Safety Assessment of Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate as Used in Cosmetics

Status:	Draft Final Report for Panel Review
Release Date:	February 10, 2023
Panel Meeting Date:	March 6-7, 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. Previous Panel member involved in this assessment: Daniel C. Liebler, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR.

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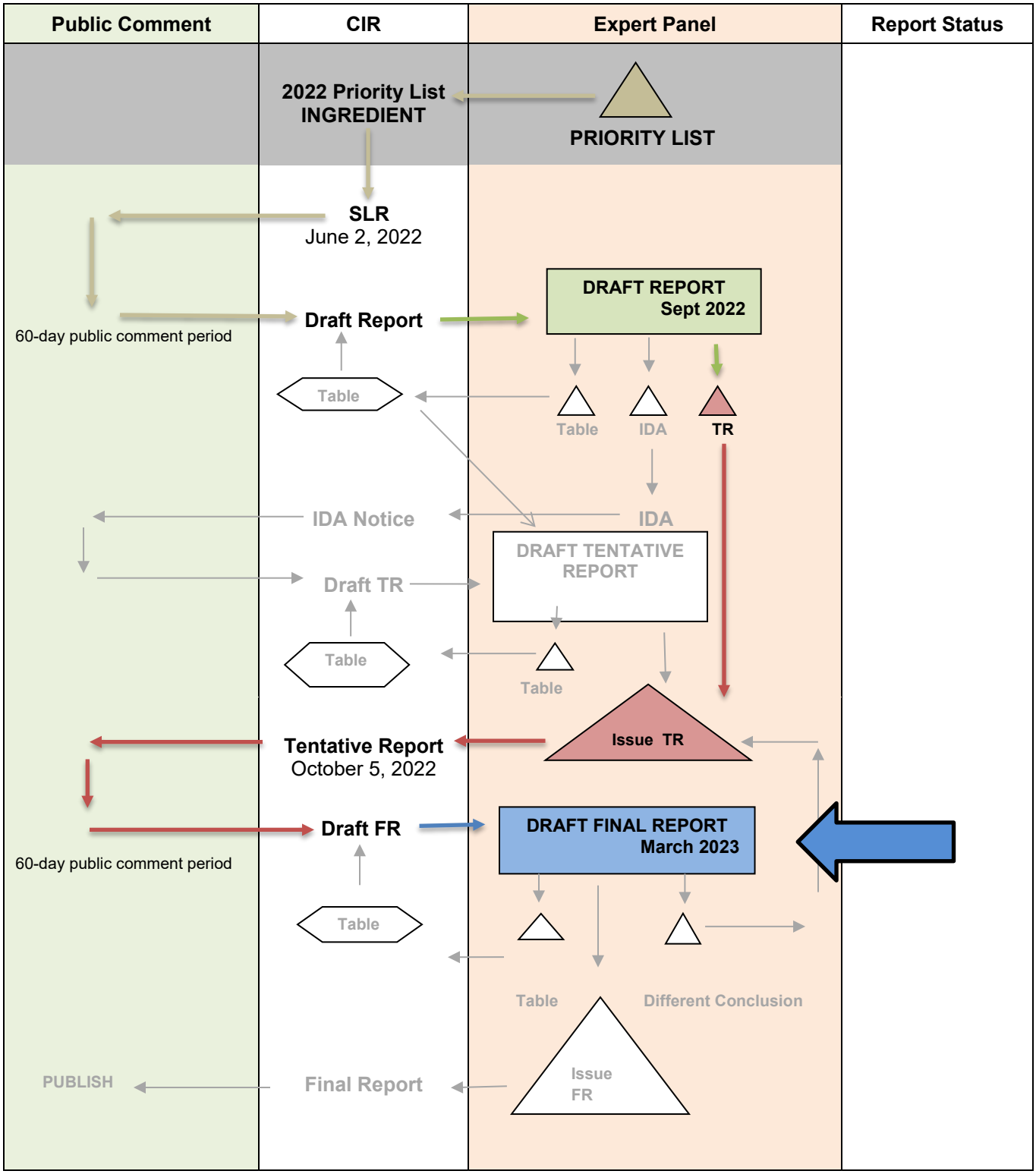
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Trisodium Ethylenediamine Disuccinate

MEETING March 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: February 10, 2023
Subject: Safety Assessment of Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate

Enclosed is the Draft Final Report on the Safety Assessment of Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate as Used in Cosmetics (*report_TrisodiumEthylenediamineDisuccinate_032023*). At the September 2023 meeting, the Panel issued a Tentative Report for public comment with the conclusion that Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate 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No unpublished data were submitted since the issuing of the Tentative Report.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_TrisodiumEthylenediamineDisuccinate_032023*) have been addressed. A comments response checklist is included (*response-PCPCcomments_TrisodiumEthylenediamineDisuccinate_032023*).

As per the Panel's request at the December 2022 meeting, an updated use table format has been implemented. The frequency and concentration of use is presented both cumulatively by likely duration and exposure and individually by product category.

Also included in this packet are the report history (*history_TrisodiumEthylenediamineDisuccinate_032023*), data profile (*dataprofile_TrisodiumEthylenediamineDisuccinate_032023*), search strategy (*search_TrisodiumEthylenediamineDisuccinate_032023*), transcripts of the previous meetings (*transcripts_TrisodiumEthylenediamineDisuccinate_032023*), and flow chart (*flow_TrisodiumEthylenediamineDisuccinate_032023).*

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: October 20, 2022

SUBJECT: Tentative Report: Safety Assessment of Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate as Used in Cosmetics (release date: October 5, 2022)

The Personal Care Products Council respectfully submits the following comments on the tentative report, Safety Assessment of Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate as Used in Cosmetics.

Abstract – Please correct: "The Panel reviewed the reviewed…"

Impurities, Tetrasodium Iminodisuccinate – It would be helpful to note that some of the impurities are residual monomers, and that both Disodium Fumarate and Sodium Aspartate have been reviewed by CIR and found safe as used.

DART – Please correct "or gestation days 6-15" ("or" should be "on"). Plasma levels of copper, iron and zinc should not be called "hematological parameters" (which are endpoints that relate to blood cells).

Summary – Please state the organ (pancreas) in which "single cell death, fatty infiltration" were observed.

Table 5, first oral study – As OECD TG 423 states the route is gavage, please delete "(method of oral administration not reported)".

Table 6, third row – In the Dose/Concentration/Procedure column "concentrations" should be changed to "doses" as the units are mg/kg bw/d.

Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate - March 2023 – Priya Cherian	
Comment Submitter: Personal Care Products Council **Date of Submission: October 20, 2022**	
Comment	**Response/Action**
Abstract – Please correct: "The Panel reviewed the reviewed…"	Addressed
Impurities, Tetrasodium Iminodisuccinate – It would be helpful to note that some of the impurities are residual monomers, and that both Disodium Fumarate and Sodium Aspartate have been reviewed by CIR and found safe as used.	Addressed
DART – Please correct "or gestation days 6-15" ("or" should be "on"). Plasma levels of copper, iron and zinc should not be called "hematological parameters" (which are endpoints that relate to blood cells).	Addressed
Summary – Please state the organ (pancreas) in which "single cell death, fatty infiltration" were observed.	Addressed
Table 5, first oral study – As OECD TG 423 states the route is gavage, please delete "(method of oral administration not reported)"	Addressed
Table 6, third row – In the Dose/Concentration/Procedure column "concentrations" should be changed to "doses" as the units are mg/kg bw/d	Addressed

Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate – Report History

July 2021

- Concentration of use submitted by Personal Care Products Council

June 2022

- SLR posted

July 2022

- Comments on SLR received by Personal Care Products Council

September 2022

- Draft Report evaluated by Expert Panel
- Panel issues Tentative Report with public comment with the conclusion that Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate are safe as used in cosmetics

October 2022

- Comments on TR received

March 2023

- Panel reviews Draft Final Report

Trisodium Ethylenediamine Disuccinate Data Profile – March 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
Tetrasodium Iminodisuccinate	**X**		X				X	X			X				X	X				X			X				X		
Trisodium Ethylenediamine Disuccinate	**X**		X	X	X	X	X	X	X		X			X	X	X				X	X		X	X			X		

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

Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate – Priya Cherian

Ingredient	CAS #	InfoB	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Trisodium Ethylenediamine Disuccinate	178949-82-1; 474787-13-8	x	x		x	x	x					x							x
Tetrasodium Iminodisuccinate	144538-83-0	x	x		x	x	x					x							x

Search Strategy

- Search terms listed below were searched in combination with INCI names, CAS numbers, and chemical names
- If information was found, an "x" is noted

Search Terms

- INCI names
  - Tetrasodium Iminodisuccinate
  - Trisodium Ethylenediamine Disuccinate
- CAS numbers
  - 178949-82-1
  - 474787-13-8
  - 144538-83-0
- chemical/technical names
- toxicity
- metabolism
- irritation
- allergy
- eye
- sensitization
- manufacture
- impurities
- 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

SEPTEMBER 2022 – INITIAL REVIEW/DRAFT REPORT

Belsito Team – September 26, 2022

[Insert Belsito Team Minutes – use the Merge Formatting option for pasting in the minutes]

Cohen Team – September 26, 2022

Dr. David Cohen - OK. No let's move on to a trisodium ethylenediamine disuccinate and tetrasodium iminodisuccinate. This is a draft report. This is the first time we're reviewing it and this is this a safety assessment of these just these two ingredients. It's they're used as chelating agents. We have frequency of use and we have maximum reported concentration of .64% for trisodium ethylenediamine disuccinate. And it's used in a variety of other types of products. We have no concentration of use for tetrasodium iminodisuccinate. And the panel had issued as a safety assessment on EDTA and its salts, which were safe as used and in 2019, the panel reaffirmed that we originally had no method of manufacturing. But in wave 2. There was a source for TSID mentioned. I don't know if we actually got have that method of manufacturing and we have impurities, which included dimethyl fumarate which is a fumaric acid Ester of contact sensitizer. Dimethyl fumarate. And we have irritation and sensitization data at 5% for the trisodium ethylenediamine. So what are thoughts on their panel? Tom, you want to go first?

