Safety Assessment of 1,2,4-Trihydroxybenzene as Used in Cosmetics

Status: Draft Final Report for Panel Review
Release Date: May 10, 2024
Panel Meeting Date: June 3-4, 2024

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

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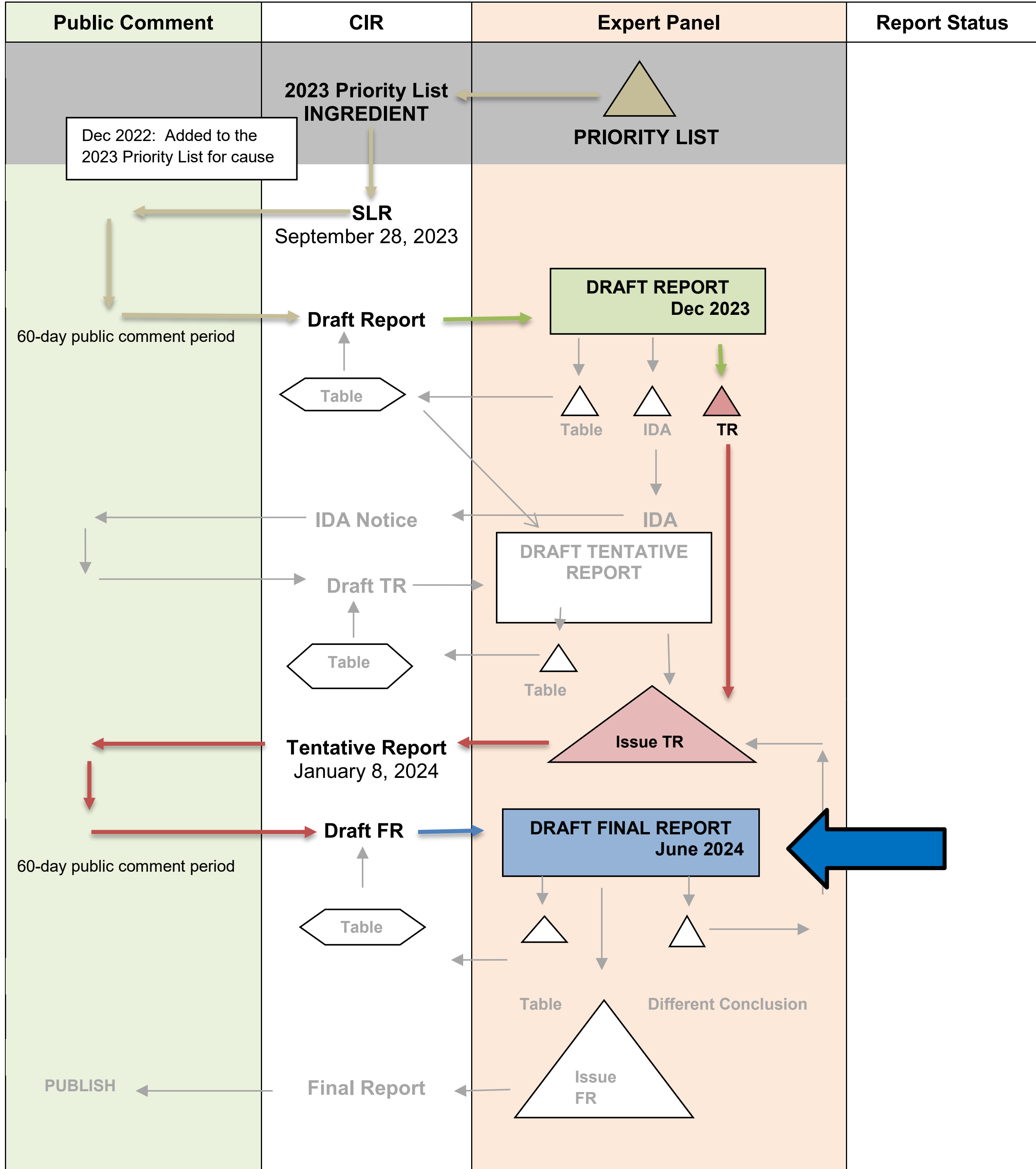
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY __ 1,2,4-Trihydroxybenzene

MEETING ___June 2024



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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, M.S., Senior Scientific Analyst/Writer, CIR
Date: May 10, 2024
Subject: Safety Assessment of 1,2,4-Trihydroxybenzene as Used in Cosmetics

Enclosed is the Draft Final Report on the Safety Assessment of 1,2,4-Trihydroxybenzne as Used in Cosmetics. (It is identified as *report_Trihydroxybenzene_062024* in the pdf document.) At the December 2023 meeting, the Panel concluded that 1,2,4-Trihydroxybenzene is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

No additional data have been received for this report. Comments provided by the Council on the Tentative Report have been addressed (*PCPCcomments_Trihydroxybenzene_062024* and *response-PCPCcomments_Trihydroxybenzene_062024*).

Other supporting documents for this report package include a flow chart (*flow_Trihydroxybenzene_062024*), report history (*history_Trihydroxybenzene_062024*), a search strategy (*search_Trihydroxybenzene_062024*), meeting transcripts (*transcripts_Trihydroxybenzene_062024*), and a data profile (*dataprofile_Trihydroxybenzene_062024*).

The Panel should carefully review the Abstract, Discussion, and Conclusion, and issue a Final Report.

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DRAFT Memorandum

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

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

DATE: February 5, 2024

SUBJECT: Tentative Report: Safety Assessment of 1,2,4-Trihydroxybenzene as Used in Cosmetics (release date January 8, 2024)

The Personal Care Products Council respectfully submits the following comments on the tentative report, Safety Assessment of 1,2,4-Trihydroxybenzene as Used in Cosmetics.

Key Issue

Genotoxicity – In the text, it would be helpful to describe the studies in two paragraphs. One paragraph for studies in which it is not known how the dosing solution was handled, and the other paragraph describing the studies in which 1,2,4-Trihydroxybenzene was in degassed solutions (to decrease autooxidation).

Combe provided expert analysis of the genotoxicity data by Prof. Helmut Greim with input from Prof Barry Halliwell, and by Dr. Marilyn J. Aardema. Information and conclusions from these analyses should be added to the genotoxicity section.

Additional Considerations

Reaction Chemistry – The sentence on metabolites of benzene ("Of the metabolites of benzene, 1,2,4-Trihydroxybenzene is the most reactive toward molecular oxygen and rapidly auto-oxidizes to its corresponding quinone via semiquinone radical intermediates.") does not belong in the Reaction Chemistry section. It should be moved to the Natural Occurrence section with the rest of the information about benzene metabolism.

Reaction Chemistry – In the last paragraph, the "s" should be removed from "1,2,4-Trihydroxybenzenes"

Cosmetic Use – In view of the reactivity of 1,2,4-Trihydroxybenzene with oxygen described in the Reaction Chemistry section, the information on how these hair dyes are packaged and sold that was provided by Combe should be added to the Cosmetic Use section. Combe stated: "hair dye formulations are filled into specialized, oxygen barrier packaging for commercialization.

Stability tests are conducted under standard conditions (both room and elevated temperatures) appropriate for cosmetic products. Final product stability meets standard requirements until the package is opened by the consumer for use. Upon dispensing and application, the combination of dyes (including 1,2,4-THB and other precursors) in the formulation undergo oxidative coupling reactions as predicted by their chemical structure."

Short-Term, Oral – The last sentence does not belong in the Oral section ("1,2,4-Trihydroxybenzene treatment also decreased fatty acid oxidation in mouse primary liver cells.") as it is describing an *in vitro* study. Is there any additional information about this study, especially the concentration of 1,2,4-Trihydroxybenzene that was tested?

Developmental and Reproductive Toxicity – The following sentence is confusing and should be revised: "Exencephaly was already noted in fetuses coming from dams treated with a non-teratogenic substance (mean incidence: 0.06%; range of incidence per study: 0.0-1.0%)." It would be clearer to state that the historical incidence of exencephaly in controls at the facility conducting the study was a mean of 0.06%; range of incidence per study of 0.0-1.0%.

Genotoxicity, Scheme 2 – Professors Griem and Halliwell indicated that the figure shown in scheme 2 came from Pellack-Walker and Blumer 1986 (appears to be reference 32 in the CIR report). Even though the figure is redrawn, it should have a reference.

Carcinogenicity, Oral – A 4-week study should not be considered a carcinogenicity study.

Immunomodulatory Effects – The following is not clear: "The author stated that because long-term oral supplementation of 1,2,4-Trihydroxybenzene with diet was conducted for more than 1 wk, the effects of 1,2,4-Trihydroxybenzene may be accumulated in the mouse body." (should be revised to say the diet was supplemented with 1,2,4-Trihydroxybenzene) Did they mean that 1,2,4-Trihydroxybenzene accumulates? It is not clear how "effects" accumulate. What effects were observed? It also does not make sense for lymph node weight to be down with unaffected lymph node cell numbers, yet it also says, "the degree of DNCB-sensitized cell proliferation was increased approximately 2-fold by 1,2,4-Trihydroxybenzene".

Sensitization – Please add the units after 10.37 (µM).

Summary – Please correct "NOAEL were determined" ("were" should be "was"). Please add the gestation days of treatment to the teratogenicity study. Please include a description of the Ames assay in which ROS inhibitors were added. Please indicate that it was a study in mice that suggested effects on allergy response.

The Summary states that the second LLNA was completed "without dimethylformamide". This is not stated elsewhere in the report, nor is it stated in the SCCP or SCCS opinions. The opinions do not specifically state what solvent was used in the second LLNA. Because there were only dimethylformamide treated controls, it is likely that dimethylformamide was also the solvent in the second LLNA. It would be appropriate for the CIR report to state that the solvent in the second LLNA was not stated, but it should not state that it was completed "without dimethylformamide".

Discussion – It is the weight of the evidence of the genotoxicity studies (not “for other toxicity endpoints” as currently stated) that supports that 1,2,4-Trihydroxybenzene would not be genotoxic in vivo under the conditions of use as a hair dye ingredient. The opinions of the experts in addition to the Expert Panel for Cosmetic Ingredient Safety should also be mentioned in the Discussion.

Table 2 – For Ames tests of 1,2,4-Trihydroxybenzene in degassed water (references 4, 5), the results column should also note that the response was negative in strains TA98. TA100, TA102 and TA1535.

1,2,4-Trihydroxybenzene - June 2024 – Christina Burnett	
Comment Submitter: Alexandra Kowcz, PCPC **Date of Submission: February 5, 2024**	
Comment	**Response/Action**
Key Issue: Genotoxicity – In the text, it would be helpful to describe the studies in two paragraphs. One paragraph for studies in which it is not known how the dosing solution was handled, and the other paragraph describing the studies in which 1,2,4-Trihydroxybenzene was in degassed solutions (to decrease autooxidation). Combe provided expert analysis of the genotoxicity data by Prof. Helmut Greim with input from Prof Barry Halliwell, and by Dr. Marilyn J. Aardema. Information and conclusions from these analyses should be added to the genotoxicity section.	Paragraphs revised. It is not CIR procedure to bring in the opinions of outside experts.
Reaction Chemistry – The sentence on metabolites of benzene ("Of the metabolites of benzene, 1,2,4-Trihydroxybenzene is the most reactive toward molecular oxygen and rapidly auto-oxidizes to its corresponding quinone via semiquinone radical intermediates.") does not belong in the Reaction Chemistry section. It should be moved to the Natural Occurrence section with the rest of the information about benzene metabolism.	Suggested edit not accepted. Statement touches on in vivo occurrence, but that is not the subject of the statement.
Reaction Chemistry – In the last paragraph, the "s" should be removed from "1,2,4-Trihydroxybenzenes"	Correction made.
Cosmetic Use – In view of the reactivity of 1,2,4-Trihydroxybenzene with oxygen described in the Reaction Chemistry section, the information on how these hair dyes are packaged and sold that was provided by Combe should be added to the Cosmetic Use section. Combe stated: "hair dye formulations are filled into specialized, oxygen barrier packaging for commercialization. Stability tests are conducted under standard conditions (both room and elevated temperatures) appropriate for cosmetic products. Final product stability meets standard requirements until the package is opened by the consumer for use. Upon dispensing and application, the combination of dyes (including 1,2,4-THB and other precursors) in the formulation undergo oxidative coupling reactions as predicted by their chemical structure."	Not our normal standard procedure, but included because this ingredient is known to be reactive with air.
Short-Term, Oral – The last sentence does not belong in the Oral section ("1,2,4-Trihydroxybenzene treatment also decreased fatty acid oxidation in mouse primary liver cells.") as it is describing an in vitro study. Is there any additional information about this study, especially the concentration of 1,2,4-Trihydroxybenzene that was tested?	Last sentence deleted.
Developmental and Reproductive Toxicity – The following sentence is confusing and should be revised: "Exencephaly was already noted in fetuses coming from dams treated with a non-teratogenic substance (mean incidence: 0.06%; range of incidence per study: 0.0-1.0%)." It would be clearer to state that the historical incidence of exencephaly in controls at the facility conducting the study was a mean of 0.06%; range of incidence per study of 0.0-1.0%.	Sentence rewritten.

Genotoxicity, Scheme 2 – Professors Griem and Halliwell indicated that the figure shown in scheme 2 came from Pellack-Walker and Blumer 1986 (appears to be reference 32 in the CIR report). Even though the figure is redrawn, it should have a reference.	Edit accepted.
Carcinogenicity, Oral – A 4-week study should not be considered a carcinogenicity study.	Moved to Short-Term Toxicity section.
Immunomodulatory Effects – The following is not clear: "The author stated that because long-term oral supplementation of 1,2,4-Trihydroxybenzene with diet was conducted for more than 1 wk, the effects of 1,2,4-Trihydroxybenzene may be accumulated in the mouse body." (should be revised to say the diet was supplemented with 1,2,4-Trihydroxybenzene) Did they mean that 1,2,4-Trihydroxybenzene accumulates? It is not clear how "effects" accumulate. What effects were observed? It also does not make sense for lymph node weight to be down with unaffected lymph node cell numbers, yet it also says, "the degree of DNCB-sensitized cell proliferation was increased approximately 2-fold by 1,2,4-Trihydroxybenzene".	Paragraph revised.
Sensitization – Please add the units after 10.37 (μM).	Corrections made.
Summary – Please correct "NOAEL were determined" ("were" should be "was"). Please add the gestation days of treatment to the teratogenicity study. Please include a description of the Ames assay in which ROS inhibitors were added. Please indicate that it was a study in mice that suggested effects on allergy response.	Corrections made.
The Summary states that the second LLNA was completed "without dimethylformamide". This is not stated elsewhere in the report, nor is it stated in the SCCP or SCCS opinions. The opinions do not specifically state what solvent was used in the second LLNA. Because there were only dimethylformamide treated controls, it is likely that dimethylformamide was also the solvent in the second LLNA. It would be appropriate for the CIR report to state that the solvent in the second LLNA was not stated, but it should not state that it was completed "without dimethylformamide".	Edits accepted
Discussion – It is the weight of the evidence of the genotoxicity studies (not "for other toxicity endpoints" as currently stated) that supports that 1,2,4-Trihydroxybenzene would not be genotoxic in vivo under the conditions of use as a hair dye ingredient. The opinions of the experts in addition to the Expert Panel for Cosmetic Ingredient Safety should also be mentioned in the Discussion.	Sentence revised. The Discussion of the safety assessment is by the Expert Panel for Cosmetic Ingredient Safety. It is not procedure to bring in the opinions of outside experts to the Discussion section.
Table 2 – For Ames tests of 1,2,4-Trihydroxybenzene in degassed water (references 4, 5), the results column should also note that the response was negative in strains TA98. TA100, TA102 and TA1535.	Edits accepted.

1,2,4-Trihydroxybenzene Ingredients History

September 28, 2023– The Scientific Literature Review was issued for public comment.

December 2023 – The Panel issued a Tentative Report for public comment with the conclusion that 1,2,4-Trihydroxybenzene is safe for use as a hair dye ingredient in the present practices of use and concentration described in the safety assessment.

1,2,4-Trihydroxybenzene Data Profile* – June 2024 – Christina Burnett

				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	Other	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
1,2,4-Trihydroxybenzene CAS No. 533-73-3	X	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

1,2,4-Trihydroxybenzene

Ingredient	CAS #	INCIpedia	PubMed	FDA	EU	ECHA	SCCS	SIDS	ECETOC	HPVIS	AICIS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
1,2,4-Trihydroxybenzene	533-73-3	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Search Strategy

PubMed

((1,2,4-trihydroxybenzene) OR (533-73-3[EC/RN Number]) = 215 hits, 44 relevant
((hydroxyhydroquinone) OR (533-73-3[EC/RN Number]) = 150 hits, 41 relevant (most the same as above)

ECHA

No dossier available.

Typical Search Terms

- INCI names
- CAS numbers
- chemical/technical names
- additional terms will be used as appropriate

LINKS

Search Engines

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

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

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/infobase/
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
    - technical reports search page: https://ntrl.ntis.gov/NTRL/

Last updated: April 15, 2024.

- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

Last updated: April 15, 2024.

DECEMBER 2023 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Presentation – December 4, 2023

DR. CUEVAS: Good morning, can you all see my screen?

DR. HELDRETH: Good morning. Yes, we can.

DR. CUEVAS: And hopefully you can see my face on this wonderful Monday morning. Let me just close all that out so that we all can see each other. All right, so good morning CIR panel, CIR staff, guests. My name is AJ Cuevas, and I am the senior manager -- well, and also, Bart, thank you for the introduction -- I'm the senior manager of global product safety at Combe and thank you for this opportunity to address the panel today to speak on that mouthful that Bart just talked about. The 1,2,4-Trihydroxybenzene as a cosmetic hair ingredient.

There we go. So certainly, it's difficult to go on this trip together without understanding the direction of which we're going to go. So, before I begin, I want to lay out the map of topics we'd like to highlight. Bioavailability, mode of action, a whole bucket of genotoxicity topics, considerations that need to be discussed in regards of historical genotox data, New Alternative Method, 3D skin model data, our in vitro data for those 3D models, and we'll touch a little bit on the sensitivity and specificity of the in vitro methods.

And we'll walk through a whole new sector of in vivo data we want to highlight, and we can wrap up with a summary and our weight of evidence and conclusions. So, one more thing before we hit the road. A few house keeping items, 1,2,4-Trihydroxybenzene is quite a mouthful, so I'll affectionately refer to it as THB for the remainder of the presentation.

I'd also be remiss if I didn't mention that we, my colleagues from Combe and I are presenting a dossier in this presentation today that is a compilation of Combe's ten-year journey with THB. And this journey included reproducing historical genotox studies, filling gaps in the data to ensure safety of this ingredient, and this includes the engagement of experts and renowned subject matter experts such as Doctor's Greim and Halliwell who are renowned subject matter experts in medical toxicology and the management and disposition of ROS in biological systems respectively. You can find their detailed assessments in Exhibit C of the dossier that you all have on THB.

And additionally, Marilyn Aardema who contributed her expert guidance on the testing battery of THB in a comprehensive review of weight of evidence in the area of genotoxicity. Her independent analysis is provided in Exhibit D. And ultimately, we're here -- one more thing I would say, is I come to you standing on the shoulders of giants to present this compilation of work focused heavily on the genotox data that will hopefully show that THB when used as cosmetic hair dye is safe at the level of 2.5.

So, let's start with dermal absorption as the bioavailability of a substance informs the overall risk assessment and is a critical data piece in calculating the margin of safety. So, to understand how much a substance is bioavailable, we must first understand how much the substance is actually absorbed into the body.

So as such we investigated dermal absorption of THB in ex vivo model and we designed two studies. One with THB alone in a simple hair dye matrix and the second study with radio labeled THB and PTD. This combination provides a likely and conservative use model scenario and for what you see on the slide here the results of this combination is what we'll be discussing. However, the full details of this experiment can be found in section 3.4 of the dossier.

So, the experiment involved a 30-minute topical exposure on the skin surface. It was washed and returned to the water bath for observation for 24 hours. Radio activity was measured using liquid simulation counting and they systematically available amount is this sum of the material detected in the three compartments. The epidermis, the dermis, and the receptor fluid.

The epidermis value, we want to point out, includes the staining on the surface of the tissue. So conservatively will elevate the detective epidermal amount and as standard practice one standard deviation in risk assessment is added to this total value.

The results of this study, the radio label THB plus PTD indicated that predominantly the distribution of THB was predominantly found in the epidermis with much lower amounts detected in the dermis and receptor fluid. When we add the standard deviation which is 1.76, we had a total systemically available amount of 3.7 micrograms per equivalent centimeters squared. And this becomes the value that is used in the margin of safety calculation.

So, our findings here correlate well with the calculated value documented for THB, the Log P_{ow}, and this low value of 0.2 is characteristic of low solubility and ultimately indicating that this THB is poorly absorbed, and again more information can be found in the dossier you all have, section 3.4.

So, at this point it'd be beneficial to look at the mode of action of THB and it is well documented that THB rapidly auto-oxidizes in the presence of oxygen and in aqueous medium which generates peroxide and other Reactive Oxygenated Species. It is also well documented that genotoxic effects are observed in vitro studies from the generated peroxide and other ROS, Reactive Oxygen Species. However, these genotoxic effects seen in vitro as a result of the THB reacting are not seen in vivo;

they are only seen in vitro. And these effects are routinely mitigated by ROS scavenging mechanisms in the living organism, and we'll talk a little more about that.

