Safety Assessment of Glucosamine Ingredients as Used in Cosmetics

Status:	Draft Tentative Report for Panel Review
Release Date:	February 11, 2022
Panel Meeting Date:	March 7 – 8, 2022

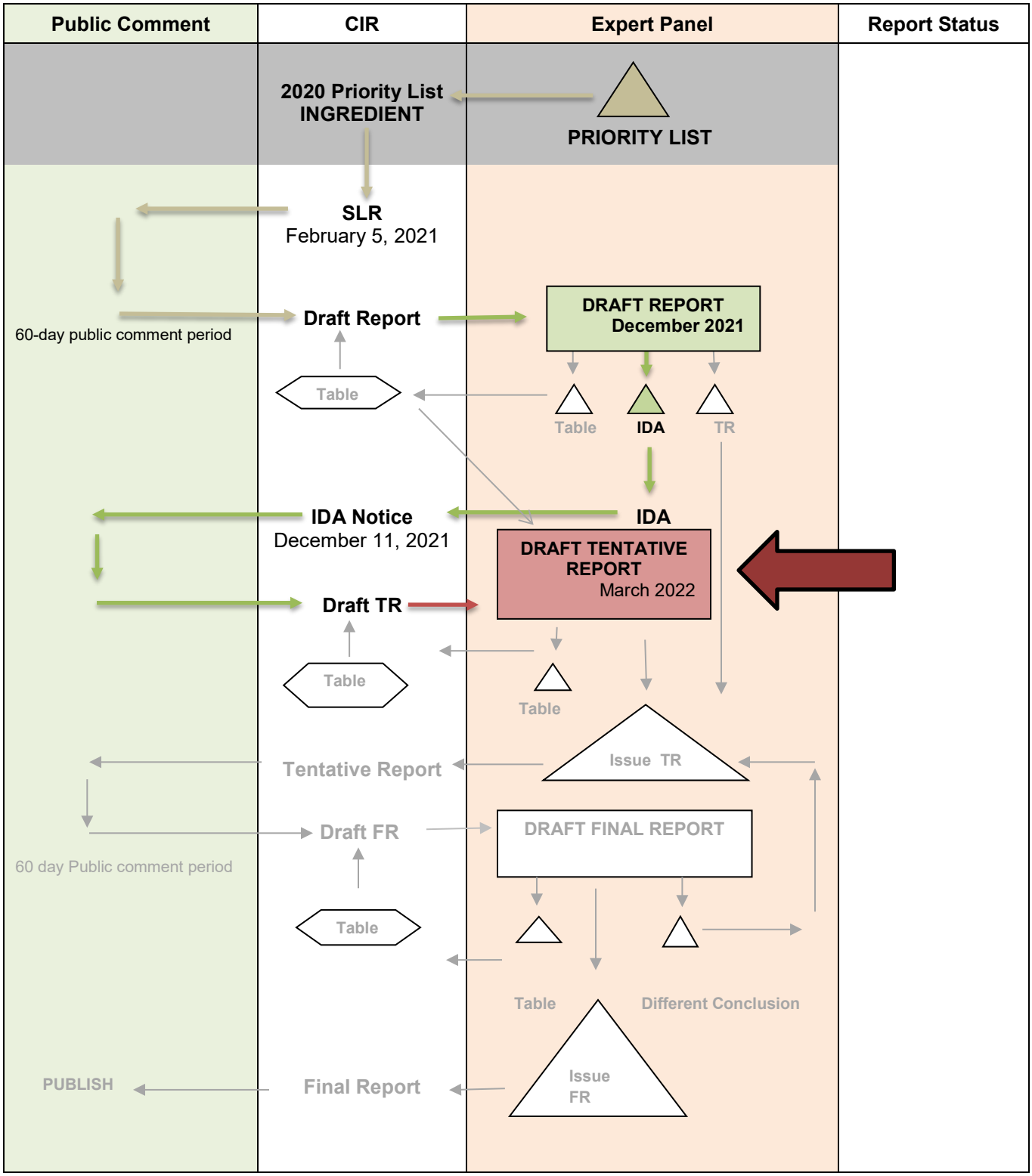
The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. Previous Panel member involved in this assessment: Lisa, A. Peterson, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Priya Cherian, Senior Scientific Analyst/Writer, CIR.

1620 L Street, NW, Suite 1200 ◇ Washington, DC 20036-4702 ◇ ph 202.331.0651 ◇ fax 202.331.0088 ◇
cirinfo@cir-safety.org

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Glucosamine Ingredients

MEETING March 2022



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, Senior Scientific Analyst/Writer, CIR
Date: February 11, 2022
Subject: Safety Assessment of Glucosamine Ingredients as Used in Cosmetics

Enclosed is the Draft Tentative Report of the Safety Assessment of Glucosamine Ingredients as Used in Cosmetics (*report_Glucosamine_032022*). The 4 ingredients reviewed in this report include Acetyl Glucosamine, Glucosamine, Glucosamine HCl, and Glucosamine Sulfate. At the December 2021 meeting, the Expert Panel for Cosmetic Ingredient Safety (Panel) issued an Insufficient Data Announcement (IDA) for this ingredient group. In order to conclude safety, the Panel requested impurities data on Acetyl Glucosamine, and irritation and sensitization data on all ingredients at the maximum concentration of use. Since the previous review of this report, the following unpublished data were received, incorporated into the text, and highlighted:

- HRIPT performed on 105 subjects using a liquid foundation containing 2% Acetyl Glucosamine (*data_Glucosamine_032022*; TKL Research 2011)
- 21-d cumulative irritation assay performed on 12 subjects using an eye cream containing 2% Acetyl Glucosamine (*data_Glucosamine_032022*; Anonymous 2006)
- In vitro ocular irritation assay performed using a face serum containing 2% Acetyl Glucosamine (*data_Glucosamine_032022*; Institute for In Vitro Sciences, Inc. 2009)

Included in this packet are the report history (*history_Glucosamine_032022*), data profile (*dataprofile_Glucosamine_032022)*, search strategy (*search_Glucosamine_032022*), transcripts for the previous meeting (*transcripts_Glucosamine_032022*), and flow chart (*flow_Glucosamine_032022*). Updated 2022 FDA VCRP data were received and incorporated into the report (*VCRP_Glucosamine_032022*). Compared to 2021 FDA VCRP data, the number of uses for Acetyl Glucosamine and Glucosamine HCl has increased by 81 uses and 8 uses, respectively. An insignificant decrease in number of uses was noted for Glucosamine.

The Panel should carefully consider and discuss the data (or lack thereof), and the draft Abstract and draft Discussion presented in this report. A Tentative Report with a safe, safe with qualifications, unsafe, insufficient data, or split conclusion should then be issued.

1620 L Street, NW Suite 1200, Washington, DC 20036
(Main) 202-331-0651 (Fax) 202-331-0088
(email) cirinfo@cir-safety.org (website) www.cir-safety.org

History – Glucosamine Ingredients

February 2021

- SLR posted
- Comments on SLR received
- Concentration of use data received
- Data received:
    - Repeat insult patch test; mask containing 0.005% Acetyl Glucosamine
    - Human maximization assay; product containing 0.25% Glucosamine HCl
    - Human maximization assay; product containing 0.01% Glucosamine

April 2021

- Data received
    - Repeat insult patch test; leave-on product containing 0.005% Glucosamine HCl

December 2021

- Panel reviews Draft Report and issues an IDA; requests impurities data on Acetyl Glucosamine, and irritation and sensitization data on all ingredients at the max use concentration of 5%

January 2022

- Unpublished data received from Council:
    - HRIPT on liquid foundation containing 2% Acetyl Glucosamine
    - 21-d cumulative irritation assay on eye cream containing 2% Acetyl Glucosamine
    - In vitro ocular irritation assay on face serum with 2% Acetyl Glucosamine
- Updated 2022 FDA VCRP data received
    - Increased uses for Acetyl Glucosamine and Glucosamine HCL
    - Decreased uses for Glucosamine
    - Still no uses reported for Glucosamine Sulfate

March 2022

- Panel reviews Draft Tentative Report

Glucosamine Ingredients Profile – March 2022 – Writer, Priya Cherian																														
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Acetyl Glucosamine	x	x		x	x						x					x		x	x		x	x		x		x			
Glucosamine	x	x		x				x						x										x				x	x
Glucosamine HCL	x	x	x	x	x	x		x			x					x								x					
Glucosamine Sulfate		x			x	x																							x

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

Glucosamine Ingredients

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Acetyl Glucosamine	10036-64-3; 72-87-7; 7512-17-6	yes	yes	no	no	no	no	no	yes	no	no	no	no	no	no	no	yes
Glucosamine	3416-24-8	yes	no	no	no	no	yes	no	yes	no	no	no	no	no	no	no	yes
Glucosamine HCL	66-84-2	yes	no	no	no	no	yes	no	yes	yes	no	no	no	no	no	no	yes
Glucosamine Sulfate	29031-19-4	yes	no	no	no	no	no	no	yes	yes	no	no	no	no	no	no	yes

Search Strategy

Search terms below were searched for in the websites listed above. If useful information was found, a "yes" is noted.

Search Terms

- INCI names
  - Acetyl Glucosamine
  - Glucosamine
  - Glucosamine HCl
  - Glucosamine Sulfate
- CAS numbers
  - 10036-64-3
  - 72-87-7
  - 7512-17-6
  - 3416-24-8
  - 66-84-2
  - 29031-19-4

- chemical/technical names
- metabolism
- dermal
- inhalation
- skin
- toxicity
- drugs
- medicine
- irritation
- ocular
- eye
- sensitization
- allergy
- manufacture
- cancer
- carcinogenicity
- mutagenicity
- pigmentation
- tyrosinase
- melanogenesis
- Ames
- Reproductive
- Teratogenicity
- Synthesis

LINKS

Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)

Pertinent Websites

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

- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

DECEMBER 2021 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito Team – December 6. 2021

DR. BELSITO: This is the first time we're looking at the four ingredients in this report. And we received lots of data from various sources. So what do we think here at this point?

DR. LIEBLER: My high level takeaway is include all the ingredients. Method of manufacture, composition and impurities are okay for all. These are absorbed, but no significant toxicity. And if the sensitization data are okay, then safe as used. We'll deal with the pigmentation issue in the discussion.

DR. BELSITO: Yeah, so I had safe as used, but need to finesse the discussion. So do we need to deal with the DART issues? Or is this dose effect? Because there were a whole bunch of different DART issues that sort of went back and forth. Paul, you're usually the person I go to for this.

DR. SNYDER: Yeah, I looked at those pretty carefully. I mean, those positive studies were oral in the diet and intraperitoneal injections. And then there was an intrauterine 60-day sustained release pellet. So I just didn't think they had much relevance to the dermal.

DR. BELSITO: Okay. And would we need to put that in the discussion?

DR. SNYDER: We could. I mean, we could state that the repro effects were seen at levels that far exceeded what would be a cosmetic use.

DR. BELSITO: Okay, so the dose that was used is irrelevant to cosmetics?

DR. SNYDER: Yeah.

DR. BELSITO: Okay. And, Dan, how were you going to deal with the pigment issue?

DR. LIEBLER: Well, I wanted to just bring that up. I think that we need to talk about it as a team. I'm trying to remember now if the study, the human with acetylglucosamine at the bottom of PDF 22. Trying to get an idea -- okay, so 4 percent niacinamide and 2 percent acetylglucosamine and this is for facial hyperpigmentation, obviously not a cosmetic use. But is that a lot higher than our maximum use concentration of acetylglucosamine for a leave-on on skin? I'm scrolling down to the use table. Acetylglucosamine, up to five percent leave-on dermal contact.

DR. BELSITO: Which is what it was in this product.

DR. LIEBLER: Yeah. Right. So this is one where I defer to you and David on the context in which somebody would be using this for facial hyperpigmentation. Is that something that's treated with acetylglucosamine? Is that relevant to a cosmetic that would contain the same amount? In other words, if you're not hyperpigmented, is this something that would cause a loss of pigmentation in somebody who, you know, was not trying to deal with hyperpigmentation? I just didn't have a good feel for the clinical context here, and whether or not that impacts our evaluation in a cosmetic use context.

DR. BELSITO: Yeah, so -- but the effect is on tyrosinase, right? So it would effect normal, not just diseased. But, I mean, I thought that we would deal with this simply by saying that effects on pigmentation are, you know, drug and not cosmetic and not under our purview. And that manufacturers should be aware of this in designing cosmetic products, or whatever that boilerplate is we've used for other ingredients. Because we don't have an NOAEL for this.

DR. LIEBLER: Yeah.

DR. BELSITO: And also, as you point out, what we're told is, it's used up to 5 percent in a face product and this is the concentration that caused the lightening. Or we don't know that it's the -- first of all, we don't know if it's the acetylglucosamine that caused it, right?

DR. LIEBLER: Right.

DR. BELSITO: It just happened to be in the product.

DR. LIEBLER: I'm looking, but I'm not sure I see anywhere where there's evidence that the glucosamine or acetylglucosamine is a, biochemically, is a tyrosinase inhibitor.

DR. BELSITO: I thought I saw that someplace.

MR. GREMILLION: Could I, I'm sorry, this is Thomas, CFA, I just wanted to ask a clarifying question. When they say it improved the appearance of facial hyperpigmentation, does that just mean it succeeded in having a lightening effect, so it's used as a skin lightener?

DR. BELSITO: Yes.

MR. GREMILLION: Okay.

DR. SNYDER: Don, on Page 22, under the Animal and Human, Acetylglucosamine.

DR. BELSITO: Yeah.

DR. SNYDER: Is that a misstatement? That third from last line of that first paragraph, "The degree of pigmentation at each time point measured after the application of acetyl glucosamine was higher than the vehicle control group." Should that -- was that supposed to be the degree of depigmentation?

DR. BELSITO: Yeah, let me -- can I just answer Dan's question first and then go back --

DR. SNYDER: Yeah, sorry, go ahead.

DR. BELSITO: -- because I'm in a separate page. Dan, the tyrosinase activity, that is in the references, Reference Number 44.

DR. LIEBLER: Oh. It wasn't brought out in the report because I missed it.

DR. BELSITO: Right. "Disruption of tyrosinase glycosylation of N-acetylglucosamine and its depigmenting effect on guinea pig skin and in human skin." So that was the reference. Sorry, Paul, what PDF page are you on?

DR. SNYDER: Page 22. Under the Animal and Human, Acetylglucosamine.

DR. BELSITO: Yeah.

DR. SNYDER: The third from last. It says, "The degree of pigmentation at each time point measured after application of acetylglucosamine was higher than the vehicle control group."

DR. BELSITO: Yeah, I had that flagged. I think it should be hypopigmentation.

DR. SNYDER: Okay, because otherwise there's no effect.

DR. BELSITO: Right, yeah.

MS. CHERIAN: It's probably a mistake. I can fix that.

DR. SNYDER: Just verify that that's correct.

MS. CHERIAN: I'm going to double-check the reference.

DR. BELSITO: Yeah. It would have to be hypopigmentation.

DR. LIEBLER: So I didn't look at the reference that Don just cited, reference 44 on tyrosinase inhibition. If there are data specifically that indicates that any of these glucosamines are inhibitors of tyrosinase, that should be brought into that section under the pigmentation issues. I don't know what it actually says in that paper that was cited. I haven't had a chance to look at it, but maybe we need to bring that out and see what it actually says.

DR. BELSITO: Okay.

DR. LIEBLER: I think Thomas has his hand up.

MR. GREMILLION: Sorry, I just needed to lower that. Sorry.

DR. LIEBLER: Okay.

DR. BELSITO: So, in the next iteration, you want a deeper dive into Reference 44, Dan?

DR. LIEBLER: Yeah. I think we should just bring out -- either there's no evidence in that paper specifically for tyrosinase inhibition by acetylglucosamine or any glucosamines. If that's the case, and Priya reads that and sees that, then I don't think we need to bring it in. But if there are data indicating tyrosinase inhibition, in other words a biochemical mechanism to link these, then that's need to be brought in. I'm comfortable certainly leaving that to Priya to read the paper and make that judgement.

DR. BELSITO: Okay.

DR. LIEBLER: Or if she has a question, she can send it to me and get my take. Send me a copy of the paper and I can -- you know, or even just the passage and I can look at it. I think, Priya, if you're comfortable doing that, then please just go ahead and look into that reference for us.

MS. CHERIAN: Great. I can do that. Thank you.

DR. BELSITO: Okay. Go ahead, Paul.

DR. SNYDER: So what is our interpretation of this lightening effect?

DR. BELSITO: That there are these reports and that we have to -- we'll deal with them the way we've dealt with other ingredients that have been reported to cause skin lightening. That that's not a cosmetic effect and that manufacturers should be aware of this in compounding cosmetic products.

DR. SNYDER: Okay, because there does appear to be an effect, in vitro and in vivo, so.

DR. BELSITO: Right, yeah.

DR. SNYDER: Okay. Okay.

DR. BELSITO: We don't know that it's from the glucosamine but, you know, there's certainly enough of a flag there. But, you know, we need to look at that boilerplate, Bart, because this I'm sure will pop up in the future and we should make sure that the language is consistent.

DR. LIEBLER: Yeah, Paul, this is the reason I asked for Priya to look at that reference. Because if there's biochemistry data to indicate that the glucosamine can inhibit tyrosinase, then that provides a mechanistic basis for raising the concern with respect to the glucosamine.

DR. SNYDER: Yeah, I mean, this was an unusual one where the in vitro data was dose dependent decrease in melanin content, which kind of struck me at first. And then that with the in vivo data, so, okay.

DR. BELSITO: Right. Okay. Any other issues with the pigmentation effect? Okay.

DR. SNYDER: No, I think I misspoke, Don, I want to go back to that repro. So the repro there was, while there was positive/negative, but the big one was that 60-day sustained release pellet in the intrauterine was negative. So that held a lot of weight for me to not be worried about the other ones that had plus/minus effects.

DR. BELSITO: Okay. So, Priya, in the discussion that the concentrations causing these effects were greater than would be expected from cosmetics and also the 60-day study gave us further assurance?

DR. SNYDER: Yeah.

DR. BELSITO: In the risk assessment, Priya -- this is PDF Page 25 -- I just thought that we needed to make clear that the number in parenthesis is the margin of safety values. Because I wasn't sure, until I read the Norwegian food safety report, that that's what those numbers refer to.

I was very confused where you say 10 percent glucosamine sulfate in a body lotion, open parenthesis, 35.0. I just didn't know what that 35.0 was. I mean, you have margin of safety MOS in parenthesis, but I thought you were just putting the acronym in there. See what I mean?

MS. CHERIAN: I see what you mean.

DR. LIEBLER: You could simply just put, in the first one, MOS equals 35.0.

DR. BELSITO: Right.

DR. LIEBLER: And then the others will be obvious.

DR. BELSITO: Okay, and also, we don't have that study per se in the report. We have a review from Anderson, et al. And, interestingly, when I looked at that review, it's not in their references. They cite this paper by Setnikar, et al. An article entitled, "Antiarthritic effects of glucosamine sulfate study in animal models." And it's in a journal called *Arzneimittelforschung,* which, I guess, translates to drug research and I couldn't find it. So we don't have any primary data on this margin of safety and I'm not even sure what the supposed adverse event was. So, I was also proposing if we do keep it in -- you know, what I mentioned -- but maybe deleting it completely. You know, there's just -- this is just a review by Anderson, and I don't know where they came up with those numbers. And it really makes something that is part of normal human body look toxic.

DR. SNYDER: Where you at, Don, what page?

DR. BELSITO: I'm on the risk assessment still.

DR. SNYDER: Okay.

DR. BELSITO: If you look at the reference for the risk assessment, which I did because I didn't understand the numbers in parenthesis. The reference is to an article by Anderson, et al, 2005. When you go to the Anderson article, it actually ends up being a review. And it cites for these margin of safety numbers, an article by Setnikar, Pacini Revel, entitled, "Antiarthritic effects of glucosamine sulfate studied in animal models." And it's from a journal *Arzneimittelforschung*, which I could not find on Columbia.

And, so, I don't know if that had the primary data for these endpoints, but I'm also not even sure what was the adverse event that they were looking at to calculate margin of safety. Right? It says calculated the margin of safety values, for what endpoint? So I really thought that that risk assessment should just go away and never even be mentioned. Comments?

DR. SNYDER: I don't understand why the Norwegian Food Safety Authority would be studying body lotion, leg cream, and face cream. I mean, the NOAEL came from a dog study, a repro -- oral dose study in dogs.

DR. BELSITO: Right.

MR. GREMILLION: Sorry, this is Thomas. Did CIR reach out to the study authors when --

MS. CHERIAN: Yes, I did try to obtain that reference and it was unobtainable.

DR. BELSITO: The Setnikar reference, is that what you're referring to, Priya?

MS. CHERIAN: Yes.

DR. BELSITO: Yeah. I mean, I couldn't get it either. And we have no primary data. And, yeah, so we know that the margin of safety was obtained from a repeated oral dose toxicity assay performed in dogs, but what was the endpoint that they saw? What was the effect? Was it weight loss, something trivial? We don't even know.

DR. SNYDER: It had to be something deemed to be adverse so I'm less worried about that other than --

DR. BELSITO: It just seems that we're quoting this and it's just very sloppy referencing., total lack of any primary data. And we're not even sure what the adverse event was.

DR. LIEBLER: Well, we can't get that information, so we need to dump it. So I think we've settled that.

DR. SNYDER: It's not going to impact our overall assessment.

DR. BELSITO: Okay. Then I would suggest we completely strike through that section. Is everyone comfortable with that?

DR. LIEBLER: Yep.

DR. KLAASSEN: Yes.

DR. SNYDER: I say more details or strike.

DR. BELSITO: Okay. Well, we're not going to get more details. I tried and Priya tried.

DR. SNYDER: I also tried to retrieve it and couldn't get it.

DR. BELSITO: Okay. More details or strike, Priya, I wouldn't spend a lot of time because I spent way too much time.

MS. CHERIAN: Okay.

DR. BELSITO: So we dealt with the DART, pigmentation. So I think it's safe as used and in the discussion we've already talked about the DART issues. We're going to try and get a boilerplate for the pigment issues. We're going to strike through the Norwegian margin of safety calculations in the absence of knowing anything about it. Is there anything else that needs to go into the discussion? Or is anyone uncomfortable with the safe as used?

DR. LIEBLER: I'm fine with it. I think we've covered the points. I think Priya's got the comments.

DR. SNYDER: I'm good.

MS. CHERIAN: I just have one comment. We have irritation and sensitization data, but we don't have it at the max use concentration of 5 percent. Is that okay?

DR. BELSITO: Yes.

MS. CHERIAN: Okay.

DR. BELSITO: I mean, there have been -- we have it and there are no clinical reports. There's nothing about these that concern me. I put with -- oh, we also, Priya? Yeah, this is it. So if you go to PDF Page 24, you have the data there, so there's a DPRA, there's a Keratino-Sens and there's an h-CLAT and they're negative. So we got three out of three in vitros, and sort of the rule of thumb is two out of three makes it a non-sensitizer. So we have the in vitro data to support that it's not a sensitizer and there's a lack of clinical reports. So I'm not concerned.

MS. CHERIAN: Okay, thank you.

DR. BELSITO: In the case reports, I mean, with these asthma and that, I mean, we don't even know what the excipients were in those, so I didn't make much of those and I didn't think we needed to even discuss that. It does have some aerosol use, we're happy with the safe as used, or do we need the boilerplate about the data insufficient for aerosol use?

DR. SNYDER: I'm frantically trying to go to the table.

DR. BELSITO: Table is on PDF 30 and it's deodorant underarm to 0.01 percent.

DR. SNYDER: Yeah, again, this is one where there's a distinct absence of any toxicity through oral studies.

DR. BELSITO: Right.

DR. SNYDER: I think this is one we craft that way to say that, you know, the 0.01 percent would result in a low exposure even in a deodorant spray, with a particle size distribution -- and put that in there that we know from our resource document. And then say the absence of any systemic toxicity data or chemical characteristics of the -- chemical/physical characteristics of the ingredient, there was no concern for inhalation.

DR. BELSITO: Okay, because we have absolutely zero inhalation data.

DR. SNYDER: Yeah, but this is glucosamine, I don't --

DR. BELSITO: Yeah. I'm fine with it, I'm just pointing out. Okay. We're going to, unless I hear otherwise, Priya, we're going with safe as used. We need to put in the discussion why we're not asking for respiratory data. And a boilerplate on skin lightening and delete the margin of safety unless you can get more supporting data. And the DART, again, the dose is irrelevant and then the 60-day study. Anything else for the discussion?

DR. KLAASSEN: I think the fact that this is a dietary supplement also gives some degree of satisfaction that it's not a major problem.

DR. BELSITO: Yeah, I mean, it's used by millions of people, right, including myself, so. But the FDA does not specifically regulate dietary supplements, right?

DR. KLAASSEN: Right.

DR. BELSITO: They only -- and if there are consumer reports of adverse events.

DR. KLAASSEN: Right.

DR. BELSITO: Okay. So I'm not sure dietary supplement helps our argument in terms of any founded science, but --

DR. EISENMANN: One note that deodorant is a not spray. I think she would've indicated if it was a spray. I just looked it up in my Use table.

DR. BELSITO: Okay. So then the spray use was just, it's .07, but not deodorant. So deodorant, underarm, when you report that, Carol, if it were a spray you'd put spray?

DR. EISENMANN: I would put spray and I have it listed deodorants, not spray, 0.01, on the table that I provided quite a while ago.

DR. BELSITO: Okay. Okay, it's not in table three, though. Maybe that would be helpful to put in deodorant, not spray? Underarm; not spray? Okay. Anything else on these glucosamines? No? Priya, you have your marching orders?

MS. CHERIAN: I do.

DR. BELSITO: Okay, great. Okay, it's 12:01, lunch time?

DR. SNYDER: Did we skip sugarcane, or did I fall asleep?

DR. BELSITO: You fell asleep.

DR. SNYDER: Okay.

DR. BELSITO: It was -- you went into hyperglycemic coma, Paul.

DR. SNYDER: I probably jumped ahead because there was nothing there.

DR. BELSITO: Yeah.

DR. SNYDER: It was no comments, safe as used, done.

DR. BELSITO: Yeah, exactly, that's what happened.

DR. SNYDER: Okay.

DR. BELSITO: Okay. So we'll see you all at 1:00?

DR. SNYDER: Yes, sir.

DR. BELSITO: Okay, enjoy your lunch.

DR. KLAASSEN: Bye.

Cohen Team – December 6, 2021

DR. COHEN: Okay. Next is glucosamine. And this is Priya's. This is a draft report. It's the first time we're reviewing this, and the safety assessment is for four derived ingredients. It's used as a skin conditioning agent. We have frequency of use. It's in a number of leave-on products and some rinse-off products. We have max use for acetyl glucosamine in a lipstick at 2 percent, 2 percent in an eye lotion. And (audio skip 00:28:36) the recitation from just the review in the schedule.

DR. SHANK: Where is this again, please? Table 3?

DR. COHEN: Yeah. Table 3.

DR. SHANK: And your question is what?

DR. COHEN: That max use, is it 5 percent for acetyl glucosamine?

DR. PETERSON: That's what the table says.

DR. COHEN: Yeah. Yeah. Okay.

DR. CHERIAN: It's 5 percent.

DR. COHEN: It's 5 percent. Okay. So, why don't I open it up to the group for method of manufacturing and impurities. There was some material there. Lisa, you want to just comment on what you felt we need?

DR. PETERSON: I guess I thought we needed impurities for acetyl glucosamine. That was the need from the chemistry perspective. I had some questions about whether we needed to worry about nitrosamines. I mean, there's not going to be any stable nano compounds expected to be formed, but I saw we had put this in other compound statements. So I guess it's not -- once I thought it through, I wasn't concerned about it but I just thought it might be worth bringing up.

DR. COHEN: In what context would we bring it up?

DR. PETERSON: Just making sure it's not in -- I think it's the wrong issue for this compound because sometimes nitrosamines can be formed depending on what the other ingredients are in the mixture, like if there's nitrate, and there are some other compounds we're reviewing that the nitrosamines issue comes up to make sure that they're not formulated in a way that nitrosamines can be formed. I think this is a case where I'm overreacting because it's a primary amine and any nitrosamine would not be stable.

DR. SLAGA: Right.

DR. COHEN: Ron, what thought -- it looks like this gets in. When they put it on a knee, they were able to actually get it into the synovial fluid from the surface. What are your thoughts on what we need on this since it's our first time out?

DR. SHANK: I had two needs. For skin sensitization, the data we have are well below the maximum use concentrations, so we need skin sensitization for these compounds at maximum use concentrations. The difference is quite large. Acetyl glucosamine, we have data at 0.005. The maximum concentration is 5 percent. And for the glucosamine hydrochloride we have it at 0.25 with the max use is 5. So, we need skin sensitization at the higher concentrations.

The other need I have is in report there's a reference number 442N acetyl glucosamine suppressing melanogenesis. But there are no details given. I went to the paper, all I could get was the abstract, that didn't help. So I think we need to see the whole paper and then (audio skip) intraperitoneal injection. So that would produce blood concentrations far greater than (audio skip). And glucosamine penetrates the skin very slowly, so I think in the discussion we should mention that the DART data in the report don't reflect what would occur with cosmetic use. Those are the only comments I had on the report.

DR. COHEN: Tom.

DR. SLAGA: Yes, I had the same issue with the sensitization data. The rest of the data, it's not an irritant, it's not genotox. It's also an antimutagen and anticar- (audio skip), so I'm kind of safe with all of those aspects. So, I feel the sensitization data is the only thing needed.

DR. COHEN: The irritation information that we have is -- at least in HRIPT is very low concentration. It's several orders of magnitude below max use, so maybe we just go out with irritation and sensitization at max use and see what we can get?

DR. SLAGA: Fine, yeah.

DR. SHANK: Yes.

DR. COHEN: And do we have a boilerplate or comments regarding pigmentation?

DR. SLAGA: No.

DR. SHANK: No.

DR. COHEN: Okay. Look, I have the issue of reducing hyperpigmentation as well.

DR. PETERSON: I thought we had in the past discussed that you could put that's not a -- sorry, I have some trouble speaking this morning.

DR. BERGFELD: It's not a cosmetic effect.

DR. PETERSON: It's not a cosmetic -- it's not supposed to be a cosmetic effect. It would be considered a drug if it was a skin whitener or something like that.

DR. SHANK: It would be a toxic -- it would be an adverse effect.

DR. COHEN: We would have it at least in the discussion, right?

DR. BERGFELD: Absolutely.

DR. SHANK: Yes.

DR. PETERSON: Yeah, I put that up there that there was an issue with skin lightening. I also had a question about the reference that was raised by council. They said that it was considered unreliable, and I guess I was wondering what the data were. Why was it considered unreliable, and if something's considered unreliable, should we really include it in the report? Because a piece of that data is not really -- it's just additive to several other things where the data seems to be more reliable. So, I was curious about why it was highlighted for being unreliable.

MS. CHERIAN: Do you know its reference?

DR. PETERSON: It is reference 20 -- look at council's note. Reference 2, Table 4.

DR. SHANK: In the text of the report can you tell me what page --

DR. PETERSON: Oh, sure.

DR. SHANK: -- the data are presented?

DR. PETERSON: Table 4 is on PDF page 30.

DR. COHEN: I remember seeing that. I just can't -- where was it about that reliability?

DR. PETERSON: It's the last line of Table 4.

DR. COHEN: Last line Table 4. Okay.