Dr. Tom Slaga - Alright, yeah. We do we did get irritation and sensitization data. And that appeared for me anyway. Looked OK. And we have toxicity, which is negative. And the weight of evidence is negative, I should say. So we have a little least a little data to go with this so. You know. I don't know if there is. See.

Dr. David Cohen - We have impurities and.

Dr. Tom Slaga - Yeah, we have impurities, you mentioned about the method of manufacturing but I don't know. I really didn't have any problem with this one, but.

Dr. David Cohen - Priya did that reference from wave.

Dr. Tom Slaga - And similar paths they've been. Done. You know safe, so similar type. So I, you know, I think we possibly could go even safe as used.

Dr. David Ross - So when I looked at it, I yeah, I thought that I thought the acute and the subchronic was fine. I thought that looked great. A lot we got an awful lot of information on the trisodium. We haven't got much information.

Dr. Tom Slaga - Right.

Dr. David Ross - Now on the trisodium. Looking at this, there was a, one through just one isolated readout on gen tox, but it was under specific conditions with lots of other negative genetials.

Dr. Tom Slaga Right.

Dr. David Ross - So I thought that was just fine. The only thing on the trisodium that I flagged was that

You know I'm, you know, realize the ocular doesn't have many uses. I think we only List 3 uses in ocular of 228 total uses on the trisodium. So it's pretty minimal but.

Dr. Tom Slaga - Yeah.

Dr. David Ross - When I went back to the European document. Which said for ocular I sodium was non irritating. When you go into the methods, they only use 4 milligrams in the eyes of rabbits, and the comment in that document was that it was a very small amount. The Australian documents that it was slightly irritated, slightly irritating so I'm not even sure we've got a maximum concentration of use on ocular, do we? No, we don't. So that would be good to know. If you could get that.

Dr. David Cohen - Well, what if Max use by Ocular was Max use?

Dr. David Ross - Yeah. Well, we, you know, we don't have .64%, I don't think we have that data the data in the Australian study which concluded it was slightly irritating, had no concentrations on it. So we could just figure out what the concentrations were used there. I think we might be in good shape, but I didn't see that. Now on the tetrasodium, I mean there's very little data. So I mean, you know, there's no use concentrations, as David said.

Dr. David Cohen - Right.

Dr. David Ross - Method of manufacture. The purity is only 72%. You know which got my attention. There's no dark. The animal dermal this, there's one study, but again no concentration reported. The human dermal non human sensitization non an ocular was non irritating in one study, but no concentration noted again, so you know exactly. And I'm not sure whether you can read across. I don't know about the collision potential of both of them. I thought one was different to the other. I might be wrong there, but. If it is different.

Dr. Tom Slaga - Should be able to read across, shouldn't we?

Dr. David Ross - If we do.

Dr. David Cohen - Yes.

Dr. David Ross - If you can't do read across then.

Dr. Tom Slaga - Yeah.

Dr. David Ross - But the question is, are they both good coladas and I don't because that would make biological difference. I don't know the answer.

Dr. David Cohen - Susan, what do you what are your thoughts about read across and the prior assessments of EDTA?

Dr. Susan Tilton - One thing I wanted to just comment on follow up to David. Was just about. The lack of use data reported for tetrasodium, so I don't know if we can assume. That use is similar to try sodium concentrations used. That was one concern I had and trying to compare across the chemicals.

Was can we assume similar use? And they are listed as having different impurities. So I mean that that is a concern. And we don't have manufacturing for either ingredient.

Dr. David Cohen - Priya went and that reference and Wave 2 did we get an adequate method of manufacturing?

Priya Cherian (CIR)- I can read you what it says for manufacturing right now. It's pretty short, but it says that those products are made of maleic acid anhydride, ammonia water and occasionally sodium hydroxide. And in the process of manufacturing polyaspartic acid polypTacsuan mine is generated first his is converted into salts of polyaspartic acid by subsequent hydrolysis. And then these ingredients are directly synthesized of the above mentioned raw materials.

Dr. David Cohen - Was does that help? So if we were, if we were presented with that method of manufacturing with the current impurities, like what, you know, the fact that it's the tetrasodium is 72% pure, and then there's some fumaric, aster and some other impurities. What would we be putting an out IDA out for? What do what do we asking for? If we go out with an IDA?

Dr. David Ross – If asking for IDA on tetrasodium, you'd be asking for just about everything because there's not much in there. I mean, if you can do read across. That I think it's pretty straightforward. Again, As Susan said with some issues.

Dr. David Cohen - Yeah, but I think the whole idea of grouping this together is for us to have some freedom to operate about, read across, right. We don't. We don't always get.

Dr. Tom Slaga - Right.

Dr. David Cohen - You know every detail of use of every derived ingredient and the I think the whole idea of putting them together is to use the chemistry, know how of the group. And look at impurities. Look at method of manufacturing. Look for other adverse effects that could happen and see if we could bring the group forward because it's common for that table to not have exes across every column.

Dr. David Ross - What about?

Dr. David Cohen - So.

Dr. David Ross - Different impurities that you yourself pointed out, David. You're right, 70.

Dr. David Cohen - Yeah. Yeah. So, you know, the question is you know, can, does Homeric acid,

can that become a fumaric acid Ester under certain conditions? And that is a known context sensitizer.

What are the, I just, I don't know that you guys might be able to tell me they're not the same thing, so it can it become that and could we just put that in the discussion that that's an issue?

Dr. Wilma Bergfeld - Or should be formulated to be non sensitizing?

Dr. David Cohen - But we don't have any. We don't have any date on, on, on. We don't have any data that this is sensitizing right. And then we have no uses for Tetrasodium so. I suppose that trisodium we really wouldn't be hampering much because there isn't any sensitization data that we've seen that's very concerning and it's at a considerable concentration over Max use so. Help me out here. We're presenting this tomorrow. Are we going out as safe as used or do we want an IDA to and are we going to ask for? What are we asking for? Sensitization data on the tetrasodium that's not in use?

Dr. David Ross - I would just like to say that I think we do need some data on the ocular with the trisodium. Whether it's picking out the existing concentration of use.

Dr. David Cohen - OK.

Dr. David Ross - Speaking about the concentrations that we use.

Dr. Tom Slaga - It's a obviously a draft report, so. We have any concerns we should go out. What IDA.

Dr. David Cohen - OK.

Dr. David Ross - We could get the concentrations that we use in the Australian study. I didn't see that. Maybe I just missed it.

Priya Cherian (CIR)- Doctor Ross, if. If the concentrations were noted, then it would be stated so you can request ocular irritation data, but it would have to be new data that came in.

Dr. Susan Tilton - OK.

Dr. David Cohen - So did. So, David, what? What do you think? If we go out with an IDA and we don't get that data back or we're sort of in the same just the similar discussion that we had with glycol lactones, right?

Dr. David Ross - Yeah. Well, you know the issue with this compound is there's two studies in there already. You know, one from the EU and one from Australia. The only thing we're lacking is details and.

Dr. David Cohen - We can ask for the details of that study, can't we?

Dr. Tom Slaga - Yeah.

Dr. Wilma Bergfeld - Priya says she's giving you what she has.

Dr. David Ross - Yeah.

Dr. David Cohen - Yeah.

Dr. David Ross - You know in the European study the amount they put into the outlet that was formula. There was a small amount (*inaudible) slightly irritating. You know we have 3 uses, I believe listed in the use table in this area in eye area.

Dr. David Cohen - OK.

Dr. David Ross - The question would.

Dr. David Cohen - But. Look at the draft reports frequently are devoid of data, and we put out an IDA and we either get nothing or we get a flood of information on it. So. Well, I mean. This is not this is not a unique circumstance.

Dr. Tom Slaga - No.

Dr. David Cohen - So you want to go out for an IDA for more concentration for the ocular data or further information about that Australian Study?

Dr. Susan Tilton - I would support that. I mean, I made note as a lack of the lack of details for those studies too. And especially as it relates to Trisodium.

Dr. David Cohen - We're all the uses.

Dr. Tom Slaga - Yeah.

Dr. David Cohen - OK.

Dr. David Ross - I guess if you if you look at that you know I'm having a little bit of bias remorse here. So if you, if you if we get that Australian study concentration right or someone comes back with a (*inaudible) test or rabbit test denies. We actually don't know what the concentration of use. The trisodium is.

Dr. Tom Slaga - If we don't get any data. What do we do? When can we go out as safe when formulated to be non irritating? If slight hear your irritation is a problem? I don't think we went out for only related for the eye though. That's the.

Dr. David Ross - Yeah.

Dr. Tom Slaga - Have we? I don't think so.

Dr. David Cohen - Monice is there a an eye provision?

Monice Fiume (CIR) - So safe, when formulated to be non-irritating, it covers both skin and eye. The discussion may address where the concern lies. Susan and **Dr. David Ross** I do want to part of I know that there is no reported concentration of use right now and that some of the uses listed for the one don't reflect in the other. If a report goes safe as used, we do know if an ingredient we can know that concentration of use were not received for the other ingredient. But the assumption is that it's used at the same concentrations of use as the ingredient that is in use, and that's what's being evaluated.

Dr. David Ross – Monice, we don't have a concentration for either.

Dr. Wilma Bergfeld - On the eye.

Dr. Susan Tilton C - Ocular.

Monice Fiume (CIR) - So that.

Dr. David Cohen - Right, but it.

Monice Fiume (CIR) - You can you can ask for that information, right?

Priya Cherian (CIR)- Yeah.

Dr. David Cohen - It wouldn't exceed.

Priya Cherian (CIR)- I don't know if this will help, but I was looking at the study on ECA on trisodium ethylenediamine and it's as details on test material. It says white solid. So I'm assuming that that's going to be the unchanged product and it says no vehicle. So it's probably undiluted powder.

Dr. David Cohen - For which chemical?

Priya Cherian (CIR)- trisodium ethylenediamine disuccinate.

Dr. David Cohen - Ohh. well, David, that is that change your concern?

Dr. David Ross - Was that the Australian study?

Priya Cherian (CIR)– It was the study on ECHA, the ECHA study.

Dr. David Ross - Yeah, that's when they use the small right? 4 milligrams?

Dr. David Cohen - She talking about four milligrams of neat material.

Priya Cherian (CIR)- It's 55 milligrams.

Dr. David Ross - Yeah.

Dr. David Cohen - And that's.

Dr. David Ross – 55 milliograms? On you said?