So, we want to note here that hydrogen peroxide was in the same boat, so despite significant in vitro data showing genotoxic effects from hydrogen peroxide. The SCCS has reviewed this data and approved hydrogen peroxide back in 2005 for cosmetic use based on in vivo data that showed the genotoxic effect was an artifact of ROS and attenuated by scavenging mechanisms in the living organism.

So, some in vitro studies have documented THB to be genotoxic because of what we just discussed. The generation of reactive oxygen species such as peroxide and this is referenced in the SLR, so thank you CIR staff for that. And these effects are not confirmed in vivo, and this is because in vivo there are biological processes such as these ROS scavenging mechanisms that protect the living organism, also noted in the SLR.

And there's quite a bit of research that documents the attenuation affect of genotoxic effects with the addition of ROS scavengers such as superoxide dismutase, catalase, peroxidase, men- -- there's a few others. When those scavengers are added to the in vitro media, these scavengers quench the peroxide to water and oxygen which prevents the ROS from doing any damage to the living organism.

We also want to point out the SCCS and their final opinion of THB. There were also studies that document this attenuation of genotoxic affect when the ROS scavengers are added. So, hence, the living organism -- in the living organism there are protective mechanisms to protect the organism from those genotoxic effects and there's a lot more information on this particular topic in 5.5 of our dossier.

So, before we dive into the genotox data, we want to point out a few things. There's historic published literature on THB that's very well documented that there's in vitro genotoxic effects but these may not be necessarily suitable for identifying hazard in regulatory risk assessment purposes. Deficiencies in these type of studies are noted.

They can be that THB is not well characterized, unvalidated methodologies are used in the particular study. There's lack of solvent information, lack of dosing solution analysis and ultimately not being GLP. And those places doubt on the results and also make it very difficult to identify hazard in a risk assessment process.

So, at this point, with knowing what we just spoke on with the last slide, how do we build our weight of evidence to truly identify the hazard? So, this becomes a little difficult with the prohibition with animal testing in Europe for cosmetic products and ingredients. This has complicated the problem in conducting studies that are reliable for genotoxicity hazard identification.

So recognizing this, many of you probably know the cosmetic Europe groups set up a genotoxicity task force made up of subject matter experts and external non-industry subject matter experts to tackle this exact challenge to identify and validate non-animal methodologies that minimize the potential for misleading positive results that are seen in the in vitro assay such as hydrogen peroxide or a THB that cannot be necessarily replicated in vivo.

And so, 3D Skin models have been identified and they have many advantages. They use primary human keratinocytes. They are metabolically competent and mimic in vivo assays. They use dermal exposures which are most relevant to predict toxicity for cosmetic use -- and maybe not a shout out to our industry -- but also minimize the need to conduct in vivo follow ups for non-cosmetic uses in regards of compliance to the three R's being able to use that data.

Okay. So, before you dive into this slide if you just take a moment to listen to this one point and that's given that we saw a very weak positive in our repeat Ames Assay and the negative in the in vitro micronucleus study, details we're not discussing here but I'm just pointing out how we got to this step and those details can be found in 3.7.1. But because of the discordant data, we undertook a third assay which could potentially provide greater clarity and now you land on this slide which is the 3D Phenion Full-Thickness skin model was selected by the Cosmetic Europe task force as being a suitable model for the adapted Comet Assay which can identify DNA damage in vitro.

So, at the time this model was employed hair dyes had not been tested and since THB was prone to skin staining, we wanted to ensure that the color would not potentially interfere with the color metric measurements. So, as such, cytotoxicity was measured using a few different markers.

So, two experiments were completed per protocol and under GLP using a 48-hour exposure with and without aphidicolin and ultimately to improve sensitivity of the assay. And the results documented that THB did not induce DNA damage at any dose with or without aphidicolin. And as noted here more information can be found on this in section 3.7 of the dossier.

To add weight of evidence, Combe also undertook an additional study using another NAM model that was being explored by the Cosmetic Europe Genotoxicity Task Force. The Reconstructed Skin Micronucleus Assay. When this study was done under GLP using the protocol as defined of the task force at the time and cytotoxicity was measured using relative viable cell counts and a dose range finding study was completed and two experiments were done at all proposed concentrations using repeat daily applications.

And the conclusions of this study also confirmed that THB did not induce a statistically significant increase in micronucleated PCE's at any dose in either of the experiments. Also, more information can be found in the same section 3.7.

So, let's talk about these NAM 3D skin models. What have they -- these models -- been up to since Combe collected this data. So subsequently since we submitted our dossier to CIR last January, we have learned that these two NAM skin models that we just discussed have completed their validation through the OECD process. The Cosmetic Europe Task Force published their data in two papers in early 2021 and one of the experts I want to pull attention to down here, one of the experts of one of the papers on the RSMN is Marilyn Aardema who you will be able to see her expert opinion in the dossier as we discussed at the beginning of this talk.

But ultimately, overall, it's quite remarkable to see that the overall accuracy, sensitivity, and specificity of these tests are quite favorable, and both models have been accepted -- as mentioned -- into the OECD protocols as a tier two test guidelines and they will be undergoing in the development program.

If we pause here for a second and think of our four different in vitro methods that are commonly used to characterize hazard, two are long-timer tier one OECD methods the Ames and the in vitro micronucleus and two have just recently made it into the tier two -- OECD tier two. So, Power, et al published a paper in 2013 exploring the guard rails that should be in order -- sorry -- guard rails that should be in place for data for it to be considered high quality and high value. And they propose the sum of the sensitivity and specificity for a given test should be between 1.5 and 2 to be considered high quality and high value.

So, a value of two is considered ideal where a classification is correct a hundred percent of the time. However, this is definitely not the real-world setting. No testing model is perfect in its predictive capacity. So, if we look at this table on this slide, specificity and sensitivity are summarized here. We see AMES in the in vitro micronucleus value is under the 1.5 minimum whereas the NAM values are both above 1.5.

And I'm not saying that Ames or the in vitro micronucleus do not add value by any means. I'm not saying this. But they may not always be conclusive and especially for this particular end point. So, we can conclude with reasonable confidence that the data from the 3D NAM models can be considered to add relevant significant new information informing our overall weight of evidence of the safety of THB.

So, to pause here for a second and point out that we provided a great deal of data to SCCS, data that you all have in your dossiers today, we provided the repeat Ames Assay, we also provided the Ames assays with the ROS scavengers to show the genotoxic effect was removed. We provided the NAMs 3D skin model data that we just discussed and despite this, SCCS concluded that genotoxicity could not be ruled out by putting more weight of evidence on the historical in vitro literature alone.

SCCS did note that due to the historical in vitro data, that the only way to confirm the absence of genotoxicity would be -- probably guessed it -- to conduct an in vivo study which was done in the approval for hydrogen peroxide in cosmetics. However, today, this was not an option as SCCS cannot review such data due to the animal testing ban on cosmetics.

However, Combe desired to do the right thing. We wanted to make sure we objectively showed that THB is safe for use and in order to accomplish this we commissioned an in vivo micronucleus study in mice. But, again, just to reiterate that study was not reviewed by SCCS. They cannot review that data due to the ban. Which is exciting because we present this data to you all today.

So, in 2019, Combe commissioned an in vivo mouse micronucleus assay to confirm our assessment regarding genotoxicity. And the objectives of this study was to repeat the historical in vivo micro assay that existed on THB that was conducted in the '90s in mice using the same route of exposure from the 1990 study.

However, since that time the OECD guideline had been updated since the '90s when the original study was conducted. So, when we commissioned our study, we made sure it was per the current OECD guideline. And our method of exposure was the same as the '90s study, the intra-peritoneal injection. The max tolerated dose was selected after the range finding assay in both male and female mice.

The pivotal study moved forward in male mice based on the clinical observations in mortality showing in males to be more sensitive. And lastly, the study included 50 and 25 percent doses of the MTD. There was feedback. Did someone have a question or was that just on my end? I assume --

DR. HELDRETH: That's somebody logging in.

DR. CUEVAS: Okay. Thanks. All right. So, to get to our results the principal investigator selected 25 milligrams per kilogram body weight as the maximum tolerated dose and per the OECD guideline, the two additional doses being 50 and 25 percent of the MTD were also dosed as per protocol. The study concluded there are no statistically significant increases in micronucleated PCEs at any dose and this study confirms the historical results from the 1993 study.

So, this data that compliments our overall evidence -- and ultimately the star of our data package that you all get to review -- confirms that THB is non-genotoxic in vivo and doing so in an OECD approved bioassay performed under GLP. So, this is

obviously a small snapshot of what you have all in your dossier that we submitted but this summary table you can see the list of the OECD complaint GLP study that were undertaken to speak to the genotoxic endpoints.

In summary, we can think of this information we have regarding reliability of the tier one studies like the Ames as you see listed at the top may not tell the whole story but as mentioned in our dossier the repeat Ames without and then with reactive oxygen scavengers in which this data confirm that the weak positive result in the Ames was eliminated due to the presence of the scavengers as you see here. We undertook two 3D skin methods which are now validated and undergoing OECD guideline development and both of these relevant exposures yielded negative results.

And lastly, we show the two in vivo studies showing concordant results showing the absent of the genotoxic effect. The original study in ’93 and the main attraction of this talk, which you will have the opportunity to review, the GLP in vivo study conducted in 2019 per current OECD guideline that confirmed the absence of genotoxic hazard.

So, here we are, the last slide. And as I told you at the beginning of the presentation the goal was for us to walk together through the vast body of data that Combe has collected over the last ten years. And the data presented here, and also in the dossier that you’ll have to review, confirm that THB is safe as a hair ingredient. And THB demonstrates genotoxic effects in some in vitro studies but the inclusion of ROS scavengers in our Ames assay and also others documented in the literature indicate the genotoxic effect is eliminated.

Many historical literature data may be unreliable for regulatory risk assessment due to deficiencies in chemical characterization, dosing, non GLP, et cetera. Subject matter expert’s assessments included in the dossier also concluded that THB does not pose a risk, a genotoxic risk, and like hydrogen peroxide triggers formation of ROS via identical mechanisms.

We have four in vitro GLP studies that confirm THB is non-mutagenic and non-genotoxic. In vitro GLP NAM models, the 3D Comet and the 3D RSMN show THB to be non-mutagenic and non-genotoxic and these 3D NAM models have now been accepted into the OECD guideline development.

And then THB ultimately has been confirmed to be non-mutagenic/non-genotoxic in the two GLP in vivo studies. And overall, our weight of evidence concludes that THB does not pose a genotoxic risk to the consumer when used as a hair ingredient up to 2.5 percent.

And with that I open it up for myself and other colleagues from Combe who are on the call today with us for questions.

DR. BELSITO: Yeah, can you hear me?

DR. HELDRETH: Yes.

DR. BELSITO: Yeah. Don Belsito. I just want to make clear that the 3D Comet and 3D RSMN have been validated and are under consideration by OECD, but they have not yet been accepted and they don’t have official test guidelines. Is that correct?

DR. CUEVAS: That is correct. They’ve been accepted and they’re in the development process through OECD.

DR. BELSITO: But no official test guidelines?

DR. CUEVAS: There’s a --

DR. BELSITO: There’s a protocol but it hasn’t been --

DR. CUEVAS: There’s a protocol, that is correct. Like they have not been assigned an official protocol number. OECD number.

DR. BELSITO: Thank you.

DR. ROSS: This is David Ross. I had a question. Just a comment since my surname is Ross when I teach this, I always refer to Reactive Oxygen Species rather than ROS being the negative actors here. But could I just clarify that the micronucleus test -- the first one -- was peer reviewed by SCCS, that was correct?

DR. CUEVAS: The first -- these ones here?

DR. ROSS: Yeah, the ’93 study. That was in the SCCS opinion was it not?

DR. CUEVAS: Yes. That is correct. If you look at -- if you minus this bottom line, everything above this 1993 and above was part of the SCCS submission.

DR. ROSS: And at the top dose you didn’t see anything in the study if I recall correctly.

DR. CUEVAS: Say again, I’m sorry.

DR. ROSS: At the top dose you did not see any positive results with micronucleus in that ‘93 study?

DR. CUEVAS: Yes. There was -- yep. And there was also a -- that study identified the MTD as 50 micrograms per kilogram -- sorry, milligrams. Milligrams.

DR. ROSS: 50 megs and that's what you used in the test. Yeah. Yeah. Could I ask you one other question? You know you discussed the detoxification roles of catalase and removing hydrogen peroxide in in vitro assays, and I'm totally in agreement with that. There was one test which was listed in the dossier which was done by this group out of Berkeley and that was using human blood. And that was positive. Human blood also has high catalase. So, I wondered if you had any explanation for that one.

DR. CUEVAS: That question I will pass to my colleague Pushpa. Rao. Dr. Pushpa. Oh, Bart, is it possible to --

DR. HELDRETH: Yes, just give me one second. I'm pushing over to --

DR. CUEVAS: Sorry.

DR. HELDRETH: No, no. No worries. Alright, Pushpa should be able to turn on her camera and mic now.

DR. RAO: Hello.

DR. ROSS: Yeah.

DR. HELDRETH: We can hear you.

DR. RAO: Can you tell me which study you're referring to please?

DR. ROSS: Oh, I believe it was reference 33 I think.

DR. RAO: From the SLR?

DR. ROSS: That was our dossier, actually. Let me have a look. It was the only study that was using human blood. And, you know, I just look at the concen- -- sorry, I'm in your dossier right now. I just look at the concentrations of catalase in human blood and that one just seemed like an outlier. I wondered if you'd considered that.

DR. RAO: I don't think we really paid that much attention to it. Do you remember the name of the authors?

DR. ROSS: I'm getting there. Sorry. There's a lot of material here. Yeah, it's -- what we -- here. I think it looks like it's environmental molecular mutagenesis. Anyway, I mean, my question was had you considered that and obviously you hadn't. You hadn't. I mean, you've got lots of other data in there, I think, which is --

DR. RAO: Right, which contradicts what they found. Yes.

DR. ROSS: Okay. I get it. Thank you.

DR. RAO: Yep.

DR. COHEN: It's David Cohen. I had a quick question about the absorption data that you presented a little earlier. You said you presented the data with PTD when it was mixed, right. My recollection from our draft report is that absorption on the Franz cell chambers, the dermatomed material, there wasn't a difference when it was alone or with PTD. But can you corroborate that because in our dossier it suggests that THB is intended to be used in the presence of primary intermediates like PPD. So, did you recognize any change if it's alone or with the primary intermediate?

DR. RAO: There is a slight difference. There is a slight difference. You see a little bit of increase which I think is related to staining on the epidermis and because it was slightly higher, although it's hard to correlate that with actual -- you know, because there is variability inherent in the process. We use the higher value as being more conservative.

DR. ROSS: So, could I just follow up on David's question there with respect to -- could you educate me a little bit here with respect to how THB is used? I mean, in your products is it most readily used with PPD or PTD or is it used alone and -- so it looked like from figure 3 in your dossier you've got dying by itself of hair.

DR. RAO: It can. So, normally in products it's used in combination with other primary intermediates and couplers and because of the way that the substitution pattern is, the 1,4 kind of allows it to behave as a primary intermediate and the 2,4 meta substitution allows it to behave kind of like a coupler. Sorry about the --

DR. ROSS: You know, I got the answer.

DR. RETTIE: This is Allan Rettie. Can I follow up on Dave's question there? I was curious if THB is ever formulated in aromatic amine. But even if it is, wouldn't it also be formulated with ammonia and if so then --

DR. RAO: (Audio skip).

DR. RETTIE: -- yes go ahead?

DR. RAO: Sorry, (audio skip) with ammonia.

DR. RETTIE: Yeah, I mean, as I'm sure you're aware, lots of hair dyes are formulated in base to open up the follicle, I guess, is what I read.

DR. RAO: Right.

DR. RETTIE: And I guess it came across some information that THB alone can interact with ammonia and make a dimer/trimer that by itself perhaps would be the colorant. And I just wondered if that was ever the case where THB was formulated without PDD or PTD but with ammonia. Is ammonia always in there?

DR. RAO: It's not, actually.

DR. RETTIE: Well, ammonia can substitute chemically for the aromatic amine and cross link to form a trimer, actually, a phenoxazine like trimer.

DR. RAO: Yeah. When we (audio skip) as the alkalizing --

DR. RETTIE: I'm sorry, I missed that. You dropped out.

DR. RAO: Sorry. We -- monoethanolamine as the alkalizing agent and not ammonia. I think the reaction is much too rapid with ammonia and it's hard to stabilize it in a formulation. So, using the monoethanolamine and manufacturing under nitrogen the product is sealed in the tube, and it's only exposed to air when the product is opened and applied to the hair. So that's the way that it's stabilized.

DR. ROSS: But there will be an amine in there, so I think --

DR. CUEVAS: It's the monoethanolamine which is the alkalizing agent and as someone was saying, it swells the hair shaft and allows the dyes to penetrate the cortex.

DR. ROSS: Could I just get back to David Cohen's point and that was absorption plus or minus PTD and I think the absorption in the absence of PTD was a little less which you commented on, and you used the absorption with PTD as a conservative approach in the margin of safety calculation which I think is reasonable. Personally, I'd like to see the absorption in the absence of PTD as well. That's going to be less so we might be the margin of safety both ways, actually. But yeah.

DR. RAO: Yeah. And that makes complete sense but given that it's normally formulated with other hair dye ingredients, it's not used by itself, we use the PTD combination.

DR. ROSS: So just to get the definitive answer to that, then, just so I understand. It's always formulated with PTD is that correct?

DR. RAO: Or other primary intermediates like N, N-bis hydroxyethyl PPD.

DR. ROSS: Okay.

DR. RAO: Or para-aminophenol.

DR. TILTON: Hi, this is Susan Tilton. I just had a clarification with regard to the Comet assay that was run on the 3D Full-Thickness model. Did you say that this was also run under condition that would allow the detection of single strand breaks?

DR. RAO: I believe --

DR. TILTON: (Audio skip) reactive intermediates.

DR. RAO: Yes. So, the test article is prepared under nitrogen so it stabilizes until the dosing. At which point it's exposed to the skin and the atmosphere -- oxygen -- and whatever reactions are going to take place would be taking place. You know, reactive intermediates formed and if they are going to do any damage, they have the ability during the 48-hour exposure to affect DNA in whatever way that they can, actually.

DR. TILTON: Okay. Also, a follow up about the in vivo study from 2019. So, there is indication that (audio skip) of toxicity mortality in the dose ranging study. Could you just talk a little bit more about that?

DR. RAO: Yeah. So, you notice that the maximum tolerated dose was 25 milligrams per kilogram in our study and that was the selection by the principal investigator. The historical study was done at 50 milligrams per kilogram, and I think that's a function of the clinical signs and symptoms that the investigators saw in the mice, and she felt that 50 was too high.

Although they survived, they looked very -- how did she call it? She said they looked rough. There was piloerection, hunching, squinty eye. A lot of clinical observations which guided her selection to the lower dose. So, we went along with that and now we have the data from the 50 milligrams per kilogram as well as 25 and you know the 50 and 25 percent of the MTD.

DR. TILTON: All right. Thank you.

DR. BELSITO: Is Tom Slaga on the call?

DR. SLAGA: Yes, I'm here.

DR. BELSITO: Yeah, Tom, what are your thoughts? We haven't heard from you.

DR. SLAGA: Well, it's always been very interesting, the difference between looking at something totally in vitro versus a model that resembles more in vivo like it's been presented here because if you have a full thickness human skin you do have detoxification mechanisms that counter act the formation of free radicals whereas in vitro, you know, it's kind of a naked system that, you know, year and a half changes and reactivities that you may not see in vivo.

Years ago, worked with a epoxide of a polycyclic benzene -- or benzo(a)pyrene. And the difference in reactivity in vitro versus putting it on a skin and the reactivity could be finished within a half hour or hour in vitro, but in vivo you still have a reactive condition for a long period of time. So, things are different in vivo, and it may not -- there may be protective mechanisms against reactive things.

The one thing that I -- there's, you know, the weight of evidence as it's presented is always very, very important and you just -- although the Ames Assay in vitro is very predictive of carcinogenicity -- of a carcinogenic effect -- lot of times in vivo there's ways to counteract that and so we have to look at the complete weight of evidence.

The one thing that I was a little confused about, the 1,2,4-Trihydroxybenzene is -- the positioning of the 1,2,4-hydroxy group makes a reactive center on the five carbon of the benzene ring and I don't know if you put an inhibitor of free radical formation to prevent the genotoxicity that was shown here, if it also prevents that reactive carbon center because of the positioning of the hydroxy groups which can lead to DNA damage and strand breakage.

So I'd like the speaker maybe to address that aspect of it too.

DR. RAO: I think if the five position is blocked the three position is the next option and that is open. So you have to block both three and the five positions in order to prevent reaction at those two centers.

DR. SLAGA: Okay.

DR. COHEN: Tom --

DR. SLAGA: Yeah.

DR. COHEN: -- with regard to your statement about the quenching of Reactive Oxygen Species, isn't that very heterogeneous in people? I mean, that's not a uniform capacity across all people, right? I mean, some are better at it than others. You used a benzo(a)pyrene there. There are polymorphisms in epoxide hydrolases. I mean, not everyone metabolizes the same way.

DR. SLAGA: Right, right. I agree.

DR. COHEN: So, are we making assumptions that just because it's in vivo that this catalase and superoxide dismutase, that everyone's going to be able to do it the same way?

DR. SLAGA: Right.

