Safety Assessment of Hyaluronates as Used in Cosmetics

Status:	Final Report for Panel Review
Release Date:	May 19, 2023
Panel Meeting Date:	June 12-13, 2023

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

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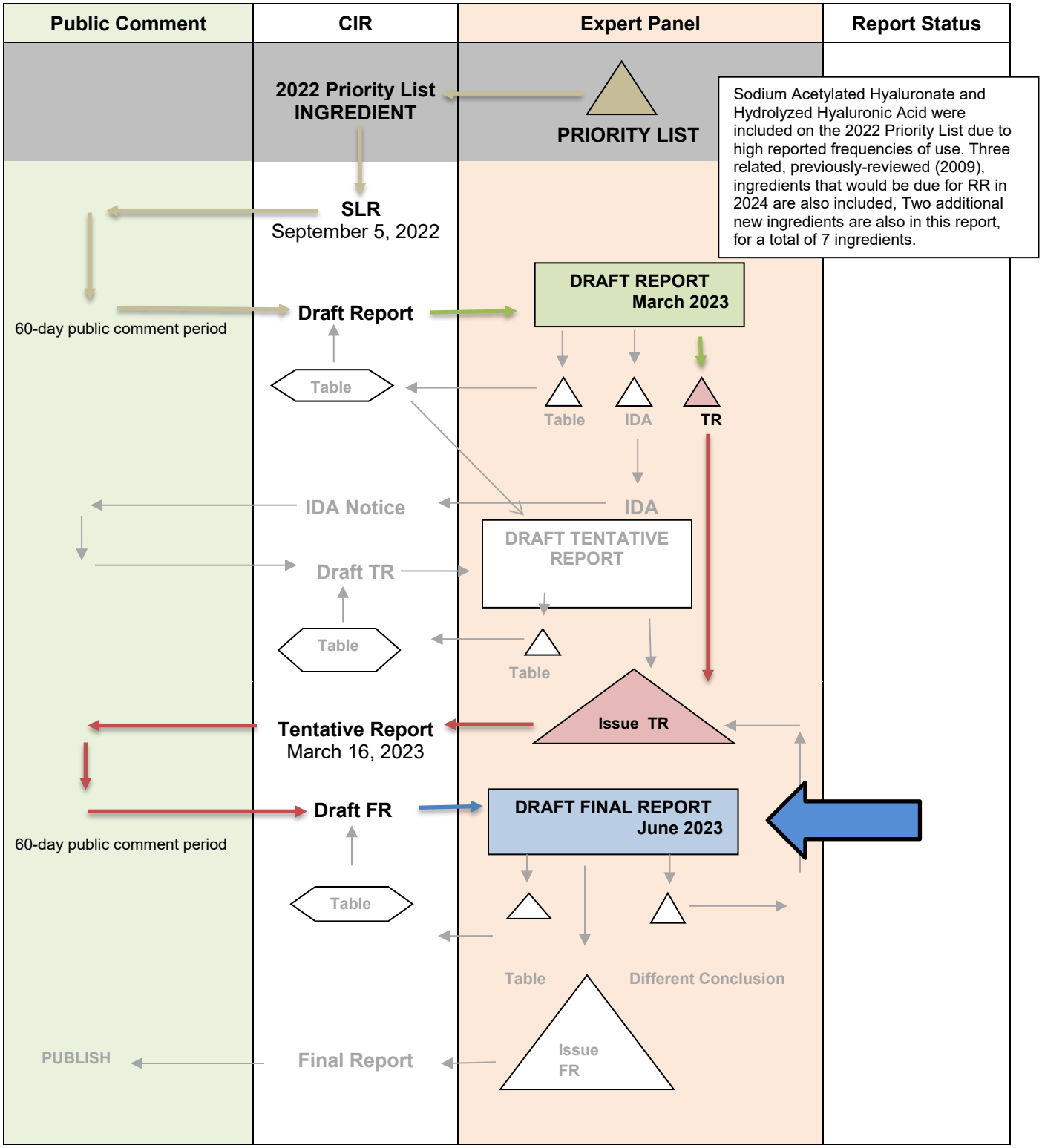
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Hyaluronates

MEETING June 2023



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR
Date: May 19, 2023
Subject: Safety Assessment of Hyaluronates

Enclosed is the Draft Final Report on the Safety Assessment of Hyaluronates as Used in Cosmetics (*report_Hyaluronates_062023*). At the March 2023 meeting, the Panel issued a Tentative Report for public comment with the conclusion that Hyaluronic Acid, Hydrolyzed Calcium Hyaluronate, Hydrolyzed Hyaluronic Acid, Hydrolyzed Sodium Hyaluronate, Potassium Hyaluronate, Sodium Acetylated Hyaluronate, and Sodium Hyaluronate are safe in cosmetics in the present practices of use and concentration described in the safety assessment.

No unpublished data were submitted since the issuing of the Tentative Report. Comments on the Tentative Report that were received from Council (*PCPCcomments_ Hyaluronates_062023*) have been addressed. A comments response checklist is included (*response-PCPCcomments_ Hyaluronates_062023*).

Also included in this packet are the report history (*history_ Hyaluronates_062023*), data profile (*dataprofile_ Hyaluronates_062023*), search strategy (*search_ Hyaluronates_062023*), transcripts (*transcripts_ Hyaluronates_062023*), and flow chart (*flow_ Hyaluronates_062023).*

The Panel should carefully consider the Abstract, Discussion, and Conclusion presented in this report. If these are satisfactory, the Panel should issue a Final Report.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: March 27, 2023

SUBJECT: Tentative Report: Safety Assessment of Hyaluronates as Used in Cosmetics (release date: March 16, 2023)

The Personal Care Products Council respectfully submits the following comments on the tentative report, Safety Assessment of Hyaluronates as Used in Cosmetics.

Introduction – It is not clear why the parenthetical Hydrolyzed Calcium Hyaluronate and Hydrolyzed Sodium Hyaluronate follows "acetylated esters derived from Hyaluronic Acid. Perhaps it should be in the previous sentence following "two hydrolyzed salts of hyaluronic acid".

Method of Manufacture, Hyaluronic Acid – Please correct "grounded" to "ground"

Non-Cosmetic Use – Please correct "in" to "an" in the following: "as in inactive ingredient". Please add "21" before "CFR 522.1145". It should be made clear that FDA approved Hyaluronic Acid dermal fillers are all cross-linked, while it is Hyaluronic Acid itself that is injected as a treatment for arthritis.

Short-Term and Subchronic; Table 8 – Please revise "were given up to 48 mg/kg bw/d of a 1% Sodium Hyaluronate ophthalmic solution". The rats were treated with 48 mg/kg/day Sodium Hyaluronate as a 1% solution. Table 8 should also make it clear that the stated doses represent Sodium Hyaluronate not the dosing solution.

Immunogenicity, old report study – The spelling error (albumen to albumin) still needs to be corrected. Please see this article that explains the difference between albumen and albumin https://pubs.asahq.org/anesthesiology/article/98/5/1304/40329/Egg-Allergy-and-Blood-Products-You-Say-Albumen-I .

Dermal Irritation and Sensitization – In the text, please identify the type of *in vitro* dermal irritation assays that were performed.

Immediate and Delayed Hypersensitivity to Intracutaneous Hyaluronic Acid; Summary – Since only dermal fillers were tested, this study is not relevant to cosmetic use of Hyaluronic Acid and Hyaluronates and it should be deleted. If it is left in the report, the heading should be revised to Hyaluronic Acid Dermal Fillers, to make it clear what was tested. If the study is left in the report, the conclusion should also be stated: "Neither type I or type IV hypersensitivity plays a role in late inflammatory reactions to Hyaluronic Acid dermal fillers".

Summary – The last paragraph of the Summary should make it clear that FDA approved Hyaluronic Acid dermal fillers are cross-linked, while Hyaluronic Acid itself is used to treat osteoarthritis.

Discussion – Please search dermal filler in the FDA catalog devices@fda https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm and look at the Hyaluronic Acid fillers. If you click on a Hyaluronic Acid filler and then look under labelling, you will find that the Hyaluronic Acid fillers approved by FDA are all cross-linked. Therefore, the statement that "the majority of Hyaluronic Acid fillers contain cross-linked hyaluronates" is not correct. Based on FDA's catalog, all FDA approved Hyaluronic Acid dermal fillers are crosslinked.

Table 6 – In the Dose/Protocol column of the second study, please correct "imagine plates" to "imaging plates". In the Results column of the third study, please add "h" after the last "168".

Table 10 – Please clarify if 20 mg represents 20 mg of the 0.2% Hyaluronic Acid containing formula, or 20 mg of Hyaluronic Acid.

Table 12 – This table is misleading. Although the heading says "Hyaluronic Acid dermal fillers", all the entries just say "Hyaluronic Acid". For each entry, please make it clear that the patients were all treated with Hyaluronic Acid dermal fillers which are all crosslinked Hyaluronic Acid not Hyaluronic Acid itself, e.g., change Hyaluronic Acid to Hyaluronic Acid dermal filler, or change Hyaluronic Acid to dermal filler.

Hyaluronates - June 2023 – Priya Cherian	
Comment Submitter: Personal Care Products Council **Date of Submission: March 27, 2023**	
Comment	**Response/Action**
Introduction – It is not clear why the parenthetical Hydrolyzed Calcium Hyaluronate and Hydrolyzed Sodium Hyaluronate follows "acetylated esters derived from Hyaluronic Acid. Perhaps it should be in the previous sentence following "two hydrolyzed salts of hyaluronic acid".	Addressed
Method of Manufacture, Hyaluronic Acid – Please correct "grounded" to "ground"	Addressed
Non-Cosmetic Use – Please correct "in" to "an" in the following: "as in inactive ingredient". Please add "21" before "CFR 522.1145". It should be made clear that FDA approved Hyaluronic Acid dermal fillers are all cross-linked, while it is Hyaluronic Acid itself that is injected as a treatment for arthritis.	Addressed
Short-Term and Subchronic; Table 8 – Please revise "were given up to 48 mg/kg bw/d of a 1% Sodium Hyaluronate ophthalmic solution". The rats were treated with 48 mg/kg/day Sodium Hyaluronate as a 1% solution. Table 8 should also make it clear that the stated doses represent Sodium Hyaluronate not the dosing solution.	Addressed
Immunogenicity, old report study – The spelling error (albumen to albumin) still needs to be corrected. Please see this article that explains the difference between albumen and albumin https://pubs.asahq.org/anesthesiology/article/98/5/1304/40329/Egg-Allergy-and-Blood-ProductsYou-Say-Albumen-I .	Addressed
Dermal Irritation and Sensitization – In the text, please identify the type of in vitro dermal irritation assays that were performed.	Addressed
Immediate and Delayed Hypersensitivity to Intracutaneous Hyaluronic Acid; Summary – Since only dermal fillers were tested, this study is not relevant to cosmetic use of Hyaluronic Acid and Hyaluronates and it should be deleted. If it is left in the report, the heading should be revised to Hyaluronic Acid Dermal Fillers, to make it clear what was tested. If the study is left in the report, the conclusion should also be stated: "Neither type I or type IV hypersensitivity plays a role in late inflammatory reactions to Hyaluronic Acid dermal fillers"	The Panel should address whether or not they would like this study to be deleted.
Summary – The last paragraph of the Summary should make it clear that FDA approved Hyaluronic Acid dermal fillers are cross-linked, while Hyaluronic Acid itself is used to treat osteoarthritis.	Addressed
Discussion – Please search dermal filler in the FDA catalog devices@fda https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm and look at the Hyaluronic Acid fillers. If you click on a Hyaluronic Acid filler and then look under labelling, you will find that the Hyaluronic Acid fillers approved by FDA are all cross-linked. Therefore, the statement that "the majority of Hyaluronic Acid fillers contain cross-linked hyaluronates" is not correct. Based on FDA's catalog, all FDA approved Hyaluronic Acid dermal fillers are crosslinked.	Addressed
Table 6 – In the Dose/Protocol column of the second study, please correct "imagine plates" to "imaging plates". In the Results column of the third study, please add "h" after the last "168"	Addressed
Table 10 – Please clarify if 20 mg represents 20 mg of the 0.2% Hyaluronic Acid containing formula, or 20 mg of Hyaluronic Acid	20 mg in this study refers to 20 mg of the 0.2% HA containing formulation
Table 12 – This table is misleading. Although the heading says "Hyaluronic Acid dermal fillers", all the entries just say "Hyaluronic Acid". For each entry, please make it clear that the	Addressed

patients were all treated with Hyaluronic Acid dermal fillers which are all crosslinked Hyaluronic Acid not Hyaluronic Acid itself, e.g., change Hyaluronic Acid to Hyaluronic Acid dermal filler, or change Hyaluronic Acid to dermal filler.	

Hyaluronates – History

July 2021

- Concentration of use received for Hydrolyzed Calcium Hyaluronate, Hydrolyzed Hyaluronic Acid, Hydrolyzed Sodium Hyaluronic Acid, and Sodium Acetylated Hyaluronate

January 2022

- Concentration of use received for Hyaluronic Acid, Sodium Hyaluronate, and Potassium Hyaluronate

October 2022

- SLR posted on CIR website
- Comments received on SLR from PCPC

November 2022

- Unpublished data received:
    - In vitro dermal and ocular irritation assays on several trade name mixtures containing 1-3% Hyaluronic Acid
    - In vitro dermal and ocular irritation assays on trade name mixture containing 0.5% Sodium Hyaluronate
    - HRIPT on formula containing 0.2% Sodium Acetylated Hyaluronate
    - HRIPT on formula containing 0.2% Hyaluronic Cid
    - HRIPT on formula containing 1.5% Sodium Hyaluronate

December 2022

- Unpublished data received:
    - Composition, impurities, manufacturing, and summarized toxicity data on Sodium Hyaluronate
    - Composition, impurities, manufacturing, and summarized toxicity data on Hydrolyzed Sodium Hyaluronate
    - Composition and Manufacturing Data on Hydrolyzed Hyaluronic Acid

January 2023

- Composition, manufacturing, instruction, and summarized safety data on Sodium Hyaluronate

March 2023

- Panel reviews Draft Report on 7 hyaluronate ingredients
- Panel issues a Tentative Report for public comment

April 2023

- Comments on TR received from PCPC

June 2023

- Panel reviews Draft Final Report

Hyaluronates Data Profile – June 2023 – 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/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Hyaluronic Acid	**XO**	**XO**	XO		XO	X		O			O								X	O	O	X		X		X	X		X
Hydrolyzed Calcium Hyaluronate	**X**																												
Hydrolyzed Hyaluronic Acid	**X**	**X**	X																										
Hydrolyzed Sodium Hyaluronate	**X**	**X**	X												X				X		X			X	X	X			
Potassium Hyaluronate	**XO**																												
Sodium Acetylated Hyaluronate	**X**																							X					
Sodium Hyaluronate	**XO**	**X**	X					X			X			XO	X	X			X		O	X		X	X	X			X

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

* "O' indicates that data were available in a category for the ingredient (data is in italics in report as it is summarized from the previous report on Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate published in 2009

Hyaluronates

Ingredient	CAS #	InfoB	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Sodium Acetylated Hyaluronate		x	x			x													x
Hydrolyzed Hyaluronic Acid		x				x													
Hydrolyzed Calcium Hyaluronate		x				x													
Hyaluronic Acid	9004-61-9	x	x			x	x												x
Sodium Hyaluronate	9067-32-7	x	x		x	x	x												x
Potassium Hyaluronate	31799-91-4	x				x													
Hydrolyzed Sodium Hyaluronate		x				x													

An "x" indicates that relevant data were found in the database/website

Search Strategy

Ingredient names and CAS numbers were searched in combination with the search terms listed below.
*these terms were searched from 2003 onwards for previously reviewed ingredients (Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate)

Typical Search Terms

- INCI names
- CAS numbers
- chemical/technical names
- metabolism
- impurities
- composition
- dermal
- inhalation
- skin
- toxicity
- drugs
- medicine
- clinical
- case report
- irritation
- ocular
- eye
- sensitization
- allergy
- manufacture
- pharmacokinetics
- cancer
- carcinogenicity
- mutagenicity
- Ames
- Reproductive
- Teratogenicity
- Synthesis

LINKS

Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)
- Toxnet (https://toxnet.nlm.nih.gov/); (includes Toxline; HSDB; ChemIDPlus; DART; IRIS; CCRIS; CPDB; GENE-TOX)

appropriate qualifiers are used as necessary
search results are reviewed to identify relevant documents

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;,
- EAFUS: http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true
- 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
- http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- OTC ingredient list: https://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm135688.pdf
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/

- HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/

- 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/
- IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
- 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
- NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.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

MARCH 2023 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito Team – March 6, 2023

DR. BELSITO: Okay. So now we're going to move on to hyaluronates. Hold on to your hats. This was another report that drove me crazy.

This is a draft report on the safety of hyaluronates as used in cosmetics. There are seven that are being reviewed in this report. I won't read them all. Sodium acetylated hyaluronate and hydrolyzed hyaluronic acid were included on the '22 priority list due to frequency of use. And, then, three related ingredients previously reviewed by the panel in 2009 were added in. Those are hyaluronic acid and the potassium and sodium salts for rereview.

And, then, we thought it was appropriate to add -- what did we add -- three more hyaluronate-derived ingredients that were in the dictionary. So we did that, and we received a good amount of data. We'll go from there.

So, in the introduction, should we mention the additional hyaluronic acid derivatives that were added as read across? Or is that not necessary? Because we say that the sodium acetylated and hydrolyzed hyaluronic acid were because of frequency of use, and then we decided to bring in three that have been previously reported. But we don't say anything about the additional ones that we added to the list, why they're there. I just throw that out for comment.

DR. RETTIE: If I'm reading it, don't we say that these ingredients were added in --

DR. SNYDER: Because they were read across, Don, they were ingredients in the hyaluronate class category that were up for rereview.

DR. RETTIE: Yeah.

DR. BELSITO: Well, they were going up for rereview because it's -- well, they'd be up for rereview in another year, I guess.

DR. RETTIE: It says would soon be considered for a rereview.

DR. BELSITO: Right. So that's why we added those in. But, then, we don't mention anything about the sodium acetylated hyaluronate. Why was that added? Why was the hydrolyzed sodium hyaluronate added? I mean, they were added because we felt we could read across to them. But I'm just saying we went in all this detail about five ingredients, but we don't say anything about the other two that we added in the introduction.

DR. SNYDER: Well, I thought we added those based on frequency of use on our priorities from last year.

DR. RETTIE: Yeah.

DR. SNYDER: And, then, when we did that, then the grouping in with these other ones seemed like they fit in.

DR. RETTIE: Yeah.

DR. BELSITO: Right. My point is we don't mention that. We only mentioned that the three that we previously reviewed in 2009 fit in. We don't mention the other two. Should we?

DR. SNYDER: In that first paragraph we do. We say it was based on frequency of use.

DR. RETTIE: First sentence.

DR. BELSITO: Yeah. Frequency of use for sodium acetylated and hydrolyzed hyaluronic.

DR. RETTIE: Yeah.

DR. BELSITO: Then we say we decided to add in hyaluronic acid and potassium and sodium salts because they would be soon due for rereview. We don't say why we added in hydrolyzed sodium hyaluronate or sodium acetylated hyaluronate. And we added them in presumably because we felt they were in the dictionary and we could read across to them. But we don't make any mention of that in the introduction. Should we?

DR. RETTIE: Well, it's really only hydrolyzed sodium hyaluronate that is not mentioned because sodium acetylated hyaluronate is the first one that we mentioned in that paragraph.

DR. BELSITO: Oh, I'm sorry.

DR. RETTIE: So I think we're down to one.

DR. BELSITO: Then there are two others that were added because they're all --

DR. SNYDER: They were the salts of hyaluronic acid. Yeah.

DR. BELSITO: Right.

MS. CHERIAN: If it makes it easier, in that first paragraph in the last sentence, when it says, additionally, two hydrolyzed salts of hyaluronic acid are included in this grouping, after that, I can put in parentheses the missing two.

DR. BELSITO: Okay. Yeah, that would be good.

MS. CHERIAN: Okay.

DR. BELSITO: You're putting that where, Priya?

MS. CHERIAN: In the last sentence of that first paragraph -- or actually, it's the second-to-last sentence. Where it says, additionally, two hydrolyzed salts of hyaluronic acid are included in this grouping.

DR. BELSITO: I'm not seeing that, but I trust you. It's in the first paragraph?

DR. RETTIE: Yeah.

MS. CHERIAN: Yes.

DR. BELSITO: Again, I'm sorry to sound really stupid here, but I'm still not seeing it in the first paragraph. I have, the Panel had concluded three ingredients as safe as described in the 2009 safety assessment, along with corresponding minutes, have been included herein, is what I got.

DR. RETTIE: It's different from I'm looking at on the first paragraph.

DR. BELSITO: Okay. For some reason, ingredients in the safety assessment -- I have something different.

DR. SNYDER: Yeah, you do. Very different.

DR. BELSITO: Okay. Well, I'm not going to worry about it. You know what? I'm not in the report. That would help, wouldn't it? You're right. Sorry. I was looking at the wrong page. This is PDF Page 3. Thank you.