Priya Cherian (CIR)- On the trisodium ethylenediamine the ECHA data.

Dr. David Ross - 55 milligrams.

Priya Cherian (CIR)- Yes.

Dr. David Ross - Well, I think, yeah, I think if we have that and I think if it's neat material, yeah, probably would change my conclusion, yeah.

Priya Cherian (CIR)- I can edit the study to say, unchanged substance the powder was installed into the eye.

Dr. David Ross - OK.

Dr. David Cohen - I unchanged or undiluted or?

Priya Cherian (CIR)– (*inaudible).

Dr. David Ross – (*inaudible).

Dr. David Cohen - OK. So has this report taken a change of fortune here and gone to safe as used?

Dr. David Ross - I think it's.

Dr. Susan Tilton - Yes, if we.

Dr. David Cohen - A reversal.

Dr. Susan Tilton - I'm going to say if we can put it in the context that Monice stated. That for the

Tetrasodium ingredient we are assuming similar use and concentrations as trisodium.

Dr. David Ross - Yeah, that would be OK with me. And I think better conclusion. They're safe as used when formulated to be non irritating. There was a lovely, there was a lovely comment in one of the discussions from Ron Shank, who said, yeah, we're just going to formulate these things to be safe as used to be non-toxic and we can all go home. I mean, I don't know which. I mean, it was a lovely comment from Ron, yeah.

Dr. David Cohen - That is great. OK. So we're going to go out with safe as used. We have the provisions already discussed. Tom any many closers comments on this? Tom, anything, are you OK with the safe as used, Tom?

Dr. Tom Slaga - Oh yes.

Dr. David Cohen - OK.

Priya Cherian (CIR)- Doctor Cohen, so in the discussion, do you want me to make a comment about the fumaric acid as an impurity and how that might be a contact sensitizer or do you not want to address them?

Dr. David Cohen - Well, I guess the question I had was, will fumaric acid become a fumaric acid Ester?

Under use concentration under use regular use. And David, I got the impression that that's not going to happen.

Dr. David Ross - I don't know this conditions of the you know, I guess it going to the Esther and the biological conditions, I don't know happen. I suppose enzymatically but look I'd have to look that up.

Dr. David Cohen - OK, we.

Dr. David Ross - Susan.

Dr. Susan Tilton - And we do have. Some dermal irritation studies that would suggest that it is non irritating.

Dr. David Cohen - It's the tetrasodium, though, that has the Fumaric acid and we have our dermal data on. Ohh.

Dr. Susan Tilton - There is for both.

Dr. David Cohen - Yeah. Just looking back

Priya Cherian (CIR)- There's animal sensitization data for?

Dr. David Cohen - Yeah. Right. That's what I was looking at. I'm going back to these very nice tables.

Yeah, the only the human was the only one we had for humans were tested for trisodium. OK, I think we can go out with this and maybe Pryia, I'll ask the Belsito team what their thoughts are about that further discussion. Fermeric acid. OK. I don't think we have to. We don't have to decide that now. I think we'll go out with safe as used and I'll open it up for commentary and. We'll get collaborative discussion going on that.

Full Panel – September 27, 2022

Dr. Wilma Bergfeld - All right. Any other points of discussion? Hearing none, all those opposed? Abstaining? Approved as an IDA. All right, moving on to the last chemical and this particular advancing group, Doctor Cohen, that Trisodium Ethylenediamine Disuccinate.

Dr. David Cohen - And Trisodium Ethylenediamine Disuccinate. This is the first time we're reviewing this. And it's these two safety ingredients which these derived ingredients, which are used as chelating agents. We have reported uses from the 2022 VCRP. We have Max concentration of .64% for trisodium ethylene diamine disuccinate. .64% and .56 in a moisturizing product of node in 2002. The panel, published in safety assessment on EDTA. A similar to trisodium ethylenediamine disuccinate. We received in Wave 2 a reference for method of manufacturing. And we came to a conclusion that these were safe as used.

Dr. Don Belsito - 2nd.

Dr. Wilma Bergfeld -2nd. Any comment or discussion points to be made here?

Dr. Don Belsito - Yeah, this is the type of data we should get when we first look at a cosmetic ingredient.

Dr. Curtis Klaassen - Wishful thinking?

Dr. Wilma Bergfeld - That your comment is that your comment, Don.

Dr. Don Belsito - Yes, that's my comment. Thank you. Really helps us.

Dr. Wilma Bergfeld - Yeah, really helps. Any other comments? I'm sorry. I'll cut someone off.

Dr. Curtis Klaassen - I just said wishful thinking.

Dr. Wilma Bergfeld - Yeah, yes, yes, another wave 2,3,4,5. Alright, come to the point of voting all those opposed? Abstaining? Approve. Safe. OK. Now moving on to the next very large group. We have the coral xylenol. Doctor Belsito. That's a rereview.

Safety Assessment of Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate as Used in Cosmetics

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ABBREVIATIONS

CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
CIR	Cosmetic Ingredient Review
CLP	classification, labeling, and packaging
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
DART	Developmental and Reproductive Toxicity
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
HRIPT	human repeated insult patch test
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
Log K_{ow}	n-octanol/water partition coefficient
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOEL	no-observed-effect-level
NOAEL	no-observed-adverse-effect-level
NR	none reported
OECD	Organisation for Economic Cooperation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
TG	test guidelines
VCRP	Voluntary Cosmetic Registration Program
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook*

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate as used in cosmetics. These ingredients are reported to function in cosmetics as chelating agents. The Panel reviewed the available data to determine the safety of these ingredients, and concluded that Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate are safe in cosmetics in the present practices of use and concentration as described in the safety assessment.

INTRODUCTION

This assessment reviews the safety of Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), both of these ingredients are reported to function as chelating agents in cosmetics (Table 1).[1]

These ingredients are being grouped together due to structural similarities as amine succinate dimers. In 2002, the Panel published a safety assessment on EDTA (a structural isomer of Trisodium Ethylenediamine Disuccinate) and its salts.[2] These ingredients were considered to be safe as used in cosmetics. In 2019, the Panel reaffirmed the original conclusion of safety. The full report on these ingredients can be accessed on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/ingredients). In addition, it should be noted that residual monomers present as impurities in Tetrasodium Iminodisuccinate (i.e., Disodium Fumarate[3] and Sodium Aspartate[4]; see the Impurities section) are cosmetic ingredients that have been previously reviewed by the Panel and considered safe as used in cosmetics.

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

Much of the data included in this safety assessment were found on the European Chemicals Agency (ECHA)[5] and National Industrial Chemicals Notification and Assessment Scheme (NICNAS; now known as the Australian Industrial Chemicals Introduction Scheme)[6,7] websites. Please note that the ECHA and NICNAS websites provide summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when ECHA or NICNAS is cited.

CHEMISTRY

Definition and Structure

The ingredients in this report are structurally related as amine succinate dimers (Figures 1 and 2). The primary structural difference, therein, is the presence of an ethylamine bridge, present only in Trisodium Ethylenediamine Disuccinate. These structural similarities ("disuccinates") confer the ability to act as chelating agents. The definitions and CAS Nos. of the ingredients included in this review are provided in Table 1.

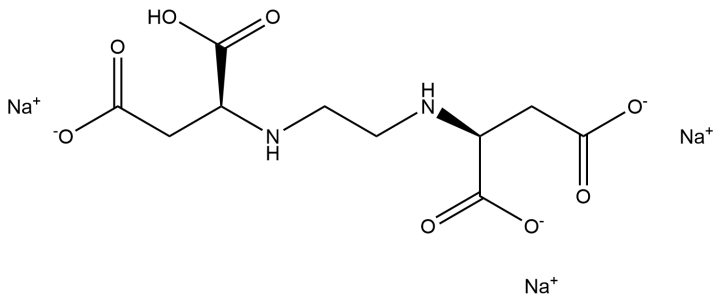


Figure 1. Trisodium Ethylenediamine Disuccinate

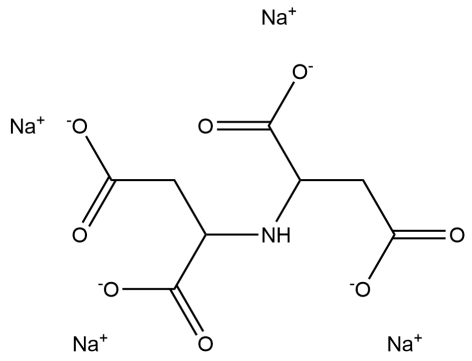


Figure 2. Tetrasodium Iminodisuccinate

Chemical Properties

Both of these ingredients are water-soluble solids. For example, Trisodium Ethylenediamine Disuccinate (CAS Nos.: 178949-82-1; 474787-13-8) is a solid, granular ingredient, that is highly water-soluble, with a reported water solubility of ≥ 1000 g/l (at 20° C and pH 7), and a low octanol/water partition coefficient (-4.7).[5,7] Other physical and chemical properties of Trisodium Ethylenediamine and Tetrasodium Iminodisuccinate (CAS No.: 144538-83-0) can be found in Table 2.

Method of Manufacture

The method below is general to the processing of Tetrasodium Iminodisuccinate. No methods specific to cosmetic ingredient manufacture were found in the literature or submitted as unpublished data.

Tetrasodium Iminodisuccinate

Tetrasodium Iminodisuccinate is synthesized from maleic anhydride, ammonia water, and sodium hydroxide.[8]

Impurities

Trisodium Ethylenediamine Disuccinate

According to a NICNAS safety assessment, Trisodium Ethylenediamine Disuccinate has a purity of 93%.[7] Impurities of this ingredient were reported to be ethylene dibromide (< 0.0000001%) and aspartic acid (3.95%).

Tetrasodium Iminodisuccinate

A NICNAS safety assessment on Tetrasodium Iminodisuccinate reported a 72.1% purity level for this ingredient.[6] Impurities present in this ingredient include residual monomers fumaric acid, disodium salt (5.6%), aspartic acid, disodium salt (10.6%), and water (8.9%).