DR. COHEN: Okay.

DR. BERGFELD: I don't understand the conclusion of that remark, Tom. Does that mean we still don't know about genotoxicity?

DR. COHEN: So what are we doing, Tom? I mean, does the in vivo model always just assuage your concerns if you have something in the in vitro model? Tom?

DR. SLAGA: I didn't hear that. I'm sorry. I --

DR. COHEN: I'm just saying knowing this heterogeneity amongst people, does the in vitro always just assuage your concerns if it, you know?

DR. SLAGA: There is quite a difference among humans and animals and we all know that if you amplify the protective mechanisms in vivo it's very difficult to induce cancer in those models if you amplify everything to take care of free radicals in that.

But it varies. The people that smoke and get cancer at a very early age probably have a very poor detoxification system. I'm just rattling now. And those people with strong detoxification systems are the people that can smoke and live to be a hundred, you know, if they don't get hit by a car.

Anyway, that varies a lot. And if you use mice that have a highly amplified detoxification system, it's really hard to give them cancer by chemicals and free radical generating compounds and that.

DR. BERGFELD: Tom, does time of application play a part in this? I mean the mice were 48 hours, the humans are 30 minutes or less.

DR. COHEN: Well, it's got to have some impact, right? I mean --

DR. BERGFELD: Yeah, yeah.

DR. COHEN: -- you're straining the system and eventually you can (audio skip) quenching systems. You can overwhelm them.

DR. ROSS: I think you're getting to that original question of the quinone in the five position. Did I not see data in here that you ameliorated the Ames effects by using glutathione. Was that not in the dossier?

DR. RAO: Yes, it's in there. So we used two possible quenchers. The catalase in one and glutathione in the other and glutathione wasn't as effective at quenching as the catalase was.

DR. ROSS: Yeah, so the thiones are going to get rid of your reactive quinones that are generated in that reaction and getting to David's point. Yeah, David, there isn't a bunch of polymorphisms that are going to be relevant in vivo and it's not just catalase. As you pointed out, there's SOD but you've also got glutathione peroxidase. You also have quinone reductases coupled with glucuronidation and sulfation. So I think you'd be better off broadening the discussion here to the whole range of potential detoxification systems that are in there.

But yes, you are correct. There's going to be some major polymorphisms and sensitivity among the human population dependent on those polymorphisms. Yeah.

DR. COHEN: Thanks.

DR. BERGFELD: Any other questions? Any other clarifications needed here? I ask if you all are going to be on our team meetings to discuss this ingredient?

DR. RAO: Yes.

DR. BERGFELD: Yes. Okay. Bart, do you think we should conclude this discussion then?

DR. HELDRETH: Yes, I absolutely do. Thank you, Dr. Bergfeld. Thank you AJ and Pushpa both. This was very informative, and we appreciate your input here. All right. So, if we're fortunate we'll get to ask them more questions, maybe later on today.

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DR. BELSITO: Okay. So then to the other hair dye 1,2,4-trihydroxybenzene. So bottom line here is do we accept the fact that in vivo there are mechanisms of dealing with the oxidative stress that is caused the in vitro genotoxes to be positive -- well, positive and negative? Or do we ask for more in vivo genotox?

DR. HELDRETH: I see that AJ is in the room if you have further questions on it for her.

DR. SNYDER: I'll probably defer to Tom's opinion. And David's reporting on this one.

DR. BELSITO: Well, I asked Tom point blank. He seemed rather wishy-washy this morning. I mean, he didn't clearly say, well yeah. I mean, I think that we have -- in the report we have two negative in vitros and then there was a third. We had the two 3D negative that were presented this morning. I mean, I'm pretty sure they'll get OECD TGs within the next year or two. I mean, I can tell you that the fragrance industry is using them and relying on them as well for toxicity -- for genotox.

DR. RETTIE: There were a couple of instances in the report on PDF pages 16 and 17 where they referred to mis-dosing to explain severe toxicities that were observed and I kind of wondered what that was.

DR. KLAASSEN: What they're talking about there is -- I think that was an oral administration.

DR. BELSITO: Yeah.

DR. KLAASSEN: And often what happens when you put a tube down a rodent's esophagus sometimes it gets into the lung and so you get lung damage. And I think that's what they were talking about there. And that's not all that unusual.

DR. RETTIE: I remember when I was gavaging hairless mice in my doctoral work. I did a lot of that.

DR. BELSITO: Okay.

DR. KLAASSEN: My feeling on the genotoxicity is, yes, I do note that there's some positive and there are some negatives, but the positives were kind of weak. They weren't strong positives and I think the negatives kind of overwhelm the positives. I'm taking that together with the dosage, et cetera. I can live with it.

DR. RETTIE: And there was a negative in vivo micronucleus.

DR. BELSITO: Yeah, there were two negative in vivos. One was new, the SCCS did not look at, the other they discounted because it was only a single dose although it was apparently the maximumly tolerated dose that was used if I recall correctly.

DR. KLAASSEN: Right. And I believe the in vivo is better than the in vitro test. If I had to compare them and Tom kind of said that this morning also. So that's where I'm at on it.

DR. BELSITO: Yeah. I mean, I basically said that if we're comfortable with the in vivos and okay with genotox then it's safe. Although it is a strong sensitizer based off the LLNA but that's never a reason not to approve a hair dye.

DR. KLAASSEN: Right. Okay. Who's reporting on this tomorrow?

DR. SNYDER: David.

DR. BELSITO: Okay. So, we'll get Tom's opinion right off the top. But basically, Curt, you're saying you're okay. Allan and Paul, I haven't heard from you.

DR. SNYDER: Yeah, I'm fine with the approach presented.

DR. RETTIE: Yep.

DR. BELSITO: Okay. So then let's just go through the document. I had a few questions here. This is PDF page 13. The paragraph under the figure says, "if 1,2,4-THB is partially oxidized prior to coupling the rates of active hair dye formation may be different from traditional hair dyes." That partial oxidation, would that be intentional or a result of improper storage? I wasn't really understanding why this paragraph was there at all.

DR. HELDRETH: This ingredient is a unique hair dye ingredient in the fact that it doesn't absolutely require peroxide for that oxidization to --

DR. BELSITO: Right. It auto-oxidizes, so that's my point. Is that because of improper storage or is it intentional?

DR. RETTIE: Just an exposure to air while it's being manipulated, I think.

DR. BELSITO: Okay.

DR. HELDRETH: Pushpa, do you want to add anything to that.

DR. RAO: Yeah.

DR. RETTIE: (Audio skip) can probably clarify that tomorrow, he spent half his life on benzene and benzene metabolites.

DR. RAO: Yeah.

DR. BELSITO: And then --

DR. RAO: Sorry, go ahead.

DR. BELSITO: No, I mean, if we're still on that discussion mine was a new point.

DR. RAO: Okay. I just wanted to share with this team the clarification that we offered Dr. Ross and Dr. Cohen earlier today. There were a couple of questions that Dr. Ross asked and just expanded on the answers. And also, a point that Dr. Cohen had brought up.

So, the first question Dr. Ross asked was about the paper, Reference Number 33 in Table 2 of the SLR. It was human peripheral blood lymphocytes where THB was tested and hydroquinone was tested and they got a positive reaction. And his contention was that with in blood there's significant amounts of catalase and did we have a response to that.

And actually, I didn't recall at the time, but when we repeated the Ames and micronucleus because we didn't have the data -- the raw data in hand -- we did the in vitro micronucleus using human peripheral blood lymphocytes and I guess the difference is that -- and Dr. Cohen brought this up -- is that the serum is not used in that model. And that's where the catalase would be. But in our hands, we got a negative, so we weren't able to duplicate what the team at Berkeley did.

So, when we did the in vitro micronucleus, we got a negative. So, the next point was, he asked about the alkalizing agent and using ammonia and I did mention that we don't use ammonia in our formulations, we use monoethanolamine and using THB alone would give you variations of brown in a hair dye and in our formulations, we mix with other primary intermediates and couplers to provide the different shades. So monoethanolamine is the alkalizing agent and the reaction with PTD or NNBIS, hydroxyethyl PPD or para-aminophenol, resorcinol -- so it's a mixture of primary intermediates and couplers.

And I just wanted to expand on that and share with you as well. And lastly, I think Dr. Cohen asked about the variability between individuals and their ability to manage reacto-oxygen -- the competence of their individual systems. And we're not arguing that, that's undeniable, but we just wanted to point out that interindividual variability is part of the margin of safety calculation and there's an uncertainty factor of ten that's taken for that as well as the ten that's used for cross species correlation.

DR. RETTIE: So, are you saying that there's no catalase in human lymphocytes?

DR. RAO: I'm not saying that, but I think that what happened -- when we did the in vitro micronucleus we prepared the test article under a nitrogen blanket, the solvents were degassed, and when the test article was dosed and exposed to atmospheric oxygen -- nitrogen is quickly replaced by oxygen since oxygen is heavier -- then the normal actions would take place. And I

think the rate of reaction far exceeds the rate of absorption dermally and so coupling reactions are going at a faster rate than a flux across membranes.

DR. RETTIE: Yeah. Just a quick search showed one reference here, at least, the presence of catalase in human lymphocytes and I would've thought it was there.

DR. RAO: Yeah.

DR. RETTIE: All right.

DR. RAO: Well, I didn't actually question it, but Dr. Cohen offered that if serum was removed then the catalase is not as versatile and I'm sure he can expand on that tomorrow.

DR. RETTIE: And that may be. I don't know this.

DR. RAO: Yep. That's not my wheelhouse so I can only comment on the results that we achieved, and I think it's the color formation when we applied to 3D skin models and even in the in vitro media there was staining of the media.

DR. BELSITO: Well, I mean, human serum does contain catalase but also monocytes contain catalase and lymphocytes do too. So, I mean, obviously in the presence of serum there would be more catalase, right, because you got catalase from both sources. But I think we're fine there. It sounds like we're --

DR. RAO: I just wanted to make sure you have the same information.

DR. BELSITO: Right. Thank you. Are we all comfortable with the impurities that are given because there were two different sets of impurities. Did that bother anyone?

DR. SNYDER: Not me.

DR. BELSITO: Okay. And, Christina, one thing I didn't understand is it said -- this is on PDF page 15 -- the dermal penetration in vitro, the fourth line down it says the study was performed at test guideline 428(draft). What did you mean by draft? TG428 is not in draft.

MS. BURNETT: It's probably how it's cited in the original copy of the citation so I will go back and check. But, yeah, the source might've said draft before it was finalized so I just carried it over.

DR. BELSITO: Okay.

DR. EISENMANN: So maybe the study was done before -- I don't know the date of the study. The study was done before the OECD guideline was finalized is what I took it to mean.

DR. BELSITO: Okay.

MS. BURNETT: Yeah. Let me --

DR. EISENMANN: The study was done in 2014.

MS. BURNETT: -- the citations are for the SCCP/SCCS opinions from 2006 and 2012. So, if it was finalized in '14 it was a draft at that time. I can remove the word draft.

DR. BELSITO: Yeah.

DR. RAO: I think the dermal absorption in the 2012 opinion was the previous defender.

DR. BELSITO: Okay.

DR. RAO: Because we did our study in 2014.

DR. RETTIE: I found that there's THB in roasted coffee beans, up to two milligrams a cup.

DR. RAO: And green tea.

DR. BELSITO: Yes, I know. Please. It had me worried.

DR. RETTIE: Knock it back.

DR. RAO: It's in green tea as well.

DR. BELSITO: I don't drink that, just coffee. Just a question on the dermal penetration. It says -- this is the first paragraph in the in vitro where it says that -- last two lines -- "the SCCP commented that degradation of circa 8 percent within one week of the test substance in the test formulation was indicated even though it was stored in an inert atmosphere." So, does that mean that the test was actually 92 percent of what's reported, and so the absorption would've been somewhat higher? What is that saying?

DR. RAO: I think that's referring to the previous dermal absorption studies where the absorption was very, very low. In our study the test article, as I said, was in degassed solvents and stored under nitrogen sealed vial until it was dosed, so we did not get degradation until the actual exposure.

DR. BELSITO: Okay.

DR. RAO: I think if you put it in a normal solvent system where you have dissolved oxygen, you're going to get some degradation and that's why we went to the extent of degassing all the solvents to make sure that it was stable.

DR. BELSITO: So, yours was the second in vitro study?

DR. RAO: Exactly.

DR. BELSITO: Okay. Then let me get rid of that comment.

DR. KLAASSEN: I have a question about the margin of safety calculation on page 20. So, the CIR staff did this calculation, I guess I had, first of all, kind of a procedural question. Are we going to start doing this kind of on all chemicals from now on? I mean, we've had margins of safety before. I think most of them have been calculated we got from the literature, but this one we calculated ourselves and maybe this is kind of a new direction for us, and I just want to make sure that we agree with that.

And the second thing, that if we do decide to calculate margins of safety, I object to five significant figure, which is a 1397.8, I would rather have it be 1400. Nobody can pipet to five significant figures.

DR. RETTIE: Well said.

DR. HELDRETH: Yeah, we have no problem reducing the sig figs on that. So following Dr. Don Bjerke's presentation at our last panel meeting, we took that as an initiative to bring in more risk into our safety assessments formally for two reasons.

One is to be a little more transparent about how the Panel is looking at risk, but then secondly with the advent of all these new approach methodologies and the non-animal models we were told that utilizing margin of safety and eventually some of these next generation margin of safety assessments helps us to build confidence in those methodologies so we -- you will see in all of the draft reports here where there wasn't already an available margin of safety Jinqiu has provided our draft calculation of it and we leave it up to the Panel to decide if they like what's there.

It's always the Panel's purview to decide if there's value there and if some of these in silico endpoints that go into this calculation are not reliable in the Panel's eyes, we can always ask for experimental data, but we wanted to provide this here as a tool for the Panel being more transparent on this and for future use with some of these new approach methodologies.

DR. BELSITO: I think it's fine.

DR. KLAASSEN: Yeah, I'm okay with it. I just wanted to make sure how consistent we're going to be, et cetera.

DR. RETTIE: Did we see a circumstance where we run a margin of safety calculation and get a value above a hundred and yet our conclusion for one reason for one reason or another is insufficient. Do the two clash off against each other? Do we send mixed messages?

DR. BELSITO: We would never be in that position because if there were an endpoint that was insufficient, we wouldn't know whether that would be the limiting factor that would control the margin of safety, right? You look at the endpoint that has the lowest safety value to it. And if we're missing something then we wouldn't be able to calculate a margin of safety.

DR. RETTIE: Okay. I just wanted to air it out.

DR. HELDRETH: Don Bjerke, did you have a question or comment?

DR. BJERKE: Yeah, I think there's a time and a place to calculate a margin of safety for skin sensitization. Typically, we've done this historically for preservatives as well as some of the hair dyes. And so, what we need is a defined no expected sensitization induction level and then we apply the appropriate default uncertainty factors or sensitization assessment factors, as they're called, for the particular application.

So, if the data's there I fully encourage you to calculate a margin of safety for skin sensitization. If not -- if their potency isn't defined -- then it becomes a little bit more difficult. But I think the Panel is definitely moving in the right direction in doing it as appropriate based on the data.

DR. BELSITO: Can we just go back to the carcinogenicity studies -- this is PDF page 17 -- because I was just a little confused here. First, so the study started with 15 male and 15 female mice. Were these all treated or were some of them controls? Was it like 25/25? And then there seemed to be a significant number of deaths that occurred so that when you got down to the final there weren't a lot of animals left and do we know what the cause of death was?

It doesn't really tell us. I mean, I'm presuming there were some controls because there's a sentence that says that at seven and nine months ten males and ten females per group were necropsied. So, I'm assuming the groups were treated in control, but I don't know. I mean, I don't think it changes anything, it's just that data from the report is really not clear.

MS. BURNETT: I will go back and check. From the one SCCS report I'm looking at right now it doesn't really say.

DR. BELSITO: But we don't have the actual study.

MS. BURNETT: I'm looking. I pulled up the first citation. So, there's three citations associated with that. So, I will check. Okay.

DR. KLAASSEN: This is not a very acceptable study in carcinogenicity in this day and age of only using 15 animals and then with all these animals dying and that's probably why the SCCS said that these data were insufficient to conclude the carcinogenicity potential of the 1,2,4-trihydroxybenzene.

DR. BELSITO: I agree.

MS. BURNETT: It is an older study. It's from 1980, it is a Burnett report and the details in the report are a little lacking.

DR. BELSITO: Well, if you can try and flesh it out as much as possible in terms of number treated, number of controls, what the cause of death was in all these animals that died off or was it that they actually slaughtered 40 animals between the seven- and nine-month necropsies. If they took ten males and ten females and there was an equal number of controls and treated that was 40 animals right there that were gone by nine months and they only started with 50. So just flesh that out.

MS. BURNETT: I will.

DR. BELSITO: And on PDF page 19, the melanogenesis inhibition, what is a suicide substrate?

DR. KLAASSEN: One that's metabolized to kill the enzyme.

DR. BELSITO: So that's a real thing?

DR. RETTIE: Yep.

DR. BELSITO: Okay. Never heard of it before.

DR. RETTIE: It's colloquial of course. Mechanism based inactivation is maybe a preferred term, but suicide inhibition really gets it across.

DR. BELSITO: Right. So, with the sensitization data -- yeah. I just put for the in vitro that it was irrelevant because only one in vitro was done so you can't make a determination and LLNA trumps the in vitro. It's just for my concern. Let me delete that. So, I have no other comments. So, we're going with safe as used and we'll see what David says tomorrow since he's reporting. Is that correct? Yes, no? Team? Allan, you're okay with that? Safe as used?

DR. RETTIE: I am.

DR. BELSITO: Okay. Curt, Paul?

DR. SNYDER: Yes, I'm fine. Yep, I'm fine.

DR. KLAASSEN: Yeah.

DR. BELSITO: Okay. Thank you.

DR. HELDRETH: So, just to clarify we're doing safe as used as a hair dye?

DR. BELSITO: Yes.

DR. HELDRETH: Because there's also this one hair coloring shampoo. I don't know if we want to say anything about that. It's got one reported use in hair shampoo (coloring).

DR. BELSITO: Well, it auto-oxidizes so wouldn't a hair shampoo be a hair dye? I don't know what to say about it, Bart. I mean, I'm a little --

DR. HELDRETH: Maybe Tony Santini might want to say something about it. Let me move him into presenter. All right, Tony, you should be able to turn on your mic and camera.

MR. SANTINI: Can you hear me?

DR. HELDRETH: Yes.

MR. SANTINI: Yes, it's a marketing thing, Dr. Belsito, correct. It is a hair dye also.

DR. HELDRETH: Great. Thank you.

DR. RAO: It's called a hair coloring shampoo.

DR. BELSITO: Okay.

DR. HELDRETH: I just called it out because, I mean, do we see a lot of other oxidative hair dyes that we put in shampoo at home?

DR. RAO: Yeah. Well, in our product I can tell you there's only three hair dyes and they're in at low levels and it's supposed to deposit small amounts of color over time with each progressive use. So, it's a progressive hair dye product.

DR. HELDRETH: Great. Thank you.

DR. RETTIE: Just for Men.

DR. BELSITO: There are two figures that were provided to us that I like and would suggest that they be in our report and that's the figure that's on PDF page 224 and the figure that is on PDF page 240. I just sort of like them as we are looking at this safety here.

DR. RAO: Yes, that's the reaction scheme compared to PPD coupling.

DR. HELDRETH: Mm-hmm.

DR. BELSITO: Right. Those two figures, I thought, were really very good and would like to see in our report.

DR. HELDRETH: Yeah, I can redraw those so that they'll look the same, but it'll be a little higher res with the next iteration.

DR. BELSITO: Great. Thanks, Bart.

DR. HELDRETH: Sure.

DR. BELSITO: That was all I have. Any other comments here.

DR. RAO: I can send you the original figures if you like, Bart.

DR. HELDRETH: I'm a chemist, I like drawing things.

DR. RAO: Okay.

DR. BELSITO: Therapeutic.

DR. HELDRETH: That's right.

DR. BELSITO: Okay.

DR. HELDRETH: It's artistry.

DR. RAO: Meditation, yeah.

DR. HELDRETH: That's right.

Cohen Team – December 4, 2023

DR. COHEN: The first of which I'll start off with is 1,2,4-Trihydroxybenzene. This is a draft report for this chemical. This ingredient is reported to function as a hair colorant in cosmetic formulations and it's an auto-oxidative dye used in permanent hair dye formulations and gradual hair coloring in shampoos. Does not require hydrogen peroxide to activate oxidation in subsequent coupling reactions. In 2023 the VCRP survey data indicated the product is used in at least 18 hair dye formulations and one hair coloring shampoo and the concentration of use was reported by the Council up to 2.5 percent.

We have method of manufacturing, composition and impurities. Interesting it's present in roasted coffee. I found that to be an interesting tidbit in the presentation. We've also had a discussion that it depended to be used in the presence of primary intermediates such as p-phenylenediamine, p-toluenediamine, and aminophenols. However, if THB can be partially oxidized prior to coupling, I guess without these -- we know this is a sensitizer in animal studies and we had a discussion about margin of safety which I'm sure we'll expand on.

We had a really excellent presentation this morning and I understand there's a motion to clarify some points from this morning and I think we can open it up now for clarification before the Panel gets to make any discussion. So, we can do that from our speaker. Is AJ on now?

DR. CUEVAS: Yes, I'm here. I'm here. Pushpa is also here.