Okay. So, for method of manufacture, we don't have sodium acetylated hyaluronate. And can we assume that calcium, potassium have the same method of manufacturing as the sodium, or do we need the acetylated hyaluronate?

DR. RETTIE: That seems fairly trivial in terms of the synthesis, but the impurities are missing. So may as well ask for MOM and impurities for the three salt forms at the same time. This is the first go around. That would be what I would think.

DR. BELSITO: Okay. Curt, Paul?

DR. KLAASSEN: Fine.

DR. SNYDER: Yeah. I don't they're going to be different, but that's all right.

DR. BELSITO: So we're asking for manufacturing, impurities for the sodium acetylated hyaluronate and the calcium and potassium hyaluronate. Is that correct?

DR. SNYDER: Yeah.

DR. RETTIE: While we're sort of on structural aspects of this, maybe I'd bring up that Figure 1 where we've got a structure for Hyaluronates. Looks like the structure for the acetylated Hyaluronate. And I wondered if that might be modified just to give a more general structure. This one would pick out, for example, the salt, where the salt would be made on the R group.

But, where the two acetyl groups are, maybe you could put an R1 in there and just specify that that's acetylated because this one's a much more specific structure for acid acetylate hyaluronate. It's held over from the previous report, which could have also been changed there. But this would be the opportunity to have a general structure.

DR. BELSITO: So what do you want there? You just want an R off of that branch rather than the acetyl group? Is that what you're saying, Allan?

DR. RETTIE: Yeah. Just put an R in the two places where there's the acetyl group now or change it to acetyl or acetyl hyaluronate. Just match up what the figure heading is with the structure is all I'm saying.

DR. BELSITO: Yeah, because it says where R is sodium, and there's no R in the current structure. I guess there is, OR up there. Yeah.

DR. RETTIE: We might have to go with RR prime or R1 and R2.

DR. BELSITO: Yeah.

DR. RETTIE: That would kind of take care of it.

DR. BELSITO: But wait a minute. Maybe the double bond CH3 is part of all hyaluronic acid. No?

DR. RETTIE: Well, that's an acyl group. And, when I looked up the structure of hyaluronic acid, there was no acyl group there.

DR. BELSITO: Okay. Bart, you're the other chemist here. What are you thinking?

DR. HELDRETH: Sorry. I was under the impression that this was the structure of hyaluronic acid. If we simply leave those as acid groups on every other mono sugar. And, then, when we get the acetylated, the primary alcohols on the other two mono sugar groups are what get acetylated. And that's why it --

DR. RETTIE: Oh, is that right? I misread that then.

DR. HELDRETH: And, then, if you look in Table 1.

DR. RETTIE: Oh, that's a ketone. You're right.

DR. HELDRETH: Mm-hmm.

DR. RETTIE: You're right. I'm wrong.

DR. HELDRETH: On Table 1, it says -- if I could look at Table 1 without coughing.

DR. RETTIE: You're right.

DR. BELSITO: Are you coughing or sneezing? I'm about to put a mask over my screen.

DR. HELDRETH: I know. For Table 1, for sodium acetylated hyaluronate, it says to see that Figure 1 where R is sodium and one or more hydroxyl groups are acetylated.

DR. RETTIE: You're correct, Bart. I missed there's no oxygen out there on the thing I was looking at. So it's not an acyl group. It's a ketone. So strike that. Figure looks fine.

DR. BELSITO: So we have this coming from rooster comb, so we're going to need the boilerplate for animal tissue in our discussion, I guess. And I guess I was really shocked that endotoxins were more likely in the animal-derived than in the bacterial-strain-derived. Was there an explanation for that?

MS. CHERIAN: Was that stated in italicized text or regular text?

DR. BELSITO: I'll have to go back, but then I had a note, can we assume any bacterial residue will be killed off in processing? But let me find the endotoxin statement. No, it's under regular text. PDF Page 22, under hyaluronic acid. It says that human umbilical cord, bovine vitreous, and rooster comb-derived hyaluronic acid preparations contained high levels of endotoxin contaminants. Bacterial-derived hyaluronic acid was nearly endotoxin free.

MS. CHERIAN: I don't think it gave an explanation. I think it just gave the data.

DR. BELSITO: I mean, that seems weird to me. No?

MS. CHERIAN: Mm-hmm.

DR. BELSITO: Where would you get endotoxins from rooster comb?

MS. CHERIAN: I'll have to go back and look at the source to find out exactly what it says.

DR. BELSITO: Yeah. I mean, Curt, Allan, Paul, what do you think of that?

DR. SNYDER: I can't explain it.

DR. BELSITO: I mean, it doesn't make sense. And do we have any definition of cosmetic grade, or are we going to make that up based upon impurities, like nucleic acid, protein and endotoxins? Because the dictionary wouldn't put limits on that, would it, Bart?

DR. HELDRETH: No. I think it's up to suppliers to provide that information for us. I'd be surprised if industry isn't making it either synthetically or from the bacterial route. One, because it's going to not have those toxin impurities, and it's probably much more efficient and cheaper to make by those pathways anyway. So I'd be a little shocked if the industry is making hyaluronic acid from roosters.

DR. BELSITO: Right. Okay. So I guess we'll get that data when we ask for manufacturing and impurities. But it would be nice to know what their definition of cosmetic-grade hyaluronic acid and derivatives mean.

DR. HELDRETH: I doubt we'll get a cosmetic grade definition because that seems to be something that industry shy away from. But what we can get, and what I think we'll likely get, is the definition of the ingredient as provided by suppliers. So the supplier may define exactly what they produce, and that's what we'll base our assessment on.

DR. BELSITO: Okay. So need cosmetic composition as supplied. Is that what we're saying?

DR. HELDRETH: That sounds right to me.

DR. BELSITO: Okay. So we have a maximum leave-on use of 7.5 percent. There's eye exposure, but we have good data for that.

DR. KLAASSEN: I have a question for my colleagues. In the ADME section on Page 24, that last paragraph of ADME, the last sentence or two, to look at the distribution of hyaluronic acid, they use 99mTc. Does that make any sense?

DR. BELSITO: Technetium. They were using radioactivity, no?

DR. KLAASSEN: Yeah, but why technetium? C14 or hydrogen, I can understand. If you got technetium there, it isn't any more hyaluronic acid, is it?

DR. BELSITO: I don't know. Good question.

DR. KLAASSEN: Allan, you see that?

DR. HELDRETH: So just pulling from a quick search of it, it's defined as a radio nuclei. That's a nuclear agent that is FDA approved for diagnostic imaging of the brain, bone, lungs, kidneys, and numerous organs.

DR. KLAASSEN: Yeah. But it's --

DR. BELSITO: Right, it's technetium scanning.

DR. RETTIE: Sorry, I was muted. Yes.

DR. KLAASSEN: Does that make any sense?

DR. RETTIE: That's something that's done -- I think they're opportunistic because they must have had some kind of bone imaging experiment going on. And, then, they sort of pivoted off that to get some information. It's certainly unusual.

DR. KLAASSEN: But they call this the distribution of hyaluronic acid.

DR. RETTIE: Right. Well, it would go with the radio treatment, wouldn't it, Curt?

DR. KLAASSEN: Yeah. I mean, if you would use C14, hyaluronic acid, you got to use one of the atoms that's in the molecule, right, to use it as a tracer. And, if you add technetium, that's not a part of hyaluronic acid. It's just kind of another test. I think it means nothing. A rapid uptake of the radioactivity was observed in the bone, muscle, et cetera. There is no technetium atom in hyaluronic acid. Is there?

DR. RETTIE: I don’t know.

DR. KLAASSEN: So, therefore, you can't use it.

DR. BELSITO: Well, does it change the way it behaves? I don't know.

DR. KLAASSEN: One assumes that -- I mean, it's not the same molecule. I mean, this is just kind of a no, no.

DR. BELSITO: Yeah. I mean, it's a medical scanning molecule.

DR. KLAASSEN: Right.

DR. BELSITO: So Allan's probably right. They were studying something else, and they decided to get some cheap data along the way.

DR. KLAASSEN: But they are interpreting --

DR. BELSITO: Are you suggesting that that entire paragraph be deleted as irrelevant?

DR. KLAASSEN: Yes. The last six lines, I think, is totally --

DR. BELSITO: So the whole distribution of technetium?

DR. KLAASSEN: Right. From there on down. Unless I'm missing something, it's absolutely crazy to me. So I would just eliminate it.

DR. BELSITO: Well, we can have that discussion with the other group tomorrow, see what they say.

DR. KLAASSEN: Yeah.

DR. RETTIE: We used to do this in hospital pharmacy all the time, but it was 30 years ago. There are technetium generators you can use. It's a very short half-life compound or half-life nuclide. So you got to use it really quickly and make it the day before. But I get what you're saying, Curt, need to look into it a bit more and have the discussion with the group tomorrow.

DR. KLAASSEN: Okay. Fine to go on.

DR. BELSITO: Above that, penetration enhancement, I had a question. Is this penetration enhancement or just use of hyaluronic acid as a vehicle? I saw it as use as a vehicle, not enhancement or penetration.

MS. CHERIAN: Are you talking about the diclofenac study?

DR. BELSITO: Yes.

MS. CHERIAN: Yeah, it was as a vehicle. It was the effect of hyaluronic acid as the vehicle, increasing the penetration of diclofenac.

DR. BELSITO: Well, do we know that it increased the penetration? Do we have a comparison to another vehicle?

MS. CHERIAN: I don't know. This was data from the other report. I'd have to look at it.

DR. BELSITO: Yeah. Because to me, as I read it, this was not -- I actually think I looked at the other report, and my conclusion was it wasn't penetration enhancement, it was just use as a vehicle. But check that.

MS. CHERIAN: Okay.

DR. SNYDER: We have strong evidence that hyaluronic acid is not absorbed, right? Minimally absorbed.

DR. BELSITO: Yeah. So maybe it is penetrated. I mean, I don't know. But it says that it gave depo effect. It says it was formed in more shallow layers of the skin, epidermis, in humans.

DR. SNYDER: Our absorption data says it's almost all contained within stratum corneum. So maybe it was a depo in the stratum corneum, but it wasn't absorbed. At least the data we have says it wasn't absorbed.

DR. BELSITO: Well, it says the drug reservoir was formed in the deeper layers of the skin, dermis, in mice, while the drug reservoir was formed in more shallow layers of the skin, epidermis, humans. And our absorption data is in what model?

DR. SNYDER: Page 24.

DR. BELSITO: Rats, gavage, oral.

DR. SNYDER: And Table 6 is a summary table.

DR. KLAASSEN: I mean, if you look at the molecule, how can it be absorbed?

DR. SNYDER: Yeah.

DR. KLAASSEN: It's huge, it's water soluble. Even without doing an experiment, you know that it's not going to be absorbed.

DR. BELSITO: So do you think it's penetration enhancement?

DR. KLAASSEN: Well, I think we need to look at that study there. There's a couple of references there, the 1 and the 26.

DR. BELSITO: Because, if it's a penetration enhancer, then that goes in our discussion. If it's not, it doesn't, right?

DR. KLAASSEN: Right. I mean, the chemical does not have to be necessarily absorbed to enhance the absorption of something else. It can just kind of alter the solubility of the other drug in such a way that it's more likely to go through the skin.

DR. BELSITO: Okay.

DR. KLAASSEN: So this could be true. When it's talking about the drug reservoir, is that having to do with hyaluronic acid or diclofenac? I assume the reservoir is diclofenac.

DR. BELSITO: Yes. That's the drug.

DR. KLAASSEN: Right.

DR. SNYDER: So Reference 1 is one of our reports, and Reference 26 is hyaluronic acid, a unique topical vehicle for the localized delivery of drugs to the skin. That sounds like it's a depo effect on the surface of the skin because it's not absorbed.

DR. BELSITO: Yeah. That's what I took away from the article, Paul.

DR. SNYDER: Yeah.

DR. BELSITO: Yeah.

DR. SNYDER: That's not dermal penetration. Penetration enhancement for sure.

DR. BELSITO: You want to send that study, Priya, to Curt so he can review it and come back with his opinion on it?

MS. CHERIAN: Sure.

DR. BELSITO: And, Paul, maybe --

DR. KLAASSEN: Yeah. Send it to --

DR. BELSITO: Send it to all of us.

MS. CHERIAN: Okay.

DR. KLAASSEN: Yeah. We're going to read it tonight.

DR. SNYDER: It contradicts all the absorption data because all the absorption data says this stuff is minimally absorbed, if at all.

DR. BELSITO: Yeah.

DR. SNYDER: Because I poo pooed all that. That carcinogenicity section is not carcinogenicity, it's tumor biology related to --

DR. BELSITO: Yeah.

DR. SNYDER: -- metastasis of tumors and stuff. But this stuff doesn't absorb. And, then, if you go to the discussion -- to the summary, that whole paragraph on Page 30, the whole discussion about the metastatic cancer stuff, it's not absorbed, it's not going to influence anything. Kind of giving too much narrative to that topic, in my opinion.

DR. KLAASSEN: Yeah. I agree. That's not a tox study. If anything, it's just --

DR. SNYDER: It's tumor biology. Yeah.

DR. KLAASSEN: Yeah. I mean, do we (audio skip). Okay (audio skip).

DR. BELSITO: So all of the short-term and subchronic toxicity we have is on sodium hyaluronate. Does that cover the other members of this group?

DR. SNYDER: Well, it's the most used. It's used in over 4,000 formulations. Almost 3,700 of those are leave-ons.

DR. BELSITO: Right. It's highest in lipstick.

DR. SNYDER: The maximum concentration is seven and a half percent.

DR. BELSITO: It's highest in lipstick at 0.39. I think it does cover, but I'm just throwing that out for you, Paul, and Curt and Allan to comment.

DR. KLAASSEN: Yes. I'm happy with that.

DR. SNYDER: With the combination of all the tox data we've seen so far being negative, and the fact that it's not absorbed, I was fine.

DR. BELSITO: Yeah. And, then, I just had to question whether we want to make any comments on the developmental repro toxicity that was taken over from the original report. Because it says that 1 percent hyaluronic acid solution and physiological saline via subcu injection, nodular hyperplasia of reticular zone cells was present in the adrenal glands. And that's weird because we didn't see anything with higher doses of sodium hyaluronate in the subchronic studies. I don't know. It's prior study, did anyone have any feelings about that?

DR. SNYDER: It's totally irrelevant. And they injected 60 milligrams per kilogram subcu.

DR. BELSITO: Okay.

DR. SNYDER: Yeah. They bypassed the skin, so I didn't put any weight on that.

DR. BELSITO: Okay.

DR. KLAASSEN: Yeah, me either.

DR. BELSITO: Okay. Then, do we put so little weight on it that we don't put it into the discussion?

DR. SNYDER: Well, I think in the discussion -- I think the discussion needs to have some language regarding intradermal because it is used as a filler. That is only important from the standpoint that it does give us some allergic information. But, as far as toxicity, all that's irrelevant to me. I mean, anything where they bypass the intact skin by injecting it intradermally or injecting it subcutaneously or whatever, all of the data that we (audio skip) been either from tox standpoint.

DR. BELSITO: Okay. Well, that gets to PCP's point about the intradermal injection for cosmetic reasons. Do we include all those case studies since it's not going to get there?

DR. SNYDER: Well, I think that and the medical device is important because, if there would be an allergy signal, you would see it, wouldn't you, with the medical devices and the intradermal injections?

DR. BELSITO: Right. You see some.

DR. SNYDER: Yeah. So put it in that context, but that's the only context that's relevant for us.

DR. BELSITO: Okay.

DR. SNYDER: I mean, that's my opinion.

DR. BELSITO: Okay.

DR. KLAASSEN: I agree with you.

DR. BELSITO: I'm good with that. Priya, on PDF 25, just right above genotox, where we're on the DART, the next to the last sentence says, fetal development and growth rates were similar between control and negative control. Do you mean control and study group?

MS. CHERIAN: Yes. I can change that.

DR. BELSITO: And, then, I agree with Paul. Those studies are not carcinogenicity studies. They're histopathological findings and malignancies.

DR. SNYDER: Yeah. It's tumor cell biology is all it is. Yeah.

DR. BELSITO: Yeah.

DR. SNYDER: I had a note that said, put it under tumor cell biology or something.

DR. BELSITO: Yeah.

MS. CHERIAN: Do you want me to keep them and put them under a different heading?

DR. SNYDER: Yes.

MS. CHERIAN: Okay.

DR. BELSITO: Yeah. I wouldn't get rid of them. But I would, as Paul said, under tumor cell biology.

DR. SNYDER: Well, the important thing is that we have no carcinogenicity studies. This looks like we have some, we don't have any. But it's not absorbed. It's fine. I thought it was okay to leave the case reports in, Don.

DR. BELSITO: Which, Paul?

DR. SNYDER: The case reports. They were plus minus. Leave them in, or take them out? There was a question whether we should leave them in.

DR. BELSITO: No, you said you wanted to leave them in and then just say the intradermal application is not relevant.

DR. SNYDER: Right. Okay. Correct.

DR. KLAASSEN: Yeah. We usually leave those in even though they don't amount to anything.

DR. BELSITO: Priya, the use in dissolving microarray patches, I think that should be placed under other clinical studies.

MS. CHERIAN: Okay.

DR. BELSITO: So, for dermal irritation and sensitization, the sodium hyaluronic is used up to 7.5 percent. The highest level tested is for 3 percent hyaluronic acid. Are we okay with that slightly more-than-two-fold difference?

DR. SNYDER: Yes. In consideration of all the intradermal stuff that was negative, there was no signals.

DR. BELSITO: Okay. So, in our discussion, we should put that we noted that the highest level tested in sensitization was 3 percent hyaluronic acid. But, in light of a lack of clinical reports for topical use and a small number of reports for adverse events with intradermal use, given the widespread use, the Panel was not of concern or something like that. The under dermal irritation and sensitization, your in vitro tests are not sensitization tests. They're hazard identification.

So I did some wordsmithing on how to refer to those in the report, mainly just by saying what the effects were, if you know, like, for DPRA, cysteine, lysine depletion. If you know for KeratinoSens what the percentage of increase in the response genes were, just put that in. Or say that the results were negative for cysteine, lysine depletion, negative for ARE-Nrf2 induction, negative for C56 CD84 -- whatever.

MS. CHERIAN: Got it.

DR. BELSITO: We have an in vitro phototox assay, 3T3, which was negative. These are ring structures. We have no absorption spectra. Do we need that? We didn't ask for it before. I'm just saying. By way of context, it's in the dermis, and UVA will get to the upper dermal levels. So, if it was a sensitizer, we'd probably be in trouble.

DR. KLAASSEN: We'd all be sensitized.

DR. BELSITO: What?

DR. KLAASSEN: I mean, we all have hyaluronic acid in our bodies.

DR. BELSITO: No, that's what I mean.

DR. KLAASSEN: Yeah.

DR. BELSITO: UVA, which is a phototox, photo allergen wavelength of light, will get to the dermis where we have hyaluronic acid. So, I don't think we need it, I'm just pointing that out. And do we discuss it at all?

DR. HELDRETH: There's no obvious chromophores in that structure.

DR. BELSITO: Okay.

DR. HELDRETH: And also, on that note, Allan was right. There was an error in my structure. It should have looked like this. I had left carbons here, so it looked like ketones when it should have been nitrogen amides. I'll make sure that gets fixed in the next iteration.

DR. BELSITO: Okay. Thanks, Bart.

DR. HELDRETH: Mm-hmm.

DR. KLAASSEN: So both of you were wrong. That makes me feel better.

DR. BELSITO: A double negative.

DR. RETTIE: Succinctly put, Curt, double negative doesn't make a positive.

DR. KLAASSEN: Double negative doesn't make a positive, not in chemistry.

DR. HELDRETH: Three wrongs don't make a right, but three lefts do.

DR. SNYDER: I just had flashbacks to organic chemistry.

DR. RETTIE: Since we're back on structure, Curt, I was looking some stuff up because I had only vague remembrance of using this stuff in radio pharmacy years and years ago. It appears that the technetium generator, once you've generated it and it's got a short half-life, you can actually bind it to drugs. So, if it stays bound to drugs, which I think is what you were questioning, then certainly you couldn't use it to follow the half-life of whatever it was bound to.

So the question probably comes down to, well, does it stay bound to hyaluronic acid? And, if it did, then you could probably use any data that you get from that. But I don't know if it does stay labeled or whether it just leaches out. I was looking for the reference. I was looking for the reference so I could look it up, but it's not here under the ADME section.

DR. KLAASSEN: Is it the 1936 reference?

DR. RETTIE: Is that Reference 28? Might be hard to find if it's '36.

DR. KLAASSEN: You're right if it bound to it. But there's two aspects to it. If it bound to it and stayed bound to it, like you said, it could be indirectly used. But the second question is, is that molecule distributed in the same way as the parent compound? It's kind of like looking at a drug and it's metabolite, right? They're two different molecules.

DR. RETTIE: Yeah. If it does stay bound to it, Curt --

DR. KLAASSEN: This is an approximation.

DR. RETTIE: If it did stay bound, Curt, wouldn't you think it would just be fine because it wouldn't massively modify the -- it's different from unlabeled hyaluronic acid, but in the same way that C14-labeled hyaluronic acid is not hyaluronic acid.

