are:* lead (14.07 mg/kg), cadmium (0.139 mg/kg), and mercury (0.014 mg/kg). It was stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities.

Also, because final product formulations may contain multiple botanicals, each possibly containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. For example, Equisetum Arvense Extract (butylene glycol extract) contains tannin (possible allergen). Additionally, the Panel was aware that variances in the composition of *Equisetum arvense,* based on the geographical area of plant growth (i.e., Asia and North America vs. Europe), have been reported. Therefore, when formulating products, manufacturers should avoid reaching levels of plant constituents that may cause sensitization or other adverse health effects.

The Panel discussed the issue of incidental inhalation exposure resulting from an *Equisetum arvense*-derived ingredient (e.g. Equisetum Arvense Extract in cologne and toilet waters, and in other fragrance preparations (concentrations unknown); Equisetum Arvense Extract in face powders (concentrations unknown)). Inhalation toxicity data were not available. However, the Panel noted that, in aerosol products, 95% - 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial 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 concentrations at which the ingredients are used, 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.

CONCLUSION

To be determined.

TABLES

Table 1. Definitions and Functions of the Ingredients in this Safety Assessment.[1]

Ingredient/CAS No.	Definition & Structures	Function(s)
Equisetum Arvense Extract 71011-23-9	Equisetum Arvense Extract is the extract of the whole herb, *Equisetum arvense*.	Skin-Conditioning Agents - Miscellaneous
Equisetum Arvense Juice 71011-23-9 (generic)	Equisetum Arvense Juice is the juice expressed from *Equisetum arvense*.	Skin-Conditioning Agents - Miscellaneous
Equisetum Arvense Leaf Extract 71011-23-9	Equisetum Arvense Leaf Extract is the extract of the leaves of *Equisetum arvense*.	Skin-Conditioning Agents - Miscellaneous
Equisetum Arvense Leaf Powder	Equisetum Arvense Leaf Powder is the powder obtained from the dried, ground leaves of *Equisetum arvense*.	Skin-Conditioning Agents - Miscellaneous
Equisetum Arvense Powder	Equisetum Arvense Powder is the powder obtained from the dried, ground whole plant, *Equisetum arvense*.	Skin-Conditioning Agents - Humectant

Table 2. Dominant Compounds in *Equisetum arvense* (native to Vojvodina, Serbia) extracts , based on solvent.[8]

Phenolic Compounds	Quantity (mg/g dry extract) in Ethyl Acetate Extract	Quantity (mg/g dry extract) in *n*-Butanol Extract	Quantity (mg/g dry extract) in Aqueous Extract
Isoquercitrin	152	382	----
Apigenin 6-*O*-glucoside	22.40	----	----
Kaempferol 3-*O*-glycoside	26.20	----	----
di-*E*-caffeoyl-meso-tartaric acid	----	100	10
Phenolic Acid 1 (unnamed)	----	----	3
Phenolic Acid 2 (unnamed)	----	----	6

Table 3. Composition Data on Equisetum Arvense Extract from Asia and Europe.[17]

Components	*Equisetum arvense* (methanol and water extract) from Asia	*Equisetum arvense* (methanol and water extract) from Europe
Apigenin 4'-*O* glucoside	Detected (quantity not stated)	Detected (quantity not stated)
Apigenin 5-*O*-(6"-O-malonylglucoside)	Detected (quantity not stated)	Detected (quantity not stated)
Apigenin 5-*O*-glucoside	>500 µg/g dry weight	>500 µg/g dry weight
5-*O*-Caffeoylshikimic acid	Detected (quantity not stated)	Detected (quantity not stated)
Chlorogenic acid	Detected (quantity not stated)	Detected (quantity not stated)
Dicaffeoyl-*meso*-tartaric acid	>500 µg/g dry weight	>500 µg/g dry weight
Equisetumpyrone	Detected in fertile sprouts only	Detected in fertile sprouts only (quantity not stated)
Genkwanin 4'-*O*-glucoside	Detected (quantity not stated)	Detected (quantity not stated)
Genkwanin 5-*O*-glucoside	Detected (quantity not stated)	Detected (quantity not stated)
Genkwanin 5-O-(6"-*O*-malonyglucoside)	Detected (quantity not stated)	Detected (quantity not stated)
Gossypetin 7-*O*-glucoside	Detected in fertile sprouts only (quantity not stated)	Detected in fertile sprouts only (quantity not stated)
Kaempferol 3-*O*-glucoside	Detected (quantity not stated)	Detected (quantity not stated)
Kaempferol 3-*O*-rutinoside-7-*O*-glucoside	Detected (quantity not stated)	Detected (quantity not stated)
Kaempferol 3-*O*-sophoroside	Detected (quantity not stated)	Detected (quantity not stated)
Kaempferol 3-O-(6"-*O*-malonylglucoside)	Detected (quantity not stated)	Detected (quantity not stated)
Kaempferol 3-O-(6"-O-malonylglucoside)-7-O-glucoside	Detected (quantity not stated)	Detected (quantity not stated)
Kaempferol 3,7-*O*-diglucoside	Detected (quantity not stated)	Detected (quantity not stated)
Luteolin 5-*O*-glucoside	>500 µg/g dry weight	Not detected
Luteolin 5-*O*-(6"-O-malonylglucoside)	Detected (quantity not stated)	Not detected
Monocaffeoyl-*meso*-tartaric acid	Detected (quantity not stated)	Detected (quantity not stated)
Protoapigenin 4-*O*-glucoside	Detected in fertile sprouts only (quantity not stated)	Detected in fertile sprouts only (quantity not stated)
Protogenkwanin 4'-*O*-glucoside	Detected in fertile sprouts only (quantity not stated)	Detected in fertile sprouts only (quantity not stated)
Quercetin 3-*O*-glucoside	Detected (quantity not stated)	>500 µg/g dry weight
Quercetin 3-*O*-sophoroside	Detected (quantity not stated)	>500 µg/g dry weight
Quercetin 3-*O*-(6"-*O*-malonylglucoside)	Detected (quantity not stated)	Detected (quantity not stated)
Quercetin 3,7-*O*-diglucoside	Detected (quantity not stated)	Detected (quantity not stated)

Table 4. Frequency (2021) and Concentration of Use (2019) According to Duration and Type of Exposure.[20,21]

	Equisetum Arvense Extract		**Equisetum Arvense Leaf Extract**		**Equisetum Arvense Powder**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals*	**186**	**0.0000011-0.4**	**12**	**NR**	**1**	**NR**
Duration of Use						
Leave-On	*125*	*0.01-0.4*	*8*	*NR*	*1*	*NR*
Rinse off	*59*	*0.0000011-0.00078*	*4*	*NR*	*NR*	*NR*
Diluted for (bath) Use	*2*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	11	NR	1	NR	NR	NR
Incidental Ingestion	NR	0.0002	NR	NR	NR	NR
Incidental Inhalation - Sprays	1;33[a];62[c]	0.0002[a]	1;3[a];1[c]	NR	NR	NR
Incidental Inhalation - Powders	2;62[c]	0.01-0.4[b]	1[c]	NR	NR	NR
Dermal Contact	138	0.00078-0.4	5	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	46	0.0000011-0.0006	6	NR	1	NR
Hair-Coloring	1	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	9	0.0002	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

	equisetum arvense (horsetail)**	
	# of Uses	Conc. (%)
Totals/Conc. Range	**3**	**NS**
Duration of Use		
Leave-On	*2*	*NS*
Rinse off	*1*	*NS*
Diluted for (bath) Use	*NR*	*NS*
Exposure Type		
Eye Area	1	NS
Incidental Ingestion	NR	NS
Incidental Inhalation - Sprays	NR	NS
Incidental Inhalation - Powders	NR	NS
Dermal Contact	1	NS
Deodorant (underarm)	NR	NS
Hair - Non-Coloring	2	NS
Hair-Coloring	NR	NS
Nail	NR	NS
Mucous Membrane	NR	NS
Baby Products	NR	NS

NR – not reported
NS = Not Surveyed
* 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.
**Not an International Nomenclature Cosmetic Ingredient (INCI) name, but uses under this name are in the VCRP
[a]It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays
[b]It is possible that these products may be powders, but it is not specified whether the reported uses are powders
[c]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

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2021 FDA VCRP Data

Equisetum Arvense Extract

Bath Oils, Tablets, and Salts	02A	1
Other Bath Preparations	02D	1
Eye Lotion	03D	6
Eye Makeup Remover	03E	1
Other Eye Makeup Preparations	03G	4
Cologne and Toilet waters	04A	1
Hair Conditioner	05A	13
Shampoos (non-coloring)	05F	23
Tonics, Dressings, and Other Hair Grooming Aids	05G	7
Other Hair Preparations	05I	3
Hair Shampoos (coloring)	06D	1
Face Powders	07B	2
Foundations	07C	1
Makeup Bases	07F	1
Bath Soaps and Detergents	10A	3
Douches	10C	1
Other Personal Cleanliness Products	10E	3
Shaving Cream	11E	1
Cleansing	12A	7
Face and Neck (exc shave)	12C	36
Body and Hand (exc shave)	12D	26
Moisturizing	12F	17
Night	12G	5
Paste Masks (mud packs)	12H	6
Skin Fresheners	12I	4
Other Skin Care Preps	12J	12
Total		**186**

Equisetum Arvense Juice - No FDA Uses

Equisetum Arvense Leaf Extract

Mascara	03F	1
Hair Conditioner	05A	1
Hair Spray (aerosol fixatives)	05B	1
Rinses (non-coloring)	05E	1
Shampoos (non-coloring)	05F	1
Tonics, Dressings, and Other Hair Grooming Aids	05G	1
Other Hair Preparations	05I	1
Cleansing	12A	1
Face and Neck (exc shave)	12C	1
Moisturizing	12F	2
Other Skin Care Preps	12J	1
Total		**12**

Equisetum Arvense Leaf Powder - No FDA Uses

Equisetum Arvense Powder		
Other Hair Preparations	05I	1
Total		**1**
Equisetum Arvense		
Eye Lotion	03D	1
Shampoos (non-coloring)	05F	1
Other Hair Preparations	05I	1
Total		**3**

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: January 12, 2021

SUBJECT: Equisetum Arvense Extract

Anonymous. 2021. Summary of Safety Studies of Equisetum Arvense Extract.

Anonymous. 2021. Full composition Equisetum Arvense Extract.

January 2021

Summary of Safety Studies – Equisetum Arvense Extract

In 1992, an hydroglycolic extract of *Equisetum arvense* was tested on animals.