DR. COHEN: Okay. Who am I --

DR. RAO: I think I wanted to answer some of the points that were raised during the morning's discussion. And I'm wondering if we can help the echo by anyone who is not speaking put themselves on mute. That might help. Okay.

Maybe I can start with Dr. Ross. I had two points that I wanted to clarify that Dr. Ross had brought up. The first one was the paper -- I think it was Reference Number 33 in the SLR, a paper by Zhang in 1998. And I couldn't put my finger on it at the time we were discussing it, but we actually are -- the in vitro micronucleus that we did with our original -- repeating the studies that had already been done, we did an AMES and in vitro micronucleus. And the in vitro micronucleus was in human peripheral blood lymphocytes.

So, we actually did that using the OECD methodology and we got a negative. So, I think that is a direct counterpoint to the Zhang paper that used human blood. And they found an effect and I can't exactly say why they saw an effect, but I think it's subject to all of the deficiencies that we had talked about with using methodologies that may not be compliant with an OECD protocol or uncharacterized test articles, you know, that kind of thing. So, it was done in an academic setting.

The other point I wanted to address was the reaction chemistry in using THB with ammonia. And we had looked at using it by itself with ammonia but the reaction is much too fast with ammonia. And it's hard to stabilize in a hair dye formulation that it's more likely to form color on the surface of the hair rather than it doesn't give it time to actually penetrate the cortex and create color inside that won't be washed out. And the use with other hair dye ingredients allows for the various shades to be formed.

So, by itself it gives you variations of brown. And if you want to have something a little bit more interesting you need to mix with other hair dye ingredients. It's more of an efficacy type of reason.

DR. ROSS: That beautiful figure that you had in Figure 3 of the dossier that you submitted to us, that was on its own or was that with PTD or PPD?

DR. RAO: Let me pull up -- Figure 3 you said?

DR. ROSS: Yeah. It looked like it was on its own to me. But there's some pretty impressive (audio skip).

DR. RAO: Yes. So, it's a typical hair dye formulation so it's not by itself.

DR. ROSS: Yeah.

DR. RAO: It is mixed with other hair color ingredients. So that study that you're referring to I think is the decay of the material in a hair dye formulation, so how quickly does it react over time? And we followed it over a 30-minute time period. And normally, according to the directions, the product is left on the hair for ten minutes. That's the recommendation, but we took the study out to 30 minutes because sometimes people leave it on longer and it's good to understand exactly how quickly is the material being used up. That was one of the questions the SCCS came to us with. How much unreacted THB is there and so on and so forth.

But in our experience, it reacts very quickly. And with stronger alkalizing agents like an ammonia or -- which is normally used in hair colors, the reaction rate is too quick and it's hard to control. So, I hope that answers your two points, Dr. Ross. If you have a follow-up please let me know.

DR. ROSS: Yeah. I mean, that's good. Thank you. I would say maybe it wasn't done to OECD guidelines that Zhang et al. It was done in a lab. But I can tell you it was a good lab.

DR. RAO: I know. I know. We're truly not casting aspersions on the academic research laboratories, but I think when we're talking about -- I mean, I was a grad student once, I know how it is. You're trying to do work that's important and describe your -- you can't publish negative results, right? That's kind of a fundamental principle.

DR. ROSS: You can.

DR. COHEN: It's harder.

DR. RAO: It's harder. Right.

DR. COHEN: It's harder.

DR. ROSS: I'll hand it back to David. He's going to move this forward.

DR. RAO: Yes. I mean, I suppose you can say our addressing the Zhang paper was to do the in vitro micronucleus in the human peripheral blood lymphocytes. So hopefully that puts that to rest?

DR. COHEN: When that's done with human peripheral blood lymphocytes they're taken out of serum, right? So, they are incubated on their own. So, none of the serum-quenching factors for reactive oxygen species are present, right?

DR. RAO: Yeah, I think you're probably right. And, I mean, if you're going to see an effect you'd see it in that milieu, right. But we didn't see an effect.

DR. COHEN: Right. No, no. I think that's a stronger point, right --

DR. RAO: Yes, yes.

DR. COHEN: -- that you actually don't -- if they're removed from circulation and you're just using them as a cell line, that they're not bathed in the milieu of having lots of available ROS quenching options.

DR. RAO: And I think that's the drawback of most in vitro systems, right, except for the 3D skin models where you have metabolically competent model, and it more closely mimics an in vivo type of an exposure. And I think that's the value of that. And it's more relevant for cosmetic ingredients because you're applying it to skin. Yeah.

DR. COHEN: Okay.

DR. RAO: And there was one point, I think, Dr. Cohen, that you had raised about interindividual variability and metabolic competence for ROS management. And I think -- I mean, that's undeniable. It's well recognized. There's a lot of variability among individuals but that's part of the margin of safety calculation is there's an uncertainty factor of ten and we also take an uncertainty factor of ten for the cross-species correlations, right?

DR. COHEN: Yeah. Completely legitimate and that's part of the uncertainty. And I think there's some data regarding those polymorphisms and the capacity to be sensitized to PPD as well. Right? So, okay.

So why don't we open this up for discussion since this is a draft report. This is the early days of this report. And, Tom, I think maybe you should open us up with comments/questions.

DR. SLAGA: Okay. Well, first of all, for a draft report there's a lot of data. I want to emphasize that. And based on what we heard today that some potential in vitro or in vivo full-thickness human skin type of studies will shed light on these things, still we have to look at all the data. All the, you know, is it a mutagen in various strains? Is it a mutagen in other in vitro and is it in vivo?

And if you take all that data together it's really quite mixed right now. You could go either way and say the weight of evidence is for genotoxicity or weight of the evidence is not for genotoxicity.

The one saving grace that we do have related to this particular compound is that it was tested as a skin cancer agent -- the induction of skin cancer -- and it was deemed negative. So, here is an in vivo assay that is an endpoint that we think is very important. So, whatever the genotoxicity is here, it's not leading -- if it is a genotox it's not leading to any skin cancer.

The other thing that would affect if it was a genotoxic agent is that how much irritation or skin hyperplasia would occur. And in this case, as an irritant, it appears to be very weak. So even if it was genotoxic, there was a possibility you wouldn't get any tumors. But anyway, the endpoint is we have to consider the skin cancer data is a large number of animals and it's very reliable. So, I'm just summarizing data, I'm not saying that it's safe right now or not safe.

It's still a very reactive compound and depending if you neutralized free radicals or not, it can go one way or another. So, obviously the dose levels used to do the skin cancer were reasonably high so I would say that it does not cause skin cancer and I believe there was an oral cancer too that they studied but it didn't really show anything really positive. I'll stop right there for more discussion to come in.

DR. COHEN: Tom, do you have any data needs yourself right now?

DR. SLAGA: No. That's the problem. What data needs would you ask for? I mean, you may want more sensitization data or something, but it appears to be not a sensitizer. Well, anyway, I don't have any data needs.

DR. ROSS: Tom, could I just respond to your point on the carcinogenicity? When I looked at that data, you know, I went to the SCCS opinion, and they said it was insufficient mainly because so few animals survived out to the --

DR. SLAGA: Yeah, that survived the whole, but they started out with a large number of animals and that's a concern. I was going to bring that up and I forgot to. There is some toxicity occurring to kill any animals and so at the endpoint there isn't many animals but --

DR. ROSS: Yeah. So, they were saying they couldn't conclude -- I'm not getting into the carcinogenesis analysis, I'll leave that up to you. And then on the oral they saw increased DNA synthesis with bromodeoxyuridine in both sex apparently. And so there were some signs there.

But, if I can get back to the, I guess, we're talking genotoxicity here and yeah. It's a whole weight of evidence thing here so I agree with what Tom said. You could go either way on here whether you're putting your emphasis on in vitro or in vivo. But what we do have are two in vivo tests that are showing very little effect, I guess at the micronucleus. And I think that was highlighted this morning by Dr. Cuevas, but yeah.

So that's where we are with that so just depends on where you come down on that, in vitro, in vivo or weight of evidence.

DR. SLAGA: That's right.

DR. COHEN: Yeah. But this is more than an academic discussion (audio skip) how do you land a plane. Why don't we hear from -- David, do you have any general comments or data needs?

DR. ROSS: I do. I think, generally I thought there was very little absorption with this compound. I mean, you're looking at 0.2/0.3 percent. I think the weight of evidence generally for genotox, it's a difficult question but the in vivo is coming in negative. Some of my major concerns were really lack of any human skin data with respect to sensitization. We know it induces DTH, delayed type hypersensitivity and it's classified as an extreme sensitizer. So, I need the guidance of my expert dermatologist in this group to tell me if that is going to be an issue or not. Because when I look at the data -- you know, we don't have HRIPT.

DR. COHEN: Remember this is a hair dye.

DR. ROSS: Yeah, I know.

DR. COHEN: This comes along a different pathway and the users are instructed to patch test this before use. I suspect we'll find sensitization.

DR. ROSS: Was this a coal tar exemption?

DR. COHEN: I'm sorry, David, what?

DR. ROSS: This was not a coal tar exemption, was it?

DR. BERGFELD: No. No, it's not.

DR. COHEN: No. So that's a very good point, although I was in my head looking at this as a hair dye. I guess the one question will be if our clearance is that it should be used with primary intermediates only then it would, by that definition, be patch tested ahead of time. But you're right, it's not a coal tar exemption.

DR. BERGFELD: David, could I interfere?

DR. COHEN: Please.

DR. BERGFELD: I wanted to -- I'm sorry.

DR. COHEN: I'll go on --

DR. BERGFELD: All right, one of the questions. Still --

DR. COHEN: Why doesn't everyone go on mute, and let's see what happens there.

DR. BERGFELD: I mean, I --

DR. COHEN: You're not on your phone, just the computer?

DR. BERGFELD: I'm just through the computer.

DR. COHEN: Can you lower the speaker only when you're talking and see if that works?

DR. BERGFELD: How did that hel- --

UNKNOWN MALE: You may have to dial out and dial back in.

DR. BERGFELD: I'll leave and come back.

DR. COHEN: All right, so we do have that issue, David. There's just nothing on that. The other question I had -- why don't we go to Susan for her general -- like, what other remarks did you have, David?

DR. ROSS: Yeah. I felt that we didn't have the sensitization data and so I raised the issue of the HRIPT, and I realized that might be difficult to get. There are other sensitization assays we can do. More QSAR type assays and the possibilities with the h-CLAT for example, human cell line activation, U937 activation. I mean, I guess there are -- you'd know more about these than I, David. Guinea pig maximization tests. Do we have additional data on sensitization because I'm not seeing a lot right now which is giving me a lot of comfort here with sensitization.

With respect to another point, This thing, you know, I've worked on phenolic metabolites of benzene most of my scientific career, particularly with respect to benzene toxicity, and this thing is a tyrosinase suicide substrate which is in our dossier. So, I think you're going to get some impacts there on melanogenesis. And that should be addressed at least in the discussion of concerns.

And then, finally, the margin of safety -- and this was in our dossier not in the Combe dossier, and I've talked with Jinqiu already with this. Clearly the 50 mg/kg was a LOAEL and not a NOAEL and so we should start with that. And the margin of safety would be coming in at 466 and 139 -- which I think Dr. Cuevas presented this morning -- rather than the one in our dossier at 1,300.

And both of those indicate an acceptable degree of risk. I mean I don't think the term safe is particularly helpful in general toxicological terms, but I think an acceptable degree of risk is probably right.

So, yeah, they were my comments. Sensitivity, tyrosinase suicide substrate, and then the MOS and then we still have to figure out which way we're going on the genotox.

DR. BERGFELD: Can I talk now?

DR. ROSS: Yes.

DR. COHEN: Yes.

DR. BERGFELD: Okay. The thing that I wondered about as a dermatologist was that these are applied for ten minutes and they -- let me see what it -- THB is coupled with other hair colorants. And this activity of the coupling takes place as soon as they're mixed or open to oxidation. So, the problem is in the whole or in the timing of that to offer risk.

If it's slow and both the coupler and the THB takes a long time for them to couple and become an inert mass of colorant, that's one thing. If they are mixed before and they are applied, they're basically inactivated as they're applied. So I'm not sure about this absorption. I mean, there's an absorption time but it's very, very short. And if the coupling also allows for the free radicals to be quenched immediately.

DR. COHEN: So, are you suggesting it's mitigating a safety risk in a carcinogenesis perspective? Because hair dye --

DR. BERGFELD: I'm talking about sensitization.

DR. COHEN: Yeah. But hair dyes also have that brief episode of exposure.

DR. BERGFELD: No, I know that. It is a hair dye but a different one, different kind.

DR. COHEN: Right. But people get pretty reactive when they're allergic to --

DR. BERGFELD: All of them. All of them.

DR. COHEN: Not all of them. But I'm saying even with a short exposure some of them can cause pretty remarkable reactions, right?

DR. BERGFELD: But we were also talking about the fact that we have phenylenediamine that's most commonly coupled with the THB to give the color, and then some modification of some other colorants in them. And I'm just thinking of it as a larger molecule now because it's got all these additives to it. And how active is that and does that have the same risk as the THB?

DR. ROSS: It's certainly going to have different reaction products when you couple, Wilma. That's obviously correct. These things are going to be larger and, in fact, if you look at the absorption, they're seeing greater absorption with PTD when they have it with PTD than they are without. And so that speaks to that.

With respect to your question about red counts, I don't have a red count for that reaction, but my assumption is it will take some time -- it may be rapid, but it will take some time. And will you get exposure to both the unreacted compound and the products that are coupling, I think you probably would.

DR. BERGFELD: It's a ten-minute application, I understood.

DR. ROSS: Yeah. Yeah.

DR. BERGFELD: Not 20 minutes, not a half hour, not an hour. So the exposure time is pretty short.

DR. ROSS: Yeah, as I commented, that's Figure 3 in their dossier. Pretty impressive. But, yeah, no. I still think you'll get exposure to both. And those coupling products -- and SCCS spoke to that, actually. They actually looked at what the coupling products were going to be, and they had a nice little figure in there of what those couplings were.

DR. COHEN: Let's go to Susan and then we'll swing around because we haven't heard from her on this.

DR. TILTON: Yeah. So, I just wanted to get back to the aspect of genotoxicity and as for a little bit of historical perspective. So, I mean, in this case we have an ingredient where we know a lot about the mechanism of action, there's quite a bit of genotoxicity data. It is certainly genotoxic through its reactive intermediates. It seems like the question is, can it cause damage under conditions in which it would be utilized, not necessarily is it genotoxic?

So, I feel like we need to make that distinction. Usually when we're talking about genotoxicity what we're really saying is yes/no. But has that distinction been considered previously? And it sounds like possibly for hydrogen peroxide, although I would say that's a little bit different. Because what we're trying to consider is whether or not it can cause damage under the conditions in which it will be utilized.

DR. COHEN: I mean, I think we at times have to thread the needle where we have some genotoxicity data and then others do not confirm that, and we wave them and make a final adjudication. So, Susan, do you have any data needs yourself on any of this?

DR. TILTON: I mean, I agree with David about the discussion regarding the margin of safety but, like Tom, I noted we actually do have quite a bit of data here. And so, I didn't note any data needs.

DR. COHEN: I guess the discussion of sensitization data is a data need.

DR. ROSS: I think it is.

DR. BERGFELD: The discussion or the test?

DR. COHEN: No, no, no. Is it an IDA?

DR. ROSS: I think it is. You need more sensitization data.

DR. BERGFELD: And concentration of use and in animals or humans or either or both?

DR. COHEN: Well, let's have something.

DR. BERGFELD: Well, I think you ask what they have.

DR. COHEN: I mean, ideally HRIPT would be the best, right?

DR. ROSS: Maybe (audio skip), Wilma. But we talked about HRIPT but then the other cell line acids and maybe some guinea pig acids, it's possible. And I don't know what's out there but there may be some of that out there already, but we didn't see it. I think we got one -- I think Pushpa has a comment here.

DR. COHEN: Yes.

DR. RAO: I just wanted to point out that there is the LLNA data, and we also did KeratinoSens.

DR. ROSS: Yeah. Yeah, the KeratinoSens was wide variation, I think. And then the LLNA data showed us (inaudible).

DR. RAO: And I am not sure of this but because it's a derivative of benzene, does it not fall under the coal tar -- is it considered a coal tar dye just as PPD or PTD?

DR. ROSS: I don't know.

DR. COHEN: Christina or Monice, any thought on that? It's funny, I reviewed it with that in my head but David made me question that very appropriately.

DR. RAO: Yeah. That's the way I was thinking of it because it was derived from benzene.

DR. ROSS: I don't think it is. I mean, the method of manufactures very different.

DR. KOWCZ: I mean, even PPD is not directly harvested from crude oil. There's the reaction process, the synthetic process that it undergoes in order to be made but it's still captured under the coal tar dye exemption.

DR. COHEN: Monice, you have any thought on it?

MS. FIUME: I was going to ask PCPC to weigh in. Cathy or Kim, I believe are in the meeting, if they could have any input on it. Because they would probably be better versed in whether or not it's considered a coal tar than I would be.

MR. SANTINI: I don't know if you can hear me or not, but I'd be happy to weigh in.

MS. FIUME: I'm sorry, who are you?

DR. KOWCZ: I think it's Tony.

MS. FIUME: Okay, I'm sorry. I didn't know who was speaking. Okay, yes, if someone from industry who's familiar with it could weigh in that would be helpful.

MR. SANTINI: I'm Tony Santini, I'm the General Counsel of Combe. I'm also the recent Chair of the PCPC LSC Committee. I have 30 plus years' experience in the hair color industry across a number of companies. And we always have considered THB to be a coal hair dye by virtue of its permanent hair coloration attributes and also, it's chemical characteristics. As a result, all of the products that contain THB abide by the coal tar exemption guidelines; namely, to do the 48-hour allergy patch test and to have other guidelines vis-a-vie symptoms and avoid incidents of application on eyebrows and eyelashes and the like. So, yes, we consider it to be a coal tar hair dye.

DR. COHEN: Okay. So, we can have that discussion tomorrow. Now, let's just say that our "data needs" are filled with sensitization data, right. You know, that's not where the -- look, I know even if I got this back and it was a sensitizer we would, under normal circumstances, say formulate to not be sensitizing. In this circumstance, it's going to come under the exemption of doing the allergy alert test which has its own major issues in my opinion, but it's there. Are we willing to take a step on the genotox and clear it?

DR. ROSS: The genotox is a separate question. If I can just get back to respond to -- I'm sorry, I didn't catch the gentleman's name who was speaking. I mean, the response was it was considered a coal tar exemption product, but in a regulatory perspective will it be labeled that way is my question? I don't know -- here what I'm saying, David? I mean, is it going to be regulatory? Or is it just something where we consider it might be that way? I mean, the question is will it really be?

DR. COHEN: I don't know if FDA's on the line, if Prashiela's on the line or not, but right. And this is an FDA exemption, not a CIR exemption.

DR. ROSS: Correct.

DR. COHEN: So, what are they calling it?

DR. ROSS: But in the absence of the coal tar, yeah, I think we need sensitization data.

DR. COHEN: Okay. I'll have that discussion tomorrow. And before I make a motion, right, I can try to clear the air on that with the group in the room. That's one reason's Don's right, these virtual ones are a little more difficult. But are we clearing this otherwise?

DR. SLAGA: Yeah.

DR. COHEN: So, Tom, you're voting to clear this as a coal tar dye? Monice?

MS. FIUME: David, if I could just interrupt for a second. Dr. Zhang from FDA is on the line and I just made her a presenter so she can respond.

DR. ZHANG: Yes, Prashiela's not here, she's on leave. I'm here with your room. I heard the discussion regarding coal tar versus non-coal tar. I'm a toxicologist usually in FDA, if FDA's trying to make such a determination, we will need more data. I need to go back to our chemist and usually it will depend on chemical structure, manufacture process and further information -- and the source of material.

So those are the information that our chemist will look at usually and I can only talk about the principles here. But thank you.

DR. COHEN: That helps a lot, though, thank you. We have -- all right, so I just want to get clarity from the group because this is a tricky one. So, Tom, in the outside of the sensitization discussion, right, are we clearing this on the toxicology other than sensitization?

DR. ROSS: On the genotox you're talking about?

DR. COHEN: Well, is there any other tox in concern other than sensitization?

DR. SLAGA: Yeah, the genotox could go either way but the fact that we have carcinogenicity data, shouldn't we use that?

DR. ROSS: Well, I'm just not sure. I mean, it's been looked at by SCCS and they felt they couldn't come to a conclusion based on that carcinogenicity data. Simply because by the end of the time, Tom, they had very few animals left. You know, we just chatted about it.

But getting back to the genotox what you have, is you've got in vitro tests that are positive and basically you've got, I guess, two in vivo tests that are negative.

DR. SLAGA: Right.

DR. ROSS: Then you've got the contention out there that it's catalase, it's glutathione peroxidase, it's glutathione, it's quinone reductases, in vivo that are making a difference. And maybe that's true.

DR. TILTON: Well, David, we also have the full-thickness 3D model studies.

DR. ROSS: Yes, that's correct, Susan. Yeah.

DR. TILTON: Yeah.

DR. COHEN: Is there any value if we clear this -- let's just assume it's coal -- I'm not digging in on that, but if it is a coal tar hair dye and our safe as used would say when used with a primary intermediate, does that mitigate any of our toxicology risk?