DR. KLAASSEN: Well, it's pretty darn close.

DR. RETTIE: Yeah.

DR. KLAASSEN: Let's see what they say tomorrow. I don't like it, but I can be talked out of it.

DR. BELSITO: Okay.

DR. RETTIE: Well, at the end of the day, when you read the paragraph, it doesn't actually give you any PK data at all. It just tells you where it ended up. So I don't think it's very helpful regardless of what it really means.

DR. KLAASSEN: And it says a lot of it goes to the bone, and that's where the technetium would go if you only gave technetium. So I'm not overly confident. Anyway --

DR. BELSITO: Okay. Well, we'll see what they say tomorrow.

DR. KLAASSEN: -- I think this is something that -- right.

DR. BELSITO: I think we're sort of beating a dead horse here right now. So, in the discussion --

DR. KLAASSEN: Yeah. (Audio skip) bar, not in a science --

DR. BELSITO: Yeah. In the discussion, we're going to need the biologic and heavy metal boilerplates. We need to figure out tonight, or tomorrow morning, whether it's a penetration enhancer or a vehicle. And, if it's a penetration enhancer, that would have to go in the discussion. Otherwise, I think pretty much the discussion is previously written into this document. The question now becomes, as we started this, we were going with insufficient for manufacturing and impurities of the acetylated and the calcium and potassium hyaluronate.

And we're asking what is the composition or cosmetic-grade product? So are we staying with that insufficient conclusion?

DR. SNYDER: This is the first time we've seen it, right?

DR. BELSITO: Yeah.

DR. RETTIE: Yeah.

DR. SNYDER: So let's ask for it, and we can change that later if we get more confidence in that no penetration enhancement. We have quite a bit of data, so.

DR. BELSITO: Right, okay. So we have our discussion pretty much written, I think, at this point. And our conclusion right now is insufficient. And the insufficiencies are manufacturing and composition of the acetylated and the calcium and potassium salts, and the definition of cosmetic-grade ingredient. Anything else?

DR. SNYDER: That sums it up.

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DR. COHEN: Let's move on to hyaluronates. So, hold on, excuse me. I'm just looking back, here. Just pardon me one second while I pull things up. You know what, I'm sorry, I pulled up an old one. This was for seven ingredients -- hold on, I apologize.

Okay, I'm back. So, this is the first time we're reviewing this draft report and this safety assessment is for seven ingredients. They're used as hair and skin conditioning agents. Sodium hyaluronate has the highest concentration of use at 7.5 percent in face and neck.

In 2005, sodium hyaluronate was reported to be used up to two percent. And sodium hyaluronate has the highest frequency of use with several thousand products. There was a notation that three of the ingredients had been previously reported by the panel in 2009. Hyaluronic acid, potassium hyaluronate and sodium hyaluronate would soon be considered for re-review so they were blended into this for just convenience, I suppose. Makes a lot of sense.

And there's a comment on the data. The crosslinked hyaluronic acids, should we include this in the safety assessment or not. These are often the injected products that we hear about all the time in cosmetic uses like as injectable fillers.

Rooster combs are used as food and hyaluronic acid seems to be ingested orally as a supplement. We have methods of manufacturing on calcium and potassium in acetylated and we don't have sensitization at max use, although I wonder what the groups comments are regarding the molecular weights and if there really is any suspicion there.

And we had Wave 3 comments that looked good, and I agreed with the previous discussion as well. So, I'll just open it up to the group now. Tom?

DR. SLAGA: Yeah, we obviously got a lot of data and going from methods of manufacturing all the way to sensitivity, there seems to be sufficient data and as you mentioned it is a natural product. It's put in as fillers for food. So, oral toxicity is not a problem and I think we have sufficient data to make a conclusion that they're all safe. You can read across with some of them -- all of them, actually, they're very similar. So, I think it's safe.

DR. COHEN: Yeah. Wilma?

DR. BERGFELD: I think it's safe. I think it's a wonderful summary and I would vote to keep the injectable information, at least summarized in it, so it isn't lost.

DR. ROSS: Sorry. My internet dropped, so I'm back. Where are we?

DR. COHEN: That's fine. I was fishing for my report anyway, so you didn't miss a lot. We're on hyaluronates.

DR. ROSS: Yeah, I guess.

DR. COHEN: This is a draft report.

DR. ROSS: Yeah.

DR. COHEN: This was a pretty comprehensive draft report.

DR. SLAGA: Yeah. Very nice. Great job.

DR. ROSS: Well, you covered uses of max -- at least my notes of increased method of manufacture and purities of air. Read across is probably okay. There's large ranges in molecular weights on these things but -- between them, but that also occurs within a single group because of their polyatomic type of nature. The skin penetration varies. I heard Tom say the tox is okay and I would agree with that. There was one comment I had, David, and I'll defer this to you and maybe to Wilma. The sodium hyaluronate is now at maximum use is 7.5 percent, you've got HRIPT at 1.5.

DR. COHEN: That was my comment when you came on.

DR. ROSS: Okay.

DR. COHEN: Is that we're far off from max use and these things are pervasive in dermatology and we don't see a lot of irritation from it. The only question is, are we going to miss irritation if we're just testing at a seventh of the max use?

DR. BERGFELD: You have eye, though. You have eye. It's not irritating at eye, yeah.

DR. ROSS: It's just sensitization needed.

DR. COHEN: We have eye.

DR. BERGFELD: Yeah. And you have the in vitro. Yeah.

DR. ROSS: So, it's just sensitization, I think.

DR. BERGFELD: But you know they use an injectable. And we don't see much trouble with the injectables even though it's a bigger product.

DR. COHEN: It is a bigger product, and the human irritation is at one percent and two percent, right? We don't get to seven.

DR. BERGFELD: Do you think the injectable would help us there? (Inaudible)?

DR. COHEN: So, I think naturally that certainly lends a lot to it. But poison ivy stroked on your skin surface causes a big problem and if you eat it, it's not that big a problem. So, are we bypassing the dendritic cells on the surface when we're injecting into the dermis? I think we might.

Look, this is a draft report, and my gut was Tom's reaction that these are safe, and I think we're somewhat influenced by our extensive experience with this. But this is going to be presented by Don tomorrow and I wonder that if I not just at least bring this up for discussion instead of going to a straight second if they have a safe as used.

DR. BERGFELD: Could be a good idea.

DR. ROSS: So on the irritation you have an epiderm out there, neat, I mean, it's in vitro, right? both with sodium hyaluronate and with hyaluronic acid. And you're right, the human is only up to 2 percent non-irritating. But you do have some in vitro to help back you up.

DR. COHEN: Yeah. Yeah. David, I think that's right and there was one part of me that went with my gut and just reviewing the size of these things. And I think probably the molecular weight probably takes the allergen component down quite a bit. But I think at the very least it should be part of the discussion.

DR. ROSS: Yeah.

DR. COHEN: And I'll just bring it up tomorrow for a group discussion. Because I had it as safe as used but I really wanted to have a review of the max use issue.

DR. TILTON: I would agree to bring it up for discussion. I would say I didn't note a lot of concerns because of the non-sensitization data and ocular and in vitro. And just the fact that even though there's a large difference in molecular weight, really dermal penetration seemed to be primarily independent of molecular weight in terms of how far it penetrated into the skin.

But I agree, we can discuss this tomorrow.

DR. COHEN: Yeah, that's helpful. Yeah, I think we all came to the same conclusion, but I want to make sure in our report that we're thorough enough not to just fast track this through without going through it in greater detail. Any other --

DR. BERGFELD: I have a comment to make. I believe that this is a reopened document with the addons. Is that correct or is it brand new? I mean, the document itself is brand new, but the item was reviewed in 2009. Is this not a reopened one with added ingredients?

DR. COHEN: Three were going to come up for re-review so they were blended into this one.

DR. BERGFELD: But it doesn't consider it a re-review as well?

DR. ROSS: Or was on the priority list, wasn't it?

MS. FIUME: Well, it's not a re-review because two of the ingredients are what participated it being added to the priority list. So because those had high enough frequency, it's not a re-review. It's just since hyaluronic acid would be coming up and it fits into this group, it was folded in to avoid the re-review in the future and to provide the supporting information.

DR. BERGFELD: So, could we present it as a hybrid? I think that it's important to make that very clear. I know it's stated in here, I did read it, yeah.

MS. FIUME: We've done this in the past, I believe. Where ingredients have had high enough frequency of use that it was added to the priority list with something that would be up for re-review in the next couple of years, had been added, and therefore supportive information and it formed a group. But I will let Bart handle that question tomorrow in full meeting as far as a new category.

DR. BERGFELD: I think it'd be fun to add it as a hybrid review with a new priority list and three re-reviews added to it. I think it'd be interesting to do that. So, it gets defined differently in these summaries.

MS. FIUME: That's true, it does get defined differently.

DR. COHEN: Any further comments on hyaluronates? Let's move on to stearalkonium chloride.

DR. ROSS: Previous document was really well done as well.

DR. COHEN: Which one are we talking about, David?

DR. ROSS: The hyaluronates. I thought the previous document was really well done.

DR. COHEN: Yeah.

DR. TILTON: And, David, I'm just going to note we're going to discuss the inclusion of the data from the injectable dermal fillers.

DR. COHEN: Yes.

DR. TILTON: So, I am okay with them being included as a summary. It certainly doesn't contribute to the assessment and so it doesn't have to be in the report.

DR. BERGFELD: I would just speak as a dermatologist, it's very helpful for us to have it in this report. And it can be reduced in size, but just the notation.

DR. COHEN: Yeah, a lot of reactions to fillers may be -- because it's very theoretical -- may be related to biofilms, changes in microbiome in the injected areas, granulomas reactions that you wouldn't see from a topical preparation. But I went back and forth on this, again, and I think if we don't have it in there at all I think it might appear a year or two or three from now that, like, hey, did they even look at this, right? Did we consider it?

So, in the discussion we can get into a little greater detail that the molecules themselves are highly crosslinked, they're injected, and there are discussions about adverse effects being related to them being fillers as opposed to necessarily the hyaluronic acid itself. Because we see reactions to non-hyaluronic acid fillers that are sometimes similar. Does that do the job, Wilma, you think, if we at least caveat it a little in the discussion?

DR. BERGFELD: Yeah. Well, you need the reference, a few references, I think.

DR. COHEN: Yeah.

DR. BERGFELD: So, it has to be outside the discussion as well. I just think to put something in there in a summary form would be good, to note that we had considered, we looked at it, blah, blah, blah. You know?

DR. COHEN: Yeah. Yeah. Your point's well taken, Susan. I want to just make -- because they're used so pervasively in dermatology and plastic surgery. And people will come in for the hyaluronic acid injections and leave with hyaluronic acid moisturizers. It's hard to disconnect a lot of that encounter. So at least, hey, we've thought about it and the discussion will a little bit more clear.

DR. BERGFELD: Could I just add to that? Most of your OTCs and your cosmetic moisturizers do include hyaluronic acid. It's a great humectant.

DR. ROSS: Yeah.

DR. COHEN: You know, that's funny, Wilma, that you mentioned that because I was looking back at the comment that says a gram could hold six liters of water. And I'm wondering, can a gram hold six liters of water or could six liters of water hold a gram of hyaluronic acid? Because it's a striking comment. Carol, is your hand up?

DR. EISENMANN: Yes. My comment is that when I was reading the first draft of this report and there were data on the crosslinked hyaluronates in the report, I looked up and yeah some of the crosslinked ingredients are in the dictionary and they're on your priority list now. To me, if you're going to include the data on the crosslinked, then maybe the ingredients need to be in the report, too, or to have separate reports.

I mean, that was my concern that if you're putting the data on the materials in this report, do the ingredients belong on the report? And that's why, to me, you either should put the data in and the ingredients in or make them two separate reports; rather than if the data's not going to support the ingredients, then maybe doesn't need to be there. And you can say you're going to discuss this in a future report, or do it all in one report.

DR. BERGFELD: What would it take to do it in one report?

DR. EISENMANN: I don't know. I mean, it'd be up to you to make that decision. But you do have the crosslinked are showing up on the 2024 priority list.

DR. COHEN: That's an interesting quandary. Any advice on this, Monice or Priya? What do we do with this?

MS. FIUME: So as far as the question of including the crosslinked, I believe that'd be up to the chemist to think if it was an appropriate grouping. And then it would also beg the question if you have crosslinked, does it provide read across or is it just in here because they all have the name hyaluronic acid in here?

So, for me, especially not being a chemist, I can't answer the question as to whether or not they should be included. That would be up to the chemist on the panel. And then how the information would apply across the board as far as read across or would they all need stand-alone full safety panels.

DR. COHEN: The problem is the crosslinked fillers are not applied to the patient in any way near the same technique that they're being used in this report here. It sounds like Carol's point's pretty important and maybe if we markedly abbreviate in the discussion this, we could allude to a future discussion about it. If we get too heavy into this people will start assuming that they're similar. You know, sticking a 25-gauge needle into someone's cheek and injecting is not the same as putting it on the surface in a moisturizer.

MS. FIUME: And I cannot recall offhand which crosslinked are on the priority list. I know we'll see it later. So if it's more appropriate to keep it there, and if it's going to be reviewed next year, if it was okay for the panel that that's a sufficient time frame to look at those, especially if there's different concerns, it may be a worthwhile reason to leave the crosslinked out of the report.

I was going to ask, based on your discussion, if Table 12, if you did want that reduced to a paragraph referring to the case reports because often in the past, we've just listed the citations in text and given a range rather than bring it into a full table if you don't feel that the entire table is needed in the report we can do that.

And then Priya, I don't know. Do you know offhand how many are crosslinked? Are they all crosslinked hyaluronic acid in that table?

MS. CHERIAN: The injectable fillers?

MS. FIUME: Yes.

MS. CHERIAN: That was the problem. That's why I left the table as is because I didn't know if all of the fillers were crosslinked or not, and so I was trying to look up on the internet. I think most dermal fillers hyaluronic acid are crosslinked, but there are some FDA-approved non-crosslinked hyaluronic acid fillers so I can't guarantee that all of them are.

DR. BERGFELD: Priya, when you looked it up did you find that the crosslinks had a longer duration?

MS. CHERIAN: Yes. Yes.

DR. BERGFELD: Yeah. I would suggest that most of the time they're using crosslink now.

MS. CHERIAN: Right.

DR. BERGFELD: For years. Improvement.

DR. COHEN: Yeah.

DR. ROSS: Is that (inaudible) -- it didn't exactly meet (inaudible).

DR. COHEN: You're breaking up a little, David. There's some like background noise.

DR. ROSS: Okay. Let's try that. Can you hear me there?

DR. BERGFELD: A little better.

DR. COHEN: That's a little better. Good.

DR. ROSS: Table 12, I felt we probably didn't need it, but we need all the references. I mean, it's taking a lot of real estate there and I'm not sure you need all of that. But that's more an editorial call. And there was just an editorial comment, Priya, that was a lot of stuff in the original report on ocular, on monkey studies. And I felt some of that would be nice to put either in the text or in the table. But anyway, you'll see that in my comments.

MS. CHERIAN: Okay. Perfect, thank you.

DR. COHEN: Look, this is a draft report. We'll have time for discussion tomorrow about how we do this and I guess we'll contemplate it more tonight. I think Table 12 could be a little distracting to the report.

DR. BERGFELD: I find it very informative as a derm.

DR. COHEN: But so, let me ask you something, Wilma. Would you look at Table 12 --

DR. BERGFELD: I'm looking at it, yeah.

DR. COHEN: -- and would you say that an over-the-counter moisturizer with hyaluronic acid -- does reading Table 12 inform you about the use of that topical product on the face?

DR. BERGFELD: No. No. No, as you just mentioned, two different injectable sites, different immune response.

DR. COHEN: Delivery systems and -- right. So, is it conflating this report if we're going to do crosslinked? As you said, most of the injectables are crosslinked, right, because they need some endurance. Will Table 12 have a better place living in that future report?

DR. BERGFELD: Well, as we've all discussed, now, I think maybe the future report. But I think you have to make mention of it somewhere in this report, its relationship to the topical.

DR. COHEN: Yeah, I think that's where we landed. And we'll hash it out with the group tomorrow because it's a draft report. We're going to see this two more times and, between actually tomorrow and the next one, we should certainly have that resolved. Right?

DR. SLAGA: That's right.

DR. COHEN: Okay. Does everyone find that acceptable? I didn't think it was going to go that long. But it was a good discussion.

DR. BERGFELD: Yes.

DR. SLAGA: Yep.

DR. BERGFELD: Did you look at the priority list, Monice, and decide which one of these chemicals or, Susan, in the future priority list, might be the crosslink? Is it the poly- -- no, it wouldn't be that one, polysaccharides.

MS. FIUME: I did. It's sodium hyaluronate crosspolymer, and then hyaluronate crosspolymer-2 and cross polymer-3.

DR. BERGFELD: Is that for next year?

MS. FIUME: Yes.

DR. BERGFELD: I don't see the sodium -- okay.

MS. FIUME: That's on the list for 2024.

DR. BERGFELD: Okay.

MS. FIUME: So currently sodium hyaluronate crosspolymer has 207 uses.

DR. BERGFELD: 207. I suspect it would be nice if you are presenting this one, David, as you mentioned that -- what they are and where they are.

DR. COHEN: Yeah. This is Don's presentation tomorrow, but I have a feeling we'll have a lot of crosstalk between the teams.

DR. BERGFELD: Okay.

DR. COHEN: Okay, if there's no further comments about hyaluronates we can move on to stearalkonium chloride.

Full Panel – March 7, 2023

DR. BERGFELD: So our next big one is the hyaluronates. Dr. Belsito.

DR. BELSITO: Yeah. One second here. So this is a draft report, a safety assessment of hyaluronates as used in cosmetics. It's basically got a blend of the fact that hydrolyzed hyaluronic acid and sodium acetylated hyaluronate were included in the 2022 priority list due to frequency of use. And then it was noted that there were three related hyaluronates, hyaluronic acid, potassium and sodium hyaluronate. It would be up for re-review in 2024. And then there were several others that were chemically related that could be thrown into the batch to be looked at all together.

And so, an SLR on the seven of these ingredients. I've mentioned five, the other two are the hydrolyzed sodium hyaluronate and hydrolyzed calcium hyaluronate, I think. And so we're looking at all of this data. We've got a huge (audio skip) of data that was submitted, as well as information from the prior report.

Of note, hyaluronic acid is a component of skin itself. And after looking at all of the data, we felt we could go safe as used for this group of ingredients.

DR. BERGFELD: And that's your motion?

DR. BELSITO: That's a motion.

DR. COHEN: Second.

DR. BERGFELD: David, second. Now we come to the discussion and edits. Anything?

DR. BELSITO: Yeah. There were a lot of edits as it would be with a first draft, and we gave them to Priya. So, we'll see what it looks like in the final iteration when we get it back. We did agree with the PCPC comments about the reports of the reactions to intradermal injections for cosmesis, but thought that those case reports should remain in the (audio skip) does not and that was the major issue.

DR. BERGFELD: David?

DR. COHEN: Yeah. Well, and of course, rooster combs are eaten, so it's a food. We didn't have sensitization at max use, although the molecular weight and it's widespread use didn't concern me as much. We went back and forth, Don, on those adverse reports of those crosslinked injectable ones.

I think, a lot of those adverse events are often related to microbiome, infection, foreign body reactions. It's very different (audio skip) that you're going to have from the topical use of it.

Don't we have crosslinked hyaluronic acids in the priorities for next year?

DR. BERGFELD: Yes.

DR. BELSITO: Yeah.

DR. BERGFELD: We do.

DR. COHEN: So we really went back and forth, Don. I can't argue that it's got no value, but I do believe that the casual reader could get very confused as to the method of use, and the products that are being injected, as being similar to what we're reporting on in this assessment and they're really not.

DR. BELSITO: I don't have a problem with that because I thought it should be deleted, too. So, I'll turn it over to Paul, who felt that it should be included, and my other teammates.

DR. BERGFELD: Paul.

DR. SNYDER: Yeah. I mean, we discussed this. I just felt that it adds additional information regarding allergic potential, (inaudible) exposure and things. We include all kinds of safety data in these reports and so it's safety data that's relevant that they're negative. Just like it would be if it was positive.

So I thought just leaving it in doesn't do any harm. I think we always included as much as we can to support our conclusion. The question I had, Don, my notes say that we came up insufficient on this one?

DR. BELSITO: I think we changed it at the last moment.

DR. SNYDER: Method of manufacture, composition and impurity for the sodium acetylated and the calcium potassium hyaluronate. And then we wanted the definition of the cosmetics rate.

DR. BELSITO: Yeah. We discussed that and then I thought Allan said that we could read-across from the others for those endpoints. So, Allan, chime in, because that's what my notes have.

DR. SNYDER: Okay. All right.

DR. RETTIE: Yeah, that was my reading on it.

DR. SYNDER: Okay. All right. I just wanted to make sure that I didn't get something wrong. All right.

DR. RETTIE: We had a fair bit of discussion about endotoxins and the big difference between the rooster comb extracts and the bacterial extracts. Did we come up with any language that we wanted in there about that?