The results are as follows:

- eye irritation: slightly irritating (4 rabbits)
- skin irritation: non-irritating (4 rabbits- non-occlusive test)
- acute oral toxicity: LD0 ≥ 20 ml/kg (10 mice)

Composition of the extract attached.

It is an extraction directly into the solvent mixture (water/glycol) by maceration.

[redacted]

[redacted]

Full composition

% predicted	INCI name	CAS No.	EINECS No.	Function
~ 2 % (dry extract)	EQUISETUM ARVENSE EXTRACT	71011-23-9	275-123-8	active
~ 66 %	PROPYLENE GLYCOL	57-55-6	200-338-0	solvent
~ 0.5 %	Parabens/Phenoxyethanol :			preservative
~ 0.36 %	Phenoxyethanol	122-99-6	204-589-7	
~ 0.08 %	Methylparaben	99-76-3	202-785-7	
~ 0.02 %	Ethylparaben	120-47-8	204-399-4	
~ 0.04 %	Propylparaben	94-13-3	202-307-7	
qsp 100 %	WATER	7732-18-5	231-791-2	solvent

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: February 3, 2021

SUBJECT: Equisetum Arvense Extract

Consumer Product Testing Co. 2017. Repeated insult patch test (nail polish containing 0.000049% Equisetum Arvense Extract).

Consumer Product Testing Co. 2017. In-use safety evaluation (nail polish containing 0.000049% Equisetum Arvense Extract).

Consumer Product Testing Co.

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Repeated Insult Patch Test
Protocol No.: CP-01.01S

TEST MATERIAL: NAIL POLISH SHADE – ENG087315, CN9-59-2

contains 0.000049% Equisetum Arvense Extract

EXPERIMENT REFERENCE NUMBER: C17-3264.01

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Michael Caswell, Ph.D., CCRA, CCRC
Vice President, Clinical Evaluations
05 Sep 2017

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations
9/5/17

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

QUALITY ASSURANCE UNIT STATEMENT

Study Number: C17-3264.01

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, CPTC Standard Operating Procedures, and the approved protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QAU to obtain custody of trial records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

William Cavaliere
Quality Assurance Representative

9/11/2017
Date

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: Two-hundred thirty (230) qualified subjects, male and female, ranging in age from 16 to 79 years, were selected for this evaluation. Two-hundred nine (209) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] to 79 years.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History Form and the understanding and signing of an Informed Consent Form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: NAIL POLISH SHADE – ENG087315, CN9-59-2

Study Schedule:

Panel #	Initiation Date	Completion Date
20170253	July 10, 2017	August 18, 2017
20170254	July 12, 2017	August 18, 2017
20170260	July 12, 2017	August 24, 2017
20170266	July 17, 2017	August 24, 2017

[a]With parental or guardian consent

Methodology:

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 1" x 1" absorbent pad portion of a clear adhesive dressing and allowed to dry overnight. This was then applied to the appropriate treatment site to form a semi-occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of one day following each Tuesday and Thursday removal, and two days following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic Day 1 and Day 3 post-application.

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	**=**	**No visible skin reaction**	**E**	**=**	**Edema**
0.5	**=**	**Barely perceptible**	**D**	**=**	**Dryness**
1	**=**	**Mild**	**S**	**=**	**Staining**
2	**=**	**Moderate**	**P**	**=**	**Papules**
3	**=**	**Marked**	**V**	**=**	**Vesicles**
4	**=**	**Severe**	**B**	**=**	**Bullae**
			U	**=**	**Ulceration**
			Sp	**=**	**Spreading**

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Adverse Events:

On 7/16/17 Subject #4, Panel 20170260, took NyQuil for nasal congestion. Because NyQuil contains an antihistamine, the Principal Investigator removed this subject from participation in this trial for taking a prohibited medication.

Amendments:

There were no amendments.

Deviations:

There were no deviations.

Results:

The results of each participant are appended (Table 1).

Observations remained within normal limits throughout the test interval.

Subject demographics are presented in Table 2.

Summary:

Under the conditions of this study, test material, NAIL POLISH SHADE – ENG087315, CN9-59-2, indicated no potential for dermal irritation or allergic contact sensitization.

[redacted]
C17-3264.01

Table 1
Panel #20170253

Individual Results

NAIL POLISH SHADE – ENG087315, CN9-59-2

Subject Number	Induction Phase									Virgin Challenge Site	
	Day1*	1	2	3	4	5	6	7	8	9	Day 1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	-------DID NOT COMPLETE STUDY------				
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0[m]	0	0	0	0	0	0	0
16	----------DID NOT COMPLETE STUDY----------											
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	----------DID NOT COMPLETE STUDY----------											
24	0	0	0	0	0	0	0	0	0	0	0	0
25	----------DID NOT COMPLETE STUDY----------											
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor

[redacted]
C17-3264.01

Table 1
(continued)
Panel #20170253

Individual Results

NAIL POLISH SHADE – ENG087315, CN9-59-2

Subject Number	Induction Phase									Virgin Challenge Site	
	Day1*	1	2	3	4	5	6	7	8	9	Day 1*	Day 3
30	DID NOT COMPLETE STUDY											
31	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	DID NOT COMPLETE STUDY											
35	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0
38	DID NOT COMPLETE STUDY											
39	DID NOT COMPLETE STUDY											
40	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	DID NOT COMPLETE STUDY				
45	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	DID NOT COMPLETE STUDY								
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0[m]	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor

[redacted]
C17-3264.01

Table 1
(continued)
Panel #20170254

Individual Results

NAIL POLISH SHADE – ENG087315, CN9-59-2

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day 1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0[m]	0	----------DID NOT COMPLETE STUDY----------						
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor

[redacted]
C17-3264.01

Table 1
(continued)
Panel #20170254

Individual Results

NAIL POLISH SHADE – ENG087315, CN9-59-2

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day 1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0
40	**0.5**	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal

Table 1
(continued)
Panel #20170260

Individual Results

NAIL POLISH SHADE – ENG087315, CN9-59-2

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day 1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	----------DID NOT COMPLETE STUDY----------									
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	----------DID NOT COMPLETE STUDY----------							
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	**0.5**	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal

Table 1
(continued)
Panel #20170260

Individual Results

NAIL POLISH SHADE – ENG087315, CN9-59-2

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day 1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	----------DID NOT COMPLETE STUDY--------					
35	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	----------DID NOT COMPLETE STUDY--------					
41	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0[m]	0	0	0	----DNC---	
46	0	0	0	0	0	0	0	0	0	0	0	0
47	----------DID NOT COMPLETE STUDY----------											
48	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor
DNC = Did not complete study

[redacted]
C17-3264.01

Table 1
(continued)
Panel #20170266

Individual Results

NAIL POLISH SHADE – ENG087315, CN9-59-2

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day 1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	----------DID NOT COMPLETE STUDY----------						
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal

Table 1
(continued)
Panel #20170266

Individual Results

NAIL POLISH SHADE – ENG087315, CN9-59-2

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day 1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	DID NOT COMPLETE STUDY									
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0
36	0	DID NOT COMPLETE STUDY										
37	DID NOT COMPLETE STUDY											
38	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal

Table 2
Panel #20170253

Subject Demographics

Subject Number	Initials	Age	Gender
1	M-S	66	M
2	AJB	52	F
3	ATA	48	F
4	PAB	47	F
5	MAC	68	F
6	ISM	26	F
7	ABT	44	M
8	D-A	63	M
9	LSL	61	F
10	PJL	62	M
11	JAW	70	F
12	MLP	61	F
13	FMH	56	M
14	JAB	34	M
15	GEC	51	F
16	A-B	41	F
17	M-C	28	F
18	JGM	29	M
19	EBM	49	M
20	BWF	49	M
21	MJM	44	M
22	MTZ	28	M
23	TMA	20	F
24	EES	53	F
25	CMW	28	F
26	EAM	19	M
27	SCS	65	F
28	LER	55	F
29	R-C	47	F

Table 2
(continued)
Panel #20170253

Subject Demographics

Subject Number	Initials	Age	Gender
30	HAD	57	F
31	DJB	58	F
32	HAD	58	F
33	SCF	47	F
34	CIT	40	M
35	CMM	49	F
36	EAM	57	F
37	JHP	49	M
38	TTR	23	M
39	JTD	23	M
40	TKP	46	F
41	KLK	30	F
42	SIP	48	F
43	DKS	29	F
44	JEP	18	M
45	TFC	55	F
46	LJK	61	F
47	RME	20	M
48	REG	61	M
49	S-N	33	F
50	C-S	47	M
51	DAN	63	F
52	KEO	26	M
53	TMQ	21	F
54	DMS	45	F
55	MAC	33	M
56	D-A	32	F
57	SAF	36	M
58	R-B	62	F
59	SEO	44	F
60	E-Q	59	M

Table 2
(continued)
Panel #20170254

Subject Demographics

Subject Number	Initials	Age	Gender
1	LAN	49	F
2	NJA	27	F
3	CAM	61	F
4	BAM	58	F
5	BCT	68	F
6	SIS	58	M
7	M-H	78	F
8	DRB	73	M
9	CJP	74	F
10	MJC	53	M
11	RDT	71	F
12	B-H	32	F
13	QTL	26	M
14	P-M	55	M
15	K-W	43	F
16	DDT	36	M
17	GDB	72	M
18	T-M	58	F
19	R-M	47	F
20	J-P	39	F
21	N-H	60	F
22	TAF	43	F
23	DQT	32	F
24	DAH	59	F
25	LMF	74	F
26	VDM	76	F
27	LAH	79	F
28	E-C	55	M
29	DSH	29	M

Table 2
(continued)
Panel #20170254

Subject Demographics

Subject Number	Initials	Age	Gender
30	MDP	34	F
31	VMA	51	F
32	LAB	70	M
33	E-F	53	F
34	SMP	61	F
35	SAL	64	M
36	COC	18	M
37	M-S	68	F
38	N-P	72	M
39	MJR	66	F
40	A-S	51	F
41	T-G	74	M
42	RMS	71	F
43	KAG	28	F
44	LDW	59	M
45	P-E	35	M
46	JGS	64	M
47	MNC	56	F
48	TMD	45	F
49	J-A	41	F
50	K-D	58	F
51	SAM	46	F
52	SLN	22	F
53	CDW	33	M
54	TNT	36	F
55	N-S	27	F
56	EMS	74	F