DR. ROSS: I'm not sure it does. You don't really know the exact structures of all the intermediates that are formed. You have this (audio skip) of coupled quinone products. And it's sometimes difficult to figure out what's formed in these reactions. You do see greater absorption with the coupler. So, yeah, I'm not sure if you can use that argument.

DR. COHEN: Okay.

DR. BERGFELD: Well, does the company know what's reactive and left? I mean, the company has studied this.

DR. COHEN: They may be so fleeting, those compounds, right?

DR. BERGFELD: Well, if they're fleeting, they may have no activity if it's a quick reaction.

DR. ROSS: Hydroxyl radical is the most reactive species -- oxygen radical species around, and that's very fleeting. So is it difference.

DR. COHEN: So, we have Tom with a yes. Susan, what do we have from you?

DR. TILTON: So, I do think that there is pretty good evidence in vivo and in the human 3D studies, that under conditions that would be more realistic compared to the in vitro studies we're not able to see damage.

DR. COHEN: Is that a yes?

DR. TILTON: I would put higher weight on those studies then.

DR. COHEN: So, your vote would be?

DR. TILTON: Would be yes.

DR. COHEN: And David?

DR. ROSS: It's difficult, in vitro versus in vivo. Generally, in these discussions I think I put more weight on the in vivo results. And that's where we would be here with, you know, they're showing negative. Plus you have the intact skin data. So I would lean that we'd have to conclude that we could clear that genotox based on the in vivo evidence.

I'm still of the opinion you need some sensitization data. The tyrosinase issue probably needs discussed. And we need to make sure the margin of safety is modified in our document.

DR. COHEN: And what was the modifications again?

DR. ROSS: Modification of the MOS, it's a LOAEL not a NOAEL.

DR. COHEN: Yeah, a NOAEL versus a LOAEL.

DR. ROSS: Yeah, and it was a three-fold extension. I think Dr. Cuevas presented it this morning. It was 488, actually. So, it's not the 1,300 that CIR staff Jinqiu had in there. And he and I talked about this before, actually, and he's totally fine with it.

DR. KOWCZ: In our dossier we used it as a LOAEL.

DR. ROSS: Yeah. Which is the right way to do it. Yeah.

DR. KOWCZ: Mm-hmm.

DR. COHEN: Okay. The reason I'm polling you all now, because this might be a live action discussion tomorrow and I don't want to go back and relitigate this whole thing again. But right now, I think we can go out as an IDA with sensitization data and what is FDA's assessment of this product? If it is a coal tar derivative hair dye, we may not need the sensiti- -- I could stage it. Is this a coal tar exemption? If yes, I think we may be okay with the sensitization, if it's a sensitizer. And if it's not assigned to that exemption then we would need further material. Is that reasonable?

DR. ROSS: Yeah.

DR. BERGFELD: I think that your discussion will have to contain a lot of this in brevity, including the fact that it's a quick coupling and a short duration on the skin.

DR. COHEN: I think the exposure is quick, but the discussion should be long.

DR. ROSS: Yeah.

DR. BERGFELD: Whatever.

DR. ROSS: I think you've got all the difficult ones tomorrow, David.

DR. COHEN: I do. I did notice that. All right. So, are we comfortable with that?

DR. ROSS: Yeah.

DR. COHEN: Okay.

DR. GRIFFIN: Can I just ask one quick question before we move on? In the discussion this morning, Dr. Belsito had a question or made a point that the non-animal methods have not been OECD approved. I guess my follow-up question would be does moving forward -- does that mean CIR is prematurely approving those methods without reviewing them or evaluating them? I'm just curious.

DR. COHEN: Is that sensitization you're talking about?

DR. GRIFFIN: No, I think this would go back to the tox data. The non-animal.

DR. TILTON: (Audio skip) genotox.

DR. GRIFFIN: Yeah. Right, right.

DR. COHEN: Oh, the genotox. Okay, I'm sorry. Understood. Tom, what do you think? You're on mute, Tom.

DR. SLAGA: I'll go with what David said in the last discussion.

DR. ROSS: Courtney, if I could just answer your question. I think.

DR. GRIFFIN: I'm sorry, what did you say, Dr. Ross?

DR. ROSS: Yeah, I'll have a go at answering your question.

DR. GRIFFIN: Okay, perfect.

DR. ROSS: I think if we went with the weight of evidence on the in vivo then --

DR. SLAGA: Yeah. In vivo, that's what I meant. Yeah.

DR. ROSS: And then the vivo micronucleus is OECD approved.

DR. SLAGA: Right.

DR. ROSS: So you wouldn't need the necessarily intact skin models which were so nicely presented this morning.

DR. SLAGA: Right.

DR. ROSS: So, I don't think we're getting out ahead of things here. I mean, we hate to do that.

DR. GRIFFIN: Perfect. Thank you.

Full Panel – December 5, 2023

DR. COHEN: This is a Draft Report for 1,2,4-Trihydroxybenzene. And this resulted in a rather robust discussion yesterday morning, with long deliberations regarding this chemical in the afternoon. This ingredient is reported to function as a hair coloring in cosmetic formulation. It is an auto-oxidative dye used in permanent hair dye formulations and gradual hair coloring shampoos, and does not require hydrogen peroxide to activate oxidation subsequent coupling reactions.

In 2023 the VCRP survey data demonstrated its use in 18 hair dye formulations, and one hair shampoo. The concentration of use survey, conducted by the Council, revealed a concentration up to 2.5 percent in hair dyes and colors. We have been presented with a rather robust package for review. Of note it's interesting that it is present in roasted coffee up to 1.7 milligrams per cup.

This ingredient is intended to be used in the presence of primary intermediates such as p-phenylenediamine, p-toluenediamine, and p-aminophenol to name a few. And this was discussed yesterday as well. We discussed the use of the product with ammonia alone, which does not yield adequate linger time to penetrate the hair fiber. We understand this is a sensitizer in animal studies. We also received data that FDA considers this a coal tar hair dye, and it's regulated as such. And our motion is safe as used as hair dye. And we can have further discussion on other points.

DR. BELSITO: Second.

DR. BERGFELD: Any further discussion, or elaboration?

DR. BELSITO: We were told that the shampoo is actually a dye formulation, so it is not a shampoo. It is intended as a hair dye. It's just a gradual dying process.

DR. COHEN: Right, you lather it in and then you rinse it out.

DR. BELSITO: Right, since it auto-oxidative. But that should probably be in the Discussion.

DR. ROSS: Yeah, I agree with that. That's an important part, Don. I think, David, we also discussed putting in the Discussion the amendment to the MOS where the dose was actually a LOAEL and it was not a NOAEL. And I think the lower margin of safety, which I think was in the Combe Dossier that was presented to us. The 466, I believe, is what we should go with.

DR. COHEN: Did you make a mention about our adjustment factor three for that?

DR. ROSS: Yeah, there's an adjustment factor of three going from a LOAEL to a NOAEL.

DR. COHEN: You also mentioned that it's a suicide substrate for tyrosinase.

DR. ROSS: Yeah, I thought that should be in the Discussion, the possible depigmentation.

DR. BELSITO: The most critical part of the Discussion is the in vitro verses the in vivo genotox, and why the in vitro can be falsely positive. I also thought that there were two figures that were provided to us in the additional data that should be brought into the report. The first one is on PDF Page 224, that large figure where you have the auto-oxidation and the final reaction products, and then the figure on PDF Page 240, which shows the toxification and detoxification pathways.

DR. COHEN: What about them, Don?

DR. BELSITO: I think they should be incorporated into the report.

DR. COHEN: I think we also spent a fair amount of time that there were a substantial number of animal deaths at the in vivo tests.

DR. BELSITO: Yeah, we asked for clarification because it was 50 males, 50 females, but it was not clear to us how many of those were controls. And then there were ten males and females that were sacrificed at seven and nine months. Was that a total of 20, or were there controls so it was a total of 40 and only 20 were left at the -- 10, 20, 40, there were only 60 left at the conclusion. Christina, have you figured that out?

MS. BURNETT: Yes, I was able to get the study this morning and there is another control group of 50 of each sex. So it was 50 males, 50 females for the treatment group and 50 males, 50 females for the control group.

DR. BELSITO: Do they know why there were so many deaths?

MS. BURNETT: No, the only thing in the results section it talks about the survival of the animals. And it says after 12 months there was 84 to 88 percent. After 18 months there was 60 to 65 percent survival. And then it said it compared favorably with other studies conducted by this institute.

DR. COHEN: We have a hand raised that may clarify.

DR. RAO: I just wanted to point out that carc study that was in the 2006 SECP opinion. That was a hair dye formulation, and one of the components was 2, 4 Diaminoanisole, which is a category two carcinogen. And the THB was only in at half a percent. So, I think the study is flawed and doesn't really reflect the effects of THB alone. Since it's a complex mixture, you don't know what is causing the toxicity, especially when you have the 2 4 d amine amosol.

DR. COHEN: So we have seconded Don, and further discussion points.

DR. BERGFELD: Now, what are we going to do with this last report -- Dr. Rao?

DR. BELSITO: What we're being told is that -- so, what you're saying is, for the carcinogenicity study the test material was not just simply 1,2,4-THB; it was combined with others. So that would need to be added into that section. But in the end, the conclusion that there were too few animals to make a conclusion will stand because there really were.

DR. BERGFELD: So we'll make that editorial changes. Any other comments?

DR. KLAASSEN: I think it also needs to be made clear that this wasn't a classical toxicology study to look at the carcinogenicity of 1,2,4 but of two different chemicals. So it's kind of almost worthless for our purposes.

MS. BURNETT: The original study actually was looking at seven oxidative hair dye formulations, and they list them off under code names. I have to go and look for another paper that actually describes what the code names are, in order to find out what exactly was in the formulation that was tested.

DR. KLAASSEN: Yeah, okay, but, I think it's important that if it's two different chemicals, it tells us almost nothing about this one chemical.

MS. BURNETT: Correct.

DR. COHEN: This is a Draft Report; we should be able to review this again with more unpacked information. We should make a special note in the letter to the Panel that we should look out for this in three months.

DR. BELSITO: Correct, and we can always strike this from the document at that point if we feel that it doesn't necessarily add any constructive information.

DR. BERGFELD: Bart, do you want to comment on that?

DR. HELDRETH: No, I think that's perfectly fine. If we're going with a safe as a hair dye conclusion here, then this will go out as a Tentative Report. And it'll come back to the Panel again. Just, categorically, we've been trying to give longer time for comment. So we'll probably wait to bring this back until June instead of March per se.

DR. BERGFELD: Okay. So with the sort of edits that have been put in there, and also the fact that the study right now is of questionable value, we still want to move ahead with a vote or we want to table this until then? We have a second on it.

DR. ROSS: Can I add one point, Wilma?

DR. BERGFELD: Sure David.

DR. ROSS: As Doc commented, the big issue was the weight of evidence on the genotoxicity, the in vitro verses the in vivo. I was just thinking this morning, it would be nice to have the catalase and other detoxification enzymes content in the skin, in the report. And I did a quick Google search, and yeah, upon that search you can find it. And it looks quite decent, but it'd be nice to have that in the report as well.

DR. BERGFELD: So, again, we have some things being added. Do we want to send it out just as a comment before these are added or changed? Bart?

DR. KLAASSEN: Yes, I think it can be sent out. What Dave is just suggesting is kind of additional information; it's not going to alter anything.

DR. ROSS: I'd agree with that, Curt.

DR. BERGFELD: All right. Then I'll call the question. All those opposed? Abstaining? Unanimously approved, with the comments that have been recommended for embellishment of certain parts of the document.

Safety Assessment of 1,2,4-Trihydroxybenzene as Used in Cosmetics

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ABBREVIATIONS

ARE	antioxidant responsive element
BrdU	bromodeoxyuridine
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Cu^{2+}	copper (II)
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
DMSO	dimethyl sulfoxide
DNCB	2,4-dinitrochlorobenzene
EC1.5	interpolated concentration resulting in a 1.5-fold luciferase induction
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EC_3	estimated concentrations of an SI of 3
ED_{50}	median effective dose
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
Fe^{3+}	iron (III)
FISH	fluorescence in situ hybridization
FOX	ferrous oxidation-xylenol orange
GC	gas chromatography
HPLC	high-performance liquid chromatography
$[^3H]$TTP	tritiated thymidine triphosphate
IC_{50}	50% inhibitory concentration
IgE	immunoglobulin E
IU	international units
LLNA	local lymph node assay
LNC	lymph node cell
LOAEL	lowest-observed-adverse-effect-level
LPS	lipopolysaccharide
MOS	margin of safety
MS	mass spectrometry
NMR	nuclear magnetic resonance spectroscopy
NOAEL	no-observed-adverse-effect-level
Nrf2	nuclear factor erythroid 2-related factor 2
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered saline
PoD	point of departure
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCE	sister chromatid exchange
SED	systemic exposure dose
SI	stimulation index
TBARS	thiobarbituric acid-reactive substances
TG	test guideline
TGx	toxicogenomics
TMA	trimellitic anhydride
UV	ultraviolet light
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 1,2,4-Trihydroxybenzene, which is an oxidative dye reported to function as a hair dye in cosmetic products. The Panel reviewed the available data to determine the safety of this ingredient. The Panel concluded that 1,2,4-Trihydroxybenzene is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This assessment reviews the safety of 1,2,4-Trihydroxybenzene as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), this ingredient, also called 1,2,4-benzenetriol, 4-hydroxycatechol, or THB, is reported to function as a hair colorant.[1]

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 extensive search of the world's literature; a search was last performed April 2024. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Expert Panel for Cosmetic Ingredient Safety (Panel) typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Much of the published data included in this safety assessment was found in the opinions of the Scientific Committee on Consumer Products (SCCP)[2] and Scientific Committee on Consumer Safety (SCCS).[3,4] Please note that these opinions provide summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when the SCCP and SCCS are cited.

CHEMISTRY

Definition and Structure

According to the *Dictionary*, 1,2,4-Trihydroxybenzene (CAS No. 533-73-3) is the phenol that conforms to the structure in Figure 1.[1, CIR staff]

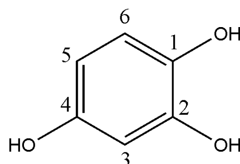
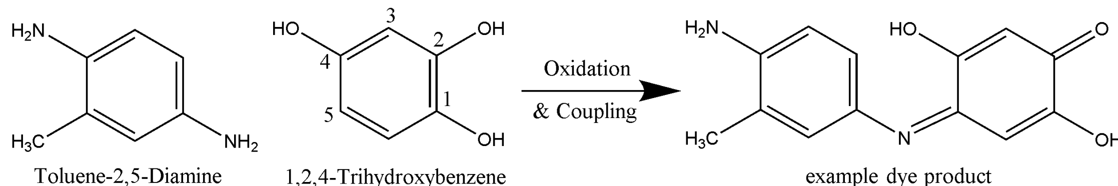


Figure 1. 1,2,4-Trihydroxybenzene

1,2,4-Trihydroxybenzene is an oxidative dye used in permanent hair dye formulations and gradual hair coloring shampoos and does not require hydrogen peroxide to activate oxidation and subsequent coupling reactions.[4] This ingredient is intended to be used in the presence of primary intermediates such as *p*-phenylenediamine, *p*-toluenediamine, *p*-aminophenol, etc. However, if 1,2,4-Trihydroxybenzene is partially oxidized prior to coupling, the rates of active hair dye formation may be different from traditional oxidative hair dyes. While this ingredient can be used alone, as an "auto-oxidative" hair dye, it is commonly used with reactants, much like traditional oxidative hair dyes.

Reaction Chemistry

The hydroxyl substituent pattern of 1,2,4-Trihydroxybenzene affects its reactivity.[4] The hydroxyl groups direct the substitution reaction on the benzene ring, making the C5 position on the ring (see Scheme 1) most likely to participate in coupling reactions. 1,2,4-Trihydroxybenzene reacts in the presence of oxygen and primary intermediates to form coupled products, without peroxide.[4,5] In formulation, once the reaction mixture is exposed to air, the oxidative coupling of 1,2,4-Trihydroxybenzene with an available primary intermediate proceeds rapidly, slowed only by temperature and pH adjustment.



Scheme 1. Example dye formation with 1,2,4-Trihydroxybenzene

1,2,4-Trihydroxybenzene, at physiological pH, can be oxidized spontaneously (auto-oxidation) or enzymatically (through the action of myeloperoxidase).[6] Auto-oxidation is the "uncatalyzed" oxidation of a substance exposed to oxygen in air; however, these reactions are usually metal catalyzed, as redox active metals can serve as free radical initiators. 1,2,4-

Trihydroxybenzene oxidation can be catalyzed by iron and copper salts, with copper (II) (Cu^{2+}) being a more active catalyst than iron (III) (Fe^{3+}). Of the metabolites of benzene, 1,2,4-Trihydroxybenzene is the most reactive toward molecular oxygen and rapidly auto-oxidizes to its corresponding quinone via semiquinone radical intermediates.

The auto-oxidation of 1,2,4-Trihydroxybenzene produces reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and hydroxyl radicals.[6] In the process, 1,2,4-Trihydroxybenzene is oxidized to 2-hydroxy-1,4-benzoquinone. (See Figure 2.)

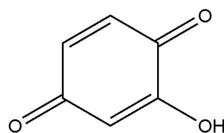


Figure 2. 2-hydroxy-1,4-benzoquinone

The quantitative determination of hydrogen peroxide formation from 50 or 100 µM 1,2,4-Trihydroxybenzene (98.1% pure) in various solvent media was performed using the ferrous oxidation−xylenol orange (FOX) assay.[4,5] The formation of hydrogen peroxide was measured as a function of concentration and time in phosphate buffer at pH 7.4. The quantitative generation of hydrogen peroxide from 1,2,4-Trihydroxybenzene was also evaluated in various solvents (deionized water, phosphate-buffered saline (PBS), serum-free Roswell Park Memorial Institute (RPMI) medium, and RPMI 1640 complete medium used for preparing dosing solutions commonly reported for evaluation of genotoxic effects in vitro and in vivo of 1,2,4-Trihydroxybenzene. The time point of 30 min was assumed as the time between preparation of dosing solution to actual dosing. The control was 30% (w/w) hydrogen peroxide solution.

PBS provided the most efficient matrix for the generation of hydrogen peroxide. In this solvent, 1 mole of 1,2,4-Trihydroxybenzene generated 0.9 moles of hydrogen peroxide. Deionized water was the least efficient solvent in generating hydrogen peroxide, likely due to the lack of a buffering capacity to maintain a pH. The authors of the study concluded that 1,2,4-Trihydroxybenzene is a spontaneous hydrogen peroxide releasing compound in "cell-free" solution on exposure to atmospheric oxygen. The release of hydrogen peroxide is facile, quantitative, and is time, solvent, pH and buffer, ionic salts, and transition metal dependent. The efficiency of the dosing solvents in catalyzing hydrogen peroxide generation from 1,2,4-Trihydroxybenzene was determined as follows: PBS > serum free RPMI > phosphate buffer > RPMI > water.[4,5]

In a time-dependent hair color-simulating consumer usage study, a representative gel-cream-based hair color formulation with 2% 1,2,4-Trihydroxybenzene (pH 9) was made in de-aerated water purged with nitrogen and tested on 90% virgin gray hair swatches.[4,5] A control formulation was made under the same conditions without 1,2,4-Trihydroxybenzene. A reverse phase high-performance liquid chromatography (HPLC) method was utilized to determine the amount of 1,2,4-Trihydroxybenzene reacted at time 0, 5, 15, and 30 min. This experiment was performed in duplicate. The resulting data showed that more than 70% of 1,2,4-Trihydroxybenzene reacted within the first 5 min and ~95% of the test material has reacted by the end of 15 min. Less than 3% of the original 2% formulation of 1,2,4-Trihydroxybenzene remained after 30 min. The study authors concluded that 1,2,4-Trihydroxybenzene in a representative formulation, when applied to hair, undergoes rapid oxidation when exposed to the atmosphere. The results of the study suggested that the consumer exposure to 1,2,4-Trihydroxybenzene is expectedly low, not only based on its limited skin permeability, but also based on the rapid decline in concentration in alkaline medium in the presence of hair under normal use conditions.[4,5] The SCCS was concerned about the proportion of unreacted 1,2,4-Trihydoxybenzene in the final hair dye formulation and that the transformation of 1,2,4-Trihydroxybenzene results in the generation of (semi)quinones.[4]

Chemical Properties

Chemical properties for 1,2,4-Trihydroxybenzene are summarized in Table 1. The molecular weight of 1,2,4-Trihydroxybenzene is 126.11 g/mol and the melting point is 139 - 150 ˚C.[2,3] 1,2,4-Trihydroxybenzene is a light-medium beige powder with an estimated log P_{ow} of 0.2 and an ultraviolet light (UV) spectrum peak of 291 nm.[2-5]

Method of Manufacture

1,2,4-Trihydroxybenzene may be produced by mixing *p*-quinone with acetic anhydride and concentrated sulfuric acid at below 40° C.[7] The resulting compound is precipitated by water or alcohol to yield 1,2,4-triacetoxybenzene. This acetate is then mixed with cold, absolute alcohol and concentrated hydrochloric acid before heating in an inert gas stream on a water bath at 80° C. The solvent and acid are removed in a vacuum prior to solidifying with chloroform and nucleation (glass scratching).