DR. BELSITO: Yeah. We had the biologic source boilerplate going into the discussion. We had the heavy metal boilerplate. But Priya was going to look and see because it seemed weird to me that the bacterial derived ones had less endotoxin and the ones that were derived from organs had endotoxin. I didn't understand how that happened.

MS. CHERIAN: I did look and see, there was no explanation for why. It was just results.

DR. RETTIE: I also thought the penetration enhancement, again, if we come up with language that we wanted to put into summary or discussion about that.

DR. BELSITO: We thought it was vehicle and not penetration enhancement. I thought that was the conclusion of our discussion. David, did you discus that? Just like te carcinogenicity was not carcinogenicity.

DR. COHEN: Yes. But I don't remember where we landed. David, do you?

DR. BERGFELD: David Ross?

DR. ROSS: Oh, the vehicle? I don't think we discussed the vehicle, but there was some differences with vehicle as Don points out. (Audio skip) vehicle more than anything else.

DR. BELSITO: But that information under penetration and enhancement for diclofenac, we thought was a vehicle effect and not actually penetration and enhancement by the hyaluronic acid. We thought it just allowed it to depo in the skin.

DR. SNYDER: Yeah, that's actually confirmed. Priya sent us that reference, that (inaudible) reference and I confirmed that this morning when I looked at it.

DR. BELSITO: Yeah.

DR. ROSS: A quick point. I thought we discussed -- and this is from the previous discussion on Table 12, the injectables, taking out the table but leaving the references. I don't know where --

DR. COHEN: That's exactly right. What we thought was we could expand a little bit in the discussion, leave the references in for Table 12, make a discussion point about it. Indicate that it's injectable and that some of these adverse effects have occurred. These are case reports of cosmetic injections, them having a poor outcome. But it's not clear that they're just from the hyaluronic acid. We see these kind of reactions from other fillers. I think it's a big table that's pretty confusing for the reader in this report.

DR. BELSITO: Yeah. I mean, some of them clearly are since they're clear with hyaluronidase, but I don't have a problem, again, with summarizing it. Whether it have to go before -- it can't just occur in the discussion.

And you could say something in the discussion, the panel noted a number of case reports of adverse reactions to intradermal injection of cross-linked hyaluronic acid for cosmesis, given the fact that these bypassed the stratum corium and epidermis. And given the fact that these are highly crosslinked polymers, and not similar to the materials we're reviewing, we felt that they weren't relevant to whatever.

DR. COHEN: Yeah. I like that. I like that. And by the way, just because hyaluronidase works, doesn't mean it was allergic to that, right? I mean, if you have a defective hip, you could take the hip out.

DR. BELSITO: (Audio skip), you know, it's whatever.

DR. COHEN: I think that's a good compromise, Don.

DR. BERGFELD: Are you going to second the proposed, amended motion?

DR. BELSITO: There's no amended motion.

DR. COHEN: There's no amended.

DR. BERGFELD: Okay.

DR. BELSITO: We're just discussing editorial comments.

DR. BERGFELD: I know. Well, restate your conclusion again.

DR. BELSITO: Safe as used.

DR. BERGFELD: Okay. With a modification of the injectables.

DR. COHEN: The table.

MS. CHERIAN: Is there any specific language you'd like to see in the discussion about not having sensitization data at 7.5 percent?

DR. BELSITO: Yeah, that it's essentially not absorbed. And lack of case reports of reactions with topical application.

DR. COHEN: And molecular weight.

DR. BELSITO: Molecular weight.

DR. COHEN: Yeah. And they're widely utilized. This is widely utilized. We would see something by now, but the lack of reports, the high molecular weight, absorption, those assuage our concerns.

DR. BELSITO: And then all three of your in vitro studies to predict hazard for sensitization, the DPRA, the KeratinoSens and the h-CLAT were all negative.

DR. ROSS: And maybe the small amount added by cosmetics, natural content.

DR. BELSITO: Yeah.

DR. BERGFELD: Any other things to be added to the discussion? Priya, do you think you have what you need?

MS. CHERIAN: I've got what I need. Thank you.

DR. BERGFELD: Thank you. So, in summary then, Don, we have a motion that's been proposed as safe, and it looks like David has seconded it. We've had a long discussion of what is needed in the discussion and in the document itself about the injectables and the reasons for what we're going to do with those.

And it'd be my understanding that some of this information would float into the new priority list ingredient. Okay, anything else to be said about this ingredient? Hearing none, I'm going to move the question. All those opposing? Abstaining? It is now deemed as safe and we're going to move on. Thank you very much. Nice discussion.

Safety Assessment of Hyaluronates as Used in Cosmetics

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ABBREVIATIONS

ADME	absorption, distribution, metabolism, and excretion
ARE	antioxidant response element
BCOP	bovine corneal opacity and permeability
BDDE	1,4-butanediol diglycidyl ether
CAMVA	chorioallantoic membrane vascular assay
CAS	Chemical Abstracts Service
CD44	cluster of differentiation 44
CFR	Code of Federal Regulations
cGMP	current good manufacturing practices
cfu	colony forming units
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Da	dalton
DART	developmental and reproductive toxicity
DMSO	dimethyl sulfoxide
DPRA	direct peptide reactivity assay
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
EU	endotoxin units
FCC	*Food Chemicals Codex*
FDA	Food and Drug Administration
FIGO	International Federation of Gynecology and Obstetrics
FW	formula weight
HA	Hyaluronic Acid
h-CLAT	human cell line activation test
HRIPT	human repeated insult patch test
I-NOSE	Nasal Obstruction Symptom Evaluation Instrument
I_{max}	maximum response value
K_{ow}	n-octanol/water partition coefficient
kDa	kiloDaltons
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
Log K_{ow}	n-octanol/water partition coefficient
MBq	megabecquerels
MDa	megadaltons
MW	molecular weight
MTD	maximum tolerable dose
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
ND	not detected
Nfr2	nuclear factor erythroid 2-related factor 2
NIBUT	non-invasive break-up time
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEL	no-observed-adverse-effect-level
NOEL	no-observed-effect-level
NR	not reported
OECD	Organisation for Economic Cooperation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered saline
PSL	photo-stimulated luminescence
RFI	relative fluorescence intensity
SDS	sodium dodecyl sulfate
SPECT	single photon emission computed tomography
TG	test guidelines
US	United States
UVB	ultraviolet light B (mid-wavelength)
VCRP	Voluntary Cosmetic Registration Program
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook*

ABSTRACT

The Expert Panel for Cosmetic Safety (Panel) assessed the safety of 7 hyaluronate ingredients, of which 3 were previously reviewed, as used in cosmetic formulations. These ingredients are reported to function in cosmetics as skin and/or hair conditioning agents and humectants. The Panel noted that these ingredients may be derived from animal-sources (i.e., rooster combs), and stressed that such ingredients must be free of detectable pathogenic viruses, infectious agents, and/or biologically-derived impurities (e.g., nucleic acids, proteins, endotoxins). Additionally, industry should continue to use good manufacturing practices to limit impurities, such as heavy metals, in cosmetic formulations. The Panel reviewed all relevant data and concluded that these 7 ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This assessment reviews the safety of the following 7 ingredients as used in cosmetic formulations:

Hyaluronic Acid*
Hydrolyzed Calcium Hyaluronate
Hydrolyzed Hyaluronic Acid
Hydrolyzed Sodium Hyaluronate
Potassium Hyaluronate*
Sodium Acetylated Hyaluronate
Sodium Hyaluronate*

* previously reviewed by the Panel

Sodium Acetylated Hyaluronate and Hydrolyzed Hyaluronic Acid were included on the 2022 Priority List due to high reported frequencies of use in the US Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP). Three structurally-similar ingredients (i.e., Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate) have previously been reviewed by the Panel in a safety assessment that was published in 2009.[1] Accordingly, in that these ingredients would soon be considered for re-review, the Panel deemed it appropriate to include the 3 previously-reviewed ingredients in this safety assessment. Additionally, two hydrolyzed salts of Hyaluronic Acid are included in this grouping (Hydrolyzed Calcium Hyaluronate and Hydrolyzed Sodium Hyaluronate). Hence, all ingredients reviewed in this report are structurally similar as they are salts or acetylated esters derived from Hyaluronic Acid.

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), the majority of ingredients included in this assessment are reported to function in cosmetics as skin and/or hair conditioning agents (Table 1).[2] Sodium Acetylated Hyaluronate is reported to function in cosmetics only as a humectant.

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 search was last conducted May 2023. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

In its original 2009 review of Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate, the Panel concluded that these 3 ingredients are safe in the present practices of use and concentration, as described in the safety assessment.[1] Excerpts from the 2009 report are disseminated throughout the report, as appropriate, and are *identified by italicized text.* (This information is not included in the tables or the Summary section.) Accordingly, for these 3 ingredients, an exhaustive search of the world's literature was performed for studies dated 2004 forward (to February 2023), and relevant new data were included.

Information on cross-linked hyaluronic acid dermal fillers is available in the published literature. However, it should be noted that cross-linked hyaluronic acid ingredients are assigned separate INCI names, and these ingredients are not reviewed in this report. Accordingly, data on crosslinked hyaluronic acid ingredients are not included in this safety assessment. In addition, it should be noted that safety and efficacy data regarding Hyaluronic Acid (non-cross-linked and cross-linked) used as dermal fillers, as well in surgical procedures and arthritic therapy were found; however, with the exception of reference to studies regarding hypersensitivity reactions to injectable Hyaluronic Acid (which can be found in the Clinical Studies section), the other studies are not summarized in this report as no relevance to cosmetic use could be surmised, as exposure to Hyaluronic Acid and its derivatives would be topical when used in cosmetics.

CHEMISTRY

Definition and Structure

Hyaluronic Acid (CAS No. 9004-61-9; Figure 1) is a linear glycosaminoglycan composed of repeating disaccharides of β4-glucuronic acid-β3-*N*-acetylglucosamine.[3] The remaining ingredients in this report are derivatives of Hyaluronic Acid (e.g., Sodium Hyaluronate (CAS No. 9067-32-7) is a sodium salt of Hyaluronic Acid). The definitions of the ingredients included in this review are provided in Table 1.

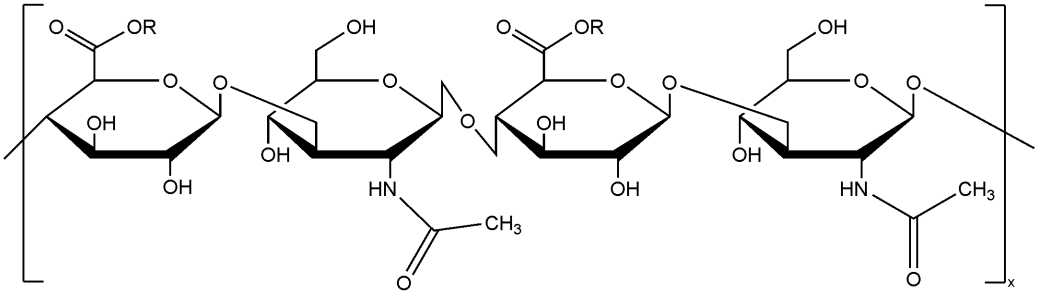

Figure 1. Hyaluronates (when R is hydrogen = Hyaluronic Acid; when R is sodium = Sodium Hyaluronate; etc.)

Chemical Properties

Hyaluronic Acid is a water-soluble substance that is available as a highly purified, freeze-dried powder or aqueous solution.[1] Hyaluronic Acid may also be presented as its potassium or sodium salt (i.e., Potassium Hyaluronate or Sodium Hyaluronate). The molecular weight (MW) of Hyaluronic Acid in cosmetics is highly variable and ranges from 5 – 1800 kiloDaltons (kDa), dependent upon manufacturing procedures. Hyaluronic Acid has a high capacity for water retention; 1 g of Hyaluronic Acid can hold up to 6 l of water.

These hyaluronates have a wide range of MW. For instance, according to the *Food Chemicals Codex* (*FCC*), the formula weight (FW) of Sodium Hyaluronate can vary from 80.2 to 4010 kDa. Other chemical properties of Hyaluronic Acid and Sodium Hyaluronate can be found in Table 2.

Method of Manufacture

Hyaluronic Acid is an ubiquitous substance that can be derived from several natural sources.[1] These sources can be found in the Natural Occurrence section of this report. According to unpublished data, Hyaluronic Acid obtained for cosmetic use is derived via either bacterial fermentation or extraction from rooster combs.[1]

Hyaluronic Acid

In order to manufacture Hyaluronic Acid from rooster combs, the frozen tissue is first thoroughly washed with water, acetone, ethanol, or a mixture of ethanol and chloroform.[4] The tissues are then ground and extracted with a solvent. Examples of solvents include distilled water, salt solutions, and aqueous-organic mixtures. The substance then undergoes purification to remove potential impurities such as proteins, peptides, lipids, nucleic acids, mucopolysaccharides, and low MW precursors. Purification can be performed via extraction using ethanol, acetone, acetic acid, or a double volume of ethanol with sodium acetate. Proteins are typically removed using a water-chloroform or chloroform-iso-amyl alcohol extraction, followed by intensive stirring. In order to remove covalently bonded peptides and proteins, proteolytic enzymes such as pepsin, trypsin, papain, or pronase, may be used. A fractional precipitation with cetylpyridinium chloride followed by dissolution with sodium chloride may be performed to remove mucopolysaccharides from the final product. Polysaccharides can be removed with ion-exchange chromatography, cellulose, and gel-filtration. Other purification methods include ultrafiltration, sorption on the activated carbon, ion-exchange resin, electrodialysis, electrophoresis, and ultracentrifusion with cesium chloride.

Hyaluronic Acid derived from bacterial strains (e.g., *Streptococcus* sp.) involve the cultivation of these bacteria in conditions where the polysaccharide capsule containing Hyaluronic Acid is formed.[4] The cultural liquid containing accumulated Hyaluronic Acid is then ultrafiltrated, precipitated with an organic solvent, and purified using similar methods as described above for rooster comb-derived Hyaluronic Acid.

Hydrolyzed Hyaluronic Acid

Hydrolyzed Hyaluronic Acid (MW = 37 – 56 megaDaltons (MDa)) is manufactured via similar methods as stated below (see manufacturing process of Hydrolyzed Sodium Hyaluronate (FW = 5 - 10 kDa)).[5] However, when manufacturing Hydrolyzed Hyaluronic Acid, the Hyaluronic Acid product is mixed with ethanol and hydrochloric acid. When the Hyaluronic Acid is degraded to the set point, the pH is adjusted via a sodium hydroxide solution. Finally, the resulting solution is dehydrated and dried, yielding the final product.

Hydrolyzed Sodium Hyaluronate

Hydrolyzed Sodium Hyaluronate (formula weight (FW) = 5 - 10 kDa) manufactured for cosmetic use may be produced from the bacterial strain *Streptococcus equi* subsp. *zooepidemicus*.[6] The process begins with the preparation of a seed broth prepared from seed culture, which is transferred from a fermenter containing sterilized fermentation medium. After fermentation, the seed broth is mixed with ethanol. The crude Sodium Hyaluronate precipitate is dissolved in water and filtered to remove impurities and inactivated fragments. The resulting filtrate is precipitated, dehydrated, and dried, yielding

the Hyaluronic Acid product. This product is dissolved in purified water and combined with hyaluronidase to create a solution that is then degraded, heated, filtered, precipitated, dehydrated, and dried.

Low-FW Hydrolyzed Sodium Hyaluronate (FW = 1 - 5 kDa) is produced via the hydrolysis by hyaluronidase from low MW Sodium Hyaluronate.[6] This process includes dissolution, enzymatic hydrolysis, inactivation, filtration, spray drying, sieving, and packaging. In order to produce Hydrolyzed Sodium Hyaluronate of a very low FW (FW < 1 kDa), Sodium Hyaluronate (FW > 1 MDa) undergoes enzymatic hydrolysis via purified water and hyaluronidase.[7] The resulting solution is ultrafiltrated and heated to denature and remove the remaining hyaluronidase. Activated carbon is then used to absorb the denatured hyaluronidase, and the residual hyaluronidase is removed via the removal of activated carbon through multistage filtration. The resulting filtrate is dried, yielding the final product.

Sodium Hyaluronate

According to a supplier, Sodium Hyaluronate, is manufactured via a similar process to that stated above for Hydrolyzed Sodium Hyaluronate (FW = 5 - 10 kDa; omitting enzymatic hydrolysis) using the bacterial strain *Streptococcus equi* subsp. *zooepidemicus*.[8,9] The manufacturing process for low MW Sodium Hyaluronate is the same; however, when manufacturing low MW Sodium Hyaluronate, the seed broth is degraded prior to mixing with ethanol.[10]

Impurities

When derived from animal sources, Hyaluronic Acid may contain several impurities.[1] These impurities include proteins, DNA, and chondroitin sulfate.

Hyaluronic Acid

The impurities (nucleic acid, protein, endotoxins) of Hyaluronic Acid obtained from several sources (e.g., *Streptococcus zooepidemicus*, rooster comb, bovine vitreous, human umbilical cord) were evaluated.[11] Nucleic acid and protein impurities were highest in human umbilical cord- and bovine vitreous-derived Hyaluronic Acid, and were lowest in bacterial- and rooster comb-derived Hyaluronic Acid. Human umbilical cord-, bovine vitreous-, and rooster comb-derived Hyaluronic Acid preparations contained high levels of endotoxin contaminants. Bacterially-derived Hyaluronic Acid was nearly endotoxin-free. The specific levels of impurities evaluated in these samples can be found in Table 3.

Hydrolyzed Hyaluronic Acid

A supplier reported that Hydrolyzed Hyaluronic Acid (MW = 37 - 56 kDa) contained < 0.5 endotoxin units (EU)/mg, < 0.05% protein, < 0.5% chlorides, < 20 ppm total metals, and < 2 ppm arsenic.[12]

Hydrolyzed Sodium Hyaluronate

The same supplier as referenced above reported that several Hydrolyzed Sodium Hyaluronate ingredients of different FWs (<1 kDa, 1 - 5 kDa, and 5 - 10 kDa) contained < 0.5 EU/mg, < 0.05% protein, < 0.5% chlorides, and ≤ 10 - 20 ppm total metals.[13]

Sodium Hyaluronate

According to a manufacturer, Sodium Hyaluronate contained < 5000 ppm residual solvents (ethanol), < 20 ppm heavy metals, < 2 ppm arsenic, and < 0.1% protein.[14] A different manufacturer reported that both Sodium Hyaluronate (FW ≥ 1 MDa) and low FW Sodium Hyaluronate (FW = 100 kDa -1 MDa) contained < 0.5 EU/mg, < 0.05% protein, < 0.5% chlorides, and ≤ 20 ppm total metals.

The *FCC* states that Sodium Hyaluronate manufactured for use in foods may not contain more than 1 mg/kg lead, 2 mg/kg arsenic, or 0.5% chloride.[15] A manufacturer of food-use Sodium Hyaluronate states that potential contaminants of Sodium Hyaluronate include microbes and heavy metals.[16] This manufacturer requires a purity level of ≥ 93% Sodium Hyaluronate, and maximum lead and arsenic levels of 1 and 2 ppm, respectively. The same manufacturer also requires bacteria counts of ≤ 500 colony forming units (cfu)/g, yeast and mold counts of ≤ 100 cfu/g, and negative test readings for *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* sp.

Natural Occurrence

Hyaluronic Acid and its derivatives can be found distributed throughout vertebrate tissues such as the brain, vitreous humor, umbilical cord, synovial fluid, skin, rooster combs, neural tissues, and epithelium.[3,17] Hyaluronic Acid is also a signaling molecule involved in biological processes such as embryonic development, wound healing, inflammation, and cancer. In addition, Hyaluronic Acid can be found in the extracellular capsule formed by gram-positive microorganisms such as *Streptococcus* sp. and *Pasteurella* sp..[4]

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US FDA and the cosmetics industry on the expected use of these ingredients in cosmetics, and does not cover their use in

airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's VCRP database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 FDA VCRP data, Sodium Hyaluronate has the highest frequency of use (4713 total formulations; 4317 leave-on formulations, 394 rinse-off formulations, and 2 formulations diluted for bath use; Table 4).[18] This use of this ingredient has increased significantly since it was last reviewed; it was reported to be used in 601 formulations in 2005.[1] Hyaluronic Acid is reported to be used in 663 formulations, Hydrolyzed Hyaluronic Acid is reported to be used in 476 formulations, and Sodium Acetylated Hyaluronate in 455 formulations. All other ingredients are reported to be used in 204 formulations or less. The results of the 2021 concentration of use survey conducted by the Council indicate Sodium Hyaluronate also has the highest concentration of use; it is used at up to 7.5% in face and neck products (not spray).[19] In 2005, Sodium Hyaluronate was reported to be used at up to 2%. Current FDA VCRP data on the 4 hyaluronate ingredients included in this report that have not been previously reviewed (i.e., Hydrolyzed Calcium Hyaluronate, Hydrolyzed Hyaluronic Acid, Hydrolyzed Sodium Hyaluronate, and Sodium Acetylated Hyaluronate) can be found in Table 5.