USE

Cosmetic

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

According to 2022 VCRP survey data, Trisodium Ethylenediamine Disuccinate is used in 228 formulations (68 leave-on formulations and 160 rinse-off formulations; Table 3) and Tetrasodium Iminodisuccinate is used in 9 formulations (4 leave-on formulations and 5 rinse-off formulations).[9] The results of the concentration of use survey conducted by the Council in 2021 indicate that the reported maximum concentration of use is 0.64% Trisodium Ethylenediamine Disuccinate in tonics, dressings, and other hair grooming aids; the greatest reported maximum concentration of use in products intended

for dermal contact is 0.56% in moisturizing products.[10] No concentration of use data were reported for Tetrasodium Iminodisuccinate.

Incidental ingestion and mucous membrane exposure may occur as Trisodium Ethylenediamine Disuccinate is reported to be used in lipsticks at 0.01% and in bath soaps and detergents at up to 0.19%. In addition, Trisodium Ethylenediamine Disuccinate is reported to be used in baby products (e.g., baby shampoos at 0.19%).

Trisodium Ethylenediamine Disuccinate is used in cosmetic sprays and could possibly be inhaled (e.g., Trisodium Ethylene Disuccinate is used in pump hair spray formulations at 0.039%). 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.

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.

Both ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[11]

Non-Cosmetic

Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate are reported to have several industrial uses. These uses include plant protection products and washing and cleaning products (e.g., detergents, stain removers, kitchen cleaners).[5,12] Tetrasodium Iminodisuccinate is exempt from the requirement of a tolerance for residues when used as an inert ingredient in antimicrobial pesticide products for which, when ready for use, the end-use concentration does not exceed 5000 ppm Tetrasodium Iminodisuccinate.[13] Trisodium Ethylenediamine Disuccinate is exempt from the requirement of a tolerance for residues when used as an inert ingredient (sequestrant or chelating agent) in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest under Environmental Protection Agency (EPA) regulations.[14]

TOXICOKINETIC STUDIES

Details regarding the dermal and oral toxicokinetic studies on Trisodium Ethylenediamine Disuccinate summarized below can be found in Table 4.

A dermal toxicokinetic assay was performed in Crl:(WI)BR rats with [^{14}C]labeled Trisodium Ethylenediamine Disuccinate (4.14 mg/kg bw in males and 5.12 mg/kg bw in females).[5] Approximately 11.1% and 5% of the applied dose was absorbed in males and females, respectively. The amount of radioactivity detected in organs ranged from 0 to 1.2%. In an oral toxicokinetic assay, [^{14}C] labeled Trisodium Ethylenediamine Disuccinate was given to Crl:(WI)BR rats, via gavage. The majority of the radioactivity was excreted (62% in males and 71% in females) via feces within 24 h following administration. The combined mean radioactivity content of blood and tissue was 0.136% and 0.153% of the administered dose in male and female rats, respectively. In a toxicokinetic assay evaluating the distribution of [^{14}C] labeled Trisodium Ethylenediamine Disuccinate, female Wistar rats were given 2053 mg/kg bw of the test substance (via gavage), and tissues (blood, liver, kidneys, ovaries, and bone marrow) were evaluated at time intervals up to 72 h post-exposure. Radioactivity was detected at low levels in all tissues analyzed, with the highest level found in the kidneys 8 h post-administration (26 µg/g). A similar assay was performed in male Wistar rats. Animals were given [^{14}C] labeled Trisodium Ethylenediamine Disuccinate at a dose of 2106 mg/kg bw, via gavage. Peak levels of radioactivity in the testes, kidneys, liver, and bone marrow were 6.8, 42, 27, and 37 µg/g tissue, respectively.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Details regarding the acute dermal, oral, and inhalation toxicity studies summarized below can be found in Table 5.

Acute dermal LD_{50}s of > 2000 mg/kg bw and > 2640 mg/kg bw were established for rats and rabbits, respectively, given Trisodium Ethylenediamine Disuccinate.[5,7] The median lethal dose (LD_{50}) was reported to be > 2000 mg/kg bw in an acute dermal toxicity assay performed in rats using Tetrasodium Iminodisuccinate.[6] In acute oral toxicity assays using Trisodium Ethylenediamine Disuccinate performed in Wistar rats and CD-1 rats, the oral LD_{50}s were reported to be > 2000 mg/kg bw and > 2700 mg/kg bw, respectively. An LD_{50} of > 2000 mg/kg bw was established in an acute oral toxicity study evaluating a 20% solution of Tetrasodium Iminodisuccinate in Wistar rats. In addition, the acute inhalation toxicity potential of Trisodium Ethylenediamine Disuccinate was evaluated in Sprague-Dawley rats via full-body inhalation methods (4-h exposure period). The median lethal concentration was reported to be > 1490 mg/m^3 air.

Short-Term and Subchronic Toxicity Studies

Details regarding the short-term and subchronic oral toxicity studies summarized below can be found in Table 6.

In one 14-d study in which male Wistar rats were treated with Trisodium Ethylenediamine Disuccinate (up to 1250 mg/kg bw/d) in the diet, no deaths or signs of toxicity were observed.[5,7] However, in another 14-d assay in which Wistar rats were given up to 5000 mg/kg bw/d Trisodium Ethylenediamine Disuccinate in the diet, a no-observed-adverse-effect-level (NOAEL) was determined to be 500 mg/kg bw/d, due to clinical signs of toxicity (e.g., loss of body weight, diarrhea, sedation) observed at higher doses. No signs of clinical toxicity were observed in male Wistar rats given up to 400 mg/kg bw/d of a 42.3% aqueous solution of Trisodium Ethylenediamine Disuccinate, in the diet, for 28 d. In a 90-d assay, Wistar Han rats were given the same test substance as above, in the diet, in doses of up to 1000 mg/kg bw/d. The NOAEL was determined to be 300 mg/kg bw/d due to increased incidence of single cell death and fatty infiltration in the pancreas, and decreases in plasma zinc, copper, and magnesium levels at higher dose levels. In a 28-d oral toxicity assay, Tetrasodium Iminodisuccinate was given to Wistar rats, via gavage, at doses up to 1000 mg/kg bw/d.[6] A no-observed-effect-level (NOEL) of 200 mg/kg bw/d was established due to lower motor activity observed in high-dose males.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Details regarding the oral developmental and reproductive toxicity studies on Trisodium Ethylenediamine Disuccinate summarized below can be found in Table 7.

The potential developmental and reproductive toxicity of Trisodium Ethylenediamine Disuccinate was evaluated in several assays performed in rats. In one assay, male and female Sprague-Dawley rats were treated with up to 700 mg/kg bw/d Trisodium Ethylenediamine Disuccinate via gavage for 70 d.[5,7] No signs of toxicity were noted in maternal rats, paternal rats, or fetuses. Developmental toxicity was evaluated in Sprague-Dawley rats given up to 994 mg/kg bw/d Trisodium Ethylenediamine Disuccinate on gestation days 6 - 15, via diet. A dose-dependent decrease in blood zinc levels were observed. The NOAEL was determined to be 551 mg/kg bw/d for both maternal and developmental toxicity in this assay due to clinical signs of toxicity, post implantation losses, and fetus malformation at high doses. However, no signs of maternal or developmental toxicity were observed in an assay performed in female Sprague-Dawley rats given up to 1000 mg/kg bw/d Trisodium Ethylenediamine Disuccinate on gestation days 6 - 15 via gavage. In a different assay performed in female Sprague-Dawley rats, animals were given up to 1000 mg/kg bw/d Trisodium Ethylenediamine Disuccinate, via gavage, on gestation days 6 - 15. No treatment-related, statistically significant changes in hematological parameters (plasma, copper, iron, zinc) were observed. An NOAEL of 400 mg/kg bw/d was established for maternal toxicity, due to clinical signs of toxicity observed at higher doses, and an NOAEL of 1000 mg/kg bw/d was determined for developmental toxicity. Several signs of maternal toxicity (e.g., emaciation, resorptions, decreased weight gain) was observed in female Sprague Dawley rats treated with up to 40,000 ppm Trisodium Ethylenediamine Disuccinate, via the diet, on gestation days 6 - 15. The NOAEL for maternal toxicity in this assay was determined to be 8000 ppm (approximately 530 mg/kg bw/d). In an assay evaluating the reproductive effects in both male and female Wistar Han rats, animals were treated with up to 1000 mg/kg bw/d Trisodium Ethylenediamine Disuccinate, via diet, for 90 d. No effects on the duration of the estrous cycle were observed in females; however, male rats treated with 1000 mg/kg bw/d displayed an increase in the number of abnormal sperm.

GENOTOXICITY STUDIES

Details regarding the in vitro and in vivo genotoxicity studies that are summarized below can be found in Table 8.

No mutagenicity was observed in Ames assays performed on Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate (both tested at up to 5000 µg/plate; with and without metabolic activation).[5-7] Positive results were observed in an in vitro mammalian chromosome aberration assay on Trisodium Ethylenediamine Disuccinate (up to 5000 µg/plate; 34% aqueous solution), when Chinese hamster ovary cells were incubated without metabolic activation for 42 h, at concentrations as low as 20 µg/ml. However, no mutagenicity was observed in the same assay when metabolic activation was used, or at shorter incubation times. Similarly, no mutagenicity was observed in an in vitro mammalian cell gene mutation assay on Trisodium Ethylenediamine Disuccinate (up to 5028 µg/ml; with and without metabolic activation; 34% aqueous solution). Both Trisodium Ethylenediamine Disuccinate (up to 2000 mg/kg bw; 42.3% aqueous solution; gavage administration) and Tetrasodium Iminodisuccinate (up to 1500 mg/kg bw; intraperitoneal injection administration) were considered to be non-clastogenic in a mammalian erythrocyte micronucleus assay and mammalian bone marrow chromosome aberration assay, respectively.

CARCINOGENICITY STUDIES

Carcinogenicity data were not found in the published literature, and unpublished data were not submitted.

DERMAL IRRITATION AND SENSITIZATION

Details regarding the animal and human dermal irritation and sensitization data that are summarized below can be found in Table 9.