Impurities

Total impurity content of 1,2,4-Trihydroxybenzene is reported to be < 2% in a batch analyzed by HPLC and < 0.2% in a batch analyzed by potentiometry.[2] The four impurities detected by HPLC were tetrahydroxybenzene; 1,1'-biphenyl-2,2',4,4',5,5'-hexol; 2-hydroxybenzo-1,4-quinone; and 1,3,2-benzodioxathiole-5,6-diol 2,2-dioxide. Residual solvents were dichloromethane (300 µg/g) and n-propanol (1500 µg/g); isopropanol and ethyl acetate were below levels of detection. Heavy metal content was characterized as the following: aluminum (3 mg/kg), chromium (3 mg/kg), iron (11 mg/kg), nickel

(2 mg/kg), zinc (2 mg/kg), and mercury (< 0.1 mg/kg).[3] Silver, arsenic, barium, bismuth, cadmium, cobalt, copper, manganese, molybdenum, lead, palladium, platinum, antimony, selenium, tin, titanium, vanadium were each < 1 mg/kg.

In the 2019 opinion by the SCCS, the purity of 1,2,4-Trihydroxybenzene, determined by gas chromatography (GC) with UV detector, was reported to be 97.8%.[4] Impurities identified by nuclear magnetic resonance (NMR) spectroscopy and GC-mass spectrometry (MS) of 3 lots of 1,2,4-Trihydroxybenzene were: benzene-1,2,4,5-tetraol; 4-mercaptophenol; hydroquinone; and 2-(2,3,4-trimethoxyphenyl)-5,6,7-trimethoxynaphthalene. The heavy metal content of the 3 lots of 1,2,4-Trihydroxybenzene was: arsenic (0.20 - 0.33 ppm), chromium (0.12 - 0.17 ppm), lead (0.03 - 0.05 ppm), mercury (0.02 - 0.03 ppm), and zinc (6.9 - 14 ppm). Cadmium was not detected.

Natural Occurrence

1,2,4-Trihydroxybenzene is a metabolite in biodegradation of many aromatic chemicals, including benzene.[8] Benzene is metabolized in the liver via benzene epoxide to phenol, which is then further hydroxylated to catechol, hydroquinone, and 1,2,4-Trihydroxybenzene.[6] Studies have detected 1,2,4-Trihydroxybenzene in the urine of humans and hamsters exposed to benzene, and in the urine of rats exposed to phenol, quinol, or catechol.[9,10] Metabolites of benzene, including 1,2,4-Trihydroxybenzene, may mediate the myelotoxicity and carcinogenicity of benzene.[11]

1,2,4-Trihydroxybenzene also occurs as a biodegradation product by fungi, yeast, and bacteria of catechin, resorcinol, and other aromatic chemicals.[12-14] Additionally, 1,2,4-Trihydroxybenzene is found in roasted coffee beans, with a typical cup of coffee containing 0.1-1.7 mg of the chemical.[15-18]

USE

Cosmetic

The safety of the cosmetic ingredient 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 this ingredient in cosmetics and does not cover its use in airbrush delivery systems. Data included herein were obtained from the FDA's Voluntary Cosmetic Registration Program (VCRP) database in 2023 (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) in 2022 - 2023 (maximum use concentrations). The data were provided by cosmetic product categories, based at that time on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP survey data, 1,2,4-Trihydroxybenzene is reported to be used in 18 hair dye formulations and 1 hair shampoo (coloring).[19] The results of the concentration of use survey submitted by the Council in 2023 indicate 1,2,4-Trihydroxybenzene is used at up to 2.5% in hair dyes and colors.[20]

Although products containing this ingredient 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 this ingredient (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.

These ingredients are considered coal tar hair dyes for which regulations require caution statements and instructions regarding patch tests in order to be exempt from certain adulteration and color additive provisions of the US Federal Food, Drug, and Cosmetic Act (FD&C Act). In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

> Caution - this product contains ingredients which may cause skin irritation on certain individuals and a preliminary test according to accompanying directions should be made. This product must not be used for dyeing the eyelashes or eyebrows; to do so may cause blindness.

Product labels shall also bear patch test instructions for determining whether the product causes skin irritation. However, whether or not patch testing prior to use is appropriate is not universally agreed upon. The Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 h after application of the test material and prior to the use of a hair dye formulation. Conversely, a report in Europe suggests that self-testing has severe limitations, and may even cause morbidity in consumers.[21,22] Hair dye products marketed and sold in the US, though, must follow the labeling requirements established by the FD&C Act.

Hair dye formulations are filled into specialized, oxygen barrier packaging for commercialization.[3] Stability tests are conducted under standard conditions (both room and elevated temperatures) appropriate for cosmetic products. Final product stability meets standard requirements until the package is opened by the consumer for use. Upon dispensing and application, the combination of dyes (including 1,2,4-Trihydroxybenzene and other precursors) in the formulation undergo oxidative coupling reactions as predicted by their chemical structure.

Under European regulations for cosmetic ingredients, 1,2,4-Trihydroxybenzene, when used as a substance in hair and eyelash dye products, is categorized in Annex II, the list of substances prohibited in cosmetic products in Europe.[23] The SCCS does not consider 1,2,4-Trihydroxybenzene safe due to potential genotoxicity when used as an auto-oxidative hair dye component in permanent hair dye formulations.[4]

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

The dermal absorption/percutaneous penetration potential of 1,2,4-Trihydroxy [U-^{14}C]benzene (93.5% radiochemical purity) through dermatomed human skin (~ 400 µm) was determined for a formulation containing 2.78% of the radiolabeled active dye.[2,3] The formulation also contained 50% PEG-6 and approximately 47% water. The study was performed in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 428. Using flow-through diffusion cells, 20 mg/cm^2 of the formulation, corresponding to 556 µg/cm^2, was applied for 30 min to 8 samples. The receptor fluid (PBS containing 0.01% sodium azide) was pumped at a rate of approximately 1.5 ml/h. Application of the test material was terminated by rinsing with water (10x), 2% sodium dodecylsulfate solution, and water (10x) again. The washing solutions were combined, and the amount of radioactivity was determined. Post-exposure time was 23.5 h. Tape stripping was then performed to determine the 24-h penetration profile. The recovery of radioactivity was 105%. Most of the test material was recovered in the wash after 30 min of exposure. Virtually no penetration of radioactivity into the receptor fluid after 24 h was observed (0.0019 μg_{eq}/cm^2 or 0.0003% of the applied dose).

In another dermal penetration study, [^{14}C]1,2,4-Trihydroxybenezene (98.3% pure; 2.00 MBq/mg) was incorporated at a final concentrations of 2.5% (w/w) into two hair dye formulations, one with and one without 2.25% *p*-toluenediamine.[4,5] The study was performed in accordance with OECD TG 428. The formulations were applied to dermatomed human skin (400 µm thick) in static glass diffusion cells at a dose of 20 mg/cm^2 of the test article; the dose of 1,2,4-Trihydryoxybenezene was approximately ~ 500 µg/cm^2. After 30 min, the skin samples were washed with a mild soap solution. After washing, the diffusion cells were returned to the water bath for the remaining 23.5 h. At the end of the experiment, the 24-h penetration profile was determined using tape stripping and a heat separation technique. The mean recovery of the applied test material without and with *p*-toluenediamine was 101% and 99.2%, respectively. The total systemically available dose (epidermis, dermis, and receptor fluid (PBS)) from the test formulation without *p*-toluenediamine was 1.13 $\pm$ 0.58 μg_{eq}/cm^2 or 0.226%. The total systemically available dose from the test formulation with *p*-toluenediamine was 1.94 $\pm$ 1.76 μg_{eq}/cm^2 or 0.393%.

Absorption, Distribution, Metabolism, and Excretion

No absorption, distribution, metabolism, or excretion studies were reported for 1,2,4-Trihydroxybenzene in the published literature and unpublished data were not submitted. Many studies are available on the absorption, distribution, metabolism, and excretion of benzene as a starting material, but not on 1,2,4-Trihydroxybenzene.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

In an acute dermal toxicity study performed in accordance with OECD TG 402, 5 male and 5 female Sprague-Dawley rats received 2000 mg/kg bw 1% 1,2,4-Trihydroxybenzene (98.1% pure) in carboxymethylcellulose/water on skin (application site not described).[2,3] The test site was semi-occluded for 24 h. The rats were observed for a period of 14 d following the single application. No mortality was observed during the study period. Hypoactivity, piloerection, and dyspnea were observed in all female rats from days 2 through 8. One of the female rats had tremors. Overall body weight gain was comparable to historical control animals in all but 1 animal; one female had slightly reduced body weight gain during the second week of the study. A black coloration of the skin was noted in all animals from day 2 until study end. Erythema was observed in 2 males on day 2 and persisted in 1 animal until day 3. Edema was recorded between days 2 and 5 in 2 males and in all females between days 2 and 6. No apparent abnormalities were noted at necropsy in any animal.

Oral

In an acute oral toxicity study, groups of 5 male and 5 female OFA Sprague-Dawley-derived rats received 100, 250, 350, 500, or 1000 mg/kg bw 1% 1,2,4-Trihydroxybenzene in carboxymethylcellulose/water via gavage.[2,3,5] The animals were observed for 14 d following the single administration. No further details were provided. The LD_{50} for both sexes was between 350 and 500 mg/kg bw for a preparation containing 1% 1,2,4-Trihydroxybenzene.

Short-Term Toxicity Studies

Oral

In an oral study, male BALB/c mice (number not reported) received diet of 400 g of normal feed mixed with 210 ml of water containing 8 g 1, 2, 4-Trihydroxybenzene (1.3% w/w) for 1 wk.[24] A control group received the feed mixed with untreated water. No observable toxic effects were observed. (No further details provided.)

In a 4-wk study, groups of 8 male KKAy mice received purified water or water with 100 or 500 mg/l 1,2,4-Trihydroxybenzene 5 times/wk.[25] Individual body weights were recorded weekly, feed intake was measured every 2-3 d, and water consumption was measured each time the water was replaced throughout the study. Metabolic rate was measured after 3 wk by indirect calorimetry. Blood, urine, and tissue samples were collected after 4 wk. Body and liver weights of the mice were not affected. Feed intake and water consumption decreased with 1,2,4-Trihydroxybenzene, with consumption levels significantly lower ($p < 0.05$) in the high dose group than in controls. Blood glucose, serum triglyceride, and non-esterified fatty acid levels did not differ among the groups. Serum and urinary hydrogen peroxide levels increased with 1,2,4-Trihydroxybenzne, with the 500 mg/l dose group significantly higher ($p < 0.01$) than the control group. Reduced blood nitric oxide metabolites ($p < 0.05$) and liver *S*-nitrosylated protein levels (not significant) and decreased whole-body fat utilization ($p < 0.001$) were observed following treatment with 1,2,4-Trihydroxybenzene, with the latter occurring in a dose-dependent manner.

In an oral study, 5 male and 5 female F344 rats received 1.5% 1,2,4-Trihydroxybenzene in diet continuously for 4 wk.[26] A control group of the same composition of animals received regular diet. At week 4, the rats were injected intraperitoneally with 50 mg/kg bw bromodeoxyuridine (BrdU) and after 1 h, the rats were then sequentially killed. The stomachs were removed for histopathological and immunohistochemical examination. The number of cells incorporating BrdU into DNA per 2000 basal cells of the forestomach and the numbers of cells labeled with BrdU per 50 pyloric glands were counted. No deaths were observed during the treatment period and there were no clinical effects of toxicity observed, with the exception of a statistically significant reduction in weight gain in both sexes treated with 1,2,4-Trihydroxybenzene. This observation was associated with a decrease in feed consumption. No erosion or ulcer formation was observed in any of the treated animals or the controls. Significantly increased DNA synthesis ($p < 0.01$) was noted in both sexes of the treated animals when compared to the control animals. 1,2,4-Trihydroxybenzene was not associated with any hyperplasia changes in glandular stomach mucosa.

Subchronic Toxicity Studies

Oral

In a 90-d gavage study, groups of 15 male and 15 female Han Wistar rats received 0, 50, 100, or 200 mg/kg bw/d 1,2,4-Trihydroxybenzene (purity not reported) in sterile water.[2,3,5] The study was performed in accordance with OECD TG 408. Clinical signs of toxicity, mortality, and water consumption were monitored daily. Examinations of individual animals for signs of reaction to the test material were performed daily immediately after dosing and approximately 1 and 3 h after dosing during the first 3 wk of the study; after this, observations were performed at approximately 15 min and 1 and 2 h after dosing until the end of the study. Prior to the commencement of treatment and weekly thereafter, each animal was subjected to a detailed clinical examination, including an evaluation of neurotoxicity. Body weight and feed consumption were recorded weekly. An ophthalmological examination was performed prior to the start of the study and in week 12. "Motor activity of the first 5 males and 5 females was measured once during week 12 of treatment." (No further details of this methodology were provided.) Hematology, blood clinical chemistry, and urinalysis were performed in week 13 of treatment. At study end, all surviving animals were killed and underwent macroscopic examination. Select organs (not described) were weighed and microscopic examination was performed of specified tissues and organs (lungs from all animals, other organs not described) from all rats that died during the study, all control and high-dose rats killed at the end of the study, and in animals where gross anomalies were noted.

During the study, 12 animals died: 1 male in each the control, low- and intermediate-dose groups and 5 males and 4 females of the high-dose group. Microscopic examination indicated that mis-dosing was the cause of death for the first 3 groups while the main cause of death for the high-dose rats was stomach ulcerations. Piloerection and salivation were observed in the 100 and 200 mg/kg bw/d dose groups. An overall slight reduction in body weight gain was observed in treated males when compared with controls from approximately 1 mo of treatment. A 14% decrease in feed consumption was observed at week 13 in high dose males, but this result was not observed in treated females. A statistically significant increase in mean red blood cell volume, mean corpuscular hemoglobin, and platelets and a statistically significant decrease in hematocrit, red blood cell count, and hemoglobin were observed in animals treated with 100 and 200 mg/kg bw/d, when compared to the controls; however, values remained within the normal range for this strain of rats. A statistically significant increase in bilirubin was observed in rats of the high dose group of both sexes; however, the color of the test compound may have interfered with the methodology used. No toxicological significance was given to the statistically significant increase in urea observed in treated females only. Statistically significant increases in the absolute weight and/or organ-to-body weight ratios were observed in treated males for the spleen (all dose levels), liver and kidney (100 and 200 mg/kg bw/d), and testes and heart (200 mg/kg bw/d). In the females, statically significant increase in the absolute weight and/or organ-to-body weight ratios were observed for the liver, spleen, and kidneys at 200 mg/kg bw/d. Ulcerations in the non-glandular gastric region were observed in 1/10 males and 1/11 females of the high dose group and in 1/14 males in the intermediate dose group at study end. The histopathological evaluation of the stomach in the remaining animals of the intermediate dose group did not reveal any further treatment-related gastric lesions. Dark-brown, micrugranular pigmentation was clearly evident in single cells or in the lumen of renal cortical tubes of 10/15 males and 10/15 females in the high dose group and in 2/15 males and 1/15 females of the intermediate dose group. The no-observed-adverse-effect-level (NOAEL) was determined by the study authors to be 50 mg/kg bw/d. However, the SCCP concluded that no NOAEL could be derived in this study as the

relative organ weight was increased significantly in the spleen of the 50 mg/kg bw/d male rats.[2,4] This increase continued in a dose-dependent manner in the male rats.[4] Absolute organ weight of the spleen was also increased in male rats, but the increase was not significant at 50 mg/kg bw/d. The value 50 mg/kg bw/d was considered to be the lowest-observed-adverse-effect-level (LOAEL) by the SCCP.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

The teratogenic potential of 1,2,4-Trihydroxybenzene (99.8% pure) was evaluated in groups of 25 mated female Sprague-Dawley (Crl CD (SD) BR) rats in accordance with OECD TG 414.[2,3,5] The rats received 0, 30, 100, or 300 mg/kg bw/d of the test material dissolved in water on gestation days 6 through 15. On day 20 of gestation, the rats were killed. The number of corpora lutea, resorptions, live and dead fetuses, and implantation sites were recorded. Live fetuses were weighed and examined externally. Half of the live fetuses per litter underwent skeletal examination and the remaining fetuses underwent soft tissue examination.

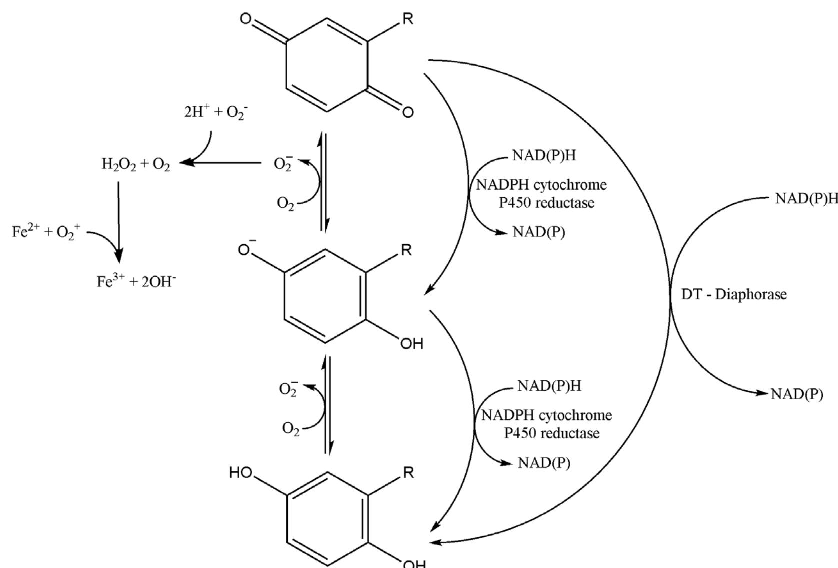
No clinical signs of toxicity or deaths occurred in the 0, 30, or 100 mg/kg bw/d groups. In the 300 mg/kg dose group, 3 females died or were killed in moribund conditions due to mis-dosing. Another female died without any clinical signs. At necropsy, gaseous dilatation of the stomach and intestine and congested lungs were noted. The mean body weight gain and feed consumption of females that survived to day 20 were similar to the control and other dose groups. In the 300 mg/kg dose group, the mean body weight gain was slightly lower than that of the controls between days 6 and 9 (not significantly) and the feed consumption was also slightly lower than that of the control females during the treatment period. No further details of these observations were provided. The litter parameters were comparable in the control and treated groups. No fetal external malformations were observed in the 0, 30, or 100 mg/kg bw/d groups; however, in the 300 mg/kg bw/d dose group, 4 fetuses from the same litter (out of 325 fetuses) had exencephaly associated with opened eyelids. Historical incidence of exencephaly in control fetuses from the test facility was noted (mean incidence: 0.06%; range of incidence per study: 0.0 - 1.0%). This incidence was slightly higher (1.2%) than that of the historical data, but it was considered a congenital malformation due to the fetuses coming from the same dam, and no other malformations were noted in any other litters. The dam showed no sign of any toxicity. No other treatment-related fetal skeletal variations, anomalies, malformations, and/or fetal soft tissue anomalies or malformations were observed. 1,2,4-Trihydroxybenzene was maternotoxic at 300 mg/kg bw/d, but not embryotoxic or teratogenic.[2,3,5]

GENOTOXICITY STUDIES

1,2,4-Trihydroxybenzene is a compound known to induce DNA damage and is considered genotoxic in vitro. This compound can result in oxidative DNA damage, which is linked to the generation of ROS.[27-32] Additionally, 1,2,4-Trihydroxybenzene has been studied for its effects on cellular structures, inducing DNA strand breaks and mutations as well as potential epigenetic modifications, potentially leading to various diseases, including cancer.[33,34] 1,2,4-Trihydroxybenzene may inhibit mitochondrial DNA replication.[35] Furthermore, several studies have elucidated the role of 1,2,4-Trihydroxybenzene in causing chromosomal damage and subsequent biological consequences.[36-38] Genotoxic effects may be modulated in vivo (Scheme 2). However, catalase, peroxidases, and other detoxification (antioxidant) enzymes in the epidermis, along with smaller antioxidant molecules, work to minimize ROS damage within skin cells.[39,40] For example, the enzyme superoxide dismutase converts harmful ROS to the less harmful ROS, hydrogen peroxide, which in turn is converted by catalase to harmless water and hydrogen.