Incidental ingestion of several of these ingredients may occur as they are reported to be used in lipstick formulations (e.g., Sodium Hyaluronate is used in lipsticks at up to 0.39%). In addition, these ingredients are also reported to be used in products that are applied near the eye; for example, Sodium Hyaluronate is used in eye shadows at up to 0.96%. Sodium Hyaluronate is also used in baby products at up to 0.005%.

Some of these hyaluronate ingredients are used in cosmetic sprays and powders, and could possibly be inhaled; for example, Sodium Hyaluronate is reported to be used at up to 0.01% in other skin care preparations (spray) and at up to 0.099% in face powders. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

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

All of the hyaluronate 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

Hyaluronic Acid and Sodium Hyaluronate are reported to be used in FDA-approved medical devices as surgical fluids, topical wound creams, osteoarthritis treatments, periodontitis treatments, and ophthalmic surgery aids; crosslinked Hyaluronic Acid is FDA-approved as a dermal filler.[21] In addition, Sodium Hyaluronate is used as an inactive ingredient in several FDA-approved medications.[22] These medications include injectable intra-articular, intramuscular, and intravitreal treatments containing Sodium Hyaluronate at up to 2.3% for various conditions such as arthritis and hypotony. Sodium Hyaluronate is also used in FDA-approved topical medications at up to 0.01% as a skin lubricant. In addition to human medicine, Sodium Hyaluronate has FDA-approved uses in veterinary medicine as an implantable or injectable treatment for joint ailments, as indicated in 21CFR 522.1145.[23]

Hyaluronic Acid is found as a natural component in foods as it is present in animal products.[16] Rooster combs, which are rich in Hyaluronic Acid, are eaten alone or in dishes such as chicken soups or stews in European countries. Sodium Hyaluronate is used as an ingredient in food (e.g., ready-to-eat cereal preparations and candies) and beverages including fruit drinks, soft drinks, milk, and milk products.[16] In addition, both Hyaluronic Acid and Sodium Hyaluronate are reported to be ingredients of dietary supplements on the market in the US.[24]

TOXICOKINETIC STUDIES

Dermal Penetration

Autoradiography was used to detect the dermal penetration of Hyaluronic Acid (in the form of [^{3}H]hyaluronan) in SKh/1 hairless mice (4 animals/group; sex not stated; one group treated with radioactive gel; one group treated without

radioactive gel).[1] Mice were treated for either 3 or 12 total applications (12 h intervals). Twelve to 16 h after the last application, animals were examined. Radioactivity was found mainly in the dermis, from the outermost layer to the lymphatic and blood vessels. In a second experiment using 10 mice (strain and sex not reported; performed according to similar procedures), radioactivity was found in the same distribution within the dermis. In both assays, grains were found in the keratinized layer of the skin and hair follicles. In a dermal penetration assay performed in 11 Sprague-Dawley rats, Hyaluronic Acid (1.35 – 4.5 kDa) was applied dermally, twice daily, for 5 d. The Hyaluronic Acid penetrated to a maximum depth of 136 µm beneath the epidermis. In a different assay, radiolabeled Hyaluronic Acid was placed on the back of one Sprague-Dawley rat (sex not stated; singular dose). After a 4-h absorption period, the test substance was found to penetrate the rat skin to a maximum depth of approximately 800 µm. Autoradiography was used to detect the dermal penetration of [^{3}H]Hyaluronic Acid gel (56.3 – 56.4 mg) in the forearm of one male subject (2 total applications 12 h apart; skin removed by biopsy 7 h after last treatment). The test substance was shown to disseminate through all layers of the skin.

Hyaluronic Acid

The dermal penetration of three Hyaluronic Acid solutions, with three different MW (20 - 50 kDa, 100 - 300 kDa, and 1000 - 1400 kDa), was evaluated via Raman microimaging.[25] Test solutions contained 1% Hyaluronic Acid in distilled water. The solution (300 µl) was placed on human dermatomed skin samples for 8 h. Control skin samples were treated with either water (negative control) or glycerin (positive control). After the diffusion period, the skin surface was cleaned, samples were frozen, 10 µm-thick transverse skin sections were obtained, and spectral images were recorded. Spectral images revealed that the Hyaluronic Acid solution with the lowest MW (20 – 50 kDa) was present in the skin section at around 100 µm (full epidermal depth). The Hyaluronic Acid solution with a MW range of 100 - 300 kDa was present at an epidermal depth of approximately 50 µm. Permeation did not exceed 25 µm for the 1 – 14 MDa Hyaluronic Acid solution. The majority of each of the Hyaluronic Acid solutions, regardless of MW, was found in the stratum corneum, around 25 µm from the skin surface.

Effect on Penetration of Other Chemicals

According to a review article evaluating Hyaluronic Acid's influence as a drug delivery system for diclofenac, it was observed that dermal penetration was dependent on animal species.[1,26] The drug reservoir was formed in the deeper layers of the skin (dermis) in mice, while the drug reservoir was formed in more shallow layers of the skin (epidermis) in humans. Hyaluronic Acid can moderate the penetration of other chemicals such as diclofenac, causing a slower absorption of the drug, and preferential accumulation in the epidermis.

Absorption, Distribution, Metabolism, and Excretion (ADME)

Details on the oral absorption, distribution, and excretion studies summarized below can be found in Table 6.

In an absorption assay in which male Sprague-Dawley rats (n = 3) were dosed with 25 mg/kg [^{14}C]Hyaluronic Acid (MW = 920 kDa) via gavage, the peak plasma radioactivity level was 7.6 µg eq/ml 8 h post-administration.[27] The highest amount of radioactivity was observed in intestinal contents 8 h post-administration. When evaluating excretion, the total excretion of radioactivity in the urine, feces, and expired air was 91.3% by 168 h post-administration. In a different assay, male Sprague-Dawley rats (6/group) were orally administered Hyaluronic Acid (MW = 300 kDa; 200 mg/kg bw), and blood, cecal content, and ventral skin were evaluated at different time intervals.[28] The recovery rate of unsaturated hyaluronic disaccharides and unsaturated hyaluronic tetrasaccharides in the serum and skin was approximately 25 and 70%, respectively. Male Sprague-Dawley rats (8/group) were given Hyaluronic Acid (MW = 300,000 Da) in distilled water (5 ml/kg bw/d; 1 and 5% concentrations; 5 d administration) via gavage, and excretion parameters were evaluated. Hyaluronic Acid was below the detection limit in the feces for all treated groups.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

No deaths were observed in an acute oral toxicity assay in which ICR mice were given > 1200 mg/kg Hyaluronic Acid.[1] No other details were provided.

Details on the acute oral toxicity studies summarized below can be found in Table 7.

Several acute oral toxicity assays were performed using Sodium Hyaluronate in mice (at up to 15,000 mg/kg bw) and rats (at up to 5280 mg/kg bw).[16] No signs of toxicity or deaths were reported in any of these assays.

Short-Term and Subchronic Toxicity Studies

No toxicity was observed in in a short-term inhalation toxicity assay performed in male Beagle dogs exposed to 10% Hyaluronic Acid formulations containing insulin. No other details were provided for this study.

Details on the short-term and subchronic oral toxicity assays summarized below can be found in Table 8.

No signs of toxicity were observed in short-term and subchronic oral toxicity assays of Sodium Hyaluronate.[16] These assays include a 30-d study in which Wistar rats (10/sex/group) were given up to 1500 mg/kg bw Sodium Hyaluronate via feed, a 90-d assay in which Sprague-Dawley rats (5 - 10/sex/group) were given up to 48 mg/kg bw/d of Sodium Hyaluronate

via gavage, a 90-d assay in which Wistar rats (10/sex/group) were given up to 1000 mg/kg bw/d Sodium Hyaluronate via feed, and a 90-d study using Wistar rats (12/sex/group) given up to 1333 mg/kg bw/d Sodium Hyaluronate in corn oil via gavage.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Several reproductive and developmental studies on subcutaneously injected Hyaluronic Acid (up to 60 mg/kg/d) and Sodium Hyaluronate (up to 50 mg/kg/d) were performed in rats and rabbits (majority of dosing occurred on days 7 to 17 of pregnancy or day 17 of pregnancy to day 20-21 after delivery in rats, and on days 6-18 of gestation in rabbits).[1] In most assays, treatment with the test substance had no effect on mortality, necropsy observations post-delivery, food or water consumption, or fertility in dams. However, in one assay in which rats were given up to 60 mg/kg bw of a 1% Hyaluronic Acid solution in physiological saline via subcutaneous injection, nodular hyperplasia of reticular zone cells was present in the adrenal glands of treated dams (treatment on day 17 of pregnancy to day 20 after parturition). No fetal abnormalities were observed in rats or rabbits.

Sodium Hyaluronate

A sperm malformation assay was performed in adult male mice (strain not stated; 10/group).[29] Sodium Hyaluronate (20 ml/kg bw), cyclophosphamide (40 mg/kg bw (positive control), and distilled water (negative control) was given to animals via gavage, once a day, for 5 d. Mice were killed 30 d after the last administration. No other details were provided. The test substance had no influence on sperm malformation rate.

A teratogenicity assay was performed in Wistar rats (15/group) given Sodium Hyaluronate (FW = 270 kDa) via gavage in doses of 0, 170, 330, or 670 mg/kg bw/d (administration during gestation days 7 - 16).[16] Dams were euthanized and evaluated on day 20 of gestation. No statistically significant differences ($P > 0.05$) were observed in the maternal, uterine, and ovary weights or in the number of corpus lutea and nidation between test and control groups. In addition, no statistically significant differences ($P > 0.05$) were observed between the control and treated groups regarding the weight, length, and number of living embryos. No evidence of maternal or embryo toxicity resulting from the test substance administration was observed.

In a different teratogenicity assay, Sprague Dawley rats (12/group) were given Sodium Hyaluronate, via gavage, in doses of 333, 667, or 1333 mg/kg bw/d, on gestation days 7 - 16.[16] A negative control group was given water and a positive control group was given aspirin on the same gestation days. Animals were euthanized and evaluated on gestation day 20. No statistical differences in maternal body weight, number of corpus lutea, implantations, uterine weight, placental weight, live fetus rate, fetal death rate, or absorbed fetus rate were observed between test and negative control treated groups. Fetal development and growth were similar between negative control and treated groups. No evidence of maternal toxicity or teratogenicity resulting from test substance administration was observed.

GENOTOXICITY STUDIES

No genotoxicity was observed in an Ames assays evaluating Sodium Hyaluronate (up to 1%; up to 5000 µg/plate) in Staphylococcus aureus, Salmonella typhimurium and Escherichia coli or in an in vitro chromosomal aberration assay evaluating the genotoxic potential of Sodium Hyaluronate (up to 1000 µg/ml) in Chinese hamster lung fibroblasts. Negative results were also observed in an in vivo micronucleus assay using 1% Sodium Hyaluronate (up to 400 mg/kg) in CD-1 (ICR) mice and in a micronucleus assay evaluating ICR (Crj: CD-1) mice treated with 360 mg/kg Sodium Hyaluronate for up to 4 d.

Details on the genotoxicity assays summarized below can be found in Table 9.

Hydrolyzed Sodium Hyaluronate (FW = 5-10 kDa), Hydrolyzed Sodium Hyaluronate (FW < 1 kDa) and Sodium Hyaluronate were determined to be non-genotoxic in several Ames assays when tested at up to 5 mg/plate, with and without metabolic activation (assays performed in *S. typhimurium* strains TA97a, TA98, TA100, TA102, TA1535, TA537 and *E. coli* WP2 *uvrA*).[16,29-31] In addition, Sodium Hyaluronate (up to 5000 mg/kg bw) was non-mutagenic in mouse micronucleus assays.[29]

CARCINOGENICITY STUDIES

No carcinogenicity data were found in the literature, and no unpublished data were submitted.

OTHER RELEVANT STUDIES

Immunogenicity

Multiple injections of Hyaluronic Acid derived from human umbilical cords or streptococcal fermentation did not result in sensitization in immunogenicity studies performed in rabbits (administration of test substance via either subcutaneous or intramuscular route). In an antigenicity assay using injected streptococcal-derived Hyaluronic Acid in rabbits, precipitating antibodies were observed. A similar assay was performed using purified Hyaluronic Acid derived from rooster combs and

human umbilical cords in rabbits. No formations of passive cutaneous anaphylaxis reactive antibodies were observed. Antibody response by rooster comb-derived Hyaluronic Acid caused an enhanced secondary antibody response to birch pollen, egg albumin (the protein in egg whites), and dog albumin in rats. Neither commercial Sodium Hyaluronate preparations nor a crude rooster comb Sodium Hyaluronate preparation elicited a Hyaluronic Acid-specific antibody response in rabbits.

Cancer Cell Biology

Mouse melanoma cell lines with high Hyaluronic Acid production had increased lung metastasis and lower survival than melanoma cell lines with lower Hyaluronic Acid production.[1] Aneuploid human breast adenocarcinoma cells modified with antisense inhibition of hyaluronan synthase 2 expression produced more high MW Hyaluronic Acid. Injection of these cells into mice did not result in primary tumors. In other studies, well-differentiated tumors (e.g., salivary gland, stomach, colon) had intense Hyaluronic Acid-staining in the tumor cells, intratumoral and associated surrounding stroma. Poorly differentiated tumor samples (e.g., astrocytomas, infiltrating breast, stomach, gallbladder) with carcinoma or sarcoma had almost no Hyaluronic Acid when stained. Enhanced motility of human pancreatic carcinoma cells was dependent on the cluster of differentiation 44 (CD44)-hyaluronic acid interaction where low MW Hyaluronic Acid induced angiogenesis, enhanced CD44 cleavage, and promoted the migration of the tumor cells in a CD44-dependent manner. In a different study, stromal Hyaluronic Acid was not related to survival or recurrence-free survival from cutaneous melanoma. Compared with normal epidermis, in situ carcinomas and well-differentiated squamous cell carcinomas showed an enhanced Hyaluronic Acid signal on carcinoma cells, while CD44 expression resembled normal skin. Less-differentiated squamous cell carcinoma samples had reduced and irregular expression of Hyaluronic Acid and CD44 on carcinoma cells. In basal cell carcinoma samples, Hyaluronic Acid was frequently present on cell nuclei but not in the other types of samples. Hyaluronidase applied to tumors or tumor cells injected into the footpads of mice reduced growth rates in human breast carcinoma. Hyaluronic Acid levels have been found to be increased in tissues surrounding some breast cancer, gastric cancer, poorly differentiated, serous histological type, advanced stage, and large primary tumor epithelial ovarian cancer, endometrial cancer, ganglioma, thyroid cancer, and salivary gland cancer. Normal and low levels of stromal Hyaluronic Acid were associated with early International Federation of Gynecology and Obstetrics (FIGO) stage, mucinous histological-type epithelial ovarian cancer, and murine astrocytoma. Increased Hyaluronic Acid intensity in breast cancer patients was related to axillary lymph node positivity and poor survival.

Ocular Toxicity

Owl monkey and rhesus monkeys had no ill effects from repeated injection of Hyaluronic Acid into the eyes.[1] Repeated injections of Sodium Hyaluronate into the eyes of owl monkeys increased the leukocyte count up to 2000 cells/mm^3 after 48 h. The severity of haze and flare in the eyes after the injections did not increase over time, and there was no immunogenic response. This experiment was continued for up to 9 yr in 2 eyes with no adverse effects.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Hyaluronic Acid was non-irritating in a single-stimulus skin test using Japanese rabbits and Hartley guinea pigs.[1] In addition, no irritation was observed in a human closed skin patch test using Hyaluronic Acid produced via fermentation. No details were provided for either study. A skin prick test was performed in 9 subjects. The forearm of each subject was pricked with Sodium Hyaluronate (10 mg/ml), and evaluated 15 min, and 2, 6, and 24 h after pricking. No skin reactions were observed.

Details on the dermal irritation and sensitization data summarized below can be found in Table 10.

In vitro dermal irritation assays (EpiDerm™ and reconstructed human epidermis assays) performed on two trade name mixtures containing 1% Hyaluronic Acid (tested neat), a trade name mixture containing 3% Hyaluronic Acid (tested neat), Hydrolyzed Sodium Hyaluronate (FW < 1 kDa; tested at 1%), a trade name mixture containing 0.5% Sodium Hyaluronate (tested neat), and Sodium Hyaluronate (concentration not reported) yielded negative results.[29,31-35] No irritation was observed in human dermal irritation assays performed under occlusive conditions using Hydrolyzed Sodium Hyaluronate (several FW tested; 30 - 32 subjects; tested at up to 2%).[31] No responses predicting sensitization were noted in a direct peptide reactivity assay (DPRA) performed on a trade name mixture containing 1% Hyaluronic Acid (tested at up to 25 mM), in a KeratinoSens™ assay performed on a trade name mixture containing 1% Hyaluronic Acid (up to 2 mM), and in a human cell line activation test (h-CLAT) performed on Sodium Hyaluronate (tested at 1 mg/ml).[29,36,37] Similarly, no sensitization was observed in human repeat insult patch tests (HRIPTs) performed using a formula containing 0.2% Hyaluronic Acid (114 subjects; tested neat), a formula containing 0.2% Sodium Acetylated Hyaluronate (104 subjects; tested neat), Hydrolyzed Sodium Hyaluronate (FW = 5 - 10 kDa; 55 subjects; tested at 0.5%); Sodium Hyaluronate (50 - 100 subjects; tested at 0.2%), and a formula containing 1.5% Sodium Hyaluronate (198 subjects; tested neat).[29-31,38-40]

Phototoxicity

In Vitro

Summary data were provided from a supplier on 3T3 neutral red uptake phototoxicity assays performed on Hydrolyzed Sodium Hyaluronate (FW < 1 kDa; up to 128 mg/ml) and Sodium Hyaluronate (125 µg/ml).[29,31] Neither test substance was predicted to induce phototoxicity.

OCULAR IRRITATION STUDIES

Details on the ocular irritation summarized below can be found in Table 11.

No ocular irritation was observed in EpiOcular™ assays performed on 2 trade name mixtures containing 1% Hyaluronic Acid (tested neat), a trade name mixture containing 3% Hyaluronic Acid (tested neat), and a trade name mixture containing 0.5% Sodium Hyaluronate (tested neat).[32-35] Test substances were considered to be non-irritating/slightly irritating in chorioallantoic membrane vascular assays (CAMVA) performed using Hydrolyzed Sodium Hyaluronate (FW < 1 kDa; concentration tested not reported) and Sodium Hyaluronate (tested neat).[29,31] Similar results were observed in a bovine corneal opacity and permeability (BCOP) test performed using Sodium Hyaluronate (tested neat). In addition, a *Bacillus*-derived and *Streptococcus*-derived Hyaluronic Acid (up to 0.3%) was considered to be very well-tolerated when tested in the eyes of New Zealand white rabbits (3/group).[41]

CLINICAL STUDIES

Nebulized Nasal Hypertonic Solution in the Treatment of Chronic Rhinosinusitis

Hyaluronic Acid

Eighty patients with chronic rhinosinusitis were instructed to use a nasal spray containing high MW Hyaluronic Acid and sodium chloride, twice daily (2 puffs per nostril at each administration), for 20 d.[42] Patients were assessed at baseline, on day 10, and day 20. An endoscopic nasal examination and nasal obstruction symptom evaluation instrument (I-NOSE) questionnaire were performed during each visit, and safety parameters (adverse effects and local tolerability (burning, irritation, congestion) were assessed. Patients were instructed to keep diaries to log changes in symptoms. The improvement in chronic rhinosinusitis compared to baseline as measured by the I-NOSE score, was statistically significantly ($P < 0.001$). According to patient diaries, nasal blockage, congestion, drainage, and rhinorrhea was significantly improved between baseline and day 20 ($P < 0.01$). Fourteen patients experienced at least one adverse effect; however, these effects were not related to study treatment. No symptoms related to local tolerability at the site of administration were reported.

Immediate and Delayed Hypersensitivity to Intracutaneous Hyaluronic Acid

Hyaluronic Acid

Twelve patients with previously reported inflammatory responses to Hyaluronic Acid fillers were subjected to intracutaneous tests.[43] Approximately 0.1 ml of each filler was tested on the inner sides of the upper arms in a randomized manner. Tests were read after 15 min, and 2, 3, 4, and 7 d following application. Potential late reactions were monitored after 2 and 4 wk, and patients were instructed to contact study conductors in case at any later reaction. No positive reactions were observed for any of the tested Hyaluronic Acid fillers during the testing period, or during the 4- mo follow-up.

Treatment of Dry Eye

Sodium Hyaluronate

The effectiveness of Sodium Hyaluronate eye drops was evaluated in 13 patients with moderate dry eye.[44] Patients were treated with instillations of 40 µl of 0.1% Sodium Hyaluronate, 0.3% Sodium Hyaluronate, or 0.9% saline, in a randomized, double-masked manner (vehicles for treatment not stated). Symptom intensity and non-invasive break-up time (NIBUT) were evaluated at 5, 15, 30, 45, 60 min, and hourly, until 6 h after drop instillation. This process was repeated twice following an interval of approximately 7 d, but with a different treatment, so that at the end of the final visit, each subject had trialed all products. Symptoms and NIBUT improved with all treatments; however, improvement (reduction in eye irritation) was of a greater magnitude and longer duration with Sodium Hyaluronate drops. Eye drops containing 0.3% Sodium Hyaluronate performed better than 0.1% Sodium Hyaluronate ($P = 0.04$).