DR. PETERSON: It's under the dotted line. It's one "Glucosamine HCL mice strain unspecified." I mean, the data aren't -- I guess it's because it's a glucosamine HCL but --

MS. CHERIAN: It was an ECHA study, so it was, I'm assuming, summarized data.

DR. PETERSON: Ah, and that's why it's not reliable?

MS. CHERIAN: I can doublecheck in the dossier and see why it was considered unreliable.

DR. PETERSON: She doesn't really say -- I mean, the letter calling attention to it just says the reference is unreliable or the study is unreliable.

MS. FIUME: Can someone from industry -- Jay, can you respond to the concerns for that citation, please?

DR. PETERSON: You are on mute, Jay.

DR. ANSELL: I'm sorry, I was just away for a second. Could you repeat the question?

MS. FIUME: Oh, sorry, in the comments it said in Table 4 the one reference from the ECHA dossier was not considered very reliable (audio skip) considered unreliable.

DR. ANSELL: I don't have that in my notes. Linda, do you have a --

Dr. KATZ: No, we don't.

DR. SHANK: Since we don't know why -- and I don't think that table's very important in evaluating the safety -- acute oral LD50s and they're huge. Grams per kilogram bodyweight, yeah. So I wouldn't put too much time on it.

DR. PETERSON: I just thought it was interesting. It was highlighted as being unreliable.

DR. COHEN: Okay.

DR. SHANK: Why?

MS. CHERIAN: Sometimes ECHA data states in their summaries that they didn't consider a study reliable because they require certain factors like a certain number of animals or a certain strain or something like that, so it could've been that kind of issue.

DR. PETERSON: Okay.

DR. COHEN: So, we'll go out with an IDA on (audio skip) -- sensitization of (large audio skip 00:42:00).

Full Panel – December 7, 2021

DR. COHEN: So this is a draft report for glucosamine, and this is the first time we're reviewing this. This safety assessment has four derived ingredients which are used as skin conditioning agents. The frequency of use is reported, and we have max use of glucosamine up to 5 percent. And it may be used in lipstick formulations and eye lotions. Our motion is for an IDA asking for impurities for acetyl glucosamine, irritation and sensitization at max use for acetyl glucosamine.

DR. BERGFELD: Don?

DR. BELSITO: We have it safe as used.

DR. COHEN: That's why we do it this way, I guess.

DR. BELSITO: So, Dan, you want to comment on the manufacturing?

DR. LIEBLER: Let's see. So your IDA was impurities for acetyl glucosamine?

DR. COHEN: Yes.

DR. LIEBLER: Okay. Yeah. It's not listed.

DR. BELSITO: We have a method of manufacturing. You can't get anything from that?

DR. LIEBLER: It doesn't provide a strict purity specification.

DR. BELSITO: But is there anything in what's used to manufacture that you'd be concerned about?

DR. LIEBLER: There's either a chemical modification of purified glucosamine or a production from a plant source. And first time we're seeing this; right?

DR. BERGFELD: Right.

DR. COHEN: Yes.

DR. LIEBLER: Yeah. Okay. Let's just go ahead and I'll support the IDA. We can triangulate if necessary depending on what information we get.

DR. BERGFELD: Anyone else want to make a comment? Don, are you seconding it now?

DR. SNYDER: Well, Don, you said on the sensitization for this that you used the logic of the adverse outcomes pathway. It had two of three of the in vitro are negative and no clinical reports of sensitization or irritation. So that cleared the sensitization even though it wasn't at the max concentration of use.

DR. BELSITO: Right. So I'm just back to looking at --

DR. COHEN: Our human sensitization data is more than an order of magnitude off from max use.

DR. BELSITO: Yeah. But, again, David, we now have three of three in vitro tests, all of them predicting no sensitization. We have a DPRA. We have a Keratinosense and we have the h-CLAT. So, you know, it's totally predicted to be non-sensitizing, and we have acetyl glucosamine and HRIPT. Now, granted, it's not at max concentration of use, non-sensitizing. I just didn't feel we needed the data. Particularly, we have completely negative in vitro data that is accepted methodology for determining in vitro sensitizing capabilities. And we have no clinical reports.

DR. COHEN: Yeah. I don't want to rely this early on the absence of data as being an indication of its safety.

DR. BELSITO: We don't have absence of data. We have three --

DR. COHEN: Well, no, there's no clinical reports.

DR. BELSITO: We have three of three in vitro tests which are accepted methodologies for assessing sensitization.

DR. COHEN: We have one in vitro test for irritation; right? And certainly these HRIPTs might uncover some irritancy and/or sensitization at higher concentrations.

DR. BELSITO: We have an in vitro test of a neat material that was nonirritating in reconstructed human epidermis with OECD guidelines, and we have three OECD guideline in vitro sensitization test which cover three aspects of the adverse outcome pathway for sensitization that we can study in vitro and that are accepted.

DR. COHEN: So, Don, I guess it's really more a question and advice on your part is if we're relying that heavily on the in vitro and you're seeing no signs of sensitization in these in vitro irritation/sensitization, then why are we doing the human ones? And if we still want that as a corroboration, by that logic we can have people doing HRIPTs at one part per million getting a negative result and then saying okay, we have two in vitro, and we have a negative human; and that's it.

DR. BELSITO: Well, I mean, first of all, maybe you weren't part of the panel when we got the in vitro presentations, and certainly Dan and I are privy to the fact that, you know, we're using these in vitro tests for fragrances all the time. Europe has banned animal testing for cosmetic use, and they also feel that it's unethical to do HRIPTs, which have now been rephrased by the fragrance industry as not HRIPT but confirmation of no induction of sensitization.

So we as a cosmetic ingredient review panel need to begin stepping up to accepting and understanding the in vitro studies that are done for sensitization because as materials come in where we need this data, we cannot ask for animal data. If it's out there, we can certainly use it, but we cannot ask for new animal data.

DR. LIEBLER: So, David, I'm the not the dermatologist obviously, but I'm the other person who's on the RIFM panel. And I've been kind of watching how this dynamic has developed about what data are accepted to clear this sensitization endpoint on both panels. And on the CIR panel we have frequently usually had HRIPT data to clear the sensitization endpoint at concentration of use, and we usually haven't cleared ingredients that didn't meet that standard. On the other hand, on the RIFM side, we've been making increasingly heavy uses of the in vitro tests, the DPRA, the Keratinosense and the h-CLAT are kind of the trio that get used. Thanks for the slide there, Bart.

This is just from a presentation that we had. We have used those on the RIFM side to make a decision about the sensitization risk, and so this is the first time that I can recall on a CIR review where we've been presented with this situation where we've at least -- Don has suggested that I think this clears the sensitization endpoint -- first time that I can recall. And this is the logic. And I think it's changing for reasons in the RIFM panel because that's an international panel. It's dealing with not only the data situation in the U.S. but the data situation worldwide and particularly in the EU where we're going to -- we have less and less access to HRIPT data to clear fragrance materials.

So that's just my observation of what the dynamic is, and it's finally hitting us square in the face on the CIR panel with this ingredient -- with these ingredients.

DR. COHEN: Well, thank you. It makes me feel better that this is the first time it's coming up like that here. I'd love to hear that presentation and maybe other members of our team would as well because I wasn't here for it. I understand the evolution of these in vitro tests, but we're still presented with human data that's pretty far off. We're early on in the stage of this report, and I think I'd still stick with our IDA for now.

DR. LIEBLER: I can support that for the reasons that we just discussed, and I would also ask Bart if Don Bjerke's presentation was recorded?

DR. HELDRETH: Yes, it was.

DR. LIEBLER: I'm wondering if that could be made available webinar-style to members of the Panel to dial up and listen to on a cold winter's night over the holiday break to at least have a chance to listen to it, anybody who didn't hear Don's presentation when he made it. That was just the last couple of years, I guess.

DR. BERGFELD: It would be nice to have printed that slide as well sent to us.

DR. HELDRETH: I'll have a look into it.

MS. KOWCZ: And, Bart, this is Alex. We can also ask Don if he'd be opened to actually presenting it because I think it's always better to have in person because we can ask questions as they come up. Just a suggestion from the industry, from PCPC.

DR. HELDRETH: Yeah. I like that a lot. For those of you who haven't met Don Bjerke, he's a fantastic presenter and really knows his stuff, so I think that would be great if we could ask Don to come back.

MS. KOWCZ: That presentation was actually 2016 from the slide that you've shown.

DR. LIEBLER: Oh, man. Time flies.

MS. KOWCZ: Most of us were not here.

DR. BERGFELD: I suspect things have changed a little bit too.

MS. KOWCZ: Just a recommendation, Bart, if you'd like to take us up on it.

DR. LIEBLER: I support the IDA that David's asking for with the understanding that this is an area where there are shifting criteria. We're really seeing it on the RIFM side, and I think that's why Don is -- Don's not just arbitrarily saying these three in vitro tests clear it for us. But I think we need to reckon with this, and perhaps as a process that needs to take place and be evaluated on the CIR panel as well.

DR. BERGFELD: Well, it sounds like everyone agrees to that, and Bart's going to facilitate it. So may we move on?

DR. LIEBLER: Don, are you agreeing to that? The ask for sensitization data?

DR. BELSITO: I mean, I don't think we need it, but it's early in the process. I think the other team is uncomfortable with the in vitro because they're not familiar with it, so I'm fine. I'll just go on record saying if we don't get it, I will go back and support the safety as I just suggested.

DR. BERGFELD: So are you going to second the motion of the IDA?

DR. BELSITO: Yes.

DR. BERGFELD: And we assume that we will before the next meeting be able to review the presentation by Dr. Don --

MS. KOWCZ: Bjerke

DR. BERGFELD: Bjerke. Okay. And maybe some of it in print form as well could be sent to us.

DR. LIEBLER: Yes.

DR. BELSITO: Wilma, can I just interject totally off topic? Are we going to be discussing the Women's Voice of the Earth submission on MCI/MI?

DR. BERGFELD: WE don't have that on the agenda, so I'll leave that up to Bart to discuss. I know we discussed it in our team meeting.

DR. BELSITO: Yeah. So -- go ahead, Bart.

DR. HELDRETH: Yeah. I was just going to say the letter from Women's Voices for the Earth came in after we created the initial panel material, so there was no way to go back in time and put it back on the agenda. But the panel is welcome to discuss any items that come in, so if you want to interject it, that's just fine.

DR. COHEN: Should be do it in other items?

DR. BERGFELD: Yes, we could do it under other items.

DR. BELSITO: We also need a presentation on the QRA for MI because it was my recommendation that we bring that back to the panel, and I think that that is very relevant to the concerns voiced by WVE that we decided to go with the QRA approach as opposed to an outright ban as Europe did. So I don't know if we're going to be discussing it, but that would be another good presentation.

DR. BERGFELD: So let's assume that that will also occur. Okay, Bart?

DR. HELDRETH: Yes. I will ask for both.

DR. BERGFELD: Good. All right. I'm going to call the question on the glucosamine and the IDA and the listed needs. We understand that in between we're going to get this information on the in vitro testing for assessment of sensitization. So all those opposing? Abstaining? Unanimously approved. So now moving on to our next ingredient, Dr. Belsito on ginger, another botanical.

Safety Assessment of Glucosamine 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.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. Previous Panel member involved in this assessment: Lisa, A. Peterson, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Priya Cherian, Senior Scientific Analyst/Writer, CIR.

1620 L Street, NW, Suite 1200 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651 ♢ fax 202.331.0088 ♢
cirinfo@cir-safety.org

ABBREVIATIONS

AUC_{ss}	area under the curve; extent of exposure
BAL	bronchoalveolar lavage
BCOP	bovine corneal opacity and permeability
BUN	blood urea nitrogen
CAS	Chemical Abstracts Service
CI	confidence interval
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
C_{max}	peak serum concentration
C_{ss}	peak concentration
DART	Developmental and Reproductive Toxicity
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
DNFB	dinitrofluorobenzene
DPRA	Direct Peptide Reactivity Assay
ECHA	European Chemicals Agency
ET_{50}	Effective time causing 50% reduction in tissue viability
FDA	Food and Drug Administration
FITC	fluorescein isothiocyanate
FW	formula weight
GFR	glomerular filtration rate
h-CLAT	human cell line activation test
HPLC	high performance liquid chromatography
HR	hazard ratio
HRIPT	human repeated insult patch test
IC_{50}	half maximal inhibitory concentration
IgE	immunoglobulin E
IGF-1	insulin-like growth factor 1
IL	interleukin
K_{ow}	n-octanol/water partition coefficient
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LD_{50}	median lethal dose
ME	microemulsion
MnNCE	micronucleated normochromatic erythrocytes
MnPCE	micronucleated polychromatic erythrocytes
MoS	margin of safety
MW	molecular weight
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCE	normochromatic erythrocytes
NOAEL	no-observable-adverse-effect-level
NR	not reported
OECD	Organisation for Economic Cooperation and Development
OVA	ovalbumin
Panel	Expert Panel for Cosmetic Ingredient Safety
PCE	polychromatic erythrocytes
PBS	phosphate-buffered saline
SBP	systolic blood pressure
SHR	spontaneously hypertensive rats
SLS	sodium lauryl sulfate
SIAscopy™	noncontact spectrophotometric intracutaneous analysis
SIDS	screening information dataset
SPF	sun protection factor
$T_{1/2}$	elimination half life
TG	test guidelines
THP-1	human monocytic cell line
T_{max}	time to reach serum concentration
UV	ultraviolet
VCRP	Voluntary Cosmetic Registration Program

DRAFT ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Acetyl Glucosamine, Glucosamine, Glucosamine HCl, and Glucosamine Sulfate. Two of these ingredients are reported to function in cosmetics as skin-conditioning agents, one is reported to function as a pH adjuster, and the function of Glucosamine is not reported. The Panel reviewed the available data to determine the safety of these ingredients and concluded that… [to be determined].

INTRODUCTION

This assessment reviews the safety of the following 4 ingredients as used in cosmetic formulations:

Acetyl Glucosamine	Glucosamine HCl
Glucosamine	Glucosamine Sulfate

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), Acetyl Glucosamine and Glucosamine Sulfate are reported to function in cosmetics as skin-conditioning agents – miscellaneous, Glucosamine HCl is reported to function as a pH adjuster, and the function of Glucosamine is not reported (Table 1).[1] These glucosamine ingredients are being reviewed together due to structural similarities, sharing an aminomonosaccharide core group in common.

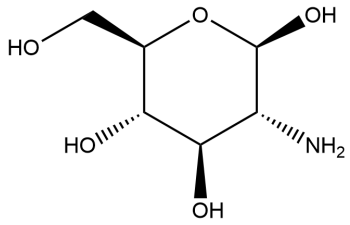
This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that 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).

Some of the data included in this safety assessment were found on the European Chemicals Agency (ECHA) website.[2,3] Please note that the ECHA website provides summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when ECHA is cited. Some types of data were found but not included, as no relevance to cosmetic use could be surmised (e.g., studies on the efficacy of Glucosamine for the treatment of arthritis).

CHEMISTRY

Definition and Structure

The definitions and structures of the ingredients included in this review are provided in Table 1. All of these ingredients share the ubiquitous aminomonosaccharide, Glucosamine (CAS No. 3416-24-8; Figure 1), as the core structure. Structurally, Glucosamine is modified glucose with an amine group replacing the hydroxyl group found on carbon two (C2).[4] Glucosamine and its salt forms, i.e., Glucosamine HCl (CAS No. 66-84-2) and Glucosamine Sulfate (CAS No. 29031-19-4), are metabolized to Acetyl Glucosamine (CAS Nos. 10036-64-3, 72-87-7, 7512-17-6) via the hexosamine pathway.[5]



Chemical Properties

Glucosamine HCl (formula weight (FW) = 215.63 g/mol; log K_{ow} = -1.91) and Glucosamine Sulfate (FW = 277.25 g/mol) are charged, highly polar, and water-soluble salts.[5] The acetylated glucosamine metabolite, Acetyl Glucosamine (MW = 222.21 g/mol; log K_{ow} = -2.2), is less polar and neutral. Available information on the chemical properties of the glucosamine ingredients are presented in Table 2.

Method of Manufacture

The methods described below are general to the processing of commercial forms of glucosamine ingredients. It is unknown if they apply to cosmetic ingredient manufacturing.

Acetyl Glucosamine

Acetyl Glucosamine may be prepared using chitin as a substrate via chemical, enzymatic, and biotransformation methods.[6] Chemical production of Acetyl Glucosamine involves the chemical degradation or dissolving of chitin with a

strong acid, such as hydrochloric acid. Another method of chemical production of Acetyl Glucosamine involves the acetylation of Glucosamine using pyridine as a solvent, in the presence of tributylamine and acetic anhydride. In addition, enzymatic hydrolysis may be performed to produce Acetyl Glucosamine. Several of these enzymes include derivatives of *Trichoderma viride*, *Aspergillus niger*, *Carica papaya* L., and *Aeronomium*. Examples of commercial crude enzymes that degrade chitin include cellulose, lysozyme, papain, and lipase. Production of Acetyl Glucosamine via biotransformation involves the degradation of chitin using whole microbes (e.g., *Aeromonas caviae*, *Chitinibacter tainanensis*). Genetically modified microorganisms (e.g., *Escherichia coli*) may also be used to produce Acetyl Glucosamine, using glucose as a substrate.

Glucosamine, Glucosamine HCl, and Glucosamine Sulfate

Commercial forms of Glucosamine are prepared mainly from the hydrolysis of chitin, which is the main component of shells from crustaceans (crab, lobster, and shrimp).[7] The produced Glucosamine can then be transformed into Glucosamine Sulfate or Glucosamine HCl. Glucosamine Sulfate is typically stabilized by co-crystallization or co-precipitation with sodium chloride. Commercial forms of Glucosamine can also be prepared from the hydrolysis of chitin with *Aspergillus niger* biomass.[8] In order to derive Glucosamine HCl, the hydrolysate is acidulated with hydrochloric acid for several hours at 100 °C. The product is then filtered to remove solid impurities. Crystals are separated and purified by centrifugation and washing with water.

Impurities

Acetyl Glucosamine

Impurities following chemical and enzymatic synthesis of β-*N*-Acetyl Glucosamine were evaluated via high resolution mass spectrometry, nuclear magnetic resonance spectroscopy, and liquid chromatograph-tandem mass spectrometry.[9] The impurities α-*N*,6-diacetylglucosamine and α-*N*-acetylglucosamine were observed to be present. β-*N*-Acetyl Glucosamine prepared via chemical and enzymatic methods contained a concentration of 146 ± 0.15 and 10.90 ± 0.02 µg/kg α-*N*,6-diacetylglucosamine, respectively. Quantification of α-*N*-acetylglucosamine was not performed, as the recovery value was too low.

Glucosamine HCl

The *United States Pharmacopeia* states that Glucosamine HCl must have a minimum of 98% purity and contain ≤ 3 ppm arsenic and ≤ 0.001 % heavy metals.[10] The purity of Glucosamine HCl sourced from *Aspergillus niger* is reported to be 83.1% free-base glucosamine.[8]

Natural Occurrence

Glucosamine is a monosaccharide that is synthesized from glucose by the hexosamine biosynthetic pathway in nearly all types of human body cells.[11] This natural compound is a constituent of mucosal secretions, skin, tendons, ligaments, and cartilage.[7] In mammals, Acetyl Glucosamine may be found as a component of glycoproteins, proteoglycans, glycosaminoglycans, and other connective tissue building blocks.[6] Acetyl Glucosamine may also be found in human milk at levels of 600 - 1500 mg/ml. Acetyl Glucosamine is the monomeric unit of chitin, which is found in arachnids, most fungal cell walls, insect exoskeletons, the shells of crustaceans, and parts of invertebrates. It may also be present as an extracellular polymer of some microbes.

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 the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category

According to 2022 VCRP survey data, Acetyl Glucosamine is reported to be used in 198 formulations (185 leave-on formulations and 13 rinse-off formulations; Table 3), and Glucosamine HCl is reported to be used in 77 formulations (64 leave-on formulations and 13 rinse-off formulations).[12] Glucosamine is reported to be used in 2 leave-on formulations. The results of the concentration of use survey reported by the Council in 2020 indicate Acetyl Glucosamine also has the highest concentration of use in a leave-on formulation; it is used at up to 5% in face and neck products (not spray).[13] No VCRP or concentration of use data were reported for Glucosamine Sulfate.

Incidental ingestion of Acetyl Glucosamine may occur, as it is used in lipstick formulations at concentrations up to 2%. In addition, Acetyl Glucosamine and Glucosamine HCl are used in formulations applied near the eye; for example, Acetyl Glucosamine is reported to be used at concentrations up to 2% in eye lotions.

Some of these glucosamine ingredients are used in formulations that could possibly be inhaled. For example, Acetyl Glucosamine is reported to be used at 0.1% in pump hair sprays and at up to 0.07% in face powders. 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 < 10 µm compared with pump sprays.[14,15] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[15,16] 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.[17-19]

All of the glucosamine ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[20]

Non-Cosmetic

In the US, Glucosamine (up to 1500 mg/d) and its metabolites are not classified as drugs, but as dietary supplements, under the US FDA Dietary Supplement Health and Education Act of 1994.[5,21] Acetyl Glucosamine and Glucosamine salts (Glucosamine Sulfate and Glucosamine HCl) are commercially available as dietary supplements, and are commonly administered in conjunction with chondroitin sulfate. According to 21 CFR 216.23, *N*-acetyl-D-glucosamine [Acetyl Glucosamine] is a bulk drug substance that may be used to compound topical drug products, in accordance with section 502A of the Federal Food, Drug, and Cosmetic Act.

In most European countries, Glucosamine is marketed as both a medicinal product and a food supplement.[7] In France, Glucosamine (in the form of the sulfate or HCl salt) is used in orally-ingested medicinal products as the only active ingredient (up to 1250 mg/d). In veterinary medicine, Glucosamine HCl is commonly used for treating osteoarthritis in dogs.[22]

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

Acetyl Glucosamine

The skin penetration of Acetyl Glucosamine was evaluated in split-thickness Caucasian cadaver skin.[23] The skin was cut and mounted in standard Franz-type diffusion cells (exposed skin surface area of 0.79 cm^2) maintained at 34 °C. The receptors were filled with phosphate-buffered saline (PBS) incorporating 1% polysorbate-20 and 0.02% sodium azide, and skin was allowed to equilibrate for 2 h. Test formulations (n = 8) contained either 2% Acetyl Glucosamine alone with the vehicle (vehicle not stated), or a combination of 4% niacinamide and 2% Acetyl Glucosamine with the vehicle. Approximately 5 µl of the test formulation was applied to the cells using a positive displacement pipette. The receptor solution was collected and replaced at 2, 4, and 6 h (termination) of study. Solutions were assayed for total radiolabel via liquid scintillation. Approximately 7% of the applied dose permeated the skin when the test substance containing only Acetyl Glucosamine was applied. Approximately 6.5% of the applied dose permeated the skin when the test substance containing both Acetyl Glucosamine and niacinamide was applied. The test substances were found to readily penetrate into and through human skin.

Glucosamine HCl

Using a saturated aqueous solution of Glucosamine HCl, in vitro permeation studies were performed on human epidermal membranes prepared by a heat separation method and mounted in Franz-type diffusion cells with a diffusional area of 2.15 ± 0.1 cm^2.[24] Studies were performed over a 48 h period by loading donor compartments with 2 ml of the Glucosamine HCl solution of each diffusion cell (n = 5), and evaluating receptor solutions for permeation. Glucosamine HCl permeated through the skin with a flux of 1.497 ± 0.42 µg cm^2/h, a permeability coefficient of 5.66 ± 1.6 x 10^{-6} cm/h, and a lag time of 10.9 ± 4.6 h.

The transdermal penetration of 5% Glucosamine HCl in different vehicles (aqueous, oil-in-water cream, liposomal suspension, liposomal gel, cubic liquid crystalline bulk phase) was evaluated in the dorsal skin of Sprague-Dawley rats mounted in Franz diffusion cells (diffusional surface area of 2.14 cm^3).[25] Epidermal sides of the skin were exposed to the various formulations of Glucosamine HCl (100 mg). Aliquots (0.5 ml) were withdrawn from the receptor compartment over a period of 12 h and evaluated for Glucosamine HCl via high-performance-liquid-chromatography (HPLC). The steady state flux of the drug through the skin for the aqueous solution, cream, liposomal suspension, liposomal gel, and cubic phase was calculated to be 56.89 ± 23.76, 58.24 ± 29.46, 57.61 ± 26.72, 57.27 ± 4.35, and 248.89 ± 64.57 µg/h/cm^2, respectively. According to study authors, the reason for the enhanced permeation of Glucosamine HCl caused by the cubic phase was likely due to the structural similarity between the cubic phase and biomembrane.

Glucosamine Sulfate

Skin permeation of Glucosamine Sulfate was evaluated in Sprague-Dawley full-thickness rat skin.[26] Freshly excised rat skin was mounted between the donor and receptor cell (area of diffusion was 2.14 cm^2). Donor cells, facing the stratum corneum surface, contained 5% Glucosamine Sulfate aqueous solution (3 ml). Receptor cells, which faced the dermis side,

were filled with normal saline solution (12 ml). At predetermined time intervals, 0.5 mL of the receptor solution was withdrawn and refilled with the same volume of fresh receptor solution. Samples were analyzed by HPLC. The skin permeation rate (amount recovered in receptor fluid) was determined to be 13.27 µg/cm^2/h.

Human

Glucosamine Sulfate

The penetration of a 10% Glucosamine Sulfate cream into the synovial fluid of patients with knee osteoarthritis (134 subjects/group) was evalauted.[27] For treated groups, cream (2 g) was placed on the knee, for 1-3 h, followed by synovial fluid collection. A control group was not subjected to any treatment, but their synovial fluid was collected. Synovial fluid from both treated and control groups was evaluated for Glucosamine concentrations via HPLC. The mean Glucosamine concentrations in treated and control patients were 100.56 ng/ml and 17.83 ng/ml, respectively ($p < 0.0001$).

Absorption, Distribution, Metabolism, and Excretion (ADME)

Animal

Oral

Glucosamine HCl

A pharmacokinetic analysis was performed via liquid chromatography-tandem mass spectrometry (LC-MS/MS) in 4 female Beagle dogs.[28] Animals were given a single oral dose of a dietary supplement containing 450 mg Glucosamine HCl. Blood samples from dogs were collected and analyzed 0, 1, 2, 4, 6, 8, 12, and 24 h post-administration. Glucosamine was detected up to 8 h post-dose, with a time to reach serum concentration (T_{max}) of 2 h and a peak serum concentration (C_{max}) of 9.69 µg/ml. The elimination half-life ($t_{1/2}$) of Glucosamine after administration of the test substance was approximately 35 min.

Glucosamine HCl and Glucosamine Sulfate

Blood levels, tissue distribution, and excretion patterns of radioactivity were studied in Sprague-Dawley rats (44 rats/ sex) after oral administration of [^{14}C]Glucosamine HCl diluted with unlabeled Glucosamine Sulfate (dose not reported).[29] Plasma, urine, feces, blood, and organs/tissues were evaluated for radiolabel concentrations. At 1 - 2 h after administration, Glucosamine radioactivity was bound to or incorporated into plasma proteins. After peaking at 2 - 4 h, radioactivity declined from plasma at a slower rate ($t_{1/2}$ = 46 h). Approximately half of the radioactivity was excreted as [^{14}C]carbon dioxide, and 40% of the radioactivity was excreted in the urine. Only 2% of the administered dose was excreted in feces. Radioactivity analysis in tissues and organs revealed that the [^{14}C] from the labeled Glucosamine quickly entered into all tissues, included cartilage, reaching a maximum at 8 h.

Human

Oral

Glucosamine HCl

Glucosamine HCl bioavailability from two different orally-administered formulations was evaluated in healthy adult males (9/group) under fasting conditions.[30] A single dose of Glucosamine HCl was administered to the volunteers via a dispersible tablet (240 mg Glucosamine HCl/tablet) or capsule (240 mg Glucosamine HCl/capsule). Subjects received either 2 Glucosamine HCl tablets or capsules with 250 ml water. Blood samples were collected before test substance administration, and at various intervals up to 12 h after administration. Plasma Glucosamine concentration was evaluated via the LC-MS/MS method. The mean C_{max}, T_{max}, and $T_{1/2}$ values were reported to be 907.1 ng/ml, 3.03 h, and 1.10 h, respectively, for the dispersible tablet formulation. For the capsule formulation, mean C_{max}, T_{max}, and $T_{1/2}$ values were reported to be 944.40 ng/ml, 3.30 h, and 1.50 h, respectively.

Glucosamine HCl and Glucosamine Sulfate

The pharmacokinetics of Glucosamine after oral administration of crystalline Glucosamine Sulfate and Glucosamine HCl were evaluated in 12 healthy volunteers (5 male and 7 female).[31] Volunteers received once-daily, oral administrations of crystalline Glucosamine Sulfate soluble powder at a dose of 1500 mg, or Glucosamine HCl capsules at a dose of 500 mg, for 3 consecutive days, alone, or in combination with chondroitin sulfate (400 mg). Glucosamine was determined at steady state in plasma collected up to 48 h after the last dose by a validated LC-MS/MS method. After Glucosamine Sulfate administration, peak concentrations (C_{ss}, max) and extent of exposure (AUC_{ss}) averaged 9.1 ± 6.3 µM and 76.5 ± 23.0 µM/h, respectively. Significantly lower plasma concentrations ($p \leq 0.005$) were determined after the administration of Glucosamine HCl alone ($C_{ss,\,max}$ and AUC_{ss} averaged 4.5 ± 1.8 µM and 21.4 ± 7.6 µM/h, respectively), or in combination with chondroitin sulfate ($C_{ss,\,max}$ and AUC_{ss} averaged 3.3 ± 1.0 µM and 13.8 ± 5.4 µM/h, respectively).

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

Details regarding the acute oral toxicity studies summarized below can be found in Table 4.

The reported median lethal dose (LD_{50}) values for Glucosamine were higher than the doses tested (> 15,000 mg/kg in mice and > 8000 mg/kg in rats and rabbits).[32] According to an ECHA dossier, the acute oral LD_{50} for Glucosamine HCl was reported to be 15,000 mg/kg bw in mice.[2]

Short-Term Toxicity Studies

Oral

Glucosamine HCl

The effect of oral Glucosamine was evaluated in male Sprague-Dawley and male spontaneously hypertensive rats (SHR; 8 rats/strain/group).[33] Four groups of both rat strains received either no treatment (control), Glucosamine (0.5%), chondroitin sulfate (0.4%), or a combination of both, for 9 wk, via diet. A concentration of 0.5% or 0.4% of Glucosamine and chondroitin sulfate roughly calculates to 1500 and 1200 mg/d, respectively. Systolic blood pressure (SBP) and body weight were evaluated weekly. Hematological and histological evaluations were performed. No statistically significant differences in body weight were observed in any of the four dietary groups. SBP of both strains consuming the two ingredients alone and in combination was statistically significantly lower than the SBP in control animals. No statistically significant histological differences were found in the hearts, kidneys, or livers among the treated and control groups. In Sprague-Dawley rats, there were no relevant trends in blood chemistries among the four groups, however BUN levels were significantly lower ($p < 0.03$) in the control group compared to the other three groups. In SHR, no hematological differences between groups were observed.