In vitro and in vivo genotoxicity studies on 1,2,4-Trihydroxybenzene summarized here are detailed in Table 2. In in vitro studies where auto-oxidation was not minimized or efforts to reduce auto-oxidization were not reported, 1,2,4-Trihydroxybenzene was mutagenic in Ames tests when tested at up to 4000 µg/plate.[2,3,41] Genotoxicity of 1,2,4-Trihydroxybenzene was observed in a gene mutation assay of mutants to 6-thioguanine (tested at up to a maximal concentration of 10 µM), DNA strand break tests (at up to 1000 µM), DNA synthetic activity inhibition assays (at up to 24 µM), and sister chromatid exchange (SCE) assays (at up to 500 µM).[3,33,41-45] 1,2,4-Trihydroxybenzene was not genotoxic in a gene mutation test at the *hprt* locus (up to 240 µg/ml) and was not clastogenic in a chromosome aberration test (at up to 20 µg/ml).[2,3] Increases in micronucleus induction were observed in Chinese hamster V79 cells (at up to 25 µM) and human TK6 lymphoblastoid cells (at up to 30 µg/ml).[41,44] 1,2,4-Trihydroxybenzene was clastogenic and aneugenic in a cytokinesis-block micronucleus test with human lymphocytes at up to 100 µM.[3,46] Chromosomal damaged was observed in fluorescence in situ hybridization (FISH) procedures when 1,2,4-Trihydroxybenzene was tested at up to 100 µM.[36-38]

In studies where degassed solutions were used, 1,2,4-Trihydroxybenzene was mutagenic in an Ames test when tested at up to 5000 µg/plate; however, the mutagenic effect of 1,2,4-Trihydroxybenzene was eliminated in another Ames test when evaluated in the presence of radical scavengers catalase (up to 20,000 IU) and L-glutathione (up to 10 µM).[4,5] Catalase also reduced the toxicity of 1,2,4-Trihydroxybenzne. In other studies with degassed solutions, 1,2,4-Trihydroxybenzene was not genotoxic in a 3D comet assay (at up to 1250 µg/ml) or in micronucleus assays with human reconstructed skin tissue and human lymphocytes (at up to 224 µg/ml). In in vivo micronucleus tests in mice, 1,2,4-Trihydroxybenzene was not genotoxic when tested intraperitoneally at up to 50 mg/kg bw.[2,47]



Scheme 2. Potential pathways, relevant to genotoxicity, for benzoquinone and 1,2,4-Trihydroxybenzene via redox cycling between the quinone and its hydroxylated counterpart.[33] This also demonstrates the concomitant production of reactive oxygen species (benzoquinone: R = H; 1,2,4-Trihydrixybenzene: R = OH)

Mechanism

Oxidant-mediated genotoxicity of 1,2,4-Trihydroxybenzene is a multifactorial process.[6] Redox reactions of 1,2,4-Trihydroxybenzene yield oxygen species, semiquinones, and quinones. Copper salts stimulate oxidation of 1,2,4-Trihydroxybenzene, leading to 1,2,4-Trihydroxybenzene-induced genotoxicity. Copper salts change the mechanism of reaction from superoxide-propagated 1-election transfer pathway to Cu^{2+}-mediated 2-electron transfer pathway during the oxidation of 1,2,4-Trihydroxybenzene.

CARCINOGENICITY STUDIES

Dermal

The dermal carcinogenicity potential of a semi-permanent hair dye formulation containing 0.5% 1,2,4-Trihydroxybenzene (purity not reported), 0.5% 2,4,5-toluenetriol, and 0.25% *p*-toluenediamine sulfate was evaluated in treatment and control groups of 50 male and 50 female Swiss Webster mice each.[2,3,48,49] The test material (0.05 ml) was applied once weekly for 23 mo on a 1 cm^2 area of clipped skin on the interscapular region. Test sites were not occluded. The mice were observed daily for mortality and clinical signs of toxicity and were weighed monthly. A weekly record was maintained for any skin lesions noted. After 9 mo of treatment, 10 males and 10 females per group were necropsied. Skin and internal organs were evaluated histologically. Four males and 4 females that received 1,2,4-Trihydroxybenzene survived until study termination, while 3 males and 8 females survived until study termination in the control groups (further information on survival rates not provided). There were no significant differences in absolute or relative liver or kidney weights in groups of 10 male and 10 female mice necropsied after 9 mo. There were no statistically significant differences in the distribution of tumors among treated and control groups. The SCCS determined that these data were insufficient to conclude on the carcinogenic potential of 1,2,4-Trihydroxybenzene.

OTHER RELEVANT STUDIES

Cytotoxicity

The cytotoxicity of 1,2,4-Trihydroxybenzene was investigated in K562 erythroleukemia cells.[50] Cultured K562 cells were exposed to 0, 0.1, 0.2, 0.3, 0.4, or 0.5 mM 1,2,4-Trihydroxybenzene for 24 h. The K562 cells showed significant inhibition of viability ($p < 0.05$) in a concentration-dependent manner.

In another study, K562 cells were treated with 0, 0.01, 0.02, 0.04, or 0.08 mM 1,2,4-Trihydroxybenzene for 24 h.[51] Eighty-five percent (85%) of the total cells were viable after treatment at concentrations less than 0.08 mM. At 0.08 mM, cell viability was slightly greater than 60%.

Cytotoxicity was also investigated using HL-60 human promyelocytic leukemic cells incubated with 10 - 100 µM 1,2,4-Trihydroxybenzene, hydroquinone, and *p*-benzoquinone for 1 - 4 h.[52] The rank order of cytotoxicity of these benzene metabolites to the cells were determined to be *p*-benzoquinone > hydroquinone > 1,2,4-Trihydroxybenzene at any given time period. In further in vitro testing, cells were exposed to 50 µM of each metabolite for 2 h. Cell viability was more than 80% (no further details provided).

Hematotoxicity

In vitro studies using mouse bone marrow adherent stromal cells and K562 cells have been used to study the mechanisms of benzene hematotoxicity.[51,53,54] 1,2,4-Trihydroxybenzene (3.1 x 10^{-6} to 500 x 10^{-6} M) was studied for its effect on the ability of stromal cells to influence granulocyte/monocyte colony growth after incubation with the test material for 3 d.[53] 1,2,4-Trihydroxybenzene inhibited colony growth at concentrations $\geq$ 100 x 10^{-6} M. K562 cells were used to determine the effects of 1,2,4-Trihydroxybenzene on erythroid differentiation.[51,54] The results of the studies on K562 cells indicated that 1,2,4-Trihydroxybenzne inhibited hemin-induced erythroid differentiation in concentration-dependent manner (tested at up to 40 µM).

Oxidative Stress

In an investigation on oxidative stress, groups of 6 mice received diet of 400 g of normal feed mixed with 210 ml of water containing 8 g 1, 2, 4-Trihydroxybenzene (1.3% w/w) for 1 - 2 wk, with or without exposure to air containing 10 ppm nitrogen dioxide.[24] Control groups received normal feed mixed with untreated water, with or without exposure to nitrogen dioxide. At the end of the exposure period, blood was collected and the heart, kidney, liver, and lungs were obtained from 1 mouse to measure thiobarbituric acid-reactive substances (TBARS) in assays with ethylenediaminetetraacetic acid (+ EDTA) and without EDTA (– EDTA). In the red blood cell membranes, there were no significant differences in the levels of TBARS in the 1,2,4-Trihydroyxbenzene or the control groups with the EDTA assay. In the organs, the level of TBARS with the - EDTA assay was significantly decreased by 1,2,4-Trihydroxybenzene in the kidney and liver. In the – and + EDTA assays of the lung, levels of TBARS from malonaldehyde derivatives plus alkadienal/alkenal derivatives were remarkably increased by 1,2,4-Trihydroxybenzene, but those from malonaldehyde derivatives alone were not. There were no effects of nitrogen dioxide inhalation on lung lipid peroxidation; lung lipid peroxidation was enhanced by 1,2,4-Trihydroxybenzene, but this effect was not greatly impacted in other tissues.

Neuroprotective Effects

1,2,4-Trihydroxybenzene (tested at 10 - 100 µM) was found to significantly inhibit lipopolysaccharide (LPS)-stimulated nitric oxide production in BV-2 microglia cells treated with 1 µg/ml LPS followed by the test material for 24 h.[55] 1,2,4-Trihydroxybenzene also inhibited inducible nitric oxide synthase mRNA and protein expression (cells treated with test material and LPS for 4 h). Additionally, 1,2,4-Trihydroxybenzene significantly reduced the generation of ROS in hydrogen-peroxide-induced BV-2 cells (treated with 1 mM hydrogen peroxide followed by test material) and in hydrogen peroxide-cell free conditions (scavenging effect of test material assessed with 1 mM hydrogen peroxide or with 0.1 mg/ml ferrous sulfate heptahydrate). The neuroprotective effect of 1,2,4-Trihydroxybenzene (30 mg/kg) was observed in the ischemic male Sprague-Dawley rat brain under middle cerebral artery occlusion in an in vivo infarction assay.

Melanogenesis Inhibition

1,2,4-Trihydroxybenzene is characterized kinetically as a tyrosinase substrate, and the action of tyrosinase on 1,2,4-Trihydroxybenzene may cause the inactivation of the enzyme through a suicide inactivation mechanism.[56] The activity of tyrosinase on 1,2,4-Trihydroxybenzene was assessed through the measurement of the formation of 2-hydroxy *p*-benzoquinone via spectrophotometric assay. The author suggested that 1,2,4-Trihydroxybenzene, being a potent suicide substrate of tyrosinase, could play a role in the depigmenting effect of hydroquinone. (Tyrosinase can catalyze hydroxylation of hydroquinone to 1,2,4-Trihydroxybenzene).[57]

Immunomodulatory Effects

The effect of 1,2,4-Trihydroxybenzene on type IV and type I allergy responses was studied using male BALB/c mice.[24] Type IV allergy responses were investigated through contact sensitization responses induced by 2,4-dinitrochlorobenzene (DNCB). Groups of 3 mice received a diet of 400 g of normal feed mixed with 210 ml of water containing 8 g 1,2,4-Trihydroxybenzene (1.3% w/w) for 1 - 2 wk, with or without exposure to air containing 10 ppm nitrogen dioxide. Control groups received normal feed mixed with untreated water, with or without exposure to nitrogen dioxide. The mice then underwent a local lymph node assay (LLNA) where both ears were treated with 25 µl of 1% (w/v) DNCB in acetone/olive oil (4:1 v/v). Lymph node weight was significantly lowered (p not reported) in mice fed a diet containing1,2,4-Trihydroxybenzene, but total lymph node cell (LNC) number was unaffected. No difference in the weight and LNC number was noted between nitrogen dioxide exposed and non-exposed groups. The degree of DNCB-sensitized cell proliferation was increased approximately 2-fold by supplementation of 1,2,4-Trihydroxybenzene in both nitrogen dioxide exposed and non-exposed groups.

Type I allergy responses were measured in serum immunoglobulin E (IgE) levels in the mice treated with the test material as described above. The mice were then sensitized using 50 µl of 1% DNCB or 25% (w/v) trimellitic anhydride (TMA) solution in acetone/olive oil on both shaved flanks. Controls were the same as described above. After 7 d, the mice received 25 µl of the same inducing agent on both ears. Seven days after the challenge, serum IgE was measured in an enzyme-linked immunosorbent assay (ELISA). 1,2,4-Trihydroxybenzene enhanced the serum IgE levels induced by DNCB and TMA, with no significant differences noted in the IgE levels between the nitrogen dioxide exposed and non-exposed groups. TMA sensitization was enhanced to a greater extent by 1,2,4-Trihydroxybenzene. Effects produced by nitrogen dioxide inhalation were observed only in control mice with TMA sensitization, but not in mice that received 1,2,4-

Trihydroxybenzene. The authors concluded that intake of a large amount of 1,2,4-Trihydroxybenzene may have an adverse effect on both type IV and type I allergy responses.[24]

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

The irritation potential of 3% 1,2,4-Trihydroxybenzene (98.1% pure) in water was assessed in 3 male New Zealand White rabbits in accordance with OECD TG 404.[2,3,5] The test material (0.5 ml) was applied to 1 rabbit for durations of 3 min, 1 h, and 4 h. In the remaining 2 rabbits, the test material was applied for durations of 1 h and 4 h, each. The test material was placed on a dry gauze pad that was then applied to the clipped flanks of the animals with a semi-occluded dressing. The sites were clipped thereafter on several days up to day 9. Untreated skin served as the control. After 3 min, very slight or well-defined erythema (grade 1 or 2) was noted from day 2 to day 6. After the 1-h exposure in the same animal, a very slight or well-defined erythema (grade 1 or 2) was noted from day 1 to day 8. In the other 2 animals, discrete erythema was noted on day 1 and 2 in 1 animal and no erythema was observed in the other animal. After the 4-h exposure, a brown coloration of the skin was noted in all animals from day 1 to day 2, 6, or 9; this could have masked very slight or well-defined erythema. No other cutaneous reactions were recorded during the study. Based on the 1-h exposure, 3% 1,2,4-Trihydroxybenzene in water was slightly irritating to rabbit skin.

Sensitization

In Vitro

In an antioxidant responsive element-nuclear factor erythroid 2-related factor 2 (ARE-Nrf2) luciferase KeratinoSens™ test, HaCaT keratinocytes were exposed to 1,2,4-Trihydroxybenzene (97.8% pure) at concentrations ranging from 0.977 - 2000 µM in 1% dimethyl sulfoxide (DMSO) in 1% Dulbecco's modified Eagle medium.[4,5] The test was performed in accordance with OECD TG 442D. 1,2,4-Trihydroxybenzene was tested in 3 definitive assays, and the positive control was cinnamic aldehyde at concentrations ranging from 4 - 64 µM. The interpolated concentration resulting in a 1.5-fold luciferase induction (EC1.5) value for the test material was 374.31 µM, and is below the threshold value of 1000 µM. For comparison, the EC1.5 value of cinnamic aldehyde was 10.37 µM. 1,2,4-Trihydroxybenzene has the potential to be a sensitizer, but is not equivalent to the potent sensitizer, cinnamic aldehyde. The SCCS noted that 1,2,4-Trihydroxybenzene was positive at a concentration of 500 µM, but the dose-response curve had a large variation in gene induction at 500 µM. According to test guidelines, the assay is positive when gene induction is statistically significant from the solvent control in at least 2 out of 3 replicates. Statistical analysis of the data for the results was not provided, and the SCCS determined the results of the assay inconclusive.

Animal

An LLNA was performed using 1,2,4-Trihydroxybenzene (98.1% pure) in accordance with OECD TG 429.[2,3] Female CBA mice were divided into groups of 4 and received 0.25, 0.5, 1, 2.5, or 5% (w/v) of the test material in dimethylformamide in experiment 1 and 0.01, 0.05, 0.1. 0.25, or 0.5% (solvent not stated) in experiment 2. The test material was applied to the ear surface (25 µl) once daily for 3 consecutive days. α-Hexylcinnamaldehyde (25% v/v) was used as the positive control. Five days after the first topical application, all animals were injected intravenously with [^{3}H]methyl thymidine and the proliferation of lymphocytes in the draining lymph nodes was measured.

No clinical signs or mortality related to treatment were observed. In experiment 1, dryness of the skin was noted on day 6 in 2/4 and 4/4 animals that received the test material at 1 and 2.5%, respectively. Additionally, a moderate increase in ear thickness (up to 45%) was observed at 2.5 and 5%, indicating irritation potential of the test material at these concentrations. No cutaneous reactions or noteworthy increases in ear thickness was observed in experiment 2.

In experiment 1, positive lymphoproliferative responses were observed at all tested concentrations, but without a clear dose-response relationship. Positive responses observed at concentrations of 0.25 and 0.5% were attributed to delayed contact hypersensitivity as there was no local irritation. The stimulation indices (SI) in experiment 1 ranged from 12.68 to 26.41 using concentrations from 0.25 to 5%. In experiment 2, a dose-related increase in SI (except for 0.1%) was noted and the threshold positive value of 3 was exceeded at 0.25%. The estimated concentration for an SI of 3 (EC_3) was calculated on the basis of the results in experiment 2 to be 0.08%. It was concluded that 1,2,4-Trihydroxybenzene induced delayed contact hypersensitivity, and based on the EC_3 value, should be categorized as an extreme sensitizer.[2,3]

OCULAR IRRITATION STUDIES

Animal

In an ocular irritation study performed in accordance with OECD TG 405, 3 male New Zealand rabbits received approximately 0.1 ml of a 3% dilution of 1,2,4-Trihydroxybenzene in water in the conjunctival sac of the left eye.[2,3] The right eyes served as the controls. The eyes were not rinsed after administration of the test material. Eyes were observed for reactions 1, 24, 48, and 72 h after instillation. Very slight chemosis and very slight redness of the conjunctiva were observed in all animals on day 1, which persisted in 2 of the 3 animals up to day 3. No other reactions were observed. It was concluded that 3% 1,2,4-Trhydroxybenzene was slightly irritating to rabbit eyes.

MARGIN OF SAFETY

A margin of safety (MOS) was calculated for 2.5% 1,2,4-Trihydroxybenzene to be 467 for a dermal exposure. This calculation is based on the LOAEL of 50 mg/kg bw/d from a 90-d oral rat study as a point of departure (PoD), an assessment factor of 3 for extrapolation from LOAEL to NOAEL, and an SED of 0.03577 mg/kg bw (skin area surface of 580 cm^2 x absorption through skin of 3.70 $\mu g/cm^2$ x 0.001 (unit conversion)/typical human bw of 60 kg).

The parameters that are used for the MOS calculation are listed below:

Systemically available dose: 3.70 $\mu g_{eq}/cm^2$ (derived from an in vitro study using frozen human dermatomed skin)[4]
Skin surface area for application: 580 cm^2 (½ area head)[58]
Dermal absorption per treatment: 3.70 $\mu g_{eq}/cm^2 \times 580\ cm^2 = 2146\ \mu g = 2.146$ mg
Human body weight: 60 kg

SED: 2.146 mg ÷ 60 kg = 0.03577 mg/kg bw/d
LOAEL: 50 mg/kg bw/d (90-d, gavage, oral, rat)[2,3]
NOAEL (extrapolated from LOAEL using an assessment factor of 3): 50 mg/kg bw/d ÷ 3 = 16.67 mg/kg bw/d

$$MOS = \frac{NOAEL}{SED} = \frac{16.67\ mg/kg\ bw/d}{0.03577\ mg/kg\ bw/d} = 466$$

The resulting MOS is greater than 100, which is generally considered to be protective. The standard of MOS value of 100 is derived from multiplying two factors: a 10-fold factor accounts for the extrapolating data from test animals to human being (interspecies extrapolation), and an additional 10-fold for accommodating differences among the human population (intra-species extrapolation).

HAIR DYE EPIDEMIOLOGY

Hair dyes may be broadly grouped into oxidative (permanent) and direct (temporary or semi-permanent) dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes consist of preformed colors. 1,2,4-Trihydroxybenzene is reported to be used in oxidative hair dye formulations. While the safety of individual hair dye ingredients is not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer. A detailed summary of the available hair dye epidemiology data is available at https://www.cir-safety.org/cir-findings.

SUMMARY

1,2,4-Trihydroxybenzene is reported to function as a hair colorant in cosmetics, according to the *Dictionary*. It is an oxidative dye used in permanent hair dye formulations and gradual hair coloring shampoos; this ingredient does not require hydrogen peroxide to activate oxidation and subsequent coupling reactions.

1,2,4-Trihydroxybenzene is a metabolite in biodegradation of aromatic compounds, such as benzene. It also occurs as a biodegradation product of fungi, yeast, and bacteria of aromatic compounds, and is found in roasted coffee beans.

According to 2023 VCRP survey data, 1,2,4-Trihydroxybenzene is reported to be used in 18 hair dye formulations and 1 hair shampoo (coloring). The results of the concentration of use survey conducted by the Council indicate 1,2,4-Trihydroxybenzene is used at up to 2.5% in hair dyes and colors. Under European regulations for cosmetic ingredients, 1,2,4-Trihydroxybenzene, when used as a substance in hair and eyelash dye products, is categorized in Annex II, the list of substances prohibited in cosmetic products in Europe due to potential genotoxicity.

In a dermal penetration study, the maximum absorption of a formulation containing 2.78% 1,2,4-Trihydroxybenzene through dermatomed human skin (~ 400 µm) was 0.17 $\mu g/cm^2$ or 0.03% after being corrected by + 2 standard deviation from 0.07 $\pm$ 0.05 $\mu g/cm^2$ (0.01 $\pm$ 0.01%) due to correction of the concentration tested (originally reported at 3% instead of 2.78%). The total systemically available dose of a hair dye formulation containing 2.5% 1,2,4-Trihydroxybenzene was 1.94 $\mu g_{eq}/cm^2$ (0.393%) with 2.25% *p*-toluenediamine and 1.13 $\mu g_{eq}/cm^2$ (0.226%) without *p*-toluenediamine.

In an acute dermal toxicity study in rats, no mortality was observed following a dermal dose of 2000 mg/kg bw 1,2,4-Trihydroxybenzene. The LD_{50} in an acute oral toxicity rat study of 1,2,4-Trihydroxybenzene was between 350 and 500 mg/kg bw for a preparation containing 1% 1,2,4-Trihydroxybenzene.

No observable toxic effects were noted in mice that received 1.3% (w/w) Trihydroxybenzene in feed mixed with water for 1 wk. In a 4-wk study in which mice received 100 or 500 mg/l 1,2,4-Trihydroxybenzene, feed intake and water consumption decreased, and serum and urinary hydrogen peroxide levels increased with the test material. Reduced blood nitric oxide metabolites and liver *S*-nitrosylated protein levels and decreased whole-body fat utilization were observed following treatment with 1,2,4-Trihydroxybenzene, with the latter occurring in a dose-dependent manner. In another 4-wk

oral dietary study, 1.5% 1,2,4-Trihydroxybenzene increased DNA synthesis in a BrDU assay, but was not associated with any hyperplasia changes in glandular stomach mucosa.

In a 90-d gavage study of 1,2,4-Trihydroxybenzene in rats, the NOAEL was determined to be 50 mg/kg bw/d, according to the researchers; this value was determined to be an LOAEL by the SCCP. Statistically significant increases in the absolute weight and/or organ-to-body weight ratios were observed in treated males for the spleen (all dose levels), liver and kidney (100 and 200 mg/kg bw/d), and testes and heart (200 mg/kg bw/d). In the females, statically significant increases in the absolute weight and/or organ-to-body weight ratios were observed for the liver, spleen, and kidneys at 200 mg/kg bw/d.

In a teratogenicity study in which gravid female Sprague-Dawley rats were dosed by gavage with up to 300 mg/kg bw/d of the test article on gestation days 6 through 15, 1,2,4-Trihydroxybenzene was maternotoxic at 300 mg/kg bw/d. Embryotoxicity and teratogenicity were not observed in the fetuses.