Two dermal irritation assays were performed using Trisodium Ethylenediamine Disuccinate in New Zealand white rabbits, under semi-occlusive conditions.[5,7] The test substance was not considered to be irritating in either study. No irritation was observed in a dermal irritation assay evaluating the irritation potential of Tetrasodium Iminodisuccinate in male Himalayan white rabbits.[6] No irritation was observed in a repeat patch test performed in 12 subjects using an aqueous solution of Trisodium Ethylenediamine Disuccinate (up to 29.41%; occlusive conditions; 24-h applications). Dermal sensitization assays were performed in albino Himalayan spotted guinea pigs using either a 50% aqueous solution of Trisodium Ethylenediamine Disuccinate, or 100% Trisodium Ethylenediamine Disuccinate moistened with water (occlusive conditions).[5,7] Both test substances were considered to be non-sensitizing; however, slight confluent erythema was observed 24 h after the challenge application in one animal treated with 100% Trisodium Ethylenediamine Disuccinate. No sensitization was observed in a guinea pig maximization assay performed using Tetrasodium Iminodisuccinate (1% intradermal injection; 25% dermal induction; 20% dermal challenge).[6] No irritation or sensitization was observed in an HRIPT performed in 111 subjects using a 5% aqueous solution of Trisodium Ethylenediamine Disuccinate, under occlusive conditions.

OCULAR IRRITATION STUDIES

Animal

Trisodium Ethylenediamine Disuccinate

In an ocular irritation assay evaluating the irritation potential of Trisodium Ethylenediamine Disuccinate performed according to OECD TG 405, the unchanged test substance was instilled into the eyes of 3 New Zealand White rabbits, and the animals were observed for 7 d.[5,7] The overall irritation score was reported to be 0.56/13, and the test substance was considered to be slightly irritating. In a different ocular irritation assay performed using Trisodium Ethylenediamine Disuccinate according to the same procedures as above, the mean irritation score was reported to be 0/110.[5]

Tetrasodium Iminodisuccinate

An ocular irritation assay was performed using Tetrasodium Iminodisuccinate in Himalayan white rabbits (n = 3), according to OECD TG 405.[6] The test substance was considered to be non-irritating to the eye (mean irritation score of 0). No details regarding this study were provided.

SUMMARY

The safety of Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate is reviewed in this safety assessment. According to the *Dictionary*, these ingredients are reported to function as chelating agents in cosmetics.

According to 2022 VCRP data, Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate are reported to be used in 228 and 9 total formulations, respectively. The results of the concentration of use survey conducted by the Council indicate Trisodium Ethylenediamine Disuccinate is used at up to 0.64% in tonics, dressings, and other hair grooming aids; the greatest reported maximum concentration of use in products intended for dermal contact is 0.56% in moisturizing products. No concentrations of use were reported for Tetrasodium Iminodisuccinate.

A dermal toxicokinetic assay was performed in Crl:(WI)BR rats using [^{14}C]labeled Trisodium Ethylenediamine Disuccinate. Approximately 11.1% and 5% of the radioactivity was absorbed in males and females, respectively. In an oral toxicokinetic assay, [^{14}C] labeled Trisodium Ethylenediamine Disuccinate was given to Crl:(WI)BR rats. The majority of the radioactivity was excreted (62% in males and 71% in females) via feces, within 24 h following administration. In a toxicokinetic assay evaluating the distribution of [^{14}C] labeled Trisodium Ethylenediamine Disuccinate in female Wistar rats, radioactivity was detected at low levels in all tissues analyzed, with the highest level of radioactivity found in the kidneys 8 h post-administration (26 µg/g tissue). A similar assay was performed in male Wistar rats. Peak levels of radioactivity in the testes, kidneys, liver, and bone marrow were 6.8, 42, 27, and 37 µg/g tissue, respectively.

Acute dermal LD_{50}s of > 2000 mg/kg bw and > 2640 mg/kg bw was established for rats and rabbits given Trisodium Ethylenediamine Disuccinate, respectively. The LD_{50} was reported to be > 2000 mg/kg bw in an acute dermal toxicity assay performed in rats using Tetrasodium Iminodisuccinate. In acute oral toxicity assays using Trisodium Ethylenediamine Disuccinate performed in Wistar rats and CD-1 rats, the oral LD_{50}s were reported to be > 2000 mg/kg bw and > 2700 mg/kg bw, respectively. An LD_{50} of > 2000 mg/kg bw was established in an acute oral toxicity study evaluating a 20% solution of Tetrasodium Iminodisuccinate in Wistar rats. In addition, the acute inhalation toxicity potential of Trisodium Ethylenediamine Disuccinate was evaluated in Sprague-Dawley rats via full-body inhalation (4-h exposure period) methods. The median lethal concentration was reported to be > 1490 mg/m^3 air.

No toxicity was observed in a 14-d oral toxicity assay using Male Wistar rats treated with Trisodium Ethylenediamine Disuccinate (up to 1250 mg/kg bw/d). However, in a different 14-d oral toxicity assay performed in Wistar rats given up to 5000 mg/d Trisodium Ethylenediamine Disuccinate, the NOAEL was determined to be 500 mg/kg bw/d due to clinical signs of toxicity observed at higher doses. No signs of clinical toxicity were observed in male Wistar rats given up to 400 mg/kg bw/d of a 42.3% aqueous solution of Trisodium Ethylenediamine Disuccinate, in the diet, for 28 d. In a 90-d assay, Wistar

Han rats were given the same test substance as above, in the diet, in doses of up to 1000 mg/kg bw/d. The NOAEL was determined to be 300 mg/kg bw/d due to increased incidence of single cell death, fatty infiltration in the pancreas, and decreases in plasma zinc, copper, and magnesium levels at higher dose levels. In a 28-d oral toxicity assay, Tetrasodium Iminodisuccinate was given to rats, via gavage, at up to 1000 mg/kg bw/d. An NOEL of 200 mg/kg bw/d was established due to lower motor activity observed in high-dose males.

The potential developmental and reproductive toxicity of Trisodium Ethylenediamine Disuccinate was evaluated in several assays performed in rats. No signs of paternal, maternal, or fetal toxicity was observed in an oral reproductive toxicity assay using Sprague-Dawley rats treated with up to 700 mg/kg bw/d Trisodium Ethylenediamine Disuccinate. Reproductive toxicity was evaluated in Sprague-Dawley rats given up to 994 mg/kg bw/d Trisodium Ethylenediamine Disuccinate on gestation days 6 - 15, via diet. The NOAEL was determined to be 551 mg/kg bw/d for both maternal and developmental toxicity. No signs of maternal or reproductive toxicity were observed in an assay performed in female Sprague-Dawley rats given up to 1000 mg/kg bw/d Trisodium Ethylenediamine Disuccinate on gestation days 6 - 15 via gavage. In a different assay performed in female Sprague-Dawley rats, animals were given up to 1000 mg/kg bw/d Trisodium Ethylenediamine Disuccinate, via gavage, on gestation days 6 - 15. An NOAEL of 400 mg/kg bw/d was established for maternal toxicity due to clinical signs of toxicity observed at higher doses and an NOAEL of 1000 mg/kg bw/d was determined for developmental toxicity. Several signs of maternal toxicity were observed in female Sprague Dawley rats treated with up to 40,000 ppm Trisodium Ethylenediamine Disuccinate via the diet, on gestation days 6 - 15. The NOAEL for maternal toxicity in this assay was determined to be 8000 ppm (approximately 530 mg/kg bw/d). In an assay evaluating the reproductive effects in both male and female Wistar Han rats, animals were treated with up to 1000 mg/kg bw/d Trisodium Ethylenediamine Disuccinate, via diet. No effects on the duration of the estrous cycle were observed in females; however, male rats treated with 1000 mg/kg bw/d displayed an increase in the number of abnormal sperm.

No mutagenicity was observed in Ames assays performed on Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate (both at up to 5000 µg/plate; with and without metabolic activation). Positive results were observed in an in vitro mammalian chromosome aberration assay on Trisodium Ethylenediamine Disuccinate (up to 5000 µg/plate; 34% aqueous solution), when Chinese hamster ovary cells were incubated without metabolic activation for 42 h. No mutagenicity was observed in the same assay when metabolic activation was used, or at lower incubation times. Similarly, no mutagenicity was observed in an in vitro mammalian cell gene mutation assay on Trisodium Ethylenediamine Disuccinate (up to 5028 µg/plate; with and without metabolic activation; 34% aqueous solution). Both Trisodium Ethylenediamine Disuccinate (up to 2000 mg/kg bw; 42.3% aqueous solution; gavage administration) and Tetrasodium Iminodisuccinate (up to 1500 mg/kg bw; intraperitoneal injection administration) were considered to be non-clastogenic in a mammalian erythrocyte micronucleus assay and mammalian bone marrow chromosome aberration assay, respectively.

No irritation was noted in two dermal irritation assays performed using Trisodium Ethylenediamine Disuccinate in New Zealand white rabbits, under semi-occlusive conditions. Similarly, no irritation was observed in a dermal irritation assay evaluating the irritation potential of Tetrasodium Iminodisuccinate in male Himalayan white rabbits. No irritation was observed in a repeat patch test performed in 12 subjects using an aqueous solution of Trisodium Ethylenediamine Disuccinate (up to 29.41%; occlusive conditions; 24-h applications). Dermal sensitization assays were performed in albino Himalayan spotted guinea pigs using either a 50% aqueous solution of Trisodium Ethylenediamine Disuccinate, or 100% Trisodium Ethylenediamine Disuccinate moistened with water (occlusive conditions). Both test substances were considered to be non-sensitizing; however, slight confluent erythema was observed 24 h after the challenge application in one animal treated with 100% Trisodium Ethylenediamine Disuccinate. No sensitization was observed in a guinea pig maximization assay performed using Tetrasodium Iminodisuccinate (1% intradermal injection; 25% dermal induction; 20% dermal challenge). No irritation or sensitization was observed in an HRIPT performed in 111 subjects using a 5% aqueous solution of Trisodium Ethylenediamine Disuccinate, under occlusive conditions.

Slight irritation was observed in an ocular irritation assay using Trisodium Ethylenediamine Disuccinate in New Zealand white rabbits; however, in a different ocular irritation assay performed in New Zealand white rabbits, using the same test substance, no irritation was reported. Tetrasodium Iminodisuccinate was considered to be non-irritating in an ocular irritation assay using Himalayan white rabbits.

DISCUSSION

This assessment reviews the safety of Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate as used in cosmetic formulations. The Panel reviewed the available impurities, systemic toxicity, dermal irritation and sensitization, and ocular irritation data, and determined Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate are safe in cosmetics in the present practices of use and concentrations described in this safety assessment.