Table 2
(continued)
Panel #20170260

Subject Demographics

Subject Number	Initials	Age	Gender
1	B-D	63	F
2	A-M	63	F
3	S-S	70	M
4	R-R	43	M
5	JMM	39	F
6	FJC	28	M
7	AML	70	F
8	T-G	28	F
9	KRB	25	M
10	DOC	39	M
11	TAK	45	F
12	M-F	57	M
13	XIQ	29	F
14	NCE	73	F
15	DRH	56	F
16	S-F	55	F
17	RIC	51	F
18	GCL	70	F
19	MJA	45	M
20	W-L	65	M
21	JLL	61	F
22	RAL	43	F
23	STC	45	F
24	V-G	52	M
25	CDC	26	M
26	GNO	25	F
27	MME	50	M
28	J-R	38	M
29	JAG	39	M

Table 2
(continued)
Panel #20170260

Subject Demographics

Subject Number	Initials	Age	Gender
30	C-H	54	F
31	SMF	56	F
32	KMB	32	F
33	A-V	28	F
34	CMS	22	M
35	FKH	19	M
36	DAB	16	M
37	AML	43	F
38	CAA	60	F
39	DMM	65	F
40	VVL	38	M
41	P-H	61	F
42	M-D	72	M
43	JVR	72	F
44	JBR	74	M
45	LAM	57	F
46	ACS	44	F
47	FAP	64	F
48	LKD	40	M
49	J-R	77	M
50	M-E	79	F
51	MTM	31	F
52	LNV	57	F
53	RAJ	39	F
54	DIW	17	F
55	HSH	47	F
56	D-D	23	F
57	DJH	54	M

Table 2
(continued)
Panel #20170266

Subject Demographics

Subject Number	Initials	Age	Gender
1	FPS	65	F
2	B-Z	73	M
3	EHB	72	F
4	R-C	78	M
5	AJL	18	M
6	JAP	76	F
7	M-B	39	M
8	AJR	77	M
9	P-G	17	F
10	P-G	17	F
11	EMR	52	F
12	I-S	78	F
13	M-D	73	F
14	G-D	77	M
15	J-H	78	M
16	RLS	56	F
17	MCL	71	F
18	ACK	66	F
19	PEC	57	F
20	PMJ	53	F
21	LTH	34	M
22	LTA	19	F
23	KMG	23	F
24	HYP	23	F
25	VDS	48	F
26	LSH	51	F
27	GSG	75	F
28	T-P	50	F
29	TES	30	M

Table 2
(continued)
Panel #20170266

Subject Demographics

Subject Number	Initials	Age	Gender
30	J-F	26	F
31	D-M	63	F
32	MSA	23	M
33	MGG	22	M
34	NSN	73	F
35	M-Q	66	F
36	PID	41	M
37	J-Q	39	F
38	TLL	48	F
39	JNM	25	M
40	B-B	67	F
41	MEH	65	F
42	OSG	34	F
43	EMS	41	F
44	CAT	60	F
45	GSM	26	M
46	GMB	48	F
47	D-H	19	F
48	O-M	56	F
49	TMR	79	F
50	JEV	37	F
51	MAT	61	F
52	ECK	62	F
53	LDA	61	M
54	C-A	45	F
55	M-Q	57	F
56	TSR	46	F
57	KNS	47	F

Consumer Product Testing Co.

EST 1975

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: In-Use Safety Evaluation – Technician Examination with a Dermatologist Review of Data
Protocol No.: BNIW02-215

TEST MATERIALS:
.01 NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.02 NAIL POLISH - (SHADE 18) ENG086172, PB-1063-1
.03 NAIL POLISH - (SHADE 28) ENG086175, PB-1065-1
.04 NAIL POLISH - (SHADE 38) ENG087315, CN9-59-2

products 02, 03 and 04 contain 0.000049% Equisetum Arvense Extract

EXPERIMENT REFERENCE NUMBER: C17-3181.01-.04

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Michael Caswell, Ph.D., CCRA, CCRC
Vice President, Clinical Evaluations
05 Sep 2017

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations
9/5/17

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QUALITY ASSURANCE UNIT STATEMENT

Trial No.: C17-3181.01-.04

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, CPTC Standard Operating Procedures, and the approved protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QAU to obtain custody of trial records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

William Cavaliere
Quality Assurance Representative

9/5/2017
Date

Objective: To determine if repeated use of a nail polish, following a weekly regimen provided by the Sponsor, elicited adverse reactions.

Participants: Thirty-four female subjects, ages 20 to 65 years, were recruited for this trial. Thirty-one subjects completed the trial. Subject #'s 13 and 18 were disqualified by the trained Clinical Technician due to unhealthy nail plates and cuticles. Subject #26 discontinued her participation due to personal reasons unrelated to test material use.

Inclusion Criteria:

a. Approximately 30 healthy female subjects, aged 18 to 79 years, inclusive;
b. Subjects who had self-perceived sensitive skin;
c. Subjects who were free of any visible skin disease which might have been confused with a reaction from the test material;
d. Subjects who completed a Medical History Form and understood and executed an Informed Consent Form; and
e. Subjects who were considered reliable and capable of following directions.

Exclusion Criteria:

a. Subjects in ill health;
b. Female subjects who were pregnant, nursing or planning a pregnancy;
c. Subjects who received medication which may have influenced the outcome of the trial; or
d. Subjects who had a history of adverse reactions to similar products.

Test Materials:

.01 NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.02 NAIL POLISH - (SHADE 18) ENG086172, PB-1063-1
.03 NAIL POLISH - (SHADE 28) ENG086175, PB-1065-1
.04 NAIL POLISH - (SHADE 38) ENG087315, CN9-59-2

Trial Schedule:

Initiation Date	Completion Date
July 14, 2017	August 11, 2017

Methodology:

Qualification (Day 0)

Subjects arrived at the Testing Facility with clean, manicured nails free of nail enamel and executed an Informed Consent Form.

Methodology (continued):

Subjects then removed any residue from the nail surface by applying nail enamel remover to each nail plate, followed by washing their hands with soap and water and drying thoroughly.

Each subject's nail cuticles and nail plates were visually examined by a trained Clinical Technician to identify any pre-existing cuticle or nail condition, which would have precluded qualification for this trial.

The trained Clinical Technician evaluated the cuticles for erythema, dryness, splitting/cracking and the nail plates for ridges, peeling and splitting using the following Evaluation Scale:

Evaluation Scale:
0=none
1=minimal
2=mild
3=moderate
4=severe

Subjects who presented a score of moderate (2) or greater for any one parameter did not qualify for the trial.

All observations were recorded on Case Report Forms.

Test Phase (Day 0)

After completion of the nail evaluations, qualified subjects were supplied with the test material and written and verbal instructions. Subjects were instructed to use the test material for 4 weeks according to the following directions:

Instructions:

Discontinue the use of your current nail enamel products, including basecoat, color nail enamel and topcoat, and use only the test material provided for the duration of the trial. Do not introduce any new hand or nail products, cleansing treatment and/or cosmetic products for the duration of the trial.

Usage Directions:

- **Wash hands with anti-bacterial soap to remove deposits that may be present on the nail.**

Methodology (continued):

- **Towel dry hands to ensure nail beds are dry before applying Nail Polish.**
- **Apply a thin coat of Nail Polish evenly on the nail.**
- **Allow 30 seconds for the Nail Polish to dry.**
- **Apply second coat of Nail Polish.**
- **Allow 30 seconds for Nail Polish to dry.**
- **Apply Nail Polish Top Coat evenly to each nail.**
- **Allow 15 minutes for Nail Polish to dry.**

Between applications remove Nail Polish by peeling off, or using your regular nail polish remover.

After using the test material, place an "X" in the box on the daily diary.

Do not let anyone else use the test material. Keep out of reach of children.

Report any adverse reactions or problems immediately to the Testing Facility staff.

To document compliance, subjects were required to maintain a daily diary of use and to return the daily diary and remaining test material at the end of the trial.

Test Phase (Week 4)

After 4 weeks of test material use, subjects returned to the Testing Facility with clean nails and received a final examination of their nail plates and cuticles by the trained Clinical Technician, as previously described.

All unused test material and daily diaries were returned to the Testing Facility at the final visit.

All daily diaries were reviewed for completeness prior to the subjects' dismissal.

All data was reviewed by a Board Certified Dermatologist.

Amendments:

OLD VERSION:

5.3 Exclusion Criteria

a. Subjects must not be in ill health, as recorded in the CPTC Medical History.

b. If female, must not be pregnant or nursing or planning a pregnancy.

c. Subjects must not be under a doctor's care or receiving medication which may influence the outcome of the study.

d. Subjects must not reported history of adverse reactions to similar formulations.

NEW VERSION:

5.3 Exclusion Criteria

a. Subjects in ill health;

b. Female subjects who are pregnant, nursing or planning a pregnancy;

c. Subjects receiving medication which may influence the outcome of the trial; or

d. Subjects who have a history of adverse reactions to similar products.

REASON FOR CHANGE: Revision to the Exclusion Criteria.

OLD VERSION:

6 Methodology

6.1 Qualification

At baseline the appropriate proposed treatment site(s) on each subject will be examined by a trained Clinical Technician for qualification. Evidence of erythema, dryness and edema or any anomaly of the test area will be evaluated and recorded on Dermatological Evaluation Forms.

Amendments (continued):

Evaluation Key:

0	None
0.5	Barely perceptible
1	Mild
2	Moderate
3	Marked
4	Severe

NEW VERSION:

6 Methodology

6.1 Qualification

Subjects will arrive at the Testing Facility with clean, manicured nails free of nail enamel and execute an Informed Consent Form (ICF) to become subjects.

The subjects will remove any residue from the nail surface by applying nail enamel remover to each nail plate. They will then wash their hands with soap and water and dry thoroughly.

Each subject's nail cuticles and nail plates will be visually examined by a trained Clinical Technician to identify any pre-existing cuticle or nail condition, which would preclude qualification for this trial.

The trained Clinical Technician will evaluate the cuticles for erythema, dryness, splitting/cracking and the nail plates for ridges, peeling and splitting using the following Evaluation Scale:

Evaluation Scale:
0=none
1=minimal
2=mild
3=moderate
4=severe

REASON FOR CHANGE: Evaluation parameters updated to correspond to the trial test area (fingernails).

Deviations: There were no deviations.

Adverse Events: There were no adverse events.

Results:

Subject demographics are presented in Table 1.

Baseline cuticle and nail plate examinations are presented in Table 2.

Final cuticle and nail plate examinations are presented in Table 3.

Summary:

Under the conditions of this trial, test materials, NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG, NAIL POLISH - (SHADE 18) ENG086172, PB-1063-1, NAIL POLISH - (SHADE 28) ENG086175, PB-1065-1 and NAIL POLISH - (SHADE 38) ENG087315, CN9-59-2, did not elicit any adverse reactions after repeated use.