Subchronic Toxicity Studies

Animal

Oral

Acetyl Glucosamine

Acetyl Glucosamine was fed to F344 rats (10 rats/sex/group) via pelleted diets containing 0, 0.625, 1.25, 2.5 or 5% Acetyl Glucosamine for 13 wk.[34] Clinical signs, food intake, hematology, serum biochemistry, and histopathology were evaluated in all animals. All animals survived until the end of the experiment. A slight, non-significant increase in body weights was observed in males receiving 0.625, 1.25, and 2.5% Acetyl Glucosamine from wk 4 until the end of the experiment. Statistically significant elevation of weight gain was observed in males receiving 0.625, 1.25 and 2.5% Acetyl Glucosamine at the terminal sacrifice, which resulted in decreased relative weights in many organs. However, no obvious indications of toxicity were observed in any of the parameters evaluated. The no-observed-adverse-effect-level (NOAEL) was determined to be > 5%.

Human

Oral

Acetyl Glucosamine

The effect of orally ingested Acetyl Glucosamine was evaluated in healthy adult humans.[35] Safety assessments were performed via physical parameters, hematology, blood biochemistry, and urinalysis. The test supplement contained green tea extract powder and either 500 (n = 22) or 1000 (n = 22) mg of Acetyl Glucosamine. The placebo supplement contained green tea extract powder without Acetyl Glucosamine (n = 24). All subjects were instructed to take the supplements, dissolved in a cup of water, once a day for 16 wk. A total of 66 adverse events occurred in 12, 10, and 9 subjects receiving placebo, 500 mg/d Acetyl Glucosamine, and 1000 mg/d Acetyl Glucosamine, respectively, and there was no significant difference in the frequency among the 3 groups. Relatively frequent adverse symptoms included cold symptoms, gastric distress, and pain. These effects were generally mild. Routine physical and cardiovascular characteristics, hematology, and blood chemistry, did not show any significant abnormalities in all three groups.

Glucosamine HCl

A 16-wk, randomized, double-blind, placebo-controlled crossover trial of a combination of Glucosamine HCl (1500 mg/d), chondroitin sulfate (1200 mg/d), and manganese ascorbate (228 mg/d) was conducted in degenerative joint disease patients.[36] Thirty-four male patients were randomized and given either the test substance (a tablet containing a combination of Glucosamine HCl, chondroitin sulfate, and manganese ascorbate), or a placebo for 8 wk. For an additional 8-wk period, the patients crossed over to the regimen not followed previously. Patients were asked to complete a survey of symptoms consistent with toxicity and to return cards for fecal occult blood testing at the end of each protocol phase. No patients reported symptoms requiring termination of study, and symptom frequency on medication was similar to that at baseline. Vital signs, occult blood testing, and hematologic parameters were similar among the placebo and medicated groups.

Chronic Toxicity Studies

Oral

Acetyl Glucosamine

The chronic toxicity potential of Acetyl Glucosamine was evaluated in F344 rats (10 rats/sex/group).[37] Acetyl Glucosamine was administered via the diet at levels of 0, 1.25, 2.5 or 5%, for 52 wk. Clinical effects, mortality, hematology,

serum biochemistry, and histopathology were evaluated. After gross examination, the brain, heart, lungs, liver, spleen, adrenals, kidneys, and testes were weighed. No toxic effects were observed in any parameter evaluated; however, slight suppression of body weight gain was observed in animals dosed with concentrations of greater than 2.5%. This effect appeared to be due to a slight reduction of caloric intake with the high concentration of test compound.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

Glucosamine

The effects of Glucosamine treatment were evaluated in 8-wk old and 16-wk old adult female C57B1/6 mice (24 mice/group).[38] Each age group received either 0 or 20 mg/kg Glucosamine in the diet for 3 wk. After the 3-wk feeding period, treated animals were given an intraperitoneal injection of a solution containing PBS and Glucosamine (20 mg/kg). Mice that received no Glucosamine treatment during the feeding period received injections of PBS only. Mice were injected for 3 consecutive days. On the third day, each female was mated with a male. All mice were again treated accordingly with an injection of the Glucosamine and PBS solution, or PBS only, on the fourth day. Females that did not successfully mate were re-introduced to males and daily injections were repeated until mating was achieved, followed by a final injection on the day following successful mating, or until mating had been attempted for a maximum of 4 nights. Pregnancy outcomes were assessed at day 18 of gestation. The total number of implantations ($p < 0.0001$) and viable fetuses ($p < 0.0001$) was lowest in the 8-wk old, Glucosamine-treated group. The number of implantations and viable fetuses among the 16-wk old Glucosamine-treated mice and control mice did not differ significantly. Fetal weight was reduced by periconception Glucosamine treatment in 16-wk-old mice ($p < 0.05$), whereas the same treatment did not affect 8-wk old mice. Glucosamine also reduced fetal length in pups derived from 16-wk-old Glucosamine-treated mice ($p < 0.05$). In addition, a significantly higher number of abnormal fetuses was present in litters of 16-wk-old Glucosamine-treated mice, compared with all other groups ($p < 0.05$).

The effects of premating Glucosamine supplementation via drinking water on Sprague-Dawley rat litter homogeneity, uterine receptivity, and maternal hormones levels, were evaluated.[39] Female rats (29 animals/group) were given either normal drinking water, or drinking water supplemented with 0.5 mM Glucosamine, from 6 to 8 wk old. After a 2-wk administration, the rats were mated. Ovaries, uteri, implantation sites, pup birth weight, maternal placental efficiency, and plasma of dams were evaluated. Variation of within-litter birth weight in the Glucosamine-treated group was 5.55%, a significantly lower variation than that of the control group (8.17%). Birth weights and absolute and relative ovary weights were statistically significantly greater in the Glucosamine-treated group compared to the control group ($p < 0.05$). In the Glucosamine-treated group, there were more successfully implanted blastocysts (13.38 ± 0.63 and 15.75 ± 0.59 in the control and treated group, respectively), with more uniform distribution along the two uterine horns compared with the control group. Maternal progesterone, estradiol, and insulin-like growth factor 1 (IGF-1) concentrations on day 19.5 of pregnancy were significantly increased in treated rats, while insulin and total cholesterol levels were significantly decreased compared with control rats.

Intrauterine

Glucosamine

The effects of intrauterine Glucosamine were evaluated in female ICR mice (3 mice/group).[40] A hysterectomy of one uterine horn was performed according to standard surgical procedures. A 60-d sustained-release Glucosamine pellet (15, 150, or 1500 µg) or placebo pellet was implanted into the top of the remaining uterine horn. Females recovered independently for 10 d, and then mated with ICR male mice. The number of pups/litter was recorded until two litters after the 60-d pellet release period. After hysterectomy and implantation of placebo pellets, litters were approximately half the size that they were before surgery (5.6 and 12.7 pups/litter, respectively). Mice that received Glucosamine pellets delivered significantly fewer live pups/litter over a 60-d pellet active period than those that received placebo pellets (15 µg Glucosamine, 2.75 ± 0.73 pups/litter; 150 µg Glucosamine, 2.13 ± 0.85 pups/litter; 1500 µg Glucosamine, 0.25 ± 0.25 pups/litter; placebo, 5.61 ± 0.66 pups). The gross morphological appearance of the pups from placebo and Glucosamine-treated mice were normal post-birth. Serum glucosamine levels were similar among placebo and treated groups. After the 60-d pellet release period, there was no statistically significant difference in litter sizes delivered by Glucosamine-treated and placebo-treated mice, except at the highest dose level.

GENOTOXICITY STUDIES

In Vitro

Acetyl Glucosamine

An Ames assay was performed according to Organization for Economic Co-Operation and Development test guideline (OECD TG) 471.[3] *Salmonella typhimurium* strains TA 1537, TA 1535, TA 98, TA 100, and TA 102 were exposed to Acetyl Glucosamine at concentrations of 156.25, 312.5, 625, 1250, 2500, and 5000 µg/plate, with and without metabolic activation.

Plates were maintained in triplicate, and the number of revertant colonies were recorded after the 48-h incubation period. The test substance was non-mutagenic to any strain of *S. typhimurium* when tested under specified experimental conditions.

Glucosamine HCl

The potential genotoxicity of Glucosamine HCl derived from *Aspergillus niger* was evaluated in an Ames assay.[8] The tester strains (*S. typhimurium* TA 98, TA 100, TA 1535, and TA 1537, and *E.coli* WP2 uvrA) were exposed to Glucosamine HCl at concentrations of 100, 333, 1000, 3300, and 5000 µg/plate, with and without metabolic activation. The test substance was considered to be non-mutagenic.

In Vivo

Glucosamine HCl

An in vivo micronucleus assay was performed in accordance with OECD TG 474.[8] Mice (number of animals and strain not reported) were dosed with *Aspergillus niger*-derived Glucosamine HCl mixed with water, via gavage. The test substance was administered in doses of 500, 1000, or 2000 mg/kg bw. There was no statistically significant increase in micronucleated polychromatic erythrocytes (PCE) or decrease in the ratios of polychromatic PCEs and normochromatic erythrocytes (NCE) at any dose level. The test substance was considered to be non-toxic to bone marrow.

ANTI-GENOTOXICITY STUDIES

In Vitro

Acetyl Glucosamine and Glucosamine

The anti-genotoxic effect of Glucosamine and Acetyl Glucosamine in human peripheral lymphocytes exposed to oxidative stress was evaluated.[41] Lymphocytes were treated with Acetyl Glucosamine or Glucosamine at concentrations of 0, 2.5, 5, 10, 20, or 50 mM. Cells were also treated with 25 µM hydrogen peroxide to induce DNA damage. Control cells were treated with the vehicle (PBS) and hydrogen peroxide. Cells were analyzed and data were presented as % DNA in tail. Acetyl Glucosamine only indicated a slight DNA protection at a concentration of 50 mM ($p < 0.01$). Glucosamine, at all concentrations, showed a significant protective activity ($p < 0.001$) against hydrogen peroxide-induced DNA damage.

In Vivo

Glucosamine

The chemoprotective ability of Glucosamine against cisplatin-induced genotoxicity was evaluated in rat bone marrow cells.[42] Male Wistar rats (5/group) were fed diets containing either 75 or 150 mg/kg Glucosamine, for 7 consecutive d. On the 7th d, 1 h after Glucosamine treatment, a single intraperitoneal dose of cisplatin (5 mg/kg) was administered. Three control groups were used, a normal control group (oral PBS treatment and injection with saline), a Glucosamine control group (oral 150 mg/kg Glucosamine treatment and injection of PBS), and a cisplatin control group (oral PBS treatment and injection of cisplatin). All animals were killed 24-h post-treatment with cisplatin, and rat bone marrow cells were collected. For each experimental group, a total of 5000 PCE and corresponding NCE were scored to determine the number of micronucleated polychromatic erythrocytes (MnPCE) and micronucleated normochromatic erythrocytes (MnNCE). Pretreatment with 75 and 150 mg/kg Glucosamine prior to cisplatin injection significantly reduced the frequency of MnPCE and MnNCE ($p < 0.05$). Treatment with Glucosamine also prevented the fall in the PCE/(PCE + NCE) ratio as compared with the cisplatin control group ($p < 0.001$). The test substance was considered to be an effective chemoprotector against cisplatin-induced DNA damage.

CARCINOGENICITY STUDIES

Acetyl Glucosamine

The carcinogenic potential of Acetyl Glucosamine was evaluated in F344 rats (50 rats/sex/group).[37] Animals were given Acetyl Glucosamine in the diet at levels of 0, 2.5, or 5%, for 104 wk. Many tumors were found in males and females in all groups; however, all tumors observed were well-known to occur spontaneously in F344 rats. No significant intergroup differences in tumor frequency or histological types were apparent. Additionally, the number of neoplastic lesions observed in animals was similar among control and treated groups. The test substance was considered to be non-carcinogenic.

ANTI-CARCINOGENICITY STUDIES

In Vitro

Glucosamine

The anti-proliferative potential of Glucosamine in human renal cancer cell lines (786-O and caki-1) was studied via an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and annexin V-fluorescein isothiocyanate (FITC) assay.[43] To evaluate cell proliferation, renal cancer cells were treated with either 0, 1, 5, or 10 mM Glucosamine, and incubated. After incubation, MTT solution was added, cells were again incubated, followed by addition of dimethyl sulfoxide and the evaluation of optical density. Glucosamine inhibited the proliferation of renal cancer cells in a dose-

dependent manner ($p < 0.05$) as compared with the control group. In order to evaluate cell apoptosis, cancer cells were serum-starved for 24 h, and treated with various doses of Glucosamine (0, 1, 5, or 10 mM) for 24 h. Cells were then collected and washed twice with PBS. Then, cells were re-suspended, stained with FITC-annexin V/PI and analyzed by flow cytometry. The apoptosis rate of both cell lines was up-regulated by the high concentration of Glucosamine (10 mM), but down-regulated by low concentrations of Glucosamine (1 and 5 mM), as compared with the control groups.

Acetyl Glucosamine, Glucosamine, and Glucosamine HCl

The growth inhibitory effects of Glucosamine, Glucosamine HCl, and Acetyl Glucosamine on human hematoma SMMC-721 cells were evaluated in vitro.[44] Tumor cells were cultured in a growth medium supplemented with 15% bovine calf serum, 100 U/ml penicillin, and 100 U/ml streptomycin at 37° C, seeded in 96-well plates, and incubated for 24 h. After incubation, cells were treated with Glucosamine, Glucosamine HCl, or Acetyl Glucosamine (10 - 1000 µg/ml), and again incubated for 24 – 120 h. Untreated cells were used as controls. Results measured by an MTT assay showed that Glucosamine HCl and Glucosamine caused a concentration-dependent reduction in hepatoma cell growth. In addition, human hepatoma cells treated with Glucosamine HCl resulted in the induction of apoptosis as assayed qualitatively by agarose gel electrophoresis. Acetyl Glucosamine did not inhibit the proliferation of SMMC-7721 cells.

Animal

Glucosamine HCl

Sarcoma 180 tumor ascites cells were subcutaneously inoculated (0.2 ml/mouse) into 8-wk-old Kunming male mice (number of animals not stated).[44] Mice were divided and given an oral dose of either saline (control group) or Glucosamine HCl dissolved in saline (125, 250, or 500 mg/kg/d). The method of oral administration was not stated. Administrations occurred once daily for 10 d. The tumor was allowed to grow on mice for 10 d before it was removed from the animal and evaluated. The anti-tumor activity of Glucosamine HCl was expressed as an inhibition ratio calculated as [(average tumor weight of control – average tumor weight of treated group)/average tumor weight of control] x 100%. Glucosamine HCl, at the intermediate dose (250 mg/kg/d), had the highest inhibition ratio (34.02%) on sarcoma 180 tumor growth. Inhibition ratios at the 125 and 500 mg/kg/d dose levels were reported to be 27.84 and 29.33%, respectively.

OTHER RELEVANT STUDIES

Effects on Pigmentation

The following studies are included in this report as they may be relevant to concerns regarding depigmentation, skin whitening, and anti-melanogenesis.

In Vitro

Acetyl Glucosamine

The effect of Acetyl Glucosamine on melanin production was evaluated in an in vitro assay using reconstituted human tanned epidermis.[45] Skin cultures were placed in 6-well tissue culture plates containing 2 ml/well of a growth medium. Administrations of either Acetyl Glucosamine (1, 3, or 5% in water) or water alone (30 µl) were applied topically, for 10 d. Culture medium and treatment was replenished daily. Skin equivalent cell cultures treated topically with 1, 3, or 5% Acetyl Glucosamine produced dose-dependent decreases in melanin content. According to the study authors, Acetyl Glucosamine can inhibit the enzymatic glycosylation of tyrosinase, resulting in pigmentation effects. In addition, pigmentation effects following Acetyl Glucosamine exposure may occur due to its effect on the expression of several pigmentation-relevant genes.

The anti-melanogenic effect of an Acetyl Glucosamine-loaded microemulsion was evaluated in B16 melanoma cells.[46] The microemulsion contained 1% Acetyl Glucosamine, 9% water, and 10% propylene glycol, 20% palm oil, and 60% of a surfactant mixture. A control solution was prepared using the same components as the test microemulsion, excluding Acetyl Glucosamine. In addition, an aqueous solution containing 1% Acetyl Glucosamine was also evaluated (untreated B16 cells used for control). B16 cells were first plated with 1 µmol/l of α-melanin stimulating hormone for 3 d, followed by incubation with microemulsions, at a 1:2000 dilution, for 24 h. Melanin content in B16 melanoma cells decreased by 21% and 44% after treatment with the microemulsion and the microemulsion control, respectively. Slight melanin reduction was noted in B16 cells treated with the aqueous Acetyl Glucosamine solution (7% reduction), compared to the untreated control.

Animal and Human

Acetyl Glucosamine

The whitening effect of Acetyl Glucosamine in skin was examined in humans (number of subjects not specified) and brown guinea pigs (strain and number of animals not specified) that were subjected to ultraviolet (UV; wavelength not provided)-induced pigmentation.[47] The 5% Acetyl Glucosamine (information regarding solution not provided) was applied to the dorsal skin of brown guinea pigs and the inner side of human forearm skin for 8 wk, twice a day. In humans, a visual reduction in hyperpigmentation was observed 2 wk after treatment with the Acetyl Glucosamine solution, compared to the vehicle-treated group, and a strong decrease in visible pigmentation was observed after 8 wk of Acetyl Glucosamine treatment. The degree of hypopigmentation at each time point measured after the application of Acetyl Glucosamine was higher than the vehicle control group. In guinea pigs, biopsy specimens were obtained from both the treated and control

groups 4 wk after topical application. Acetyl Glucosamine-treated skin had decreased levels of melanin without affecting the number of melanocytes, compared to vehicle-treated skin.

Human

Acetyl Glucosamine

The reduction of facial hyperpigmentation after use of a moisturizer containing Acetyl Glucosamine and niacinamide was evaluated in a 10-wk, randomized, double-blind, vehicle-controlled trial.[48] During a 2-wk preconditioning period, the test subjects (101 women/group) used the same commercial facial cleanser, nighttime moisturizer, and daytime moisturizing lotion. After the 2-wk period, subjects used a daily regimen of either a morning sun protection factor (SPF) 15 sunscreen moisturizing lotion and evening moisturizing cream containing 4% niacinamide and 2% Acetyl Glucosamine, or the SPF 15 lotion and cream vehicles. Product-induced changes in apparent pigmentation were assessed by capturing digital photographic images of the women after 0, 4, 6, and 8 wk of product use. Images were evaluated by algorithm-based computer image analysis for colored spot area fraction, by expert visual grading, and by chromophore-specific image analysis based on noncontact spectrophotometric intracutaneous analysis (SIAscopy^{TM}) for melanin spot area fraction, and melanin chromophore evenness. By all parameters measured, the Acetyl Glucosamine and niacinamide formulation regimen caused a more pronounced decrease in detectable areas of facial spots and the appearance of pigmentation, compared to those that used the control formulation ($p < 0.05$).

A similar study, from Japan, was performed in healthy women ($n = 25$ women/group).[23] Volunteers were instructed to apply a formulation (0.3 g) containing either the placebo control or 2% Acetyl Glucosamine, on the side of the face, twice daily, for 8 wk. Digital images of each side of the face of all subjects were captured at baseline, and at week 4 and 8. Topical 2% Acetyl Glucosamine was effective in improving the appearance of facial hyperpigmentation based on computer image analysis, with an overall directional ($p = 0.089$) spot area fraction change across the entire study.

Forty-five Caucasian women (Fitzpatrick skin types I, II, and III), aged 40 - 65 yr, with moderate skin texture and the presence of at least mild to moderate-severe hyperpigmentation on the décolletage, were used in this study.[49] Volunteers were instructed to apply a neck cream containing 8% Acetyl Glucosamine and 4% triethyl citrate, each day, for 16 wk. Skin pigmentation and texture were graded using a 0 – 5 scale with half-point increments. Irritation/tolerability parameters (dryness, itching, stinging/burning) were measured at week 0, 8, 12, and 16 using a 0 - 3 scale (none, mild, moderate, severe). Colorimetric measurements were also made at week 0, 8, and 16. A significant reduction of skin pigmentation was observed at each time point ($p < 0.001$). After 16 wk, skin pigmentation was reduced by 23%. Chromameter measurements revealed significant improvement at week 8 and 16 in brightness ($p < 0.001$) and erythema ($p < 0.05$). The test cream was well-tolerated with no signs of irritation. One subject experienced an adverse event of contact dermatitis on two separate occasions. No other adverse events were reported.

Reduction of IgE-Mediated Hypersensitivity

The following studies are included in this report as they may be helpful in addressing cosmetic safety concerns regarding immunoglobulin E (IgE)-mediated hypersensitivity.

Glucosamine

The effect of Glucosamine on ovalbumin (OVA)-induced atopic dermatitis was evaluated in female BALB/c mice (5 mice/group).[50] Approximately 1.5 ml of OVA and 3 ml of aluminum hydroxide gel were mixed, and 150 µl of the mixture was intraperitoneally injected into mice 3 times a week, for 3 wk. After the first week of OVA injection, mice were epicutaneously sensitized with OVA patches (1 cm x 1 cm patch containing 50 µl OVA). Patches were applied 3 times a week, for 2 wk. After atopic dermatitis was induced, mice were given 100 µl Glucosamine injections at concentrations of 1 mg/10 µl, 1 mg/5 µl, and 1 mg/2.5 µl. After a week of Glucosamine administration, 3 OVA patches were again attached during the next week. In addition, two control groups were used. One group received a PBS injection without OVA induction, and a second group received a PBS injection with OVA induction. Clinical dermatitis scores decreased with increasing Glucosamine dose ($p < 0.001$). Concentrations of tissue interleukin (IL)-13 and IL-17 decreased after Glucosamine administration (each group: $p = 0.002$ and $p < 0.001$, respectively), but the concentrations of tissue IL-4 did not show differences across groups. Serum IgE levels tended to be lower after Glucosamine administration ($p = 0.004$).

The anti-allergic effect of Glucosamine in female BALB/c mice with allergic rhinitis and asthma was studied.[11] Mice (8/group) were given an OVA intraperitoneal/intranasal challenge to induce allergic asthma and rhinitis. Thirty min prior to sensitization induction, animals were administered Glucosamine treatment, via intraperitoneal injection, at concentrations of either 1 or 5%. A negative control group received an intranasal/intraperitoneal challenge using sterile saline, and did not receive Glucosamine treatment. A positive control group received an OVA intranasal/intraperitoneal challenge, and no treatment with Glucosamine. Serum total and OVA-specific IgE, cytokine titers, and the number of inflammatory cells in bronchoalveolar lavage (BAL) fluid were evaluated. A histopathologic examination of the lung and nasal cavity was also performed. OVA-specific IgE and eosinophils in BAL fluid were significantly decreased after 5% Glucosamine treatment compared with the positive control group ($P < 0.05$). In addition, significant improvement of inflammation was apparent in groups treated with 1 and 5% Glucosamine when compared to the positive control group.

Acetyl Glucosamine and Glucosamine HCl

The anti-allergic effect of orally ingested Acetyl Glucosamine and Glucosamine HCl was evaluated in female BALB/c mice (3 animals/group).[51] The dorsal skin of each mouse was shaved and 100 µL 0.5% dinitrofluorobenzene (DNFB) in acetone-soybean oil was applied to induce sensitization. After induction, Acetyl Glucosamine or Glucosamine HCl (0.1 or 1 mg/mouse) was administered orally, once per day, for 6 d. The method of oral administration was not specified. One h after the final administration, both right and left ears were challenged with 20 µl 0.5% DNFB in acetone-soybean oil. The thickness of the right ear was measured with a dial thickness gauge 0, 6, and 24 h after DNFB challenge. In addition, the amount of histamine in the plasma of the right ear was measured. Oral administration of Acetyl Glucosamine or Glucosamine HCl significantly inhibited DNFB-induced ear swelling in mice at both 6 h and 24 h after DNFB challenge ($P < 0.05$), and reduced the concentration of histamine in both the ear and plasma of DNFB-treated mice ($P < 0.05$).

Effect of Oral Administration on Atopic Dermatitis

Glucosamine

The effect of orally-administered Glucosamine in the treatment of atopic dermatitis was evaluated in a placebo-controlled, double-blind, clinical trial. [52] Patients with atopic dermatitis received either a combination of 2 mg/kg cyclosporine and 25 mg/kg Glucosamine (n = 16; Group A), or a combination of 2 mg/kg cyclosporine and placebo (n = 17; Group B), for 8 wk. Among the 16 patients receiving Glucosamine treatment, 15 patients reported clinical improvement of atopic dermatitis symptoms. Clinical improvement was noted in 10 of 17 patients treated with the placebo. Among the 19 intention-to-treat patients in each group, three from group A and 4 from group B experienced adverse effects, with abdominal pain being the common adverse effect.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Details regarding the irritation and sensitization studies summarized below can be found in Table 5.

No irritation was noted in an vitro reconstructed human epidermis assay performed using Acetyl Glucosamine (99.42% purity).[3] Multiple in chemico/in vitro sensitization assays (direct peptide reactivity assay (DPRA), KeratinoSens™ assay, human cell line activation test (h-CLAT)) performed using Acetyl Glucosamine yielded negative results.[3] Very mild cumulative irritation was noted in a 21-d cumulative patch human dermal irritation using an eye cream containing 2% Acetyl Glucosamine (12 subjects; occlusive conditions).[53] HRIPTs performed using a mask containing 0.005% Acetyl Glucosamine (108 subjects), a liquid foundation containing 2% Acetyl Glucosamine, and a leave-on product containing 0.005% Glucosamine HCl (51 subjects) yielded negative results.[54-56] Similarly, no sensitization was noted in maximization assays performed, each in 25 subjects, using a product containing 0.01% Glucosamine and a product containing 0.25% Glucosamine HCl.[57,58]

OCULAR IRRITATION STUDIES

In Vitro

Acetyl Glucosamine

An EpiOcular™ 3-[4,5,-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) conversion assay was performed to determine the ocular irritation potential of a face serum containing 2% Acetyl Glucosamine.[59] Stratified human keratinocytes were exposed to the neat test article for 8, 16, 20, and 24 h. The effective time (ET_{50}) at which the test substance caused a 50% reduction in tissue viability was 17.2 h. The ET_{50} of the positive control was 16.3 min.

A bovine corneal opacity and permeability (BCOP) test assay was performed according to OECD TG 437.[3] Bovine corneas (3/group) were treated with either 750 µl of a saline solution containing 20% Acetyl Glucosamine, 750 µl of saline alone (negative control), or 750 µl of a saline solution containing 20% imidazole (positive control). Corneas were exposed for 4 h ± 5 min at 32 ± 1 °C. The mean in vitro irritancy scores for the test substance, negative control, and positive control were 0.42, 0.70, and 105.42, respectively.

CLINICAL STUDIES

Lack of Hypersensitivity to Shrimp-Derived Glucosamine

Glucosamine

The tolerability of shrimp-derived Glucosamine was evaluated in shrimp-allergic individuals.[60] Subjects with a history of shrimp allergy were recruited and tested for both shrimp reactivity and shrimp-specific IgE by an ImmunoCAP™ assay. Fifteen individuals with a positive skin prick test to shrimp and an ImmunoCAP™ class level of two or greater were selected for a double-blind placebo-controlled food challenge using Glucosamine-chondroitin tablets containing 1500 mg of synthetically-produced (control) or shrimp-derived Glucosamine. Immediate and delayed reactions (up to 24 h post-challenge) were evaluated via a questionnaire. All subjects tolerated the 1500 mg Glucosamine administration from the shrimp-derived and synthetic sources, without any incidences of hypersensitivity.

Case Reports

Glucosamine

A 52-yr-old with a history of long-standing intermittent asthma complained of exacerbation of underlying asthma.[61] Exacerbation was characterized by shortness of breath and wheezing. Inhaled albuterol was not sufficient to extinguish or diminish symptoms. Aside from osteoarthritis of the knees and hips, mild stage 1 hypertension, and obesity, the patient was in reasonably stable health. During the course of 3 wk, the patient's condition waxed and waned despite an increased albuterol dose. The patient mentioned that her symptoms began after beginning a Glucosamine-chondroitin sulfate preparation 3 times per day for arthritis treatment. This preparation contained 500 mg Glucosamine and 400 mg chondroitin sulfate. Within 24 h of discontinuing Glucosamine and chondroitin treatment, the patient's asthma symptoms completely subsided.

A 67-yr-old male with type-2 diabetes was given oral antidiabetic medication (500 mg metformin, twice daily).[62] The patient had also been previously taking angiotensin-converting-enzyme inhibitors for hypertension for 5 yr, and Glucosamine (1200 mg), once daily, for 3 yr, to relieve osteoarthritic knee pain. Fourteen yr after starting the diabetic medication, the patient was referred to a nephrology consultant due to non-proteinuric renal insufficiency and a reduction of the glomerular filtration rate GFR), from 86 to 46 ml/min, within 3 mo. A kidney biopsy revealed non-inflammatory, 40 – 50% fibrosis of the renal cortex associated with acute tubular necrosis. The etiological investigation was negative apart from the daily ingestion of 1200 mg Glucosamine. After stopping Glucosamine for 3 wk, GFR increased from 47.5 to 60 ml/min. Reintroduction of Glucosamine resulted in loss of kidney function after 3 wk, with GFR reduced from 60 to 53 ml/min.

Glucosamine Sulfate

A 76-yr-old woman with arterial hypertension and osteoarthritis was referred for evaluation after an episode of urticaria after drug intake.[63] The patient was prescribed Glucosamine Sulfate for osteoarthritis, and suffered from erythematous lesions and facial swelling within several hours after Glucosamine Sulfate intake. The following day, 5 min after a new dose, the patient developed tongue, facial, and throat swelling with facial erythema. She was treated in the emergency department with antihistamines and corticosteroids. Symptoms resolved within 4 h. After a washout period, a skin prick test and intradermal test with Glucosamine Sulfate was performed. The skin prick test yielded negative results, however, the intradermal test (concentration of 1.5 mg/ml) yielded positive results with a papule of 35 mm^2. The intradermal test in 10 healthy volunteers was negative.

EPIDEMIOLOGICAL STUDIES

Cancer Endpoints

Glucosamine

The association between Glucosamine use and colorectal cancer risk was examined among 113,067 volunteers in the Cancer Prevention Study II Nutrition Cohort.[64] Those with a history of colorectal cancer prior to 2001, those with inflammatory conditions, and those without sufficient information to determine exposure category for the Glucosamine variable, were excluded from this study. Participants were first asked about Glucosamine intake in 2001 (baseline). Those who reported current use were then asked to report this frequency and duration of use. At baseline, 10.7% of participants (12,060), reported current Glucosamine use on ≥ 4 d/wk for ≤ 2 yr, and 5.6% of participants (6729), reported current use on ≥ 4 d/wk for ≥ 3 yr. Glucosamine intake was surveyed and updated every 2 yr until 2011. Current use of Glucosamine, modeled using a time-varying exposure, was associate with a lower risk of colon cancer (hazard ratio (HR): 0.83, 95% confidence interval (CI): 0.71 - 0.97), compared to those who reported no ingestion of Glucosamine. This reduction in risk, however, was only observed for shorter duration use of Glucosamine (HR: 0.68, 95%, CI: 0.52 - 0.87), rather than the longer duration of use (HR: 0.99, 95% CI: 0.76 - 1.29).

Similarly, the association between lung cancer and Glucosamine was evaluated in 76,904 volunteers with no prior history of lung cancer.[65] The participants were queried on their use of Glucosamine from the years 2000 - 2010. Low use participants were considered to be volunteers who ingested Glucosamine < 4 d/wk or < 3 yr, and high use was considered to be ingestion of Glucosamine for ≥ 4 d/wk and ≥ 3 yr. Compared to non-use, use of Glucosamine was associated with a 20% reduction in lung cancer risk (HR: 0.80, 95% CI: 0.65 - 0.99) after multivariable adjustment. High 10-yr use of Glucosamine (HR: 0.77, 95% CI: 0.56 – 1.05; P-trend = 0.04) was associated with a linear 23% reduction in lung cancer risk. A large proportion of volunteers who reported Glucosamine use also used chondroitin. When the analysis of Glucosamine was restricted to non-users of chondroitin (Glucosamine-only) an inverse association with lung cancer was apparent (HR: 0.84, 95% CI: 0.61 – 1.17), and high 10-yr use of Glucosamine alone was associated with a 61% reduction in lung cancer risk (HR 0.39, 95% CI: 0.17- 0.86).