The Panel noted mutagenicity in an in vitro mammalian chromosomal aberration assay performed on Trisodium Ethylenediamine Disuccinate. However, concern for this result was mitigated as mutagenicity was only observed under specific conditions (i.e., without metabolic activation, 42-h incubation), and several other in vitro and in vivo genotoxicity assays had negative results.

In addition, the Panel noted reproductive toxicity observed in assays performed in rats orally administered Trisodium Ethylenediamine Disuccinate. The Panel determined that these effects would not be relevant to cosmetic exposure due to the high doses/concentrations used in these studies.

The Panel discussed the issue of incidental inhalation exposure resulting from these ingredients (e.g., Trisodium Ethylenediamine Disuccinate is reported to be used in pump hair spray formulations at up to 0.039%). 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 for Cosmetic Ingredient Safety concluded that Trisodium Ethylenediamine Disuccinate and Tetrasodium Iminodisuccinate are safe in cosmetics in the present practices of use and concentration described in the safety assessment.

TABLES

Table 1. Definitions, structures, and reported functions[1, CIR STAFF]

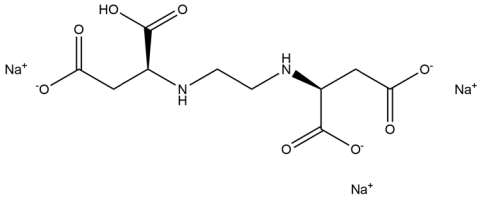
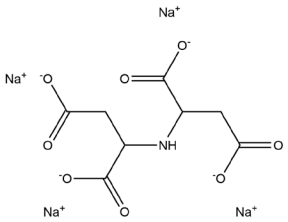
Ingredient (CAS No.)	Definition	Function
Trisodium Ethylenediamine Disuccinate (178949-82-1; 474787-13-8)	Trisodium Ethylenediamine Disuccinate is the organic compound that conforms to the formula: 	chelating agent
Tetrasodium Iminodisuccinate (144538-83-0)	Tetrasodium Iminodisuccinate is the organic compound that conforms to the formula: 	chelating agent

Table 2. Physical and chemical properties

Property	Value	Reference
Trisodium Ethylenediamine Disuccinate		
Physical Form	solid, granular	[5]
Density/Specific Gravity (@ 20ºC)	1.63	[5]
Vapor Density (mmHg)	0.014	[5]
Melting Point (ºC)	> 311	[5]
Water Solubility (g/l @ 20ºC & pH 7)	≥ 1000	[5]
Solubility in Organic Solvents (mg/100 g solvent @ 37 ºC)	≤ 0.4	[5]
log K_{ow}	< -4.7	[5]
Disassociation Constants (pK_a, pK_b) (@ 20 ºC)	7.5, 4	[5]
Mass median aerodynamic diameter (µm)	50 – 63	[5]
Tetrasodium Iminodisuccinate		
Physical Form	solid	[6]
Color	white	[6]
Melting Point (ºC) (est.)	336.12	[15]
Water Solubility (g/l @ 25ºC & pH 13.1)	564	[6]
Particle Size* (µm)	< 67 - < 281	[6]

**ingredient particle size does not necessarily translate to final formulation particle size*

Table 3. Frequency (2022)[9] and concentration (2021)[10] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Trisodium Ethylenediamine Disuccinate		Tetrasodium Iminodisuccinate	
Totals	**228**	**0.0039 – 0.64**	**9**	**NR**
summarized by likely duration and exposure*				
Duration of Use				
Leave-On	*68*	*0.01 – 0.64*	*4*	*NR*
Rinse-Off	*160*	*0.0039 – 0.51*	*5*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type*				
Eye Area	3	NR	NR	NR
Incidental Ingestion	NR	0.01	NR	NR
Incidental Inhalation-Spray	1; 12[a]; 19[b]	0.039; 0.12 – 0.64[b]	1[b]	NR
Incidental Inhalation-Powder	12[a]	0.06 – 0.19[c]	NR	NR
Dermal Contact	83	0.06 – 0.56	3	NR
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	75	0.0039 – 0.64	3	NR
Hair-Coloring	66	0.3 – 0.36	3	NR
Nail	NR	NR	NR	NR
Mucous Membrane	20	0.01 – 0.19	NR	NR
Baby Products	3	0.19	NR	NR
as reported by product category				
Baby Products				
Baby Shampoos	1	0.19		
Other Baby Products	2	NR		
Eye Makeup Preparations				
Eye Lotion	1	NR		
Other Eye Makeup Preparations	2	NR		
Fragrance Preparations				
Cologne and Toilet Water	1	NR		
Hair Preparations (non-coloring)				
Hair Conditioner	3	0.0039	2	NR
Hair Spray (aerosol fixatives)	NR	0.039		
Shampoos (non-coloring)	62	0.12 – 0.51		
Tonics, Dressings, and Other Hair Grooming Aids	7	0.34 – 0.64		
Other Hair Preparations	2	NR	1	NR
Hair Coloring Preparations				
Hair Dyes and Colors (all types requiring caution statements and patch tests)	66	NR	3	NR
Hair Rinses (coloring)	NR	0.3		
Hair Shampoos (coloring)	NR	0.36		
Makeup Preparations				
Blushers (all types)	3	NR		
Foundations	16	NR		
Lipstick	NR	0.01		
Makeup Bases	3	NR		
Other Makeup Preparations	5	NR		
Personal Cleanliness Products				
Bath Soaps and Detergents	9	0.12 – 0.19		
Douches	4	NR		
Other Personal Cleanliness Products	7	NR		
Shaving Preparations				
Aftershave Lotion			1	NR
Other Shaving Preparations	1	NR		
Skin Care Preparations				
Cleansing	5	0.093 – 0.14		
Face and Neck (exc shave)	11	0.06 – 0.8 (not spray)		
Body and Hand (exc shave)	1	0.078 – 0.19 (not spray)		
Moisturizing	9	0.12 – 0.56 (not spray)	1	NR
Night	3	0.074 (not spray)		
Paste Masks (mud packs)	2	0.063		
Skin Fresheners	NR	0.12		
Other Skin Care Preparations	2	0.074	1	NR
Suntan Preparations				
Other Suntan Preparations	NR	0.2 (not spray)		

NR – not reported

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

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

[a] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

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

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

Table 4. Toxicokinetic studies[5]

Test Substance	Animals	No./Group	Vehicle/Dose	Dose/Protocol	Results
			DERMAL		
^{14}C-labeled Trisodium Ethylenediamine Disuccinate	Crl: (WI)BR rats	5/sex	water; males: 4.14 mg/kg bw (66.7 µCi/kg bw); females: 5.12 mg/kg bw (84.0 µCi/kg bw)	OECD TG 417; The test substance (0.2 ml) was applied to shaved skin (area of 7.6 cm^2) in a glass chamber for 72 h. Urine, feces, cage washing, and expired air were collected at intervals. Levels of radioactivity were determined in adipose tissue, brain, bone marrow, femur bones, gonads, heart, gastrointestinal tracts, gastrointestinal contents, kidneys, livers, lungs, muscle, pancreas. spleens, carcasses, and skin.	Following the 72-h exposure period, 11.1% of the applied dose was absorbed in males, and 5% of the applied dose was absorbed in females. The total recovery of radioactivity in males and females was 59.1% and 62.8%, respectively. These levels mainly represented unabsorbed test material found at the skin treatment site (~40%) and dermal chamber washings (~10%). The amount of radioactivity detected in organs and tissues ranged from 0 to 1.2% in the male carcass. The combined mean blood and tissue recovery was 2.34% in males and 1.45% in females. Approximately 9% of the applied dose was excreted in males, and about 4% of the applied dose was excreted in females, during the 72-h exposure period.
			ORAL		
^{14}C-labeled Trisodium Ethylenediamine Disuccinate	Crl: (WI)BR rats	5/sex	water; 2 -3 mg/kg bw (approximately 10 µCi/rat)	OECD TG 417; A single dose of the test substance was administered via gavage while in metabolism cages. Urine, feces, cage washes, and expired air were collected at intervals. At study termination (72 h after administration), blood samples were taken, select tissue and gastrointestinal contents were taken, and levels of radioactivity were determined via liquid scintillation counting.	In the first 24 h following administration, approximately 62% and 71% of the radioactivity was excreted in the feces in male and female rats, respectively. After 72 h, approximately 75% was excreted by male and female rats (primarily in the feces, with approximately 5% excretion in expired air and urine. At least 5% of the total administered dose was considered absorbed from the gastrointestinal tract, 72 h after administration. The combined mean radioactivity content of blood and tissue was 0.136% and 0.153% of the administered dose in male and female rats, respectively.
^{14}C-labeled Trisodium Ethylenediamine Disuccinate	Wistar rats	27 female rats	water; 2053 mg/kg bw	A single dose of the test substance was given to rats via gavage. Animals were necropsied at 2, 8, 15, 24, 32, 37, 48, 56, or 72 h post-exposure. Radioactivity was measured in the blood, liver, kidneys, ovaries, and bone marrow.	Radioactivity was detected at low levels in all tissues analyzed, peaking within 24-h post-administration. Highest levels of radioactivity were found in the kidney and liver (26 and 16, µg/g tissue, respectively), peaking within 8 h post-administration. Peak levels in the blood, plasma, ovaries, and bone marrow were 13, 9.4, 6.7, and 14 µg/g tissue, respectively.
^{14}C-labeled Trisodium Ethylenediamine Disuccinate	Wistar rats	3 male rats/group	water; 2106 mg/kg bw	A single dose of the test substance was given to rats via gavage. Animals were necropsied at 2, 8, 15, 24, 32, 37, 48, 56, or 72 h post-exposure. Radioactivity was measured in the blood, plasma, testes, kidneys, liver, and bone marrow.	Levels of radioactivity peaked in the liver, kidney, testes, and bone marrow between 15 – 32 h post-administration. Peak levels in the testes, kidneys, liver, and bone marrow were 6.8, 42, 27, and 37 µg/g tissue, respectively. Blood and plasma levels were relatively constant during the first 48 h (at around 7 and 11 µg/g tissue, respectively). These levels decreased to 4.5 µg/g by study termination.