Table 1

Subject Demographics

Subject Number	Initials	Age	Test Material
1	C-M	36	.01 + .02
2	NEH	55	.01 + .03
3	J-M	31	.01 + .04
4	KMB	32	.01 + .02
5	D-W	54	.01 + .03
6	EAR	61	.01 + .04
7	JMM	48	.01 + .02
8	NSD	27	.01 + .03
9	CAN	32	.01 + .04
10	D-R	40	.01 + .02
11	RAH	64	.01 + .03
12	LMP	65	.01 + .04
13	VLW	51	DNQ
14	BJL	62	.01 + .03
15	T-Z	53	.01 + .04
16	M-R	36	.01 + .02
17	DMK	52	.01 + .03
18	RMA	43	DNQ
19	MEH	65	.01 + .02
20	BLL	21	.01 + .03
21	SEE	51	.01 + .04
22	CGP	55	.01 + .02
23	A-B	41	.01 + .03
24	ADR	45	.01 + .04
25	CLC	55	.01 + .02
26	SAF	34	.01 + .03
27	D-B	50	.01 + .04
28	RLP	50	.01 + .02
29	LAM	49	.01 + .03
30	L-A	64	.01 + .04
31	MTC	48	.01 + .02
32	HAD	58	.01 + .03
33	M-A	63	.01 + .04
34	DBJ	20	.01 + .02

Did Not Qualify: Subject #'s 13 and 18
Did Not Complete: Subject #26

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.02 = NAIL POLISH - (SHADE 18) ENG086172, PB-1063-1
.03 = NAIL POLISH - (SHADE 28) ENG086175, PB-1065-1
.04 = NAIL POLISH - (SHADE 38) ENG087315, CN9-59-2

Table 2

Baseline Cuticle Evaluations

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.02 = NAIL POLISH - (SHADE 18) ENG086172, PB-1063-1

Subject #	Finger	Erythema		Dryness		Splitting/Cracking	
		Left Hand	Right Hand	Left Hand	Right Hand	Left Hand	Right Hand
1	Thumb	0	0	0	0	1	0
1	Index	0	0	0	0	0	0
1	Middle	0	0	0	0	0	0
1	Ring	0	0	0	0	0	0
1	Pinky	0	0	0	0	0	0
4	Thumb	0	0	0	1	0	1
4	Index	0	0	0	0	0	0
4	Middle	0	0	0	0	0	0
4	Ring	0	0	0	0	0	0
4	Pinky	0	0	0	0	0	0
7	Thumb	0	0	1	1	0	0
7	Index	0	0	1	1	0	0
7	Middle	0	0	1	1	0	0
7	Ring	0	0	1	1	0	0
7	Pinky	0	0	1	1	0	0
10	Thumb	0	0	0	0	0	0
10	Index	0	0	1	1	0	0
10	Middle	0	0	1	1	0	0
10	Ring	0	0	1	0	0	0
10	Pinky	0	0	0	0	0	0
16	Thumb	0	0	0	1	0	1
16	Index	1	0	0	1	0	0
16	Middle	0	0	0	0	0	0
16	Ring	0	0	0	0	0	0
16	Pinky	0	0	0	1	0	0
19	Thumb	0	0	1	1	0	0
19	Index	0	0	1	1	0	0
19	Middle	0	0	1	1	0	0
19	Ring	0	0	1	1	0	0
19	Pinky	0	0	1	1	0	0
22	Thumb	0	0	0	1	0	1
22	Index	0	0	0	0	0	0
22	Middle	0	0	1	1	1	0
22	Ring	0	0	0	0	0	0
22	Pinky	0	0	0	0	0	0
25	Thumb	0	0	1	1	0	0
25	Index	0	0	0	1	0	1
25	Middle	0	0	1	0	1	0
25	Ring	0	0	1	0	1	0
25	Pinky	0	0	0	1	0	1
28	Thumb	0	0	1	1	0	0
28	Index	0	0	1	1	0	0
28	Middle	0	0	1	1	0	0
28	Ring	0	0	1	1	0	0
28	Pinky	0	0	1	1	0	0
31	Thumb	0	0	0	0	0	0
31	Index	0	0	0	1	0	0
31	Middle	0	0	0	1	0	0
31	Ring	0	0	0	0	0	0
31	Pinky	0	0	0	0	0	0
34	Thumb	0	0	0	0	0	0
34	Index	0	0	0	0	0	0
34	Middle	0	0	0	0	0	0
34	Ring	0	0	0	0	0	0
34	Pinky	0	0	0	0	0	0

Did Not Qualify: Subject #'s 13 and 18

Table 2
(continued)

Baseline Cuticle Evaluations

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.03 = NAIL POLISH - (SHADE 28) ENG086175, PB-1065-1

Subject #	Finger	Erythema		Dryness		Splitting/Cracking	
		Left Hand	Right Hand	Left Hand	Right Hand	Left Hand	Right Hand
2	Thumb	0	0	1	0	0	0
2	Index	0	0	1	0	1	0
2	Middle	0	0	1	0	1	0
2	Ring	0	0	1	1	0	0
2	Pinky	0	0	1	1	0	0
5	Thumb	0	0	0	0	0	0
5	Index	0	0	0	0	0	0
5	Middle	0	0	0	0	0	0
5	Ring	0	0	0	0	0	0
5	Pinky	0	0	0	0	0	0
8	Thumb	0	0	0	0	0	0
8	Index	0	0	0	0	0	0
8	Middle	0	0	0	0	0	0
8	Ring	0	0	0	0	0	0
8	Pinky	0	0	0	0	0	0
11	Thumb	0	0	1	1	1	1
11	Index	0	0	1	1	0	0
11	Middle	0	0	1	1	0	1
11	Ring	0	0	1	1	0	0
11	Pinky	0	0	1	1	1	0
14	Thumb	0	0	0	0	0	0
14	Index	0	0	1	1	0	1
14	Middle	0	0	0	0	0	0
14	Ring	0	0	0	0	0	0
14	Pinky	0	0	0	0	0	0
17	Thumb	0	0	1	1	0	0
17	Index	0	0	0	0	0	0
17	Middle	0	0	0	0	1	1
17	Ring	0	0	0	0	1	0
17	Pinky	0	0	0	0	0	0
20	Thumb	0	0	1	1	0	0
20	Index	0	0	1	1	0	0
20	Middle	0	0	1	1	0	0
20	Ring	0	0	1	1	0	0
20	Pinky	0	0	1	1	0	0
23	Thumb	0	0	0	0	0	0
23	Index	0	0	1	1	0	0
23	Middle	0	0	1	1	0	0
23	Ring	0	0	1	0	0	0
23	Pinky	0	0	0	0	0	0
26	Thumb	0	0	1	1	0	0
26	Index	0	0	1	1	0	0
26	Middle	0	0	1	1	1	1
26	Ring	0	0	1	1	0	0
26	Pinky	0	0	1	1	0	0
29	Thumb	0	0	1	1	0	0
29	Index	0	0	1	1	0	1
29	Middle	0	0	1	1	1	0
29	Ring	0	0	1	1	0	0
29	Pinky	0	0	1	1	0	0
32	Thumb	0	0	1	1	0	0
32	Index	0	0	1	1	0	0
32	Middle	0	0	1	1	0	0
32	Ring	0	0	1	1	0	0
32	Pinky	0	0	1	1	0	0

Did Not Qualify: Subject #'s 13 and 18

Table 2
(continued)

Baseline Cuticle Evaluations

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.04 = NAIL POLISH - (SHADE 38) ENG087315, CN9-59-2

Subject #	Finger	Erythema		Dryness		Splitting/Cracking	
		Left Hand	Right Hand	Left Hand	Right Hand	Left Hand	Right Hand
3	Thumb	0	0	0	0	0	0
3	Index	0	0	1	0	0	0
3	Middle	0	0	1	1	0	0
3	Ring	0	0	0	0	0	0
3	Pinky	0	0	0	0	0	0
6	Thumb	0	0	1	1	0	0
6	Index	0	0	1	1	0	0
6	Middle	0	0	1	1	0	0
6	Ring	0	0	1	1	0	0
6	Pinky	0	0	1	1	0	0
9	Thumb	0	0	0	0	0	0
9	Index	0	0	0	0	0	0
9	Middle	0	0	0	1	0	0
9	Ring	0	0	0	1	0	0
9	Pinky	0	0	0	0	0	0
12	Thumb	0	0	1	0	0	0
12	Index	0	0	0	0	0	0
12	Middle	0	0	1	1	0	0
12	Ring	0	0	0	1	0	0
12	Pinky	0	0	1	0	0	0
15	Thumb	0	0	0	0	0	0
15	Index	0	0	0	1	0	0
15	Middle	0	0	0	1	0	0
15	Ring	0	0	0	0	0	0
15	Pinky	0	0	0	0	0	0
21	Thumb	0	0	0	0	0	0
21	Index	0	0	0	0	0	0
21	Middle	0	0	0	0	0	0
21	Ring	0	0	0	0	0	0
21	Pinky	0	0	0	0	0	0
24	Thumb	0	0	0	0	0	0
24	Index	0	0	1	1	1	1
24	Middle	0	0	1	1	1	1
24	Ring	0	0	1	1	1	1
24	Pinky	0	0	0	1	0	1
27	Thumb	0	0	1	1	0	0
27	Index	0	0	1	1	1	1
27	Middle	0	0	1	1	1	1
27	Ring	0	0	1	1	1	0
27	Pinky	0	0	1	1	0	0
30	Thumb	0	0	1	1	0	0
30	Index	0	0	1	1	0	0
30	Middle	0	0	1	1	0	0
30	Ring	0	0	1	1	0	0
30	Pinky	0	0	1	1	0	0
33	Thumb	0	0	1	1	0	0
33	Index	0	0	1	1	0	0
33	Middle	0	0	1	1	0	0
33	Ring	0	0	1	1	0	0
33	Pinky	0	0	1	1	0	0

Did Not Qualify: Subject #'s 13 and 18

Table 2
(continued)

Baseline Cuticle Evaluations

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.02 = NAIL POLISH - (SHADE 18) ENG086172, PB-1063-1