RISK ASSESSMENT

Glucosamine Sulfate

The Norwegian Food Safety Authority calculated margin of safety (MoS) values for the use of 10% Glucosamine Sulfate in a body lotion (35.0), leg cream (99.0), and face cream (178.0), and from overall exposure from cosmetics (29.2).[66]

These values were calculated assuming 100% dermal absorption, a NOAEL value of 430 mg/kg/d (obtained from a repeated oral dose toxicity assay performed in dogs with a bioavailability of 20%), and a calculated relative daily exposure of 123.20, 43.50, and 24.13 mg/kg bw/d for the body lotion, leg cream, and face cream, respectively. According to this assessment, maximum use levels were reported to be 18, 10, and 3.5% in face, leg and body lotion, respectively.

SUMMARY

The safety of Acetyl Glucosamine, Glucosamine, Glucosamine HCl, and Glucosamine Sulfate as used in cosmetics is reviewed in this assessment. According to the *Dictionary*, Acetyl Glucosamine and Glucosamine Sulfate are reported to function in cosmetics as skin-conditioning agents – miscellaneous, and Glucosamine HCl is reported to function as a pH adjuster. The function of Glucosamine is not reported

According to 2022 VCRP survey data, Acetyl Glucosamine, Glucosamine HCl, and Glucosamine are reported to be used in 198, 77, and 2 formulations, respectively. The results of the concentration of use survey conducted by Council indicate that Acetyl Glucosamine has the highest concentration of use in a leave-on formulation; it is used at up to 5% in face and neck products (not spray). Glucosamine Sulfate is not reported to be in use.

The skin penetration of Acetyl Glucosamine was evaluated in split-thickness Caucasian cadaver skin. Approximately 7% of the applied test substance (which contained 2% Acetyl Glucosamine) permeated the skin after 6 h. An in vitro permeation assay was also performed with Glucosamine HCl in human epidermal membranes. Over a 48-h period, Glucosamine HCl permeated through the skin with a flux of 1.497 ± 0.42 µg/cm^2/h, a permeability coefficient of $5.66 \pm 1.6 \times 10^{-6}$ cm/h, and a lag time of 10.9 ± 4.6 h. The dermal penetration of 5% Glucosamine HCl in different vehicles was evaluated in rat skin. Transdermal flux of Glucosamine HCl was greatest in the cubic liquid crystalline formulation (248.89 ± 64.57 µg/h/cm^2). The skin permeation rate of Glucosamine Sulfate was determined to be 13.27 µg/cm^2/h when evaluated in Sprague-Dawley full-thickness rat skin. The amount of Glucosamine in synovial fluid was measured in osteoarthritis patients following an application of 10% Glucosamine Sulfate cream. A mean Glucosamine concentration of 100.56 ng/ml was observed in the synovial fluid of treated patients.

Female Beagle dogs were given a single dose of 450 mg Glucosamine HCl, and a pharmacokinetic analysis was performed. Glucosamine was detected in the blood up to 8 h post-dose, with a T_{max} of 2 h and a C_{max} of 9.69 µg/ml. [^{14}C]Glucosamine HCl diluted with unlabeled Glucosamine Sulfate was given to Sprague-Dawley rats to examine excretion patterns of radioactivity. Radioactivity analysis in tissues and organs revealed that the [^{14}C] from the labeled Glucosamine quickly entered into all tissues, included cartilage, reaching a maximum at 8 h. Bioavailability was also evaluated in humans. Healthy adult males, under fasting conditions, were given a single oral dose of 480 mg Glucosamine HCl in a dispersible tablet or capsule form. The mean C_{max}, T_{max}, and $T_{1/2}$ values were reported to be 907.1 ng/ml, 3.03 h, and 1.10 h, respectively, for the dispersible tablet form, and 944.40 ng/ml, 3.30 h, and 1.50 h, respectively, for the capsule form. The pharmacokinetics of Glucosamine after a single oral administration of Glucosamine Sulfate and Glucosamine HCl were evaluated in 12 healthy volunteers. Glucosamine was determined at steady state in plasma collected up to 48 h after the last dose by a validated LC-MS/MS method. After Glucosamine Sulfate administration, peak concentrations and extent of exposure averaged 9.1 ± 6.3 µM and 76.5 ± 23.0 µM/h, respectively. Significantly lower plasma concentrations ($p \leq 0.005$) were determined after the administration of Glucosamine HCl.

The reported LD_{50} values for Glucosamine were higher than the doses tested (> 15,000 mg/kg in mice and > 8000 mg/kg in rats and rabbits). According to an ECHA dossier, the acute oral LD_{50} for Glucosamine HCl was reported to be 15,000 mg/kg bw in mice. In a 9-wk study, Glucosamine (0.5%) was fed to male Sprague-Dawley and SHR rats. The systolic blood pressure in treated rats was statistically significantly lower than control animals. No statistically significant histological differences were found in the hearts, kidneys, and livers, among the treated and control groups. Acetyl Glucosamine (up to 5%) was fed to F344 rats for 13 wk. No obvious indications of toxicity were observed in any of the parameters evaluated. The NOAEL was determined to be > 5%. The effect of orally-ingested Acetyl Glucosamine (1000 mg) was evaluated in healthy adults. Volunteers ingested the dissolved Acetyl Glucosamine in water, once a day, for 16 wk. A control group received green tea extract powder. Routine physical and cardiovascular characteristics, hematology, and blood chemistry, did not show any significant abnormalities between control and treated groups. The potential toxic effects of a tablet containing Glucosamine HCl (1500 mg/d), chondroitin sulfate (1200 mg/d), and manganese ascorbate (228 mg/d) in degenerative disease patients was evaluated in a 16-wk crossover study. No patients reported symptoms requiring termination of study, and symptom frequency on medication was similar to that at baseline. Vital signs, occult blood testing, and hematologic parameters were similar among the placebo and medicated groups. The chronic toxicity potential of Acetyl Glucosamine (up to 5%) given in the diet for 52 wk was evaluated in F344 rats. No toxic effects were observed in any parameter evaluated, however, slight suppression of body weight gain was observed in animals dosed with concentrations of greater than 2.5%.

The effects of Glucosamine (20 mg) treatment via oral ingestion and peritoneal injection was evaluated in 8-wk old and 16-wk old adult female C57B1/6 mice. Mice were fed the test substance via diet for 3 wk, and injected with Glucosamine for 3 consecutive days. On the third day of injection, mice were mated. Pregnancy outcomes were assessed at day 18 of gestation. Fetal weight and length were reduced in Glucosamine-treated 16-wk old mice, compared to control animals. In

addition, a significantly higher number of abnormal fetuses was present in litters of 16-wk old Glucosamine-treated mice compared with all other groups ($p < 0.05$). The effects of premating Glucosamine supplementation via drinking water on Sprague-Dawley rat litter homogeneity, uterine receptivity, and maternal hormones levels were evaluated. Female rats were given 0.5 mM Glucosamine via drinking water for 2 wk, and then mated. Birth weights and absolute and relative ovary weights were statistically significantly greater in the Glucosamine-treated group compared to the control group ($p < 0.05$). Maternal progesterone, estradiol, and IGF-1 concentrations on day 19.5 of pregnancy were significantly increased in treated rats, while insulin and total cholesterol levels were significantly decreased compared with control rats. The effects of intrauterine Glucosamine (up to 1500 µg) were evaluated in female ICR mice. Ten d after implantation of the Glucosamine pellet, mice were mated. Mice that received Glucosamine pellets delivered significantly fewer live pups/litter over a 60-d pellet active period than those that received placebo pellets. However, after the 60-d pellet active period, there was no statistically significant difference in litter sizes delivered by Glucosamine-treated and placebo-treated mice, except at the highest dose level.

Acetyl Glucosamine (up to 5000 µg/plate) was considered to be non-mutagenic in an Ames assay using *S. typhimurium* strains TA 1537, TA 1535, TA 98, TA 100, and TA 102, with and without metabolic activation. Similarly, an Ames assay was performed on Glucosamine HCl derived from *Aspergillus niger*. Tester strains (*S. typhimurium* and *E. coli* WP2 uvrA) were exposed to up to 5000 µg/plate of the test substance, with and without metabolic activation. No mutagenicity was observed. In an in vivo micronucleus assay, mice (strain not reported) were administered *Aspergillus niger*-derived Glucosamine HCl (up to 2000 mg/kg bw) in water, via gavage. There was no statistically significant decrease in the ratios of PCE and NCE at any dose level.

In an in vitro anti-genotoxicity assay, human peripheral lymphocytes were exposed to Glucosamine or Acetyl Glucosamine at concentrations up to 50 mM. DNA damage was induced with hydrogen peroxide. Glucosamine, at all concentrations, showed a significant protective activity ($p < 0.001$) against hydrogen peroxide-induced DNA damage. Acetyl Glucosamine only indicated a slight DNA protection at the highest test concentration. The chemoprotective ability of Glucosamine (diets containing up to 150 mg/kg Glucosamine; 7 d exposure) against cisplatin-induced genotoxicity was evaluated in male Wistar rats. The test substance was considered to be an effective chemoprotector against cisplatin-induced DNA damage.

The carcinogenic potential of Acetyl Glucosamine (up to 5% in the diet; 104-wk treatment) was evaluated in F344 rats. The test substance was considered to be non-carcinogenic. The anti-proliferative potential of Glucosamine (10 mM) was evaluated in human renal cancer cell lines (786-O and caki-1) via an MTT and FITC-annexin V/PI assay. The apoptosis rate of both cell lines was up-regulated by the high concentration of Glucosamine (10 mM), but down-regulated by low concentrations of Glucosamine (1 and 5 mM), as compared with the control groups. The growth inhibitory effects of Glucosamine, Glucosamine HCl, and Acetyl Glucosamine on human hematoma SMMC-721 cells was evaluated in vitro. Tumor cells were exposed to Glucosamine, Glucosamine HCl, or Acetyl Glucosamine, at concentrations of up to 1000 µg/ml. Results measured by an MTT assay showed that Glucosamine HCl and Glucosamine caused a concentration-dependent reduction in hepatoma cell growth. In an animal anti-carcinogenicity assay, Kunming male mice were inoculated with sarcoma 180 tumor cells. Mice were orally treated for 10 d with up to 500 mg/kg Glucosamine HCl dissolved in saline... Glucosamine HCl, at the intermediate dose (250 mg/kg/d), had the highest inhibition ratio (34.02%) on sarcoma 180 tumor growth.

The effect of Acetyl Glucosamine on melanin production was evaluated in an in vitro assay. Reconstituted human tanned epidermis cells were exposed to up to 5% Acetyl Glucosamine in water for 10 d. Dose-dependent decreases in melanin content were observed. The whitening effect of Acetyl Glucosamine (5%) was evaluated in human and brown guinea pig skin subjected to UV-induced pigmentation. A visual reduction in hyperpigmentation was observed 2 wk after treatment with the Acetyl Glucosamine solution, in humans, compared to the vehicle-treated group. Acetyl Glucosamine-treated guinea pig skin had decreased levels of melanin without affecting the number of melanocytes, compared to vehicle-treated skin. Anti-melanogenic activity was evaluated using an Acetyl Glucosamine-loaded microemulsion and an aqueous solution containing 1% Acetyl Glucosamine in B16 melanoma cells. Melanin content decreased by 22% and 7%, after treatment with the microemulsion and the aqueous solution, respectively.

The reduction of facial hyperpigmentation after topical treatment on Acetyl Glucosamine was evaluated in a 10-wk trial. Volunteers (101 women/group) were instructed to apply a facial lotion containing 4% niacinamide and 2% Acetyl Glucosamine twice a day for 8 wk. A control group applied the lotion vehicle without 4% and 2% Acetyl Glucosamine. By all parameters measured, the niacinamide and Acetyl Glucosamine formulation regimen caused a significant reduction in the detectable area of facial spots and appearance of pigmentation compared to the controls ($p < 0.05$). In a similar study, from Japan, healthy women ($n = 25$ women/group) were instructed to apply a facial lotion containing 2% Acetyl Glucosamine on the side of the face, twice daily, for 8 wk. A control group applied the vehicle lotion that did not contain Acetyl Glucosamine. Topical 2% Acetyl Glucosamine reduced the appearance of facial hyperpigmentation, with an overall directional ($p = 0.089$) spot area fraction change across the entire study.

The effects of a neck cream formulation containing 8% Acetyl Glucosamine was evaluated in 45 Caucasian women. Applications of the cream occurred once a day, for 16 wk. The test cream was well-tolerated with no signs of irritation. One subject experienced an adverse event of contact dermatitis on two separate occasions. No other adverse events were reported.

The effect of Glucosamine injections (concentrations up to 1 mg/2.5 µl) on OVA-induced atopic dermatitis was evaluated in female BALB/c mice. Clinical dermatitis scores decreased with increasing Glucosamine dose ($p < 0.001$). Concentrations of tissue IL-13 and IL-17 decreased after Glucosamine administration (each group: $p = 0.002$ and $p < 0.001$, respectively), but the concentrations of tissue IL-4 did not show differences across groups. The anti-allergic effect of Glucosamine (concentrations up to 5%) in female BALB/c mice with allergic rhinitis was evaluated. OVA-specific IgE and eosinophils in BAL fluid were significantly decreased after 5% oral Glucosamine treatment compared with the positive control group. In addition, significant improvement of inflammation was apparent in groups treated with Glucosamine when compared to the positive control group. The anti-allergic effects of orally-ingested Acetyl Glucosamine and Glucosamine HCl (up to 1 mg/mouse; 6 d treatment) was also evaluated in BALB/c mice with DNFB-induced skin sensitization. Oral administration of Acetyl Glucosamine or Glucosamine HCl significantly inhibited DNFB-induced ear swelling in mice at both 6 h and 24 h after DNFB challenge ($p < 0.05$), and reduced the concentration of histamine in both the ear and plasma of DNFB-treated mice ($p < 0.05$). In vivo sensitization assays performed on humans using various test substances (a mask containing 0.005% Acetyl Glucosamine, a product containing 0.01% Glucosamine, a leave-on product containing 0.005% Glucosamine HCl, and a product containing 0.25% Glucosamine HCl) yielded negative results.

The effect of orally-administered Glucosamine (25 mg/kg) in the treatment of atopic dermatitis was evaluated in an 8-wk, placebo-controlled, double-blind, clinical trial. Among the 16 patients receiving Glucosamine treatment, 15 patients reported clinical improvement of atopic dermatitis symptoms. Three Glucosamine-treated patients reported adverse effects, with abdominal pain being the most common adverse effect.

Potential skin irritation of Acetyl Glucosamine was evaluated in an in vitro assay using 3 reconstructed human epidermis samples. Reduction of cell viability was similar in the negative control and treated groups; therefore, the substance was considered to be non-irritating. Acetyl Glucosamine was predicted to be non-sensitizing in a DPRA, KeratinoSens™ assay, and h-CLAT. Very mild cumulative irritation was observed in a 21-d cumulative patch irritation assay performed using an eye cream containing 2% Acetyl Glucosamine (12 subjects). HRIPTs performed using a mask containing 0.005% Acetyl Glucosamine (108 subjects), a liquid foundation containing 2% Acetyl Glucosamine (105 subjects), and a leave-on product containing 0.005% Glucosamine HCl (51 subjects) yielded negative results. Similarly, no sensitization was in maximization assays performed, each in 25 subjects, using a product containing 0.01% Glucosamine and a product containing 0.25% Glucosamine HCl.

In vitro ocular irritation assays were performed using a face serum containing 2% Acetyl Glucosamine and a saline solution containing 20% Acetyl Glucosamine. Neither test substance was considered to be irritating when compared to positive controls.

The tolerability of orally-ingested, shrimp-derived Glucosamine was evaluated in 15 shrimp-allergic individuals. Subjects were given either 1500 mg of synthetically-derived or shrimp-derived Glucosamine. All subjects tolerated the 1500 mg Glucosamine administration from the shrimp-derived and synthetic sources, without any incidences of hypersensitivity.

A 52-yr old complained of exacerbation of underlying asthma after beginning treatment with a Glucosamine-chondroitin sulfate preparation containing 500 mg Glucosamine. Within 24 h of discontinuing Glucosamine and chondroitin treatment, the patient's asthma symptoms completely resolved.

A 67-yr-old male with type-2 diabetes was referred to a nephrology consultant due to non-proteinuric renal insufficiency and a reduction in GFR supposedly due to Glucosamine intake for the past 3 yr. After stopping Glucosamine for 3 wk, GFR increased from 47.5 to 60 ml/min.

A 76-yr-old woman with arterial hypertension and osteoarthritis was referred for evaluation after an episode of urticaria after Glucosamine Sulfate intake. After treatment with antihistamines and corticosteroids, symptoms resolved within 4 h.

The association between Glucosamine use and colorectal cancer risk was examined among 113,067 volunteers. Participants were asked to log their Glucosamine intake from 2001 - 2011. Current use of Glucosamine, modeled using a time-varying exposure, was associated with a lower risk of colon cancer, for those using Glucosamine for a short duration (HR: 0.68, 95% CI: 0.52 - 0.87). Similarly, the association between lung cancer and Glucosamine was evaluated in 76,904 volunteers with no prior history of lung cancer. The participants were queried on their use of Glucosamine from the years 2000 - 2010. Compared to non-use, use of Glucosamine was associated with a 20% reduction in lung cancer risk (HR: 0.80, 95% CI: 0.65 - 0.99) after multivariable adjustment.

The Norwegian Food Safety Authority calculated MoS values for the use of 10% Glucosamine Sulfate in a body lotion, leg cream, face cream, and from overall exposure from cosmetics. The MoS for each of these formulation types were 35.0, 99.0, 178.0, and 29.2, respectively.

DRAFT DISCUSSION

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

This assessment reviews the safety of Acetyl Glucosamine, Glucosamine, Glucosamine HCl, and Glucosamine Sulfate as used in cosmetic formulations. The Panel concluded [TBD].

The Panel noted the reproductive effects observed in mice and rats following oral ingestion and intraperitoneal injections of Glucosamine. The Panel determined that these effects would not be relevant to cosmetic exposure as administration in these studies resulted in a much higher systemic concentration of Glucosamine than would be expected with cosmetic use.

In addition, data included in this report indicate that Acetyl Glucosamine may have a skin lightening effect. The Panel noted that skin lightening is considered to be a drug effect, and should not occur during the use of cosmetic products. Because of that caveat, the Panel's knowledge of the mechanism of action (i.e., inhibition of tyrosinase activity resulting in reduced melanin synthesis), and clinical experience, concern for this effect in cosmetics was mitigated. Nevertheless, cosmetic formulators should only use this ingredient in products in a manner that does not cause depigmentation. The safety of these ingredients was further supported by a lack of clinical reports and negative in chemico/in vitro irritation and sensitization data on Acetyl Glucosamine (tested at 99.42%).

The Panel discussed the fact that some of these ingredients are used in formulations that could result in incidental inhalation (e.g., Acetyl Glucosamine is used at up to 0.1% in pump hair sprays). Inhalation toxicity data were not available; however, the oral toxicity data that were available did not report adverse effects. Additionally, 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, the concentrations at which the ingredients are used, and a lack of systemic toxicity, 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, structures, and functions of glucosamine ingredients.[1], CIR Staff

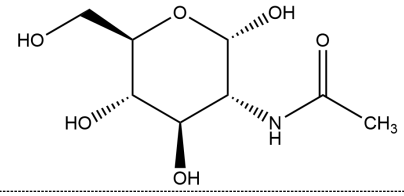
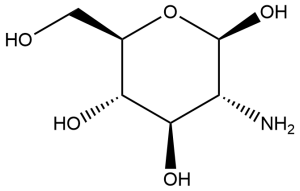
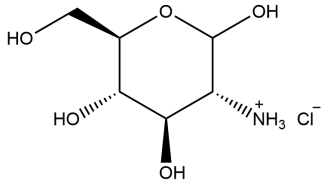
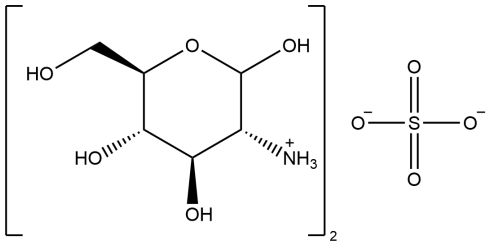
Ingredient	Definition	Function
Acetyl Glucosamine (10036-64-3; 72-87-7; 7512-17-6)	Acetyl Glucosamine is the organic compound that conforms to the structure: 	Skin-Conditioning Agents – Miscellaneous
Glucosamine (3416-24-8)	Glucosamine is the organic compound that conforms to the structure: 	Not Reported
Glucosamine HCl (66-84-2)	Glucosamine HCl is the amine salt that conforms to the structure: 	pH Adjusters
Glucosamine Sulfate (29031-19-4)	Glucosamine Sulfate is the amine salt that conforms to the structure: 	Skin-Conditioning Agents – Miscellaneous

Table 2. Chemical properties

Property	Value	Reference
Acetyl Glucosamine		
Physical Form	Solid	[3]
Color	White	[3]
Molecular Weight (g/mol)	221.21	[3]
Density (g/ml @ 20 ºC)	1.234	[3]
Vapor pressure (mmHg @ 20 ºC)	0.06	[3]
Melting Point (ºC)	162.7	[3]
Water Solubility (g/l @ 20 ºC)	256.8	[3]
log K_{ow} (@ 23.7 ºC)	-2.2	[3]
Glucosamine		
Physical Form	Solid	[67]
Molecular Weight (g/mol)	179.17	[67]
Vapor pressure (mmHg @ 25ºC)	0.0000000902	[68]
Melting Point (ºC)	88	[67]
Water Solubility (g/L)	551	[67]
log K_{ow}	-4.2	[68]
Disassociation constants (pKa)	7.58	[69]

Table 2. Chemical properties

Property	Value	Reference
Glucosamine HCl		
Physical Form	Crystalline	70
Formula Weight (g/mol)	215.63	71
Color	Off-White	70
Odor	Odorless	2
Specific Gravity (@ 38 ºC)	1.42	70
Melting Point (ºC)	190 - 194	70
Water Solubility	Soluble	2
log K_{ow}	-1.91	24
Disassociation constant (pKa) (@ 37 ºC)	7.75	24
Glucosamine Sulfate		
Physical Form	Solid	72
Color	Off-White	72
Formula Weight (g/mol)	277.25	72
Density(g/ml)	1.56	73
Boiling Point (ºC)	449.9	73
Water Solubility (g/l)	Freely soluble	73
Disassociation constants (pKa)	12.51 (estimated)	74

Table 3. Frequency (2022)[12] and concentration (2020)[13] of use

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Acetyl Glucosamine		Glucosamine		Glucosamine HCl	
Totals*	**198**	**0.001 – 5**	**2**	**0.04**	**77**	**0.0001 – 5**
Duration of Use						
Leave-On	*185*	*0.002 – 5*	*2*	*0.04*	64	*0.0001 – 0.9*
Rinse-Off	*13*	*0.001 – 5*	*NR*	*NR*	13	*0.07 – 5*
5Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	NR	*NR*
Exposure Type						
Eye Area	12	0.2 – 2	NR	NR	6	0.0001 – 0.2
Incidental Ingestion	3	0.002 – 2	NR	NR	NR	NR
Incidental Inhalation-Spray	75[a]; 72[b]	0.1; 0.005 – 0.07[b]	1[a]	NR	20[a]; 30[b]	NR
Incidental Inhalation-Powder	75[a]	0.07; 0.12 – 5[c]	1[a]	0.04[c]	20[a]	0.0006 – 0.38[c]
Dermal Contact	194	0.01 – 5	1	0.04	67	0.0001 – 5
Deodorant (underarm	NR	0.01	NR	NR	NR	NR
Hair - Non-Coloring	1	0.001 – 0.55	1	NR	10	0.55
Hair-Coloring	NR	0.01	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	4	0.002 – 2	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[a] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[b] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[c] It is possible these products are powders, but it is not specified whether the reported uses are powders
NR – not reported

Table 4. Acute oral toxicity studies

Ingredient	Animals	No. /group	Dose/Route of Administration	LD_{50}/Results	Reference
Glucosamine	Mice (strain unspecified)	NR	5000 mg/kg; gavage	LD_{50} > 5000 mg/kg	32
Glucosamine	CD-1 Mice	NR	8000 mg/kg; gavage	LD_{50} > 8000 mg/kg	32
Glucosamine	Mice (strain unspecified)	NR	15,000 mg/kg; gavage	LD_{50} > 15,000 mg/kg	32
Glucosamine	Sprague-Dawley Rat	NR	8000 mg/kg; gavage	LD_{50} > 8000 mg/kg; no adverse effects reported	32
Glucosamine	Rabbit (strain unspecified)	NR	8000 mg/kg; gavage	LD_{50} > 8000 mg/kg	32
Glucosamine HCl	Mice (strain unspecified)	NR	15,000 mg/kg (method of oral administration not specified)	LD_{50} = 15,000 mg/kg	2

NR = Not reported

Table 5. Dermal irritation and sensitization studies

Ingredient	Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION						
In Vitro						
Acetyl Glucosamine	Acetyl Glucosamine (99.42% purity)	tested neat; 16 mg	3	reconstructed human epidermis; OECD TG 439; positive control: 5% sodium dodecyl sulfate; negative control: PBS; 42 min incubation	Non-irritating	[3]
Human						
Acetyl Glucosamine	Eye cream containing 2% Acetyl Glucosamine	tested neat; 0.2 g	12	21-d cumulative patch test; patches removed and re-applied each day for 21 days (excluding weekends); occlusive conditions	Average irritation score of 0.34/4; very mild cumulative irritation	[53]
SENSITIZATION						
In Chemico/In Vitro						
Acetyl Glucosamine	Acetyl Glucosamine (99.42% purity)	tested neat	NR	DPRA; OECD TG 442C; test material exposed to model synthetic peptides containing cysteine and lysine; mean percent depletion of cysteine and lysine calculated	Non-sensitizing; mean percent depletion of cysteine and lysine was 1%	[3]
Acetyl Glucosamine	Acetyl Glucosamine (99.42% purity	0.98 to 2000 µM	3	KeratinoSens™ assay; OECD TG 442D; human epidermal keratinocytes exposed to test substance; cells analyzed for luciferase activity after 48 ± 2 h incubation period	Non-sensitizing; IC_{50} = > 2000 µM	[3]
Acetyl Glucosamine	Acetyl Glucosamine (99.42% purity	1395 - 5000 µg/ml	NR	h-CLAT; OECD TG 442E; THP-1 cells incubated with test substance for 24 h and analyzed via flow cytometry	Non-sensitizing; cell viability > 50% at all tested concentrations	[3]
Human						
Acetyl Glucosamine	Mask containing 0.005% Acetyl Glucosamine	tested neat; 2cm x 2 cm	108	HRIPT; occlusive conditions	Non-sensitizing	[54]
Acetyl Glucosamine	Liquid foundation containing 2% Acetyl Glucosamine	tested neat; 2 cm x 2 cm	105	HRIPT; occlusive conditions	Non-sensitizing	[56]
Glucosamine	Leave-on product containing 0.005% Glucosamine HCl	tested neat; 25-38 mg/cm^2	51	HRIPT; occlusive conditions	Non-irritating and Non-sensitizing	[55]
Glucosamine	Product containing 0.01% Glucosamine	tested neat; 2 cm x 2 cm	25	Maximization assay; induction phase – 0.25% SLS for 24 h; subjects then exposed to the test substance for 48-72 h (5 total induction applications); 10-d rest period; challenge phase – 5% SLS for 1 h; subject then exposed to test material for 48 h; all patches under occlusive conditions; sites evaluated 15 min, 30 min, and 24 h after patch-removal	Non-sensitizing	[57]
Glucosamine HCl	Product containing 0.25% Glucosamine HCl	tested neat; 0.05 g	25	Maximization assay performed according to the same procedures as above; occlusive conditions	Non-sensitizing	[58]

DPRA = direct peptide reactivity assay; h-CLAT = human cell line activation test; HRIPT = human repeated insult patch test; IC_{50} = half maximal inhibitory concentration; OECD TG = Organisation for Economic Cooperation and Development test guidelines; PBS = phosphate-buffered saline; SLS = sodium lauryl sulfate; THP-1 = human monocytic cell line

REFERENCES

1. Nikitakis J, Kowcz A. wINCI: *International Cosmetic Ingredient Dictionary and Handbook*. http://webdictionary.personalcarecouncil.org/jsp/Home.jsp. Washington, DC: Personal Care Products Council. Last Updated: 2021. Accessed: November 5, 2021.

2. European Chemicals Agency (ECHA). Glucosamine hydrochloride. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/11641/7/9/1. Last Updated: 2020. Accessed: October 7, 2020.

3. European Chemicals Agency (ECHA). N-acetyl-β-D-glucosamine. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/30216. Last Updated: 2020. Accessed: October 7, 2020.

4. Kelly GS. The role of glucosamine sulfate and chondroitin sulfates in the treatment of degenerative joint disease. *Alternative Medicine Review.* 1998;3(1):27-39.

5. Garner ST, Jr., Israel BJ, Achmed H, Capomacchia AC, Abney T, Azadi P. Transdermal permeability of N-acetyl-D-glucosamine. *Pharm Dev Technol.* 2007;12(2):169-174.

6. Chen J, Shen C, Liu C. *N*-Acetylglucosamine: production and applications. *Mar Drugs.* 2010;8:2493-2516.

7. French Agency for Food, Environmental, and Occupational Health & Safety. 2019. Opinion of the French Agency on Food, Environmental, and Occupational Health & Safety on the risks associated with the consumption of food supplements for joint conditions containing glucosamine and/or chondroitin sulphate. (ANSES Opinion Request No 2015-SA-0069.)

8. European Food Safety Authority (EFSA). Opinion on the safety of glucosamine hydrochloride from *Aspergillus niger* as food ingredient. *The EFSA Journal.* 2009;1099:1-19.

9. Kim YS, Lee SJ, Choi JY, et al. Determination of process-related impurities in N-acetylglucosamine prepared by chemical and enzymatic methods: structural elucidation and quantification. *Arch Pharm Res.* 2016;39(7):937-945.

10. U.S. Pharmacopeia. United States Pharmacopeia - National Formulary Online. https://www.usp.org/. Last Updated: 2021. Accessed: August 10, 2021.

11. Jung AY, Heo MJ, Kim YH. Glucosamine has an antiallergic effect in mice with allergic asthma and rhinitis. *Int Forum Allergy Rhinol.* 2017;7(8):763-769.

12. US Food and Drug Administration (FDA) Center for Food Safety & Applied Nutrition (CFSAN). 2022. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients. (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2022; received January 11, 2022). College Park, MD.

13. Personal Care Products Council. 2020. Concentration of Use by FDA Product Category: Glucosamine Ingredients. (Unpublished data submitted by the Personal Care Products Council on February 28, 2020.)

14. Johnsen MA. The influence of particle size. *Spray Technology and Marketing.* 2004;14(11):24-27.

15. Rothe H, Fautz R, Gerber, E, et al. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. Netherlands National Institute for Public Health and Environment; Bilthoven, Netherlands. *Toxicol Lett.* 2011;205(2):97-104.