Treatment of Rosacea

Sodium Hyaluronate

The effect of a cream containing 0.2% Sodium Hyaluronate (containing low MW Hyaluronic Acid) was evaluated in 14 patients with mild to moderate facial rosacea.[45] Patients were instructed to apply the cream, following cleansing, on the whole face, twice daily, for 4 wk. After 4 wk, patients continued the cleansing regimen for an additional 4 wk, but discontinued the use of the cream containing Sodium Hyaluronate. Patients were evaluated for papules, pustules, erythema, edema, telangiectasia, burning, stinging, and/or dryness at baseline and at 2-wk intervals following administration. No patients experienced adverse effects throughout the study. The largest reduction in erythema was observed at the 2-wk visit

(48.3% reduction). At the 4-wk visit, it was reported that treatment with 0.2% Sodium Hyaluronate cream resulted in a reduction of papules, erythema, burning/stinging, and dryness in all patients.

Use in Dissolving Microarray Patches

Hyaluronic Acid

Dissolving microarray patches containing 30% Hyaluronic Acid (in distilled water) were placed under and at the corner of the eyes of 30 female subjects aged 35 - 60.[46] Patches were applied 3x/wk for 4 wk. Safety was assessed by the degree of adverse effects, including facial itching, prickling, burning, erythema, edema, and swelling. These parameters were evaluated by participant questionnaires. No adverse effects on the skin or eyes were reported throughout the study.

Case Reports

Numerous case reports were found in the literature regarding hypersensitivity/adverse reactions to Hyaluronic Acid used as an injectable dermal filler. A summary of these studies has been provided and can be found in Table 12. In addition, it should be noted that case reports were also found on the adverse effects of Hyaluronic Acid following other methods of administration (e.g., intra-articular injections for osteoarthritis, injection during surgical procedures). These studies were not summarized in the table, as their relevance to the cosmetic use of Hyaluronic Acid is not likely; however, two case report regarding photodermatitis following the intra-articular administration of Hyaluronic Acid in the knee has been summarized below, as it may have relevance in evaluating the photosensitivity-inducing potential of Hyaluronic Acid. In addition, a case report regarding an anaphylactic response in an elderly patient following oral exposure to Hyaluronic Acid has also been included.

A 71-yr-old man with a history of osteoarthritis reported previous treatment with three-series 2 ml Hyaluronic Acid injections in the knee for 5 yr with no adverse reactions.[47] The patient switched to a single 6 ml Hyaluronic Acid injection and immediately developed pain and swelling at injection site. These effects were also seen 5 mo later following a second injection of 6 ml Hyaluronic Acid. Six mo after the second injection, the patient received another 6 ml Hyaluronic Acid injection in the knee and developed a similar localized inflammatory reaction with chills. Eight days later, the patient developed erythematous, pruritic, scaly papules and plaques near the injection site. Several weeks later, he presented with photo-distributed scale and scattered excoriations on the bilateral cheeks and all four extremities. A similar reaction was observed in a 65-yr-old woman who also switched from three-series 2 ml Hyaluronic Acid injections in the knee, with no adverse effects for 13 yr, to a singular 6 ml Hyaluronic Acid injection. Following the singular injection, the patient displayed a localized inflammatory reaction at the injection site, followed by the development of photo-distributed erythematous macules and papules with pustules and scale on the face and all four extremities. Both patients recovered following treatment with triamcinolone and prednisone.

Upper airway angioedema was observed in a 100-yr-old woman following application of a spray containing xylitol and Hyaluronic Acid (0.01%) to the inner lower lip and gums to treat gingival sores for the third time in 2 d.[48] The previous two applications were much smaller in quantity. Following admission to the emergency department, the patient became dyspneic and hypoxemic, with edema of the lip, lower face, and epiglottis. The patient recovered following treatment with oxygen, epinephrine, methylprednisolone, diphenhydramine, ranitidine, and icatibant.

SUMMARY

The safety of Hyaluronic Acid and 6 hyaluronate ingredients are reviewed in this safety assessment. The majority of these ingredients are reported to function in cosmetics as skin and/or hair conditioning agents. Sodium Acetylated Hyaluronate is reported to function in cosmetics as a humectant. In cosmetics, these hyaluronates are derived from either bacterial fermentation or rooster combs. Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate have previously been reviewed by the Panel and were considered safe in the present practices of use and concentration as described in the safety assessment published in 2009.

According to 2023 VCRP survey data, Sodium Hyaluronate is reported to be used in 4713 formulations (4317 leave-on formulations, 394 rinse-off formulations, and 2 formulations diluted for bath use), and Hyaluronic Acid is reported to be used in 663 formulations (571 leave-on formulations, 89 rinse-off formulations, and 3 formulations diluted for bath use). Additionally, Hydrolyzed Hyaluronic Acid is reported to be used in 476 formulations and Sodium Acetylated Hyaluronate in 455 formulations. All other ingredients are reported to be used in 204 formulations or less. The results of the 2021 concentration of use survey conducted by the Council indicate Sodium Hyaluronate also has the highest concentration of use in a leave-on formulation; it is used at up to 7.5% in face and neck products (not spray).

A dermal penetration assay was performed in human dermatomed skin samples using Hyaluronic Acid solutions of three different MW (20 - 50 kDa, 100 - 300 kDa, and 1 – 1.4 MDa). Hyaluronic Acid solutions, from lowest to highest MW, were present at epidermal depths of 100 µm, 50 µm, and 25 µm, respectively. Regardless of the MW of the Hyaluronic Acid solution, the majority quantity of Hyaluronic Acid was found in the stratum corneum, approximately 25 µm from the skin surface.

In an absorption assay in which male Sprague-Dawley rats (n = 3) were dosed with 25 mg/kg [^{14}C]Hyaluronic Acid (MW = 920 kDa) via gavage, the peak plasma radioactivity level was 7.6 µg eq/ml 8 h post-administration. When evaluating excretion, the total excreted radioactivity in the urine, feces, and expired air was 91.3% by 168 h post-administration. In a different assay, male Sprague-Dawley rats were orally administered Hyaluronic Acid (MW = 300 kDa; 200 mg/kg bw), and blood, cecal content, and ventral skin were evaluated at different time intervals. The recovery rate of unsaturated hyaluronic disaccharides and unsaturated hyaluronic tetrasaccharides in the serum and skin was approximately 25 and 70%, respectively. Male Sprague-Dawley rats were given Hyaluronic Acid (MW = 300 kDa) in distilled water (5 ml/kg bw/d; 1 and 5% concentrations; 5 d administration) via gavage, and excretion parameters were evaluated. Hyaluronic Acid was below the detection limit in the feces for all treated groups.

No signs of toxicity were observed in several acute oral toxicity assays performed using Sodium Hyaluronate in mice (at up to 15,000 mg/kg bw) and rats (at up to 5280 mg/kg bw). Similarly, no signs of toxicity were observed in 30- and 90-d oral toxicity assays performed in rats given Sodium Hyaluronate (up to 1333 mg/kg bw/d).

Sodium Hyaluronate (20 ml/kg) did not have an influence on sperm malformation in adult male mice. No maternal toxicity or teratogenicity resulting from Sodium Hyaluronate (up to 1333 mg/kg bw/d) were observed in two developmental toxicity assays using rats. In both assays, animals were treated via gavage, on gestation days 7 - 16. All measured parameters (e.g., ovary weights, number of living embryos, implantations) were similar among control and treated groups.

Hydrolyzed Sodium Hyaluronate and Sodium Hyaluronate were determined to be non-genotoxic in several Ames assays performed using strains of *S. typhimurium*, at concentrations up to 5 mg/plate, with and without metabolic activation. Similarly, no mutagenicity was observed in micronucleus assays using mice given up to 5000 mg/kg bw Sodium Hyaluronate.

In vitro dermal irritation assays performed on two trade name mixtures containing 1% Hyaluronic Acid (tested neat), a trade name mixture containing 3% Hyaluronic Acid (tested neat), Hydrolyzed Sodium Hyaluronate (FW < 1 kDa; tested at 1%), a trade name mixture containing 0.5% Sodium Hyaluronate (tested neat), and Sodium Hyaluronate (concentration not reported) yielded negative results. No irritation was observed in human dermal irritation assays performed under occlusive conditions using Hydrolyzed Sodium Hyaluronate (tested at up to 2%). No responses predicting sensitization was noted in a DPRA performed on a trade name mixture containing 1% Hyaluronic Acid (tested at up to 25 mM), in a KeratinoSens™ assay performed on a trade name mixture containing 1% Hyaluronic Acid (up to 2 mM), or in an h-CLAT performed on Sodium Hyaluronate (tested at 1 mg/ml). Similarly, no sensitization was observed in HRIPTs performed using a formula containing 0.2% Hyaluronic Acid (tested neat), a formula containing 0.2% Sodium Acetylated Hyaluronate (tested neat), Hydrolyzed Sodium Hyaluronate (FW = 5 - 10 kDa; tested at 0.5%); Sodium Hyaluronate (tested at 0.2%), and a formula containing 1.5% Sodium Hyaluronate (tested neat). No potential for phototoxicity was observed in in vitro phototoxicity assays performed on Hydrolyzed Sodium Hyaluronate (FW < 1 kDa; up to 128 mg/ml) and Sodium Hyaluronate (125 µg/ml).

In vitro ocular irritation assays performed on several test substances (trade name mixtures containing 1% Hyaluronic Acid, trade mixture containing 3% Hyaluronic Acid, Hydrolyzed Sodium Hyaluronate, Sodium Hyaluronate (100%), and a trade name mixture containing 0.5% Sodium Hyaluronate) yielded slightly irritating/non-irritating or non-irritating results. In addition, a *Bacillus*-derived and *Streptococcus*-derived Hyaluronic Acid (up to 3%) was considered to be very well-tolerated when tested in the eyes of rabbits.

Eighty patients with chronic rhinosinusitis were treated with a nasal spray containing high MW Hyaluronic Acid and sodium chloride (2 puffs/nostril/d for 20 d). A statistically significant improvement in rhinosinusitis symptoms was observed at the end of treatment compared to baseline ($P < 0.01$). No study-related adverse effects were observed. The treatment was considered to be well-tolerated.

Twelve patients with previously-reported inflammatory responses to Hyaluronic Acid fillers were subjected to intracutaneous tests using 6 different types of Hyaluronic Acid fillers. No positive reactions were observed for any of the tested Hyaluronic Acid fillers during the testing period, or during the 4-mo follow-up

The effect of Sodium Hyaluronate eye drops was evaluated in 13 patients with dry eye. Patients were treated with instillations of 40 µl of 0.1% Sodium Hyaluronate, 0.3% Sodium Hyaluronate, or 0.9% saline, in a randomized, double-masked manner (vehicles for treatment not stated). Symptoms of dry eye improved with the use of all treatments; however, improvement was greatest with the use of 0.3% Sodium Hyaluronate drops.

The effect of a cream containing 0.2% Sodium Hyaluronate was evaluated in 14 patients with facial rosacea. Use of the cream (2x/d for 4 wk) resulted in a reduction in rosacea symptoms. No adverse effects were reported throughout the study.

The safety of dissolving microarray patches containing Hyaluronic Acid (30% in distilled water) placed under the eyes was evaluated in 30 subjects (patches applied 3x/wk for 4 wk). No adverse dermal or ocular effects were reported throughout the study.

Case reports were found in the literature regarding hypersensitivity/adverse reactions to Hyaluronic Acid used as an injectable dermal filler and injections used as treatments for osteoarthritis. Two case reports stated that treatment of osteoarthritis with three-series 2 ml Hyaluronic Acid injections was performed without adverse effects; however, switching to

a single 6 ml Hyaluronic Acid injection did result in adverse effects. It should be noted that all FDA-approved Hyaluronic Acid dermal fillers are cross-linked, and cross-linked Hyaluronic Acid ingredients have separate INCI names, and are not reviewed in this report. In another case report, upper airway angioedema was observed in a 100-yr-old woman after use of a spray containing xylitol and Hyaluronic Acid (0.01%) to the inner lower lips and gums; the patient recovered following treatment.

DISCUSSION

Sodium Acetylated Hyaluronate and Hydrolyzed Hyaluronic Acid were included on the 2022 Priority List due to high reported frequencies of use in FDA VCRP. In 2009, the Panel published a final report on 3 structurally-similar ingredients (Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate) and concluded that these ingredients were safe as used in cosmetic products. As these ingredients would soon be considered for re-review, the Panel deemed it appropriate to include the 3 previously-reviewed ingredients in this safety assessment. Also included in this ingredient group are Hydrolyzed Calcium Hyaluronate and Hydrolyzed Sodium Hyaluronate. Furthermore, the Panel reviewed the available data, and concluded that all 7 hyaluronate ingredients reviewed in this report are safe in cosmetics in the present practices of use and concentration. The safety of these ingredients was supported by available toxicity data, the presence of Hyaluronic Acid as an endogenous substance in the skin, and the extensive use of these ingredients with few reported adverse effects.

The Panel noted sensitization studies included in this report were not performed at maximum use concentrations. However, the Panel determined that additional studies were not needed to determine the safety of this ingredient group because these ingredients have large molecular weights (and as such, are not expected to absorb into the skin), and because although these ingredients are widely utilized, and there are a lack of case reports following topical application. The Panel did note case reports of hypersensitivity reactions following use of Hyaluronic Acid dermal fillers, but stated these effects would not be relevant to cosmetic safety as dermal fillers are administered via intradermal injection, and therefore bypass the stratum concern. Concern was further mitigated as FDA-approved Hyaluronic Acid fillers contain cross-linked hyaluronates, which chemically differ from the non-cross-linked ingredients reviewed in this report.

The Panel was concerned with the risks inherent in using animal-derived ingredients (i.e., rooster combs), namely the transmission of infectious agents and biologically-derived impurities (e.g., nucleic acids, proteins, endotoxins). The Panel stressed that these ingredients must be free of detectible pathogenic viruses, infectious agents, and/or biologically-derived impurities. Suppliers and users of these ingredients must accept responsibility for assuring that these ingredients are risk-free. Tests to assure the absence of a pathogenic agent in the ingredients or controls to assure derivation from pathogen-free sources are two approaches that should be considered.

In addition, the Panel expressed concern regarding heavy metals that may be present in these ingredients. The Panel stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit these impurities in cosmetic formulations.

The Panel discussed the issue of incidental inhalation exposure resulting from these ingredients (e.g., Sodium Hyaluronate is reported to be used at up to 0.01% in spray other skin care preparations). Inhalation toxicity data were limited; however, the Panel noted that in aerosol products, the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which the ingredients are used in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

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

CONCLUSION

The Expert Panel concluded that the 7 following hyaluronate ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment:

Hyaluronic Acid
Hydrolyzed Calcium Hyaluronate
Hydrolyzed Hyaluronic Acid
Hydrolyzed Sodium Hyaluronate
Potassium Hyaluronate
Sodium Acetylated Hyaluronate
Sodium Hyaluronate

TABLES

Table 1. Definitions and reported functions of the hyaluronate ingredients[2]

Ingredient	Definition	Function
Hyaluronic Acid (9004-61-9)	Hyaluronic Acid is the natural mucopolysaccharide formed by bonding *N*-acetyl-D-glucosamine with glucuronic acid. *See Figure 1, wherein "R" is hydrogen.*	Skin-Conditioning Agents - Miscellaneous; Viscosity Increasing Agents - Aqueous
Hydrolyzed Calcium Hyaluronate	Hydrolyzed Calcium Hyaluronate is the hydrolysate of the calcium salt of Hyaluronic Acid derived by acid, enzyme, or other method of hydrolysis. *See Figure 1, wherein 2 "R" are replaced by 1 calcium cation.*	Skin-Conditioning Agents - Miscellaneous
Hydrolyzed Hyaluronic Acid	Hydrolyzed Hyaluronic Acid is the hydrolysate of Hyaluronic Acid derived by acid, enzyme, or other method of hydrolysis.	Hair Conditioning Agents; Skin-Conditioning Agents - Humectant
Hydrolyzed Sodium Hyaluronate	Hydrolyzed Sodium Hyaluronate is the hydrolysate of Sodium Hyaluronate derived by acid, enzyme, or other method of hydrolysis.	Skin-Conditioning Agents - Miscellaneous
Potassium Hyaluronate (31799-91-4)	Potassium Hyaluronate is the potassium salt of Hyaluronic Acid. *See Figure 1, wherein R is potassium.*	Skin-Conditioning Agents - Miscellaneous
Sodium Acetylated Hyaluronate	Sodium Acetylated Hyaluronate is the acetyl ester of Sodium Hyaluronate. *See Figure 1, wherein "R" is sodium, and one or more hydroxyl groups are acetylated.*	Humectants
Sodium Hyaluronate (9067-32-7)	Sodium Hyaluronate is the sodium salt of Hyaluronic Acid. *See Figure 1, wherein "R" is sodium.*	Skin-Conditioning Agents - Miscellaneous

Table 2. Chemical properties of Hyaluronic Acid and Sodium Hyaluronate

Property	Value	Reference
Hyaluronic Acid		
Physical Form	powder	[1]
MW (kDa)	5 - 1800	[1]
Sodium Hyaluronate		
Physical Form	powder	[1]
Color	white	[1]
Odor	faint odor	[1]
FW (kDa)	80.2 - 4010	[15]

FW = formula weight; MW = molecular weight

Table 3. Molecular weight (MW) and impurities measurements of Hyaluronic Acid derived from different sources (per 1 mg Hyaluronic Acid)[11]

	Human umbilical cord	Bacterially-derived	Bacterially-derived*	Rooster comb	Bovine vitreous
MW (x 10^6 Da)	1.3 ± 0.1	1.6	1.4	1.4	0.4
Endotoxin (EU/mg HA)	> 100	< 0.02	0.022	23	> 100
Total Protein (µg/ml HA)	47.7 ± 3	1.1	ND	1.0	36.2
RNA (µg/mg HA)	6.7 ± 0.1	ND	ND	ND	1.9
DNA (µg/mg HA)	16.8 ± 4.5	ND	ND	ND	1.1

*two different bacterially-derived (*Streptococcus zooepidemicus*) samples were tested; EU = endotoxin units; HA = Hyaluronic Acid; ND = not detected

Table 4. Frequency (2023; 2005) and concentration (2021; 2005) of use according to likely duration and exposure and by product category

	Hyaluronic Acid				Potassium Hyaluronate				Sodium Hyaluronate			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023[18]	2005[1]	2021[19]	2005[1]	2023[18]	2005[1]	2022[49]	2005[1]	2023[18]	2005[1]	2022[49]	2005[1]
Totals*	**663**	**223**	**0.000002 – 0.83**	**0.00005 – 1**	**68**	**11**	**NR**	**NR**	**4713**	**601**	**0.00001 – 7.5**	**0.000001 – 2**
summarized by likely duration and exposure**												
Duration of Use												
Leave-On	*571*	*194*	*0.000002 – 0.3*	*0.00005 – 1*	*60*	*10*	*NR*	*NR*	*4317*	*552*	*0.00001 – 7.5*	*0.000001 – 2*
Rinse-Off	*89*	*29*	*0.002 – 0.83*	*0.001 – 0.3*	*8*	*1*	*NR*	*NR*	*394*	*49*	*0.0001 – 0.12*	*0.000001 – 0.5*
Diluted for (Bath) Use	*3*	*NR*	*0.0089*	*NR*	*NR*	*NR*	*NR*	*NR*	*2*	*NR*	*NR*	*0.001 – 0.5*
Exposure Type*												
Eye Area	47	33	0.001	0.001 – 0.07	NR	NR	NR	NR	278	49	0.0001 – 0.96	0.0001 – 0.7
Incidental Ingestion	1	NR	0.003 – 0.05	0.01	NR	NR	NR	NR	237	96	0.24 – 0.39	0.0002 – 0.5
Incidental Inhalation-Spray	3; 260[a]; 191[b]	57[a]; 21[b]	NR	0.001[a]; 0.001 – 1[b]	30[a]; 22[b]	4[a]; 6[b]	NR	NR	9; 1707[a]; 1454[b]	1; 180[a]; 73[b]	0.01; 2[a]	0.000001 – 1[a]; 0.0001 – 2[b]
Incidental Inhalation-Powder	3; 191[b]; 4[c]	6; 21[b]	0.003 – 0.3[c]	0.00005; 0.001 – 1[b]	22[b]	6[b]	NR	NR	32; 1454[b]; 9[c]	16; 73[b]	0.001 – 0.099; 0.00002 – 7.5[c]	0.0005 – 0.5; 0.0001 – 2[b]; 0.5[c]
Dermal Contact	635	216	0.000002 – 0.83	0.00005 – 1	68	11	NR	NR	4371	482	0.00001 – 7.5	0.000001 – 2
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	6[a]	NR	0.013	0.5[a]
Hair - Non-Coloring	26	4	0.0036	NR	NR	NR	NR	NR	83	12	0.005 – 2	0.001 – 0.5
Hair-Coloring	NR	1	0.002	NR	NR	NR	NR	NR	2	2	NR	0.5
Nail	NR	2	NR	0.001 – 0.01	NR	NR	NR	NR	2	NR	0.025	0.01 – 0.5
Mucous Membrane	27	1	0.003 – 0.05	0.01	3	NR	NR	NR	275	97	0.01 – 0.39	0.0002 – 0.5
Baby Products	5	NR	NR	0.001	NR	NR	NR	NR	14	NR	0.005	0.5
as reported by product category												
Baby Products												
Baby Shampoos	1	NR	NR	NR					NR	NR	NR	0.5
Baby Lotions/Oils/Powders/Creams	4	NR	NR	NR					9	NR	NR	0.5
Other Baby Products	NR	NR	NR	0.001					5	NR	0.005	0.5
Bath Preparations (diluted for use)												
Bath Oils, Tablets, and Salts	1	NR	NR	NR					NR	NR	NR	0.5
Bubble Baths	1	NR	NR	NR					NR	NR	NR	0.001 – 0.5
Bath Capsules									NR	NR	NR	0.5
Other Bath Preparations	1	NR	0.0089	NR					2	NR	NR	0.001 – 0.5
Eye Makeup Preparations												
Eyebrow Pencil	2	NR	NR	NR					1	3	NR	0.5
Eyeliner	1	NR	NR	NR					12	4	NR	0.001 – 0.5
Eye Shadow	1	15	NR	0.02					21	11	0.097 – 0.96	0.0001 – 0.5
Eye Lotion	15	5	0.001	NR					106	6	0.1	0.001 – 0.7
Eye Makeup Remover	1	2	NR	0.001					8	NR	0.12	NR
Mascara									18	9	0.0001 – 0.1	0.0001 – 0.5
Other Eye Makeup Preparations	28	11	NR	0.07					112	16	0.001 – 0.1	0.0001 – 0.5
Fragrance Preparations												
Cologne and Toilet Water									2	NR	NR	NR
Perfumes									1	1	NR	0.5
Powders (dusting/talcum, excl aftershave talc)	NR	1	NR	NR					NR	1	NR	0.5
Sachets									ǂ	NR	ǂ	0.5
Other Fragrance Preparation	2	NR	NR	NR					5	NR	NR	0.0002
Hair Preparations (non-coloring)												
Hair Conditioner	11	2	0.0036	NR					21	5	NR	0.001 – 0.5