Subject #	Finger	Ridges		Peeling		Splitting	
		Left Hand	Right Hand	Left Hand	Right Hand	Left Hand	Right Hand
1	Thumb	0	0	0	0	0	0
1	Index	0	0	0	0	0	0
1	Middle	0	0	0	0	0	0
1	Ring	0	0	0	0	0	0
1	Pinky	0	0	0	0	0	0
4	Thumb	0	0	0	0	0	0
4	Index	0	0	0	0	0	0
4	Middle	0	0	0	0	0	0
4	Ring	0	0	0	0	0	0
4	Pinky	0	0	0	0	0	0
7	Thumb	1	1	0	0	0	0
7	Index	1	1	0	0	0	0
7	Middle	1	1	0	0	0	0
7	Ring	1	1	0	0	0	0
7	Pinky	1	1	0	0	0	0
10	Thumb	1	1	0	0	0	0
10	Index	1	1	0	0	0	0
10	Middle	1	1	0	0	0	0
10	Ring	1	1	0	0	0	0
10	Pinky	1	1	0	0	0	0
16	Thumb	1	1	0	0	0	0
16	Index	1	1	0	0	0	0
16	Middle	1	1	0	0	0	0
16	Ring	1	1	0	0	0	0
16	Pinky	1	1	0	0	0	0
19	Thumb	1	1	0	0	0	0
19	Index	1	1	0	0	0	0
19	Middle	1	1	0	0	1	0
19	Ring	1	1	0	0	0	0
19	Pinky	1	1	0	0	1	0
22	Thumb	0	0	0	0	0	0
22	Index	0	0	0	0	0	0
22	Middle	0	0	0	0	0	0
22	Ring	0	0	0	0	0	0
22	Pinky	0	0	0	0	0	0
25	Thumb	0	0	1	0	0	0
25	Index	0	0	0	0	0	0
25	Middle	0	0	0	0	0	1
25	Ring	0	0	0	0	0	1
25	Pinky	0	0	0	0	0	0
28	Thumb	1	1	0	0	0	0
28	Index	1	1	0	0	0	0
28	Middle	1	1	0	0	0	0
28	Ring	1	1	0	0	0	0
28	Pinky	1	1	0	0	0	0
31	Thumb	1	1	1	1	1	1
31	Index	1	1	1	1	1	1
31	Middle	1	1	0	0	0	0
31	Ring	1	1	1	1	1	1
31	Pinky	1	1	1	1	1	1
34	Thumb	0	0	0	0	0	0
34	Index	0	0	1	0	0	0
34	Middle	0	0	0	0	0	0
34	Ring	0	0	0	0	0	0
34	Pinky	0	0	0	0	0	0

Did Not Qualify: Subject #'s 13 and 18

Table 2
(continued)

Baseline Cuticle Evaluations

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.03 = NAIL POLISH - (SHADE 28) ENG086175, PB-1065-1

Subject #	Finger	Ridges		Peeling		Splitting	
		Left Hand	Right Hand	Left Hand	Right Hand	Left Hand	Right Hand
2	Thumb	1	1	0	0	0	0
2	Index	1	1	0	0	0	0
2	Middle	1	1	0	0	0	0
2	Ring	1	1	0	0	1	0
2	Pinky	1	1	0	0	0	0
5	Thumb	0	0	0	0	0	0
5	Index	0	0	0	0	0	0
5	Middle	0	0	0	0	0	0
5	Ring	0	0	0	0	0	0
5	Pinky	0	0	0	0	0	0
8	Thumb	0	0	0	0	0	0
8	Index	0	0	0	0	0	0
8	Middle	0	0	0	0	0	0
8	Ring	0	0	0	0	0	0
8	Pinky	0	0	0	0	0	0
11	Thumb	1	1	0	0	0	0
11	Index	1	1	0	0	0	0
11	Middle	1	1	0	0	0	0
11	Ring	1	1	0	0	0	0
11	Pinky	1	1	0	0	0	0
14	Thumb	1	1	0	0	0	1
14	Index	1	1	0	0	0	0
14	Middle	1	1	0	0	0	0
14	Ring	1	1	0	0	0	0
14	Pinky	1	1	0	0	0	0
17	Thumb	1	1	0	0	0	0
17	Index	1	1	0	0	0	0
17	Middle	1	1	0	0	0	0
17	Ring	1	1	0	0	0	0
17	Pinky	1	1	0	0	0	0
20	Thumb	0	0	0	0	0	0
20	Index	0	0	0	0	0	0
20	Middle	0	0	0	0	0	0
20	Ring	0	0	0	0	0	0
20	Pinky	0	0	0	0	0	0
23	Thumb	0	0	0	0	0	1
23	Index	0	0	0	0	0	0
23	Middle	0	0	0	0	0	0
23	Ring	0	0	0	0	0	0
23	Pinky	0	0	0	0	0	0
26	Thumb	0	0	0	0	0	0
26	Index	0	0	0	0	0	0
26	Middle	0	0	0	0	0	0
26	Ring	0	0	0	0	0	0
26	Pinky	0	0	0	0	0	0
29	Thumb	1	1	0	0	1	0
29	Index	1	1	0	0	0	0
29	Middle	1	1	0	0	0	0
29	Ring	1	1	0	0	0	0
29	Pinky	1	1	0	0	0	0
32	Thumb	1	1	0	0	1	0
32	Index	1	1	0	0	0	1
32	Middle	1	1	0	0	1	0
32	Ring	1	1	0	0	1	0
32	Pinky	1	1	0	0	0	0

Did Not Qualify: Subject #'s 13 and 18

Table 2
(continued)

Baseline Cuticle Evaluations

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.04 = NAIL POLISH - (SHADE 38) ENG087315, CN9-59-2

Subject #	Finger	Ridges		Peeling		Splitting	
		Left Hand	Right Hand	Left Hand	Right Hand	Left Hand	Right Hand
3	Thumb	0	0	0	0	0	0
3	Index	0	0	0	0	0	0
3	Middle	0	0	0	0	0	0
3	Ring	0	0	0	0	0	0
3	Pinky	0	0	0	0	0	0
6	Thumb	1	1	0	0	0	0
6	Index	0	0	0	0	0	0
6	Middle	0	0	0	0	0	0
6	Ring	1	1	0	0	0	0
6	Pinky	0	1	0	0	0	0
9	Thumb	0	0	0	0	0	0
9	Index	0	1	0	0	0	0
9	Middle	0	1	0	0	0	0
9	Ring	0	1	0	0	0	0
9	Pinky	1	0	0	0	0	0
12	Thumb	1	1	0	0	0	0
12	Index	1	1	0	0	0	0
12	Middle	1	1	0	0	0	0
12	Ring	1	1	0	0	0	0
12	Pinky	1	1	0	0	0	0
15	Thumb	0	0	0	0	0	0
15	Index	0	0	0	0	0	0
15	Middle	0	0	0	0	0	0
15	Ring	0	0	0	0	0	0
15	Pinky	0	0	0	0	0	0
21	Thumb	0	0	0	0	0	0
21	Index	0	0	0	0	0	0
21	Middle	0	0	0	0	0	0
21	Ring	0	0	0	0	0	0
21	Pinky	0	0	0	0	0	0
24	Thumb	0	0	0	0	0	0
24	Index	0	0	0	0	0	0
24	Middle	0	0	0	0	0	0
24	Ring	0	0	0	0	0	0
24	Pinky	0	0	0	0	0	0
27	Thumb	1	1	0	0	0	0
27	Index	0	0	0	0	0	0
27	Middle	0	0	0	0	0	0
27	Ring	1	0	0	0	0	0
27	Pinky	1	0	0	0	1	0
30	Thumb	1	1	0	1	0	0
30	Index	1	1	0	0	0	0
30	Middle	1	1	0	0	0	0
30	Ring	1	1	0	0	0	0
30	Pinky	1	1	0	0	0	0
33	Thumb	1	1	0	0	0	0
33	Index	1	1	0	0	0	0
33	Middle	1	1	0	0	0	0
33	Ring	1	1	0	0	0	0
33	Pinky	1	1	0	0	0	0

Did Not Qualify: Subject #'s 13 and 18

Table 3

Final Cuticle Evaluations

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.02 = NAIL POLISH - (SHADE 18) ENG086172, PB-1063-1

Subject #	Finger	Erythema		Dryness		Splitting/Cracking	
		Left Hand	Right Hand	Left Hand	Right Hand	Left Hand	Right Hand
1	Thumb	0	0	0	0	0	0
1	Index	0	0	0	0	0	0
1	Middle	0	0	0	0	0	0
1	Ring	0	0	0	0	0	0
1	Pinky	0	0	0	0	0	0
4	Thumb	0	0	0	0	0	0
4	Index	0	0	0	0	0	0
4	Middle	0	0	0	0	0	0
4	Ring	0	0	0	0	0	0
4	Pinky	0	0	0	0	0	0
7	Thumb	0	0	0	0	1	1
7	Index	0	0	0	1	0	1
7	Middle	0	0	0	1	0	1
7	Ring	0	0	0	1	0	1
7	Pinky	0	0	1	0	1	0
10	Thumb	0	0	0	1	0	1
10	Index	0	0	0	1	0	1
10	Middle	0	0	0	0	0	0
10	Ring	0	0	0	0	0	0
10	Pinky	0	0	0	1	1	1
16	Thumb	0	0	1	1	0	0
16	Index	0	0	0	0	0	0
16	Middle	0	0	1	0	0	0
16	Ring	0	0	0	1	0	0
16	Pinky	0	0	0	0	0	0
19	Thumb	0	0	0	0	0	0
19	Index	0	0	0	0	0	0
19	Middle	0	0	0	0	0	0
19	Ring	0	0	0	0	0	0
19	Pinky	0	0	0	0	0	0
22	Thumb	0	0	0	0	0	0
22	Index	0	0	0	0	0	0
22	Middle	0	0	0	0	0	1
22	Ring	0	0	1	0	1	0
22	Pinky	0	0	0	0	0	0
25	Thumb	0	0	0	0	0	0
25	Index	0	0	0	0	0	0
25	Middle	0	0	0	0	0	0
25	Ring	0	0	0	0	0	0
25	Pinky	0	0	0	0	0	0
28	Thumb	0	0	1	0	0	0
28	Index	0	0	0	0	0	0
28	Middle	0	0	0	1	0	0
28	Ring	0	0	0	0	0	0
28	Pinky	0	0	0	0	0	0
31	Thumb	0	0	0	0	0	0
31	Index	0	0	0	0	0	0
31	Middle	0	0	0	0	0	0
31	Ring	0	0	0	0	0	0
31	Pinky	0	0	0	0	0	0
34	Thumb	0	0	0	0	0	0
34	Index	0	0	0	0	0	0
34	Middle	0	0	0	0	0	0
34	Ring	0	0	0	0	0	0
34	Pinky	0	0	0	0	0	0

Did Not Qualify: Subject #'s 13 and 18
Did Not Complete: Subject #26

Table 3
(continued)

Final Cuticle Evaluations

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.03 = NAIL POLISH - (SHADE 28) ENG086175, PB-1065-1