16. Bremmer HJ, Prud'homme de Lodder L, van Engelen J. Cosmetics Fact Sheet: To assess the risks for the consumer, Updated version for ConsExpo4. Bilthoven, Netherlands 2006. RIVM 320104001/2006. 2006. http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Accessed June 25, 2019. Pages1-77.

17. CIR Science and Support Committee of the Personal Care Products Council (CIR SCC). 11-3-2015. Cosmetic powder exposure. Unpublished data submitted by the Personal Care Products Council.

18. Aylott R, Byrne G, Middleton J, Roberts M. Normal use levels of respirable cosmetic talc: preliminary study. *Int J Cosmet Sci.* 1979;1(3):177-186.

19. Russell R, Merz R, Sherman W, Siverston J. The determination of respirable particles in talcum powder. *Food Cosmet Toxicol.* 1979;17(2):117-122.

20. European Commission. CosIng database; following Cosmetic Regulation No. 1223/2009. http://ec.europa.eu/growth/tools-databases/cosing/. Last Updated: 2020. Accessed: October 10, 2020.

21. Dahmer S, Schiller RM. Glucosamine. *Am Fam Physician.* 2008;78(4):471-476.

22. Bhathal A, Spryszak M, Louizos C, Frankel G. Glucosamine and chondroitin use in canines for osteoarthritis: a review. *Open Vet J.* 2017;7(1):36-49.

23. Bissett DL, Robinson LR, Raleigh PS, et al. Reduction in the appearance of facial hyperpigmentation by topical N-acetyl glucosamine. *J Cosmet Dermatol.* 2007;6(1):20-26.

24. Tekko I, Bonner MC, Williams AC. An optimized reverse-phase high performance liquid chromatographic method for evaluating percutaneous absorption of glucosamine hydrochloride. *J Pharm Biomed Anal.* 2006;41(2):385-392.

25. Han IH, Choi S, Nam DY, et al. Identification and assessment of permeability enhancing vehicles for transdermal delivery of glucosamine hydrochloride. *Arch Pharm Res.* 2010;33(2):293-299.

26. Kanwischer M, Kim SY, Kim JS, Bian S, Kwon KA, Kim DD. Evaluation of the physicochemical stability and skin permeation of glucosamine sulfate. *Drug Dev Ind Pharm.* 2005;31(1):91-97.

27. Kong M, Hashim KB, Lin P, et al. Penetration of topical glucosamine sulfate into the synovial fluid of patients with knee osteoarthritis: a non-randomized, open-label, single dose, bioavailability study. *Journal of Biosciences and Medicines.* 2019;7(11):76-90.

28. Martinez SE, Lillico R, Lakowski TM, Martinez SA, Davies NM. Pharmacokinetic analysis of an oral multicomponent joint dietary supplement (Phycox(®)) in dogs. *Pharmaceutics.* 2017;9(3):30.

29. Setnikar I, Giachetti C, Zanolo G. Absorption, distribution and excretion of radioactivity after a single intravenous or oral administration of [14C] glucosamine to the rat. *Pharmatherapeutica.* 1984;3(8):538-550.

30. Wu H, Liu M, Wang S, et al. Comparative fasting bioavailability and pharmacokinetic properties of 2 formulations of glucosamine hydrochloride in healthy Chinese adult male volunteers. *Arzneimittelforschung.* 2012;62(8):367-371.

31. Persiani S, Rovati L, Pastorini E, Locatelli M. Pharmacokinetics of glucosamine in man after oral administration of crystalline glucosamine sulfate or glucosamine hydrocholoride alone or in combination with chondroitin sulfate. Osteoarthritis and Cartilage 15 (Suppl C):C223; 2007.

32. Anderson JW, Nicolosi RJ, Borzelleca JF. Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. *Food Chem Toxicol.* 2005;43(2):187-201.

33. Echard BW, Talpur NA, Funk KA, Bagchi D, Preuss HG. Effects of oral glucosamine and chondroitin sulfate alone and in combination on the metabolism of SHR and SD rats. Mol Cell Biochem. 2001 Sep:225(1):85-91.

34. Lee KY, Shibutani M, Takagi H, et al. Subchronic toxicity study of dietary N-acetylglucosamine in F344 rats. *Food Chem Toxicol.* 2004;42(4):687-695.

35. Kubomura D, Ueno T, Yamada M, Tomonaga A, Nagaoka I. Effect of N-acetylglucosamine administration on cartilage metabolism and safety in healthy subjects without symptoms of arthritis: A case report. *Exp Ther Med.* 2017;13(4):1614-1621.

36. Leffler CT, Philippi AF, Leffler SG, Mosure JC, Kim PD. Glucosamine, chondroitin, and manganese ascorbate for degenerative joint disease of the knee or low back: a randomized, double-blind, placebo-controlled pilot study. *Mil Med.* 1999;164(2):85-91.

37. Takahashi M, Inoue K, Yoshida M, Morikawa T, Shibutani M, Nishikawa A. Lack of chronic toxicity or carcinogenicity of dietary N-acetylglucosamine in F344 rats. *Food Chem Toxicol.* 2009;47(2):462-471.

38. Schelbach CJ, Robker RL, Bennett BD, Gauld AD, Thompson JG, Kind KL. Altered pregnancy outcomes in mice following treatment with the hyperglycaemia mimetic, glucosamine, during the periconception period. *Reprod Fertil Dev.* 2013;25(2):405-416.

39. Feng C, Yuan T, Wang S, et al. Glucosamine supplementation in premating drinking water improves within-litter birth weight uniformity of rats partly through modulating hormone metabolism and genes involved in implantation. *Biomed Res Int.* 2020;2020:1630890.

40. Tsai J-H, Schulte M, O'Neill K, Chi MMY, Frolova AI, Moley KH. Glucosamine inhibits decidualization of human endometrial stromal cells and decreases litter sizes in mice. *Biology of reproduction.* 2013;89(1):16-16.

41. Jamialahmadi K, Soltani F, Nabavi Fard M, Behravan J, Mosaffa F. Assessment of protective effects of glucosamine and N-acetyl glucosamine against DNA damage induced by hydrogen peroxide in human lymphocytes. *Drug Chem Toxicol.* 2014;37(4):427-432.

42. Cheki M, Jafari S, Najafi M, Mahmoudzadeh A. Glucosamine protects rat bone marrow cells against cisplatin-induced genotoxicity and cytotoxicity. *Anticancer Agents Med Chem.* 2019;19(14):1695-1702.

43. Wang LS, Chen SJ, Zhang JF, Liu MN, Zheng JH, Yao XD. Anti-proliferative potential of Glucosamine in renal cancer cells via inducing cell cycle arrest at G0/G1 phase. *BMC Urol.* 2017;17(1):38.

44. Zhang L, Liu W, Han B, Peng Y, Wang D. Antitumor activities of D-glucosamine and its derivatives. *J Zheijang Univ Sci B.* 2006;7(8):608-614.

45. Bissett DL, Farmer T, McPhail S, et al. Genomic expression changes induced by topical N-acetyl glucosamine in skin equivalent cultures in vitro. *J Cosmet Dermatol.* 2007;6(4):232-238.

46. Wuttikul K, Boonme P, Thammarat C, Khongkow P. N-acetylglucosamine microemulsions: Assessment of skin penetration, cytotoxicity, and anti-melanogenesis. *J Cosmet Dermatol.* 2021;20(1):304-309.

47. Hwang JS, Lee HY, Lim TY, Kim MY, Yoon TJ. Disruption of tyrosinase glycosylation by N-acetylglucosamine and its depigmenting effects in guinea pig skin and in human skin. *J Dermatol Sci.* 2011;63(3):199-201.

48. Kimball AB, Kaczvinsky JR, Li J, et al. Reduction in the appearance of facial hyperpigmentation after use of moisturizers with a combination of topical niacinamide and N-acetyl glucosamine: results of a randomized, double-blind, vehicle-controlled trial. *Br J Dermatol.* 2010;162(2):435-441.

49. Schlessinger J, Green B, Edison BL, Murphy L, Sabherwal Y. Scientific poster: A firming neck cream containing N-acetyl glucosamine significantly improves signs of aging on the challenging neck and décolletage. March 20-24, 2015. San Francisco, CA.

50. Yoon HS, Byun JW, Shin J, Kim YH, Choi GS. Therapeutic Effect of Glucosamine on an Atopic Dermatitis Animal Model. *Ann Dermatol.* 2019;31(5):538-544.

51. Sakai S, Sugawara T, Kishi T, Yanagimoto K, Hirata T. Effect of glucosamine and related compounds on the degranulation of mast cells and ear swelling induced by dinitrofluorobenzene in mice. *Life Sci.* 2010;86(9-10):337-343.

52. Jin S, Lim W, Sung NH, Cheong KA, Lee A. Combination of glucosamine and low-dose cyclosporine for atopic dermatitis treatment: A randomized placebo-controlled, double-blind, parallel clinical trial. *Dermatol Ther.* 2015;28(1):44-51.

53. Anonymous. 2006. 21-Day cumulative irritation patch test (eye cream with 2% Acetyl Glucosamine).

54. Anonymous. 2018. Repeat insult patch test (mask contains 0.005% Acetyl Glucosamine). (Unpublished data submitted by the Personal Care Products Council on February 19, 2021.)

55. Anonymous. 2012. Clinical safety evaluation repeated insult patch test (leave-on product containing 0.005% Glucosamine HCl). (Unpublished data submitted by Personal Care Products Council on April 29, 2021.)

56. TKL Research. 2011. Human repeat insult patch test (liquid foundation with 2% Acetyl Glucosamine).

57. Anonymous. 2005. An evaluation of the contact sensitization potential of a topical coded product in human skin by means of the maximization assay (product contains 0.01% Glucosamine). (Unpublished data submitted by Personal Care Products Council on February 19, 2021.)

58. Anonymous. 2007. An evaluation of the contact sensitization potential of a topical coded product in human skin by means of the maximization assay (product contains 0.25% Glucosamine HCl). (Unpublished data submitted by Personal Care Products Council on February 19, 2021.)

59. Institute for In Vitro Sciences Inc. 2009. Tissue equivalent assay with Epiocular™ cultures (face serum with 2% Acetyl Glucosamine).

60. Villacis J, Rice TR, Bucci LR, et al. Do shrimp-allergic individuals tolerate shrimp-derived glucosamine? *Clin Exp Allergy.* 2006;36(11):1457-1461.

61. Tallia AF, Cardone DA. Asthma exacerbation associated with glucosamine-chondroitin supplement. *J Am Board Fam Pract.* 2002;15(6):481-484.

62. Gueye S, Saint-Cricq M, Coulibaly M, et al. Chronic tubulointerstitial nephropathy induced by glucosamine: a case report and literature review. *Clin Nephrol.* 2016;86(2):106-110.

63. Matheu V, Gracia Bara MT, Pelta R, Vivas E, Rubio M. Immediate-hypersensitivity reaction to glucosamine sulfate. *Allergy.* 1999;54(6):643.

64. Kantor ED, Newton CC, Giovannucci EL, McCullough ML, Campbell PT, Jacobs EJ. Glucosamine use and risk of colorectal cancer: results from the cancer prevention study II nutrition cohort. *Cancer Causes Control.* 2018;29(3):389-397.

65. Brasky TM, Lampe JW, Slatore CG, White E. Use of glucosamine and chondroitin and lung cancer risk in the vitamins and lifestyle (VITAL) cohort. *Cancer Causes Control.* 2011;22(9):1333-1342.

66. Norwegian Food Safety Authority. Risk Profile *Glucosamine (GlcN)*. Oslo, Norway 2012. https://www.mattilsynet.no/kosmetikk/stoffer_i_kosmetikk/risk_profile_glucosamine_2012.9871/binary/Risk%20Profile%20Glucosamine%20(2012). Pages1-21.

67. Human Metabolome Database (HMDB). Metabocard for glucosamine (HMDB0001514). https://hmdb.ca/metabolites/HMDB0001514. Last Updated: 2020. Accessed: November 20, 2020.

68. 2004. Estimation Programs Interface Suite™ Ver 3.12. Washington, DC, USA: United States Environmental Protection Agency.

69. Bichsel Y, Von Gunten U. 2000. Environ Sci Technol Vol 34. 2784-2791.

70. Technical Services Branch of the USDA National Organic Program. Technical Evaluation Report: Glucosamine Hydrochloride Handling/Processing. https://www.ams.usda.gov/sites/default/files/media/Glucosamine%20Hydrochloride%20TR.pdf. Last Updated: 2010. Accessed: January 29, 2020.

71. National Center for Biotechnology National Library of Medicine. D-(+)-Glucosamine hydrochloride. https://pubchem.ncbi.nlm.nih.gov/compound/D-_-Glucosamine-hydrochloride. PubChem. Last Updated: 2020. Accessed: November 20, 2020.

72. ThermoFisher Scientific. Safety Data Sheet - D-Glucosamine sulfate. https://www.fishersci.com/store/msds?partNumber=AAJ6627106&productDescription=D-GLUCOSAMINE+SULFATE+98%25+5G&vendorId=VN00024248&countryCode=US&language=en. Last Updated: 2020. Accessed: November 20, 2020.

73. Guidechem Chemical Trading Guide. D-Glucosamine sulfate. https://www.guidechem.com/encyclopedia/d-glucosamine-sulfate-dic21885.html#Properties. Last Updated: 2020. Accessed: November 20, 2020.

74. PerkinElmer Inc. ChemDraw Pro. 14.0. Waltham, MA: 2021.

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, 2022

SUBJECT: Acetyl Glucosamine

TKL Research. 2011. Human repeat insult patch test (liquid foundation with 2% Acetyl Glucosamine).

Anonymous. 2006. 21-Day cumulative irritation patch test (eye cream with 2% Acetyl Glucosamine).

Institute for In Vitro Sciences, Inc. 2009. Tissue equivalent assay with Epiocular™ cultures (face serum with 2% Acetyl Glucosamine).

TKL RESEARCH

HUMAN REPEAT INSULT PATCH TEST

liquid foundation with 2% Acetyl Glucosamine

TKL STUDY NO. [redacted]

[redacted]

CONDUCTED FOR:

[redacted]

DATE OF REPORT:

March 16, 2011

Version 1.0

TABLE OF CONTENTS

APPENDICES

SIGNATURES

This study was conducted in compliance with the requirements of the protocol and TKL's Standard Operating Procedures, and in the spirit of GCP ICH Topic E6.[1] The report accurately reflects the raw data for this study.

Jonathan S. Dosik, MD
Dermatologist
Principal Investigator

03/15/11
Date

Kathleen Georgeian
Director, Dermatologic Safety Testing

3/16/11
Date

Michelle Medina
Manager, Dermatologic Safety Testing

3/16/11
Date

STATEMENT OF QUALITY ASSURANCE

This report has been reviewed by the TKL Research, Inc (TKL) Corporate Quality Assurance Department and the report accurately reflects the raw data for this study.

Quality Assurance

3/17/11
Date

[1] ICH Topic E6 "Note for guidance on Good Clinical Practices (CPMP/ICH/135/95)" – ICH Harmonised Tripartite Guideline for Good Clinical Practices having reached Step 5 of the ICH Process at the ICH Steering Committee meeting on 1 May 1996.

TITLE OF STUDY

Human Repeat Insult Patch Test

SPONSOR

[redacted]

STUDY MATERIAL

[redacted] Liquid Foundation with 2% acetyl glucosamine

DATE STUDY INITIATED

December 6, 2010

DATE STUDY COMPLETED

January 14, 2011

DATE OF REPORT

March 16, 2011

INVESTIGATIVE PERSONNEL

Jonathan S. Dosik, MD - Dermatologist
Principal Investigator

Kathleen Georgeian
Director, Dermatologic Safety Testing

Michelle Medina
Manager, Dermatologic Safety Testing

CLINICAL SITES

TKL RESEARCH, INC
48 Franklin Turnpike
Ramsey, NJ 07446

SUMMARY a liquid foundation containing 2% acetyl glucosamine

One product, [redacted], was evaluated neat to determine its ability to sensitize the skin of volunteer subjects with self-assessed sensitive skin using an occlusive repeated insult patch study. Of the enrolled study population 97.32% of subjects had self-assessed sensitive skin. This is a deviation from the protocol-specified 100% of subjects with self-assessed sensitive skin. One hundred five (105) subjects completed the study.

The dermatologist was present at the final visit.

Under the conditions employed in this study, there was no evidence of sensitization to [redacted]. a liquid foundation containing 2% acetyl glucosamine.

1.0 OBJECTIVE

The objective of this study was to determine the ability of the study material to cause sensitization by repeated topical applications to the skin of humans under controlled patch study conditions.

2.0 RATIONALE

Substances that come into contact with human skin need to be evaluated for their propensity to irritate and/or sensitize. Once an appropriate pre-clinical safety evaluation has been performed, a reproducible, standardized, quantitative patch evaluation procedure must be used to demonstrate that a particular material can be applied safely to human skin without significant risk of adverse reactions. The method herein employed is generally accepted for such a purpose.

Repeated insult patch evaluation is a modified predictive patch study that can detect weak sensitizers that require multiple applications to induce a cell-mediated (Type IV) immune response sufficient to cause an allergic reaction. Irritant reactions may also be detected using this evaluation method, although this is not the primary purpose of this procedure. Results are interpreted according to interpretive criteria based upon published works, as well as the clinical experience of TKL. These interpretive criteria are periodically reviewed and amended as new information becomes available.

3.0 STUDY DESIGN

3.1 STUDY POPULATION

A sufficient number of subjects were enrolled to provide 100 completed subjects. In the absence of any sensitization reactions in this sample size (100 evaluable subjects), a 95% upper confidence bound on the population rate of sensitization would be 3.5%.

3.1.1 Inclusion Criteria

Individuals eligible for inclusion in the study were those who:

1. Were males or females, 18 years of age or older, no more than 20% of the panel of subjects over the age of 65;
2. Were in general good health as determined by the Medical and Dermatological History Questionnaire (Appendix A of Protocol, see Appendix IV of this report);
3. Read, understood, and signed an informed consent agreement after being advised of the nature of the study;
4. Were willing to refrain from using lotions, creams, powders, or other skin preparations on the skin in the test area for the duration of the study; and
5. Were willing to refrain from exposing skin sites to the sun or going to tanning beds for the duration of the study.

3.1.2 Exclusion Criteria

Individuals excluded from participation in the study were those who:

1. Had a clinically significant active dermatitis or skin disease anywhere on the body (excluding facial acne);

2. Had a history of psoriasis, eczema, or skin cancer;

3. Had a condition or were taking medication(s) which, in the judgment of the Investigator or Designate, made the subject ineligible or placed the subject at undue risk;

4. Had received treatment (chemotherapy, radiation, immune suppressant medications) for any type of cancer within the last 6 months;

5. Had a mastectomy or axillary lymph nodes removed;

6. Had an autoimmune or immune deficiency disease (eg, lupus, myositis, Crohns disease, autoimmune thyroid diseases, autoimmune hepatitis);

7. Were taking any immunosuppressant medication;

8. Had insulin-dependent diabetes;

9. Had asthma or any other chronic respiratory condition requiring daily therapy;

10. Were using on a routine or frequent basis antihistamines or any systemic or topical anti-inflammatory medications (eg, ibuprofen, corticosteroid). Maximum acceptable dosage was determined by written laboratory guidelines;

11. Had used a topical anti-inflammatory in the patch area within the last 2 weeks;

12. Were receiving allergy injections, expected to start injections before the conclusion of the study, or had the final injection within a week of the study start;

13. Were participating in another dermal study of any kind;

14. Were participating in any clinical study, which in the judgment of the Investigator or Designate, could have potentially affected responses in either study;

15. Had a confirmed skin allergy as a result of participation in a patch study;

16. Had a known sensitivity or allergy related to the substance(s) evaluated;

17. Had a known sensitivity or allergy to adhesives, surgical tapes, bandages, etc; and/or

18. Had scars, moles, sunburn, tattoos, etc in the patch area.

3.1.3 Informed Consent

A properly executed informed consent document was obtained from each subject prior to entering the study. The signed informed consent document is maintained in the study file. In addition, the subject was provided with a copy of the informed consent document (see Appendix III).

3.2 DESCRIPTION OF STUDY

3.2.1 Outline of Study Procedures

Subjects participated in the study over a 6-week period involving 3 phases: (1) Induction, (2) Rest, and (3) Challenge. Prior to study entry, the subjects were screened to assure that they met the inclusion/exclusion criteria. Informed consent was obtained. Each subject was provided with a schedule of the study activities. All subjects were told to avoid wetting the patches and were asked not to engage in activities that caused excessive perspiration. They were instructed to notify the staff if they experienced any discomfort beyond mild itching or observed any adverse changes at the patch sites, while on the study or within 2 weeks of completing the study.

The Induction Phase consisted of 9 applications of the study material and subsequent evaluations of the patch sites. Prior to application of the patches, the sites were outlined with a skin marker, eg, gentian violet. The subjects were required to remove the patches approximately 24 hours after application. They returned to the facility at 48-hour intervals to have the sites evaluated and identical patches applied to the same sites. Patches applied on Friday were removed by subjects after 24 hours. The sites were evaluated on the following Monday, ie, 72 hours after patch application.[2]

Following the ninth evaluation, the subjects were dismissed for a Rest Period of approximately 10-15 days. Subjects who were absent once during the Induction Phase received a make-up (MU) patch at the last Induction visit. The MU applications were graded 48 hours later at the MU visit, or were recorded as N9G (no ninth grading).

The Challenge Phase was initiated during the sixth week of the study. Identical patches were applied to sites previously unexposed to the study material. The patches were removed by subjects after 24 hours and the sites graded after additional 24-hour and 48-hour periods (ie, 48 and 72 hours after application). Re-challenge was performed whenever there was evidence of possible sensitization.

To be considered a completed case, a subject must have had 9 applications and no fewer than 8 subsequent readings during Induction, and a single application and 2 readings during Challenge. Only completed cases were used to assess sensitization.

3.2.2 Definitions Used for Grading Responses

The symbols found in the data listings accompanying this report were used to express the response observed at the time of examination:

The following numerals were used to express the response observed at the time of examination. This scale was used only for grading the degree of erythema (redness).

[2] A Monday or Friday holiday could result in evaluation at 96 hours after patch application.

ERYTHEMA SCALE

Numerical Equivalent	Response
0	No visible erythema
1	Mild erythema (faint pink to definite pink)
2	Moderate erythema (definite redness)
3	Severe erythema (very intense redness)

DESIGNATIONS FOR ELEVATED RESPONSES

Symbol	Response
E	Edema – definite swelling
P	Papules – many small, red, solid elevations; surface of reaction has granular feeling
V	Vesicles – small, circumscribed elevations having translucent surfaces so that fluid is visible (blister-like). Vesicles are no larger than 0.5 cm in diameter.
B	Bullae – vesicles with a diameter of >0.5 cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.

OTHER RESPONSE CHARACTERISTICS

S	Spreading – evidence of the reaction beyond the pad area (does not include obvious signs of leakage of test substance away from pad).
W	Weeping – evidence of release of fluid from a vesicular or bullous reaction.

OTHER RECORDING DESIGNATIONS:

-	Subject absent
A	Marked reaction to adhesive (patch relocated)
X	Succeeding patch not applied and succeeding grade was for residual reaction. At challenge, an "X" denoted that the patch was not applied.
N9G	No Ninth Grade. Subject wore 9 induction patches but was not present for scoring following ninth application.

4.0 NATURE OF STUDY MATERIAL

4.1 STUDY MATERIAL SPECIFICATIONS

Identification : [redacted] a liquid foundation with 2% acetyl glucosamine
Amount Applied : 0.2 mL
Special Instructions : Applied to patch pad no longer then 15 minutes prior to patch application.

4.2 STORAGE, HANDLING, AND DOCUMENTATION OF STUDY MATERIAL

Receipt of the material used in this study was documented in a general logbook, which serves as a permanent record of the receipt, storage, and disposition of all study material received by TKL. On the basis of information provided by the sponsor, the study material was considered reasonably safe for evaluation on human subjects. A sample of the study material was reserved and will be stored for a period of 6 months. All study material is kept in a locked product storage room accessible to clinical staff members only. At the conclusion of the clinical study, the remaining study material was discarded or returned to the sponsor and the disposition documented in the logbook.

4.3 APPLICATION OF STUDY MATERIAL

Study material was applied to the patch as instructed. The patch was applied to the infrascapular area of the back, either to the right or left of the midline, or to the upper arm.

4.4 DESCRIPTION OF PATCH CONDITIONS

Material evaluated under occlusive patch conditions is applied to a 2 cm x 2 cm Webril pad attached to a non-porous, plastic film adhesive bandage (3M medical tape). The patch is secured with hypoallergenic tape (Micropore), as needed.

Material evaluated under semi-occlusive patch conditions is applied to a 2 cm x 2 cm Webril pad. The pad is affixed to the skin with hypoallergenic tape (Micropore).

5.0 INTERPRETATION

Sensitization is characterized by an acute allergic contact dermatitis. Typical sensitization reactions begin with an immunologic response in the dermis resulting in erythema, edema formation, and secondary epidermal damage (vesiculation), sometimes extending beyond the patch site and often accompanied by itching. Sensitization reactions tend to be delayed. The reaction typically becomes evident between 24 and 48 hours, peaks at 48-72 hours and subsequently subsides. The reaction is often greater at 72 hours than at 48 hours. The severity of the reaction is generally greater during the challenge phase of a Repeated Insult Patch Test (RIPT) than that seen during induction.

Irritant reactions are characterized as a non-immunologic, localized, superficial, exudative, inflammatory response of the skin due to an externally applied material. The typical initial reaction does not develop much edema or vesiculation but results in scaling, drying, cracking, oozing, crusting, and erosions. The reaction is usually sharply delineated, not spreading beyond the patch site. Irritant reactions are typically evident by 24 hours and diminish over the next 48-72 hours. Removal of the offending agent results in gradual improvement of the epidermal damage. The reaction seen at 72 hours is, therefore, less severe than that seen at 48 hours. Finally, the severity of

the reaction experienced in the challenge phase is generally similar to that seen during Induction.

If the results of the study indicate the likelihood of sensitization, the recommended practice is to re-challenge the subjects who have demonstrated sensitization-like reactions to confirm that these reactions are, indeed, associated with the product. Our preferred re-challenge procedure involves the application of the product to naïve sites, under both occlusive and semi-occlusive patch conditions. Use of the semi-occlusive patch condition helps to differentiate irritant and sensitization reactions. Generally speaking, if a product is a sensitizer it will produce a similar reaction under both occlusion and semi-occlusion. Whereas, if the product has caused an irritant reaction, the reactions will be less pronounced under the semi-occlusive condition.

6.0 PROTOCOL

See Protocol - Appendix IV.

7.0 DOCUMENTATION AND RETENTION OF DATA

The case report forms (CRFs) are designed to identify each subject by subject number and initials, and to record demographics, examination results, adverse events, and end of study status. Originals or copies of all CRFs, correspondence, study reports, and all source data will be kept on hard-copy file for a minimum of 20 years from completion of the study. Storage is maintained either at a TKL facility in a secured room accessible only to TKL employees, or at an offsite location which provides a secure environment with burglar/fire alarm systems, camera detection and controlled temperature and humidity. Documentation will be available for the sponsor's review on the premises of TKL.

8.0 RESULTS AND DISCUSSION

One hundred twelve (112) subjects between the ages of 18 and 75 were enrolled and 105 completed the study (see Tables 1 and 2 in Appendix I and Data Listings 1 and 2 in Appendix II). The following table summarizes subject enrollment and disposition:

Number enrolled:		112
Number discontinued:		7
Lost to follow-up:	5	
Voluntary withdrawal:	1	
Protocol violation: (lymph nodes removed)	1	
Number completed:		105

Source: Table 1, Appendix I

A total of 97.32% of the enrolled subjects had self-assessed sensitive skin. This is a deviation from the protocol-specified 100% of subjects with self-assessed sensitive skin.

There were no adverse events reported during the study.

Due to inclement weather, TKL Research was closed on December 27, 2010 (Monday). The subjects removed the product on December 25, 2010, ie, 24 hours after the product application on December 24, 2010. Sixty-six (66) subjects who did not miss any Induction visits received a no ninth grade (N9G) for their last Induction visit on December 28, 2010. Thirty-three (33) subjects, who missed one Induction visit in addition to the site closure, returned to the site on December 28, 2010 for the evaluation (96 hours) and new product application. This is deviation from the protocol-specified product applied on Friday to be evaluated on Monday at 72-hour interval. These subjects received a MU patch application on December 28, 2010 and were requested to return on December 29, 2010 for MU patch evaluation, ie, 24 hours after the MU patch application. This is a deviation from the protocol-specified 48-hour evaluation visit to the facility after patch application. The subjects either returned for the evaluation or received a N9G.

The change in the study schedule did not affect the conduct of the study and all subjects who did not discontinue prior to the Challenge visit were considered eligible for the Challenge Phase of the study.

The dermatologist was present at the final visit.

The summary of response data are provided in Appendix I. Individual dermatological response grades and sensitivity scale are provided in Data Listing 3, Appendix II.

9.0 CONCLUSION

Under the conditions employed in this study, there was no evidence of sensitization to [redacted]. a liquid foundation containing 2% acetyl glucosamine.

10.0 REFERENCES

Schwartz L, Peck SM. The patch test in contact dermatitis. Publ Health Pep 1944; 59:2.

Draize JH, Woodward G, Calvary HO. Methods for the study of irritation and toxicology of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944; 82: 377-390.

Lanman BM, Elvers WB, Howard CS. The role of human patch testing in a product development program. Joint Conf Cosmet Sci Toilet Goods Assoc 1968; 135-145.

Marzulli FN, Maibach HI. Contact allergy: predictive testing in man. Contact Dermatitis 1976; 2:1.

Zhai H, Maibach HI. Dermatotoxicology. 6th ed. New York:Hemisphere, 1996.

Stotts J. Planning, conduct and interpretation of human predictive sensitization patch tests. In:Drill VA, Lazar P, eds. Current Concepts in Cutaneous Toxicity. New York: Academic Press, 1980: 41-53.

Griffith JF. Predictive and diagnostic testing for contact sensitization. Toxicol Appl Pharmacol, Suppl 1969; 3:90.

Gerberick GF, Robinson MK, Stotts J. An approach to allergic contact sensitization risk assessment of new chemicals and product ingredients. American Journal of Contact Dermatitis 1993; 4(4): 205-211.

APPENDIX I

SUMMARY TABLES

TKL Study No. [redacted]

Table 1: Summary of Subject Enrollment and Disposition

	N (%)
Subjects enrolled	112
Subjects completed induction phase	106 (94.6)
Subjects completed all phases	105 (93.8)
Total subjects discontinued	7 (6.3)
Lost to follow-up	5 (4.5)
Voluntary withdrawal	1 (0.9)
Protocol violation	1 (0.9)

Note: All percentages are relative to total subjects enrolled.

See data listing 1 for further detail.

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Table 2: Summary of Subject Demographics
All Enrolled Subjects

Age	
N (%) 18 to 44	39 (34.8)
N (%) 45 to 64	53 (47.3)
N (%) 65 and up	20 (17.9)
Mean (SD)	50.3 (12.6)
Median	48.8
Range	18.9 to 75.7
Gender	
N (%) Male	21 (18.8)
N (%) Female	91 (81.3)
Race	
Amer Ind	1 (0.9)
Asian	3 (2.7)
Black	2 (1.8)
Caucasian	99 (88.4)
Hispanic	7 (6.3)

See data listing 2 for further detail.