Table 4. Frequency (2023; 2005) and concentration (2021; 2005) of use according to likely duration and exposure and by product category

	Hyaluronic Acid				Potassium Hyaluronate				Sodium Hyaluronate			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023[18]	2005[1]	2021[19]	2005[1]	2023[18]	2005[1]	2022[49]	2005[1]	2023[18]	2005[1]	2022[49]	2005[1]
Hair Spray (aerosol fixatives)	1	NR	NR	NR					1	NR	NR	0.5
Hair Straighteners									2	1	NR	0.5
Permanent Waves	NR	1	NR	NR					NR	NR	NR	0.5
Rinses (non-coloring)									2	NR	NR	0.001 – 0.5
Shampoos (non-coloring)	6	NR	0.0036	NR					25	6	0.01	0.001 – 0.5
Tonics, Dressings, and Other Hair Grooming Aids	3	1	NR	NR					8	NR	2	0.02 – 0.5
Wave Sets									NR	NR	NR	0.5
Other Hair Preparations	4	NR	NR	NR					24	NR	0.005	0.5
Hair Coloring Preparations												
Hair Dyes and Colors (all types requiring caution statements and patch tests)									1	NR	NR	0.5
Hair Tints	NR	NR	0.002	NR					NR	NR	NR	0.5
Hair Rinses (coloring)	NR	1	NR	NR					NR	NR	NR	0.5
Hair Shampoos (coloring)									1	NR	NR	NR
Hair Color Sprays (aerosol)									NR	NR	NR	0.5
Hair Lighteners with Color									NR	NR	NR	0.5
Hair Bleaches									NR	NR	NR	0.5
Other Hair Coloring Preparation									NR	2	NR	0.5
Makeup Preparations												
Blushers (all types)	NR	7	NR	0.02					13	20	0.05	0.001 – 0.5
Face Powders	3	5	NR	0.00005					32	15	0.001 – 0.099	0.0005 – 0.5
Foundations	2	24	0.000002 – 0.1	0.002					81	27	0.015 – 0.2	0.001 – 0.5
Leg and Body Paints									NR	1	NR	0.001 – 0.5
Lipstick	1	NR	0.003 – 0.05	0.01					229	96	0.24 – 0.39	0.0002 – 0.5
Makeup Bases	4	22	0.1	NR	3	NR	NR	NR	29	15	NR	0.002 – 0.5
Rouges									1	10	0.001	0.0001 – 0.5
Makeup Fixatives									6	3	NR	0.05 – 0.5
Other Makeup Preparations	4	NR	NR	0.001					76	17	0.025 – 0.1	0.0001 – 0.5
Manicuring Preparations (Nail)												
Basecoats and Undercoats									NR	NR	NR	0.5
Cuticle Softeners	NR	1	NR	0.001					NR	NR	NR	0.01 – 0.5
Nail Creams and Lotions									NR	NR	NR	0.5
Nail Extenders									1	NR	NR	0.5
Nail Polish and Enamel									NR	NR	NR	0.5
Nail Polish and Enamel Removers									NR	NR	NR	0.5
Other Manicuring Preparations	NR	1	NR	0.01					1	NR	0.025	0.5
Oral Hygiene Products												
Dentifrices									3	NR	NR	NR
Mouthwashes and Breath Fresheners									4	NR	NR	NR
Other Oral Hygiene Products									1	NR	NR	NR
Personal Cleanliness Products												
Bath Soaps and Detergents	16	NR	NR	NR	3	NR	NR	NR	17	1	0.01	0.001 – 0.5
Deodorants (underarm)									6	NR	0.013 (not spray)	0.5
Douches	1	NR	NR	NR								
Feminine Deodorants									2	NR	NR	0.001

Table 4. Frequency (2023; 2005) and concentration (2021; 2005) of use according to likely duration and exposure and by product category

	Hyaluronic Acid				Potassium Hyaluronate				Sodium Hyaluronate			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023[18]	2005[1]	2021[19]	2005[1]	2023[18]	2005[1]	2022[49]	2005[1]	2023[18]	2005[1]	2022[49]	2005[1]
Other Personal Cleanliness Products	6	1	NR	NR					17	NR	NR	0.5
Shaving Preparations												
Aftershave Lotion	1	1	NR	NR					10	6	0.1	0.001 – 0.5
Beard Softeners									NR	NR	NR	0.5
Mens Talcum									NR	NR	NR	0.5
Preshave Lotions (all types)									NR	NR	NR	0.5
Shaving Cream	NR	3	NR	0.3					6	NR	NR	0.001 – 0.5
Shaving Soap									NR	NR	NR	0.5
Other Shaving Preparations	3	NR	0.008	NR					5	2	0.01 (razor lube strip)	0.5
Skin Care Preparations												
Cleansing	34	6	NR	0.001	5	NR	NR	NR	173	20	0.0001 – 0.1	0.000001 – 0.5
Depilatories									1	NR	NR	0.5
Face and Neck (exc shave)	175	8	0.003 – 0.3 (not spray)	0.1	15	6	NR	NR	1246	48	0.005 – 7.5 (not spray)	0.005 – 1
Body and Hand (exc shave)	16	12	0.05 (not spray)	0.001 – 1	7	NR	NR	NR	206	25	0.00002 – 0.86 (not spray)	0.0001 – 2
Foot Powders and Sprays	NR	1	NR	NR					NR	NR	NR	1
Moisturizing	212	37	0.08 – 0.2 (not spray)	0.001 – 0.1	27	4	NR	NR	1482	151	0.001 – 0.4 (not spray)	0.000001 - 1
Night	19	17	0.15 (not spray)	0.02	2	NR	NR	NR	131	11	0.00001 – 0.3 (not spray)	0.0001 - 1
Paste Masks (mud packs)	11	13	0.83	0.001	NR	1	NR	NR	107	12	0.024	0.005 – 0.5
Skin Fresheners	20	NR	NR						67	10	0.01	0.05 – 0.5
Other Skin Care Preparations	47	23	NR	0.001	5	NR	NR	NR	358	38	0.02 – 0.1 0.01 (spray)	0.001 - 1
Suntan Preparations												
Suntan Gels, Creams, and Liquids	1	1	NR	NR					5	4	NR	0.000001 – 1
Indoor Tanning Preparations	3	1	NR	0.001[a]					5	1	NR	0.001 – 0.5
Other Suntan Preparations	2	NR	NR	NR					5	3	NR	0.001 – 0.5

NR – not reported

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

**likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] 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

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

‡ Sachets are no longer listed as a product category in the VCRP.

Table 5. Frequency (2023) and concentration (2021) of use of ingredients not previously reviewed according to likely duration and exposure and by product category

	Hydrolyzed Calcium Hyaluronate		Hydrolyzed Hyaluronic Acid		Hydrolyzed Sodium Hyaluronate		Sodium Acetylated Hyaluronate	
	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Totals*	**2**	**NR**	**476**	**0.002 – 0.2**	**204**	**0.0015 – 0.15**	**455**	**0.002 – 0.1**
summarized by likely duration and exposure**								
Duration of Use								
Leave-On	*2*	*NR*	*408*	*0.01 – 0.2*	*191*	*0.0015 – 0.15*	*414*	*0.002 – 0.1*
Rinse-Off	*NR*	*NR*	*68*	*0.002 – 0.01*	*13*	*NR*	*41*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type*								
Eye Area	NR	NR	9	0.01	11	NR	45	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	68	NR
Incidental Inhalation-Spray	2[a]	NR	216[a]; 128[b]	0.02	89[a]; 74[b]	NR	136[a]; 79[b]	0.0085 – 0.1[a]
Incidental Inhalation-Powder	NR	NR	4; 128[b]; 4[c]	0.01[c]	1; 74[b]	0.15[c]	11; 79[b]; 3[c]	0.1[c]
Dermal Contact	2	NR	460	0.01 – 0.2	202	NR	383	0.002 – 0.1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	16	0.002 – 0.02	2	NR	2	NR
Hair-Coloring	NR	NR	NR	0.002	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	11	NR	3	NR	74	NR
Baby Products	NR	NR	7	NR	NR	NR	6	NR
as reported by product category								
Baby Products								
Baby Shampoos								
Baby Lotions/Oils/Powders/Creams			4	NR			3	NR
Other Baby Products			3	NR			3	NR
Bath Preparations (diluted for use)								
Bath Oils, Tablets, and Salts								
Bubble Baths								
Bath Capsules								
Other Bath Preparations								
Eye Makeup Preparations								
Eyebrow Pencil							1	NR
Eyeliner							8	NR
Eye Shadow							13	NR
Eye Lotion			2	NR	8	NR	13	NR
Eye Makeup Remover								
Mascara							2	NR
Other Eye Makeup Preparations			7	0.1	3	NR	8	NR
Fragrance Preparations								
Cologne and Toilet Water								
Perfumes								
Powders (dusting/talcum, excl aftershave talc)								
Sachets								
Other Fragrance Preparation								
Hair Preparations (non-coloring)								
Hair Conditioner			6	NR			1	NR
Hair Spray (aerosol fixatives)								
Hair Straighteners								
Permanent Waves								
Rinses (non-coloring)								

Table 5. Frequency (2023) and concentration (2021) of use of ingredients not previously reviewed according to likely duration and exposure and by product category

	Hydrolyzed Calcium Hyaluronate		Hydrolyzed Hyaluronic Acid		Hydrolyzed Sodium Hyaluronate		Sodium Acetylated Hyaluronate	
	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Shampoos (non-coloring)			4	0.002				
Tonics, Dressings, and Other Hair Grooming Aids			1	0.02				
Wave Sets								
Other Hair Preparations			5	NR	2	NR	1	NR
Hair Coloring Preparations								
Hair Dyes and Colors (all types requiring caution statements and patch tests)			NR	0.002				
Hair Tints								
Hair Rinses (coloring)								
Hair Shampoos (coloring)								
Hair Color Sprays (aerosol)								
Hair Lighteners with Color								
Hair Bleaches								
Other Hair Coloring Preparation								
Makeup Preparations								
Blushers (all types)							4	NR
Face Powders			4	NR	1	NR	11	NR
Foundations							43	0.002
Leg/Body Paints								
Lipstick							68	NR
Makeup Bases			3	NR	3	NR	4	NR
Rouges								
Makeup Fixatives			1	NR	1	NR	1	NR
Other Makeup Preparations			4	NR	1	NR	4	NR
Manicuring Preparations (Nail)								
Basecoats and Undercoats								
Cuticle Softeners								
Nail Creams and Lotions								
Nail Extenders								
Nail Polish and Enamel								
Nail Polish and Enamel Removers								
Other Manicuring Preparations								
Oral Hygiene Products								
Dentifrices								
Mouthwashes and Breath Fresheners								
Other Oral Hygiene Products								
Personal Cleanliness Products								
Bath Soaps and Detergents			8	NR	3	NR	4	NR
Deodorants (underarm)								
Douches								
Feminine Deodorants								
Other Personal Cleanliness Products			3	NR			2	NR
Shaving Preparations								
Aftershave Lotion								
Beard Softeners								
Mens Talcum								
Preshave Lotions (all types)								
Shaving Cream								

Table 5. Frequency (2023) and concentration (2021) of use of ingredients not previously reviewed according to likely duration and exposure and by product category

	Hydrolyzed Calcium Hyaluronate		Hydrolyzed Hyaluronic Acid		Hydrolyzed Sodium Hyaluronate		Sodium Acetylated Hyaluronate	
	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Shaving Soap								
Other Shaving Preparations								
Skin Care Preparations								
Cleansing			41	0.01	9	NR	30	NR
Depilatories								
Face and Neck (exc shave)			114	0.01 (not spray)	63	0.15 (not spray)	63	0.1 (not spray)
Body and Hand (exc shave)			14	NR	11	NR	16	NR
Foot Powders and Sprays								
Moisturizing	2	NR	187	0.1 – 0.2 (not spray)	74	0.0015 (not spray)	122	0.0085 – 0.1 (not spray)
Night			14	0.15 (not spray)	7	NR	9	NR
Paste Masks (mud packs)			6	NR	1	NR	4	NR
Skin Fresheners			14	NR	15	NR	5	NR
Other Skin Care Preparations			31	0.2			12	0.1
Suntan Preparations								
Suntan Gels, Creams, and Liquids								
Indoor Tanning Preparations								
Other Suntan Preparations								

NR – not reported

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

**likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] 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

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

Table 6. Oral ADME studies

Parameter Measured	Test Substance	Animals	No./Group	Vehicle/Dose	Dose/Protocol	Results	References
Absorption	[^{14}C]Hyaluronic Acid (MW = 920 kDa)	Male Sprague-Dawley rats	3	Distilled water; 25 mg/kg	Animals were given a single oral dose of the test substance via gavage. Administered radioactivity was 2.04 megabecquerel (MBq)/kg bw. The transition of plasma ^{14}C radioactivity was evaluated by collecting blood from the tail vein of treated animals at 5, 15, and 30 min, and 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, and 168 h post-administration.	The peak plasma radioactivity level was 7.6 µg eq/ml, at 8 h. The half-life was approximately 1.9 d. The area under the concentration-time curves of plasma was determined to be 309 µg of eq/ml/h.	[27]
Distribution	[^{14}C]Hyaluronic Acid (MW = 920 kDa)	Male Sprague-Dawley rats	1/group	Distilled water; 25 mg/kg	Animals were given a single dose of the test substance via gavage, and killed under anesthesia at 8, 24, or 96 h after administration. Whole-body autoradiographs were prepared from radioactivity images recorded on imaging plates.	^{14}C was detected in the skin as follows: 2.36 PSL/mm^2 at 8 h, 3.81 PSL/mm^2 at 24 h, and 1.98 PSL/mm^2 at 96 h after administration. ^{14}C was detected in the blood as follows: 2.12 PSL/mm^2 at 8 h, 1.68 PSL/mm^2 at 24 h, and 0.84 PSL/mm^2 at 96 h after administration. Radioactivity was higher in the skin than in the blood at 24 and 96 h post-administration. In other tissues, the highest levels of radioactivity were observed in the intestinal contents 8 h post-administration (710 PSL/mm^2). Readings in the pancreas (17.45 PSL/mm^2), harderian gland (12.27 PSL/mm^2), liver (9.22 PSL/mm^2), and mandibular gland (7.49 PSL/mm^2) were also high 8 h post-administration. At 96 h post-administration, all radioactivity dropped.	[27]
Distribution	[^{14}C]Hyaluronic Acid (MW = 920,000 kDa)	Male Sprague-Dawley rats	3	Distilled water; 25 mg/kg	Animals were given a single oral dose of the test substance via gavage, and housed in metabolic cages. ^{14}C-excretion rates in the urine, feces, and expired air were evaluated at predetermined times (0-168 h post-administration). Animals were killed at the end of the study to measure the residual radioactivity in the body.	Radioactivity was excreted in the urine as follows: 2.5% of the dose by 24 h, 2.9% by 96 h, and 3% by 168 h. In feces, radioactivity was excreted as follows: 7.8% by 24 h, 11.6% by 96 h, and 11.9% by 168 h. In expired air, radioactivity was excreted as follows: 70.7% of the dose by 24 h, 75.4% by 96 h, and 76.5% by 168 h. The total excretion rate in the urine, feces, and expired air was 91.3% of the administered dose by 168 h post-administration. Approximately 8.8% of the dose remained in the carcass 168 h post-administration.	[27]
Metabolism/ Distribution	Hyaluronic Acid (MW = 300 kDa)	Male Sprague-Dawley rats	6/group	Vehicle not stated; 200 mg/kg bw	After overnight fasting, rats were given a single dose of the test substance (method of oral administration not stated). Samples of cecal content, blood, and shaved ventral skin were collected 0, 2, 4, 6, and 8 h after administration. Unsaturated Hyaluronic Acid disaccharides (u-HA2) and tetrasaccharides (u-HA4) in the serum and the supernatant of homogenized skin were analyzed via liquid chromatography-tandem mass spectrometry (LC/MS/MS).	Hyaluronic Acid is degraded by intestinal bacteria and oligosaccharide Hyaluronic Acid is absorbed in the small intestines and widely distributed. Oligosaccharide (di-, tetra-, hexa-, octa-, and decasaccharides) Hyaluronic Acid was observed in cecal content 2 h after test substance administration. The recovery rate of u-HA2 and u-HA4 in the serum and skin was approximately 25 and 70%, respectively. U-HA2 was observed in the serum 2 h after test substance administration, and peaked in concentration after approximately 6-8 h post-administration. U-HA4 was observed in the serum 8 h after test substance administration. Both u-HA2 and u-HA4 were observed in the skin 6 h after test substance administration, and peaked after 8 h.	[28]

Table 6. Oral ADME studies

Parameter Measured	Test Substance	Animals	No./Group	Vehicle/Dose	Dose/Protocol	Results	References
Excretion	Hyaluronic Acid (MW = 300 kDa)	Male Sprague-Dawley rats	8/group	Distilled water; 5 ml/kg bw/d; 1 and 5%	Animals were orally administered the test substance (method of oral administration not stated) for 5 d. Control animals received distilled water only. On the last 3 d of treatment, feces were collected, freeze-dried, and ground for analysis. Hyaluronic Acid concentration was measured using a hyaluronan assay kit with a Hyaluronic Acid binding protein.	Hyaluronic Acid was below the detection limit (10 µg/3 d) in all groups.	[28]

kDa = kilodalton; MW = molecular weight; PSL = photo-stimulated luminescence; SPECT = single photon emission computed tomography

Table 7. Acute oral toxicity studies on Sodium Hyaluronate[16]

Test Article	Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/Results
Sodium Hyaluronate (FW = 1800-2100 kDa)	NR	ICR mice (number of animals not stated)	500 mg/kg bw	single dose; method of oral administration not stated	LD_{50} > 500 mg/kg bw
Sodium Hyaluronate	peanut oil	Kunming mice (10/sex)	2000 mg/kg bw	single dose; gavage; 14-d evaluation	LD_{50} > 2000 mg/kg bw; no signs of toxicity or deaths
Sodium Hyaluronate	peanut oil	Kunming mice (20/sex)	1000, 2150, 4640, or 10,000 mg/kg bw	single dose; gavage; 14-d evaluation	LD_{50} > 10,000 mg/kg bw; no signs of toxicity or deaths
Sodium Hyaluronate	corn germ oil	Kunming mice (10/sex)	15,000 mg/kg bw	single dose; gavage; 14-d evaluation	LD_{50} > 15,000 mg/kg bw; no signs of toxicity or deaths
Sodium Hyaluronate (FW = 1800 – 2100 kDa)	NR	Rats (strain and number of animals not stated)	200 mg/kg bw	single dose; method of oral administration not stated	LD_{50} > 200 mg/kg bw; no signs of toxicity or deaths
Sodium Hyaluronate (FW = 270 kDa)	distilled water	Wistar rats (10/sex)	5280 mg/kg bw	single dose; gavage	MTD > 5280 mg/kg bw; no signs of toxicity or deaths