OECD TG = Organisation for Economic Cooperation and Development test guidelines

Table 5. Acute toxicity studies

Test Article	Vehicle	Animals	No./Sex/Group	Concentration/Dose	Protocol	LD_{50}//LC_{50}/Results	Reference
DERMAL							
Trisodium Ethylenediamine Disuccinate	Water	Wistar rats	5	2000 mg/kg bw	OECD TG 402; test substance applied to clipped skin on back of rats; semi-occlusive dressing; 24 h exposure period; 14-d observation period	> 2000 mg/kg bw; no clinical signs of systemic toxicity, irritation at treatment site, body weight changes, macroscopic abnormalities, or deaths	5,7
Trisodium Ethylenediamine Disuccinate	Water	New Zealand White rabbits	5	2640 mg/kg bw	OECD TG 402; test substance applied to the dorso-lumbar region of rabbits under semi-occlusive conditions; 24 h exposure period; 14-d observation period	> 2640 mg/kg bw; no clinical signs of systemic toxicity, irritation at treatment site, body weight changes, macroscopic abnormalities, or deaths	5
Tetrasodium Iminodisuccinate	Water	Wistar rats	3	2000 mg/kg bw	Animals exposed to test substance under semi-occlusive conditions; exposure period length not stated	> 2000 mg/kg bw; no systemic effects or skin corrosivity	6
ORAL							
Trisodium Ethylenediamine Disuccinate	Water	Wistar rats	5	2000 mg/kg bw	OECD TG 401; test substance administered to animals via gavage; 14-d observation period	> 2000 mg/kg bw; no body weight changes, deaths, or macroscopic abnormalities	5,7
Trisodium Ethylenediamine Disuccinate	Water	CD-1 rats	5	2700 mg/kg bw	OECD TG 401; test substance administered to animals via gavage; 14-d observation period	> 2700 mg/kg bw; no deaths observed, pilo-erection in all animals noted for first few hours after dosing, enlarged cervical lymph nodes noted in five males and two females, nephrotic effects noted in several males	5
Tetrasodium Iminodisuccinate	NR	Wistar rats	3	20%; 2000 mg/kg bw	OECD TG 423; test substance administered to animals via gavage	> 2000 mg/kg	6
INHALATION							
Trisodium Ethylenediamine Disuccinate	Clean air	Sprague-Dawley rats	5	1490 mg/m^3	OECD TG 401; animals were exposed to air containing Trisodium Ethylenediamine Disuccinate via a whole-body chamber; 4 h exposure period	> 1490 mg/m^3 air	5

LC_{50} = median lethal concentration; LD_{50} = median lethal dose; NR = not reported; OECD TG = Organisation for Economic Cooperation and Development test guidelines

Table 6. Repeated dose oral toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Doses	Protocol	Results	Reference
Trisodium Ethylenediamine Disuccinate	Diet	Male Wistar rats (5/group)	14 d	0, 750, 1000, or 1250 mg/kg bw/d	Animals were treated via diet for 14 d. Observations for clinical toxicity were performed throughout study. Internal organs were macroscopically examined following necropsy.	No deaths or toxicity were observed. The NOAEL was determined to be 1250 mg/kg bw/d.	5
Trisodium Ethylenediamine Disuccinate	Diet	Wistar rats (5/sex/group)	14 d	0, 50, 500, 2500, or 5000 mg/kg bw/d	Animals were treated via diet for 14 d. Clinical signs of toxicity were observed throughout the study. Internal organs were macroscopically examined following necropsy.	Loss of body weight, reduced food and water consumption, diarrhea, and hunched posture, was seen in animals dosed with 2500 mg/kg bw/d and higher. One male treated with 5000 mg/kg bw/d died during the study. Sedation was observed in animals treated with 5000 mg/kg bw/d. No microscopic or macroscopic signs of toxicity were observed. The NOAEL was determined to be 500 mg/kg bw/d.	5
Trisodium Ethylenediamine Disuccinate (42.3% aqueous solution)	Diet	Male Wistar Han rats (5/group)	28 d	0, 50, 150, 300, or 400 mg/kg bw/d	OECD TG 407; Animals were treated via diet for 28 d and observed throughout the study for signs of toxicity. Urine and feces were collected over the last 3 d to analyze mineral content (calcium, sodium, potassium, magnesium, zinc, phosphorous, manganese, and copper). Blood was sampled before study termination. Ophthalmoscopic examinations were performed before study, and on day 21. Macroscopic and microscopic evaluations were performed following study termination.	No deaths were observed throughout the study, and no signs of clinical toxicity or adverse effects were noted upon necropsy. A dose-related, statistically-significant increase in the zinc content of urine was evident; however, this increase was compensated for by a decreased zinc output in feces in the 300 and 400 mg/kg bw/d treated groups. No other dose-dependent, statistically-significant changes in mineral levels were observed. The NOAEL was determined to be 400 mg/kg bw/d.	5,7
Trisodium Ethylenediamine Disuccinate (42.3% aqueous solution)	Diet	Wistar Han rats: -20/sex/group at 0 and 1000 mg/kg bw/d (10/sex/group kept for a 4-wk recovery period) -10/sex/group at 50, 300, and 700 mg/kg bw/d	90 d	0, 50, 300, 700, or 1000 mg/kg bw/d	Animals given the test substance via diet. Satellite groups of 10 animals/sex were given the control or high-dose diets for 90 d, and allowed to recover for 28 d. Animals were observed for toxicity throughout the study, and blood samples were analyzed at 4 and 13 wk (or 17 wk for the recovery groups). Ophthalmoscopic examinations took place at 4 and 13 wk (and at 17 wk in the recovery groups). Macroscopic and microscopic evaluations were performed following study termination.	No deaths were observed, and no treatment-related abnormalities were observed in ophthalmoscopic data, organ weights, urinalysis, or clinical chemistry. Increased incidence of single cell death and fatty infiltration in the pancreas was observed at 700 mg/kg bw/d and higher. At week 13, in animals treated with 1000 mg/kg bw/d, a significant decrease in plasma zinc levels in male and female animals, were observed, compared to controls. A significant reduction in plasma copper and magnesium levels were observed in male animals treated with 1000 mg/kg bw/d, at week 13, compared to controls. The NOAEL was determined to be 300 mg/kg bw/d. Reproductive toxicity parameters evaluated in this study can be found in Table 7.	5,7
Tetrasodium Iminodisuccinate	Water	Wistar rats (5/sex/group)	28 d	0, 40, 200, or 1000 mg/kg bw/d; two recovery groups treated with 0 or 10,000 mg/kg bw/d	OECD TG; 28-d exposure period (animals dosed 7d/wk); 14-d observation period for control and high-dosed rats	No deaths reported. Lower motor activity was observed in high-dose males. A reduction in levels of alanine aminotransferase was observed in high-dose males, but this effect was not dose-dependent. High-dose recovery animals displayed lower relative thymus weights upon necropsy; however, this effect was not observed in the high dose treatment group. No histopathological changes were noted. An NOEL of 200 mg/kg bw/d was determined due to low motor activity at the highest dose.	6

NOEL = no-observed-effect-level; NOAEL = no-observed-adverse-effect-level; OECD TG = Organisation for Economic Cooperation and Development test guidelines

Table 7. Oral developmental and reproductive toxicity studies

Ingredient	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Trisodium Ethylenediamine Disuccinate	Water	Sprague-Dawley rats (25/sex/group)	0, 90, 250,or 700 mg/kg bw/d	Animals were treated with the test substance via gavage for 70 d before mating, and throughout mating, pregnancy, and weaning. Animals were observed for mortality, clinical signs of toxicity, body weight gain, feed consumption, changes in the estrus cycle, precoital index, mating index, fertility, gestation index, number and sex of offspring, litter size and viability, and lactation index. Culling of several of the offspring was performed 4 d after birth, and these offspring were evaluated for external and internal abnormalities, as well as hydrocephaly. Post-necropsy evaluations were performed 28 d after delivery. Reproductive organs were weighed and sperm evaluations were performed.	No signs of developmental or reproductive toxicity were observed in any treated group. Zinc levels in serum were elevated in males in all treatment groups, and in highest-dosed females. The NOAEL was determined to be 700 mg/kg bw/d.	[5]
Trisodium Ethylenediamine Disuccinate	Diet	Female Sprague-Dawley rats (34 rats/group)	0, 132, 551, or 994 mg/kg bw/d	Pregnant female rats were given the test substance on gestation days 6 to 15. Four rats per group (satellite group) were killed on gestation day 16 for blood analysis of zinc, iron, and copper. The remaining animals were killed on gestation day 20. Animals were observed for gross abnormalities, and the uterus was examined for fetuses, implantations, resorptions, and corpora lutea. Fetuses were weighed and examined.	Reduced body weight gain and food consumption was observed in maternal rats treated with 994 mg/kg bw/d. A dose-dependent decrease in blood zinc levels was observed (statistically significant at doses of 551 and 994 mg/kg bw/d). Mean gravid uterine weights were significantly reduced in animals treated with 994 mg/kg bw/d. A statistically significant increase in post-implantation losses was observed in the high-dose group, reducing the number of live male fetuses. Fetuses in the high-dose group had a range of external, visceral, and/or skeletal malformations and developmental variations. The NOAEL was determined to be 551 mg/kg bw/d for both maternal and developmental toxicity.	[5,7]
Trisodium Ethylenediamine Disuccinate	Water	Female Sprague-Dawley rats (9/group)	0, 50, 200, 400, 600, or 1000 mg/kg bw/d	Pregnant females were given the test substance via gavage on gestation days 6-15. Three rats from each group were killed on gestation day 16, and blood samples were analyzed for zinc, copper, and iron. Remaining animals were killed on gestation day 20, and evaluated for gross abnormalities, viable fetuses, resorptions, implantations, and corpora lutea. Fetuses were weighed and examined for soft-tissue and skeletal effects.	No evidence of treatment-related maternal or developmental toxicity was observed. The maternal and developmental NOAEL was determined to be 1000 mg/kg bw/d.	[5]