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[redacted]
TKL Study No. [redacted]

Table 3: Summary of Dermatologic Response Grades
Number of Subjects by Product

Product = [redacted] liquid foundation with 2% acetyl glucosamine

	Induction Reading										Challenge Phase 48 hr		Challenge Phase 72 hr	
Response	1	2	3	4	5	6	7	8	9	Make Up	O	N	O	N
0	108	100	100	98	97	101	92	88	36	28	104	104	104	104
1	0	5	6	7	6	5	9	13	3	3	1	1	1	1
Total evaluable	108	105	106	105	103	106	101	101	39	31	105	105	105	105
Number absent	4	7	5	5	5	2	6	6	67	.	0	0	0	0
Number discontinued	0	0	1	2	4	4	5	5	6	.	7	7	7	7

Maximum Elicited Response During Induction
All Subjects Completing Induction (N=106)

Response	n(%) Subjects
0	89 (84.0%)
1	17 (16.0%)

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TKL Study No [redacted]

Table 3.1: Key To Symbols and Scores

[redacted] **Grading Scale**

ERYTHEMA SCALE	**and Definition of Symbols**
0	No visable erythema
1	Mild erythema (faint pink to definite pink)
2	Moderate erythema(definite redness)
3	Severe erythema (very intense redness)
	DESIGNATIONS FOR ELEVATED RESPONSES:
E	Edema, definite swelling
P	Papules - many small, red, solid evaluations; surface of reaction has granular feeling.
V	Vesicles - small, circumscribed elevations having translucent surfaces so that fluid is visible (blister-like). Vesicles are no longer than 0.5 cm in diameter.
B	Bullae - vesicles with a diameter > 0.5 cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.
	OTHER RESPONSE CHARACTERISTICS:
S	Spreading - evidence of the reaction beyond the pad area (does not include obvious signs of leakage of test substance away from pad)
W	Weeping - evidence of release of fluid from a vesicular or bullous reaction.
	OTHER RECORDING DESIGNATIONS
-	Subject absent
A	Marked reaction to adhesive (patch relocated)
X	Succeeding patch not applied and succeeding grade is for residual reaction At challenge an 'X' denotes that the patch was not applied.
N9G	No ninth grading

APPENDIX II

DATA LISTINGS

Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
001	12/06/10	12/06/10	01/11/11	01/13/11	I9	S	39
002	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
003	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
004	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
005	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
006	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
007	12/06/10	12/06/10	--	12/22/10	I6	V	17
008	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
009	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
010	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
011	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
012	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
013	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
014	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
015	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
016	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
017	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
018	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
019	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
020	12/06/10	12/06/10	--	12/13/10	I2	L	8
021	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
022	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
023	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
024	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
025	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
026	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
027	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
028	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
029	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
030	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
031	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
032	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
033	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
034	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
035	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
036	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
037	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
038	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
039	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
040	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
041	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
042	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
043	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
044	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
045	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
046	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
047	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
048	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
049	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
050	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
051	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
052	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
053	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
054	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
055	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
056	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
057	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
058	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
059	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
060	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
061	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
062	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
063	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
064	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
065	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
066	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
067	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
068	12/06/10	12/06/10	--	12/28/10	I8	L	23
069	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
070	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
071	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
072	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
073	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
074	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
075	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
076	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
077	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
078	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
079	12/06/10	12/06/10	--	12/17/10	I4	L	12
080	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
081	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
082	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
083	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
084	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
085	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
086	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
087	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
088	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
089	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
090	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
091	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
092	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
093	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
094	12/06/10	12/06/10	--	12/17/10	I4	L	12
095	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
096	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
097	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
098	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
099	12/06/10	12/06/10	--	12/15/10	I3	L	10
100	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
101	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
102	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
103	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
104	12/06/10	12/06/10	01/11/11	01/14/11	C	C	40
105	12/08/10	12/08/10	01/11/11	11/14/11	C	C	342
106	12/08/10	12/08/10	01/11/11	11/14/11	C	C	342
107	12/08/10	12/08/10	01/11/11	11/14/11	C	C	342
108	12/08/10	12/08/10	01/11/11	11/14/11	C	C	342
109	12/08/10	12/08/10	01/11/11	11/14/11	C	C	342
110	12/08/10	12/08/10	01/11/11	11/14/11	C	C	342
111	12/08/10	12/08/10	01/11/11	11/14/11	C	C	342
112	12/08/10	12/08/10	01/11/11	11/14/11	C	C	342

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
001	46.2	Male	Caucasian
002	43.9	Female	Caucasian
003	69.5	Female	Caucasian
004	47.7	Female	Caucasian
005	34.6	Female	Caucasian
006	35.1	Female	Caucasian
007	64.0	Female	Caucasian
008	44.4	Female	Caucasian
009	72.7	Female	Caucasian
010	35.7	Female	Caucasian
011	42.6	Female	Caucasian
012	52.3	Female	Caucasian
013	69.8	Female	Caucasian
014	75.7	Female	Caucasian
015	49.5	Female	Caucasian
016	41.5	Female	Caucasian
017	38.6	Female	Caucasian
018	65.8	Female	Caucasian
019	39.8	Male	Amer Ind
020	20.3	Female	Caucasian
021	49.3	Female	Caucasian
022	34.8	Female	Caucasian
023	73.4	Female	Caucasian
024	64.4	Male	Caucasian
025	58.8	Female	Caucasian
026	53.1	Female	Caucasian
027	39.7	Female	Hispanic
028	46.0	Female	Caucasian
029	46.1	Male	Black
030	50.2	Male	Caucasian
031	65.1	Female	Caucasian
032	47.2	Female	Caucasian
033	45.6	Female	Caucasian
034	46.4	Female	Caucasian
035	59.7	Female	Caucasian
036	60.1	Female	Hispanic
037	41.4	Female	Caucasian

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
038	58.5	Female	Caucasian
039	47.6	Female	Caucasian
040	46.4	Female	Caucasian
041	58.4	Male	Caucasian
042	60.3	Female	Caucasian
043	59.7	Female	Caucasian
044	67.6	Female	Caucasian
045	50.4	Male	Caucasian
046	40.6	Female	Hispanic
047	38.5	Female	Caucasian
048	53.2	Female	Caucasian
049	41.5	Female	Caucasian
050	44.3	Male	Caucasian
051	41.2	Female	Caucasian
052	18.9	Male	Caucasian
053	47.2	Male	Caucasian
054	50.1	Female	Caucasian
055	48.1	Female	Caucasian
056	47.3	Female	Caucasian
057	27.1	Female	Caucasian
058	30.2	Female	Caucasian
059	51.7	Female	Hispanic
060	48.3	Female	Caucasian
061	33.2	Female	Asian
062	41.7	Female	Asian
063	44.6	Female	Asian
064	46.7	Male	Caucasian
065	54.2	Female	Caucasian
066	75.0	Male	Caucasian
067	42.6	Male	Caucasian
068	55.4	Female	Caucasian
069	49.7	Female	Caucasian
070	68.1	Female	Caucasian
071	68.5	Female	Caucasian
072	73.3	Male	Caucasian
073	67.3	Female	Caucasian
074	27.0	Female	Caucasian

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
075	43.0	Female	Caucasian
076	47.3	Female	Caucasian
077	49.5	Female	Caucasian
078	40.7	Male	Caucasian
079	42.5	Female	Caucasian
080	55.3	Female	Caucasian
081	49.2	Female	Caucasian
082	55.2	Female	Caucasian
083	45.1	Female	Caucasian
084	30.1	Female	Caucasian
085	34.1	Female	Hispanic
086	58.2	Female	Caucasian
087	53.6	Male	Caucasian
088	52.1	Female	Caucasian
089	69.0	Female	Caucasian
090	50.6	Female	Hispanic
091	73.7	Female	Caucasian
092	68.2	Male	Caucasian
093	67.5	Female	Caucasian
094	37.3	Male	Caucasian
095	66.1	Female	Caucasian
096	52.9	Female	Caucasian
097	71.7	Female	Caucasian
098	42.1	Male	Caucasian
099	39.4	Male	Caucasian
100	50.9	Female	Caucasian
101	37.7	Female	Caucasian
102	43.7	Female	Caucasian
103	74.8	Female	Caucasian
104	32.9	Female	Hispanic
105	50.4	Female	Caucasian
106	46.3	Female	Caucasian
107	32.4	Female	Black
108	60.4	Male	Caucasian
109	43.9	Female	Caucasian
110	57.8	Female	Caucasian
111	57.0	Female	Caucasian
112	54.0	Female	Caucasian

[redacted]
TKL Study No. [redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted] liquid foundation with 2% acetyl glucosamine

Subject No.	Induction Reading										Challenge Phase 48 hr		Challenge Phase 72 hr	
	1	2	3	4	5	6	7	8	9	MU	O	N	O	N
001	0	0	0	0	(-)	0	0	0	0	0	(-)	(-)	(-)	(-)
002	0	0	0	0	0	0	0	0	N9G		0	0	0	0
003	0	0	0	0	0	0	0	0	N9G		0	0	0	0
004	0	0	0	0	0	0	0	0	N9G		0	0	0	0
005	0	0	0	0	0	0	0	0	N9G		0	0	0	0
006	0	0	0	0	0	0	0	0	N9G		0	0	0	0
007	0	0	0	0	0	0	(-)	(-)	(-)		(-)	(-)	(-)	(-)
008	0	0	0	0	0	0	0	0	N9G		0	0	0	0
009	0	0	0	0	0	0	0	0	N9G		0	0	0	0
010	0	0	0	0	0	0	0	0	N9G		0	0	0	0
011	0	0	(-)	0	0	0	0	0	0	0	0	0	0	0
012	0	0	0	0	0	0	0	0	N9G		0	0	0	0
013	0	0	0	0	0	1	1	1	N9G		0	0	0	0
014	0	0	0	0	0	0	0	0	N9G		0	0	0	0
015	0	0	0	0	(-)	0	0	0	0	0	0	0	0	0
016	0	0	0	0	0	0	0	0	N9G		0	0	0	0
017	0	0	0	0	0	0	0	0	N9G		0	0	0	0
018	0	0	0	0	0	0	1	1	N9G		0	0	0	0
019	0	0	0	0	0	0	0	0	N9G		0	0	0	0
020	(-)	0	(-)	(-)	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(-)
021	0	0	0	0	0	0	0	0	N9G		0	0	0	0
022	0	0	(-)	0	0	0	0	0	0	0	0	0	0	0
023	0	0	(-)	0	0	0	0	0	0	0	0	0	0	0

See Table 3.1 for Key to Symbols and Scores

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[redacted]
TKL Study No [redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted] liquid foundation with 2% acetyl glucosamine

	Induction Reading										Challenge Phase 48 hr		Challenge Phase 72 hr	
Subject No.	1	2	3	4	5	6	7	8	9	MU	O	N	O	N
024	0	0	0	0	0	0	0	0	N9G		0	0	0	0
025	0	0	0	1	1	1	1	(-)	0	0	0	0	0	0
026	0	0	0	(-)	0	0	0	0	0	0	0	0	0	0
027	0	0	0	(-)	0	0	0	0	0	0	0	0	0	0
028	0	0	0	0	0	0	0	1	N9G		0	0	0	0
029	0	0	0	0	0	0	0	(-)	0	0	0	0	0	0
030	0	0	0	0	0	0	0	0	N9G		0	0	0	0
031	0	0	0	0	0	0	0	0	N9G		0	0	0	0
032	0	0	0	0	0	0	0	0	N9G		0	0	0	0
033	0	0	0	0	0	0	0	0	N9G		0	0	0	0
034	0	0	0	0	0	0	0	0	N9G		0	0	0	0
035	0	0	0	0	0	0	0	0	N9G		0	0	0	0
036	0	0	0	0	0	0	0	0	N9G		0	0	0	0
037	0	0	0	0	0	0	0	0	N9G		0	0	0	0
038	0	0	0	0	0	0	0	0	N9G		0	0	0	0
039	0	0	0	0	0	0	0	0	N9G		0	0	0	0
040	0	0	0	0	0	0	(-)	0	0	0	0	0	0	0
041	0	0	0	0	0	0	0	0	N9G		0	0	0	0
042	0	0	0	0	(-)	0	0	0	0	0	0	0	0	0
043	0	0	0	0	0	0	0	0	N9G		0	0	0	0
044	0	0	0	0	0	0	0	0	N9G		0	0	0	0
045	0	0	0	0	0	0	0	0	N9G		0	0	0	0
046	0	0	0	(-)	0	0	0	0	0	0	0	0	0	0

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[redacted]
TKL Study No. [redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted] liquid foundation wiht 2% acetyl glucosamine

	Induction Reading										Challenge Phase 48 hr		Challenge Phase 72 hr	
Subject No.	1	2	3	4	5	6	7	8	9	MU	O	N	O	N
047	0	0	0	0	0	0	(-)	0	0	0	0	0	0	0
048	0	0	0	0	0	0	0	0	N9G		0	0	0	0
049	0	0	0	0	0	(-)	0	0	0	0	0	0	0	0
050	0	0	0	0	0	0	0	(-)	0	0	0	0	0	0
051	0	0	0	0	0	0	0	1	N9G		0	0	0	0
052	0	1	1	1	1	1	1	1	N9G		1	1	1	1
053	0	0	0	0	0	0	0	1	N9G		0	0	0	0
054	0	0	0	0	0	0	0	0	N9G		0	0	0	0
055	0	1	1	1	(-)	0	0	0	0	0	0	0	0	0
056	0	0	0	0	0	0	0	0	N9G		0	0	0	0
057	0	(-)	0	0	1	0	1	1	1	1	0	0	0	0
058	0	0	0	0	0	0	0	0	N9G		0	0	0	0
059	0	0	0	0	0	0	0	0	N9G		0	0	0	0
060	0	0	0	(-)	0	0	0	0	0	0	0	0	0	0
061	0	0	0	0	0	0	0	0	N9G		0	0	0	0
062	0	0	0	0	0	0	0	0	N9G		0	0	0	0
063	0	0	0	0	0	0	0	(-)	0	0	0	0	0	0
064	0	0	0	0	0	0	0	0	N9G		0	0	0	0
065	0	0	0	0	0	0	0	0	N9G		0	0	0	0
066	0	0	0	0	0	0	0	(-)	0	0	0	0	0	0
067	0	(-)	0	0	0	0	0	0	0	0	0	0	0	0
068	0	0	0	0	0	0	(-)	0	(-)		(-)	(-)	(-)	(-)
069	0	0	0	0	0	0	0	0	N9G		0	0	0	0

Generated on 01/18/11:12:18 by DETAIL1.SAS/USES: RESPONSE, PRODLIST

[redacted]
TKL Study No. [redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted] liquid foundation with 2% acetyl glucosamine

	Induction Reading										Challenge Phase 48 hr		Challenge Phase 72 hr	
Subject No.	1	2	3	4	5	6	7	8	9	MU	O	N	O	N
070	0	0	0	0	0	0	0	0	N9G		0	0	0	0
071	0	0	0	0	0	0	0	0	N9G		0	0	0	0
072	0	0	0	0	0	0	0	0	N9G		0	0	0	0
073	0	0	0	0	0	0	1	1	N9G		0	0	0	0
074	0	(-)	0	0	0	0	0	0	0	0	0	0	0	0
075	0	0	0	0	0	0	(-)	0	0	0	0	0	0	0
076	0	(-)	0	0	0	0	0	1	1	1	0	0	0	0
077	0	0	0	0	0	0	0	0	N9G		0	0	0	0
078	0	0	0	0	0	0	0	0	N9G		0	0	0	0
079	(-)	0	0	0	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(-)
080	0	0	0	0	0	0	0	0	N9G		0	0	0	0
081	0	1	1	1	1	1	1	1	N9G		0	0	0	0
082	0	1	1	1	0	0	0	0	N9G		0	0	0	0
083	0	0	0	0	0	0	0	0	N9G		0	0	0	0
084	0	0	0	0	0	0	0	(-)	0	0	0	0	0	0
085	0	1	1	1	1	0	0	0	N9G		0	0	0	0
086	0	0	0	0	0	(-)	0	0	0	0	0	0	0	0
087	0	0	0	0	0	0	0	0	N9G		0	0	0	0
088	0	0	0	0	0	0	0	0	N9G		0	0	0	0
089	0	0	0	0	0	0	(-)	0	0	0	0	0	0	0
090	0	(-)	0	0	0	0	0	0	0	0	0	0	0	0
091	0	0	0	0	0	0	0	0	N9G		0	0	0	0
092	0	0	1	1	1	1	1	1	N9G		0	0	0	0

Generated on 01/18/11:12:18 by DETAIL1.SAS/USES: RESPONSE, PRODLIST

[redacted]
TKL Study No. [redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted] liquid foundation with 2% acetyl glucosamine

Subject No.	Induction Reading										Challenge Phase 48 hr		Challenge Phase 72 hr	
	1	2	3	4	5	6	7	8	9	MU	O	N	O	N
093	0	0	0	0	0	0	0	0	N9G		0	0	0	0
094	0	0	(-)	0	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(-)
095	0	0	0	0	0	0	(-)	0	0	0	0	0	0	0
096	0	0	0	0	0	0	0	0	N9G		0	0	0	0
097	0	0	0	0	0	0	0	0	N9G		0	0	0	0
098	0	0	0	0	0	0	0	0	N9G		0	0	0	0
099	0	(-)	0	(-)	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(-)
100	0	0	0	(-)	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	(-)	0	0	1	1	1	0	0	0	0
102	0	0	0	0	0	0	1	1	N9G		0	0	0	0
103	0	0	0	0	0	0	0	0	N9G		0	0	0	0
104	0	0	0	0	0	0	0	0	N9G		0	0	0	0
105	0	0	(-)	0	0	0	0	0	0	N9G	0	0	0	0
106	0	0	0	0	0	0	0	0	0		0	0	0	0
107	(-)	0	0	0	0	0	0	0	0	N9G	0	0	0	0
108	(-)	0	0	0	0	0	0	0	0	N9G	0	0	0	0
109	0	(-)	0	0	0	0	0	0	0	N9G	0	0	0	0
110	0	0	0	0	0	0	0	0	0		0	0	0	0
111	0	0	0	0	0	0	0	0	0		0	0	0	0
112	0	0	0	0	0	0	0	0	0		0	0	0	0

Generated on 01/18/11:12:18 by DETAIL1.SAS/USES: RESPONSE, PRODLIST

ADDENDUM TO REPORT NO. [redacted]

21-DAY CUMULATIVE IRRITATION PATCH TEST

eye cream with 2% Acetyl Glucosamine

[redacted]

[redacted]

[redacted]

[redacted]

PERFORMED BY:

[redacted]

[redacted]

21-DAY CUMULATIVE IRRITATION PATCH TEST

ADDEMDUM TO [redacted]

CORRECTION TO SUMMARY:

This addendum is being issued to correct an error in the summary of the report. The summary report read -
"Under the conditions of the 21-day repeat application test with semi-occluded patches"..
The summary should have listed occluded patches as the type of patch used for this study.

SUMMARY:

"Under the conditions of this 21-day repeat application patch test with occluded patches the accumulative average of the test material examined can be described as very mild.:

[redacted]

21-DAY CUMULATIVE IRRITATION PATCH TEST

REPORT [redacted]

[redacted]

[redacted]

[redacted]

PERFORMED BY:

[redacted]

[redacted]

21-DAY CUMULATIVE IRRITATION PATCH TEST

[redacted]

[redacted]

CONCENTRATION: 100%, As Is

VEHICLE: N/A

PREPARATION OF TEST MATERIALS: N/A

SUMMARY:

Under the conditions of this 21-day repeat application patch test with semi-occluded patches the accumulative average of the test material examined can be described as very mild.

Individual scores can be found in Table I.

[redacted]

[redacted]

PURPOSE: To assess the cumulative irritation potential of topical creams to the skin as a result of repeated exposure under occluded patch conditions.

INVESTIGATIVE FACILITY: [redacted]

TEST LOCATION: [redacted]

INVESTIGATOR: [redacted]

STUDY MANAGER: [redacted]

STUDY COORDINATOR/GRADER: [redacted]

SPONSOR AND MONITOR: [redacted]

TEST DATES: 6/5 – 6/23/06

NUMBER OF PANELIST COMPLETING THE STUDY: 12

[redacted]

PROTOCOL:

The study protocol, [redacted] was followed with the modifications or deviations cited below. See Appendix I for the complete protocol.

DEVIATIONS/MODIFICATIONS TO PROTOCOL:

Test material was applied to occluded patches; fabrics were not used for this study. Patches were applied daily Mon – Fri for test weeks 1 & 2 and Mon – Thurs for week 3 (totaling 14 patch applications).

#1 – Removed top patch (Tx = A) at 11:00 PM on Tues. (6/6/06, first application) due to burning and stinging.
#2 – Removed bottom patch (Tx = A) at 9:00 PM on Thurs evening (6/15/06) due to excessive itching.

SUBJECT INFORMATION:

Number of subjects screened/excluded at initial interview:	13/0
Number of subjects starting study:	13 (9 females/4 males)
Number of subjects who withdrew:	1*

* #1 – Dropped from study on Thursday, 6/15/06 - forgot.

MISCELLANEOUS INFORMATION: None

RECORD OF MONITORING VISITS: This study was not monitored.

[redacted]

Treatment D Identification Number:	[redacted] eye cream with 2% acetyl glucosamine
Product Name:	[redacted]
Color:	White Pearl
Physical Form:	Cream
Concentration Tested:	100%, As Is
Concentration Relative to Use Concentration:	1x
Vehicle:	N/A
Test Material Preparation:	N/A
Patch Type:	Occlusive, Kendall Health Care Products (consisting of a nonwoven ~ 4 cm^2 x 1.65 mm thick cotton pad [e.g. Webril]
Amount Placed on Patch Pad:	0.2 gm
Method of Application:	Pipette
Patch Site:	Outer aspect of upper arm.
ADVERSE EVENTS:	None

APPENDIX I

[redacted]

[redacted] **Cumulative Irritancy Patch Test** [redacted]

Revision #: 2 **Procedure #:** [redacted]

Supersedes: [redacted]

Originator: [redacted] **Page: 1 of 10**

1.0 PURPOSE

1.1 To assess the cumulative mildness/irritation [redacted] on human skin as a result of repeated exposure.

2.0 SCOPE

2.1 This protocol is written for [redacted] Product Safety and must be followed for all [redacted] Cumulative Irritancy Patch Tests [redacted]

3.0 REFERENCES

3.1 Phillips, L., Steinberg, M., Maibach, H., Akers, W. Toxicology & Applied Pharmacology, 21, 369-382 (1972),

4.0 RESPONSIBILITIES

4.1 It is the responsibility of the person performing the [redacted] Cumulative Irritancy Patch Test [redacted] to follow this SOP.

5.0 PROCEDURE

5.1 Test Materials/Equipment:

[redacted]

5.1.2 Blenderm tape

5.1.3 Alcohol swabs

5.1.4 Gentian violet or Sharpie permanent markers (for reference dots)

5.1.5 Selectapette or other appropriate pipette system

[redacted]

5.1.7 Patch Test Grading Scale (0-4)

5.1.8 Optional: Minolta ChromaMeter (Model #CR-231)

5.2 Test Samples:

5.2.1 [redacted] Refer to any special instructions supplied by the toxicologist.

5.3 Panelists:

5.3.1 Twelve healthy adult volunteers between the ages of 18 and 65 are required. Eligibility of a volunteer is determined upon completion of a questionnaire (see Attachment I). A volunteer is rejected if he/she has been on a patch test within the last two months, meets one of the exclusion criteria (Attachment II), or has a skin condition such as sunburn, acne, skin disease, abrasions, scare tissue, tattoos on the test site, or active skin responses.

5.3.2 Informed Consent/Test Description:

Each subject participating in the study must read and sign an informed consent sheet (see Attachment III). This sheet provides a fair explanation of the procedures to be followed, a description of the attendant discomforts and risks, and a description of benefits to be expected, if any. In addition , the subject is given the opportunity to discuss the procedure involved in the test and given the opportunity to withdraw his/her consent and to discontinue participation in the test for any reason.

5.3.3 Instructions:

In written form, the subject is provided with the details involving his/her participation in the test including scheduled visits and procedures to follow if adverse reactions are experienced (see Attachment IV).

5.4 Procedure:

5.4.1 Patch Preparation:

[redacted]

5.4.2 Patch Applications:

5.4.2.1 Wipe test site with alcohol swab to remove surface oils. (Initial application ONLY).

5.4.2.2 Place reference dots on the skin to each side of the top test site.

5.4.2.3 Apply patches in straight lines to outer aspect of the upper arms or to the interscapular regions of the upper back. Choice of site is determined by the number of samples to be tested. Two-to-four patches may be applied to each arm; eight patches may be applied to the back. Randomize according to the Test Layout Diagram.

5.4.2.4 Secure the patches with Dermicel cloth tape if necessary.

5.4.2.5 After the patches are worn for 24 hours, they are removed and the area washed with water to remove any residue. The skin site is allowed to remain unpatched for 24 hours before reapplication.

5.4.2.6 Skin grades are taken before each reapplication of patches and 24 hours after removal of the third application, using the 0-4 scale described in Attachment V. ChromaMeter*. Patches are not reapplied to any test site which receives a grade of 2 or more; patching of the reference material is also discontinued at this time.

Optional: Instrumental Analysis - Minolta Chromameter (Model CR-231) Calibrate instrument daily, prior to use, using the white standard plate. Establish the L, a, and b values for each patch test site by placing the probe in the center of the patch application site in a manner that prevents occlusion/blanching or reddening of the test site area.

5.4.2.7 Patches are applied, removed, and graded on this schedule:

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Week #1					Apply Patches	Remove Patches
Week #2	Grade Reactions Reapply Patches	Remove Patches	Grade Reactions Reapply Patches	Remove Patches	Grade Reactions Reapply Patches	
Week #3	Grade Reactions Reapply Patches	Remove Patches	Grade Reactions Reapply Patches	Remove Patches	Grade Reactions	

OPTION: Continue above schedule for 21 days.

5.4.2.8 Care should be taken when reapplying the patches to place the patch pads directly over the sites previously exposed to the product. Reapplying reference dots is helpful in this alignment.

5.4.3 Grading:

5.4.3.1 The grading is done by an individual who is familiar with the evaluation of skin reactions and the 0-4 grading scale (see Attachment V), and use of the Minolta Chromameter* if included. The grades are recorded in a systematic way in the laboratory notebook. Any unavoidable deviation from the protocol (patches loose, fall off, etc.) is recorded with the grades. The reason any subject discontinues participation in a study shall be recorded.

5.4.3.2 A test site is not re-patched if it reaches a grade of 2 or more. This cut-off grade is carried through and used in the final evaluation of data.

5.4.3.3 For each test material, the average of all grades (12 subjects, 3 grading sessions) is calculated and reported as the average skin grade. Descriptive words are corrected with values of the average skin grade as follows:

0.00-0.49 - Very Mild

0.50-0.99 - Mild

1.00-1.49 - Slightly irritating

1.50-1.99 - Mildly irritating

2.00-2.99 - Moderately irritating

3.00-4.00 - Severely irritating

5.5 Experimental Design:

This procedure is a modification of that described by Dr. B. M. Lanman at the Joint Conference on Cosmetic Sciences, April 21-23, 1968, in Washington, D.C. This procedure was further modified in the procedure of Phillips, L., Steinberg, M., Maibach, H., and Akers, W., Toxicology and Applied Pharmacology, 21, 369-382 (1972).

5.6 Changes in Protocol:

If changes or modifications in the approved protocol are requested, the revisions and reasons for change are to be documented on the [redacted] The study placement form is to become part of the permanent file for that study. Similarly, the Principal Investigator is to be notified as soon as possible whenever an event occurs that is unexpected and may have an effect on the validity of the study.

[redacted]

[redacted]

6.0 ATTACHMENTS / DEFINITIONS

Attachment #	Description
I	Questionnaire for [redacted] Test Participants
II	Exclusion Criteria for Skin Irritation Testing
III	Repeat Application Patch Test Descritpion & Voluntary Consent Form
IV	Instruction Sheet - Repeat Application Patch Test
V	[redacted] Patch Test Grading Scale

7.0 UPDATE

7.1 Converted [redacted]

7.2 Converted to new protocol numbering system [redacted]

Revision #2 Updates:

7.3 Consent Form (Attachment III) revised to reflect panelist rights as outlined in the HIPAA Privacy Act.

7.4 Instruction Sheet (Attachment IV) modified to outline daily compensation for panelist participation.

75. Converted to global numbering system.

8.0 APPROVALS

Originator	Date
Quality Assurance	Date

ATTACHMENT I

QUESTIONNAIRE FOR [redacted] TEST PARTICIPANTS

1. Name _____ Date _____

2. Phone _____ Room _____

4. Age: 18-25 ____ 26-35 ____ 36-45 ____ 46-55 ____ 56-65 ____ 65+ ____

5. Sex: Female ____ Male ____

6. Ethnic Background: Caucasian[] Black[] Asian[] Hispanic[] Other:_____

For Test Eligibility

7. Have you participated in any skin test or other product use study including non-P&G tests) within the last 2 months? (If yes, please specify.)
No____ Yes___
Type Test: _____ Test Coordinator: _____ Date Completed: _____

8. Do you regularly take or use immunosuppressive or anti-inflammatory medications(i.e. Prednisone, steroids, aspirin, arthritis medication, etc.) (If yes, please specify.)
No_____ Yes ____, Specify:_____

9. Are you currently taking injectable insulin or any oral hypoglycemic agent for the treatment of diabetes? **No____ Yes ____, Specify:** _____

10. Are you currently applying any anti-inflammatory creams (i.e. Aspercreme) or other medicated lotions to your hands, arms or upper body? (If yes, please specify.)
No ____ Yes ____, Specify: _____

11. Are you being treated for cancer, or have you been treated for cancer within the last six months. **No____ Yes** _____

12. Do you presently have any of the following skin problems:

	Yes	No	Areas Affected If Present	Prescription or non-prescription medications or creams currently using
Psoriasis	____	____	_____	_____
Eczema	____	____	_____	_____
Skin Cancer	____	____	_____	_____
Other Skin Problems	____	____	_____	_____

Describe: _____

13. Do you have a medically confirmed allergy to any soap or cleaning product?
No ____ Yes ____

14. Are you currently participating in [redacted] skin prick testing program?
No ____ Yes ____
If yes, did you test positive to any enzyme or other test antigen?
No ____ Yes ___ (Please list)
_____

Participant Signature_____

For Laboratory Use Only

Accepted_____ Excused_____ Reason_____

Comments_____ Initials_____ Date_____

ATTACHMENT II

EXCLUSION CRITERIA FOR SKIN IRRITATION TESTING

Non-optional Criteria

1. Current use of an immunosuppressive drug (i.e. Prednisone) or as needed for an organ transplant.
2. Active psoriasis, active eczema, skin cancer, or other active skin eruptions at testing site, or disease(s) of the skin that might interfere with the evaluations made in this study or expose panelists to unacceptable risks.
3. Current routine or frequent use of a topical or systematic anti-inflammatory drug for a defined medical condition such as arthritis or back pain; e.g. aspirin, ibuprofen, corticosteroid.
4. Being treated for any kind of cancer, or has been treated for cancer, within the last six months.
5. Confirmed sensitization from previous patch test participation.
6. Has a tattoo or had one removed at the treatment application site.
7. Medically confirmed allergy to any soap, cleaning product, or ingredient in assigned test product(s).
8. Currently participating in another clinical, efficacy, or product use study which, in the opinion of the Clinical Investigator, may inhibit the accurate interpretation of test results.
9. Has a condition or used a medication which, in the Investigator's judgment, makes the subject ineligible or places the subject at undue risk.
10. Erythema greater than a score of "1" due to sunburn or other active condition at the patch application site.
11. Has participated on a similar clinical study within the past two months.