Subject #	Finger	Erythema		Dryness		Splitting/Cracking	
		Left Hand	Right Hand	Left Hand	Right Hand	Left Hand	Right Hand
2	Thumb	0	0	0	0	0	0
2	Index	0	0	1	1	0	0
2	Middle	0	0	1	1	0	0
2	Ring	0	0	0	0	0	0
2	Pinky	0	0	0	0	0	0
5	Thumb	0	0	0	0	0	0
5	Index	0	0	0	0	0	0
5	Middle	0	0	0	0	0	0
5	Ring	0	0	0	0	0	0
5	Pinky	0	0	0	0	0	0
8	Thumb	0	0	0	0	0	0
8	Index	0	0	0	0	0	0
8	Middle	0	0	0	0	0	0
8	Ring	0	0	0	0	0	0
8	Pinky	0	0	0	0	0	0
11	Thumb	0	0	0	1	0	0
11	Index	0	0	0	0	0	0
11	Middle	0	0	0	0	0	0
11	Ring	0	0	0	0	0	0
11	Pinky	0	0	0	0	0	0
14	Thumb	0	0	0	0	0	0
14	Index	0	0	0	1	0	0
14	Middle	0	0	0	0	0	0
14	Ring	0	0	0	0	0	0
14	Pinky	0	0	1	0	1	0
17	Thumb	0	0	1	1	1	1
17	Index	0	0	1	1	0	0
17	Middle	0	0	1	1	0	0
17	Ring	0	0	0	1	0	0
17	Pinky	0	0	0	0	0	0
20	Thumb	0	0	0	0	0	0
20	Index	0	0	0	0	0	0
20	Middle	0	0	0	0	0	0
20	Ring	0	0	0	0	0	0
20	Pinky	0	0	0	0	0	0
23	Thumb	0	0	1	1	1	1
23	Index	0	0	0	0	0	0
23	Middle	0	0	0	0	0	0
23	Ring	0	0	0	0	0	0
23	Pinky	0	0	0	0	0	0
29	Thumb	0	0	0	0	0	0
29	Index	0	0	0	0	0	0
29	Middle	0	0	1	0	0	0
29	Ring	0	0	0	0	0	0
29	Pinky	0	0	0	0	0	0
32	Thumb	0	0	0	0	0	0
32	Index	0	0	0	0	0	0
32	Middle	0	0	0	0	0	0
32	Ring	0	0	0	0	0	0
32	Pinky	0	0	0	0	0	0

Did Not Qualify: Subject #'s 13 and 18
Did Not Complete: Subject #26

Table 3
(continued)

Final Cuticle Evaluations

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.04 = NAIL POLISH - (SHADE 38) ENG087315, CN9-59-2

Subject #	Finger	Erythema		Dryness		Splitting/Cracking	
		Left Hand	Right Hand	Left Hand	Right Hand	Left Hand	Right Hand
3	Thumb	0	0	0	0	0	0
3	Index	0	0	0	0	0	0
3	Middle	0	0	0	0	0	0
3	Ring	0	0	0	0	0	0
3	Pinky	0	0	0	0	0	0
6	Thumb	0	0	0	1	0	0
6	Index	0	0	1	1	0	0
6	Middle	0	0	1	0	0	0
6	Ring	0	0	0	0	0	0
6	Pinky	0	0	0	0	0	0
9	Thumb	0	0	0	0	0	0
9	Index	0	0	0	1	0	1
9	Middle	0	0	0	0	0	0
9	Ring	0	0	0	0	0	0
9	Pinky	0	0	0	0	0	0
12	Thumb	0	0	0	0	0	0
12	Index	0	0	0	0	0	0
12	Middle	0	0	0	0	0	0
12	Ring	0	0	0	0	0	0
12	Pinky	0	0	0	0	0	0
15	Thumb	0	0	0	0	0	0
15	Index	0	0	0	0	0	0
15	Middle	0	0	0	0	0	0
15	Ring	0	0	0	0	0	0
15	Pinky	0	0	0	0	0	0
21	Thumb	0	0	0	0	0	0
21	Index	0	0	0	0	0	0
21	Middle	0	0	0	0	0	0
21	Ring	0	0	0	0	0	0
21	Pinky	0	0	0	0	0	0
24	Thumb	0	0	0	0	0	0
24	Index	0	0	0	0	0	0
24	Middle	0	0	0	0	0	0
24	Ring	0	0	0	0	0	0
24	Pinky	0	0	0	0	1	0
27	Thumb	0	0	1	1	0	0
27	Index	0	0	1	1	1	0
27	Middle	0	0	1	1	0	0
27	Ring	0	0	1	1	1	0
27	Pinky	0	0	0	0	0	0
30	Thumb	0	0	0	0	0	0
30	Index	0	0	0	0	0	0
30	Middle	0	0	0	0	0	0
30	Ring	0	0	0	0	0	0
30	Pinky	0	0	0	0	0	0
33	Thumb	0	0	0	0	0	0
33	Index	0	0	1	0	0	0
33	Middle	0	0	0	1	0	0
33	Ring	0	0	0	0	0	0
33	Pinky	0	0	0	1	0	0

Did Not Qualify: Subject #'s 13 and 18
Did Not Complete: Subject #26

Table 3
(continued)

Final Nail Plate Evaluations

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.02 = NAIL POLISH - (SHADE 18) ENG086172, PB-1063-1

Subject #	Finger	Ridges		Peeling		Splitting	
		Left Hand	Right Hand	Left Hand	Right Hand	Left Hand	Right Hand
1	Thumb	0	0	4	4	0	0
1	Index	0	0	3	1	0	0
1	Middle	0	0	3	3	0	0
1	Ring	0	0	4	3	0	0
1	Pinky	0	0	3	3	0	0
4	Thumb	0	0	0	0	0	0
4	Index	0	0	0	0	0	0
4	Middle	0	0	0	0	0	0
4	Ring	0	0	0	0	0	0
4	Pinky	0	0	0	0	0	0
7	Thumb	1	1	0	0	0	0
7	Index	1	1	0	0	0	0
7	Middle	1	1	0	0	0	0
7	Ring	1	1	0	0	0	0
7	Pinky	1	1	0	0	0	0
10	Thumb	1	1	0	0	0	0
10	Index	1	1	0	0	0	0
10	Middle	1	1	0	0	0	0
10	Ring	1	1	0	0	0	0
10	Pinky	1	1	0	0	0	0
16	Thumb	1	1	0	0	0	0
16	Index	1	1	0	0	0	0
16	Middle	1	1	0	0	0	0
16	Ring	1	1	0	0	0	0
16	Pinky	1	1	0	0	0	0
19	Thumb	1	1	0	0	0	0
19	Index	1	1	0	0	0	0
19	Middle	1	1	0	0	0	0
19	Ring	1	1	0	0	1	0
19	Pinky	1	1	0	0	1	0
22	Thumb	0	0	0	0	0	0
22	Index	0	0	0	1	0	1
22	Middle	0	0	0	0	0	0
22	Ring	0	0	0	0	0	0
22	Pinky	0	0	0	1	0	0
25	Thumb	0	0	1	0	0	0
25	Index	0	0	0	0	0	0
25	Middle	0	0	1	0	0	0
25	Ring	0	0	0	0	0	0
25	Pinky	0	0	1	1	0	0
28	Thumb	1	1	0	0	1	0
28	Index	1	1	0	0	0	0
28	Middle	1	1	0	0	0	0
28	Ring	1	1	0	0	0	0
28	Pinky	1	1	0	0	0	0
31	Thumb	1	1	0	0	0	0
31	Index	1	1	0	0	0	0
31	Middle	1	1	0	0	0	0
31	Ring	1	1	0	0	0	0
31	Pinky	1	1	0	0	0	0
34	Thumb	0	0	0	0	0	0
34	Index	0	0	0	0	0	0
34	Middle	0	0	0	0	0	0
34	Ring	0	0	0	0	0	0
34	Pinky	0	0	0	0	0	0

Did Not Qualify: Subject #'s 13 and 18
Did Not Complete: Subject #26

Table 3
(continued)

Final Nail Plate Evaluations

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.03 = NAIL POLISH - (SHADE 28) ENG086175, PB-1065-1

Subject #	Finger	Ridges		Peeling		Splitting	
		Left Hand	Right Hand	Left Hand	Right Hand	Left Hand	Right Hand
2	Thumb	1	1	0	0	0	0
2	Index	1	1	0	0	0	0
2	Middle	1	1	0	0	0	0
2	Ring	1	1	0	0	0	0
2	Pinky	1	1	0	0	0	0
5	Thumb	0	0	0	0	0	0
5	Index	0	0	0	0	0	0
5	Middle	0	0	0	0	0	0
5	Ring	0	0	0	0	0	0
5	Pinky	0	0	0	0	0	0
8	Thumb	0	0	0	0	0	0
8	Index	0	0	0	0	0	0
8	Middle	0	0	0	0	0	0
8	Ring	0	0	0	0	0	0
8	Pinky	0	0	0	0	0	0
11	Thumb	1	1	0	0	0	0
11	Index	1	1	0	0	0	0
11	Middle	1	1	0	0	0	0
11	Ring	1	1	0	0	0	0
11	Pinky	1	1	0	0	0	0
14	Thumb	1	1	0	0	0	0
14	Index	1	1	0	0	0	0
14	Middle	1	1	0	0	0	0
14	Ring	1	1	0	0	0	0
14	Pinky	1	1	0	0	0	0
17	Thumb	1	1	0	0	0	0
17	Index	1	1	0	0	0	0
17	Middle	1	1	0	0	0	0
17	Ring	1	1	0	0	0	0
17	Pinky	1	1	0	0	0	0
20	Thumb	0	0	0	0	0	0
20	Index	0	0	0	0	0	0
20	Middle	0	0	0	0	0	0
20	Ring	0	0	0	0	0	0
20	Pinky	0	0	0	0	0	0
23	Thumb	0	0	0	0	0	0
23	Index	0	0	0	0	0	0
23	Middle	0	0	0	0	0	0
23	Ring	0	0	0	0	0	0
23	Pinky	0	0	0	0	0	0
29	Thumb	1	1	0	0	1	0
29	Index	1	1	0	0	0	0
29	Middle	1	1	0	0	0	0
29	Ring	1	1	0	0	0	0
29	Pinky	1	1	0	0	0	0
32	Thumb	1	1	0	0	0	0
32	Index	1	1	0	0	0	0
32	Middle	1	1	0	0	0	0
32	Ring	1	1	0	0	0	0
32	Pinky	1	1	0	0	0	0

Did Not Qualify: Subject #'s 13 and 18
Did Not Complete: Subject #26

Table 3
(continued)