1,2,4-Trihydroxybenzene is a compound known to induce DNA damage and is considered genotoxic in vitro. This compound can result in oxidative DNA damage, which is linked to the generation of ROS. In in vitro studies where auto-oxidation was not minimized or efforts to reduce auto-oxidization were not reported, 1,2,4-Trihydroxybenzene was mutagenic in Ames tests when tested at up to 4000 µg/plate. Genotoxicity of 1,2,4-Trihydroxybenzene was observed in a gene mutation assay of mutants to 6-thioguanine (tested at up to a maximal concentration of 10 µM), DNA strand break tests (at up to 1000 µM), DNA synthetic activity inhibition assays (at up to 24 µM), and SCE assays (at up to 500 µM). 1,2,4-Trihydroxybenzene was not genotoxic in a gene mutation test at the *hprt* locus (up to 240 µg/ml) and was not clastogenic in a chromosome aberration test (at up to 20 µg/ml). Increases in micronucleus induction were observed in Chinese hamster V79 cells (at up to 25 µM) and human TK6 lymphoblastoid cells (at up to 30 µg/ml). 1,2,4-Trihydroxybenzene was clastogenic and aneugenic in a cytokinesis-block micronucleus test with human lymphocytes at up to 100 µM. Chromosomal damaged was observed in FISH procedures when 1,2,4-Trihydroxybenzene was tested at up to 100 µM.

In studies where degassed solutions were used, 1,2,4-Trihydroxybenzene was mutagenic in an Ames test when tested at up to 5000 µg/plate; however, the mutagenic effect of 1,2,4-Trihydroxybenzene was eliminated in another Ames test when evaluated in the presence of radical scavengers catalase (up to 20,000 IU) and L-glutathione (up to 10 µM). Catalase also reduced the toxicity of 1,2,4-Trihydroxybenzne. In other studies with degassed solutions, 1,2,4-Trihydroxybenzene was not genotoxic in a 3D comet assay (at up to 1250 µg/ml) or in micronucleus assays with human reconstructed skin tissue and human lymphocytes (at up to 224 µg/ml). In in vivo micronucleus tests in mice, 1,2,4-Trihydroxybenzene was not genotoxic when tested intraperitoneally at up to 50 mg/kg bw.

Oxidant-mediated genotoxicity of 1,2,4-Trihydroxybenzene is a multifactorial process. Redox reactions of 1,2,4-Trihydroxybenzene yield oxygen species, semiquinones, and quinones. Copper salts stimulate oxidation of 1,2,4-Trihydroxybenzene, leading to 1,2,4-Trihydroxybenzene-induced genotoxicity.

No conclusion as to the carcinogenic potential of a hair dye formulation containing 0.5% 1,2,4-Trihydroxybenzene could be made in a 2-yr dermal study of mice. 1,2,4-Trihydroxybenzene was cytotoxic in a dose-dependent manner in K562 cells, but it was determined to be less cytotoxic than other benzene metabolites in HL-60 cells. 1,2,4-Trihydroxybenzene has been studied for its role in benzene hematotoxicity, oxidative stress, neuroprotective effects, and potential melanogenesis inhibition. Based on a study in mice, 1,2,4-Trihydroxybenzene may have an adverse effect on both allergen-sensitized type IV and type I allergy responses.

In a dermal irritation study, 3% 1,2,4-Trihydroxybenzene was slightly irritating to rabbit skin. 1,2,4-Trihydroxybenzene was predicted to be a sensitizer in an ARE-Nrf2 luciferase KeratinoSens™ test, and was categorized as an extreme sensitizer in an LLNA when tested at up to 5% in dimethylformamide and at up to 0.5% (solvent not stated). In ocular studies, 3% 1,2,4-Trihydroxybenzene was slightly irritating to rabbit eyes.

An MOS for 2.5% 1,2,4-Trihydroxybenzene was determined to be 466 for a dermal exposure. This calculation is based on the LOAEL of 50 mg/kg bw/d from a 90-d oral rat study as a PoD, an assessment factor of 3 for extrapolation from LOAEL to NOAEL, and an SED of 0.03577 mg/kg bw/d. The MOS value is greater than 100, a figure generally accepted as the threshold for considering an ingredient safe to use.

The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer.

DISCUSSION

1,2,4-Trihydroxybenzene is reported to function as an oxidative hair dye in hair coloring products. The Panel noted that while results of in vivo micronucleus studies were negative, in vitro genotoxicity studies yielded mixed results; however, concern for these mixed results was mitigated by the weight-of-evidence of the negative genotoxicity results in vivo under conditions of use, the negative results for other toxicity endpoints, and slow absorption through the skin. Additionally, enzymes present in the skin deactivate harmful ROS species following dermal exposure. The Panel considered these findings, coupled with the short exposure time as a rinse-off product, and determined that the data are sufficient to conclude that 1,2,4-Trihydroxybenzene is safe for use as an oxidative hair dye ingredient in the present practices of use and concentration.

The Panel also noted that 1,2,4-Trihydroxybenzene is a potent suicide substrate of tyrosinase, i.e., the action of tyrosinase on 1,2,4-Trihydroxybenzene may cause the enzyme's own inactivation. As tyrosinase is a key enzyme involved in the synthesis of the pigment melanin, the inactivation of tyrosinase by 1,2,4-Trihydroxybenzene may play a role in skin depigmentation. The Panel noted that depigmentation is considered to be a drug effect in the US, and should not occur during the use of cosmetic products. Cosmetic formulators should only use 1,2,4-Trihydroxybenzene in products in a manner that does not cause depigmentation.

The Panel recognizes that hair dyes containing this ingredient, as coal tar hair dye products, are exempt from certain adulteration and color additive provisions of the FD&C Act, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures. The Panel considered concerns that such self-testing might induce sensitization, but agreed that there was not a sufficient basis for changing this advice to consumers at this time.

In considering hair dye epidemiology data, the Panel concluded that the available epidemiology studies are insufficient to scientifically support a causal relationship between hair dye use and cancer or other toxicological endpoints, based on lack of strength of the associations and inconsistency of findings. Use of direct hair dyes, while not the focus in all investigations, appears to have little evidence of any association with adverse events as reported in epidemiology studies.

The Panel's respiratory exposure resource document (available at https://www.cir-safety.org/cir-findings) 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 1,2,4-Trihydroxybenzene is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Chemical properties

Property	Value	Reference
Physical Form	light-medium beige powder	4
Molecular Weight (g/mol)	126.11	2
Melting Point (ºC)	139 - 150	2,3
Water Solubility (g/l @ 20 ºC)	486	3
Other Solubility (g/100 ml @ 22 ºC)	ethanol: > 1, < 10 DMSO: > 10, < 20	3
log P_{ow}	0.2 (estimated)	2
UV/Visible Spectrum (λ_{max}; nm)	291	3

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
			IN VITRO			
1,2,4-Trihydroxybenzene (purity not stated)	2.5 - 40 µg/plate (without metabolic activation); 1 – 2000 µg/plate (with metabolic activation)	not reported	*Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA104, TA1535	Bacterial reverse mutation test performed with and without metabolic activation	Mutagenic; test substance induced gene mutation in strain TA104 with metabolic activation	41
1,2,4-Trihydroxybenzene; 98.1% pure	6.25 - 4000 µg/plate	purified water	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; with and without S9 metabolic activation	Mutagenic; test material induced gene mutations in strains TA98 and TA100 without metabolic activation; toxic effects (i.e., reduction in the number of revertant colonies and/or thinning of the bacterial lawn) was observed at higher concentration with and without metabolic activation in nearly all strains used	2,3
1,2,4-Trihydroxybenzene; 97.8 - 99.5% pure	6.7 - 5000 µg/plate	degassed water	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; with and without S9 metabolic activation	Mutagenic; test material induced positive mutagenic response in strain TA1537 without metabolic activation; results were negative for strains TA98, TA100, TA102, and TA1535	4,5
1,2,4-Trihydroxybenzene; 98.1% pure	100 - 500 µg/plate	degassed water	*S. typhimurium* strain TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; without S9 metabolic activation; study evaluated the effect of radical scavengers, catalase (1000 - 20,000 IU) and L-glutathione (5 – 15 µM), with the test material	Mutagenic; effect observed in the study described above was repeated in test strain without metabolic activation; mutagenic effect was eliminated in presence of 5 and 10 µM L-glutathione and in the presence of 1000 - 20,000 IU of catalase	4,5
1,2,4-Trihydroxybenzene; 99.4% pure	Test 1: up to 20 µg/ml without metabolic activation and up to 160 µg/ml with metabolic activation Test 2: up to 22.5 µg/ml without metabolic activation and up to 240 µg/ml with metabolic activation	not reported	L5178Y mouse lymphoma cells	Mammalian cell gene mutation test at the *hprt* locus in accordance with OECD TG 476; with and without metabolic activation; appropriate negative and positive controls used	Not genotoxic; no statistically significant increase in mutant frequency was observed at any dose level tested, with or without metabolic activation; positive and negative controls yielded expected results	2,3

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
1,2,4-Trihydroxybenzene (purity not stated)	At least 5 concentrations used; optimal/maximal concentration of 10 µM; no further details provided	not reported	Chinese hamster V79 cells	Gene mutation assay; cells were incubated with test material for 24 h; frequency of mutants to 6-thioguanine (7 µg/ml) determined after an expression period of 6 d	Genotoxic; gene mutations induced at optimal/maximal concentration of 10 µM	41
1,2,4-Trihydoxybenzene; purity not reported	6, 12, or 24 µM	not reported	ICR mouse bone marrow cells	DNA strand break test; cells treated with test material for 1 h; alkaline DNA elution method (pH > 9.5) used; fractions collected every 24 min over a total of 120 min; cells exposed to test material also evaluated for protective effects of glutathione (350 µg/ml) and catalase (130 IU/ml) tested in parallel	Genotoxic; concentration-dependent increase in alkali-labile DNA single strand breaks observed, with a 42% increase at the highest concentration tested; double-strand breaks were not observed, but a significant pH-dependent increase in DNA elution rate was observed in treated cells when the elution pH increased from 9.6 to 12.6; DNA damage by test material was 53% blocked by glutathione and completely blocked by catalase	3,42
1,2,4-Trihydroxybenzene; 99.4% pure	Test 1: 1.25 - 5 µg/ml without metabolic activation and 3.75 - 15 µg/ml with metabolic activation Test 2: 2.5 - 7.5 µg/ml without metabolic activation and 10 - 20 µg/ml with metabolic activation	not reported	human lymphocytes	Mammalian chromosome aberration test in accordance with OECD TG 473, with and without S9 metabolic activation; appropriate negative and positive controls used	Not clastogenic; test material did not induce any significant increase in aberrant cell frequency, with or without metabolic activation; however, test concentrations did not induce required degree of cytotoxicity and an insufficient number of cells was evaluated in some cases	2
1,2,4-Trihydroxybenzene; purity not reported	24 µM	not reported	Crl:COBS CD-1 ICR BR mice bone marrow cells	DNA synthetic activity inhibition assay; DNA synthesis evaluated via the addition of [^{3}H]thymidine into DNA	Genotoxic; the test substance inhibited 64% of nuclear DNA synthetic activity; IC_{50} determined to be 19.4 µM	43
1,2,4-Trihydroxybenzene; purity not reported	0 - 24 µM	not reported	cell-free DNA assay system	DNA synthetic activity inhibition assay; test substance incubated with reaction mixture containing DNA polymerase (either DNA polymerase α or DNA polymerase I) for 30 min, followed by the addition of ^{3}H-TTP; filtered precipitated DNA evaluated for radioactivity	Genotoxic; when DNA polymerase α was used as the source of DNA polymerase, a dose-related inhibition of DNA synthesis was observed (IC_{50} = 15 µM); no inhibitory effect observed when DNA polymerase I was used	43
1,2,4-Trihydroxybenzene; purity not reported	1 - 1000 µM	not reported	L5178YS mouse lymphoma cells	DNA strand break test; cells exposed to test substance, followed by alkaline denaturation method, including hydroxylapatite chromatography, to separate single- and double-stranded DNA in order to examine DNA strand breaks	Genotoxic; the test substance produced DNA breaks in a dose-related fashion; ED_{50} values for induction of single-stranded DNA was 55µM	33
1,2,4-Trihydroxybenzene (purity not stated)	At least 5 concentrations used up to 25 µM, no further details provided	not reported	Chinese hamster V79 cells	Micronucleus assay; cells were incubated with test material for 24 h	Genotoxic; elevated frequencies of micronucleated cells observed at 25 µM	41

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
1,2,4-Trihydroxybenzene; 98.1% pure	Range finding: 0.50 - 200 µg/cm^2 Test 1: 1.5 - 200 µg/cm^2 Test 2: 12 – 224 µg/cm^2 Test 3: 3 - 224 µg/cm^2	degassed acetone	MatTek EpiDerm™ human reconstructed skin tissue	Micronucleus assay; 3 tissue models per concentration in Tests 1, 2, and 3; tissue was exposed to 10 µl of test material in solution and incubated for 24 h twice (48 h total) before harvesting in Tests 1 and 2; Test 3 was confirmatory and 72 h in duration	Not genotoxic; induction of micronuclei did not occur in reconstructed skin	4,5
1,2,4-Trihydroxybenzene; 97.8% pure	Range finding: 0.126 - 1260 µg/ml, with and without metabolic activation 4 h exposure + 20 h: 1.26 - 150 µg/ml, with and without metabolic activation 24 h exposure: 0.1 - 100 µg/ml without metabolic activation	degassed water	human lymphocytes	Micronucleus test in accordance with OECD TG 487, with and without S9 metabolic activation; appropriate negative and positive controls used	Not genotoxic; percentage of cells with micronuclei in treated group not significantly increased relative to vehicle control at any dose level, with or without metabolic activation; positive and negative controls yielded expected results; substantial cytotoxicity (55 ± 5% or greater reduction in cytokinesis-blocked proliferation index relative to vehicle control at dose levels ≥ 50 µg/ml in non-activated 4 h treatment group, at dose levels ≥ 100 µg/ml metabolic activated 4 h treatment group, and at dose levels ≥ 30 µg/ml in the non-metabolic activated 24 h treatment group	4,5
1,2,4-Trihydroxybenzene; purity not reported	15, 20, 30 µg/ml	DMSO and water	human TK6 lymphoblastoid cells	Flow cytometry assay used to measure relative survival, apoptotic/necrotic cells, and micronucleus induction in parallel with application of TGx-28.65 genomic biomarker; lymphoblastoid cell exposures to test substance occurred in the presence of 2% hepatic S9 fraction; vehicle used as negative control; benzo[*a*]pyrene used as positive control; TK6 cells exposed for 4 h, rinsed, re-suspended, and re-incubated for additional 3 - 4 h for gene expression analysis and 20 h for flow cytometry analysis note: TGx-28.65 genomic biomarkers were developed by the authors were previously based on a database of gene expression profiles derived from human TK6 cells exposed to 28 well-known compounds; the biomarker comprises 65 genes that can classify chemicals as DNA damaging or non-DNA damaging	Genotoxic; dose-dependent declines in relative survival and increase in apoptosis; strong significant increase in micronucleus induction at all concentrations; the test substance was considered to be genotoxic at all three test concentrations. Controls gave expected results. TGx-28.65 analysis classified the test substance as genotoxic.	44

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
1,2,4-Trihydoxybenezene; purity not reported	10, 25, 50 or 100 µM	not reported	human lymphocytes	Cytokinesis-block micronucleus test in combination with a fluorescence in situ hybridization technique with specific centromeric probes for chromosomes 7 and 8; lymphocytes were treated for 48 h with test material; 1000 binuclear cells scored for presence of micronuclei	Clastogenic and aneugenic; concentration-dependent and statistically significant increase in number of lymphocytes with micronuclei observed; concentration-dependent and statistically significant induction of aneuploidy of chromosomes 7 and 8 observed, with aneuploidy 8 being more frequent; non-disjunction of chromosomes 7 and 8 also observed	3,46
1,2,4-Trihydroxybenzene (purity not stated)	Optimal/maximal concentration was 10 µM, no further details provided	not reported	Chinese hamster V79 cells	SCE assay; cells were incubated with test material for 27 h; a total of 30 metaphases were scored for SCE per data point	Genotoxic; statistically significant ($p > 0.01$) number of SCE per cell above background at an optimal/maximal concentration of 10 µM	41
1,2,4-Trihydroxybenzene; 99% pure	5, 50, 70, 100, 300, or 500 µM	RPMI 1640 medium	human lymphocytes	SCE assay; lymphocytes treated with test material for 48 h in presence of BrdU	Genotoxic; concentration-dependent decrease of mitotic activity observed; concentration-dependent increase in SCE observed	3,45
1,2,4-Trihydroxybenzene; 97.8% pure	Range finding: 0.1 - 100 mg/ml or 1.6 - 1600 µg/cm^2 Test 1: 0.125 - 1 mg/ml or 2 - 16 µg/cm^2 Test 2: 0.25 - 1.25 mg/ml or 4 - 20 µg/cm^2	degassed acetone	Phenion® full thickness human skin model consisting of human primary keratinocytes and fibroblasts from single donor origin	3D skin comet assay; application volume was 16 µg/cm^2; total exposure time was 48 h; negative and positive controls were run in parallel	Not genotoxic; test material did not induce DNA damage to human skin cells after topical application; controls yielded expected results; cytotoxicity observed at and above 50 µg/cm^2 in adenylate kinase release and at 16 µg/cm^2 based on lactate dehydrogenase release during range finding study, in test 2, cytotoxicity observed at 20 µg/cm^2 based on measurement of intracellular adenosine triphosphate	4,5
1,2,4-Trihydroxybenzene; 99% pure	0, 5, 10, 20, 50. 80, or 100 µM	PBS	human HL60 cells	FISH procedure to detect aneuploidy; cells treated with test material at 24 h after culture initiation in duplicate and harvested after 48 h of exposure; centromeric probes specific for chromosomes 7 and 9 utilized; microtubule staining was also performed after 1 h of exposure	Genotoxic; treatment with 5 µM test material increased hyperdiploidy for chromosome 9 approximately 3-fold and 50 µM increased hyperdiploidy approximately 4-fold; similar results observed with chromosome 7 probe; majority of hyperdiploidy induced was trisomy; staining with anti-tubulin antibodies showed that the test material disrupted microtubule organization	37
1,2,4-Trihydroxybenzene; 99% pure	10, 25, and 50 µM	PBS	human peripheral lymphocytes	FISH procedure; cells treated with test material at 24 h after culture initiation in duplicate and harvested after 48 h of exposure; metaphase spreads prepared and hybridized with centromeric probes for chromosomes 1, 5, and 7, and sequence specific probes for 5q31 and 7q36-qter	Genotoxic; test material significantly increased monosomy 5 and 7 by 3-5 fold ($p < 0.0001$); test material also significantly increased the rate of del(5q) and del(7q) by 8-12 fold ($p < 0.0001$); chromosome 7 was especially susceptible to aneusomy induction at lower dose: treatment at 10 µM increased monosomy of chromosome 7, but not of chromosome 1 or 5	36

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
1,2,4-Trihydroxybenzene; 99% pure	10, 25, and 50 µM	PBS	human peripheral blood	Blood exposed to test substance, and ploidy status of 9 different chromosomes evaluated using FISH of metaphase spreads; 48-h chemical exposure	Genotoxic; test substance produced a dose-dependent increase in monosomy of chromosomes 5, 7, 8, and 9, but not of chromosomes 1 and 21; more profound effect observed on monosomy 5 and 7 compared to other chromosomes, and at low levels of exposure; the test substance also induced trisomy in all evaluated chromosomes in a dose-dependent manner	38
IN VIVO						
1,2,4-Trihydroxybenzene; 99.4% pure	50 mg/kg bw	water	5 Swiss OF1 mice per sex	Mammalian erythrocyte micronucleus test in accordance with OECD TG 474; single intraperitoneal dose; appropriate negative and positive controls used	Not genotoxic; number of micronucleated polychromatic erythrocytes did not differ statistically from the vehicle control values; ratio of polychromatic to normochromatic erythrocytes decreased significantly ($p < 0.05$) 24 h after treatment and ($p < 0.001$) 48 h after treatment, indicating a toxic effect of the test material to bone marrow cells; SCCP noted test not in accordance with current OECD TG as only 1 dose was tested	2
1,2,4-Trihydroxybenzene; 99% pure	6.3, 12.5, or 25 mg/kg (low and mid-concentrations did not meet acceptance criterion for % of targe, actual values achieved were 5.32 and 8.15 mg/kg); range finding doses were 25, 50, and 100 mg/kg	degassed deionized water	groups of 6 Hsd:ICR (CD-1) male mice; range finding study used groups of 3 males and 3 females mice of same strain	Mammalian erythrocyte micronucleus test in accordance with OECD TG 474; mice received single intraperitoneal injection (5 ml/kg); blood samples taken 1-h post dosing in 3 mice/dose group; micronucleated cells scored at 24 and 48 h post-dosing; appropriate negative and positive controls used	Not genotoxic; no statistically significant increase in the incidence of micronucleated polychromatic erythrocytes observed at either time point relative to the vehicle control; positive control yielded expected results; no detectable presence of test article in blood samples; in range finding study, all but 1 female died in the high dose group and mice in mid-dose group had severe clinical signs of toxicity	5,47

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