ATTACHMENT III

VOLUNTARY CONSENT FOR PARTICIPATION IN A 21-DAY CUMULATIVE IRRITATION PATCH TEST

Nature & Purpose of Study:
You are being asked to participate in a research study. Before you agree to participate you need to read and understand the following information regarding this study. You are encouraged to ask as many questions as necessary to be sure you understand what will be expected of you during this study and what you can expect as a result of your participation. Bear in mind that your participation is completely voluntary and you can withdraw from the study at any time.

To facilitate detecting differences in mildness/irritancy for both products and ingredients patch testing has been designed to exaggerate the product exposure conditions that the homemaker would ordinarily experience. All materials have been reviewed by a toxicologist to insure that adequate safety data exists to justify human exposure. The test materials are not identified for the test subjects.

Test Products:
The products may be considered antimicrobial pesticides under the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA). The active ingredient in the product which kills bacteria is *(insert active ingredient here)*.

Study Procedures:
Test materials are placed on non-woven cotton pads backed by surgical tape and applied to the upper back area on each side of the midline or to the outer aspect of the upper arm. Patches are applied six times and worn for twenty-four hours each time. Patches that are applied on Friday are to be removed by the test subjects on Saturday. Patches are reapplied on Monday, Wednesday and Friday and will be removed in the Skin Lab on Tuesday and Thursday for the next two weeks. Skin reactions will be graded before each application of patches and on Friday after the final application. Patches will not be reapplied to any test site which, in the judgment of the grader, would be inappropriate.

Risks:
The test subjects can expect to experience skin irritation resembling a mild sunburn from some of the test materials. Skin reactions which develop are usually confined to the area covered by the patches. The most severe reaction anticipated in this test would be severe redness accompanied by localized swelling and small watery blisters. Skin reactions may be accompanied by localized soreness and itching. Reactions ordinarily begin to subside within two to three days. In some rare instances localized discoloration of the skin has persisted for a prolonged period.

Emergency Contacts:
If you have any questions regarding your rights as a research subject, please contact [redacted]. If you have questions regarding this study or if an unexpected skin problem or other side effects arise between 7:00am-4:00pm, please contact the [redacted] immediately at [redacted]. Professional medical care to treat any skin problem or other side effects associated with this study will be made available at no expense to you. If you experience a medical problem after business hours, contact your Primary Care Physician or Urgent Care facility, then inform the [redacted] immediately on the next business day.

ATTACHMENT III (con't)

Confidentiality:
If you agree to take part in this study your personal data will be collected and processed for research purposes in connection with this study. Personal data associated with this study may include such items as name (first and last), age, sex, health status, and any other information supplied to us in any screeners. [redacted] or its appointed sub-contractors who will control the use of the data, will take all necessary steps to ensure your personal data are protected. You will not be identified by name or identified in any report or publication. In some cases, it may be necessary to use your information for submissions to regulatory agencies.

Termination of Paticipation/Right to Withdraw:
Your participation in this study may be terminated without your consent if you fail to report for scheduled appointments, or otherwise violate any terms of this agreement. If this occurs, you will be compensated for that portion of the study already completed. If you are excused at the Investigator's discretion due to clinical indications at the test site, you will receive full compensation. Your participation is voluntary and you may withdraw your consent and discontinue participation in the study at any time without jeopardizing your employment or eligibility for participating in future skin lab studies.

Availability of Information:
Any new information which is discovered during the study and which may influence your willingness to continue in the study will be made available to you.

Consent:
I have read and understand the above description of the Repeat Application Patch Test and have been given the opportunity to discuss with Company personnel any aspect of the test which is unclear to me. I understand that I may discontinue participation in the study at any time without jeopardizing my employment or eligibility for participating in future studies. I am aware that this work is not being done for my benefit but for the expected advancement of knowledge.

Furthermore, I will inform the Scientific Investigator of this study if I begin to take any medication (prescription or over the counter) or use any creams which contain an anti-inflammatory or immunosuppressive agent during this study.

By signing this document, I grant access to my medical records and study data as described above. I have received a copy of this informed consent.

Printed Name of Participant:	**Signature:**

Witness Signature: _____ Date: _____

[redacted] Study #: _____ Scientific Investigator: _____

ATTACHMENT IV

INSTRUCTION SHEET FOR A REPEAT APPLICATION PATCH TEST

You will wear these patches for 24 hours each time.

Tomorrow at this time you will remove the patches and rinse the patch sites with clear water to remove any residue.

Monday at your scheduled time, return to the [redacted] for grading and reapplication of patches.

Tuesday at your scheduled time, return to the [redacted] to have your patches removed and patch sites rinsed.

Wednesday at your scheduled time, return to the [redacted] for grading and reapplication of patches.

Thursday at your scheduled time, return to the [redacted] to have your patches removed and patch sites rinsed.

Friday at your scheduled time, return to the [redacted] for final grading and bonus distribution.
(The above schedule will be followed for 14 or 21 days, depending on study length.)
Please try not to remove the non-permanent marker on your arm which defines the patch area for grading and reapplication purposes.

If at any time the patches sting, burn or itch excessively, **remove them and note the time.** Rinse the patch sites with clear water to remove any residue.

Please **do not use any creams, oils or lotions** in the patch area for the duration of the study.
Do not expose the patch sites to the sunlight at any time. Any irritation you may receive from the patches will be similar in nature to a sunburn, and the ultraviolet rays of the sun will only compound the reaction. ABSOLUTELY NO SWIMMING!

You may discontinue participation in the study at any time. Should you elect to discontinue you will be compensated with [redacted] according to the days you completed as follows:

Day 1 - Day 14/21 = [redacted]

Total compensation for completion of study - 14 days = [redacted]

- 21 days = [redacted]

Please be on time for your scheduled appointment. If you will be delayed or have questions, call [redacted].

Thank you for your participation!

ATTACHMENT V

[redacted] PATCH TEST GRADING SCALE

0	No apparent cutaneous involvement.
1/2	Greater than 0, less than 1.
1	Faint but definite erythema, no eruptions or broken skin or no erythema but definite dryness; may have epidermal fissuring.
1-1/2	Greater than 1, less than 2.
2	Moderate erythema, may have a few papules or deep fissures, moderate-to-severe erythema in the cracks.
	Cut-off Grade - Patches are not reapplied.
2-1/2	Greater than 2, less than 3.
3	Severe erythema (beet redness), may have generalized papules or moderate-to-severe erythema with slight edema (edges well defined by raising).
3-1/2	Greater than 3, less than 4.
5	Generalized vesicles or eschar formations or moderate-to-severe erythema and/or edema extending beyond the area of the patch.
NOTE:	The degree of reaction expressed by such descriptive terms as "moderate" and "severe" is, in itself, subjective. Such terminology can be accurately understood only through experience.
	Any reaction of greater severity than Grade 4 should be described in detail. Unusual reactions not described by the scale should also be described.

Typical Examples of Half-Grade Scores

1/2	Faint, barely perceptible erythema or slight dryness glazed appearance).
1-1/2	Well-defined erythema or faint erythema with definite dryness may have epidermal fissuring.
2-1/2	Moderate erythema with barely perceptible edema or severe erythema not involving a significant portion of the patch (halo effect around the edges), may have a few papules or moderate-to-severe erythema.
3-1/2	Moderate-to-severe erythema with moderate edema (confined to patch area) or moderate-to-severe erythema with isolated eschar formations or vesicles.

Study#: [redacted]

Table I: Individual Panelist Scores

Pan #	TX.	6/6/2006	6/7/2006	6/8/2006	6/9/2006	6/12/2006	6/13/2006	6/14/2006	6/15/2006	6/16/2006	6/19/2006	6/20/2006	6/21/2006	6/22/2006	6/23/2006	Avg. 'A'	Avg. 'B'	Avg. 'C'	Avg. 'D'
1	[redacted]																		
	D	0	0.5	0.5	1	1	1	1	.	.	.	.	.	.	.				0.36
2	[redacted]																		
	D	0	0	0	0	0	0	0.5	0.5	0	0	0	0	0.5	0.5				0.14
3	[redacted]																		
	D	0	0	0	0	0	0	0	0	0	0	0	0.5	0.5	0.5				0.11
4	[redacted]																		
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0				0.00
5	[redacted]																		
	D	0	0	0	0	0.5	0.5	0.5	1	0	0	0	0.5	0	0				0.21
6	[redacted]																		
	D	0	0	0	0	0	0.5	1	1	1	1	1	1	1	1				0.61
7	[redacted]																		
	D	0	0	0.5	1	0.5	0.5	0	0	0	0	0	0	0	0				0.18
8	[redacted]																		
	D	0.5	0	0	0.5	0	0	0	0.5	1	1	1	1	1	1				0.54
9	[redacted]																		
	D	0	0	0	0.5	0	0	0	0	0	0.5	0.5	0.5	0.5	0.5				0.21
10	[redacted]																		
	D	0	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1	1				0.43
11	[redacted]																		
	D	0	0	0	1	1	0.5	1	1	1	0.5	0.5	1	1	1				0.68
12	[redacted]																		
	D	0	0	0	0.5	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5	0.5				0.29
13	[redacted]																		
	D	0	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5				0.36
														Overall Averages	[redacted]				0.34

Issue Date: May 25, 1993
(Revised 6/03)

[redacted]

[redacted] Study Placement

Study Description	Study Protocol #	[redacted]	Concentration (wt/wt., wt/vol.)	Test Concentration relative to usage (1x, 10x, etc.)
21 day Cum Irr	[redacted]	[redacted]		

eye cream with 2% acetyl glucosamine

D added test material [redacted] Cream 100% undiluted 1X ALM 5-30-06

Are any modifications in the above study protocol(s) requested? Yes __X__ No __

If yes, please specify below the study protocol number and modification(s) requested:
The products will be applied to the skin under occlusive patch conditions for a period up to 14 days (14 patches over 21 days). Patches will be applied Monday through Friday during the test period. The amount of product applied under patch will be approximately 0.2 ml (or 0.2 g). The standard grading scale will be used.

Safety Assessment
All test materials have been assessed and approved for consumer exposures consistent with this study design. If any effects occur, they are expected to be consistent with those seen with other skin care and personal cleansing products and will be limited to mild to moderate skin irritation that is transient in nature. If a moderate level of irritation occurs (score >2), exposures will be stopped and skin assessment will continue until symptoms are resolved.

Please check the appropriate boxes:

[ ] Test substance is a regulated substance.
[ ] Test substance is FIFRA regulated. Active ingredient in the product is _____.
[ ] Test material requires special handling precautions. Please Specify:
[ ] Additional safety related comments:

[redacted]

[redacted]

IN-PROCESS OR FINISHED PRODUCT

COSMETIC [X]
OTC DRUG [ ]
IND/NDA DRUG [ ]

PRODUCT NAME: [redacted]

MASTER FORMULA #: [redacted]

[redacted] COMPETITOR [ ]

COUNTRY OF MANUFACTURE: US

[redacted]	*	TRADE OR COMMON NAME	CTFA NAME	CAS #	PERCENT COMPOSITION As Added	PERCENT COMPOSITION Chem Content
		[redacted]				
		N-Acetyl Glucosamine	N-Acetyl Glucosamine	7512-17-6	2.00	2.00
		[redacted]				

*Active Ingredient

Compiled by (print): _____ Signature/Date: _____

Verified by (print)**: _____ Signature/Date: _____

**cosm-optional

Comments: _____

_____

FINAL REPORT

Study Title

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Test Articles

[redacted] face serum with 2% acetyl glucosamine

Authors

Erica Dahl, Ph.D., D.A.B.T.
Allison Hilberer, B.A.
Amanda Kong, B.S.
Nicole Barnes, B.S.

Study Completion Date

17 June 2009

Performing Laboratory

Institute for In Vitro Sciences, Inc.
30 W. Watkins Mill Road, Suite 100
Gaithersburg, MD 20878

Study Number

[redacted]

[redacted]

Laboratory Project Number

[redacted]

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

SUMMARY

IIVS Test Article Number	Sponsor's Designation	Conc.	t_{50} (hours)		pH
			Preliminary (7 April 09)	Trial 1 (8 April 09)	
[redacted]	[redacted]	[redacted]	[redacted]		
		Neat	>16	17.2	5.0-5.5
Positive Control	0.3% Triton®-X-100	NA	21.9 minutes	16.3 minutes	NA

NA - Not Applicable

TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

face serum w/ 2% acetyl glucosamine

The Tissue Equivalent Assay With Epiocular™ Cultures, of the test articles, [redacted] and [redacted], was conducted in compliance with the U.S. FDA Good Laboratory Practice Regulations as published in 21 CFR 58, the U.S. EPA GLP Standards 40 CFR 160 and 40 CFR 792, and the principles presented in the OECD series on Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test articles have not been determined by the testing facility.

The stability of the test articles under the test conditions has not been determined by the testing facility and is not included in the final report.

Erica Dahl, Ph.D., D.A.B.T.
Study Director

6/17/09
Date

QUALITY ASSURANCE STATEMENT

Study Title: Tissue Equivalent Assay with EpiOcular™ Cultures

Study Number: [redacted]

Study Director: Erica Dahl, Ph.D., D.A.B.T.

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLP Standards (40 CFR 792 and 40 CFR 160) and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected and report dates of QA inspections of this study:

Phase Inspected	Audit Date(s)	Reported to Study Director	Reported to Management
Protocol and Initial Paperwork	06-Apr-09	06-Apr-09	07-Apr-09
Definitive Assay – Removal & Transfer of Tissues – 45 min., 20 & 24 hr. time points	08-Apr-09	08-Apr-09	17-Apr-09
Draft Report and Data	22-May-09	22-May-09	26-May-09
Final Report	17-June-09	17-June-09	17-June-09

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Amanda K. Ulrey, RQAP-GLP
Quality Assurance

17-June-2009
Date

SIGNATURE PAGE

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Initiation Date:	6 April 2009
Completion Date:	17 June 2009
Sponsor:	[redacted]
Sponsor's Representative:	[redacted]
Testing Facility:	Institute for In Vitro Sciences, Inc. 30 W. Watkins Mill Road, Suite 100 Gaithersburg, MD 20878
Archive Location:	Institute for In Vitro Sciences, Inc. Gaithersburg, MD 20878
Study Director:	Erica Dahl, Ph.D., D.A.B.T. 6/17/09 Date
Laboratory Supervisor:	Nathan R. Wilt, B.S.

TEST ARTICLE RECEIPT

IIVS Test Article Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions*
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	cloudy light brown semi-viscous liquid	1 Apr 09	room temperature

* - Protected from exposure to light

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

INTRODUCTION

The EpiOcular™ Human Cell Construct (MatTek Corporation) was used to assess the potential ocular irritancy of the test articles. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) conversion assay, which measures the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, the succinate dehydrogenase reduction of MTT) to a blue formazan precipitate, was used to assess cellular metabolism after exposure to a test article for various exposure times[1]. The duration of exposure resulting in a 50% decrease in MTT conversion in test article-treated EpiOcular™ human cell constructs, relative to control cultures, was determined (t_{50}).

The purpose of this study was to evaluate the potential toxicity of the test articles, supplied by [redacted] as measured by the conversion of MTT by EpiOcular™ human cell constructs after exposure to each test article for various exposure times. The laboratory phase of the study was conducted from 7 April 2009 to 9 April 2009 at the Institute for In Vitro Sciences, Inc. After a time range finding assay, the test article was tested in a valid definitive assay to determine the time of exposure to the test articles, which resulted in the t_{50} endpoint.

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[1] Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. **Biochemica** 4:14-19.

MATERIALS AND METHODS

Receipt of the EpiOcular™ Human Cell Construct Model

Upon receipt of the EpiOcular™ Human Cell Construct Kit (MatTek Corporation), the solutions were stored as indicated by the manufacturer. The EpiOcular™ human cell constructs were stored at 2-8°C until used. On the day of dosing an appropriate volume of EpiOcular™ human cell construct assay medium was removed and warmed to approximately 37°C. Nine-tenths mL of assay medium were aliquoted into the wells of 6-well plates. The six-well plates were labeled to indicate test article and exposure time. The samples were inspected for air bubbles between the agarose gel and Millicell® insert prior to opening the sealed package. Cultures with air bubbles covering greater than 50% of the Millicell® area were not used. The 24-well shipping containers were removed from the plastic bag and their surfaces were disinfected with 70% ethanol. The EpiOcular™ human cell constructs were transferred aseptically into the 6-well plates. The EpiOcular™ human cell constructs were then incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for at least one hour. The medium was then aspirated and 0.9 mL of fresh medium were added to each assay well below the EpiOcular™ human cell construct. The plates were returned to the incubator until treatment was initiated. Upon opening the shipping bag, any remaining unused tissues were briefly gassed with an atmosphere of 5% CO_2/95% air and placed back at 2-8°C for later use.

Test Article Preparation

As instructed by the Sponsor, each test article was administered to the test system without dilution.

Assessment of Direct Test Article Reduction of MTT

Each test article was added to a 1.0 mg/mL MTT (Sigma) solution in warm Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM L-glutamine (MTT Addition Medium) to assess its ability to directly reduce MTT. Approximately 100 µL of each test article were added to 1 mL of the MTT solution and the mixtures were incubated in the dark at 37°C for approximately one hour. If the MTT solution color turned blue/purple, the test article was presumed to have reduced the MTT.

In cases where the test article was shown to reduce MTT, only those test articles that remained bound to the tissue after rinsing, resulting in a false MTT reduction signal, could present a problem. The test articles, [redacted] were not observed to reduce MTT in the absence of viable cells.

pH Determination

The pH of each neat liquid test article was measured using pH paper (EMD Chemicals Inc.). Initially, each test article was added to pH paper with 0-14 pH range in 1.0 pH unit increments to approximate a narrow pH range. Next, each test article was added to pH paper with a narrower range of 0-6 and/or 5-10 pH units with 0.5 pH unit increments, to obtain a more accurate pH value. The pH values obtained from the narrower range pH paper are presented in Table 1.

Time Range Finding Assay

A time range finding assay was performed to establish an appropriate exposure time range to be used in the definitive assay for each test article. Four exposure times 1, 4, 8, and 16 hours were tested in the time range finding assay. One culture was treated per exposure time with 100 µL of the appropriate test article or control. The negative control, 100 µL of sterile, deionized water (Quality Biological), was exposed for 16 hours. The positive control, 100 µL of 0.3% Triton®-X-100 (Fisher), was exposed for 15 and 45 minutes (one culture per exposure time).

After the appropriate exposure time, the EpiOcular™ cultures were extensively rinsed with Calcium and Magnesium-Free Dulbecco's Phosphate Buffered Saline ($Ca^{++}Mg^{++}$-Free DPBS). After rinsing, the tissues were transferred to 5 mL of Assay Medium for a 10 to 20 minute soak at room temperature to remove any test article absorbed into the tissue. A 1.0 mg/mL solution of MTT in warm MTT Addition Medium was prepared no more than 2 hours before use. Three-tenths mL of MTT solution were added to designated wells in a prelabeled 24-well plate. The EpiOcular™ constructs were transferred to the appropriate wells after rinsing with $Ca^{++}Mg^{++}$-Free DPBS. The trays were incubated at 37±1°C for approximately three hours in a humidified atmosphere of 5±1% CO_2 in air.

After the incubation period with MTT solution, the EpiOcular™ cultures were blotted on absorbent paper, cleared of excess liquid, and transferred to a prelabeled 24-well plate containing 2.0 mL of isopropanol in each designated well. The plates were sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time was harvested. The plates were then shaken for at least two hours at room temperature.

At the end of the extraction period, the liquid within the Millicell® inserts was decanted into the well from which the Millicell® insert was taken. The extract solution was mixed and 200 µL were transferred to the appropriate wells of a 96-well plate. Two hundred µL of isopropanol were added to the two wells designated as the blanks. The absorbance at 550 nm (OD_{550}) of each well was measured with a Molecular Devices Vmax plate reader.

Definitive Assay

Based on the results of the time range finding assay, four exposure times were chosen for the definitive assay. The EpiOcular™ culures were tested in duplicate with the test article [redacted] for 8, 16, 20, and 24 hours. The negative control (100 µL of sterile, deionized water) was exposed in duplicate for 0.25, 4, 8, and 24 hours. The positive control (100 µL of 0.3% Triton®-X-100) was exposed for 15 and 45 minutes. The procedures used to conduct the definitive assay were essentially the same as for the time range finding assay with the exception that at least duplicate cultures were dosed per exposure time.

Presentation of Data

The raw absorbance values were captured. The mean OD_{550} value of the blank wells was calculated. The corrected mean OD_{550} value of the negative controls was determined by subtracting the mean OD_{550} value of the blank wells from their mean OD_{550} values. The corrected

OD_{550} value of the individual test article exposure times and the positive control exposure times was determined by subtracting the mean OD_{550} value of the blank control from their OD_{550} values. The individual % of Control values were averaged to get the mean % of Control value. All calculations were performed using an Excel spreadsheet. The following percent of control calculations were made:

$$\% \text{ of Control} = \frac{\text{corrected } OD_{550} \text{ of Test Article or Positive Control Exposure Time}}{\text{corrected mean } OD_{550} \text{ of the appropriate time matched Negative Control}} \text{ X } 100$$

Exposure time response curves were plotted with the mean % of Control on the ordinate and the test article or positive control exposure time on the abscissa. The t_{50} value was interpolated from each plot. To determine the t_{50}, the two consecutive points were selected, where one exposure time resulted in a relative survival greater than 50%, and one exposure time resulted in less than 50% survival. The two selected points were used to determine the slope and the y-intercept for the equation $y=m(x) + b$. Finally, to determine the t_{50}, the equation was solved for $y=50$. When all of the exposure time points show greater than 50% survival, the t_{50} value is presented as greater than the longest test article exposure time.

Criteria for a Valid Test

The assay results were accepted when the positive control, 0.3% Triton®-X-100, caused a t_{50} value within two standard deviations of the historical mean. The corrected mean OD_{550} value for the minimum negative control exposure time should be within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time (up to 4 hours).

Deviations

During the transfer of the EpiOcular™ cultures from the shipping container to the 6 well plates in the definitive assay, non-sterile paper towels were used to remove excess agar clinging to the bottom of the millicell insert. This is a deviation from the protocol, which requires the cultures to be transferred aseptically. This may have introduced contamination to the culture medium. However, the positive and negative controls met the quality control requirements, therefore the outcome of the study was not affected.

RESULTS AND DISCUSSION

Time Range Finding Assay

A time range finding assay was performed, consisting of four exposure times 1, 4, 8, and 16 hours for the test articles, [redacted] The exposure time response curves are included in Appendix B. Based upon the results of the time range finding assay, four exposure times were selected for each test article for the definitive assay (see Materials and Methods). The t_{50} results for the time range finding assay (Preliminary) are reported in Table 1.

Definitive Assay

The EpiOcular™ culures were tested in duplicate with the test article [redacted] for 8, 16, 20, and 24 hours.. The negative control was also exposed in duplicate for 0.25, 4, 8, and 24 hours. The t_{50} results of the definitive Tissue Equivalent Assay With EpiOcular™ Cultures for the test articles and the positive control, 0.3% Triton®-X-100 (Trial 1), are summarized in Table 1. The exposure time response curves are included in Appendix B. Since the positive control fell within two standard deviations of the historical mean (15.5 – 39.2 minutes), and the corrected mean OD_{550} value for the minimum negative control exposure time (1.712) was within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time (up to 4 hours) (1.582), the assay results were accepted. Finally, none of the test articles were observed to directly reduce MTT in the absence of viable tissue.

Table 1

IIVS Test Article Number	Sponsor's Designation	Conc.	t_{50} (hours)		pH
			Preliminary (7 April 09)	Trial 1 (8 April 09)	
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
		Neat	>16	17.2	5.0-5.5
Positive Control	0.3% Triton®-X-100	NA	21.9 minutes	16.3 minutes	NA

NA - Not Applicable

APPENDIX A

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

1.0 PURPOSE

The purpose of this study is to evaluate the potential toxicity of the test article. In the Tissue Equivalent Assay, stratified human epithelial cell cultures (MatTek EpiOcular™) are exposed to topically applied test articles to evaluate potential ocular toxicity. Cell viability is determined by conversion of 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) in the treated cultures, and is expressed as a percentage relative to untreated (negative control) cultures. The endpoint of the Tissue Equivalent Assay, the t_{50} value, is the time (generally in minutes or hours) of exposure to test article required to reduce cell viability (MTT metabolism) to 50% of negative control levels as calculated from time-response curves.

2.0 SPONSOR

See Protocol Attachment 1

3.0 IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

3.1 Test Article(s): See Protocol Attachment 1

3.2 Controls:

Positive:	0.3% Triton®-X-100
Negative:	Sterile, deionized water (or other solvent as appropriate)
	blank control (MTT reading only)

3.3 Determination of Strength, Purity, etc.

3.3.1 IIVS will make every attempt to secure documentation of the analytical purity and composition of the test article and the stability and strength of the dosing solutions from the Sponsor. If the Sponsor is unwilling or unable to provide such information, IIVS will retain documentation supporting attempts to obtain this information with the study file and an exception will be noted in the Statement of Compliance in the Final Report.

3.3.2 The Institute for In Vitro Sciences, Inc. (IIVS) will be responsible for the documentation of the analytical purity and composition of the Triton®-X-100 used for the stock or working dilution of the positive control. This may be accomplished by maintaining a certificate of analysis from the supplier.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1	Name:	Institute for In Vitro Sciences, Inc.
4.2	Address:	30 West Watkins Mill Road, Suite 100 Gaithersburg, MD 20878
4.3	Study Director:	Erica Dahl, Ph.D.

5.0 TEST SCHEDULE

5.1	Proposed Experimental Initiation Date:	8 April 2009
5.2	Proposed Experimental Completion Date:	15 April 2009
5.3	Proposed Report Date:	17 June 2009

6.0 TEST SYSTEM

The EpiOcular™ human cell construct, obtained from the MatTek Corporation, will be used in this study. The use of EpiOcular™ cultures offers features appropriate for a model for ocular irritation. First, the model is composed of stratified human keratinocytes in a three-dimensional structure. Secondly, test materials can be applied topically to the model so that water insoluble materials may be tested. Prior to use, each plate (6, 12, and 24-well) will be uniquely identified with a number written in permanent marker, on the plate and its cover, the test article number, and the exposure time.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The experimental design of this study consists of a determination of the direct MTT reduction potential and the pH followed by performance of a time range finding assay and a definitive assay. The toxicity of the test article will be evaluated by the exposure time required to reduce cell viability to 50% of controls (t_{50}). Viability will be determined by the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, by the succinate dehydrogenase reduction of MTT) in control and test article-treated cultures (Berridge, et al., 1996). Data will be presented in the form of relative survival (relative MTT conversion) versus test article exposure time.

One of two exposure time ranges may be used. The standard exposure time range extends up to four hours and is used for most materials to be tested. For extremely mild materials, such as those that might be applied around or in the eyes, a long exposure assay might be used. For the long exposure study, exposure times of up to 24 hours may be used. In general, the standard exposure range will be used, unless the Sponsor specifies an alternative exposure time range, or if the Study Director determines that the class of test articles warrants the use of an alternative exposure time range.

7.1 Media and Reagents

7.1.1 Assay Medium: supplied by MatTek Corporation

7.1.2 EpiOcular™ Tissue: OCL-200 supplied by MatTek Corporation

7.1.3 Dulbecco's Modified Eagle's Medium (DMEM) containing 2mM L-glutamine (MTT Addition Medium)

7.1.4 3-[4,5 - dimethylthiazol-2-yl] - 2,5 - diphenyltetrazolium bromide (MTT) 10X stock solution: 10 mg/mL MTT in PBS

7.1.5 Ca^{++} and Mg^{++}-Free Dulbecco's Phosphate Buffered Saline – ($Ca^{++}Mg^{++}$-Free DPBS) (pH 7.0 ± 0.5)

7.1.6 Extraction Medium: Isopropanol

7.1.7 Sterile Deionized Water (Quality Biological or equivalent)

7.2 Preparation and Delivery of Test Article

Test articles will generally be tested neat. End use concentrations or other forms may be used as directed by the Sponsor. One hundred µL of pipettable substances, such as liquids, gels, creams, and foams, will be applied directly on the tissue so as to cover the upper surface. To aid in filling the pipet for pipettable materials that are viscous, the test article may first be transferred to a syringe. The pipet tip of the positive displacement pipet will be inserted into the dispensing tip of the syringe so that the material can be loaded into the displacement tip under pressure. Simultaneously, the syringe plunger is depressed as the pipet piston is drawn upwards. If air bubbles appear in the pipet tip, the test article should be removed (expelled) and the process repeated until the tip is filled without air bubbles. This method should be used for any materials that cannot be easily drawn into the pipet such as gels (e.g., toothpastes, mascaras, and face creams), and solid test articles that are creamed, like lipsticks and antiperspirants/deodorant sticks. A dosing device (a flatheaded cylinder of slightly less diameter than the inner diameter of the tissue insert) may be placed over the test article to assure even spreading, if required. Materials which are too viscous to spread over the tissue will first be spread onto the flat end of a dosing device. The dosing device will be placed into the Millicell® to bring the test article in contact with the tissue. When the test article must first be applied to a dosing device, approximately 30 µL or 30 mg of material will be applied to the dosing device so as to cover the dosing surface. The sample should be spread to form a relatively smooth even layer on the surface of the dosing device to maximize uniform tissue contact. Solids such as lipsticks or antiperspirant/deodorant sticks can be presoftened by creaming a portion in a weigh boat. The softened portion can be transferred to a syringe affixed with a three way stopcock attached to a second syringe. The sample is pushed from syringe to syringe until it is of a

consistency which can be pipetted. Dry powders will be ground with a mortar and pestle, if needed, and passed through a #40 copper sieve. Powders will be placed directly onto the culture at approximately 30 mg/culture. The exact exposure conditions used for other test article forms will be determined after consultation with the Sponsor and/or the Study Director. All exposure conditions will be documented in the study workbook.

The stability of the test article under the actual experimental conditions will not be determined by IIVS.

7.3 Route of Administration

Test article will be administered by topical application to the cell cultures.

7.4 pH Determination

The pH of the neat liquid test article (and/or dosing solution as appropriate) will be determined, if possible. The pH will be determined using pH paper (for example, with a pH range of 0 – 14 to estimate, and/or a pH range of 5 – 10 to determine a more precise value). The typical pH increments on the pH paper used to report the pH are approximately 0.3 to 0.5 pH units. The maximum increment on the pH paper is 1.0 pH units.

7.5 Controls

Two types of control treatments are used in this assay. The negative control cultures (negative control) are treated with sterile, deionized water or other solvent (if employed in its assay). Negative control cultures are dosed and exposed in parallel with the test article and positive control cultures. The exposure times used for the negative controls are selected to address the range of exposure times used for the test article and positive control cultures. Positive control cultures are treated with 0.3% Triton®-X-100 prepared in sterile, deionized water and are exposed for 15 and 45 minutes.

Time range finding assay: The assay will include a negative control, positive control and blank control (plate reading step). Each test group will be tested with at least a single culture. The negative control will be tested for one or more exposure times, which will generally be chosen to address the longest test article or positive control exposure time. At least one culture will be used for each of the two positive control exposure times.