Final Nail Plate Evaluations

.01 = NAIL POLISH TOP COAT - ENG086731, CN1-18-4ENG
.04 = NAIL POLISH - (SHADE 38) ENG087315, CN9-59-2

Subject #	Finger	Ridges		Peeling		Splitting	
		Left Hand	Right Hand	Left Hand	Right Hand	Left Hand	Right Hand
3	Thumb	0	0	0	0	0	0
3	Index	0	0	0	0	0	0
3	Middle	0	0	0	0	0	0
3	Ring	0	1	0	0	0	0
3	Pinky	0	0	0	0	0	0
6	Thumb	1	1	0	0	0	0
6	Index	1	1	0	0	0	0
6	Middle	1	1	0	0	0	0
6	Ring	1	1	0	0	0	0
6	Pinky	1	1	0	0	0	0
9	Thumb	0	0	0	0	0	0
9	Index	1	1	0	0	0	0
9	Middle	1	1	0	0	0	0
9	Ring	1	1	0	0	0	0
9	Pinky	1	1	0	0	0	0
12	Thumb	1	1	0	0	0	0
12	Index	1	1	0	0	0	0
12	Middle	1	1	0	0	0	0
12	Ring	1	1	0	0	0	0
12	Pinky	1	1	0	0	0	0
15	Thumb	0	0	0	0	0	0
15	Index	0	0	0	0	0	0
15	Middle	0	0	0	0	0	0
15	Ring	0	0	0	0	0	0
15	Pinky	0	0	0	0	0	0
21	Thumb	0	0	0	0	0	0
21	Index	0	0	0	0	0	0
21	Middle	0	0	0	0	0	0
21	Ring	0	0	0	0	0	0
21	Pinky	0	0	0	0	1	0
24	Thumb	0	0	0	0	0	0
24	Index	0	0	0	0	0	0
24	Middle	0	0	0	0	0	0
24	Ring	0	0	0	0	0	0
24	Pinky	0	0	0	0	0	0
27	Thumb	1	1	0	0	0	0
27	Index	1	1	0	0	0	0
27	Middle	1	1	0	0	0	0
27	Ring	1	1	0	0	0	0
27	Pinky	1	1	0	0	0	0
30	Thumb	1	1	0	0	0	0
30	Index	1	1	0	0	0	0
30	Middle	1	1	0	0	0	0
30	Ring	1	1	0	0	0	0
30	Pinky	1	1	0	0	0	0
33	Thumb	1	1	0	0	0	0
33	Index	1	1	0	0	0	0
33	Middle	1	1	0	0	0	0
33	Ring	1	1	0	0	0	0
33	Pinky	1	1	0	0	0	0

Did Not Qualify: Subject #'s 13 and 18
Did Not Complete: Subject #26

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: February 24, 2021

SUBJECT: Equisetum Arvense Extract

Anonymous. 2020. Human repeated insult patch test (product containing 0.60% Equisetum Arvense Extract).

[redacted]

T E S T R E P O R T
[redacted]

HUMAN REPEATED INSULT PATCH TEST (HRIPT) STUDY REPORT

[redacted] **MASK, F#**[redacted]

contains 0.60% Equisetum Arvense Extract

[redacted]

FEBRUARY 2020

[redacted]

HUMAN REPEATED INSULT PATCH TEST
(HRIPT)
STUDY REPORT

PRODUCT MANUFACTURED BY	:	[redacted]
RECEIPT DATE	:	10/01/2020
STUDY PERIOD	:	13/01/2020 - 21/02/2020
LAB ID	:	[redacted]
PRODUCT NAME	:	[redacted] MASK, F[redacted]
BRAND	:	[redacted]
LOT	:	NOT LISTED
STUDY SPONSOR	:	[redacted]
METHOD	:	Human Repeated Insult Patch Test

ASSESSMENT OF DERMAL SENSITIZATION POTENTIAL OF A PREPARATION
HUMAN REPEATED INSULT PATCH TEST ON 100 VOLUNTEERS

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[redacted]

[redacted]

REGULATORY, CONFIDENTIALITY AND ARCHIVING

Regulatory

The study has been conducted by suitably trained, qualified and experienced personnel in accordance with the Declaration of Helsinki (1964) and subsequent revisions (World Medical Association, 1989, Council for International Organizations of Medical Sciences and the World Health Organization, 1993) and taking into consideration the requirements of Directives 2001/20/EC and 2005/20/EC relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use and the COLIPA Guidelines edited on 1997 for the "Product Test Guidelines for the Assessment of Human Skin Compatibility".

Precautions have been taken to avoid the possibility that participants in the study might experience undesirable effects.

Ethical requirements which have been taken into consideration in the planning of the study include:
i) participants are informed volunteers, selected after application of inclusion/non inclusion criteria
ii) participants are aware of the purpose and nature of the study and of any foreseeable risks involved in participation in the study and have given written informed consent before the study starts
iii) a safety evaluation has been conducted on the product tested, before the study starts
iv) the test procedures conforms to national regulations
v) the Ethical Review Committee include medical, non-medical, appropriate experts and lay members; it has consider the general ethics of the test and verified that the safety and integrity of the participants in the test are protected, taking into account information on the ingredient(s)
vi) all reasonable care has been taken to avoid causing excessive skin reactions or other adverse health effects in the participants during the study
vii) safety procedures are in place in the event of any unexpected/adverse reactions, including appropriate medical cover
viii) volunteers are rewarded for their time, inconvenience, etc., but the reward is not so great that it would persuade them to participate.

Confidentiality

Requirements of Law 2472/1997 on the Protection of Individuals with regard to the processing of personal data are taken into consideration. Processing of volunteers personal data is carried out by doctors or other persons rendering medical services, provided that the Controller is bound by medical confidentiality or other obligation of professional secrecy, provided for in Law or code of practice, and data are neither transferred nor disclosed to third parties. Processing is carried out within the laboratory premises and relates to personal data of the volunteers, provided that the latter have given their consent and that such data are neither transferred nor disclosed to third parties. The anonymity of the volunteers is respected within all studies carried out in our laboratories. Each volunteer can be identified by the Investigator, the doctors and all the persons in charge of the study, thanks to his personal volunteer's code.

Archiving

The laboratory book which contains all the information (raw data and results) regarding the study and the study reports are kept in the laboratory archives during 2 years.

[redacted]

[redacted]

TYPE AND OBJECTIVE OF THE STUDY

The purpose of this study is to determine the dermal sensitization potential of a product. The HRIPT is performed to confirm the safe use of potentially sensitizing substances in consumer products, preparations such as cosmetics or household products.

PANEL STUDIED, INCLUSION / NON INCLUSION CRITERIA

Number of volunteers
A number of 100 volunteers has been recruited to satisfy the objectives of the test.

Panel characteristics
Volunteers are selected on the basis of inclusion and non-inclusion criteria. The volunteers satisfy all the inclusion criteria and are not in conflict with any of the non-inclusion criteria and had a medical examination (health certificate) and a dermatological examination. The volunteers are clearly informed, verbally and in writing, regarding the nature of the study, the timetable, constraints and possible risks. They give their written informed consent before participation in the study.

Inclusion criteria

- ✓ Informed volunteers who agree to follow the conditions specified
- ✓ where appropriate of relevant age : 18-70 years old
- ✓ where appropriate of relevant gender : female and/or male
- ✓ where appropriate of relevant origin and health
- ✓ free from any dermatological problems on the area studied
- ✓ meet the specific study criteria on skin type
- ✓ proof of home address & social security number
- ✓ able to understand the Greek language and the study requirements

Non inclusion criteria

- ✓ volunteers who does not meet the inclusion criteria
- ✓ pregnancy or nursing condition
- ✓ irritated skin on test site(s)
- ✓ blemishes, marks (e.g. tattoos, scars, sunburn) on the test site(s)
- ✓ medication which may affect skin response and/or past medical history
- ✓ presenting skin pathology which may interfere with the aim(s) of the study
- ✓ presenting contact allergy to one of the ingredients of the tested product
- ✓ participation in another simultaneous study
- ✓ participation in a previous study without an appropriate rest period between studies
- ✓ minors or majors protected by the law and people admitted in a sanitary or social institution for other purpose than research
- ✓ persons deprived of liberty by legal or administrative decision, patients in emergency situation
- ✓ volunteers who refused to give their free and informed consent.

Study constraints

During the length of the study, the volunteers are asked:

- ✓ Not to put any product, also water on the patches area.
- ✓ Not to have a bath, neither to expose themselves to UV.
- ✓ To avoid all intense sportive activities that could remove the patch.
- ✓ Not to take aspirin, anti-histaminics, corticoids, anti-inflammatories and any other treatment decreasing or avoiding inflammations or allergies or interfering with the skin metabolism.

Volunteers withdrawals

Participants will be withdrawn for the following reasons:

- ✓ they do not follow the conditions of the Study Information Sheet;
- ✓ they suffer any illness or accident or develop any condition during the study which could affect the outcome of the study;
- ✓ they no longer wish to participate in the study.

[redacted]

[redacted]

METHOD PRINCIPLE

Human Repeated Insult Patch Test (HRIPT)

The methodology used by the laboratory is an adaptation from that described by Marzulli and Maibach Human 'Repeated Insult Patch Test for delayed contact hypersensitivity: Marzulli F.N., Maibach H.I., Contact allergy : predictive testing in man, Contact Dermatitis, '1976, 2, pp. 1-17.

Table 1. Methodology used

Test	No Subjects	Induction Site	No of exposures	Duration of exposure (h)	Frequency of exposure	Rest (days)	Challenge
Adaptation of the Modified Draize human sensitisation test	min 100	Lower or upper back	9	48	Continuous	14	48 h patch test (followed by evaluations at 0, 24, 48, 72 and 96 hours)

According to the protocol the products to be tested are applied on 100 volunteer test subjects. The application is effected under occlusive conditions by the application of patches for a defined period of time. The applications are repeated 9 times on the same site (induction site) over a period of 3 consecutive weeks, period necessary to induce a possible allergy (induction phase).

After a minimal 2-week (rest period) with no product application, a single application of the product is effected, again under patch, to the induction site and to a virgin site and for a defined time, enabling to reveal a possible induced allergy (challenge).

A skin examination of the application site is performed by the dermatologist before the 1st product application of the induction phase, after each patch removal, the application of the challenge and its removal.