Table 7. Oral developmental and reproductive toxicity studies

Ingredient	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Trisodium Ethylenediamine Disuccinate	Water	Female Sprague-Dawley rats (36/group)	0, 50, 400, or 1000 mg/kg bw/d	Animals treated according to the same procedure as above; however, 6 animals/group were killed on gestation day 16	Rats treated with 1000 mg/kg bw/d displayed reduced carcass weight, a decrease in food consumption during treatment period, increased incidence of soft stools, and decreased defecation. No treatment-related statistically significant changes in plasma, copper, iron, or zinc levels were observed. No differences were noted in treatment and control groups regarding implantations, number of fetuses, post-implantation losses, or fetal body weights. Various skeletal defects were observed in fetuses of dams treated with 1000 mg/kg bw/d (presacral vertebrae, unossified sternebrae, 7th cervical rib). These variations were interpreted as evidence of developmental delay, and not as permanent malformations. An NOAEL of 400 mg/kg bw/d was determined for maternal toxicity, and an NOAEL of 1000 mg/kg bw/d was determined for developmental toxicity.	5,7
Trisodium Ethylenediamine Disuccinate	Diet	Female Sprague-Dawley rats (5/group)	0, 2000, 8000, 16,000, 24,000, or 40,000 ppm The approximate intake levels at 8000, 16,000, 24,000, and 40,000 ppm were 530, 981, 830, and 1029 mg/kg bw, respectively (intake levels corresponding to the 2000 ppm concentration were not provided)	Pregnant rats were given the test substance via diet on gestation days 6-15. Clinical signs of toxicity were evaluated throughout the treatment period. After 20 d, animals were killed, and uterine examinations were performed.	Two animals in the highest dose group died. Reduced food consumption and decreased weight gain was noted in animals treated with 16,000 ppm and above. Emaciation was noted in animals treated with 24,000 ppm and above. One animal treated with 40,000 ppm produced no feces. No viable fetuses were observed in animals treated with 40,000 ppm. The mean number of live fetuses per animal was reduced to 5 in animals treated with 24,000 ppm, compared to 14.5 in the control group. Gravid uterine weights were reduced at 16,000 and 24,000 ppm. The mean number of total resorptions in animals treated with 0, 2000, 8000, 16,000, and 24,000 ppm was 0.5, 0.2, 1.6, 0.8, and 7.67, respectively. Late resorptions were observed in animals treated with 24,000 and 40,000 ppm. Post-implantation losses were 14-fold higher in animals treated with 24,000 ppm, compared to controls. Study authors stated that because of the reduced food consumption, it was difficult to determine if effects were related to treatment or poor nutrition. The NOAEL for maternal toxicity was determined to be 8000 ppm (approximately 530 mg/kg bw/d).	5
Trisodium Ethylenediamine Disuccinate	Diet	Wistar Han rats: -20/sex/group at 0 and 1000 mg/kg bw/d (10/sex/group kept for a 4-wk recovery period) -10/sex/group at 50, 300, and 700 mg/kg bw/d	0, 50, 300, 700, or 1000 mg/kg bw/d	Animals (10/sex) were given the test substance in the diet for 90 d. Satellite groups of 10 animals/sex were given the control or high-dose diets for 90 d, and allowed to recover for 28 d. Estrous cycle duration was determined in female rats over the last month of the study. A sperm analysis and histopathological analysis of the testes were performed at study termination.	No adverse effects on the duration of the estrous cycle were observed at any dose level. In males treated with 1000 mg/kg bw/d, an increase in the number of abnormal sperm (but no effects on motility or concentration), atypical residual bodies of minimal severity and incidence, were observed. Results regarding other subchronic toxicity parameters evaluated in this study can be found in Table 6.	5

NOAEL = no-observed-adverse-effect-level; OECD TG = Organisation for Economic Cooperation and Development test guidelines

Table 8. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
IN VITRO						
Trisodium Ethylenediamine Disuccinate (34% aqueous solution)	Water	0, 33, 100, 333, 1000, 3333, 5000 µg/plate	*S. typhimurium*: TA98, TA100, TA1535, TA1537, TA1538, *Escherchia coli*: WP2 (pKM101) and WP2 uvrA (pKM101)	OECD TG 471; Ames assay performed with and without metabolic activation	Non-mutagenic	5,7
Trisodium Ethylenediamine Disuccinate	Water	0, 50, 150, 500, 1500 and 5000 ug/plate	*S. typhimurium*: TA1535, TA1537, TA1538, TA98, TA100	OECD TG 471: Ames assay performed with and without metabolic activation	Non-mutagenic	5
Trisodium Ethylenediamine Disuccinate (34% aqueous solution)	Water	0, 5, 10, 20, 40, 79, 157, 313, 625, 1250, 2500 and 5000 µg/ml	Chinese hamster ovary cells	OECD TG 473; In vitro mammalian chromosome aberration assay performed with and without metabolic activation; cells incubated without metabolic activation for 6, 18, or 42 h, and with metabolic activation for 6 h	No statistically significant increases in structural or numerical aberrations were observed in 6 and 18 h treatments. A statistically significant increase in structural aberrations at 40 µg/ml in the 42-h study ($p < 0.025$) (performed without metabolic activation), and statistically significant dose response ($p < 0.05$), was observed. In the 42-h treatment study, there was also a statistically significant increase in numerical chromosome aberrations with 20 and 40 µg/ml ($p < 0.05$) (performed without metabolic activation).	5,7
Trisodium Ethylene diamine Disuccinate (34% aqueous solution)	Culture medium	0, 2765, 3017, 3268, 3519, 3771, 4022, 4273, 4524, 4776, and 5028 µg/ml	Mouse lymphoma L5178Y cells	OECD TG 472: In vitro mammalian cell gene mutation test performed with and without metabolic activation	Non-mutagenic	5,7
Tetrasodium Iminodisuccinate	NR	50 – 5000 µg/plate	*Salmonella typhimurium:* TA1535, TA1537, TA98, TA100, TA102	OECD TG 471; Ames assay performed with and without metabolic activation	Non-mutagenic	6
IN VIVO						
Trisodium Ethylenediamine Disuccinate (42.3% aqueous solution)	Water	0, 200, 670, 2000 mg/kg bw	Sprague-Dawley rats (15-20/sex/group)	OECD TG 475; Mammalian bone marrow chromosome aberration assay; animals given a singular dose of the test substance via gavage	Non-clastogenic	5,7
Tetrasodium Iminodisuccinate	Water	0 – 1500 mg/kg bw	Mouse/HSD/Win: NMRI (5/sex/group)	OECD TG 474: Mammalian erythrocyte micronucleus test; singular intraperitoneal injection	Non-clastogenic	6

OECD TG = Organisation for Economic Cooperation and Development test guidelines

Table 9. Dermal irritation and sensitization

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION					
ANIMAL					
Trisodium Ethylenediamine Disuccinate	500 mg; area of 6 cm^2	3 New Zealand white rabbits	OECD TG 404; test substance moistened with water was applied to the intact skin of the dorsal trunk region, under semi-occlusive conditions; 4-h exposure period; site evaluated 1, 24, 48, and 72 h after patch removal	Non-irritating	5,7
Trisodium Ethylenediamine Disuccinate	660 mg; area of 5 cm^2	3 New Zealand white rabbits	OECD TG 404; test substance moistened with water was applied to the intact skin of the dorsal trunk region, under semi-occlusive conditions; 4-h exposure period; site evaluated 30 min, 24, 48, and 72 h after patch removal	Non-irritating	5
Tetrasodium Iminodisuccinate in water	NR	3 male Himalayan white rabbits	OECD TG 404; Animals exposed to test substance under semi-occlusive condition; observation period of 3 d; 4-h exposure period	Non-irritating	6
HUMAN					
Trisodium Ethylenediamine Disuccinate	0.4 ml; 2.94, 14.7, and 29.41% aq.	12 subjects	Repeat patch test; patches applied on Friday, Monday, and Wednesday (24-h applications; occlusive conditions), leaving at least 24 h after removal of previous patch; sites evaluated before each patch application, and 24 h after removal of third patch	Non-irritating	5
SENSITIZATION					
ANIMAL					
Trisodium Ethylenediamine Disuccinate	50% aq. for induction and challenge; 500 mg	Albino Himalayan spotted guinea pigs (10/sex/group for treated groups; 5/sex for control group)	OECD TG 406, modified Buehler assay; animals exposed to the test substance, on clipped shoulder region, for 6 h, under occlusive conditions. Two more applications were performed at 7-d intervals. After a 2-wk non-treatment period, animals were exposed to a challenge dose (occlusive application for 6 h), and observed for 48 h. Control animals received the challenge dose only.	Non-sensitizing	5,7
Trisodium Ethylenediamine Disuccinate	100%; 500 mg	Albino Himalayan spotted guinea pigs (10/sex/group for treated groups; 5/sex for control group)	OECD TG 406, modified Buehler assay; animals exposed to the test substance moistened with water, on clipped shoulder region, for 6 h, under occlusive conditions. Two more applications were performed at 7-d intervals. After a 2-wk non-treatment period, animals were exposed to a 100% challenge dose (occlusive application for 6 h), and observed for 48 h. Test sites were re-challenged 7 d later. Control animals received a single challenge dose only.	8/9 tested animals showed slight patchy erythema at 24 h following 1st challenge, and 15 animals showed slight patchy erythema 24 h after 1st challenge 9/10 and 3/10 negative controls showed slight patchy erythema 24 h after challenge and re-challenge, respectively One animal in the treated group was found dead on day 20, but cause of death was undetermined Slight confluent erythema was observed 24 h after challenge application in one animal. The test substance was considered to be non-sensitizing by EU CLP regulation.	5,7

Table 9. Dermal irritation and sensitization

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Tetrasodium Iminodisuccinate	intradermal injection: 1%; topical induction: 25%; topical challenge: 20%	Guinea pig/Hsd Poc:DH (20 in test group; 10 in control group)`	Guinea pig maximization test performed according to OECD TG 406; no details provided	Non-sensitizing	[6]
			HUMAN		
Trisodium Ethylenediamine Disuccinate	5% aqueous	111 subjects	The induction phase consisted of 9 24-h applications, under occlusive patches, over a 3-wk period. Between 12-20 d after the last induction exposure, a challenge dose of the same test substance was applied to the same area, under occlusive conditions, for 24 h. Test areas were examined 48 and 96 h after application of challenge dose.	Non-irritating and non-sensitizing	[5,6]

CLP = classification, labeling, and packaging; EU = European Union; NR = not reported; OECD TG = Organisation for Economic Cooperation and Development test guidelines

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