Definitive assay: Generally, at least two negative control exposure times will be used. At least duplicate cultures will be used for each control time. One negative control exposure time will be selected to fit the range of the shortest test article or positive control exposure times (the minimum negative control exposure time will be 15 minutes). The second negative control exposure time will be selected to

match the longest test article or positive control exposure time (whichever is longer, up to 240 minutes). On occasion, the second negative control exposure time may be selected to fit the longest test article exposure time of a test article run concurrently, but from an independent study. For the long exposure assay (exposures of greater than 240 minutes), multiple negative control exposure times may be selected to fit the range of test article exposure times. If all exposure times are one hour and less, a single negative control exposure time may be used. Additional negative control exposure times may be selected at the discretion of the Study Director. At least two cultures will be used for each of the two positive control exposure times.

7.6 Assessment of Direct Test Article Reduction of MTT

It is necessary to assess the ability of each test article to directly reduce MTT. A 1.0 mg/mL MTT solution will be prepared in warm MTT Addition Medium as described in §7.8. Approximately 100 µL (liquid test articles) or 30 mg (solid test articles) will be added to 1 mL of the MTT solution and the mixture incubated in the dark at 37°C for approximately one hour. If the MTT solution color turns blue/purple, the test article is presumed to have reduced the MTT. Water insoluble test materials may show direct reduction (darkening) only at the interface between the test article and the medium.

7.7 Receipt of the EpiOcular™ Model

Upon receipt of the EpiOcular™ assay materials, the solutions will be stored as indicated by the manufacturer. The cell cultures will be stored at 2-8°C until used. Cultures should generally be used within 2 days of receipt from the manufacturer.

On the day of dosing, an appropriate volume of EpiOcular™ assay medium will be removed and warmed to approximately 37°C. Nine tenths (0.9) mL of assay medium will be aliquoted into the wells of 6-well plates. Each culture will be inspected for air bubbles between the agarose gel and Millicell® insert prior to opening the sealed package. Cultures with air bubbles under greater than 50% of the Millicell® area will not be used. The 24-well shipping containers will be removed from the plastic bag and the surface disinfected with 70% ethanol. An appropriate number of cultures will be transferred aseptically from the 24-well shipping containers into the 6-well plates. The EpiOcular™ cultures will be incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for at least one hour prior to dosing. The medium will be aspirated and 0.9 mL of fresh medium will be added to each assay well below the tissue prior to dosing. Note: The refeeding step may occur at less than 1 hour but the tissue should be allowed at least one hour of incubation to become fully metabolically active before dosing. Upon opening the bag, any unused tissues remaining on the shipping agar at the time of tissue transfer will be briefly gassed with an atmosphere of 5% CO_2/95% air, and the bag will be sealed and stored at 2-8°C for subsequent use.

7.8 Time Range Finding Assay

At least four exposure times will be evaluated for each test article. For the standard assay, the exposure times for the time range finding assay will generally be 10, 30, 60 and 180 minutes unless the Sponsor requests other specific exposure times. The maximum exposure time will be 240 minutes. For the long term exposure assay, the exposure times for the time range finding assay will generally be 1, 4, 8, and 16 hours.

Each test article and control exposure time will be tested by treating at least one culture. One hundred µL of each pipettable test article will be delivered with a positive displacement pipet onto the culture. Powders will be placed directly onto the culture at approximately 30 mg/culture. Those materials which are too viscous to spread directly on the culture may be spread onto a dosing device. Approximately 30 µL or 30 mg will be applied to the dosing device for each culture, as indicated in §7.2. Exposure times of ten minutes or greater will be incubated at 37±1°C and 5±1% CO_2 in air.

At the end of the treatment time, the test article will be removed by extensively rinsing both sides of the culture with $Ca^{++}Mg^{++}$ Free-DPBS. The process will be performed until the culture appears free of test article. If it is not possible to remove all of the visible test material, this will be noted in the study workbook. After rinsing, the culture will be transferred to 5 mL of Assay Medium for a 10 to 20 minute incubation at room temperature. This rinse is intended to remove any test article absorbed into the culture.

A 10X stock of MTT prepared in PBS (filtered at time of batch preparation) will be thawed and diluted in warm MTT Addition Medium to produce the 1.0 mg/mL solution no more than two hours before use. Alternatively, a 1.0 mg/mL MTT solution will be prepared in warm MTT Addition Medium and filtered through a 0.45 µm filter to remove undissolved crystals. Three hundred µL of the MTT solution will be added to each designated well of a prelabeled 24-well plate. Excess Assay Medium will be removed and then the EpiOcular™ cultures will be transferred to the appropriate wells of the MTT plate. The 24-well plates will be incubated at 37±1°C for approximately 3 hours in a humidified atmosphere of 5±1% CO_2 in air.

After the three hour incubation, the EpiOcular™ tissues will be blotted on absorbent paper, cleared of excess liquid, and transferred to a prelabeled 24-well plate containing 2.0 mL of isopropanol in each designated well. The plates will be sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time is harvested. If necessary, plates may be stored overnight (or up to 20 hours after the last exposure time is harvested) in the refrigerator prior to extracting the MTT. The plates will then be shaken for at least 2 hours at room temperature. At the end of the extraction period, the liquid within the Millicell® inserts will be decanted into the well from which the Millicell® insert was taken. The extract solution will be mixed

and 200 µL transferred to the appropriate wells of a 96-well plate. Two hundred µL of isopropanol will be added to the wells designated as blanks. The absorbance at 550 nm (OD_{550}) of each well will be measured with a Molecular Devices Vmax plate reader.

The range of exposure times for the definitive assay will be chosen to determine the t_{50} (the exposure time to the test article that reduces MTT metabolism by 50%). Based on the results of the time range finding assay, two exposure times will be chosen that should result in expected survivals lower than 50%, and two exposure times will be chosen that should result in expected survivals greater than 50%. If a test article fails to cause 50% toxicity in the time range finding assay, the maximum exposure time will be 240 minutes, or 24 hours, depending on the assay selected. In some cases, the exposure times for the definitive assay may be selected based on visual observations of the relative MTT reduction in the tissues.

7.9 Killed Controls for Assessment of Residual Test Article Reduction of MTT

In cases where the test article is shown to directly reduce MTT, only test articles that remain bound to the tissue after rinsing, resulting in a false MTT reduction signal, present a problem. To demonstrate that possible residual test article is not acting to directly reduce the MTT, a functional check is performed in the preliminary assay to show that the test material is not binding to the tissue and leading to a false MTT reduction signal.

To determine whether residual test article is acting to directly reduce the MTT, a freeze-killed control tissue is used. Freeze-killed tissue is prepared by placing untreated EpiOcular™ constructs in the –20°C freezer at least over night, thawing to room temperature, and then refreezing. Once refrozen, the tissue may be stored indefinitely in the freezer. To test for residual test article reduction, killed tissues are treated with the test article in the normal fashion. Generally, each test article will be evaluated for at least the shortest and longest exposure times (or longest exposure time if all exposure times are one hour and less) in single replicate killed tissues. All assay procedures will be performed as for the viable tissue. At least one killed control treated with sterile deionized water (negative killed control) will be tested in parallel since a small amount of MTT reduction is expected from the residual NADH and associated enzymes within the killed tissue.

If little or no MTT reduction is observed in the test article-treated killed control, the MTT reduction observed in the test article-treated viable tissue may be ascribed to the viable cells. If there is appreciable MTT reduction in the treated killed control (relative to the amount in the treated viable tissue), additional steps must be taken to account for the chemical reduction in the Definitive Assay or the test article may be judged untestable in this system.

If required in the Definitive Assay, the killed control testing will be similar to the procedure used in the time range finding assay. Additional test article-treated killed

control tissues may be used to better cover the range of exposure times. The data from the killed controls will be analyzed as described in §7.11.

7.10 Definitive Assay

The definitive assay with generally four to five exposure times will be performed exactly like the time range finding assay with the exception that cultures will be tested in duplicate for each exposure time. If the test article(s) are found to be non-toxic in the time range finding assay, then fewer than four exposure times may be chosen for the definitive assay. At least duplicate cultures will be tested at each of the positive control exposures. At least duplicate cultures will be treated with negative or solvent control for each exposure time (see §7.5). The determination of the t_{50} will be based upon the results of the definitive assay. At the Study Director's option, a second definitive assay may be performed.

7.11 Presentation of Data

The raw absorbance values will be captured, and the following calculations made:

The mean OD_{550} of the blank control wells will be calculated. The corrected mean OD_{550} of the exposure time control(s) will be determined by subtracting the mean OD_{550} of the blank control from their mean OD_{550}s. The corrected OD_{550} of the individual test article exposure times and the positive control exposure times will be determined by subtracting the mean OD_{550} of the blank control from their OD_{550}s. When applicable, corrected OD_{550} values will be calculated for the test and control article-treated killed controls, as well. Generally, all calculations will be performed using an Excel spreadsheet.

Corr. test article exposure time OD_{550} = Test article exp. time OD_{550} – Blank mean OD_{550}

If killed controls (KC) are used, the following additional calculations will be performed to correct for the amount of MTT reduced directly by test article residues. The OD_{550} value for the negative control killed control will be subtracted from the OD_{550} values for each of the test article-treated killed controls (at each exposure time), to determine the net OD_{550} values of the test article-treated killed controls.

Net OD_{550} for each test article KC = Raw OD_{550} test article KC – Raw OD_{550} negative control KC

The net OD_{550} values represent the amount of reduced MTT due to direct reduction by test article residues at each exposure time tested. The net OD_{550} values will be subtracted from the corrected mean OD_{550} values of the viable test article-treated tissues, at each corresponding exposure time, to obtain a final corrected OD_{550} value. These final corrected OD_{550} values will then be used to determine the % of Control viabilities at each exposure time.

Final Corrected OD_{550} = Corrected test article OD_{550} (viable) – Net OD_{550} test article (KC)

Finally, the following % of Control calculations will be made:

$$\% \text{ of Control} = \frac{\text{corrected } OD_{550} \text{ of each Test Article or Positive Control exposure time}}{\text{corrected mean } OD_{550} \text{ of Negative Control}} \times 100$$

The individual % of Control values are then averaged to get the mean % of Control per exposure time. Viability calculations for test articles treated in the long exposure time assay may be performed by comparing the corrected OD_{550}s of each test article exposure time to the appropriate exposure time control(s).

Exposure time response curves may be plotted with the % of control on the ordinate and the test article exposure time on the abscissa. Other plot forms may be used as requested by the Sponsor. The t_{50} will be mathematically interpolated from each plot. To determine the t_{50}, two adjacent points will be selected, one that shows greater than 50% survival and one that shows less than 50% survival. The two selected points will be used to determine the slope and the y-intercept for the equation $y = m(x) + b$. Finally, to determine the t_{50}, the equation will be solved for $y = 50$. If all of the exposure time points show greater than 50% survival, the t_{50} will be listed as greater than the longest exposure time. If the shortest test article exposure time shows less than 50% relative survival, the plot will be extended to include the t_0 point which will be given a value of 100%. In this case, the t_{50} will be determined between the t_0 and the shortest exposure time. At the Study Director's option, additional assays may be performed to produce the final t_{50} value.

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

The definitive assay will be accepted if the positive control compound, 0.3% Triton®-X-100, causes a t_{50} within two standard deviations of the historical mean. The historical mean is updated every three months. Since the shortest positive control exposure time is 15 minutes, t_{50} values of less then 15 minutes will be considered unacceptable. The corrected mean OD_{550} value for the minimum negative control exposure time must be within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time for assays up to 240 minutes.

9.0 REPORT

A report of the results of this study will be prepared by the Testing Facility and will accurately describe all methods used for generation and analysis of the data. A summary will be prepared reporting the t_{50} values for each assay with each test article as well as the positive control data. A copy of the protocol used for the study and any significant deviation(s) from the protocol and SOPs of the Testing Facility will appear as a part of the final report.

10.0 RECORDS AND ARCHIVES

A separate working notebook will be used to record the materials and procedures used to perform this study. Upon completion of the final report, all raw data, reports and specimens will be retained in the archives for a period of either a) 5 years, b) the length of time specified in the contract terms and conditions, or c) as long as the quality of the preparation affords evaluation, whichever is applicable.

11.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

The regulatory compliance requirements for this study are detailed in the Protocol Attachment 1.

The Quality Assurance Unit will review the study protocol, perform at least one in-process laboratory inspection, and audit the raw data workbook and all reports of the study to assure compliance with the appropriate regulations specified in the Protocol Attachment 1.

12.0 PROTOCOL AMENDMENTS

When it becomes necessary to change the approved protocol for a specific study, verbal agreement to make this change should be made between the Study Director and Sponsor. As soon as practical, this change and the reason for it should be put in writing and signed by both the Study Director and the Sponsor. This document is then attached to the protocol as an amendment.

13.0 REFERENCES

MTT Effective Time 50 (ET-50) Protocol, MatTek Corporation

Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. **Biochemica** 4:14-19.

14.0 APPROVAL

(See Sponsor's Protocol Attachment 1)
SPONSOR REPRESENTATIVE

IIVS STUDY DIRECTOR

4/6/09
DATE

PROTOCOL ATTACHMENT - 1 (Page 1 of 2)

In Vitro Eye Irritation Tests
[Cytosensor Microphysiometer/Tissue Equivalent Assay/Bovine Corneal Opacity Assay]
Protocol Design Specifications and Sponsor Information

Sponsor:

[redacted]

Testing Facility:
INSTITUTE FOR IN VITRO SCIENCES, INC.
30 West Watkins Mill Road, Suite 100
Gaithersburg, MD 20878
TEL: (301) 947-6523
FAX: (301) 947-6538

Sponsor's Representative: (full address; please type/ print)

[redacted]

Alternate Contact:

[redacted]

Please Indicate Test Requirements:

		IIVS Protocol Number	Test Article Required Solid	Test Article Required Liquid
[ ]	Cytosensor Microphysiometer Assay	[redacted]	30g	50 ml
[ ]	Tissue Equivalent Assay, EpiOcular		5g	10 ml
[X]	Long Term Exposure Assay (up to 24 hours) option For TEA, EpiOcular			
[ ]	Bovine Corneal Opacity & Permeability Assay with Two Time Exposures and Optimal Histology		10g	15 ml
[ ]	Bovine Corneal Opacity and Permeability Assay with Extended Post-Exposure Intubation Period(s) and Optional Histology		10g	15 ml

[redacted]

IIVS Project # (Completed by IIVS):
Study File # (Completed by IIVS):

[redacted]

Study design to meet the scientific requirements of the following authorities (mark all that apply):

[X] FDA [ ] EPA-TSCA [ ] EPA-FIFRA [ ] CPSC [ ] DOT [X] OECD [ ] EU [ ] CEPA

[ ] JMHW [ ] MAFF [ ] MITI [ ] Other (please specify) _____

GLP Compliance:

Study will be conducted in accordance with the GLP requirements of the following agency(s): FDA and OECDE. If none specified, OECD GLPs will be followed.

QAU Master Schedule:

[ ] This study is regulated by, or is intended to be submitted to, the following agencies:
[ ] EPA/FIFRA [ ] EPA/TSCA [ ] FDA [ ] Other (list): _____.
[X] This study is not regulated.

PROTOCOL ATTACHMENT -1 (Page 2 of 2)
Cytosensor Microphysiometer and Tissue Equivalent Assay
Protocol Design Specifications and Sponsor Information

[redacted] **IIVS Project #** [redacted]

Test Article(s):

TSIN	Color	Physical Form	Expiration Date
[redacted] face serum w/ 2% acetyl glucosamine	Off-white/light brown	Lotion	March 2010

Test Article Storage Conditions: (Note:a all test articles stored protected from white light)

[X] Room Temperature (approximately 20 to 25°C)
[ ] Refrigerated (approximately 4°C)
[ ] Frozen (approximately -20°C)
[ ] Other _____

Special Instructions for Test Article Preparation:

[X] None
[ ] As Follows:

Concentration Analysis of Test Article-Vehicle Mixture(s):

[X] Not required
[ ] Required
Return ___ ml of highest and lowest concentration test article solutions to Sponsor's Representative
Storage conditions: [ ] Room temperature [ ] Refrigerated [ ] Frozen [ ] Other _____
Shipping conditions: [ ] Frozen [ ] Ambient [ ] Other _____

Classified as: (Check all that apply) [X] Non-hazardous - No Special Instructions
[ ] Flammable [ ] Poison [ ] Other

[X] Dispose of Unused Samples, after 1 year, as follows: in regular trash

[ ] Return Unused or Concentration Analysis Samples To: (Return of samples will be approximately one month after submission of final report.

[redacted]

APPENDIX B

EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 7-Apr-09
TEST MATERIAL: [redacted]
TEST ARTICLE: [redacted]

Study No. [redacted]

t_{50}= > 16 Hours

PRELIMINARY
CONCENTRATION: 100%

TIME EXPOSURE (Hours)	PERCENT VIABLE
1	99.2
4	103.3
8	97.0
16	51.8

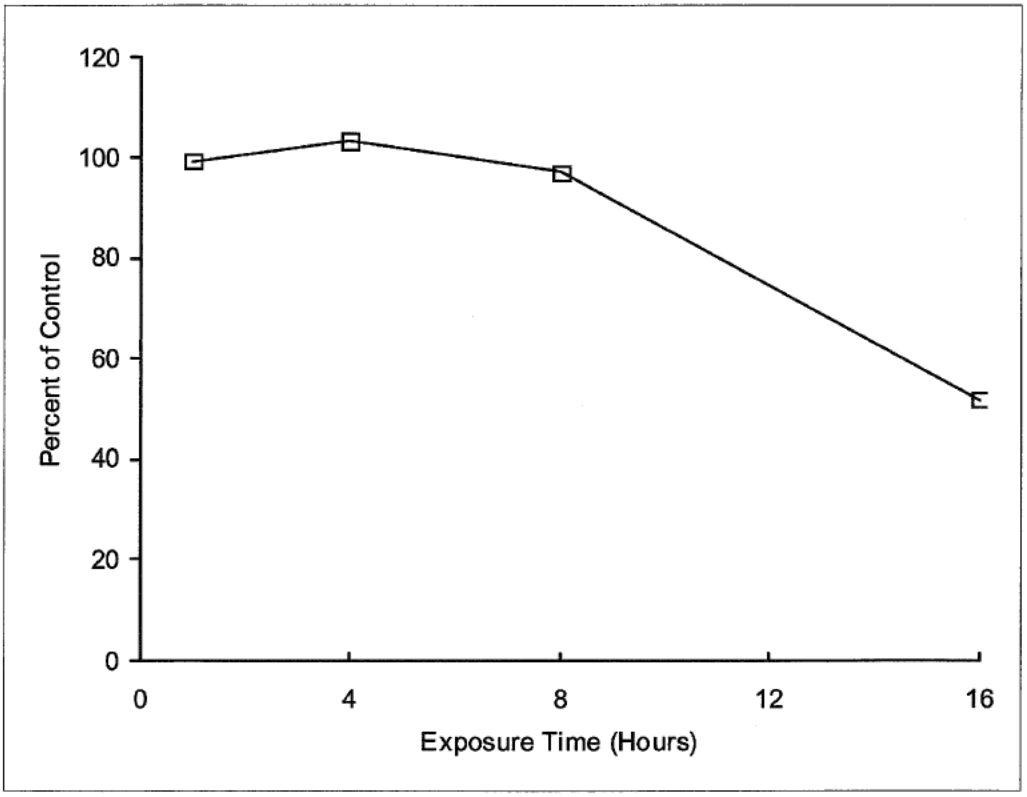
y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	16.0	1	51.8
2	16.0	2	51.8
3	#DIV/0!	3	50
	slope =		#DIV/0!
	y intercept =		#DIV/0!

Face serum with 2% acetyl glucosamine

[redacted]

CONCENTRATION: 100%
PRELIMINARY



Study No. [redacted]

EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 7-Apr-09

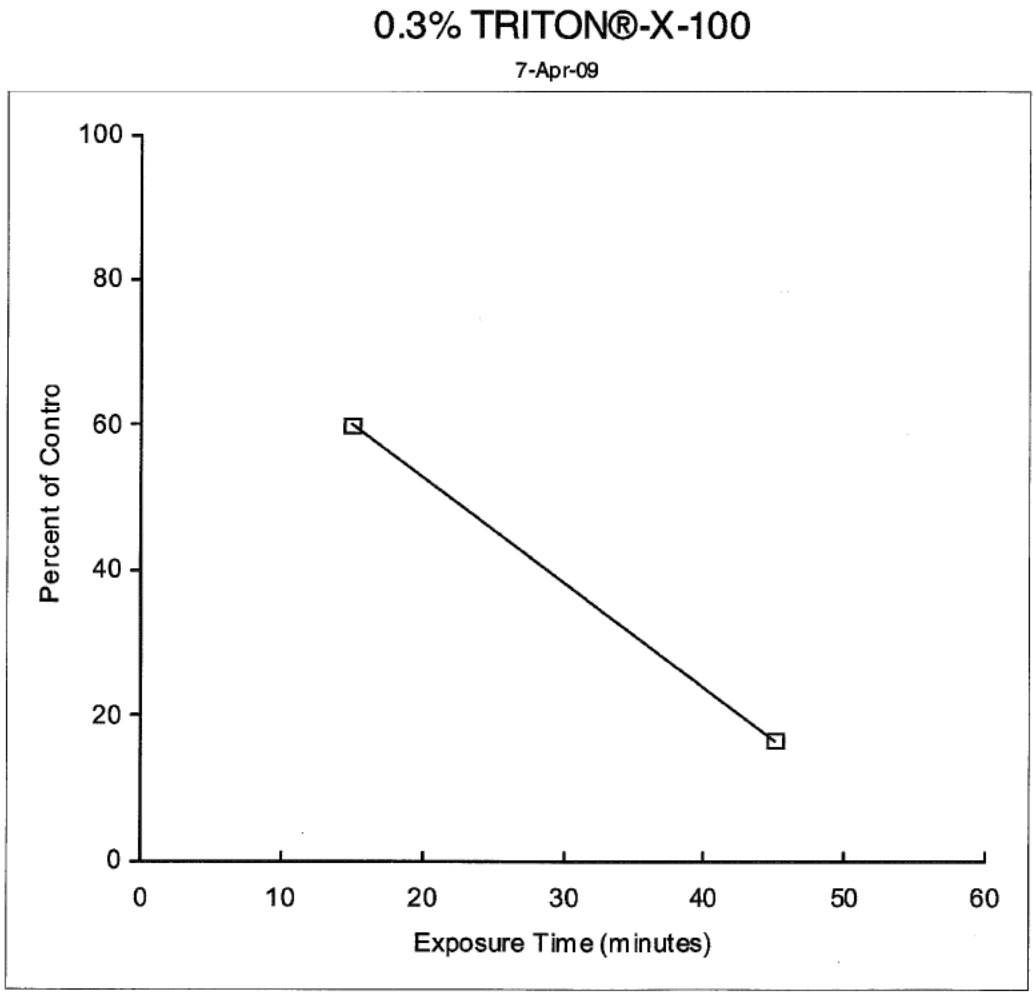
TEST MATERIAL: 0.3% TRITON®-X-100

t_{50} = 21.9 Minutes

TIME EXPOSURE (Minutes)	PERCENT VIABLE
15	60.0
45	16.6

y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	15.0	1	60
2	45.0	2	16.6
3	21.912442	3	50
	slope =		-1.446667
	y intercept =		81.7



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 8-Apr-09
TEST MATERIAL:
TEST ARTICLE:

Study No. [redacted]

t_{50}= 17.2 Hours

TRIAL 1
CONCENTRATION: 100%

TIME EXPOSURE (Hours)	PERCENT VIABLE
8	82.7
16	59.9
20	26.3
24	5.0

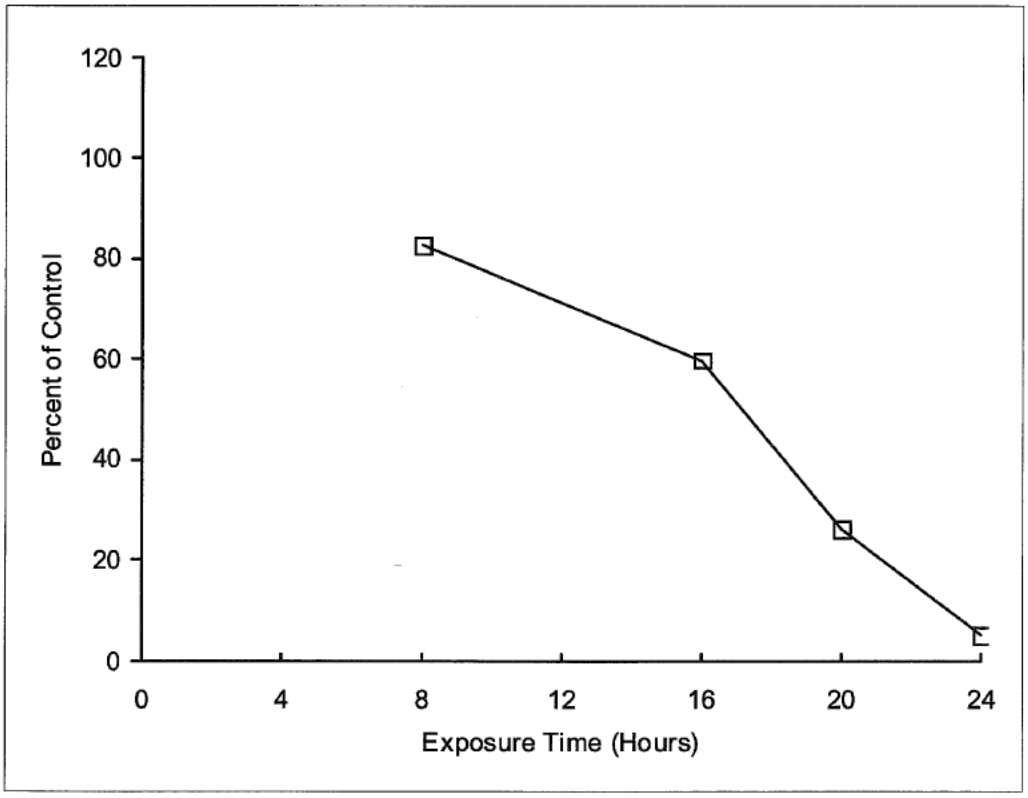
y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	16.0	1	59.9
2	20.0	2	26.3
3	17.178571	3	50
	slope =		-8.4
	y intercept =		194.3

Face serum with 2% acetyl glucosamine

[redacted]

CONCENTRATION: 100%
TRIAL 1



Study No. [redacted]

EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 8-Apr-09

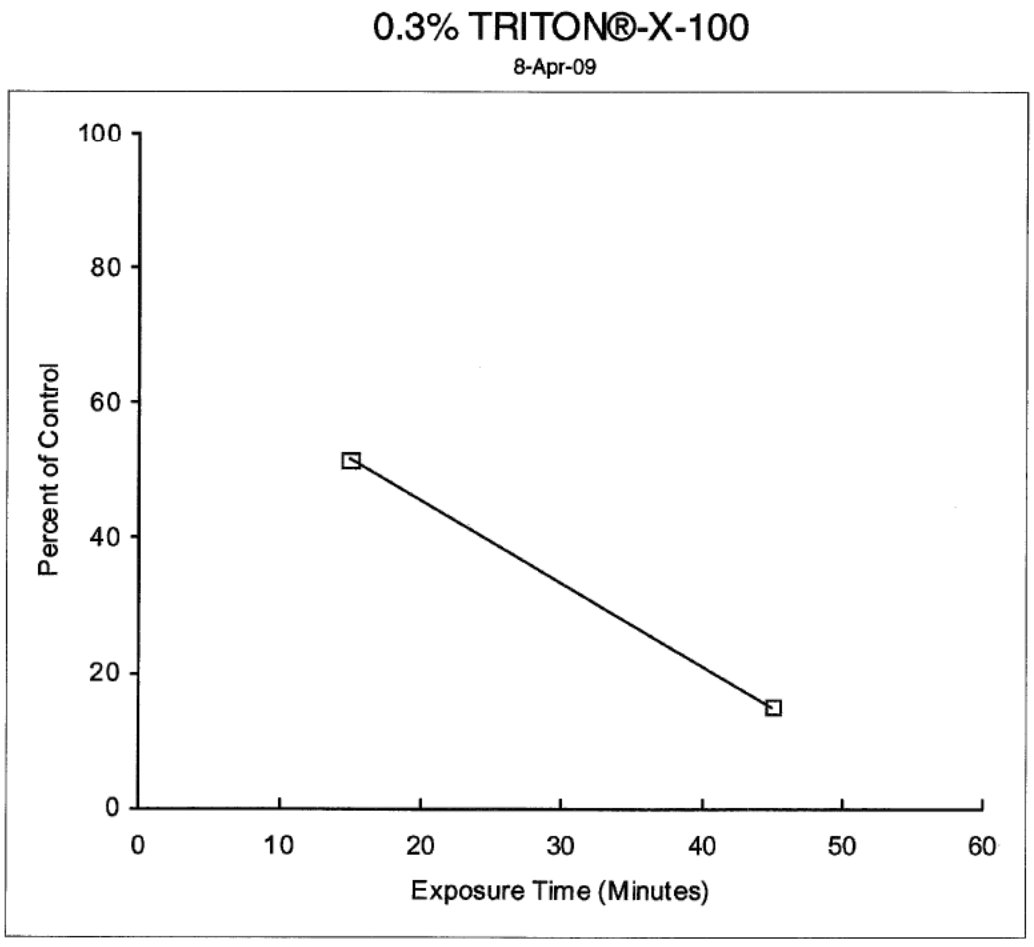
TEST MATERIAL: 0.3% TRITON®-X-100

t_{50} = 16.3 Minutes

TIME EXPOSURE (Minutes)	PERCENT VIABLE
15	51.6
45	15.1

y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	15.0	1	51.6
2	45.0	2	15.1
3	16.315068	3	50
	slope =		-1.216667
	y intercept =		69.85



[redacted]

Chg	Formulation Table/Base Formula Tracking Code	[redacted]	[redacted] Description	[redacted]	[redacted]	Min	Target	Max	Unit of Measure	Material Function (MF) Position Indicator (PI)	Comments
[redacted]											
		[redacted]	Acetyl Glucosamine	[redacted]	[redacted]		2.00000		Percent weight by weight	MF: PI:	
[redacted]											

2022 FDA VCRP data – Glucosamine ingredients Priya Cherian

Acetyl Glucosamine

Eye Lotion	8
Other Eye Makeup Preparations	4
Tonics, Dressings, and Other Hair Grooming Aids	1
Lipstick	3
Makeup Bases	3
Makeup Fixatives	2
Other Makeup Preparations	3
Other Personal Cleanliness Products	1
Cleansing	9
Face and Neck (exc shave)	58
Body and Hand (exc shave)	17
Moisturizing	57
Night	8
Paste Masks (mud packs)	3
Skin Fresheners	6
Other Skin Care Preps	15

Total: 198

Glucosamine

Tonics, Dressings, and Other Hair Grooming Aids	1
Face and Neck (exc shave)	1

Total: 2

Glucosamine HCL

Eye Lotion	4
Other Eye Makeup Preparations	2
Hair Conditioner	3
Shampoos (non-coloring)	2
Tonics, Dressings, and Other Hair Grooming Aids	2
Other Hair Preparations	3
Foundations	1
Makeup Bases	2
Shaving Cream	1
Other Shaving Preparation Products	1
Cleansing	3
Face and Neck (exc shave)	20

Moisturizing	26
Night	2
Paste Masks (mud packs)	3
Other Skin Care Preps	2

Total: 77

Glucosamine Sulfate

Total: 0