Table 1. HRIPT time table

<table>
<tr><th>W1</th><th>W2</th><th>W3</th><th>W4</th><th>W5</th><th>W6</th></tr>
<tr><td colspan="3">Induction phase</td><td colspan="2">Rest Period</td><td rowspan="2">Challenge</td></tr>
<tr><td colspan="3" rowspan="2">The applications are repeated 9 times on the same site (induction site) over a period of 3 consecutive weeks, period necessary to induce a possible allergy.</td><td colspan="2" rowspan="2">No product application</td></tr>
<tr><td>Single application</td></tr>
</table>

[redacted]

EQUIPMENT

The equipment used for the occluded patch is composed of a small plastic cavity of 0.64 cm^2 with a filter tissue at the bottom which is made to receive the product to test. All this is fixed to a hypoallergenic non woven adhesive tape.

DOSE LEVEL

The amount of test material applied to each patch 0.02ml is sufficient to fill the chamber and saturate the pad without overflowing from it when applied to the skin.

TEST MATERIAL APPLICATION

The area on which the patch is applied is previously cleaned up with demineralised water and dried with cellulose cotton wool tissue.

The patches are put on the back of the volunteer.

The products are tested pure or diluted depending on their type and their use.

- ✓ Mostly, the products are tested pure.
- ✓ Rinse-off products are tested diluted at 5%.
- ✓ Detergents are tested diluted at 10%.
- ✓ Hydrophilic products are diluted in demineralised water
- ✓ Lipophilic products are diluted in mineral oil.
- ✓ Powders are put pure in the patch small cavity and then moistened sufficiently with a drop of mineral oil in order to ensure good contact with the skin and avoid the product dispersion while applying the patch.

The patches thus prepared are left in contact 48 hours.

NEGATIVE CONTROLS

Whilst this activity is always be on a case-by-case basis and will depend on the nature and type of study, the most common approach is to compare the results obtained for the test materials with those of suitable positive and/or negative controls, or with similar materials.

A "negative" control is a patch without any product, applied in the same conditions as the product to be tested:

- ✓ if the product is tested pure: empty patch.
- ✓ if the product is tested diluted: patch with 0.02ml of the solvent used (demineralised water or mineral oil).

VISUAL ASSESSMENT

Treatment sites are assessed before the first application of test material (baseline) and after treatment at 30 minutes after patch removal during the induction period.

After a rest period of 2 weeks a patch was applied on a previously unpatched and patched skin site. This site was evaluated on 30 minutes, 24, 48, 72 and 96 hours after removal.

Skin reactions are scored throughout the test by the same experienced assessor who made the baseline assessment and under the same lighting source, following a pre-defined irritation and sensitization scoring scales.

[redacted]

RECORDINGS

EXAMPLE OF SCORING SCALE

ERYTHEMA

0 = no evidence of erythema

0.5 = minimal or doubtful erythema

1 = slight redness, spotty and diffuse

2 = moderate, uniform redness

3 = strong uniform redness

4 = fiery redness

DRYNESS (SCALING)

0 = no evidence of scaling

0.5 = dry without scaling; appears smooth and taut

1 = fine/mild scaling

2 = moderate scaling

3 = severe scaling with large flakes

OEDEMA

- = absence of oedema

+ = presence of oedema

EXAMPLE OF SENSITISATION SCORING SCALE

According to the I.C.D.R.G. (International Contact Dermatitis Research Group).

-	Negative
+?	Doubtful reaction
+	Weak reaction
++	Strong reaction
+++	Extreme
NT	Not tested
IR	Irritant reaction of different types

In case of negative evidence of any effect, the indication "-" is recorded.

[redacted]

TEST MATERIAL

DISTRIBUTOR	:	[redacted]
PRODUCT MANUFACTURED BY	:	[redacted]
RECEIPT DATE	:	10/01/2020
STUDY PERIOD	:	13/01/2020 - 21/02/2020
LAB ID	:	[redacted]
PRODUCT NAME	:	[redacted] MASK, F#[redacted]
BRAND	:	[redacted]
PRODUCT TYPE	:	RINSED, HAIR MASK
LOT	:	NOT LISTED
STUDY SPONSOR	:	[redacted]
TEST METHOD	:	Human Repeated Insult Patch Test
PANEL	:	100 healthy adult volunteers
APPLICATION AREA	:	On the back
QUANTITY OF PRODUCT	:	0.02 ml

Panel description

This study included 100 healthy adult volunteers.
A number of 100 subjects satisfactorily completed the test procedure.
Subject skin characteristics are described on the results table.
None of the volunteers selected took a treatment contraindicated with the study.

Study withdrawals

No withdrawal occurred.
No irritation or sensitization reactions occurred on the subjects that decided to withdraw.

Skin reactions

No skin reaction was noticed by the dermatologist on the reference area for all subjects.

Skin reactions

Results obtained for each volunteer.

[redacted]

RESULTS

In case of negative evidence of any effect, the indication "-" is recorded.

VOL ID	VOLUNTEER CODE	SEX	AGE	SKIN	Events occurred during the applications of induction period																																		Allergic reaction ICDRG scale			
					1				2				3				4				5				6				7				8				9							
					E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A
1	1004	F	55	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	1028	F	51	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	1029	F	45	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	1115	F	44	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	1130	F	33	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	1141	F	50	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	1182	F	38	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	1191	F	27	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	1202	F	57	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	1248	F	33	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	1265	F	50	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	1283	M	43	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	1284	F	54	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	1287	F	29	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	1292	M	38	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	1298	M	36	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	1317	F	31	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	1324	M	27	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	1329	M	37	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	1338	F	42	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	1359	F	63	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	1390	F	32	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	1404	F	30	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	1414	F	31	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	1416	F	45	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	1442	F	26	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	1445	M	39	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	1470	F	66	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	1471	F	44	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	1473	F	34	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	1476	F	45	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	1533	F	26	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	1546	F	32	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	1548	F	61	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	1562	F	31	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	1568	F	30	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	1572	F	50	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	1573	F	36	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	1577	F	33	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	1591	F	22	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	1593	F	24	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	1611	F	44	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	1612	F	26	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	1618	F	34	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	1625	M	41	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	1667	F	24	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	1669	F	32	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	1684	F	48	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	1685	F	22	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	1688	F	58	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	1705	F	33	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	1729	F	55	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
53	1731	F	58	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
54	1734	F	69	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55	1741	F	37	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
56	1750	F	20	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57	1760	F	64	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58	1793	F	20	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[redacted]

[redacted]

RESULTS (continued)

VOL ID	VOLUNTEER CODE	SEX	AGE	SKIN	Events occurred during the applications of induction period																																					
					1				2				3				4				5				6				7				8				9				Allergic reaction ICDRG scale			
					E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A	E	D	O	A
59	1800	F	47	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	1832	F	35	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
61	1847	F	39	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62	1865	F	39	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
63	1887	F	33	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
64	1888	F	26	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
65	1903	F	53	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66	1925	F	51	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
67	1928	M	28	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
68	1971	F	61	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
69	1991	F	48	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70	2000	F	28	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
71	2004	F	28	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
72	2016	F	25	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
73	2020	F	25	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
74	2021	F	36	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75	2033	M	27	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
76	2042	F	53	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
77	2044	F	51	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
78	2049	M	38	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
79	2052	F	56	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	2060	F	32	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
81	2079	F	47	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
82	2080	F	23	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
83	2104	F	18	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
84	2108	F	27	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
85	2125	F	23	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
86	2139	F	24	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	2141	F	25	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	2142	F	61	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89	2166	F	63	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	2167	F	46	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
91	2184	F	61	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
92	2187	F	65	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
93	2188	F	52	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
94	2200	F	22	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
95	2210	M	29	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
96	2218	F	43	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
97	2222	F	28	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
98	2233	F	25	Sensitive skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
99	2234	F	21	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	2235	F	47	Normal skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: *E = Erythema, D = Dryness (scaling), O = Oedema, A = Allergic reaction*

The number of volunteers that presented a slightly irritant reaction was 0 percent (0%).
On 0 volunteers (0%) noticed slight erythema & dryness.
In this clinical study there was no evidence of oedema.

[redacted]

[redacted]

STUDY SUMMARY / ABSTRACT

ASSESSMENT OF DERMAL SENSITIZATION POTENTIAL OF A PREPARATION HUMAN REPEATED INSULT PATCH TEST ON 100 VOLUNTEERS

DISTRIBUTOR	:	[redacted]
PRODUCT MANUFACTURED BY	:	[redacted]
RECEIPT DATE	:	10/01/2020
STUDY PERIOD	:	13/01/2020 - 21/02/2020
LAB ID	:	[redacted]
PRODUCT NAME	:	[redacted] MASK, F#[redacted]
BRAND	:	[redacted]
PRODUCT TYPE	:	RINSED, HAIR MASK
LOT	:	NOT LISTED
STUDY SPONSOR	:	[redacted]
TEST METHOD	:	Human Repeated Insult Patch Test
PANEL	:	100 healthy adult volunteers
APPLICATION AREA	:	On the back
QUANTITY OF PRODUCT	:	0.02 ml

METHODOLOGY ABSTRACT : Treatment sites are assessed before the first application of test material (baseline). Negative controls are used to facilitate evaluation. In an induction phase, a three times weekly exposure over three weeks is performed by subjecting the volunteers to a continuous patch exchange. The patches are reapplied to the same site, and only if moderate inflammation has developed, the next patch is moved to an adjacent skin site. After a resting phase of two weeks, challenge is performed on naive skin. In the challenge phase, single exposure is performed and potential skin reactions observed. Ref: Adaptation of the Modified Draize human sensitisation test, Marzulli and Maibach, 1973 and 1974.

RESULT : Throughout the study, the product induced no reaction or irritation. The number of volunteers that presented an allergic reaction was 0 percent (0%).

CONCLUSION : According to the experimental conditions of the study, the test product, can be considered as “Non Sensitizing”, or “Hypoallergenic” or “Formulated to minimize the risks of allergy under normal way of use”.

The samples will be stored by the laboratory during 1 month from the end test date.
The study report and raw data will be stored by the laboratory during 2 years.

[redacted]

[redacted]

DISCUSSION AND CONCLUSION

In the experimental conditions, after assessment of the skin reactions before the first application, during the induction period and after the challenge phase, on 100 healthy adult volunteers and according to the scale used for the interpretation of the results, the [redacted] MASK, F#[redacted] can be considered as “Non Sensitizing”, or “Hypoallergenic” or “Formulated to minimize the risks of allergy under normal way of use”.

Investigating doctor : [redacted]

Printed name : [redacted]

Date : 21/02/2020

[redacted]

Dermatologist - Venereologist

RESULTS AUTHENTICITY

The study concerned by this report was carried out under my responsibility, according to the experimental protocol and the quality plan of the QACS Ltd laboratory, and follows the good clinical practices.
All the observations and data recorded during this trial are reported in this study report.

I certify the rereading of this report and do agree with its content

Study Manager : [redacted]

Printed name : [redacted]

Date : 21/02/2020