LD_{50} = median lethal dose; MTD = maximum tolerable dose; MW = molecular weight; FW = formula weight; NR = not reported

Table 8. Oral repeated dose toxicity studies on Sodium Hyaluronate[16]

Test Article	Vehicle	Animals/Group	Study Duration	Dose*	Protocol	Results
Sodium Hyaluronate	feed	Wistar rats (10/sex/group)	30 d	0, 167, 500, or 1500 mg/kg bw	rats given test substance via feed; body weight changes, hematological, biochemistry, and macroscopic parameters were evaluated	no deaths or changes in body weight, food consumption, or weight gain; all blood chemistry parameters were within normal ranges; no changes in organ weight/histopathological parameters; NOAEL = 1500 mg/kg bw/d
Sodium Hyaluronate	NR	Sprague-Dawley rats (5-10/sex/group)	90 d	0, 3, 12, or 48 mg/kg bw/d	rats given test substance via gavage; body weight, food efficiency, urinalysis, and gross pathological and histopathological parameters evaluated; satellite group consisted of 5 rats/sex given 48 mg/kg bw/d for 90 d, followed by a 28 d recovery period	no dose-dependent changes in body weight, histopathological parameters, or hematological parameters were observed; NOAEL = 48 mg/kg bw/d
Sodium Hyaluronate (FW = 2270 kDa)	feed	Wistar rats (10/sex/group)	90 d	0, 330, 670, or 1000 mg/kg bw	rats given test substance via feed; evaluated for 28 d following treatment period; body weight changes, hematological, biochemistry, and macroscopic, and gross pathological parameters were evaluated	no deaths or changes in body weight, food consumption, weight gain, hematological parameters, or histopathological parameters; NOAEL = 1000 mg/kg bw/d
Sodium Hyaluronate	corn germ oil	Sprague-Dawley rats (12/sex/group)	90 d	0, 667, 100, or 1333 mg/kg bw/d	rats given test substance via gavage; body weight changes, hematological, biochemistry, and macroscopic, and gross pathological parameters were evaluated	no changes in behavior, feeding, body weight, food consumption, hematological parameters, organ weights, or macroscopic/histological parameters were observed; NOAEL = 1333 mg/kg bw/d

FW = formula weight; NOAEL = no-observed-adverse-effect-level; NR = not reported

Table 9. Genotoxicity studies[16,29-31]

Test Substance	Test Concentration/Dose	Vehicle	Test System	Procedure	Results
			IN VITRO		
Hydrolyzed Sodium Hyaluronate (FW = 5-10 kDa)	up to 5 mg/plate (specific concentrations not stated)	NR	*S. typhimurium* strains TA97a, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
Hydrolyzed Sodium Hyaluronate (FW < 1kDa)	up to 5 mg/plate (specific concentrations not stated)	NR	*S. typhimurium* strains TA97a, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	1 mg/plate	NR	*S. typhimurium* strains TA97a, TA98, TA100, TA102, and TA1535	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	up to 1 mg/plate (specific concentrations not stated)	NR	*S. typhimurium strains TA97a, TA98, TA100, TA102, and TA1535*	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	0.008, 0.04, 0.2, 1, and 5 mg/plate	NR	*S. typhimurium* strains TA97a, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	0.2, 0.5, 1, 2.5, and 5 mg/plate	NR	*S. typhimurium* strains TA97, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	up to 5 mg/plate (specific concentrations not stated)	NR	*S. typhimurium* strains TA97a, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	without metabolic activation/*S. typhimurium* and *E. coli*: 313, 625, 1250, 2500, 5000 µg/plate with metabolic activation/ *S. typhimurium*: 39.1, 78.1, 156, 313, 625, 1250 µg/plate with metabolic activation/*E. coli*: 313, 625, 1250, 2500, 5000 µg/plate	NR	*S. typhimurium strains TA98, TA100, TA1535, TA1537* and *E. coli* WP2 *uvrA*	Ames assay performed with and without metabolic activation	Non-genotoxic
			IN VIVO		
Sodium Hyaluronate	20 ml/kg bw	NR	mice (strain not reported; 5/sex/group)	Mouse micronucleus assay; cyclophosphamide used as positive control; distilled water used as negative control; administrations via gavage	non-mutagenic
Sodium Hyaluronate	440, 880, 1760 mg/kg bw	NR	KS mice (5/sex/group)	Mouse micronucleus assay; cyclophosphamide used as positive control; distilled water used as negative control; administrations via gavage	non-mutagenic
Sodium Hyaluronate	1250, 2500, and 5000 mg/kg bw	NR	KS mice (5/sex/group)	Mouse micronucleus assay; cyclophosphamide used as positive control; corn germ oil used as negative control; administrations via gavage	non-mutagenic

NR = not reported

Table 10. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/ Dose	Test Population	Procedure	Results	Reference
IRRITATION						
IN VITRO						
Trade name mixture containing 1% Hyaluronic Acid	NR	100%: 30 µl	3	EpiDerm™ assay; 60 min incubation period; sterile PBS and sterile deionized water used as negative controls; 5% SDS solution and methyl acetate used as positive control; cell viability evaluated via MTT assay	Non-irritating	[32]
Trade name mixture containing 1% Hyaluronic Acid	NR	100%: 30 µl	3	Same as above	Non-irritating	[33]
Trade name mixture containing 3% Hyaluronic Acid	NR	100%: 30 µl	3	Same as above	Non-irritating	[34]
Hydrolyzed Sodium Hyaluronate (FW < 1 kDa)	NR	1%	NR	OECD TG 439; reconstructed human epidermis assay	Non-irritating	[31]
Trade name mixture containing 0.5% Sodium Hyaluronate	NR	100%; 30 µl	3	EpiDerm™ assay; 60 min incubation period; sterile PBS and sterile deionized water used as negative controls; 5% SDS solution and methyl acetate used as positive control; cell viability evaluated via MTT assay	Non-irritating	[35]
Sodium Hyaluronate	NR	NR	NR	OECD TG 439; reconstructed human epidermis assay	Non-irritating	[29]
HUMAN						
Hydrolyzed Sodium Hyaluronate (FW = 1-5 kDa)	NR	0.5%	32	Human skin closed patch test; test substance applied to skin of individuals with sensitive skin for 24 g	Non-irritating	[31]
Hydrolyzed Sodium Hyaluronate (FW = 1-5 kDa)	NR	0.5 and 2%	30	Human skin closed patch test; applications on healthy skin; no other details provided	Non-irritating	[31]
Hydrolyzed Sodium Hyaluronate (FW = 5-10 kDa)	NR	1%	NR	Human skin closed patch test; no details provided	Non-irritating	[31]
Hydrolyzed Sodium Hyaluronate (FW < 1 kDa)	NR	1%	30	Human skin closed patch test; applications on healthy skin; no other details provided	Non-irritating	[31]
SENSITIZATION						
IN CHEMICO/IN VITRO						
Trade name mixture containing 1% Hyaluronic Acid	NR	5 (0.05 ml) and 25 mM (250 µl)	3/concentration tested	OECD TG 442C; DPRA; 24 h incubation period; mean percent depletion of cysteine and lysine evaluated; positive control: cinnamic aldehyde in acetonitrile; negative control: peptide in buffer	Mean percent depletion of cysteine and lysine was 3.11%; prediction of non-sensitizing	[36]
Trade name mixture containing 1% Hyaluronic Acid	NR	0.00098 – 2mM; 0.05 ml	3/concentration tested	OECD TG 442D: ARE-Nrf2 Luciferase Test Method; KeratinoSens™ cell line; positive control: cinnamic aldehyde; negative control: DMSO	I_{max} of 0.35 compared to I_{max} of 31.5 and 0.33 for positive and negative control, respectively; prediction of non-sensitizing	[37]
Sodium Hyaluronate	NR	1 mg/ml	NR	OECD TG 442E; h-CLAT	Mean RFI_{CD54} < 200 and mean RFI_{CD86} < 150; cell activity > 50%; prediction of non-sensitizing	[29]

Table 10. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/ Dose	Test Population	Procedure	Results	Reference
			HUMAN			
Formula containing 0.2% Hyaluronic Acid	NR	100%; 20 mg	115	HRIPT; occlusive conditions; 9 applications over a 3-wk period for induction period; challenge phase (48-h patch application) after a 10-15 d rest period; challenge patches evaluated immediately after removal, and 24, 48 and 72 h after patch removal	Non-irritating and non-sensitizing	[38]
Formula containing 0.2% Sodium Acetylated Hyaluronate		100%; 0.02 ml	104	HRIPT; occlusive conditions; 9 applications over a 3-wk period for induction period; challenge phase (48-h patch application) after a 14-d rest period; challenge patches evaluated 15 min and around 48 h after patch removal	Non-irritating and non-sensitizing	[39]
Hydrolyzed Sodium Hyaluronate (FW = 5-10 kDa)	NR	0.5%	55	HRIPT; no details provided	Non-irritating and non-sensitizing	[31]
Sodium Hyaluronate	NR	0.2%	50	HRIPT; no details provided	Non-sensitizing	[30]
Sodium Hyaluronate	NR	0.2%	100	HRIPT; no details provided	Non-irritating and non-sensitizing	[29]
Formula containing 1.5% Sodium Hyaluronate	NR	100%; 0.2 ml	198	HRIPT; occlusive conditions; 9 applications over a 3-wk period for induction period; challenge phase (48-h patch application) after a 10-15 d rest period; challenge patches evaluated immediately after removal, and 24, 48 and 72 h after patch removal	Non-irritating and non-sensitizing	[40]

ARE = antioxidant response element; DMSO = dimethyl sulfoxide; DPRA - direct peptide reactivity assay; h-CLAT - human cell line activation test; HRIPT = human repeated insult patch test; I_{max} = maximum response value; MTT = 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; NR = not reported; Nfr2 = nuclear factor erythroid 2-related factor 2; OECD TG = Organisation for Economic Cooperation and Development Test Guidelines; PBS = phosphate-buffered saline; RFI = relative fluorescence intensity; SDS = sodium dodecyl sulfate

Table 11. Ocular irritation studies

Test Article	Vehicle	Test Concentration/ Dose	Test Population	Procedure	Results	Reference
IN VITRO						
Trade name mixture containing 1% Hyaluronic Acid	NR	100%; 50 µl	2	EpiOcular™ assay; 90 min incubation period; sterile PBS and sterile deionized water used as negative controls; 5% SDS solution and methyl acetate used as positive control; cell viability evaluated via MTT assay	Non-irritating	[32]
Trade name mixture containing 1% Hyaluronic Acid	NR	100%; 50 µl	2	Same as above	Non-irritating	[33]
Trade name mixture containing 3% Hyaluronic Acid	NR	100%; 50 µl	2	Same as above	Non-irritating	[34]
Hydrolyzed Sodium Hyaluronate (FW < 1kDa)	NR	NR	NR	CAMVA (no details provided)	Non-irritating/slightly irritating	[31]
Trade name mixture containing 0.5% Sodium Hyaluronate	NR	100%; 50 µl	2	EpiOcular™ assay; 90 min incubation period; sterile PBS and sterile deionized water used as negative controls; 5% SDS solution and methyl acetate used as positive control; cell viability evaluated via MTT assay	Non-irritating	[35]
Sodium Hyaluronate	NR	100%	NR	CAMVA (no details provided)	Non-irritating/slightly irritating	[29]
Sodium Hyaluronate	NR	100%	NR	BCOP test (no details provided)	Non-irritating/slightly irritating	[29]
ANIMAL						
Bacillus-derived and *Streptococcus*-derived Hyaluronic Acid	NR	0.1 and 0.3%; 25 µl	New Zealand white rabbits (3/group)	Test substances were placed on the right eye, 4x/d, for 3 d. After the last instillation, rabbits were sedated, and eyes were evaluated via fluorescent imaging	The test substance was considered to be very well-tolerated	[41]

BCOP - bovine corneal opacity and permeability; CAMVA - chorioallantoic membrane vascular assay; MTT = 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; NR = not reported; PBS = phosphate-buffered saline; SDS = sodium dodecyl sulfate

Table 12. Case reports of hypersensitivity following injectable Hyaluronic Acid dermal fillers

Patient	Case report summary	Reference
50-yr-old female	-patient had previous Hyaluronic Acid dermal filler injections in glabellar region and nasolabial fold with no adverse effects -1 yr later, patient received same treatment, and presented with erythematous, livedoid rash 3 d after injections -rash cleared within 10 d of treatment with antibiotics and steroids -patient repeated injections 3 yr later with no adverse effects	50
47-yr-old female	-facial Hyaluronic Acid dermal filler injections at different locations of the face -12 mo later, patient complained of abdominal pain, asymmetric erythematous swelling of the lips, and pain and tingling of lips -angioedema resolved with injection of hyaluronidase	51
54-yr-old female	-dermal filler Hyaluronic Acid gel injections at multiple areas of face, once in April 1998 and again in November 1988, with no adverse effects -patient received Hyaluronic Acid dermal filler injections in melolabial folds in June 1999, and developed indurated and erythematous papulocystic nodules 2 wk after injections -patient treated with corticosteroids and warm compresses, and nodules improved -2 wk later, patient returned with recurrent inflammation of the right mesolabial fold with tender nodules -patient again treated with corticosteroids and warm compresses, and experienced rapid resolution of symptoms	52
59-yr-old female	-dermal filler Hyaluronic Acid injections in melolabial folds, glabella, lips, and perioral rhytids -2 d after injections, patient noted significant swelling and pain at injection sites -5 d after injections, patient admitted to hospital for significant facial swelling -treated with corticosteroids and an immunosuppressant	53
52-yr-old female	-left and right upper lip injections of Hyaluronic Acid dermal filler -5 min after injections, patient experienced worsening edema and erythema -patient treated with dexamethasone sodium phosphate, prednisone, and valacyclovir -the following day, the patient still had severe edema and fissures on lip mucosa -patient instructed to apply an emollient ointment, and reported improved symptoms	54
56-yr-old female	-melolabial fold injections of Hyaluronic Acid dermal filler -27 d after injections, patient developed erythematous indurated papules at injection sites -treatment with steroids resolved symptoms	55
65-yr-old female	-prior to injections, skin prick tests of Hyaluronic Acid dermal filler performed and yielded negative results -lip, nasolabial fold, and perioral rhytide injections of Hyaluronic Acid dermal filler with no adverse effects -a second treatment was performed 3 mo later with no adverse effects -a third treatment was performed 6 mo later with no adverse effects -1 mo after the third treatment, patient re-treated, and presented with erythema, edema, and induration of injection regions 6 wk after 4th series of injections -symptoms improved with steroid treatment	56
54-yr-old female	-patient reported previous facial injections of Hyaluronic Acid dermal filler (every 4 mo) with no adverse effects -10 d after an injection of Hyaluronic Acid dermal filler to melolabial folds, patient reported granulomatous reaction -symptoms improved with betamethasone treatment	57
28-yr-old female	-swelling, pain, and tenderness at injection sites 3 mo after chin injections of Hyaluronic Acid dermal filler -antibiotics did not resolve symptoms -treatment with corticosteroid, antihistamine, and clindamycin resolved symptoms	58
29-yr-old female	-asymmetry, edema, and inflammatory nodules seen at injection sites 112 d after facial injections of Hyaluronic Acid dermal filler -treatment with steroids, antihistamines, and antibiotics resolved symptoms	59
49-yr-old female	-facial edema observed 28 d after glabella and eye area injections of Hyaluronic Acid dermal filler -treatment with corticosteroids resolved symptoms	59
52-yr-old female	-inflammatory nodules, pustules, and fever observed 2 d after glabella injections of Hyaluronic Acid dermal filler -treatment with steroids, antibiotics, and coloplast cream resolved symptoms	59
56-yr-old female	-pruritis and blisters 14 d after facial Hyaluronic Acid dermal filler injections -treatment with steroids, antihistamines, saline dressings, and betamethasone resolved symptoms	59
42-yr-old-female	-inflammatory nodules 1 yr after facial Hyaluronic Acid dermal filler injections -treatment with moxypen cefamezin resolved symptoms	59
60-yr-old female	-patient reported 2 previous series of Hyaluronic Acid dermal filler injections with no adverse effects -erythema, pain, and edema observed 14 d after 3rd round of Hyaluronic Acid dermal filler injections in the cheeks -patient treated with antibiotics, pulsed light therapy, and physical therapy	60
30-yr-old female	-patient reported previous facial Hyaluronic Acid dermal filler injections with no adverse effects -5 yr after previous injections, patient was treated with Hyaluronic Acid dermal filler injections in the cheeks, mandible, and chin -the following day, patient reported sore throat and treated with antibiotics -by day 10, patient presented with erythema and edema of lip and chin, treated with corticosteroids -by day 18, patient presented with painful, palpable, subcutaneous collections at the chin, cheekbone, and mandible -patient treated with antibiotics and incision/drainage of collections -patch and intradermal testing to evaluate the potential of a hypersensitivity reaction to Hyaluronic Acid dermal filler was performed 3 mo later, and resulted in negative results	60
56-yr-old female	-swelling 4 mo after injections of Hyaluronic Acid dermal filler to cheeks -1 yr later, patients re-treated with injections in the cheeks, and developed facial swelling 4 mo after treatment -patient treated with antibiotics and hyaluronidase	61

Table 12. Case reports of hypersensitivity following injectable Hyaluronic Acid dermal fillers

Patient	Case report summary	Reference
57-yr-old female	-patient reported previous treatment with Hyaluronic Acid dermal filler to the perioral area, on 2 occasions, with no adverse effects -patient experienced erythema, warmth, and rigidity at injection sites 3 wk after 3rd series of facial Hyaluronic Acid dermal filler injections -3 mo later, a nontender deep, firm, palpable thickening over both zygomatic arches was apparent -patient treated with antibiotics and hyaluronidase -a recurrent episode occurred 3 mo later, and was again treated with hyaluronidase	61
32-yr-old female	-patient reported previous Hyaluronic Acid dermal filler injections lips with no adverse effects, and acute swelling after injection to hands -erythema and swelling at injection sites 6 mo after treatment with Hyaluronic Acid dermal filler injections in cheeks -patient treated with hyaluronidase -recurrent redness and swelling occurred 2 mo later, and was again treated with hyaluronidase	61
48-yr-old female	-patient reported previous Hyaluronic Acid dermal filler injection treatment in marionette lines with no adverse effects -swelling of cheeks 1 wk after Hyaluronic Acid dermal filler injections to cheeks -treatment with corticosteroids -flare-ups occurred 3 and 4 mo post-injection, treated with corticosteroids	61
54-yr-old female	-redness and swelling of the nasolabial folds after Hyaluronic Acid dermal filler injections -severe palpable and painful erythematous nodular papulocystic lesions 3 mo after injections -patient surgically treated	62
48-yr-old female	-blue/gray coloring of lips, cheek, and nose 8 h after Hyaluronic Acid dermal filler injections -treatment with nitroglycerin and hyperbaric chamber	63
41-yr-old female	-erythematous nodules at injection sites 5 wk after melolabial, glabellar, and periorbital area injections of Hyaluronic Acid dermal filler -treatment with antibiotics and steroids	64
49-yr-old female	-asymptomatic hard lesions along melomental folds 4 mo after lower facial injections of Hyaluronic Acid dermal filler -treatment with corticosteroids and hyaluronidase -patch tests performed were negative at 48 and 96 h -intradermal injection into forearm was negative at 20 min and 96 h, but turned positive 2 mo later	65
72-yr-old female	-well-defined, millimetric, firm nodules on lips and oral mucosa 5 mo after Hyaluronic Acid dermal filler injections -treatment with corticosteroids and hyaluronidase	66
45-yr-old female	-glabellar, neck, eyelid injections of Hyaluronic Acid dermal filler and *Botulinum* toxin -1 mo after injection, patient developed facial pain, erythema, and edema -patient's symptoms improved following treatment with pain medication, antibiotics, and steroids	67
53-yr-old female	-patient reported previous Hyaluronic Acid dermal filler injection to nasolabial folds and lips -asymmetry of nasolabial fold, palpable pea sized-lesions 1 yr after Hyaluronic Acid dermal filler injections -patient treated with antibiotics and ibuprofen	68
40-yr-old female	-dusky, red, firm, linear rash 4 mo after injection of a mixture of Hyaluronic Acid dermal filler gel and acrylic hydrogel to the nasolabial folds -treatment with betamethasone	69
66-yr-old female	-patient reported 12 treatments of facial Hyaluronic Acid dermal filler injection over the course of 5 yr -patient reported an increasing number of hard lumps in areas that were repeatedly treated with Hyaluronic Acid dermal filler gel and acrylic hydrogel -eventually developed into symmetrical linear purple plaques, nodules, induration of lips -treatment with steroids	70
65-yr-old female	-patient reported 3 treatments with a mixture of Hyaluronic Acid dermal filler gel and acrylic hydrogel -hard subcutaneous nodules in nasolabial folds, upper lip, and glabella 2 yr after last treatment -treatment with steroids	70

*It should be noted that dermal fillers are derived from one of two methods: a non-animal method (bacterial fermentation using *Streptococcus*) or via extraction of chicken/rooster combs.[71]

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